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ORIGINAL CONTRIBUTION

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# Tocopherol metabolites 2, 5, 7, 8-tetramethyl-2-(2′-carboxyethyl)-6-hydroxychroman (α-CEHC) and 2, 7, 8-trimethyl-2-(2′-carboxyethyl)-6-hydroxychroman (γ-CEHC) in human serum after a single dose of natural vitamin E

**Summary** *Background* α- and γ-Tocopherol are vitamin E compounds in human blood and tissues. α-CEHC (2,5,7,8-tetramethyl-2-(2′-carboxyethyl)-6-hydroxychroman) and γ-CEHC (2,7,8-trimethyl-2-(2′-carboxyethyl)-6-hydroxychroman) have been identified as water-soluble metabolites which are excreted with the urine in humans. *Aim of the study* To assess over-time changes of serum levels of α- and γ-CEHC in humans after a single dose of vitamin E from a natural source. *Methods* Twenty-one healthy subjects ingested a single dose of vitamin E (306 mg of RRR-α-tocopherol and 1.77 mg of γ-tocopherol). Blood was collected before (baseline) and 2, 6, 12, 24, 35, 50, and 74 h after ingestion. Serum was separated and levels of α- and γ-tocopherol and α- and γ-CEHC were determined by HPLC. *Results* After vitamin E ingestion, a statistically significant increase was observed for α-tocopherol and α-CEHC. Maximum serum levels for both compounds were measured 12 h after application (33.3 ± 11.1 μmol α-tocopherol /L and 42.4 ± 18.3 nmol α-CEHC /L); baseline values were reached again after 72 h. While γ-tocopherol levels decreased during the study period, an increase in the metabolite γ-CEHC was observed. The optical isomer formed in the metabolism of RRR-α-tocopherol was assigned as S-α-CEHC. *Conclusions* α-CEHC levels increase after administration of a single dose of natural vitamin E in humans. The appearance of the metabolite in blood parallels that of the parent compound. The γ-tocopherol analog appears to be metabolized more efficiently than α-tocopherol.

**Key words** Bioavailability – α-CEHC – γ-CEHC – optical isomers – α-tocopherol – γ-tocopherol

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## Introduction

Among the different vitamin E homologues, α-tocopherol is the predominant vitameric compound in human blood and tissues [1]. The uptake and transport of vitamin E follows the pathway of lipid absorption, and in the liver a specific transfer protein preferentially selects the RRR-α-tocopherol isomer for incorporation into lipoproteins [for review, see 2]. Other optical isomers or vitamers like γ-tocopherol appear in the blood only in small amounts, apparently being metabolized and excreted. In recent years, the metabolism of tocopherol has been reinvestigated and new metabolites were identified [3–5]. It was demonstrated that oxidative cleavage of the phytyl side chain yielding carboxylic acid derivatives is a major pathway of vitamin E metabolism; it seems that several enzymes including cytochrome-P450-dependent monooxygenases are involved in the reaction sequence that generates these metabolites [6–8]. Such kinds of metabolites, which still contain an intact chroman system but exhibit side chains of different length with an ω-carboxyl group, have been identified in vitro and in vivo [5, 7, 9, 10]. Among them, α-CEHC (2,5,7,8-tetramethyl-2-(2′-carboxyethyl)-6-hydroxychroman) and γ-CEHC (2,7,8-trimethyl-2-(2′-carboxyethyl)-6-hydroxychroman) are major metabolic derivatives of α- and γ-tocopherol and have been detected in urine and blood of humans and animals [11–13]. α-CEHC has been identified in human urine after high intake of α-tocopherol [3]; γ-CEHC

was first isolated from human urine while searching for a natriuretic hormone [14]. γ-CEHC shows interesting physiological properties which might be relevant in context with effects of the parent γ-tocopherol. Its natriuretic activity is mediated via the inhibition of 70 pS channels of the thick ascending limb of Henle's loop. In vitro experiments show that the compound inhibits cyclooxygenase activity in macrophages and epithelial cells [15]. Animal experiments demonstrate protective effects of γ-CEHC against metal-induced nephrotoxicity [16] which was discussed in context with possible antioxidant properties of the metabolite.

α- and γ-CEHC have been detected in nanomolar amounts in human blood. However, the time-dependent serum responses of the compounds following oral administration have not been evaluated. In the present study we investigated the serum levels of α- and γ-CEHC following the ingestion of a single dose of vitamin E from a natural source.

