Urinary α-tocopherol metabolites in α-tocopherol transfer protein-deficient patients

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Abstract Patients with α-tocopherol transfer protein (α-TTP) defects experience neurological symptoms characteristic of vitamin E deficiency and depend on continuous high α-tocopherol supplements. We investigated the excretion of 2,5,7,8-tetramethyl-2(2′-carboxyethyl)-6-hydroxychroman (α-CEHC), a urinary metabolite of α-tocopherol, as a putative marker for the α-tocopherol status of α-TTP-deficient patients and control subjects. In three patients vitamin E supplementation was stopped for short periods of time, during which plasma α-tocopherol concentrations and urinary α-CEHC excretion were measured. In the patients, plasma α-tocopherol decreased below normal (<5 μmol/l) but α-CEHC excretion remained above the range of unsupplemented control subjects (0.118–0.306 mg/day, n = 6). In healthy subjects, however, α-CEHC excretion was increased only after surpassing a plasma α-tocopherol threshold of 30–40 μmol/l. Such a threshold did not exist in patients. The general mechanism of α-tocopherol degradation did not appear to differ between patients and control subjects. The presumed mechanism of ω- and subsequent β-oxidation was supported by the detection of α-CPHC, an α-CEHC homolog with a side chain longer by 3 carbon atoms, both in supplemented patients and in control subjects.—Schuelke, M., A. Elsner, B. Finckh, A. Kohlschütter, C. Hübner, and R. Brigelius-Flohé. **Urinary α-tocopherol metabolites in α-tocopherol transfer protein-deficient patients.** ***J. Lipid Res.*** **2000.** 41: **1543–1551.**

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Ataxia with isolated vitamin E deficiency (AVED) is a rare inherited disease characterized by a defect in the α-tocopherol transfer protein (α-TTP) (1, 2). α-TTP is primarily expressed in liver cytosol (3) and functions there as a sorting protein. It specifically selects α-tocopherol from all incoming tocopherols (4) for incorporation into very low density lipoprotein (VLDL) and subsequent release into the circulation (5). Apart from liver, α-TTP mRNA has been found in the Purkinje cell layer of the cerebellar cortex, in spleen, lung, and kidney from rodents (6). α-TTP protein has been detected in human cerebellar Purkinje cells but only in patients with diseases that are associated with oxidative stress or various vitamin E deficiency states (7). The specific enrichment of (*RRR*)-α-tocopherol in the fetal circulation points to the presence of α-TTP also in the placenta (8, 9), although this has not been directly demonstrated. Its role in these target tissues is unknown but it may be involved in appropriate vitamin E utilization.

Functionally relevant mutations in the α-TTP gene lead to a severe neurological disorder characterized by spinocerebellar dysfunction with progressive ataxia (10), in some cases associated with retinitis pigmentosa (11, 12). So far, 27 affected families with 13 different mutations have been described. The most frequent 744delA frameshift mutation (2, 13) truncates the protein by 11% and leads to an early onset of AVED. Other truncation mutations, 513–514insTT (14), 485delT (14), and 552G→A (15, 16), have similar consequences.

The result of a mutated α-TTP gene is a severe vitamin E deficiency syndrome despite normal intestinal absorption and chylomicron metabolism (17). The inability of mutant α-TTP to incorporate α-tocopherol into VLDL causes clinical symptoms that are similar to those observed in other vitamin E deficiency states such as abetalipoproteinemia (18, 19) or cholestatic liver disease (20). In line with the view that the clinical symptoms of AVED result from an impaired vitamin E supply, ataxia, dysmetria, dysarthria, increased daytime sleepiness, and mental symptoms can be ameliorated by supplementation with high doses of vitamin E up to 40 mg/kg body weight (12, 16, 21). These high doses, 250- to 350-fold the recommended daily allowance, normalize the plasma α-tocopherol levels to 30–40 μmol/l (16),

Abbreviations: α-CEHC, 2,5,7,8-tetramethyl-2(2′-carboxyethyl)-6-hydroxychroman; α-TPP, α-tocopherol transfer protein; AVED, ataxia with isolated vitamin E deficiency.

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probably by a direct exchange between chylomicrons and lipoproteins, thus bypassing the sorting function of α-TTP in the liver.

The main urinary metabolite of α-tocopherol, 2,5,7,8-tetramethyl-2(2′-carboxyethyl)-6-hydroxychroman (α-CEHC), results from a shortening of the phytyl side chain (22). Its chroman ring is left unchanged, indicating that the vitamin has not been used for its presumed role to scavenge oxidants or radicals. Also, α-CEHC is only marginally excreted in the urine of healthy subjects unless a certain plasma α-tocopherol threshold is exceeded (22, 23). On the basis of these observations we reasoned that α-tocopherol might be degraded only after the optimum plasma level has been met. As a consequence, α-CEHC excretion could be taken as a marker for (super)optimal α-tocopherol supply. In this study, however, we show that AVED patients have a high α-CEHC excretion despite low α-tocopherol plasma levels. Therefore, α-CEHC cannot be used to monitor therapy. We further describe a novel urinary α-tocopherol metabolite, 2,5,7,8-tetramethyl-2-(4′-carboxypentyl)-6-hydroxychroman (α-CPHC), which is the direct precursor of α-CEHC in the presumed β-oxidation of the α-tocopherol side chain.

