methods

Quantitation of rat liver vitamin E metabolites by LC-MS during high-dose vitamin E administration

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Abstract To evaluate vitamin E metabolism, a method was developed to quantitate liver α- and γ-tocopherol metabolites, α-carboxyethyl hydroxychroman [α-CEHC; 2,5,7,8-tetramethyl-2-(2′-carboxyethyl)-6-hydroxychroman] and γ-CEHC [2,7,8-trimethyl-2-(2′-carboxyethyl)-6-hydroxychroman], respectively. Vitamin E supraenriched livers were obtained from rats that were injected with vitamin E daily for 18 days. Liver samples (~50 mg) were homogenized, homogenate CEHC-conjugates were hydrolyzed, CEHCs were extracted with ethyl ether, and then CEHCs were quantitated using liquid chromatography-mass spectrometry (LC-MS). Precision, based on intersample variability, ranged from 1% to 3%. Recovery of α- and γ-CEHCs added to liver homogenates ranged from 77% to 87%. Detection limits of α- and γ-CEHC were 20 fmol, with a linear detector response from 0.025 to 20 pmol injected. Corresponding with an increase in liver α-tocopherol, the MS peak for liver α-CEHC (mass-to-charge ratio 277.8) increased 80-fold (0.18 ± 0.01 to 15 ± 2 nmol/g). Liver α-CEHC concentrations were correlated with serum α-CEHC, liver α-tocopherol, and serum α-tocopherol ($P < 0.001$ for each comparison). α-CEHC represented 0.5–1% of the liver α-tocopherol concentration. Thus, LC-MS can be successfully used to quantitate α- and γ-CEHC in liver samples. These data suggest that in times of excess liver α-tocopherol, increased metabolism of α-tocopherol to α-CEHC occurs.—Leonard, S. W., E. Gumpricht, M. W. Devereaux, R. J. Sokol, and M. G. Traber. **Quantitation of rat liver vitamin E metabolites by LC-MS during high-dose vitamin E administration. *J. Lipid Res.* 2005.** 46: **1068–1075.**

Supplementary key words α-carboxyethyl hydroxychroman • γ-carboxyethyl hydroxychroman • mass spectrometry • vitamin E metabolism • α-tocopherol • γ-tocopherol • liquid chromatography-mass spectrometry

Vitamin E is a term encompassing a group of lipid-soluble, chain-breaking antioxidants that include α-, β-, γ-, and δ-tocopherol and the corresponding tocotrienols (1). Vitamin E differs from other fat-soluble vitamins in that it is not accumulated in the liver to toxic levels during times of excess intake (1). A high rate of vitamin E metabolism and/or excretion may be important in preventing adverse effects (2), but this hypothesis has not yet been tested. Tocopherols and tocotrienols are not only excreted directly but can be metabolized in the liver by hepatocytes (3–5); other tissues involved in vitamin E metabolism have not been specifically identified.

α- and γ-tocopherols are converted to their respective carboxyethyl hydroxychroman (CEHC) derivatives, α-CEHC [2,5,7,8-tetramethyl-2-(2′-carboxyethyl)-6-hydroxychroman] and γ-CEHC [2,7,8-trimethyl-2-(2′-carboxyethyl)-6-hydroxychroman] (**Fig. 1**). The specific steps involved in vitamin E metabolism have been identified in vitro using HepG2 cells. These steps initially involve ω-oxidation by cytochrome P450 enzymes followed by β-oxidative degradation (3, 4, 6).

To evaluate in vivo metabolism of tocopherols, metabolite concentrations have been measured in human urine (5, 7–13), human plasma (5, 13–19), and rat bile (20, 21). Recently, CEHCs have been reported to have potential health benefits (22–25) as well as antioxidant functions (26, 27). These latter functions are questionable because of the extremely low CEHC concentrations in human plasma (14, 15, 18, 22). There have also been discussions of the use of metabolite concentrations found in urine and plasma as indicators of vitamin E status (7, 8, 14, 28, 29), but, as this area is still fairly new and evolving, there is no clear evidence to support the use of metabolites as vitamin E status indicators.

To our knowledge, there are no reports on tissue metabolite concentrations. Based on tissue culture studies of metabolite formation by hepatocytes (3–5), it is likely that CEHCs are produced in the liver. Therefore, we have de-

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Abbreviations: CEHC, carboxyethyl hydroxychroman; LC-MS, liquid chromatography-mass spectrometry; MDR1, multidrug resistance protein-1; *m/z*, mass-to-charge ratio; SXR, steroid and xenobiotic receptor; UGT1A1, UDP-glucuronosyltransferase-1A1.

