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Tocopherols and Tocotrienols Are Bioavailable in Rats and Primarily Excreted in Feces as the Intact Forms and 13′-Carboxychromanol Metabolites

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ABSTRACT

Background: Vitamin E α-, γ-, or δ-tocopherol (αT, γT, δT) and γ- or δ-tocotrienol (γTE, δTE) are metabolized to hydroxychromanols and carboxychromanols including 13′-carboxychromanol (13′-COOH), 11′-COOH, and carboxyethyl hydroxychroman (CEHC), some of which have unique bioactivities compared with the vitamers. However, the bioavailability of these metabolites has not been well characterized.

Objective: We investigated the pharmacokinetics (PK) of vitamin E forms and metabolites in rats.

Methods: Six-week-old male Wistar rats received 1-time gavage of γT-rich tocopherols (50 mg/kg) containing γT/δT/αT (57.7%, 21.9%, and 10.9%, respectively) or δTE-rich tocotrienols (35 mg/kg) containing δTE/γTE (8:1). We quantified the time course of vitamin E forms and metabolites in the plasma and their 24-h excretion to the urine and feces. The general linear model repeated measure was used for analyses of the PK data.

Results: In the rats' plasma, C_{max} of γT or δTE was 25.6 ± 9.1 μM (T_{max} = 4 h) or 16.0 ± 2.3 μM (T_{max} = 2 h), respectively, and sulfated CEHCs and sulfated 11′-COOHs were the predominant metabolites with C_{max} of 0.4–0.5 μM (T_{max} ~5–7 h) or ~0.3 μM (T_{max} at 4.7 h), respectively. In 24-h urine, 2.7% of γT and 0.7% of δTE were excreted as conjugated CEHCs. In the feces, 17–45% of supplemented vitamers were excreted as unmetabolized forms and 4.9–9.2% as unconjugated carboxychromanols, among which 13′-COOHs constituted ~50% of total metabolites and the amount of δTE-derived 13′-COOHs was double that of 13′-COOH derived from γT.

Conclusions: PK data of vitamin E forms in rats reveal that γT, δT, γTE, and δTE are bioavailable in the plasma and are mainly excreted as unmetabolized forms and long-chain metabolites including 13′-COOHs in feces, with more metabolites from tocotrienols than from tocopherols. *J Nutr* 2020;150:222–230.

Keywords: carboxychromanol, γ-tocopherol, δ-tocotrienol, vitamin E, excretion, pharmacokinetic, metabolism, antioxidant, inflammation

Introduction

The vitamin E family has 8 naturally occurring fat-soluble antioxidants including α-, γ-, and δ-tocopherol (αT, γT, and δT) and γ-, and δ-tocotrienol (γTE and δTE) (1) (Figure 1). All the vitamin E forms possess a chromanol ring and a 13-carbon-length side chain. Tocopherols have a saturated side chain (Figure 1A), whereas tocotrienols have an unsaturated side chain (2) (Figure 1B). Among the different isoforms, αT is the predominant vitamin E in the plasma and tissues owing to its high binding affinity to hepatic α-tocopherol transfer protein, which transports αT and, to a lesser extent, other vitamin E forms (2–4). In contrast, other vitamin E forms are substantially metabolized in the liver by cytochrome P450-4F2 via ω-hydroxylation and dehydrogenation to generate 13′-hydroxychromanol (13′-OH) and 13′-carboxychromanol (13′-COOH), which is further degraded via β-oxidation to medium- or short-chain carboxychromanols including the terminal metabolite, 3′-COOH or 2-(β-carboxyethyl)-6-hydroxychroman (CEHC) (2, 5) (Figure 1B). Although tocotrienols and tocopherols are similarly metabolized, catabolism of tocotrienols likely involves additional enzymes such as enoyl-CoA isomerases and dienoyl-CA reductases for the metabolism of the double bonds (Figure 1B) (6, 7). In addition, conjugation of carboxychromanols occurs parallel with β-oxidation to generate sulfated long-chain and short-chain carboxychromanols (6, 8, 9) (Figure 1). The terminal metabolite CEHCs and conjugated metabolites are mainly excreted in the urine, whereas longer-chain

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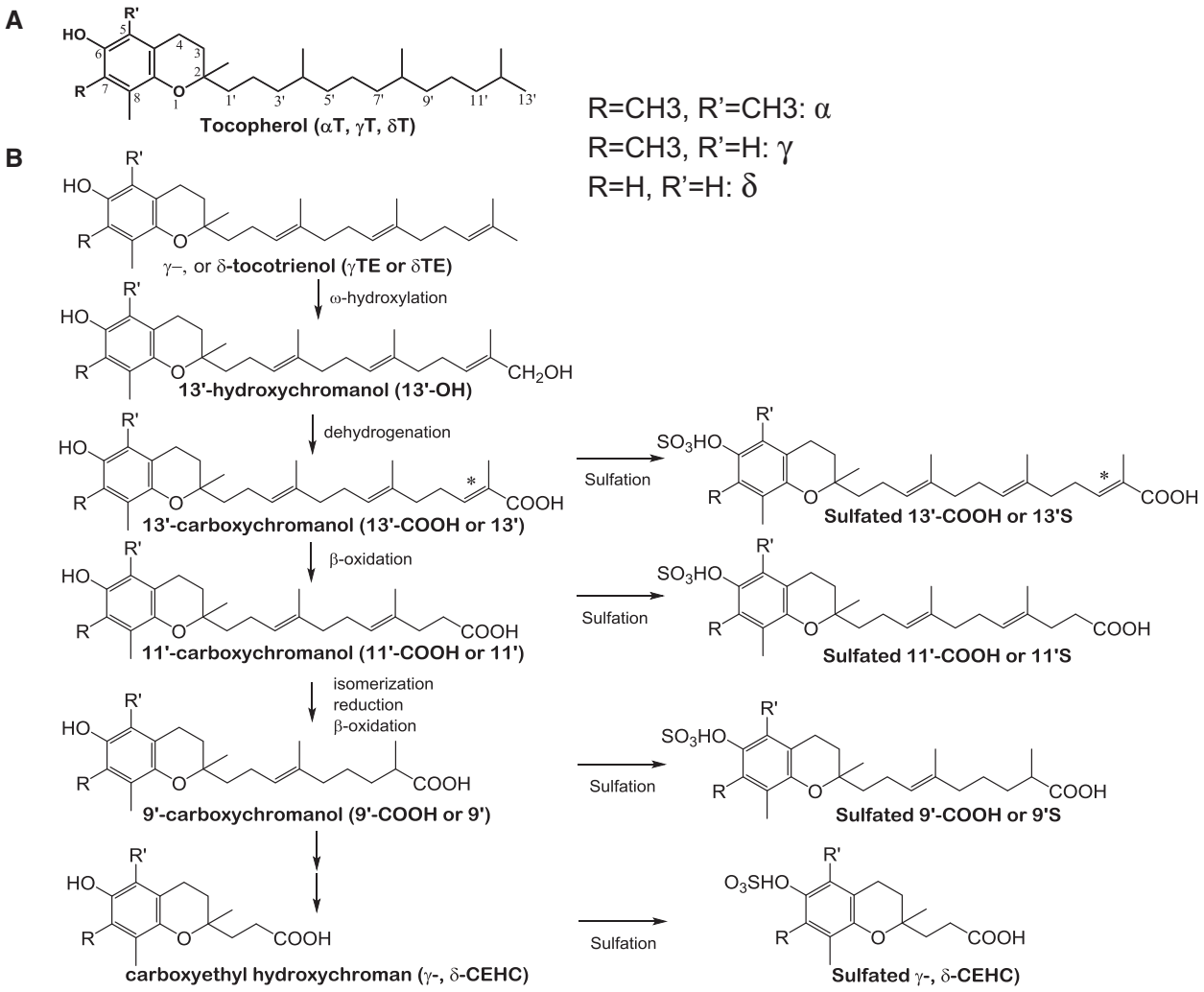

