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γ-Tocopherol Biokinetics and Transformation in Humans

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Background: The uptake and biotransformation of γ-tocopherol (γ-T) in humans is largely unknown. Using a stable isotope method we investigated these aspects of γ-T biology in healthy volunteers and their response to γ-T supplementation.

Methods: A single bolus of 100 mg of deuterium labeled γ-T acetate (d^2-γ-TAC, 94% isotopic purity) was administered with a standard meal to 21 healthy subjects. Blood and urine (first morning void) were collected at baseline and a range of time points between 6 and 240 h post-supplemetation. The concentrations of d^2 and d^0-γ-T in plasma and its major metabolite 2,7,8-trimethyl-2-(b-carboxyethyl)-6-hydroxychroman (γ-CEHC) in plasma and urine were measured by GC-MS. In two subjects, the total urine volume was collected for 72 h post-supplementation. The effects of γ-T supplementation on α-T concentrations in plasma and α-T and γ-T metabolite formation were also assessed by HPLC or GC-MS analysis.

Results: At baseline, mean plasma α-T concentration was approximately 15 times higher than γ-T (28.3 vs. 1.9 μmol/l). In contrast, plasma γ-CEHC concentration (0.191 μmol/l) was 12 fold greater than α-CEHC (0.016 μmol/l) while in urine it was 3.5 fold lower (0.82 and 2.87 μmol, respectively) suggesting that the clearance of α-CEHC from plasma was more than 40 times that of γ-CEHC. After d^2-γ-TAC administration, the d^2 forms of γ-T and γ-CEHC in plasma and urine increased, but with marked inter-individual variability, while the d^0 species were hardly affected. Mean total concentrations of γ-T and γ-CEHC in plasma and urine peaked, respectively, between 0–9, 6–12 and 9–24 h post-supplementation with increases over baseline levels of 6–14 fold. All these parameters returned to baseline by 72 h. Following challenge, the total urinary excretion of d^2-γ-T equivalents was approximately 7 mg. Baseline levels of γ-T correlated positively with the post-supplementation rise of $(d^0 + d^2) - \gamma - T$ and γ-CEHC levels in plasma, but correlated negatively with urinary levels of $(d^0 + d^2)$-γ-CEHC. Supplementation with 100 mg γ-TAC had minimal influence on plasma concentrations of α-T and α-T-related metabolite formation and excretion.

Conclusions: Ingestion of 100 mg of γ-TAC transiently increases plasma concentrations of γ-T as it undergoes sustained catabolism to CEHC without markedly influencing the pre-existing plasma pool of γ-T nor the concentration and metabolism of α-T. These pathways appear tightly regulated, most probably to keep high steady-state blood ratios α-T to γ-T and γ-CEHC to α-CEHC.

Keywords: Vitamin E; γ-Tocopherol; α-Tocopherol; Vitamin E metabolites; Carboxyethyl-hydroxychroman; CEHC

INTRODUCTION

γ-Tocopherol (γ-T) has long been considered to be of minor importance compared to α-T due to its lower plasma concentration and antioxidant activity.[1,2] As a consequence, few studies have focused on γ-T uptake and there is poor understanding of its metabolic fate in humans. Recently, evidence has accumulated suggesting a range of biological roles for γ-T and this has awakened interest in the biokinetics and metabolism of this tocopherol vitamer which predominates in nature and in many dietary regimens.[1,3] These biological roles include the control of the nitric oxide-related toxicity[4,5] and a possible inhibitory effect on platelet aggregation and thrombogenesis.[6] In addition, low γ-T concentrations and a high α- to γ-T ratio have been observed in coronary heart disease patients and in populations with high cardiovascular risk.[7–9] In a recent study, γ-T has been shown to be much more powerful than α-T in selectively inhibiting prostate cancer cell proliferation.[10] Moreover, γ-T may have other biological functions through the action of its

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metabolite 2,7,8-trimethyl-2-(b-carboxyethyl)-6-hydroxychroman (γ-CEHC), such as the possible regulation of the activity of the proinflammatory enzyme cyclooxygenase,[11] natriuretic activity,[12] antioxidant function and protection against the metal-induced nephrotoxicity in rats.[13]

The pathway through which tocopherols are metabolized to their respective CEHC metabolites was first described in the early 1980s in rats given large doses of δ-T,[14] and involves the shortening of the pythyl chain by cytochrome P450-mediated ω-oxidation and subsequent β-oxidation in hepatic cells without modification of the chroman ring.[15] Recent *in vitro* studies[16,17] carried out on hepatoma cell lines have demonstrated that minor metabolites such as the 2-(b-carboxymethylbutyl)-6-hydroxychroman (CMBHC) can be formed. However, in agreement with findings in these and other studies,[18,19] the formation and urinary excretion of CEHC appears to represent a preferential route of elimination for tocopherols and tocotrienols.