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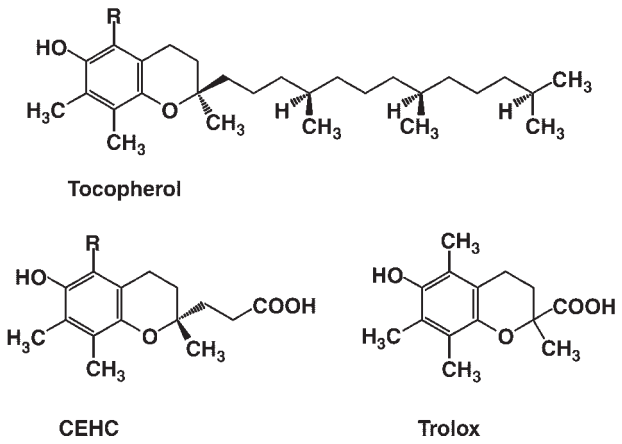

Fig. 1. Structures of tocopherols, carboxyethyl hydroxychromans (CEHCs), and trolox. R: CH_3 = α-tocopherol or α-CEHC; H = γ-tocopherol or γ-CEHC.

veloped a method to determine α- and γ-CEHC concentrations in rat liver samples. The technique for loading rat liver with vitamin E using intramuscular or subcutaneous injections of α-tocopherol has been reported (30). We used these highly vitamin E-enriched liver samples to assess the relationships between α- and γ-tocopherols and α- and γ-CEHC concentrations.

MATERIALS AND METHODS

Materials

Standards of α-CEHC and γ-CEHC (LLU-α) were gifts from W. J. Wechter of Loma Linda University. α-Tocopherol standard was a gift provided by Dr. James Clark of Cognis Nutrition and Health (LaGrange, IL).

A veterinary injectable form of D-α-tocopherol was obtained from Schering-Plough Animal Health (Vital E®-300). Vital E®-300 is a nonaqueous solution containing 300 IU/ml D-α-tocopherol [manufacturer's description; analyzed to contain α-tocopherol (163 mg/ml) and γ-tocopherol (7 mg/ml)] compounded with 20% ethanol and 1% benzyl alcohol in an emulsifiable base for use in swine, cattle, and sheep.

HPLC-grade methanol, hexane, ethanol, and glacial acetic acid were obtained from Fisher (Fair Lawn, NJ). γ-Tocopherol, ascorbic acid, potassium hydroxide, butylhydroxy toluene, lithium perchlorate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox, 98% purity), and β-glucuronidase (type H-1; contains minimum 300,000 U/g β-glucuronidase activity and minimum 10,000 U/g sulfatase activity) were from Sigma-Aldrich (St. Louis, MO). Diethyl ether was obtained from Mallinckrodt Baker, Inc. (Phillipsburg, NJ).

Study design and serum and liver tocopherol measurements

The study design and results have been reported previously (30). Briefly, male Sprague-Dawley rats (8 weeks old) were administered a daily dose of 10 mg/100 g body weight (~25 mg/injection) of Vital E®-300 for up to 18 days, either intramuscularly at different sites in the quadriceps muscle or subcutaneously under the abdominal skin, while eating a standard rat chow (diet 2018; Harlan Teklad, Madison, WI) containing 30 IU vitamin E/kg diet. On days 3 (T3), 6 (T6), 9 (T9), and 18 (T18), 12 h after the previous injection, animals were killed, serum was obtained, and livers were removed. Serum and liver α- and γ-tocopherol concentrations were determined using HPLC as described by Podda et al. (31).

Serum and liver CEHC extraction

Serum CEHCs were extracted using a modified method of Lodge et al. (11). Briefly, internal standard (trolox) was added to 0.5 ml of serum, as was 10 μl of a 1% ascorbic acid solution. Because CEHCs are excreted as sulfate or glucuronic acid conjugates, samples were hydrolyzed by the addition of 100 μl of enzyme solution (1 mg of β-glucuronidase in 100 μl of 10 mM potassium phosphate buffer, pH 6.8). After a 30 min incubation at 37°C, the samples were acidified by the addition of 10 μl of 12 M HCl. CEHCs were subsequently extracted with 5 ml of diethyl ether. An aliquot of the ether fraction was collected and dried under N_2, and the residue was resuspended in 1:1 (v/v) water-methanol containing 0.05% (v/v) acetic acid and 0.05% (w/v) ascorbic acid.