FIGURE 1 Molecular structures of vitamin E forms and vitamin E metabolism. (A) Structures of αT, γT, and δT. (B) Structures of γTE and δTE, and their metabolism. Tocopherols and tocotrienols are metabolized by ω-hydroxylation and dehydrogenation to form 13′-OH and 13′-COOH, which is then further metabolized by β-oxidation to form 11′-COOH, 9′-COOH, and the terminal metabolite CEHC as well as conjugated carboxychromanols. Compared with tocopherols, catabolism of tocotrienols involves isomerization and reduction, in addition to β-oxidation. *This position may have either a double bond or a saturated bond. CEHC, 2-(β-carboxyethyl)-6-hydroxychroman, also called 3′-COOH; COOH, carboxychromanol; 13′-OH, 13′-hydroxychromanol; αT, α-tocopherol; γT, γ-tocopherol; γTE, γ-tocotrienol; δT, δ-tocopherol; δTE, δ-tocotrienol.

carboxychromanols appear to be excreted in feces after supplementation of γT and δT (10–15).

Although most research has focused on vitamin E forms, short- and long-chain metabolites have been shown to have stronger bioactivities than the vitamers (2, 16). For instance, γ-CEHC (or 3′-COOH), but not tocopherols, appears to have natriuretic activity (17). We have demonstrated that 13′-COOHs are potent dual inhibitors of cyclooxygenases (18) and 5-lipoxygenase, and for these activities, 13′-COOHs are much stronger than vitamin E forms (19, 20). Further, 13′-COOHs induce apoptosis and autophagy in human cancer cells, but not normal cells, and suppress colon tumor development in mice (16, 20). Despite these interesting activities of vitamin E metabolites, the amounts of long-chain metabolites formed in vivo have not been well characterized. In this study, using our own developed LC-tandem MS (LC/MS/MS) method that allows simultaneous quantification of hydroxychromanols, short- and long-chain carboxychromanols, and sulfated carboxychromanols (14), we investigated the pharmacokinetics (PK) including excretion of vitamin E forms and the formation of their metabolites in response to a single gavage of γT-rich mixed tocopherol (γTmT) and δTE-rich tocotrienol (δTE/γTE) in rats.

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Abbreviations used: CEHC, 2-(β-carboxyethyl)-6-hydroxychroman, also called 3′-COOH; C_{max}, maximum plasma concentration; COOH, carboxychromanol; LC/MS/MS, LC-tandem MS; PK, pharmacokinetics; T_{max}, time at which C_{max} was observed; 13′-OH, 13′-hydroxychromanol; α-CMBHC, 2-(4-carboxy-4-methylbutyl)-6-hydroxy-2,5,7,8-tetramethylchroman; αT, α-tocopherol; γT, γ-tocopherol; γTE, γ-tocotrienol; γTmT, γ-tocopherol-rich mixed tocopherol; δT, δ-tocopherol; δTE, δ-tocotrienol; δTE/γTE, δ-tocotrienol-rich tocotrienol.

Methods

Materials

γTmT that contains αT (10.9%), γT (57.7%), and δT (21.7%) was a gift from BASF. The δTE/γTE (~8:1) that contains to-

tal tocotrienols at 70% was provided by American River Nutrition. γ-CEHC (≥98%), α-CEHC, and (±)-αT-5′-COOH [2-(4-carboxy-4-methylbutyl)-6-hydroxy-2,5,7,8-tetramethylchroman (α-CMBHC)] were purchased from Cayman Chemicals. δT-13′-COOH and δTE-13′-COOH, which are long-chain metabolites from δT and δTE, respectively, were synthesized according to a published procedure (21). All other chemicals were purchased from Sigma.