MATERIALS AND METHODS

Patients

Previous experience had shown that short interruptions of vitamin E supplementation did not adversely affect patient health. Therefore we measured α-tocopherol plasma levels and urinary α-tocopherol metabolites in three AVED patients before, during, and after a short withdrawal of their vitamin E supplementation during a regular clinical check-up and readjustment of α-tocopherol dosage. Initial supplementation was 1,800 mg of all-*rac*-α-tocopheryl acetate per day, 2,070 mg of (*RRR*)-α-tocopherol per day, and 744 mg of all-*rac*-α-tocopheryl acetate per day, each for at least 1 year in patients 1 to 3, respectively. We did not obtain consent to equilibrate all patients on all-*rac*-α-tocopheryl acetate for a prolonged time before the withdrawal because patient 2 did not want to deviate from his trusted vitamin E brand for too long. For the same reasons patient 3 did not want to change from all-*rac*-α-tocopherol to (*RRR*)-α-tocopherol. Patient 2 and 3 underwent this study once, patient 1 underwent the study twice with an interval of 1 year between the tests (**Fig. 1A** and **B**). The withdrawal period, during which we did not observe any change of clinical symptoms, lasted 5 days for patient 1, and 7 days for patients 2 and 3. Resupplementation with all-*rac*-α-tocopheryl acetate in all three patients was restarted incrementally. Patient 1 received 400, 1,200, and 1,800 mg/day, patient 2 received 1,380, 2,070, and 2,760 mg/day, and patient 3 received 1,240, 1,860, and 2,480 mg/day. In patients 2 and 3 each

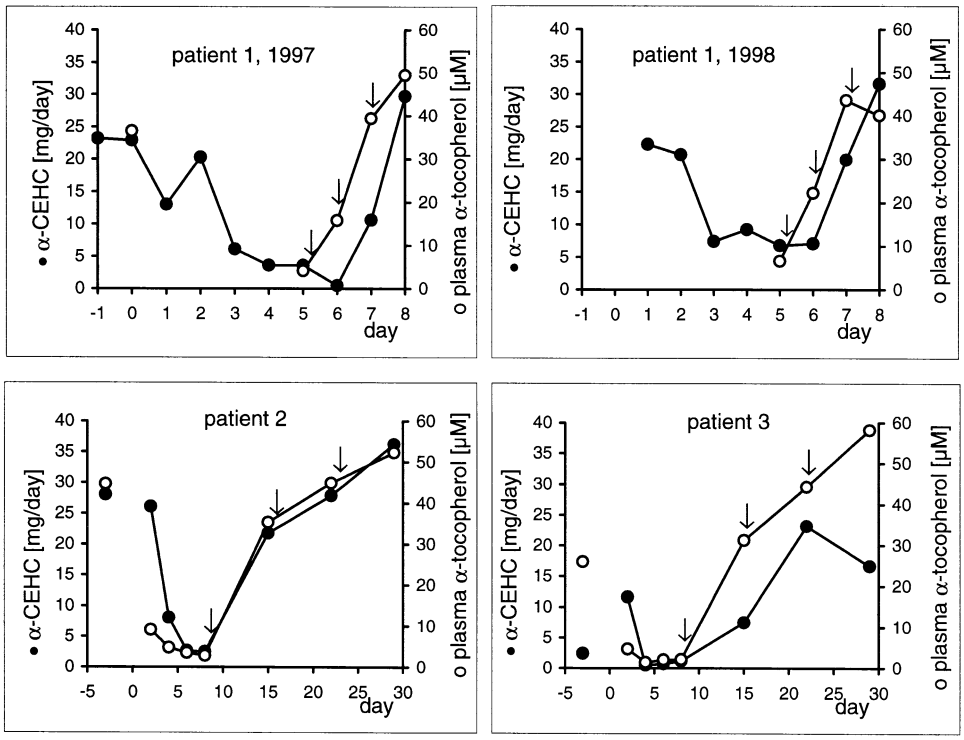


Fig. 1. Plasma α-tocopherol and urinary α-CEHC in AVED patients during withdrawal from vitamin E supplementation. Patients were supplemented with vitamin E as described in Materials and Methods until withdrawal on day 0. From patient 1 blood and 24-h urine were collected 1 day before withdrawal (−1) and at day 0. Supplementation was interrupted for 5 days, and then it was restarted on day 5 with 2 × 200 mg, on day 6 with 3 × 400 mg, and on day 7 with 3 × 600 mg of all-*rac*-α-tocopheryl acetate as indicated by the arrows. The first study was done in summer 1997 and repeated in the same way 1 year later (1998). From patients 2 and 3 blood and 24-h urine samples were collected 3 days before supplementation was terminated (day −3). The withdrawal period lasted 8 days, and then patients were supplemented with 20, 30, and 40 mg/kg body weight each dosage for 7 days. For plasma α-tocopherol and urinary α-CEHC measurements as well as further experimental details see Materials and Methods.

dose corresponded to 20, 30, and 40 mg/kg body weight, respectively. Thereafter, supplementation with the highest dosage was continued in all patients.

