Dietary flavonoids with a catechol structure increase α-tocopherol in rats and protect the vitamin from oxidation in vitro

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Abstract To identify dietary phenolic compounds capable of improving vitamin E status, male Sprague-Dawley rats were fed for 4 weeks either a basal diet (control) with 2 g/kg cholesterol and an adequate content of vitamin E or the basal diet fortified with quercetin (Q), (−)-epicatechin (EC), or (+)-catechin (C) at concentrations of 2 g/kg. All three catechol derivatives substantially increased concentrations of α-tocopherol (α-T) in blood plasma and liver. To study potential mechanisms underlying the observed increase of α-T, the capacities of the flavonoids to *i*) protect α-T from oxidation in LDL exposed to peroxyl radicals, *ii*) reduce α-tocopheroxyl radicals (α-T˙) in SDS micelles, and *iii*) inhibit the metabolism of tocopherols in HepG2 cells were determined. All flavonoids protected α-T from oxidation in human LDL ex vivo and dose-dependently reduced the concentrations of α-T˙. None of the test compounds affected vitamin E metabolism in the hepatocyte cultures. In conclusion, fortification of the diet of Sprague-Dawley rats with Q, EC, or C considerably improved their vitamin E status. The underlying mechanism does not appear to involve vitamin E metabolism but may involve direct quenching of free radicals or reduction of the α-T˙ by the flavonoids.—Frank, J., A. Budek, T. Lundh, R. S. Parker, J. E. Swanson, C. F. Lourenço, B. Gago, J. Laranjinha, B. Vessby, and A. Kamal-Eldin. **Dietary flavonoids with a catechol structure increase α-tocopherol in rats and protect the vitamin from oxidation in vitro. *J. Lipid Res.* 2006. 47: 2718–2725.**

Supplementary key words (+)-catechin • cytochrome P_{450} • (−)-epicatechin • hepatocytes • HepG2 • quercetin • tocopherol-ω-hydroxylase • tocopheroxyl radical

Of the eight natural substances exerting vitamin E activity (α-, β-, δ-, and γ-tocopherols and α-, β-, δ-, and γ-tocotrienols), α-tocopherol (α-T) has traditionally been regarded as the most important vitamer because it exerts the highest biological activity of all vitamers when assessed in animal model systems (1). All forms of vitamin E are equally well absorbed in the small intestine. From the intestinal mucosal cells, vitamin E enters the circulation via the lymphatic system incorporated into lipoproteins and is eventually transported to the liver, from which α-T is preferentially secreted into the blood (1). Tocopherol-ω-hydroxylase catalyzes the initial step in the degradation of vitamin E to its water-soluble carboxyethyl hydroxychroman urinary metabolites and has a higher catalytic activity toward the non-α-vitamers (2); thus, it may be responsible for the several times higher concentrations of α-T compared with the other vitamers in blood and most other tissues.

α-T, the major lipid-soluble antioxidant in blood and tissues (3), can scavenge reactive species and therefore is thought to have an important function in the prevention of degenerative diseases (4). High blood concentrations of vitamin E, for instance, have been associated with a reduced risk of heart disease and cancer (5, 6). In accordance, reduced vitamin E concentrations, especially of γ-T, were reported for patients with cancer of the upper aerodigestive tract (7) and cardiovascular diseases (8). Hence, increasing vitamin E levels in blood and tissues may be helpful in the prevention of these conditions. Yet, the intake of high doses of supplemental vitamin E has been associated with increases in all-cause mortality (9) and heart failure (10). These highly controversial findings, however, are currently a matter of debate (11) and are awaiting clarification. Nevertheless, the identification of

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Abbreviations: C, (+)-catechin; CYP, cytochrome P_{450}; EC, (−)-epicatechin; Q, quercetin; α-T, α-tocopherol; α-T˙, α-tocopheroxyl radical; UV, ultraviolet.

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alternative strategies to improve vitamin E status, preferably by simple means such as dietary intervention, may prove important for optimal nutrition.

Plant foods and beverages contain a great number of potentially bioactive compounds acting, similarly to α-T, as strong antioxidants in vitro, among which the flavonoids are the most abundant. Flavonoids, including the flavonol quercetin (Q) and the flavanols (−)-epicatechin (EC) and (+)-catechin (C), are ubiquitously distributed in plant foods (12, 13). In humans, the daily consumption of flavonols and flavanols has been estimated to be up to 30 and 124 mg, respectively (14, 15). Flavonoids in general, and Q, EC, and C in particular, have a number of reported functions in vitro and in vivo that are hypothesized to promote health. In vitro, they have the ability, inter alia, to scavenge reactive oxygen and nitrogen species, chelate metal ions, inhibit redox-sensitive transcription factors (e.g., nuclear factor-κB), inhibit the expression of free radical-generating enzymes (e.g., inducible nitric oxide synthase) (16), and, thus, function as strong antioxidants (17). Furthermore, previous studies support the notion of a regeneration of α-T from its α-tocopheroxyl radical (α-T$^{\bullet}$) at water-lipid interfaces by dietary phenols (18) in a way reminiscent of that of vitamin C (19). Accordingly, Q, EC, and C have been shown to reduce chemically generated α-T$^{\bullet}$ in SDS micelles and organic solutions (20, 21). In agreement with ex vivo findings that C preserves α-T in human plasma (22), we previously reported that it increases α-T levels in rats (23).