Animal experiments and sample collection

All the animal experiments were approved by the Purdue Animal Care and Use Committee. Male Wistar rats (6 wk old) were purchased from Charles River. Rats were fed ad libitum with Teklad rodent diet (8604, Envigo), which on average contains ~40–60 mg αT/kg diet. We conducted 2 independent studies. In each study, there were 2 groups of rats ($n = 3$ per group) receiving either γTmT or δTE/γTE via gavage. In the first study, we focused on the PK along with urine and fecal excretion between 8 and 24 h. In the second study, we included collection of urine and feces at 0–8 h, 8–24 h, and 24–48 h. Specifically, after 1 wk adaptation, rats were grouped by body-weight match. Plasma and 24-h fecal and urinary samples were collected for baseline measures. Twenty-four hours after baseline collection, rats were given a single gavage of γTmT (γT/δT/αT at 57.7%, 21.9%, and 10.9%) at 50 mg/kg body weight or δTE/γTE (8:1, wt:wt) at 35 mg/kg body weight, which are equivalent to 483 mg γTmT or 338 mg δTE/γTE for a human of 60 kg body weight, respectively (22). These vitamin E forms were delivered in 0.5 mL tocopherol-stripped corn oil (Dyets Inc.) as the vehicle. In the first study, plasma samples were collected repeatedly at 1, 2, 4, 6, and 8 h via a saphenous vein after gavage of γTmT or δTE/γTE. Fecal and urine samples were collected between 8 and 24 h using metabolic cages. Twenty-four hours postgavage, rats were killed and the plasma was collected. To monitor the excretion of vitamin E forms and metabolites at different times, we performed the second study with the same design and focused on collecting urine and fecal samples at baseline and postsupplementation at 0–8, 8–24, and 24–48 h. Our analyses showed that excretion of vitamin E forms or metabolites was at its minimum at 0–8 h or after 24 h. Therefore, we reported the mean of both studies ($n = 6$) with respect to 24-h excretion of vitamin E forms and metabolites in feces and urine samples.

Extraction of vitamin E forms and metabolites in the plasma and feces

Vitamin E forms and metabolites in the plasma and feces were extracted with methanol and hexane as previously described (14).

Enzyme digestion of extracted vitamin E metabolites including conjugated CEHCs in the urine

One hundred microliters of urine samples were added with δT-13′-COOH or δTE-13′-COOH (1 μM) and extracted by 500 μL working methanol (0.2 mg/mL ascorbic acid). The extraction was repeated 1 more time with 200 μL working methanol. The combined methanol layer was dried under nitrogen. As for samples subjected to enzyme hydrolysis, extracted metabolites were dissolved in 5 μL ethanol and reconstituted in 0.1 M sodium acetate at pH 5 containing 30 U sulfatase (Sigma S9626) and 40 U glucuronidase (Sigma, G0751) (6, 23). Samples were incubated with periodic mixing on a vortex for 18–24 h at 37°C, and then extracted with 5 parts of working methanol (containing 0.2 mg/mL ascorbic acid). The methanol layer was subsequently dried in nitrogen gas. Before LC/MS/MS analysis, samples were reconstituted in ethanol, and α-CMBHC (5 μM) was added as an additional internal standard for injection. It should be noted that, in most studies, injections were consistent between samples and, therefore, α-CMBHC was not used for calculation of concentrations of analytes.

Analysis of vitamin E forms by HPLC with electrochemical detection

Tocopherols and tocotrienols were analyzed by HPLC with electrochemical detection as previously described (8, 13).

Analysis of vitamin E metabolites by LC/MS/MS

The LC/MS/MS analysis was done with an Agilent 1200 LC system coupled to an Agilent 6460 QQQ mass spectrometer equipped with a jet stream ESI source, as previously described (14).

PK analysis

PK parameters were estimated based on the plasma concentration–time data using standard noncompartmental methods (24). AUC was calculated using the log-linear trapezoidal rule to determine the degree of exposure after the administration of vitamin E forms. Other PK parameters determined in this study included observed maximum plasma concentration (C_{max}), time at which C_{max} was observed (T_{max}), and elimination half-life ($T_{1/2}$) or time at which the plasma concentration was reduced by half after reaching C_{max} (25). The decline of vitamin E and metabolites is nonlinear; thus, polynomial fitting curves were applied to estimate $T_{1/2}$. The recovery rate in the plasma of a specific analyte = AUC × V/total supplement, where V = total blood (16 mL for 250 g of rat, https://www.nc3rs.org.uk/rat-decision-tree-blood-sampling). Percentage excretion of vitamin E forms and metabolites = [(total vitamin E forms and corresponding metabolites in feces and urine of supplemented animals) − baseline]/total supplement.

Statistical analysis

The general linear model repeated measure with Bonferroni post hoc test was used for comparing baseline and subsequent time points of plasma samples in the rat study. Student's *t* test was used for comparing γTmT or δTE/γTE supplement with the respective baseline for urine and fecal samples. The normality of the data was evaluated by the Shapiro–Wilk test. If data were not normally distributed, log transformation was performed to normalize unequal variances between groups. All the statistical analyses were performed using SPSS version 24 (IBM) and values of $P < 0.05$ were considered to be statistically significant. All results are expressed as mean ± SEM.

Results

PK of tocopherols and tocotrienols in rat plasma

After a single gavage with γTmT, plasma concentrations of γT and δT elevated to reach C_{max} and then decreased at a relatively rapid pace initially followed by a slow decline phase, as shown in **Figure 2**A, B. In contrast, plasma concentrations of αT did not significantly change (not shown) compared with the baseline (21.0 ± 2.2 μM), probably because of the relatively low amount of αT (1.2 mg) in the supplement but high baseline intake due to high contents of αT in the diet, which contained ≤60 mg/kg (≤0.6 mg/d) of this vitamin E. Upon supplementation, δTE and γTE reached C_{max} quickly within 2 h and declined rapidly without a slow decrease phase (Figure 2C, D). Considering similar administering of δTE (31.1 mg/kg) and γT (29.5 mg/kg), the bioavailability of γT is much higher than δTE in the plasma, as indicated by the C_{max} and AUCs (**Tables 1** and **2**).

Time-course formation of vitamin E metabolites in rat plasma

Using our own developed LC/MS/MS methodology (14), we were able to characterize PK profiles of unconjugated and sulfated vitamin E metabolites after administration of γTmT and δTE/γTE, as summarized in Tables 1 and 2, respectively. Sulfated CEHCs and sulfated 9′- and 11′-COOHs were the predominant metabolites from tocopherols and tocotrienols, and it took 3–7 h for these compounds to reach C_{max} (Tables 1 and 2, **Figure 3**). Upon reaching C_{max}, most of these metabolites showed relatively slow decline (Figure 3). Unlike other vitamin E forms, the metabolites of αT were not detectable in the plasma.