The patients' written informed consents for the study were obtained according to the Declaration of Helsinki. During the withdrawal period the patients were closely monitored by a physician.

Patient 1. This 32-year-old man is the first patient described to suffer from genetic vitamin E deficiency. He was first investigated at the age of 12 years because of ataxia, sensory neuropathy, and lipopigment deposition (24). Apart from his neurological condition, he was healthy and never showed any sign of a nutritional disorder. Mutation analysis in his α-TTP gene revealed a complex 4-bp insertion between positions 530 to 532 (530AG→GTAAGT; family 13 in ref. 2). Since the age of 14 years he regularly takes high oral supplements of all-*rac*-α-tocopheryl acetate (1.8 g/day in three doses). The dosage was titrated to achieve blood levels within or moderately above the normal range (21). The patient was studied with respect to his ability to discriminate between stereoisomers of α-tocopherol and was found to be a nondiscriminator (patient 6 in ref. 25). He has been monitored for 20 years by now, has remained neurologically stable, and is presently in good condition.

Patient 2. At the time of the study the boy was 14 years old. Symptoms started at the age of 9 years with increased clumsiness progressing to overt ataxia and deteriorating school performance. He was hospitalized at the age of 12 years with symptoms of dysarthria, ataxia, dystonia, impaired short-term memory, and confused behavior. Clinical examination revealed a positive Romberg test and absent tendon reflexes. Cerebellar signs comprised intentional tremor, dysmetria, and dysdiadochokinesia. Vitamin E levels (0.6 μmol/l) were below normal (15–25 μmol/l). Molecular analysis showed a 552G→A mutation in the α-TTP gene leading to skipping of exon 3 and subsequent truncation of the protein by 57%. High-dose vitamin E supplementation with 40 mg/kg in the form of (*RRR*)-α-tocopherol improved cognitive function rapidly. The neurological recovery was, however, slow and incomplete (16).

Patient 3. At the time of this study patient 3 was a 20-year-old woman. Progressive ataxia started at the age of 8 years. Clinical examination at the age of 19 years revealed absent deep tendon reflexes and vibratory sense in upper and lower limbs. Her mental status was normal. Plasma vitamin E levels (2.0 μmol/l) were decreased. Molecular analysis revealed a compound heterozygous mutation (421G→T, 513–514insTT) in the α-TTP gene (M. Schuelke, unpublished data). Under appropriate vitamin E supplementation (12 mg/kg body weight all-*rac*-α-tocopheryl acetate) progression of the disease stopped, but no apparent clinical improvement could be observed.

Control subjects

For control subjects, blood was taken from six unsupplemented healthy volunteers and urine collected for the next 24 h. In addition, two volunteers were supplemented with 400 mg of (*RRR*)- or all-*rac*-α-tocopherol for 5 days. Before and after vitamin E supplementation blood was taken as usual. Stored urine powder from volunteers participating in the study described in ref. 22 and receiving 50, 150, 350, and 800 mg of (*RRR*)-α-tocopherol daily for 7 days was reanalyzed for α-tocopherol metabolites (see below).

Biochemical analyses

α-Tocopherol was measured in 50-μl aliquots of plasma derived from 10 ml of EDTA-anticoagulated blood, using high-performance liquid chromatography (HPLC) analysis with electrochemical detection (26).

Urinary tocopherol metabolites were measured in lyophilized 40-ml aliquots of 24-h urine. The dry material was stored under nitrogen at −80°C. For analysis, 30 μg of Trolox (Aldrich, Steinheim, Germany) was added to 100 mg of powder as internal standard. Samples were extracted and hydrolyzed, and the resulting CEHC compounds were derivatized to trimethylsilyl ether esters according to ref. 22. Tocopherol metabolites were analyzed by gas chromatography/mass spectrometry (GC/MS) with an SSQ 710 MAT from Finnigan MAT (Bremen, Germany) with a Varian (Santa Clarita, CA) gas chromatograph as described (22, 23) with a scan range from 50 to 500 atomic mass units. Selected masses for the trimethylsilyl ether esters monitored were as follows: 394 m/z for Trolox, 408 m/z for γ-CEHC, 422 m/z for α-CEHC, and 464 m/z for α-CPHC. Concentrations were calculated from the peak areas of the respective CEHCs in relation to the peak area of Trolox and the response factor.