Despite the great number of in vitro studies published on the effects of Q, EC, and C, the literature on their effects in vivo is limited. Therefore, we studied the effects of these major dietary flavonoids on the concentration of vitamin E in rats. In a search for potential mechanisms of polyphenol-vitamin E interactions, we determined the protection of vitamin E in human LDL, the reduction of α-T$^{\bullet}$ in micellar solutions, and the effects of these flavonoids on the activity of vitamin E-metabolizing enzymes in hepatocyte cultures. This experimental strategy allows the direct comparison of the efficacy of these three related catechol-type flavonoids, which differ only marginally in the spatial configuration of their chemical structures, to interact with vitamin E in a range of in vivo and in vitro model systems.

MATERIALS AND METHODS

To study the interactions of dietary catechols with vitamin E, the project was divided into an in vivo part, to establish potential physiological effects, and several in vitro studies (using human LDL, SDS micelles, and HepG2 cells as model systems), to investigate the mechanisms underlying the observed in vivo effects.

Experimental animals and diets

Thirty-two male, 21 d old Sprague-Dawley rats with a mean body weight of 63 g (B&K Universal AB, Sollentuna, Sweden) were used for this study. The rats were housed individually in Macrolon IV cages (Ehret GmbH and Co., Emmendingen, Germany) with aspen wood bedding (Beekay bedding; B&K Universal AB) in a conditioned room at 23°C and 50% relative humidity with 12 h of light (7:00 AM to 7:00 PM) and 12 h of darkness. Each cage was equipped with a water bottle with metal lid, a feed container attached to a stainless-steel plate to avoid overthrowing and spilling, two black plastic tubes that the rats used for resting and hiding, and a table tennis ball for playing. The rats had free access to feed and water throughout the experiment, which was carried out in accordance with the guidelines of and approved by the Ethical Committee for Animal Experiments in the Uppsala region.

The basal diet was prepared from (all values in g/kg diet): maize starch, 528; casein (vitamin-free), 200; rapeseed oil, 100; sucrose, 80; cellulose powder, 40; mineral and trace element premix (Lactamin, Lidköping, Sweden), 40; vitamin premix (vitamin E-free; Lactamin), 10; and cholesterol, 2. The composition of the vitamin premix was as follows (mg/kg diet): retinol, 23.8; cholecalciferol, 3.0; thiamin, 4.0; riboflavin, 14.8; pyridoxine, 6.2; calcium pantothenate, 24.6; niacin, 40.0; cobalamin, 20.0; menadione, 3.1;biotin, 15.0; ascorbic acid, 1,429.0; inositol, 30.0; choline chloride, 2,000.0; folic acid, 0.5; and corn starch, 6,385.9. All vitamin E in the diet originated from the rapeseed oil (Izegem), which was obtained from a local grocery store. Tocopherol and tocotrienol concentrations in the oil were as follows (μg/g): α-T, 212; γ-T, 345; and δ-T, 8; α-, γ-, and δ-tocotrienols were present at concentrations of <5 μg/g. The mineral and trace element premix contained (mg/kg diet): KH_2PO_4, 13,653.2; $CaCO_3$, 14,365.3; KCl, 996.8; NaCl, 7,189.6; $MgSO_4 \times 1\ H_2O$, 2,023.6; $FeC_6H_5O_7 \times 5\ H_2O$, 1,333.2; MnO, 109.7; $Cu_2C_6H_4O_7 \times 2.5\ H_2O$, 25.2; $Zn_3(C_6H_5O_7)_2 \times 2\ H_2O$, 14.0; $CoCl_2 \times 6\ H_2O$, 0.8; $KAI(SO_4)_2 \times 2\ H_2O$, 3.2; NaF, 10.0; KIO_3, 3.6; $Na_2B_4O_7 \times 10\ H_2O$, 0.8; Na_2SeO_3, 2.7; $Na_2MoO_4 \times 2\ H_2O$, 0.4; and corn starch, 267.9. Cholesterol and the phenolic compounds EC (Chemical Absract Service No. 490-46-0), (+)-catechin (CAS No. 154-23-4), and Q dihydrate (CAS No. 6151-25-3) were added to the basal diet at concentrations of 2 g/kg and purchased from Sigma Chemical Co. (St. Louis, MO).