For liver tissue, samples (~50 mg) were homogenized with a Polytron homogenizer (Brinkmann, Westbury, NY) using a 7 mm foam reducing generator in a 10 ml screw-cap tube containing 2 ml of PBS with 0.05% EDTA, 100 μl of 1% ascorbic acid (10 mg/ml), and internal standard (trolox). The CEHC conjugates were then hydrolyzed by the addition of 100 μl of enzyme solution (1 mg of β-glucuronidase in 100 μl of 10 mM potassium phosphate buffer, pH 6.8). Subsequently, samples were incubated and extracted as described for serum. For liver samples, the ether suspensions were transferred to microcentrifuge tubes and spun at 16,000 *g* for 5 min at 10°C, and the supernatant was transferred to injection vials.

CEHC measurement by LC-MS

Liquid chromatography. Extracted CEHCs were analyzed by liquid chromatography-mass spectrometry (LC-MS) using a Waters (Milford, MA) 2690 Separations Module. Instrument control and acquisition were done using Waters Masslynx version 3.4 software. The column used was a SymmetryShield™ RP-18 column (3.0 × 150 mm, 3.5 μm particle; Waters) with a SymmetryShield™ Sentry™ RP-18 precolumn (3.9 × 20 mm, 3.5 μm particle; Waters). The solvents used for the gradient were methanol and water, both containing 0.05% acetic acid, as described by Himmelfarb et al. (32). The system was first equilibrated with 50% methanol for 1 min, followed by a linear gradient to 80% methanol in 6 min at a flow rate of 0.20 ml/min. These conditions were maintained for 15 min, followed by a 5 min wash period with 95% methanol, at which time the original conditions were established and run for 5 min before injection of the next sample.

Mass spectrometry. Samples were analyzed using a Micromass (Manchester, UK) ZQ 2000 single-quadrupole mass spectrometer with an electrospray ionization probe set to negative ionization mode. The capillary voltage was set to 2.5 kV, and the sample cone voltage was −30 V. The desolvation temperature was set at 150°C. The desolvation gas (nitrogen) was set to 160 l/h, the nebulizer gas (nitrogen) at 80 psi., and the cone gas (nitrogen) at 50 l/h. Single-ion recording mass-to-charge ratio (*m/z*) data were obtained for α-CEHC (*m/z* 277.8, molecular weight 278.3), γ-CEHC (*m/z* 263.8, molecular weight 264.3), and trolox (*m/z* 249.8, molecular weight 250.3). The dwell time for each of the ions was set at 0.20 s. Typical retention times were 14.2, 14.6, and 15.4 min for trolox, γ-CEHC, and α-CEHC, respectively. Deuterium-labeled CEHCs are not commercially available; thus, quantitation was performed using an internal standard of trolox, which was shown previously to be an effective internal standard for CEHC analysis (11). Sample CEHC concentrations were calculated from the peak area of the corresponding ion to that of the trolox peak. The working linear range for quantitation was 0.2–

20 pmol injected, and the lower limit of detection was 0.08 pmol injected (this is approximately equivalent to 1 nmol/l serum).

Statistical data analyses

Statistical analysis was performed using GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA). An unpaired Student's *t*-test or one-way ANOVA was carried out using log-transformed data to normalize unequal variances between groups. A value of $P < 0.05$ was considered statistically significant. Post hoc tests were performed using Bonferroni's multiple comparison when overall group effects were found to be significant. Data are expressed as means ± SEM. The data from intramuscularly and subcutaneously injected animals were pooled at each time point, because differences between administration techniques were not statistically significant. Sample sizes were n = 4 at T0 and n = 8 at all other time points. Serum tocopherol analysis was limited by sample size, such that at T6, n = 7, at T9, n = 4, and at T18, n = 2. Note that on day 18, only serum was available from the intramuscularly injected animals.

RESULTS

Liver LC-MS CEHC method development

Authentic α- and γ-CEHCs, ranging from 0.025 to 20 pmol, injected simultaneously generated a linear MS response (data not shown). The detection limit was 20 fmol. A typical chromatogram documents the separation of α-CEHC (*m/z* 277.8), γ-CEHC (*m/z* 263.8), and trolox (*m/z* 249.8) (**Fig. 2A**). A representative rat liver sample before α-tocopherol treatment (T0) demonstrates the low hepatic CEHC concentrations in rats consuming a standard chow diet (Fig. 2B). However, after 3 days of vitamin E injections, liver α-CEHC increased ~80-fold (from 0.18 ± 0.01 to 15 ± 2 nmol/g liver) (compare Fig. 2B, C). Figure 2 also demonstrates that the chromatographic peaks are correctly identified. The same trolox amounts were added to the standard and the two samples (Fig. 2A–C), and the peak heights were similar in all three chromatograms, suggesting that there was little sample interference or loss of trolox during sample preparation.