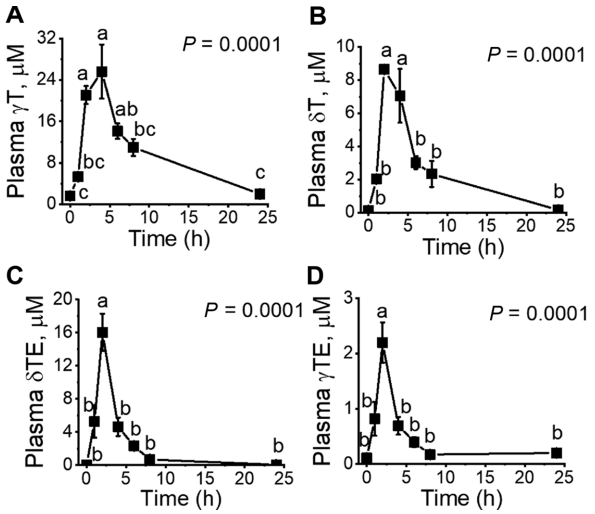

FIGURE 2 Pharmacokinetics of vitamin E forms in rat plasma after supplementation with a single dose of γTmT (28.9 mg γT/kg; 11 mg δT/kg; 5.5 mg α-tocopherol/kg) or δTE/γTE (31.1 mg δTE/kg; 3.9 mg γTE/kg). (A, B) The time course of plasma γT and δT in response to γTmT. (C, D) The time course of plasma δTE and γTE in response to δTE/γTE. General linear model repeated measure with Bonferroni post hoc test was used for data analyses. Data are expressed as mean ± SEM, $n = 3$. Labeled means without a common letter differ, $P < 0.05$. Abbreviations of vitamin E forms and metabolites are shown in Figure 1. γT, γ-tocopherol; γTE, γ-tocotrienol; γTmT, γ-tocopherol-rich mixed tocopherol; δT, δ-tocopherol; δTE, δ-tocotrienol; δTE/γTE, δ-tocotrienol-rich tocotrienol.

Excretion of vitamin E forms and metabolites in rat feces

We monitored 24-h excretion of vitamin E forms and their metabolites in feces before (baseline) and after supplementation.

TABLE 1 Pharmacokinetic parameters of plasma γT, δT, and their metabolites after 1-time supplement with γTmT (28.9 mg γT/kg; 11 mg δT/kg; 5.5 mg α-tocopherol/kg) in rats[1]

	AUC, μM × h	C_{max}, μM	T_{max}, h	$T_{1/2}$,[2] h
γT	207.5 ± 24.4	25.6 ± 5.2	4.0 ± 0.0	6.4 ± 0.3
δT	48.9 ± 8.1	8.64 ± 0.20	2.7 ± 0.7	4.3 ± 0.2
γ-CEHC	2.24 ± 0.02	0.14 ± 0.02	4.7 ± 1.8	30.1 ± 12
SO_3-γ-CEHC	5.47 ± 0.75	0.43 ± 0.07	7.3 ± 0.7	8.2 ± 1.1
SO_3-δ-CEHC	1.99 ± 0.26	0.20 ± 0.01	6.0 ± 1.2	6.9 ± 1.0
γT-9′S	2.21 ± 0.44	0.24 ± 0.04	6.0 ± 1.2	3.6 ± 0.3
δT-9′S	0.21 ± 0.02	0.036 ± 0.00	7.3 ± 0.7	1.4 ± 0.0
γT-11′S	3.52 ± 0.52	0.34 ± 0.04	6.0 ± 2.0	4.6 ± 0.3
δT-11′S	0.23 ± 0.03	0.036 ± 0.01	4.7 ± 0.7	4.0 ± 2.6
γT-13′S	1.02 ± 0.24	0.26 ± 0.07	4.0 ± 0.0	6.1 ± 2.1
δT-13′S	0.24 ± 0.04	0.10 ± 0.01	4.7 ± 0.7	3.3 ± 1.1
γT-13′	0.032 ± 0.021	0.010 ± 0.01	4.7 ± 0.7	1.9 ± 0.2
δT-13′	0.11 ± 0.04	0.021 ± 0.0	4.0 ± 0.0	3.3 ± 0.5
γT-13′-OH	0.044 ± 0.020	0.019 ± 0.0	4.0 ± 0.0	1.7 ± 0.0
δT-13′-OH	0.041 ± 0.011	0.014 ± 0.0	4.0 ± 0.0	1.9 ± 0.3

[1] Values are mean ± SEM, $n = 3$. Abbreviations of vitamin E forms and metabolites are shown in Figure 1. CEHC, 2-(β-carboxyethyl)-6-hydroxychroman, also called 3′-COOH; C_{max}, maximum plasma concentration; COOH, carboxychromanol; T_{max}, time at which C_{max} was observed; 9′S, sulfated 9′-COOH; 11′S, sulfated 11′-COOH; 13′-OH, 13′-hydroxychromanol; 13′S, sulfated 13′-COOH; γT, γ-tocopherol; δT, δ-tocopherol.

[2] Elimination half time ($T_{1/2}$) is the time taken for the plasma concentration to fall by half after reaching C_{max}.

TABLE 2 Pharmacokinetic parameters for plasma tocotrienols and metabolites after a single gavage of δTE/γTE (31.1 mg δTE/kg; 3.9 mg γTE/kg) in rats[1]

	AUC, μM × h	C_{max}, μM	T_{max}, h	$T_{1/2}$,[2] h
δTE	43.6 ± 7.72	16.0 ± 2.3	2.0 ± 0.0	1.4 ± 0.1
γTE	8.84 ± 1.59	2.20 ± 0.37	2.0 ± 0.0	1.7 ± 0.1
SO_3-δ-CEHC	4.57 ± 0.79	0.51 ± 0.09	3.3 ± 0.7	5.1 ± 1.5
SO_3-γ-CEHC	1.25 ± 0.33	0.084 ± 0.01	4.0 ± 2.0	14.1 ± 3.7
δTE-9′	0.044 ± 0.01	0.01 ± 0.00	4.0 ± 1.6	4.7 ± 2.8
δTE-9′S	0.34 ± 0.05	0.05 ± 0.01	5.3 ± 0.7	3.4 ± 0.7
γTE-9′S	0.58 ± 0.10	0.05 ± 0.0	6.0 ± 1.2	6.6 ± 2.4
δTE-11′S	2.12 ± 0.24	0.32 ± 0.06	4.7 ± 0.7	4.1 ± 1.2
γTE-11′S	2.62 ± 0.30	0.18 ± 0.0	7.3 ± 0.7	14 ± 4.0
δTE-13′S	0.10 ± 0.0	0.03 ± 0.03	4.0 ± 0.0	2.6 ± 0.4
δTE-13′	0.10 ± 0.05	0.018 ± 0.0	2.0 ± 0.0	3.5 ± 0.2
δTE-13′-OH	0.24 ± 0.06	0.04 ± 0.01	3.3 ± 0.7	1.9 ± 0.2