Study design and sample collection

The 32 rats were divided into groups of eight animals with similar mean body weights and fed their respective diets for 30 days. Body weights were measured weekly. At the end of the experiment, the rats were food-deprived for 12 h before intraperitoneal injection of an overdose of sodium pentobarbital and euthanized by exsanguination. Blood samples were withdrawn from the heart, collected in tubes containing EDTA as anticoagulant, and centrifuged (1,000 *g*, 10 min), and the blood plasma was transferred to test tubes with screw caps and stored at −20°C until analyzed. Liver tissues were excised, weighed, and stored in 2-propanol at −80°C until analyzed.

Extraction and analyses of tissue lipids

For the extraction of plasma tocopherol, blood plasma (500 μl) was mixed with ethanol containing 0.005% butylated hydroxytoluene (500 μl) and extracted with hexane (2 ml) after manual shaking for 3 min. The lipids from livers were extracted according to the method developed by Hara and Radin (24) as described previously (25). Briefly, the liver tissue was homogenized in hexane/2-propanol (3:2, v/v) and centrifuged, and the lipid extract was collected in a separatory funnel. The extract was washed with aqueous sodium sulfate, the supernatant was evaporated, and the lipids were weighed and dissolved in 10 ml of hexane. The extract was stored at −20°C until analyzed. Plasma and liver concentrations of tocopherols, cholesterol, and tri-

acylglycerols were determined by standard methods as described previously (25).

Protection of α-T in human LDL by flavonoids

LDLs were isolated from plasma of healthy volunteers by density gradient ultracentrifugation as described previously (26). Briefly, plasma was centrifuged for 3 h at 290,000 *g* and 15°C, and the LDL fraction was collected and washed by ultrafiltration. Protein concentrations were measured according to the method of Lowry et al. (27) using BSA as the standard. Incubation of LDL with 4 μM α-T in the absence (control) or presence of 2 μM EC, C, or Q was carried out at 37°C in a closed glass vessel protected from light under a stream of N_2. LDL particles were exposed to a constant flux of peroxyl radicals [generated from thermal decomposition of 2,2′-azobis(2-methylpropionamidine) dihydrochloride]. Aliquots were removed at 0, 5, 10, 20, and 30 min, and oxidation was immediately stopped by the addition of butylated hydroxytoluene. α-T concentrations in LDL were determined by HPLC as described previously (28). Experiments were performed in duplicate.

In a second experiment, human plasma was incubated with 50 μM EC or C before LDL isolation, and then the isolated LDLs were exposed to a constant flux of peroxyl radicals identical to the treatment of control samples described above.

Quantification of α-T$^{\bullet}$ in micellar solutions

A micellar solution of SDS was prepared in 50 mM phosphate buffer (pH 7.4). α-T (in ethanol) was dispersed in SDS micelles to a final concentration of 2 mM. α-T$^{\bullet}$ was generated by exposure of the micelles to ultraviolet (UV) irradiation for 3 min. Immediately upon terminating the UV irradiation, EC, C, or Q was added to the micelles at concentrations of 0, 5, 10, 25, 50, and 100 μM. Micellar solutions were then transferred to bottom-sealed Pasteur pipettes and immediately inserted into the electron paramagnetic resonance cavity of a Bruker EMX electron paramagnetic resonance spectrometer, and spectra were recorded using the following instrument settings: microwave frequency, 9.8 GHz; microwave power, 20 mW; modulation frequency, 100 kHz; modulation amplitude, 2 G; time constant, 0.65 s. The time between deactivation of the UV irradiation and recording of the electron paramagnetic resonance spectra was 2 min for all samples. α-T$^{\bullet}$ concentrations are given as 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide equivalents. All measurements were performed in triplicate.

Tocopherol-ω-hydroxylase activity

The effect of EC, C, and Q on tocopherol-ω-hydroxylase activity was evaluated in a hepatocyte cell culture assay. HepG2 cells (subclone C3A; American Type Culture Collection, Manassas, VA) were grown in DMEM containing 10% FBS under conditions recommended by the supplier and used at 3–5 days after confluence. Test compounds, in ethanol stock solutions, were first added drop-wise to FBS, which was then diluted 10-fold with DMEM for a final concentration of 20 μM. Cells were preincubated with medium containing the test compounds for 4 h, after which the medium was changed to one containing 10 μM δ-T and 20 μM EC, C, or Q, or 1 μM sesamin. Sesamin (Cayman Chemical, Ann Arbor, MI) was used as a positive control. After 48 h of incubation, the concentrations of 3′- and 5′-δ-carboxychromanol metabolites in the medium were determined by gas chromatography-mass spectrometry of their trimethylsilyl ethers, using d_9-α-3′-carboxychromanol as the internal standard, as described previously (29). Total cell protein was determined by dye binding (Bio-Rad Protein Assay; Bio-Rad Laboratories, Hercules, CA). Experiments were replicated three times, and representative results are shown. δ-T was used as a substrate for assays of tocopherol-ω-hydroxylase activity because it is a substantially better substrate than α-T and therefore offers greater sensitivity in assays of enzyme activity and inhibition. Concentrations of cell-associated δ-T were quantified by gas chromatography-mass spectrometry as described previously (29).