## Subjects and methods

### Subjects

The study was performed according to the Helsinki Declaration of 1975 as revised in 1983. Written, informed consent was obtained from each participant.

Twenty-one healthy, free-living subjects (14 m and 7 f) were included in the study. Thirteen subjects were smokers, eight non-smokers. None of the participants ingested vitamin supplements during the 4 months preceding or during the study. Demographic characteristics as well as BMI of the subjects are shown in Table 1.

All subjects received a single dose of natural vitamin E (Hermes, Munich, Germany) which contained 306 mg of RRR-α-tocopherol and 1.77 mg of γ-tocopherol. Capsules were taken together with the main meal. Blood was obtained by venipuncture before ingestion of the vitamin (baseline) and 2, 6, 12, 24, 35, 50, and 74 h after ingestion. Blood was allowed to clot for 10 min and centrifuged for 15 min at 4,500 rpm for serum preparation. Samples were stored at –40 °C until analyzed.

In a single experiment, one participant took 180 mg of synthetic all-rac-α-tocopherol-acetate (Capsules, Bad Heilbrunner, Bad Heilbrunn, Germany) every day for three days. Blood was collected 12 h after ingestion of the last capsule and serum was prepared as described above.

**Table 1** Characteristics of the subjects studied

| Parameter | All subjects (n = 21) | Smokers (n = 13) | Non-smokers (n = 8) |
|---|---|---|---|
| Age (years) | 31.4 ± 9.6 | 30.4 ± 10.6 | 33.0 ± 8.2 |
| Gender (M/F) | 14/7 | 10/3 | 4/4 |
| BMI | 24.1 ± 5.2 | 24.6 ± 5.9 | 23.2 ± 3.8 |

### Chemicals

Acetic acid, n-hexane, dichloromethane, ascorbic acid, butylated hydroxytoluene (BHT), EDTA, and α-tocopherol were obtained from E. Merck (Darmstadt, Germany).

### Sample preparation and analyses

Samples were prepared and analyzed for α- and γ-CEHC and α- and γ-tocopherol as described earlier [11]. The concentrations of the analytes were calculated according to the external standard mode.

Selected blood samples were additionally analyzed for the composition of α- and γ-CEHC from different optical isomers. For the separation of the optical isomers, a ChiraDex (250 × 4 mm) 5 µm column (E. Merck, Darmstadt, Germany) was used with a mobile phase consisting of acetonitrile/water/formic acid 30/70/0.01 (v/v/v). The flow rate was 1 ml/min; electrochemical detection was as described earlier [11]. It should be noted that, under the present conditions, the analytical column was not stable for longer than 6 d; the efficacy of isomer separation decreased rapidly after 6 h of use likely due to instability of the column material at low pH.

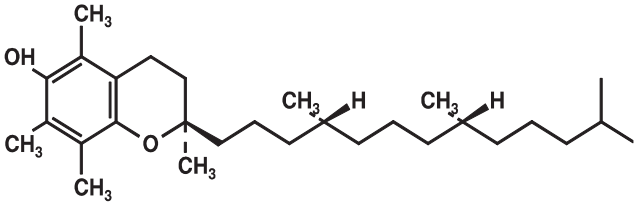
### Statistical analysis

Statistical analysis was performed with the program SPSS 10.0. All data are presented as mean ± S. D. Nonparametric Wilcoxon rank-sum test was used for comparisons between time points. Significance was accepted if the null hypothesis was rejected at the $p < 0.05$ level.

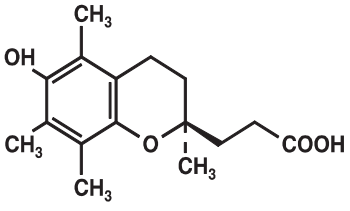
## Results

The structures of RRR-α-tocopherol and S-α-CEHC are shown in Fig. 1. Assuming that the configuration at the C-2 position is not affected – as already described for γ-CEHC [17] –, S-α-CEHC is the optical isomer which is formed in the metabolism of RRR-a-tocopherol.