[1] Values are mean ± SEM, $n = 3$. Abbreviations of vitamin E forms and metabolites are shown in Figure 1. CEHC, 2-(β-carboxyethyl)-6-hydroxychroman, also called 3′-COOH; C_{max}, maximum plasma concentration; COOH, carboxychromanol; T_{max}, time at which C_{max} was observed; 9′S, sulfated 9′-COOH; 11′S, sulfated 11′-COOH; 13′-OH, 13′-hydroxychromanol; 13′S, sulfated 13′-COOH; γTE, γ-tocotrienol; δTE, δ-tocotrienol; δTE/γTE, δ-tocotrienol-rich tocotrienol.

[2] Elimination half time ($T_{1/2}$) is the time taken for the plasma concentration to fall by half after reaching C_{max}.

Supplementation of γTmT resulted in 12-, 14-, and 2.1-fold elevation of γT, δT, and αT, respectively, in the feces compared with the baseline amounts (Table 3). Due to extremely low δTE in the basal diet, fecal excretion of δTE and γTE increased >380- and 4.7-fold, respectively, in response to δTE/γTE supplementation compared with the baseline (Table 4). Our calculation of excretion percentage showed that 45%, 37%, 34%, 36%, and 17% of αT, γT, δT, γTE, and δTE, respectively, were excreted as the intact vitamers in feces during the 24-h period (Tables 3 and 4).

As for the metabolites, unlike the observation in the plasma where sulfated metabolites dominated, the main metabolites found in feces were unconjugated carboxychromanols and hydroxychromanols, which were markedly elevated in response to supplementation of vitamin E forms. 13′-COOHs were the most abundant metabolites in feces and accounted for ~50% of total metabolites found in feces in response to the supplements (Tables 3 and 4). Interestingly, total excretion of δTE-13′-COOHs (combining isoforms with 2 or 3 double bonds) was twice as much as that of γT-13′-COOH ($P < 0.05$), and total fecal metabolites from δTE tended to be ~50% higher than those from γT ($P = 0.08$), despite similar amounts of supplementation of these 2 isoforms. Overall, we estimated that 4.9%, 6.8%, 9.2%, and 7.5% of γT, δT, γTE, and δTE, respectively, were excreted as metabolites in feces (Tables 3 and 4).

Excretion of metabolites in rat urine

Unlike the prevalent presence of unconjugated long-chain metabolites in feces, conjugated CEHCs constituted the major metabolites in the urine and were markedly elevated in response to supplementation of γTmT and δTE/γTE (Table 5). Total excreted CEHCs (conjugated or unconjugated) from tocopherols were >3-fold higher than those from tocotrienols ($P < 0.05$). Urinary excretion of conjugated CEHCs from γT or δT, as measured after glucuronidase and sulfatase digestion, was much higher than that of sulfated CEHCs,

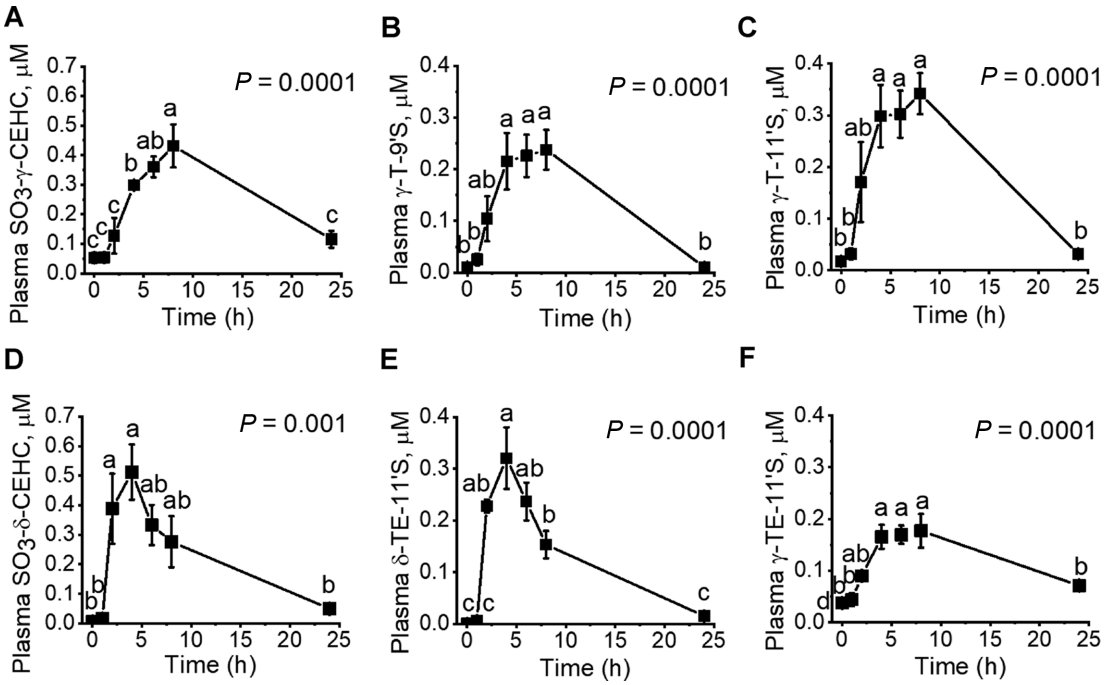

FIGURE 3 Pharmacokinetics of major vitamin E metabolites in rat plasma after supplementation with a single dose of γTmT (28.9 mg γT/kg; 11 mg δT/kg; 5.5 mg α-tocopherol/kg) or δTE/γTE (31.1 mg δTE/kg; 3.9 mg γTE/kg). (A, B, C) The time course of sulfated γ-CEHC, γT-9′S, and γT-11′S in response to the supplementation of γTmT. (D, E, F) The time course of sulfated δ-CEHC, δTE-11′S, and γTE-11′S in response to the supplementation of δTE/γTE. General linear model repeated measure with Bonferroni post hoc test was used for data analyses. Data are expressed as mean ± SEM, $n = 3$. Labeled means without a common letter differ, $P < 0.05$. Abbreviations of vitamin E forms and metabolites are shown in Figure 1. CEHC, 2-(β-carboxyethyl)-6-hydroxychroman, also called 3′-COOH; COOH, carboxychromanol; 9′S, sulfated 9′-COOH; 11′S, sulfated 11′-COOH; γT, γ-tocopherol; γTE, γ-tocotrienol; γTmT, γ-tocopherol-rich mixed tocopherol; δT, δ-tocopherol; δTE, δ-tocotrienol; δTE/γTE, δ-tocotrienol-rich tocotrienol.