TABLE 1. Plasma α-tocopherol levels and urinary α-CEHC excretion in unsupplemented healthy subjects and AVED patient 2 before supplementation

	Plasma α-Tocopherol	Urinary α-CEHC
	μM	*mg/day*
Healthy subjects	22.8 ± 6.55 (n = 6)	0.26 ± 0.067 (n = 6)
AVED patient 2	0.6	0.41

RESULTS

α-CEHC excretion in unsupplemented subjects

Urinary excretion of α-CEHC was low in healthy subjects unless they were supplemented with α-tocopherol

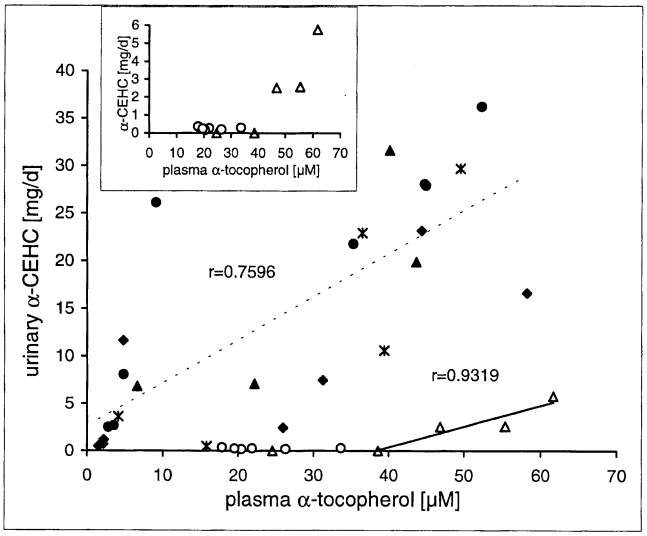


Fig. 2. Relationship between α-CEHC excretion and plasma α-tocopherol levels in patients and healthy subjects. Individual values were obtained from patients as described in the legend to Fig. 1, from control subjects as described in Materials and Methods, and from supplemented volunteers (22). Open circles, unsupplemented healthy subjects (mean in Table 1); open triangles, supplemented volunteers, recalculated from ref. 22; asterisks, patient 1, 1997; open triangles, patient 1, 1998; solid circles, patient 2; open diamonds, patient 3. (– – –) Trend line for all patients; (—) trend line for control subjects after supplementation. The inset shows the values for control subjects only at a more sensitive scale for α-CEHC excretion.

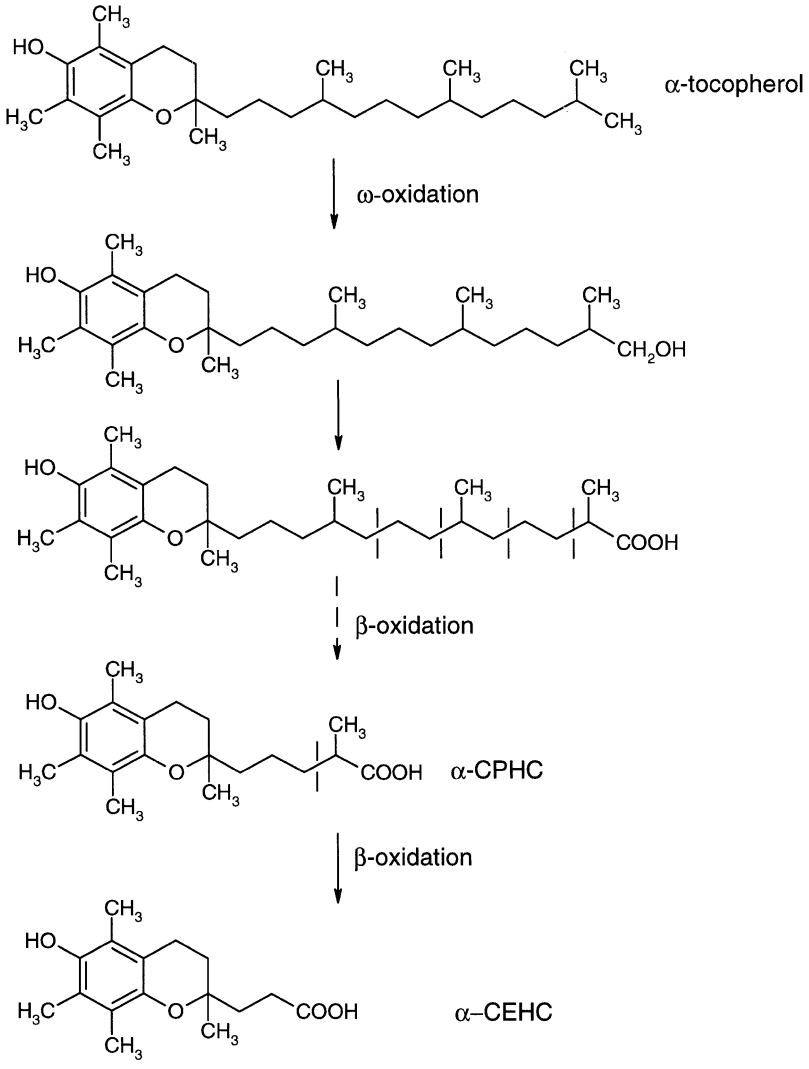
(22). After increasing the plasma α-tocopherol level by supplementation α-CEHC increased and became consistently measurable. Unsupplemented volunteers excreted 0.118–0.306 mg of α-CEHC per day, resulting in an average of 0.26 ± 0.069 mg/day, n = 6 (**Table 1**). In AVED patient 2, however, with whom we had the chance to measure α-CEHC before any vitamin E supplementation, urinary α-CEHC was slightly above the average of control subjects (Table 1). The patient excreted α-CEHC despite a plasma α-tocopherol level far below the level of healthy subjects (0.6 μmol/l). This shows that, at least in this patient, α-CEHC excretion is not determined by the plasma α-tocopherol level.