Fig. 2 shows HPLC chromatograms (optical isomers not separated) of two serum samples obtained from the same subject before (baseline; lower trace) and 12 h after administration (upper trace) of a vitamin E supplement composed of 306 mg of RRR-α-tocopherol and 1.77 mg of γ-tocopherol. Before intake, the α-CEHC signal is low compared to the signal of γ-CEHC. Twelve




RRR-alpha-Tocopherol




S-alpha-CEHC

**Fig. 1** Chemical structures of RRR-α-tocopherol and of S-α-CEHC.

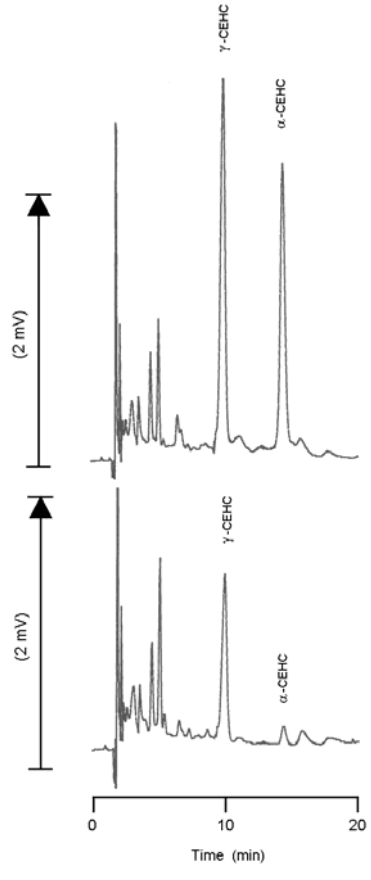



**Fig. 2** HPLC chromatogram of a serum sample obtained from the same subject before (lower trace) and 12 h after (upper trace) α-tocopherol ingestion.

hours after intake, the signal intensity of both metabolites has increased.

The time course of serum α-tocopherol and α-CEHC levels (mean ± SD for all participants) after administration of the supplement is shown in Fig. 3. Before vitamin E ingestion, the mean level of α-tocopherol was 22.3 ± 7.6 μmol/L. The concentrations increased after ingestion of vitamin E and reached a maximum at t = 12 h of 33.3 ± 11.1 μmol/L; baseline levels were approximately reached again after 72 h with 23.8 ± 9.0 μmol/L.

The levels of α-CEHC in serum followed the time course of α-tocopherol. Baseline levels before application of vitamin E were 15.1 ± 7.0 nmol/L. The level increased after ingestion of the supplement to reach a maximum at t = 12 h of 42.4 ± 18.3 nmol/L; 21.5 ± 9.2 nmol/L was found at t = 72 h which is somewhat higher than baseline values. α-Tocopherol administration induced significant over-time changes of both α-tocopherol and α-CEHC concentrations (non-parametric Wilcoxon rank-sum test: $p < 0.001$).

It is interesting to note that the mean serum levels of α-tocopherol in smokers were always lower than in non-smokers, and the mean levels of α-CEHC were higher in smokers than in non-smokers (data not shown). However, this difference was statistically not significant when the levels were corrected for BMI.

In addition to α-tocopherol, low amounts of γ-tocopherol were also present in the supplement used in this study. The concentration time courses of γ-tocopherol and γ-CEHC are presented in Fig. 4. γ-Tocopherol levels were similar (2.2 –2.6 μmol/L) before and at t = 2 h after ingestion of the supplement. Between 2 and 24 h they decreased to a level of about 1.7 μmol/L which was still de-

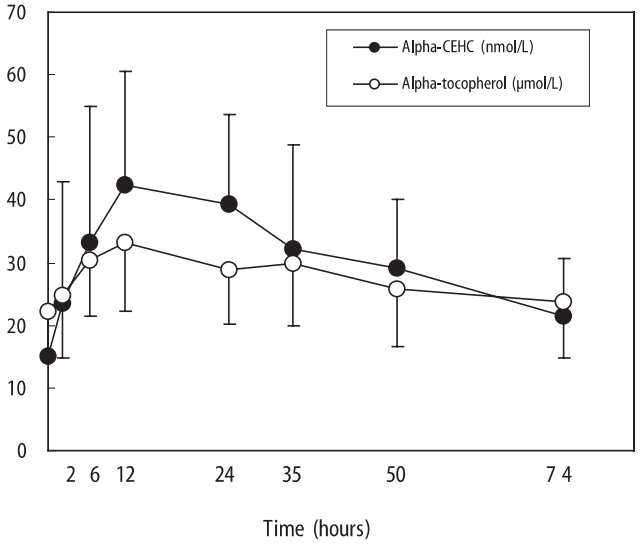



**Fig. 3** Over-time changes of serum α-tocopherol (open circles) and α-CEHC (closed circles) levels after ingestion of 306 mg of RRR-α-tocopherol and 1.77 mg of γ-tocopherol in a single dose.