Precision and recovery

The coefficients of variation for five repeat injections of two liver samples were 2–3% for α-CEHC and 1% for γ-CEHC. An experiment to test recovery through the entire analytical procedure was performed by adding 115 pmol of α-CEHC and 144 pmol of γ-CEHC to four identical liver samples before homogenization. A control liver sample with no added CEHCs was analyzed simultaneously, and the background CEHC amounts were subtracted. Recoveries of added α- and γ-CEHC were 77 ± 6% and 87 ± 1%, respectively. The release of CEHC from glucuronide or sulfate conjugates was also tested in six liver samples (four T3 and two T6). The samples were analyzed in parallel, with and without the addition of β-glucuronidase. After enzyme hydrolysis, α- and γ-CEHC increased 29 ± 5% and 43 ± 6%, respectively.

Serum tocopherol and CEHC concentrations

Pharmacological amounts of vitamin E were administered daily to rats for up to 18 days. Serum α-tocopherol concentrations dramatically increased from T0 to T3 (from 12 ± 1 to 246 ± 38 μM; $P < 0.001$) and remained increased through T9 (193 ± 37 μM) (**Fig. 3A**) (30). Despite daily vitamin E injections through T18, serum α-tocopherol concentrations decreased from T9 to T18 (36 ± 4 μM; $P < 0.05$). To evaluate why the serum α-tocopherol concentrations decreased, serum α-CEHC concentrations were measured. Similar to serum α-tocopherol, serum α-CEHC concentrations increased significantly after vitamin E injections (from 0.03 ± 0.003 to 2 ± 0.2 μM at T0 and T3,

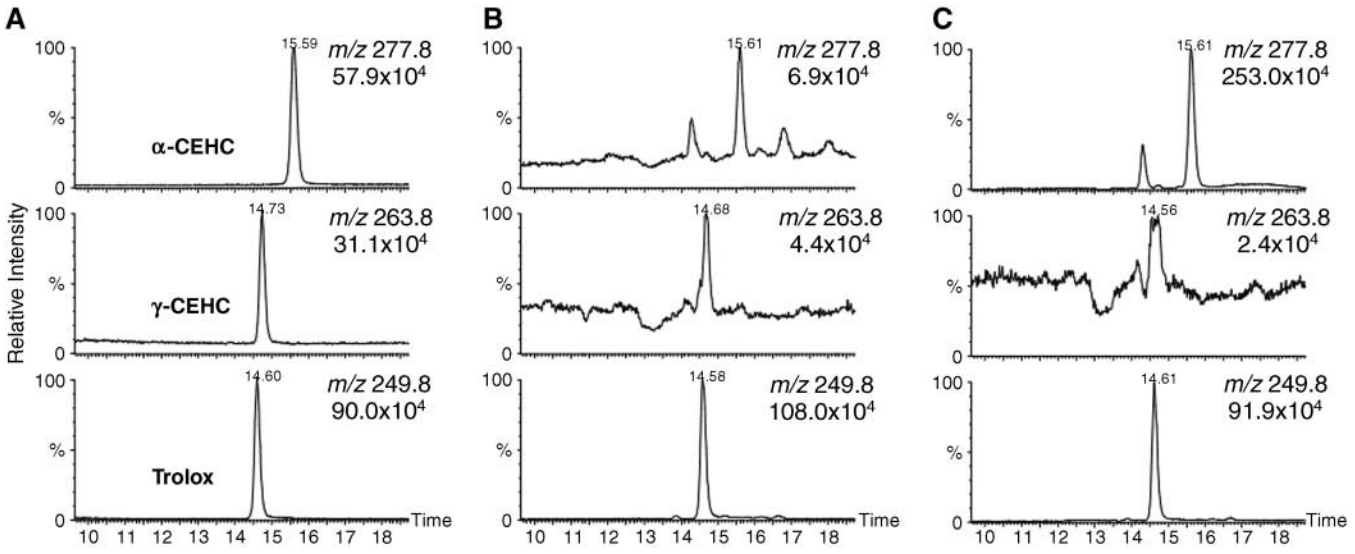

Fig. 2. Single-ion recording mass spectral data of vitamin E metabolites from a standard (A), a representative rat liver sample at baseline (B), and a representative rat liver sample after vitamin E injections (C). The α-tocopherol metabolite α-CEHC [mass-to-charge ratio (*m/z*) 277.8] was shown to increase after 3 days of vitamin E injections (C vs. B), whereas the metabolite of γ-tocopherol, γ-CEHC (*m/z* 263.8), did not. The internal standard, trolox (*m/z* 249.8), remained consistent in all traces.