Plasma α-tocopherol and urinary α-CEHC in AVED patients during withdrawal and resupplementation of α-tocopherol

To study the fate of α-tocopherol in α-TTP deficiency further, plasma α-tocopherol levels and α-CEHC excretion were monitored in three patients under supplementation with vitamin E and during an intermediate withdrawal period. During the supplementation period, plasma α-tocopherol was in the normal range in all three patients (Fig. 1). The lowest level was found in patient 3 (26 μM), which corresponds to a comparatively low supplementation with vitamin E (744 mg/day, compared with 1,800 and 2,070 mg/day in patients 1 and 2, respectively). All patients excreted between 10 and 30 mg of α-CEHC per day (Fig. 1).

During the withdrawal period, plasma α-tocopherol declined rapidly, reaching minimal levels already after 2 days in patients 2 and 3 (Fig. 1C and D). The declining kinetics were not documented in patient 1 but levels were similarly low after 5 days during both withdrawal periods. Urinary α-CEHC reached minimum levels 4 days after the supplementation was terminated in all three patients.

After resupplementation, plasma α-tocopherol levels increased quickly and correlated with the amount of intake. In patients 1 and 2 they reached the starting levels. In patient 3 plasma α-tocopherol levels were higher than she started with according to the higher resupplementation dosage (see Materials and Methods). α-CEHC excretion after resupplementation paralleled α-tocopherol intake and plasma levels.



Scheme 1. Proposed degradation of α-tocopherol by ω- and subsequent β-oxidation.

A threshold for α-CEHC excretion exists only in healthy subjects

Having observed that the excretion of high amounts of α-CEHC is possible despite an extremely low plasma α-tocopherol level in AVED patients, the question arose whether there is a threshold in patients at all. To this end, all available α-CEHC data were correlated with the respective α-tocopherol content in plasma. As depicted in **Fig. 2**, healthy subjects did not excrete substantial amounts of plasma α-tocopherol below a threshold of 30–40 μmol/l. This becomes more clear when the scale of the y axis is adjusted to the absolute amounts of control subjects only (see inset in Fig. 2). α-CEHC levels in patients were distinctly higher than in control subjects even when the plasma α-tocopherol concentration had fallen below 5 μM. This shows that only in healthy subjects does a threshold for α-CEHC excretion exist.

α-CPHC, a new α-tocopherol metabolite discovered in AVED patients

If the α-tocopherol side chain is degraded by ω-oxidation followed by β-oxidation as suggested (27), not only α-CEHC should be produced but also precursors with an additional 3, 5, 8, and 10 carbon atoms, depending on the branching points (**Scheme 1**). Indeed, a urinary metabolite with a side chain longer by three carbon atoms was detected in supplemented patients. It appeared at a retention time of 12.80 min in the system applied. The mass spectrum revealed an m/z of 464 (**Fig. 3**), corresponding to disilylated 2,5,7,8-tetramethyl-2-(4′-carboxypentyl)-6-hydroxychroman (Fig. 3). In analogy to α-CEHC the new metabolite was abbreviated α-CPHC. Alternatively, the metabolite can be named α-CMBHC for α-(4′-carboxy-4′-methyl)-hydroxychroman. The fragment ion at m/z 237 reflects fragmentation between carbons 3 and 4 and carbon 2 and oxygen, as was also observed with α-CEHC (22) and with the corresponding metabolite of γ-tocopherol, γ-CEHC (28). α-CPHC excretion in patients showed the same time course as α-CEHC (**Fig. 4A–C**). The daily excretion, however, was less than that of α-CEHC and extremely low in patient 1 during both periods of monitoring.

To determine whether healthy individuals also produce α-CPHC, we reanalyzed urine powders from volunteers supplemented with different doses of (*RRR*)-α-tocopherol (22) for α-CPHC. As shown in **Table 2**, α-CPHC was also detected in the urine of these subjects after supplementation with high dosages of vitamin E. α-CPHC was in the range of 3–6% of the amount of α-CEHC in both healthy subjects and patients (Table 2). This proportion was exceeded only in one case (the first urine sample of patient 3, which was extraordinarily low in α-CEHC). In accordance with the tiny absolute values, the α-CPHC percentages were lower in patient 1. Similarly, the amount of α-tocopherol that was degraded into α-CEHC (1–3%) did not differ between patients and control subjects (Table 2).