As a consequence of these studies, it is now appreciated that α-T is retained *in vivo* by hepatic cells in preference to γ-T which is largely subject to metabolic processing. However, γ-T has a relatively high abundance in nature compared with other tocopherols,[1–3] and the recent discovery of the exclusive biological properties of its CEHC derivative suggested that a sustained catabolism and consequent high γ- to α-CEHC ratio in plasma might be biologically beneficial. These and other aspects related with γ-T bioavailability, metabolism and excretion in humans remain to be fully established. In particular, little information is available in humans about changes occurring in γ-CEHC levels and possible effects on levels and metabolism of pre-existing pools of γ-T and α-T in response to supplements of γ-T. Conversely, it is known that supplemental α-T can increase the catabolism of γ-T.[20] To address some of these issues we carried out an *in vivo* stable isotope study to determine the concentrations of γ-T and α-T as well as their corresponding CEHC metabolites in plasma and urine at different time-points during post-supplementation. The primary goals of this study were (a) to describe the biokinetics and transformation of the supplemental and constitutive pools of γ-T, (b) to assess the inter-individual differences in the response to the supplemental γ-T and (c) to evaluate the effect of the supplementation of γ-T on the plasma levels and catabolism of α-T.

MATERIALS AND METHODS

Subjects and Sample Collection

Twenty one healthy non-smoking subjects (10F/11M) aged between 22 and 47 (mean 32.6 ± 8.3 years) and with a body mass index ranging between 19.1 and 35.7 (mean 22.9 ± 3.4) were enrolled for the study. None were receiving medication or taking vitamin supplements for 4 weeks prior to the start, or during the study. Each volunteer ingested 100 mg of d^2-γ-TAC (94 % isotopic purity) in a single dose with a standard breakfast, comprising of two rounds of lightly buttered toast and tea with milk. Blood samples (10 ml in heparinized vacutainer tubes) and the first morning void urine were collected immediately before (baseline) and 6, 9, 12, 24, 72, 168 and 240 h post-supplementation. In two male volunteers, other than samplings as above, the total urine produced was collected on the day when d^2-γ-TAC bolus was administered and on each of the following 2 days. Plasma was obtained by centrifugation (3000 rpm × 15 min at 4°C) and immediately stored at −80°C until processing. Urine samples were stored at −20°C.

Plasma Vitamin E Assay

The plasma concentrations of γ- and α-T were measured by HPLC analysis as previously described.[21] Plasma total cholesterol (Cho) was assessed by an enzymatic colorimetric test (Roche Diagnostic). Plasma tocopherol data were corrected for Cho concentration and expressed as μmol/mmol Cho. In some experiments, deuterated and undeuterated forms of tocopherols were measured in plasma by GCMS analysis (see below).

Urinary Vitamin E Metabolite Analysis

Internal standards d^3-α-quinone lactone (d^3-α-QL) and d^9-α-CEHC were added to an aliquot of urine (4 ml). Complete enzymatic hydrolysis of the glucuronide conjugate of the metabolites was carried out using 650 units of *E. coli* β-glucuronidase (Sigma) in 200 μl of 0.25 M sodium acetate buffer. The samples were then flushed with nitrogen and incubated at 30°C in the dark for 14 h. The resulting solution was acidified to pH 1.5 and extracted with hexane/*tert*-butyl methyl ether (99:1). Following centrifugation, the organic layer was removed and evaporated under nitrogen. The residue was dissolved in anhydrous pyridine and silylated at 65°C for 2 h with 50μl N, O-bis (trimethylsilyl) trifluoroacetamide and 1% trimethylchlorosilane (Pierce Chemical Co., Rockford, IL, USA). The solvents were evaporated under nitrogen and the residue dissolved in hexane for GC-MS analysis.

The disilyl derivatives of CEHC and QL were quantified by GC-MS using a Hewlett-Packard 6990 GC coupled to a Hewlett-Packard 5973 Mass Selective Detector. The carrier gas used was helium. The separation of the compounds of interest was achieved using a HP1 cross-linked methylsiloxane

column with dimensions 25 m × 0.2 mm ID and film thickness 0.33 μm. The oven temperature was set to 50°C for 2 min followed by an increase of 50°C/min to 240°C that was held for 4 min before a further increase of 25°C/min to 285°C that was held for 6 min. The injection volume was 1 μl. The transfer line temperature was 290°C and the MS source temperature 230°C. In selected ion mode, the following ions corresponding to the molecular ion of the metabolites were monitored: m/z 276 (d^0-QL), 279 (d^3-QL), 282 (d^6-QL), 285 (d^9-QL), 408 (d^0-γ-CEHC), 410 (d^2-γ-CEHC), 422 (d^0-α-CEHC), 425 (d^3-α-CEHC), 428 (d^6-α-CEHC) and 431 (d^9-α-CEHC). Concentrations of d^0-QL and CEHC were calculated from the peak area relative to the corresponding internal standard (d^3-QL and d^9-CEHC, respectively). The concentration of the d^0-γ-CEHC was calculated using a response factor and the d^9-α-CEHC internal standard.