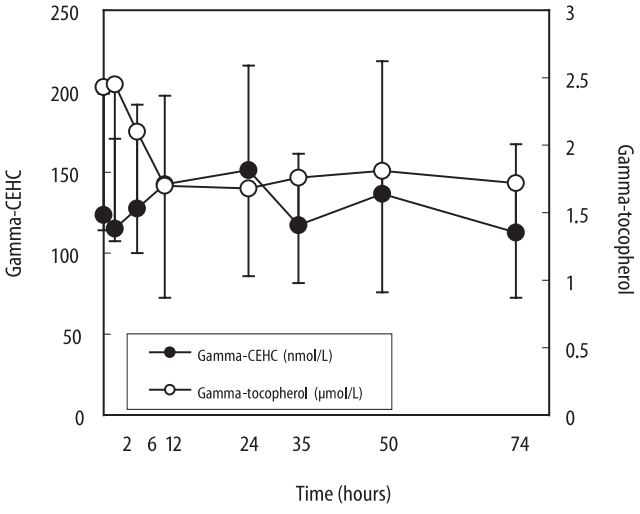



**Fig. 4** Over-time changes of serum γ-tocopherol (open circles) and γ-CEHC (closed circles) levels after ingestion of 306 mg of RRR-α-tocopherol and 1.77 mg of γ-tocopherol in a single dose.

tected 72 h after the start of the experiment. While γ-tocopherol decreased, increases in γ-CEHC levels were measured. Before vitamin E supplementation, serum γ-CEHC levels were 123.7 ± 74.7 nmol/L. γ-CEHC serum concentration increased and reached a maximum of 151.3 ± 64.6 nmol/L at t = 24 h; at t = 72 h the level was comparable to the baseline.

Overall concentration changes were found to be significant both for γ-tocopherol ($p < 0.001$) and γ-CEHC ($p < 0.02$).

By means of HPLC, applying a chiral stationary phase, it was possible to separate the two optical isomers of α-CEHC and γ-CEHC. Fig. 5A shows a chromatogram were a mixture of racemic standards of both these compounds were analyzed. Both isomeric forms are present in about equal amounts in the synthetic standards. Since none of the optical isomers was available as a pure standard compound, the isomers could not be assigned directly. The HPLC trace (with the chiral phase) of a selected serum sample from the study were RRR-α-tocopherol was applied is shown in Fig. 5B. It is obvious that only one optical isomer of each metabolite is dominating the chromatogram. This is the isomeric form that is formed in the metabolism of the RRR-isomer of the parent compound and was thus tentatively assigned as the S-isomer, S-α-CEHC. If a racemic α-tocopherol derivative is given, both isomers of α-CEHC appear in serum (Fig. 5C).