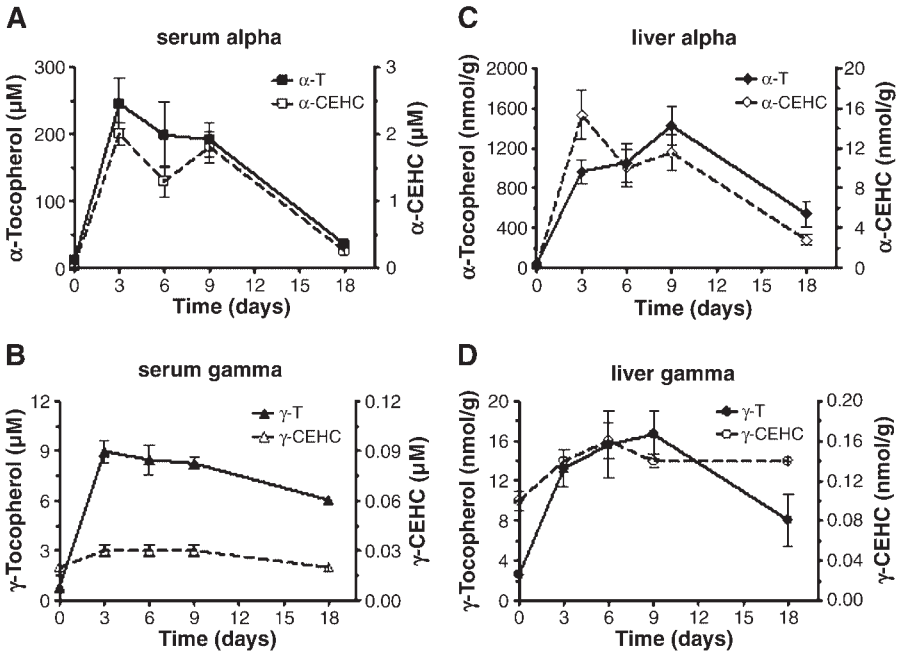

Fig. 3. Kinetic analysis of rat serum and liver α-tocopherol (α-T) and α-CEHC (A, C) and γ-tocopherol (γ-T) and γ-CEHC (B, D) levels. As described in Materials and Methods, rats were injected daily with emulsified vitamin E for 18 d and analysis was performed on samples collected on days 0, 3, 6, 9, and 18 (the number of animals used for analysis is described in Statistical Data Analyses). Data are expressed as means ± SEM.

respectively; $P < 0.001$) (Fig. 3A). In fact, the serum α-CEHC increase was greater than the α-tocopherol increase (60-fold compared with 20-fold). Consistent with the serum α-tocopherol responses, serum α-CEHC concentrations decreased significantly from T9 to T18 (from 1.81 ± 0.23 to 0.26 ± 0.05 μM; $P < 0.001$), suggesting a decrease in vitamin E metabolite formation.

Serum γ-tocopherol (Fig. 3B) also increased with vitamin E injections from T0 to T3 (from 0.83 ± 0.11 to 9 ± 1 μM; $P < 0.001$). This increase reflects the 4% γ-tocopherol in the vitamin E solution used for injection because serum γ-tocopherol represented 3.5% of the total vitamin E increase from T0 to T3. Unlike serum α-tocopherol, γ-tocopherol remained increased through T18 (6 ± 0.1 μM), consistent with the constant proportion of γ-tocopherol injected throughout the study. Surprisingly, serum γ-CEHC did not increase with serum γ-tocopherol increases but remained constant throughout the study (Fig. 3B).

Liver tocopherol and CEHC concentrations

Liver α-tocopherol also increased with vitamin E injections from T0 to T3 (43 ± 3 to 965 ± 121 nmol/g; $P < 0.001$) (Fig. 3C). This ~20-fold increase in liver α-tocopherol was similar to the observed serum α-tocopherol increase. Liver α-tocopherol concentrations remained increased at T9 (1,423 ± 187 nmol/g), then decreased at T18 (539 ± 130 nmol/g; $P < 0.01$, T9 compared with T18).