Metabolite concentration was expressed as μmol/g creatinine (Cr). The concentration of Cr in urine samples was determined spectrophotometrically using a Sigma Diagnostics creatinine kit and following manufacturers instructions (555A, Sigma Chemical Co. Louis, MO).

Plasma Vitamin E and Metabolite Analysis

The GCMS analysis of vitamin E isomers and metabolites in plasma was carried out according to the procedure recently reported.[22] Briefly, deuterated internal standards (namely, d^3-QL, d^2-γ-CEHC, d^9-α-CEHC and d^9-α-T) at a final concentration of 0.5 or 0.1 μM in a total volume of 200 μl of acetonitrile, 25 μl of 10 mg/ml ascorbic acid and 1500 UI of *E. coli* β-glucuronidase (Sigma, Dorset, UK) in 200 μl of 0.25 M sodium acetate buffer (pH 6.2) were added in sequence to 500 μl of plasma in 25 ml glass culture tubes and gently mixed. Then the tubes were flushed with oxygen-free nitrogen and incubated at 34°C for 30 min. This procedure resulted in the complete cleavage of glucuronic acid esters of CEHC.

A single-step liquid–liquid extraction protocol was carried out on acidified samples (500 μl of plasma plus 20 μl of 100% acetic acid) by mixing with 10 ml of hexane/dichloromethane (1/1 vol/vol) containing 1% (wt/vol) BHT. The samples were vortexed for 1 min at room temperature. After centrifugation (3200 rpm for 15 min at 10°C), the organic supernatants were collected and completely dried. Metabolites and vitamers were derivatized and measured essentially with the same procedure described for urinary metabolite analysis.

The following ions corresponding to the molecular ion of metabolites and vitamin forms were monitored in selected ion mode: m/z 276 (d^0-QL), 279 (d^3-QL), 408 (d^0-γ-CEHC), 410 (d^2-γ-CEHC), 422 (d^0-α-CEHC), 431 (d^9-α-CEHC), 474.4 (d^0-δ-T), 488.4 (d^0-γ-T), 490.4 (d^2-γ-T), 502.4 (d^0-α-T), 511.4 (d^9-α-T).

Concentrations of the d^0 forms of QL, α-CEHC and α-T were calculated from the peak area relative to the corresponding internal standard. In samples from subjects supplemented with d^2-γ-T acetate, the concentrations of the d^0-γ-CEHC and d^0-γ-T were calculated using d^9-α-CEHC as internal standard and corrected for the appropriate response factors. Disilyl-d^2-γ-CEHC peak area was corrected for contributions by heavy isotopes of carbon and silicon present in unlabeled d^0-γ-CEHC. This procedure gave a satisfactory recovery of metabolites and the possibility to perform in the same sample a reliable quantification of α, γ and δ forms of vitamin E.

Renal Clearance of Vitamin E Metabolites

Renal clearance data were calculated as ratio between the urinary recovery in the 24 h and the concentration in plasma of CEHC using the formula: $[\text{CEHC urine}]_{\mu M} \times \text{ml urine}/[\text{CEHC plasma}]_{\mu M} \times 1440$ min. This parameter expresses the volume of plasma cleared from CEHC in unit time (in ml/min) and is a direct function of the capability of the renal filter to remove blood CEHC.

Data and Statistical Analysis

Data were assessed for distribution and expressed as mean ± SD or median (range). Statistical analysis was carried out by Student t-test for repeated measures or one-way ANOVA test. Regression analysis of data was performed using the software Microcal™ Origin® 6.0 (Microcal Software Inc., Northampton, MA). Values of $p < 0.05$ were considered significant.

RESULTS

Baseline Concentrations of Tocopherols and Corresponding CEHCs in Healthy Volunteers

At baseline plasma tocopherol concentrations were markedly in favor of α-T with an approximate 15-fold excess over γ-T levels (28.3 vs. 1.86 μmol/l) (Table I). In contrast, mean plasma concentrations of γ-CEHC (0.191 μmol/l) were 12-fold higher than α-CEHC (0.016 μmol/l)., In urine, however, this situation was reversed as the concentration of α-CEHC (2.87 μmol/l) was 3-fold greater than γ-CEHC (0.824 μmol/l; Table I).

Change in γ-T and γ-CEHC Concentrations after d^2-γ-TAC Supplementation

Plasma and urinary γ-T and its metabolite responses to administration 100 mg of d^2-γ-TAC are shown in Fig. 1.