DISCUSSION

Vitamin E status of AVED patients

Patients with a defect in the α-TTP gene are usually treated with high doses of vitamin E to overcome the consequences of a deficiency in target tissues. The optimum dosage being unknown, we decided to monitor the vitamin E status of three AVED patients by determining the level of the urinary α-tocopherol metabolite, α-CEHC, which has been discussed as a tool to define optimum supplementation (22). For this reason patients supplemented with α-tocopherol for at least 1 year were withdrawn from

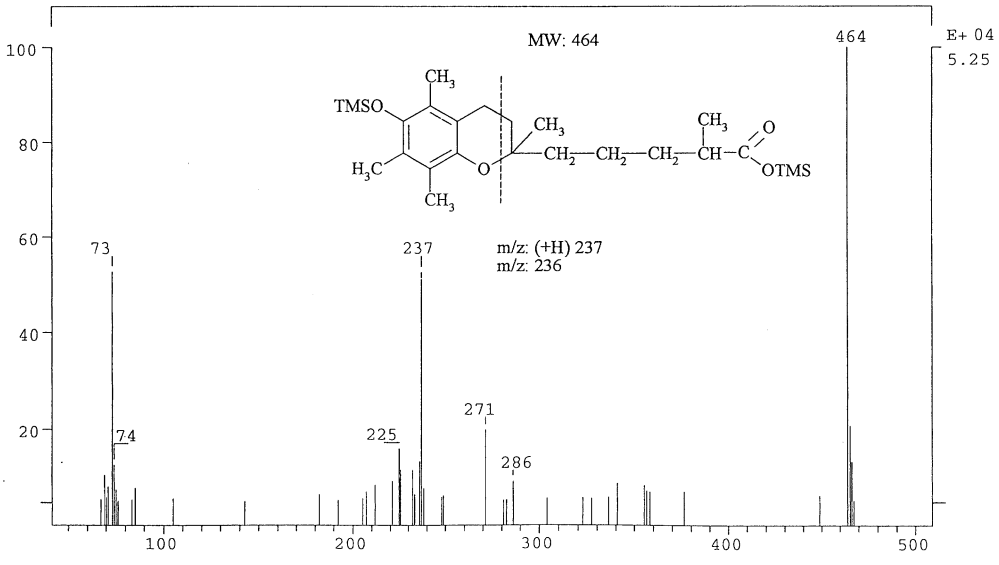


Fig. 3. Mass spectrum and structure of α-CPHC. Mass spectrum (EI) of a peak appearing in urine samples of supplemented patients eluting at 12.80 min. The spectrum corresponds to the disilylated α-carboxypentyl-6-hydroxychroman (α-CPHC), molecular ion m/z 464. The fragment ion at m/z 237 resulted from fragmentation of the chroman ring (22, 28) and the subsequent uptake of one hydrogen. Conditions as described in Materials and Methods.

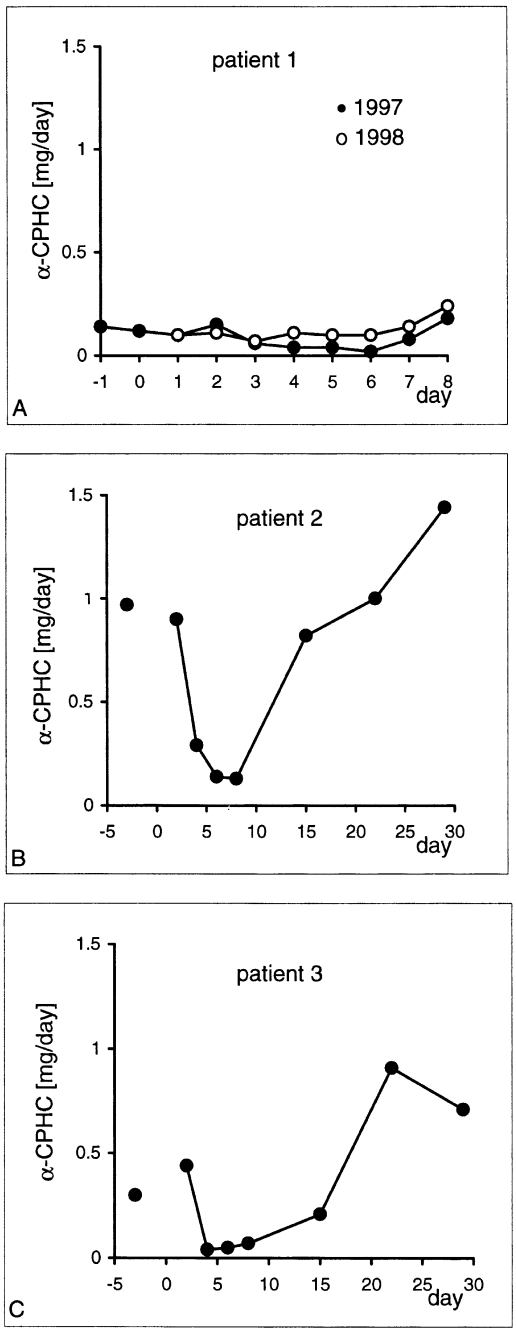


Fig. 4. Urinary excretion of α-CPHC. Patients were supplemented as described in legend to Fig. 1. α-CPHC was measured in dried 24-h urine aliquots as described in Materials and Methods.

supplementation for a short period, and the vitamin status in plasma and, for the first time, the urinary excretion of α-CEHC were measured. As observed previously (29), plasma vitamin E levels decreased rapidly when supplementation was stopped. Thus, the patients must be supplemented continuously. The equally rapid increase in the α-tocopherol plasma level after resupplementation in all patients shows that the tremendous amounts of ingested vitamin E can be exchanged to some extent between chylomicrons and plasma lipoproteins without being incorporated into VLDL in the liver, as suggested (30). By this proposed mechanism the maintenance of a plasma level within normal limits is possible.