After vitamin E injections, from T0 to T3, liver α-CEHC concentrations increased 75-fold (from 0.2 ± 0.01 to 15 ± 2 nmol/g, respectively; $P < 0.001$) (Fig. 3C). Similar to serum α-CEHC, despite daily vitamin E injections, liver α-CEHC concentrations decreased significantly from T9 to T18 (from 12 ± 2 to 3 ± 0.5 nmol/g; $P < 0.001$), also suggesting a decrease in α-tocopherol metabolism.

Liver γ-tocopherol concentrations, similar to serum γ-tocopherol, increased with vitamin E injections from T0 to T3 (from 3 ± 0.2 to 13 ± 2 nmol/g, respectively; $P = 0.01$) (Fig. 3D). However, liver γ-tocopherol concentrations remained increased at T9 (17 ± 2 nmol/g), then decreased at T18 (8 ± 3 nmol/g; $P < 0.01$, T9 compared with T18). Despite daily vitamin E injections, liver γ-CEHC remained constant throughout the study (Fig. 3D), similar to serum γ-CEHC (Fig. 3B).

Although the fold increases in α-CEHC concentrations from T0 to T3 were greater than those for α-tocopherol (serum α-CEHC, 60-fold compared with α-tocopherol, 20-fold; liver α-CEHC, 80-fold compared with α-tocopherol, 20-fold), α-CEHC as a percentage of α-tocopherol was approximately the same for both serum and liver. The percentage α-CEHC range was 0.5–1.0% for serum and 0.4–1.7% for liver. The γ-CEHC as a percentage of γ-tocopherol range was 0.3–2.0% for serum and 0.8–4.0% for liver.

Serum and liver CEHC correlations

Increased serum and liver α- and γ-tocopherol concentrations were expected to increase the production of vitamin E metabolites. Consistent with this hypothesis, we found that serum α-CEHC was correlated with serum and liver α-tocopherol as well as with liver α-CEHC ($P < 0.0001$ for each), the latter suggesting that serum α-CEHC arises from the liver α-CEHC (**Fig. 4**). Liver α-CEHC was also correlated with liver and serum α-tocopherol (Fig. 4) ($P < 0.0001$ for both).

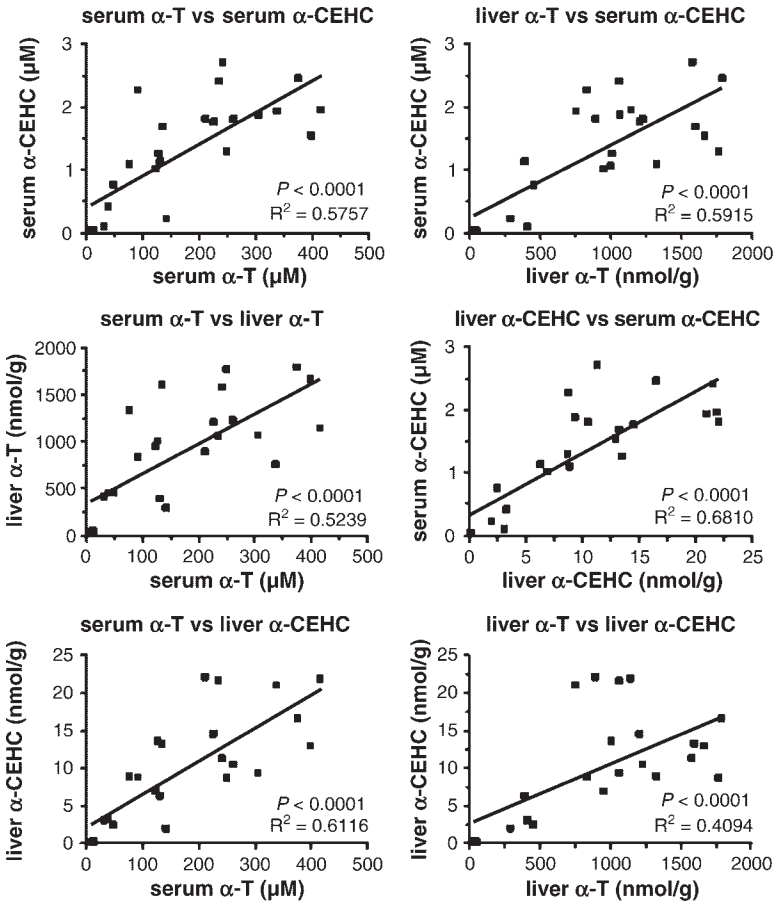

Fig. 4. Correlations of serum and liver α-tocopherol (α-T) and α-CEHC concentrations. All correlations performed were found to be significant, showing a strong relationship between serum and liver α-CEHC and α-tocopherol concentrations. The correlations were performed on all samples collected over 18 days from rats injected daily with vitamin E, as described in Materials and Methods.