TABLE I Plasma levels of tocopherols, and plasma and urinary tocopherol metabolites in the 21 healthy volunteers at the inclusion in the study (before supplementation)

Plasma vitamins	(μmol/l)	(μmol/mmol Cho)
α-T	28.27 ± 4.26	5.77 ± 0.87
γ-T	1.86 ± 0.64	0.38 ± 0.13
Plasma metabolites	**(μmol/l)**	**–**
α-CEHC	0.016 ± 0.004	–
γ-CEHC	0.191 ± 0.081	–
Urinary metabolites	**(μmol/l)**	**(μmol/g Cr)**
α-CEHC	2.87 ± 2.55	2.213 ± 1.961
α-QL	0.377 ± 0.298	0.289 ± 0.229
γ-CEHC	0.824 ± 0.489	0.634 ± 0.376

T, tocopherol; α-QL, α-tocopheronolactone; α-CEHC, 2,5,7, 8-tetramethyl-2-(2′-carboxyethyl)-6-hydroxychroman; γ-CEHC, 2,7, 8-trimethyl-2-(2′-carboxyethyl)-6-hydroxychroman.

Marked inter-individual variations were observed for each of these in response to oral loading with d^2-γ-TAC. Mean plasma γ-T concentration peaked at, or close to, the first time point assessed in the study (i.e. 6 h post-supplementation) with a 7.7 fold increase over baseline levels, while the plasma and urinary CEHC peaked, respectively, between 6–12 and 9–24 h with 6 and 14-fold increases, respectively. At these time points, the maximal concentration observed for total ($d^0 + d^2$)-γ-T in plasma and γ-CEHC in plasma and urine were 14.3 ± 5.6 μmol/l (range 7.4–26.6), 1.15 ± 0.59 (0.70–2.47) and 6.47 ± 3.82 (2.68–38.70), respectively.

The relative contribution of d^0 and d^2 species to the increase of γ-T and γ-CEHC after d^2-γ-TAC administration are shown in Fig. 2. The d^2 form of these compounds showed mean peak concentrations of, respectively, (in μmol/l) 11.52 ± 2.88, 0.85 ± 0.30 and 4.44 ± 1.55. d^0-γ-T and d^0-γ-CEHC concentrations in plasma and urine increased only slightly after d^2-γ-T supplementation and all these responses had dissipated 72 h post-supplementation. These data indicate that the plasma clearance of γ-CEHC is significantly increased following supplementation, peaking at 12 h (+45% over the baseline clearance data; $p < 0.01$).

Baseline γ-T concentration correlated positively with γ-T levels 6 h post-supplementation ($r = 0.481$, $p = 0.027$), but negatively with the concentration of total urinary γ-CEHC at 12 h post-supplementation ($r = -0.438$, $p = 0.047$; Fig. 3). The total amount of γ-CEHC appearing in plasma related directly to the increase of total γ-T concentration (0.513, $p < 0.01$, not shown).