Urinary metabolites in healthy subjects and in AVED patients

CEHCs most likely are the terminal products of phytyl tail degradation by ω- and subsequent β-oxidation as suggested by Chiku, Hamamura, and Nakamura (27) for δ-tocopherol. If so, homologs with longer residual tails could be expected (Scheme 1). We indeed discovered the immediate precursor of α-CEHC, α-CPHC (see Fig. 3), which supports the hypothesis of the side-chain degradation by ω- and β-oxidation. Although the absolute amount of α-CPHC generally increased with increasing intake of α-tocopherol, the relation of α-CPHC to α-CEHC was the same in patients 2 and 3 and healthy subjects. Only patient 1 accumulated less α-CPHC, resulting in a lower α-CPHC/α-CEHC ratio. α-CEHC consistently accounted for 1–3% of ingested α-tocopherol in patients and control subjects. This merits consideration with respect to the form of α-tocopherol applied. Patients 1 and 3 supplemented with all-*rac*-α-tocopherol excreted 2% of this dosage as α-CEHC. Patient 2 was supplemented with (*RRR*)-α-tocopherol before the withdrawal period. From this dosage he excreted 2% as α-CEHC, too. He was resupplemented with all-*rac*-α-tocopherol and again excreted 2% as α-CEHC. This is in contrast to the data provided by Kaneko et al. (31). Rats fed with 2 mg of (*RRR*)-α-[^{14}C]tocopherol or (*SRR*)-α-[^{14}C]tocopherol excreted 1.3% of the (*RRR*)-isomer dose and 7.8% of the (*SRR*)-isomer dose as α-CEHC in the urine. This confirms our own findings that all-*rac*-α-tocopherol was degraded to a higher degree than (*RRR*)-α-tocopherol in healthy volunteers (32). The identical amount of (*RRR*)- and all-*rac*-α-tocopherol degraded to α-CEHC in patients can be explained only by the lack of ability of patients to discriminate between the stereoisomers of α-tocopherol due to a nonfunctioning α-TTP. They obviously use all-*rac*-α-tocopherol as efficient as the (*RRR*)-isomer form. The principal mechanism of degradation, however, that is, ω- and β-oxidation of the side chain, appears not to differ between AVED patients and subjects with a functional α-TTP.

A threshold for α-CEHC excretion does not exist in patients

We started the study with the hypothesis that apart from the α-tocopherol plasma level, the excretion of α-CEHC might be a marker for the individual vitamin E status as has been suggested (22). The observation that in healthy subjects α-CEHC excretion only rises with α-tocopherol intake when a certain threshold of plasma α-tocopherol was exceeded, provided the basis for this hypothesis. If this also applied to AVED patients, α-CEHC excretion should start rising when normal plasma levels were reached or surpassed. However, this turned out not to be the case. Patient 2, who was examined before any supplementation started, excreted more α-CEHC than healthy subjects despite a vanishing plasma α-tocopherol concentration. All AVED patients excreted substantial amounts of

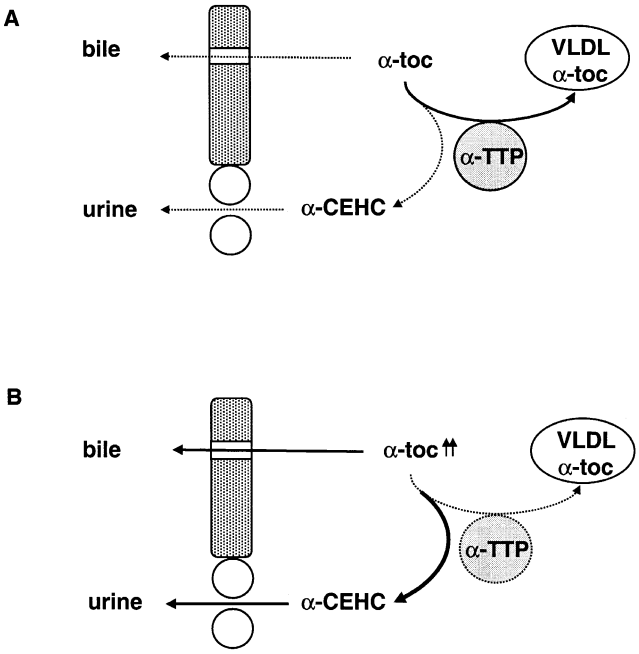
TABLE 2. Formation of urinary α-tocopherol metabolites in healthy subjects and AVED patients in dependence of the α-tocopherol intake