Although the increase in serum and liver γ-tocopherol was not as great as that of α-tocopherol, serum γ-CEHC was correlated with both serum ($P = 0.0057$) and liver ($P < 0.0001$) γ-tocopherol (**Fig. 5**). However, liver γ-CEHC was not correlated with either serum γ-tocopherol or γ-CEHC (data not shown), although liver γ-CEHC was correlated with liver γ-tocopherol ($P = 0.0067$) (Fig. 5). Interestingly, serum γ-CEHC was correlated with both serum ($P < 0.005$) and liver ($P = 0.0006$) α-tocopherol concentrations (Fig. 5).

DISCUSSION

The objective of this study was to develop a method to extract and quantitate liver α- and γ-CEHCs using LC-MS. After daily vitamin E injections to rats, CEHCs were extracted from liver homogenates and analyzed by LC-MS. The MS peak corresponding to α-CEHC (*m/z* 277.8) was found to greatly increase (Fig. 2B, C, retention time 15.6 min), confirming that the chromatographic peaks were correctly identified. The CEHC method proved to be very reliable and extremely sensitive, with detection limits at low femtomole amounts injected.

Liver α-CEHC concentrations (Fig. 3C) were found to increase within 3 days of vitamin E injection, similar to liver α-tocopherol (30). Both serum α-tocopherol and α-CEHC mirrored the changes observed in the liver (Fig. 3A). The consistent responses between serum and liver tocopherols and CEHCs confirmed that liver CEHCs were in fact correctly identified, further validating the LC-MS detection method.

An interesting finding was that liver α-CEHC decreased significantly from day 9 to day 18, despite the continued daily vitamin E injections. We had previously noted the decrease in liver α-tocopherol (30) and had several different hypotheses regarding the mechanism of the decrease, such as reduced absorption, increased metabolism, or increased vitamin E excretion. The results presented here suggest that the decrease in liver vitamin E was not attributable to an increase in metabolism because α-CEHC decreased in parallel with α-tocopherol (Fig. 3), suggesting that alternative pathways may have been activated to dispose of α-tocopherol, such as biliary tocopherol excretion (33). The decrease in liver γ-tocopherol without a corresponding increase in γ-CEHC supports the idea that biliary excretion of unmodified tocopherols might have been increased. There are some experimental data to suggest that vitamin E could regulate its own concentrations. For example, α-tocopherol binds to the pregnane X receptor [also known as the steroid and xenobiotic receptor (SXR)] (34, 35). As a result of the activation of this nuclear receptor, xenobiotic metabolism could be upregulated, potentially modulating the expression of not only P450 enzymes but conjugating enzymes and transporters (2). Moreover, Zhou et al. (36) found that not only did vi-