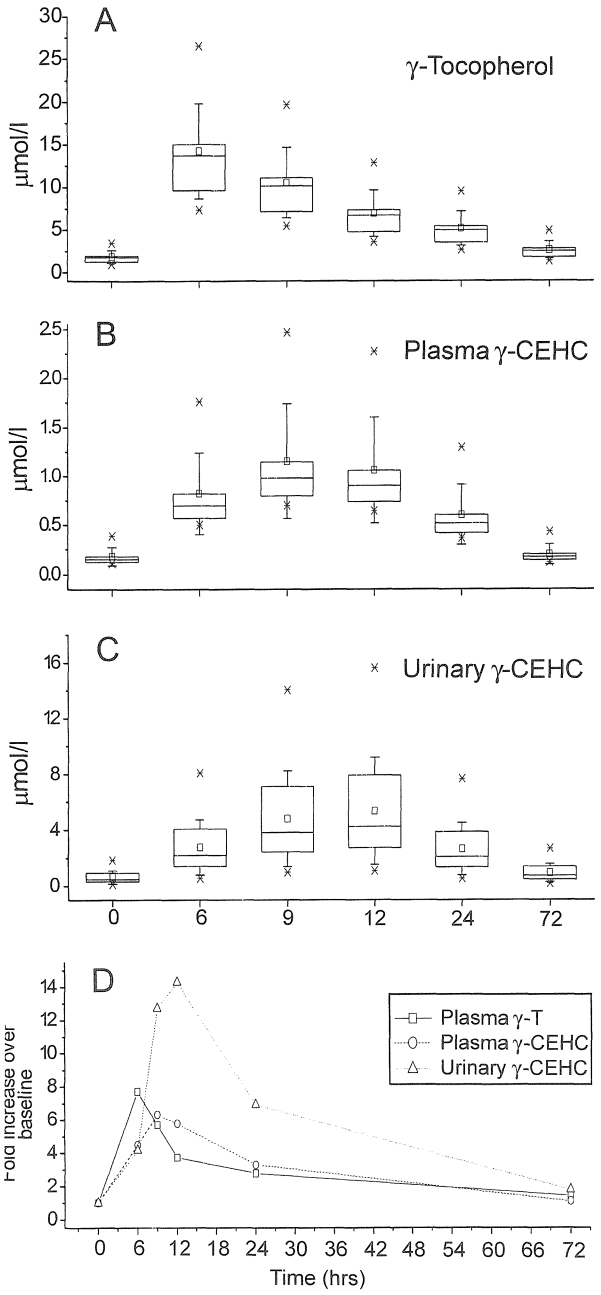

FIGURE 1 Mean levels, distribution range (panels A–C), and fold increase over baseline (panel D) of the levels (as $d^0 + d^2$ species) of plasma γ-T and plasma and urinary γ-CEHC in 21 healthy volunteers at different time points between 0 and 72 h post-supplementation with 100 mg of d^2-γ-TAC. Data in panels A–C are reported as absolute concentrations (μmol/l) to allow a comparison between the levels of γ-T and γ-CEHC. Boxes indicate 25 and 75% percentiles and median values. Asterisks show range limits. Open square symbols and vertical error bars are mean and SD. All the mean values between 6 and 24 h post-supplementation were significantly different from baseline values at the p level of 0.01 or greater.

Total Urinary Excretion of Tocopherol Metabolites

Figure 4 shows the total amounts of γ-T metabolites excreted in urine by two individuals in the 72 h following supplementation. Assuming complete conversion of d^2-γ-TAC to d^2-γ-T, these data along with the data of urinary d^2-γ-CEHC shown in panel B indicate that the total urinary excretion of d^2-γ-T equivalents was approximately 7 mg. In agreement, the integral calculus of the curve obtained by the partial urine collection in