suggesting that glucuronide but not sulfated CEHCs was the predominant form of metabolites. However, the amounts of "conjugated" CEHCs and sulfated CEHCs that resulted from δTE/γTE supplementation appeared to be comparable. Overall, 2.7% of γT, 2.2% of δT, 1.9% of γTE, and 0.7% of δTE were excreted as metabolites through the urine. These data together with the aforementioned fecal data indicate that most metabolites of vitamin E forms were excreted via feces instead of urine.

Based on plasma AUCs and fecal/urinary excretion of unmetabolized vitamers and total metabolites, we estimated total recovery of γT and δTE, which were 2 of the major forms of vitamin E in the supplements (**Table 6**). The result indicates that the overall recovery rate of γT was twice as much as that of δTE (Table 6).

Discussion

Our present study characterizes the time-course formation of short- and long-chain vitamin E metabolites after a single supplement of tocopherols or tocotrienols in rats. According to the data of AUC and C_{max}, sulfated CEHCs and sulfated long-chain carboxychromanols, i.e., sulfated 9′-COOH, sulfated 11′-COOH, and sulfated 13′-COOH, are among the major metabolites detected in rat plasma, whereas unconjugated carboxychromanols or hydroxychromanol are present at much lower concentrations than the sulfated analogs. This observation is consistent with our previous findings, where sulfated carboxychromanols appear at higher concentrations in rat plasma than other metabolites in response to γT or γTE supplementation (6, 14). According to T_{max}, 13′-COOHs and 13′-OH appear to reach C_{max} more quickly than their shorter-chain or conjugated counterparts, which is likely because 13′-COOHs and 13′-OH are the metabolites initially formed as a result of ω-oxidation (2, 5).

Our PK data allow quantitative evaluation of the relative availability of different vitamin E forms and metabolites in the plasma, except for αT, which was not elevated in this study. Based on the ratio of AUC × V to the intake of each vitamer, the relative recovery of intact vitamin E forms in the plasma follows the order of γT (19.1%) >δT (11.6%) >γTE (5.9%) >δTE (3.6%). These data are in agreement with the higher C_{max} of γT than that of δTE, despite similar amounts of intake of these 2 vitamin E forms. Thus, γT and δT are more bioavailable than their tocotrienol counterparts. Interestingly, the ratio of total metabolites' AUC × V to the intake of the corresponding vitamins follows the order of γTE (3%) >γT (1.4%) >δTE ≈δT (0.6–0.7%), which is consistent with our previous observations of higher concentrations of metabolites from γTE than those from γT, and lower γTE than γT in the plasma of rats supplemented with the same dose of these vitamin E forms (6). Furthermore, Sontag and Parker (26) demonstrated that tocopherol-ω-hydrolase metabolizes γTE more effectively than γT or δTE, and γTE can be more rapidly metabolized in HepG2 cells expressing CYP4F2.

Our study reveals new and interesting aspects of fecal and urinary excretion of tocopherols, tocotrienols, and their

TABLE 3 Twenty-four-hour fecal excretion of tocopherols and metabolites in rats after a single gavage of γTmT in comparison with baseline excretion[1]

	Baseline, nmol	γTmT supplement, nmol	Total excretion,[2] %
δT	173 ± 43	2450 ± 290*	33.9 ± 6.3
γT	583 ± 200	7030 ± 960*	37.4 ± 4.0
αT	1260 ± 290	2680 ± 320*	45.1 ± 5.3
γ-CEHC	36.7 ± 4.7	53.5 ± 9.5*	
δ-CEHC	3.26 ± 0.60	12.9 ± 1.7*	
γT-5′	0.41 ± 0.12	2.70 ± 0.37*	
γT-7′	10.0 ± 2.2	62.5 ± 10.8*	
δT-7′	0.80 ± 0.04	11.0 ± 1.8*	
γT-9′	3.38 ± 0.39	19.7 ± 2.1*	
δT-9′	1.01 ± 0.13	10.7 ± 2.7*	
γT-11′	6.46 ± 1.20	101.2 ± 13.4*	
δT-11′	2.86 ± 0.64	48.6 ± 12.1*	
γT-13′	23.7 ± 3.5	421 ± 71*	
δT-13′	14.0 ± 1.6	291 ± 86*	
γT-13′-OH	4.24 ± 0.86	126 ± 28*	
δT-13′-OH	1.02 ± 0.15	57.4 ± 21.8*	
γT-11′S	1.46 ± 0.35	46.1 ± 15.4*	
δT-11′S	n.d.[3]	14.2 ± 6.3*	
γT-13′S	14.3 ± 1.2	120 ± 12*	
δT-13′S	3.50 ± 0.20	35.6 ± 4.4*	
Total δ-metabolites	26.4 ± 3.3	482.3 ± 140.2*	6.76 ± 1.99
Total γ-metabolites	100.6 ± 15.1	952.4 ± 160.3*	4.92 ± 0.85

[1] Values are mean ± SEM, $n = 6$. Student's t test was used for comparing γTmT supplement with baseline. *$P < 0.05$, difference between baseline and γTmT supplement. Abbreviations of vitamin E forms and metabolites are shown in Figure 1.CEHC, 2-(β-carboxyethyl)-6-hydroxychroman, also called 3′-COOH; COOH, carboxychromanol; n.d., nondetectable; 11′S, sulfated 11′-COOH; 13′-OH, 13′-hydroxychromanol; 13′S, sulfated 13′-COOH; αT, α-tocopherol; γT, γ-tocopherol; δT, δ-tocopherol.
[2] Total excretion % = (24-h fecal excretion in response to γTmT supplement minus baseline excretion)/intake.
[3] Detection limit: 0.04–0.2 pmol on column.