Intake of Vitamin E	Urinary α-CEHC % Administered α-Tocopherol		Urinary α-CPHC % α-CEHC	
mg/day				
Healthy subjects				
Volumteers from ref. 22				
150	2.56 ± 1.01 (n = 6)		Not detectable	
350	1.11 ± 0.52 (n = 6)		4.6 (0.22 mg/day)	
800	1.11 ± 0.33 (n = 6)		6.0 (0.46 mg/day)	
Volunteers from this study				
400	0.9		4.4 (0.1 mg/day)	
400	1.22		4.8 (0.15 mg/day)	
	1997	1998	1997	1998
Patient 1				
Before break				
1,800	2.0		0.6	
After break				
400	0.2	2.75	4.0	1.4
1,200	1.4	2.5	0.7	0.7
1,800	2.5	2.7	0.6	0.7
Patient 2				
Before break				
2,070	2.1		3.4	
After break				
1,380	2.45		3.8	
2,070	2.09		3.6	
2,760	2.03		4.0	
Patient 3				
Before break				
744	0.3		12.2	
After break				
1,240	0.93		2.8	
1,860	1.9		3.9	
2,480	1.0		4.3	

α-CEHC even during the withdrawal phase when plasma α-tocopherol decreased far below normal values (see Fig. 2). Thus, instead of being markers for an exceeded plasma α-tocopherol threshold, the urinary metabolites α-CEHC and α-CPHC appear to reflect the α-tocopherol concentration in the liver. As hypothesized in **Scheme 2**, α-tocopherol is almost quantitatively incorporated into VLDL in unsupplemented healthy subjects by means of α-TTP (Scheme 2A). Only tiny amounts are degraded and excreted into the urine. On supplementation with higher dosages than probably can be provided by normal diet, α-TTP becomes saturated and the excess of α-tocopherol is shunted into the degrading systems (Scheme 2B). In AVED patients (Scheme 2B), α-tocopherol reaching the liver obviously is degraded without any competition by α-TTP. Thus, it is not the plasma α-tocopherol level that determines the amount of α-CEHC formation. As a consequence, α-CEHC excretion does not by any means indicate adequate vitamin E nutriture in AVED patients.

Urinary α-tocopherol metabolites as diagnostic parameters?

Methods for α-CEHC estimation are improving (33) and become more convenient for routine analyses also in plasma (34). Degradation of α-tocopherol into α-CEHC and precursors might therefore be used as an additional diagnostic parameter to differentiate between vitamin E deficiencies caused by impaired absorption of vitamin E or by a defect in the α-TTP gene. According to our findings, the excretion of substantial amounts of α-CEHC and α-CPHC associated with low plasma levels of α-tocopherol may be considered suggestive of α-TTP deficiency. The α-CEHC levels, however, are not so extraordinarily high in unsupplemented AVED patients that they could per se be taken as reliable indicators of this disease. AVED patients do not respond to supplementation with the usual moderate α-tocopherol dosages with a normalization of their α-tocopherol plasma levels, whereas the urinary metabolites are increased to an extent never observed in healthy subjects. This discrepancy might be unique for AVED patients. It is also not expected for diseases associated with impaired uptake of vitamin E such as abetalipoproteinemia (reviewed in ref. 35), cholestasis, cystic fibrosis, or other malabsorption syndromes, although this remains to be demonstrated. In all these conditions the absorption of fat and in consequence α-tocopherol is deficient, which implies that no sufficient α-tocopherol is available for degradation. Only in α-TTP deficiency is α-tocopherol normally absorbed and transported to the liver, where it is preferentially exposed to degradation because of defi-



Scheme 2. Hypothetic pathways of α-tocopherol in the liver of healthy subjects and patients with α-TTP deficiency. (A) Unsupplemented healthy subjects. α-Tocopherol provided by chylomicrons is transferred into VLDL by means of α-TTP. Incoming and transferred α-tocopherol are in a steady state, only minor amounts of α-tocopherol are degraded and excreted as α-CEHC. (B) Supplemented healthy subjects and α-TTP-deficient patients. Increasing α-tocopherol concentrations either saturate α-TTP capacity (healthy) or are unbalanced due to lack of functional α-TTP (AVED patients). In consequence, increasing amounts of α-tocopherol are degraded and excreted as α-CEHC. Accumulation of precursors, like α-CPHC, is possible. See text for further explanation.

cient utilization by α-TTP. The process of degradation, however, appears not to be different in AVED patients and healthy subjects.

In conclusion, we provide further evidence of the role of α-TTP in sorting and utilization of α-tocopherol. The capacity of α-TTP might determine the amount of α-tocopherol that can be ingested before its degradation starts to increase over basic levels. In AVED patients, who do not have a functioning α-TTP, α-tocopherol is degraded irrespective of a normalized plasma level. We further provide evidence of a degradation of the α-tocopherol side chain by ω- and β-oxidation by the identification of the three carbon atoms longer precursor of α-CEHC, α-CPHC.

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