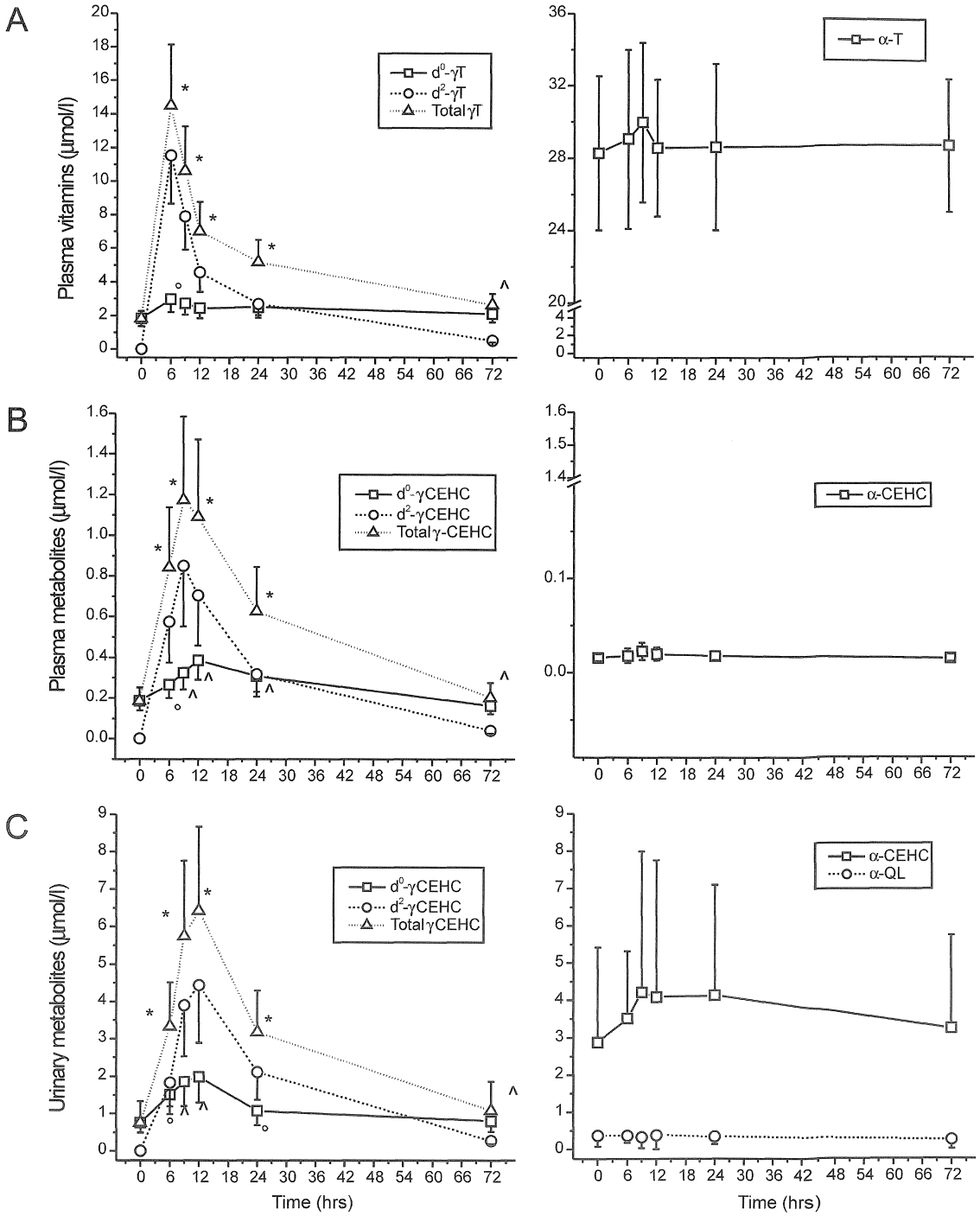

FIGURE 2 Left panels show a comparison between kinetics of mean levels of d^0, d^2 and total ($d^0 + d^2$) species of plasma γ-T (A) and plasma (B) and urinary γ-CEHC (C) in 21 healthy volunteers at different time points between 0 and 72 h post-supplementation with 100 mg of d^2-γ-TAC. The right panels show the corresponding values of alpha homologues. In the urine samples also the Simon metabolite quinone lactone (α-QL) deriving from the oxidative transformation of α-T is shown. Statistics for post-supplementation vs. baseline data: $^\circ p \leq 0.05$, $\wedge p$ between 0.01 and 0.001 and * ≤ 0.001 or greater.

these two subjects gave a total d^2-γ-CEHC excretion of 7.2 mg.

Effect of γ-T Supplementation on α-T Metabolism

The load of d^2-γ-TAC only marginally affected plasma levels of α-T (Fig. 2, Panel A). This was confirmed also from the slight increase in α-CEHC levels in plasma and urine, of urinary α-QL (Fig. 2) and plasma clearance of the α-CEHC (+24% at 24 h; p = n.s.). A positive relationship between baseline levels of α-T and baseline and 6 h post-supplementation levels of γ-T was observed ($r = 0.561$, $p < 0.01$ and $r = 0.493$, $p < 0.05$) (Fig. 3).