TABLE 4 Twenty-four-hour fecal excretion of δTE, γTE, and their metabolites in rats after a single gavage of δTE/γTE in comparison with baseline[1]

	Baseline, nmol	δTE/γTE supplement, nmol	Total excretion,[2] %
γTE	243 ± 74	1110 ± 75*	36.3 ± 0.9
δTE	8.92 ± 1.91	3430 ± 530*	17.4 ± 2.9
γ-CEHC	9.22 ± 1.55	15.9 ± 3.3*	
δ-CEHC	1.63 ± 0.22	28.6 ± 6.3*	
γTE-7′	4.92 ± 0.12	15.5 ± 1.3	
δTE-7′	n.d.[3]	9.39 ± 0.97*	
γTE-9′	4.89 ± 0.54	21.3 ± 3.6*	
δTE-9′	n.d.[3]	27.0 ± 3.1*	
γTE-11′	17.7 ± 2.0	65.2 ± 9.8*	
δTE-11′	0.81 ± 0.08	161 ± 16*	
γTE-13′ (2DB)	29.4 ± 4.4	116 ± 23*	
γTE-13′ (3DB)	6.72 ± 1.52	24.8 ± 4.3*	
δTE-13′ (2DB)	1.42 ± 0.21	452 ± 57*	
δTE-13′ (3DB)	0.54 ± 0.21	451 ± 67*	
γTE-13′OH	6.54 ± 0.89	23.9 ± 3.5*	
δTE-13′OH	0.66 ± 0.07	245 ± 28*	
γTE-13′S[4]	1.60 ± 0.32	18.0 ± 3.3*	
δTE-13′S[4]	n.d.[3]	102 ± 15*	
Total δ-metabolites	5.14 ± 0.80	1480 ± 192*	7.49 ± 0.98
Total γ-metabolites	80.9 ± 11.4	301 ± 52*	9.21 ± 1.32

[1] Values are mean ± SEM, $n = 6$. Student's t test was used for comparing δTE/γTE supplement with baseline. *$P < 0.05$, difference between baseline and δTE/γTE supplement. Abbreviations of vitamin E forms and metabolites are shown in Figure 1. CEHC, 2-(β-carboxyethyl)-6-hydroxychroman, also called 3′-COOH; COOH, carboxychromanol; DB, double bonds on the side chain of carboxychromanol; n.d., nondetectable; 13′-OH, 13′-hydroxychromanol; 13′S, sulfated 13′-COOH; γTE, γ-tocotrienol; δTE, δ-tocotrienol.
[2] Total excretion % = (24-h fecal excretion in response to δTE/γTE minus 24-h baseline excretion)/intake.
[3] Detection limit: 0.04–0.2 pmol on column.
[4] γTE-13′S and δTE-13′S were presented as the sum of 2DB and 3DB.

metabolites. First, our data indicate that a large portion of tocopherols and tocotrienols, as well as their metabolites, are excreted to feces, and the extent of excretion of these compounds depends on the specific isoform. For instance, ~37% and 45% of supplemented γT and αT, respectively, were excreted as the unmetabolized form, whereas only ~17% of δTE was found in the fecal samples. The relatively high fecal excretion of αT observed in this study might be resultant from enhanced bile excretion due to high αT in the baseline diet and generally low catabolism of this vitamin E form. Second, in contrast to the vitamers, more fecal metabolites derived from δTE (7.5%) were detected than those from γT (4.9%). In particular, fecal excretion of δTE-13′-COOH, the predominant metabolite in feces, is double that of γT-13′-COOH despite similar intakes of δTE and γT. Third, unlike feces where metabolites of δTE/γTE are higher than those of γTmT, more urinary metabolites from tocopherols are detected than those from δTE. Interestingly, most conjugated CEHCs from tocopherols are not sulfated CEHCs, suggesting potential extensive glucuronidation. On the other hand, ≤40% of CEHCs derived from tocotrienols are in the sulfated form. Overall, ~70% of γT or δT metabolites and 80–90% of metabolites from γTE and δTE appear to be excreted via feces as unconjugated ω-oxidation products, whereas only small portions of metabolites, mostly in the conjugated form, are found in the urine. Although the difference in conjugation of fecal compared with urinary metabolites may be explained by enhanced solubility of conjugated compounds for urine excretion, the mechanism related to conjugation and unconjugation of vitamin E metabolites in the liver and gut is not known and warrants investigation in the future. Regardless, our present data are consistent with those by Bardowell et al. (10), who reported higher fecal than urinary excretion of metabolites when mice were supplemented with γT and δT. Because large amounts of tocopherols, tocotrienols, and their metabolites were found in feces of rats supplemented with γTmT or δTE/γTE, we conclude that fecal excretion is the major route of elimination of vitamin E forms and metabolites when large quantities of vitamin E forms are consumed.

Our estimation of total recovery of vitamin E shows that ~65% of γT was recovered as the unmetabolized form and its metabolites, but the recovery of δTE appeared to be only ~30% (Table 6). This difference may be partially rooted in the difference in tissue retention. Although it is known that tocotrienols are low in many tissues, previous studies reported that considerable amounts of tocotrienols were found in adipose tissue or skin of rodents after administration

TABLE 5 Major metabolites detected in rat urine in response to a single gavage of γTmT or δTE/γTE in comparison with their respective baseline (in nanomoles unless otherwise indicated)[1]

	Baseline[2]	γTmT supplement	Baseline[3]	δTE/γTE supplement
δ-CEHC	0.101 ± 0.02	2.29 ± 0.32*	0.120 ± 0.037	6.89 ± 2.28*
SO_3-δ-CEHC	0.679 ± 0.101	19.8 ± 0.58*	0.751 ± 0.396	55.7 ± 0.7*
Conjugated-δ-CEHC	2.23 ± 0.71	126 ± 16*	3.77 ± 0.90	80.6 ± 18*
γ-CEHC	0.091 ± 0.021	1.01 ± 0.27*	0.177 ± 0.103	1.39 ± 0.07
SO_3-γ-CEHC	7.89 ± 0.80	63.7 ± 9.4*	12.8 ± 2.3	36.3 ± 2.5*
Conjugated-γ-CEHC	9.38 ± 4.01	413 ± 65*	16.2 ± 5.2	36.3 ± 9.4
Total δ-metabolites[4]	3.01 ± 0.69	148 ± 17*	4.6 ± 1.4	143 ± 21*
Excretion,[5] %		2.21 ± 0.26		0.71 ± 0.11
Total γ-metabolites[4]	17.4 ± 5.1	478 ± 75*	29.6 ± 8.7	74.1 ± 12*
Excretion,[5] %		2.67 ± 0.43		1.89 ± 0.31