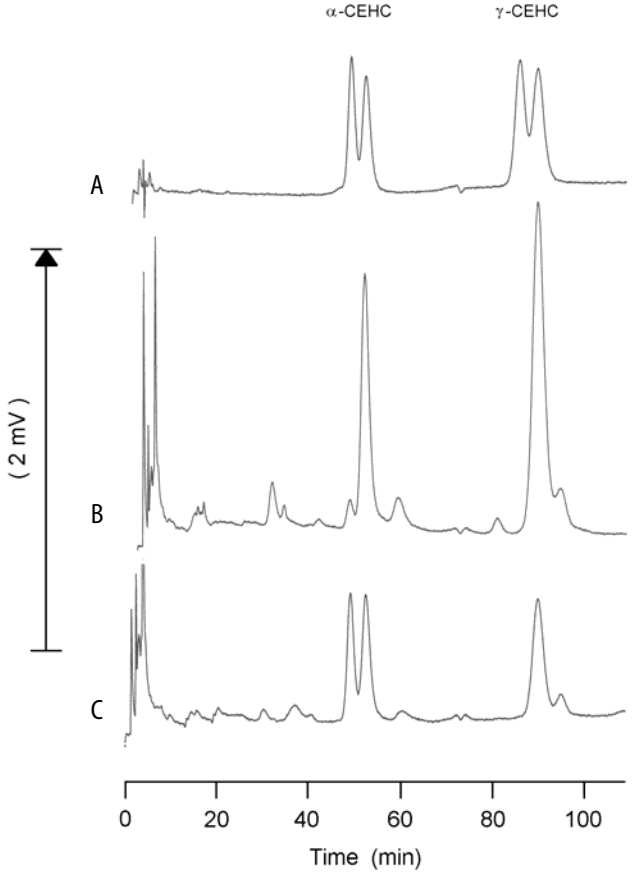



**Fig. 5** Separation of the optical isomers of α- and γ-CEHC by HPLC: **A** Standard compounds; **B** α- and γ-CEHC optical isomers in human serum after RRR-α-tocopherol administration; **C** α- and γ-CEHC optical isomers in human serum after addition of all-rac-α-tocopheryl acetate. γ-CEHC peak represents endogenous γ-CEHC only.

## Discussion

The metabolism of tocopherols in the human organism leads to the formation of two different kinds of metabolites [2]. Tocopherol quinones and analogous compounds are formed when vitamin E scavenges reactive oxygen species by transferring two electrons. In such reaction sequences the chroman ring is cleaved and a stable quinone is produced [1]. Other metabolites result from the oxidative cleavage of the phytyl side chain which leads to the formation of compounds that carry a carboxyl group and still possess an intact chroman system. α- and γ-CEHC as well as α- and γ-CMBHC (carboxy-methyl-butyl-hydroxy-chroman) are among those metabolites. Recent data suggest that α- and γ-CEHC constitute the major urinary metabolites of α- and γ-tocopherol [3, 4, 9].

Baseline γ-CEHC levels are higher than α-CEHC levels in human serum of unsupplemented persons (see Fig. 2), while the levels of α-tocopherol are higher than γ-tocopherol. These data are consistent with the literature, and the levels of CEHC and tocopherol shown here are in the range that has been reported earlier [11]. It is remarkable that the concentration of α-CEHC in serum

is about 1,500-fold lower than the parent compound. Even with ingestion of high amounts of α-tocopherol (306 mg) and an observed increase of α-tocopherol serum levels by roughly 10 μmol/L, α-CEHC levels increased by only about 30 nmol/L. This indicates that only a minor part of the dose is subjected to metabolism while most of the α-tocopherol is incorporated into lipoproteins. In the present study a vitamin E supplement from a natural source was used which also contained low amounts of γ-tocopherol. Although the dose of γ-tocopherol was 170-fold lower than α-tocopherol, the levels of γ-CEHC increased to about the same extent as α-CEHC. The levels of γ-tocopherol, however, decreased, suggesting that γ-tocopherol is more prone to metabolism than α-tocopherol. It has been shown that a tocopherol transfer protein is operative in liver and selects the RRR-α-tocopherol isomer for incorporation into the lipoproteins [17]. Other isomers are preferentially metabolized.

Metabolic oxidation of the phytyl tail results in a cleavage of the side chain. Such an enzymatic metabolism should not affect the configuration at the C-2 position; thus, it is assumed that S-α-CEHC is the optical isomer formed in the metabolism of RRR-α-tocopherol. The retention of the configuration at the C-2position has already been demonstrated for γ-CEHC [18]; S-γ-CEHC is the optical isomer that can be isolated from human urine. As expected, one major optical isomer of α- and γ-CEHC is detectable in human serum at baseline and after application of RRR-α-tocopherol. Two optical isomers, the S- and the R-form of α-CEHC were detectable when α-tocopherol was administered in a racemic form.

It is interesting to note that the levels of α-CEHC in smokers after intake of a vitamin E supplement appear to be higher than in non-smokers. In parallel, the levels of α-tocopherol are lower in smokers than in non-smokers, which is consistent with literature data [19]. It might be speculated that this is related to differences in metabolism. Cytochrome P-450-dependent monooxygenases are involved in the metabolism of tocopherols via oxidation of the phytyl side chain [7]. It is well known that smoking induces enzymes of this family which have an impact on the metabolism of xenobiotics [20]. Thus, inductory effects may be responsible for the increased levels of α-CEHC in smokers.

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