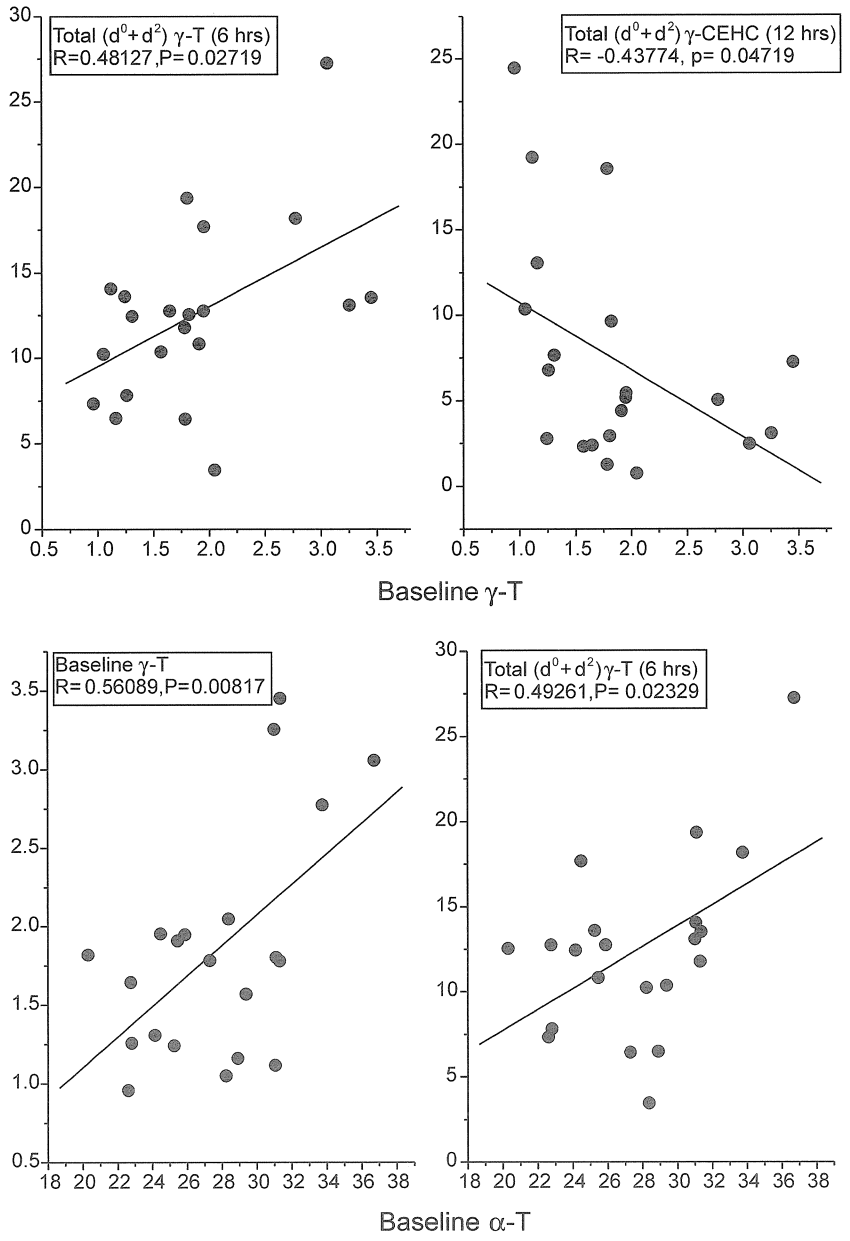

FIGURE 3 Linear regression analysis of the data (in μmol/l) of baseline α-T vs. baseline and 6 h γ-T (lower panels) and baseline γ-T vs. 6 h γ-T and 12 h total γ-CEHC (upper panels).

DISCUSSION

In this study we investigated the biokinetics and transformation of γ-T in healthy volunteers by administering 100 mg d^2-γ-TAC to each, in a single dose, with a standard meal. Following this challenge, the mean plasma concentration of γ-T peaked within 6 h and, on average, increased 7.7 fold over baseline concentration. In contrast, plasma and urine γ-CEHC concentrations reached their respective maxima between 6–12 and 9–24 h showing 6 and 14-fold increases, respectively. In contrast to these marked changes, the non-deuterated (d^0) homologues of γ-T and γ-CEHC were only slightly affected by the supplement, suggesting a low turnover rate for endogenous γ-T. At baseline, γ-T plasma concentration correlated positively with peak $d^0 + d^2$ concentrations of plasma γ-T and γ-CEHC, but negatively with peak $d^0 + d^2$ urinary γ-CEHC concentration suggesting that the renal excretion of γ-CEHC is regulated. Such a mechanism would help maintain set blood concentrations of this metabolite and could also explain the low α-CEHC/γ-CEHC urinary ratio found at baseline in this study.

Examination of the d^2-γ-T concentration curves suggests that less than 15 of the 100 mg of γ-T administered were transferred into plasma (11.5 μmol/l in an average plasma volume assumed to be 2.5 l). Although likely to be an underestimation due to tissue uptake, this value does not greatly

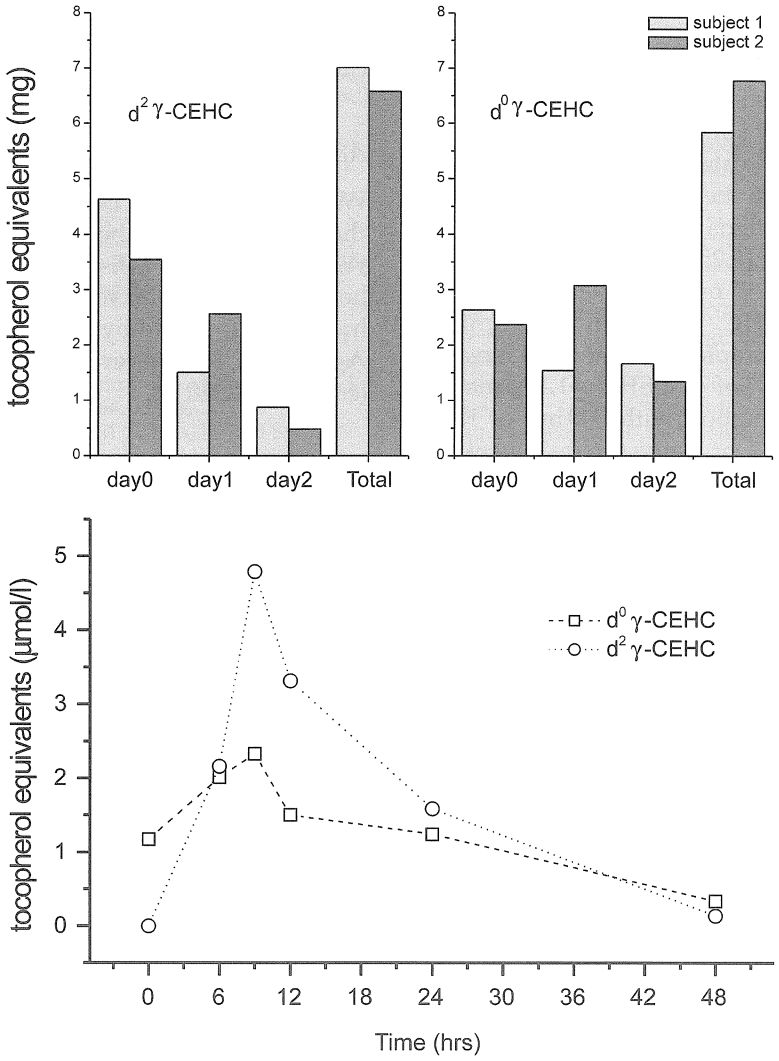

FIGURE 4 Daily amount of γ-metabolites excreted in urine in the 3 days following the supplementation with 100 mg of d^2-γ-TAC. The total urine collection was performed in two male volunteers in the day in which d^2-γ-TAC bolus was administered and in the following 2 days (top panels). An aliquot of urine was also collected at the time points of blood sampling and metabolite analysis was carried out (bottom panel) to compare the data in these two individuals with kinetics of urinary CEHC excretion of the whole population of volunteers (Fig. 2).