[1]Values are mean ± SEM, $n = 6$. Student's t test was used for comparing γTmT or δTE/γTE supplement with the respective baseline. *$P < 0.05$, difference between γTmT or δTE/γTE supplement and the respective baseline. Abbreviations of vitamin E forms and metabolites are shown in Figure 1. CEHC, 2-(β-carboxyethyl)-6-hydroxychroman, also called 3′-COOH; COOH, carboxychromanol; γTE, γ-tocotrienol; γTmT, γ-tocopherol-rich mixed tocopherol; δTE, δ-tocotrienol; δTE/γTE, δ-tocotrienol-rich tocotrienol.
[2]Baseline for γTmT-supplement rats.
[3]Baseline for δTE/γTE-supplement rats.
[4]Total metabolites: sum of δ- or γ-series metabolites.
[5]Excretion % = (24-h urine excretion of total metabolites in response to supplements minus 24-h baseline excretion)/intake.

(27–29). Future studies should be conducted to evaluate tissue distribution of tocopherols and tocotrienols including skin and adipose tissues. Moreover, it is important to note that relatively low recovery of δTE may also be caused by incomplete detection of metabolites including potential glycine and glucuronide conjugates, which warrants further investigation (30). In addition, other limitations of our present study include a relatively small sample size, data limited to the male gender, using baseline instead of vehicle control for evaluation of supplement outcomes, and using non–isotopically labeled compounds for estimation of the recovery.

Despite some limitations, our current results reveal distinct, and also similar, PK characteristics between rats and humans. For instance, we find that γT and δTE have T_{max} of 4 and 2 h in rats, respectively, whereas γT and δTE in humans have been reported to show T_{max} of ~12 h and 5.6 h, respectively (31–33). This is consistent with the notion that laboratory animals usually have a higher rate of drug elimination than humans (34). The present study shows that δTE reached C_{max} at 15.6 μM, which is higher than that reported in humans, i.e., mean C_{max} of 7–10 μM ranging from 5 to 15 μM with large individual variances (32). In addition, we observed sulfated CEHCs and sulfated 11′-COOHs to be the predominant metabolites in rat plasma, whereas unconjugated γ-CEHC reaches several micromoles per liter in the plasma of humans in response to γT supplementation (35, 36). Mahipal et al. (32) reported that δ-CEHC is the predominant metabolite found in the plasma after multiple-dose supplementation of δTE (200–1600 mg) in human subjects, but the conjugation status was not identified because sulfatase and glucuronidase were employed to remove conjugation during sample preparation. In addition, Giusepponi et al. (37) conducted a study where healthy subjects took 1000 IU RRR-αT/d for 1 wk and reported that α-CEHC, α-13′-COOH, and α-13′-OH were detected in the plasma, although whether these metabolites were in conjugated or unconjugated forms was not clear.

The bioavailability of vitamin E forms and metabolites, together with their bioactivities, determine in vivo beneficial effects of these compounds for disease prevention and therapy. The present study, along with published work (32, 33, 35, 36), show that γT and δTE can reach concentrations of 30 and 10–16 μM, respectively, in the plasma of animals and humans. At these concentrations, these vitamins have been shown to exhibit anti-inflammatory and anticancer effects in mechanistic studies (2, 38–41). Consistent with being bioavailable and bioactive, these vitamin E forms exhibit anti-inflammatory actions in rat inflammation models (42), are protective against asthma in humans (36), and prevent cancer development in cancer models (40, 43). As for the metabolites, 13′-COOHs have been demonstrated to have anti-inflammatory and anticancer activities (2, 18–20, 40, 44, 45). Our current and previous studies (13, 14) show that, although low in

TABLE 6 Twenty-four-hour recovery of γT, δTE, and their metabolites in rat plasma, urine, and feces in response to a single gavage of γTmT or δTE/γTE[1]

%	Plasma vit. E forms[2]	Plasma metabolites[2]	Fecal vit. E forms[3]	Fecal metabolites[3]	Urine metabolites[4]	Total excretion[5]	Total recovery[6]
γT	19.0 ± 2.2	1.40 ± 0.18	37.4 ± 4.0	4.92 ± 0.85	2.67 ± 0.43	45.0 ± 5.3	65.4 ± 7.7
δTE	3.56 ±0.7	0.61 ± 0.11	17.4 ± 2.9	7.49 ± 0.98	0.71 ± 0.11	25.6 ± 3.9	29.8 ± 4.8

[1]Values are mean ± SEM. vit., vitamin; γT, γ-tocopherol; γTE, γ-tocotrienol; γTmT, γ-tocopherol-rich mixed tocopherol; δTE, δ-tocotrienol; δTE/γTE, δ-tocotrienol-rich tocotrienol.
[2]The values were calculated as the ratio of plasma AUC × V to the amount of γT or δTE intake.
[3]The values were the ratio of 24-h fecal excretion of γT (or δTE or the sum of metabolites) to the amount of γT or δTE intake.
[4]The values were the ratio of 24-h urinary excretion of the sum of metabolites to the amount of γT or δTE intake.
[5]Total excretion % = (fecal vitamin E and metabolites + urine metabolites)/intake.
[6]Total recovery % = (plasma AUC × V of γT or δTE and metabolites + fecal and urinary excretion)/intake.

the blood, unconjugated 13′-COOHs are the predominant metabolites found in feces and constitute ∼50% of overall fecal metabolites. High amounts of 13′-COOHs in fecal samples suggest that these metabolites may potentially have an impact on gastrointestinal health. Indeed, we have shown that δTE-13′-COOH significantly inhibited colon cancer development in mice (20). In the future, research should be carried out to characterize the PK including excretion of vitamin E forms and metabolites in humans.

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The authors' responsibilities were as follows—both authors: designed the research, conducted the research, analyzed the data, performed statistical analysis, have primary responsibility for the final contents, and read and approved the final manuscript.

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