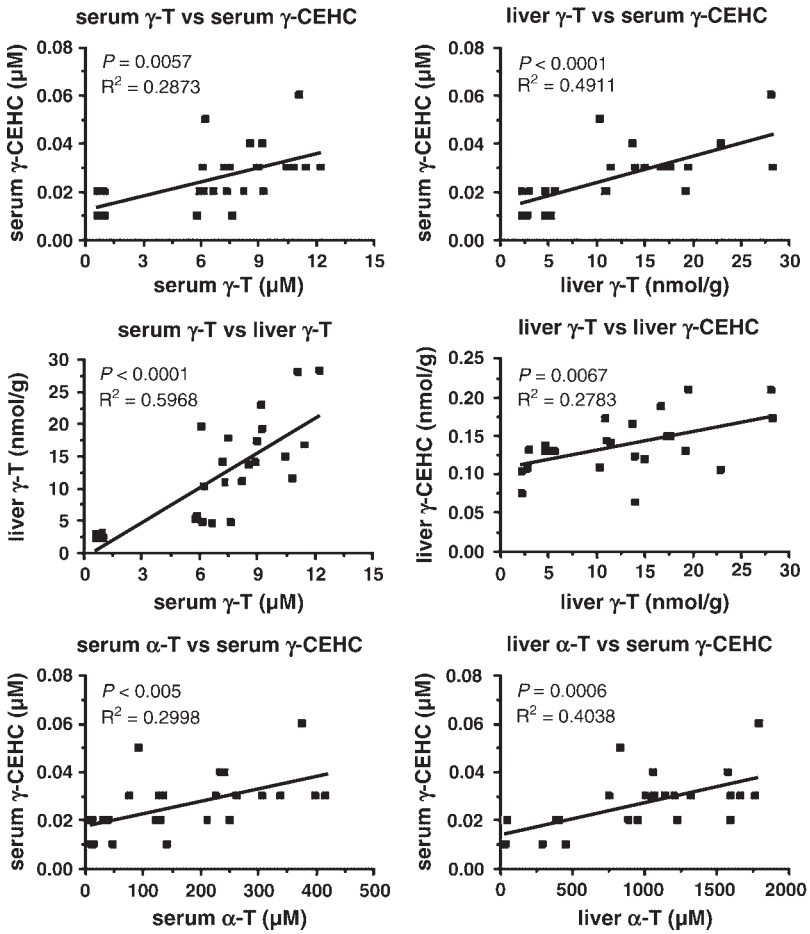

Fig. 5. Correlations of serum and liver γ-tocopherol (γ-T) and γ-CEHC concentrations. Only correlations found to be significant are shown. The correlations were performed on all samples collected over 18 days from rats injected daily with vitamin E, as described in Materials and Methods. Serum γ-CEHC was also found to be significantly correlated with serum and liver α-tocopherol (α-T), as shown in the bottom two panels.

tamin E upregulate these various systems through SXR upregulation but that liver and intestinal cells were differently regulated. In primary hepatocytes, CYP3A4 but not UDP-glucuronosyltransferase-1A1 (UGT1A1) or multidrug resistance protein-1 (MDR1) was upregulated by tocotrienols, the most potent stimulators of SXR. By contrast, the reverse was true in intestinal cells: CYP3A4 was not upregulated, whereas UGT1A1 and MDR1 were upregulated. Unfortunately, we have not measured any other possible routes for vitamin E excretion or whether there was increased accumulation of vitamin E metabolite precursors.

The increase in liver and serum γ-tocopherol concentrations after the vitamin E injections is most likely explained by the observation that the solution used for the injections contained ~4% γ-tocopherol. Quite surprisingly, neither serum nor liver γ-CEHC concentrations changed over the course of the study (Fig. 3). Although Brigelius-Flohé's group (7) has suggested that a human serum α-tocopherol concentration of 30–40 μM needs to be reached before α-CEHC can be detected in the urine, this limitation does not appear to be the case for γ-tocopherol. For example, Sontag and Parker (6) have identified CYP4F2 as the tocopherol ω-hydrolase involved in vitamin E metabolism and report that it has higher catalytic activity with respect to γ- than α-tocopherol. Moreover, Birringer et al. (3, 4) report that HepG2 cells more actively metabolize γ-compared with α-tocopherol but that upregulation of CYP3A by incubation of the cells with rifampicin could increase α-tocopherol metabolism (3). These findings suggest that α- and γ-tocopherols may be initially ω-hydroxylated by different cytochrome P450 enzymes. Consistent with this suggestion are our recent findings in γ-tocopherol-fed mice in which no regulation of Cyp4f was detected but Cyp3a varied with the liver α-tocopherol concentration (37). Therefore, it is plausible that the increased α-tocopherol concentrations in the liver increased CYP3A, leading to increased α-CEHC production, whereas the increased γ-tocopherol concentrations did not upregulate CYP4F, so γ-CEHC was unchanged. Alternatively, the enormous increase in liver α-tocopherol serving as a P450 substrate could have outcompeted the γ-tocopherol for metabolizing enzymes; therefore, no increases in γ-CEHC were observed.

A recent meta-analysis suggested that vitamin E supplements are associated with an increased risk of dying (38). Such a relationship suggests that vitamin E accumulates to toxic levels to cause an adverse effect. However, the data

shown here demonstrate that increased hepatic α-tocopherol concentrations cause an upregulation of vitamin E metabolism and α-CEHC production. Moreover, the dramatic decreases in both α-tocopherol and α-CEHC suggest that alternative xenobiotic pathways for increased vitamin E excretion may be upregulated (2). The possible upregulation of xenobiotic pathways suggests that vitamin E may cause altered drug metabolism and thus could have adverse effects in patients taking pharmaceutical agents. However, this statement is merely speculation and further studies are needed for its evaluation.

In conclusion, we have described a very sensitive and reliable method to measure liver α- and γ-CEHCs. This method will be an invaluable tool for elucidating the regulation of vitamin E metabolism. Further work is warranted to characterize the mechanism(s) for the regulation of liver and serum α- and γ-tocopherol and α- and γ-CEHCs.

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