exceed the amount of d^2-γ-T equivalents recovered (approximately 7 mg) by total urine collection across 72 h. In healthy unsupplemented US volunteers, Swanson *et al.*[17] calculated a daily urinary excretion of γ-CEHC ranging from 1.2 to 7.8 mg and estimated this to be 50% of the daily-ingested γ-T. The difference between these US data and the results of the present study may be due, in part, to the high provision of γ-T in the US diet or alternatively to the limited absorption and excretion of the γ-T when provided in supplemental format. At present, we are not aware of other studies that could help clarify the basis of this discrepancy.

Previously, Lodge and colleagues[19] investigated the urinary excretion of α- and γ-CEHC after administering the corresponding acetate forms of tocotrienol vitamers. The administration of 125 or 500 mg of γ-tocotrienol acetate increased the production of γ-CEHC 4- to 6-fold over baseline with the maximum excretion occurring within the first day. In contrast, α-CEHC excretion increased only after the ingestion of 500 mg α-tocotrienol acetate, and only a small fraction of the supplements (1–6 %) was recovered as corresponding CEHC metabolite in urine. The limited recovery of CEHC metabolites in urine may be due, in part, to the elimination of CEHC by other routes, such as in bile. Rodent biliary concentrations of γ-CEHC after γ-tocotrienol supplementation suggest that this could be an important route of elimination of γ-T.[23] Nevertheless, the possibility that some γ-CEHC is retained in tissues with, or without, further transformation cannot be ruled out at this stage.

The current study revealed considerable inter-individual differences in response to γ-TAC supplementation (Fig. 1). Marked differences in plasma γ-T and plasma and urinary γ-CEHC concentrations were observed for a range of healthy subjects even

though they were administered the supplement under similar dietary conditions. These observations are similar to those seen previously in studies examining the biokinetics of oral supplements of α-tocopherol in healthy subjects.[24]

Common uptake and catabolic pathways exist for γ-T and α-T, in which hepatic and extra-hepatic tocopherol binding proteins playing a key regulatory role (reviewed in Refs. [2,25]). The lower affinity of α-TTP for γ-T makes it more available for rapid degradation to γ-CEHC. Accordingly, administration of 100 mg of γ-T rapidly resulted in increased plasma concentrations of γ-CEHC (1.17 μmol/l). In contrast, supplementation with 300 mg of RRR-α-T in a single dose[20] or daily for 4–7 weeks[26] results in much lower plasma concentrations of α-CEHC (0.42 and 0.20 μM, respectively). For the same reason, supplementation with 100 mg of γ-TAC did not appreciably influence α-T plasma concentrations or its catabolism (as both plasma α-CEHC and urinary α-CEHC and α-QL were largely unchanged). Therefore, while supplements of α-T influence the plasma concentration of γ-T and its metabolite,[20] the converse does not occur. Thus, under normal nutritional regimens the uptake and catabolism of α-T and γ-T are regulated to maintain a high blood steady-state α- to γ-T ratio, while the clearance of the corresponding CEHC from the plasma seems set to maintain a high plasma and possibly tissue γ- to α-CEHC ratio. Accordingly, we found the baseline urinary excretion of α-CEHC to be greater than γ-CEHC, which suggests differential regulation of the renal excretion rates for these CEHC homologues. Other studies have, however, reported conflicting results [27 and references therein] such that ingested γ-T is largely degraded and excreted in urine.[17] The current results however are in agreement with previous findings from our laboratory.[22]

In summary, these data demonstrate that oral supplements of γ-T are subject to a limited release into plasma, and rapid metabolic processing and excretion through the formation of CEHC. This high metabolic rate appears to exist in order to maintain low, but relatively constant plasma pools of γ-T and γ-CEHC. Moreover, since γ-CEHC was described to play some specific biological functions as an anti-inflammatory[11] and antioxidant molecule,[13] and natriuretic factor,[12] the evidence of a sustained γ-T catabolism in humans raises the question whether γ-CEHC formation could have a precise biological meaning as opposed to just representing a catabolic route for γ-T.

The catabolism of γ-T does not seem to interfere with the catabolism of α-T. In addition, as previously reported for α-T,[24] a broad inter-individual variability in terms of uptake and transformation is observed also for γ-T.

The findings in this and other studies like it highlight the need of a better understanding of the catabolism of γ-T.

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