Statistical analyses

Statistical comparisons were made using the statistical software StatView (version 4.51; Abacus Concepts, Inc., Berkeley, CA) by way of an ANOVA with the Bonferroni-Dunn posthoc test, and effects were considered significant at 5% ($P < 0.0083$).

RESULTS

During the 30 days of this experiment, the rats generally ate all of the feed provided, amounting to a total consumption of 380 g per rat and a mean intake of Q, EC, or C of 25 mg/day. Animal performance, assessed by measuring feed consumption, weight gain, total body weight, and total and relative liver weight, was not affected by any of the test compounds (**Table 1**).

Rats fed Q, EC, or C had substantially increased concentrations of α-T in their blood plasma and liver ($P < 0.0002$) (**Table 2**), whereas γ-T concentrations were unaltered. In EC-fed rats, however, plasma and liver γ-T concentrations were slightly, although not significantly, increased. Consequently, the ratio of γ-T to α-T was significantly reduced in plasma ($P < 0.0001$) and liver ($P < 0.0003$) by Q and C but unaltered by EC. Consumption of Q, EC, or C did not change the plasma concentrations of triacylglycerols, total cholesterol, HDL-cholesterol, or VLDL+LDL-cholesterol, or the total cholesterol content of the liver (**Table 3**).

The tested flavonoids markedly protected α-T from oxidative degradation in human LDL challenged with a constant flux of peroxyl radicals in the order EC > C > Q (**Fig. 1A**). Consistent with this observation, LDLs isolated

TABLE 1. Body weights, liver weights, and liver lipid contents of rats fed control or flavonoid-supplemented (2 g/kg) diets for 4 weeks

Variable	Control	Quercetin	(−)-Epicatechin	(+)-Catechin
Body weight (g)	221 ± 7.4	217 ± 11.2	221 ± 6.9	220 ± 4.8
Liver weight (g)	10.2 ± 1.3	10.3 ± 1.1	10.8 ± 0.8	10.2 ± 0.9
Relative liver weight (g/100 g body weight)	4.6 ± 0.5	4.7 ± 0.3	4.9 ± 0.3	4.6 ± 0.4
Liver lipids (mg/g fresh weight)	102 ± 11	92 ± 15	97 ± 14	103 ± 26

Values represent means ± SD (n = 8). No statistically significant differences were observed.

TABLE 2. Plasma and liver tocopherol concentrations of rats fed control or flavonoid-supplemented (2 g/kg) diets for 4 weeks

Variable	Control	Quercetin	(−)-Epicatechin	(+)-Catechin
Plasma (μmol/l)	n = 7	n = 8	n = 8	n = 8
α-T	6.5 ± 0.9	14.3 ± 2.8[a]	11.0 ± 1.6[b]	14.3 ± 2.1[a]
γ-T	0.7 ± 0.2	0.8 ± 0.3	1.1 ± 0.3	0.6 ± 0.2
α-T + γ-T	7.2 ± 1.1	15.1 ± 3.0[a]	12.0 ± 2.0[b]	14.9 ± 2.2[a]
γ-T/α-T	0.10 ± 0.03	0.05 ± 0.01[a]	0.10 ± 0.02	0.04 ± 0.01[a]
Liver (nmol/g)	n = 8	n = 8	n = 8	n = 8
α-T	13.1 ± 2.8	35.4 ± 2.4[a]	23.5 ± 2.3[a]	36.8 ± 4.2[a]
γ-T	2.0 ± 0.9	2.1 ± 0.7	2.9 ± 0.7	2.0 ± 0.6
α-T + γ-T	15.1 ± 3.2	37.8 ± 2.1[a]	26.4 ± 2.7[a]	38.8 ± 4.6[a]
γ-T/α-T	0.16 ± 0.08	0.07 ± 0.02[b]	0.12 ± 0.03	0.05 ± 0.01[a]

α-T, α-tocopherol. Values represent means ± SD.
[a] Different from control at $P < 0.0001$.
[b] Different from control at $P < 0.001$.

from plasma preincubated with 50 μM EC or C, although devoid of detectable amounts of the flavanols, were protected from free radical-induced oxidative degradation of α-T (Fig. 1B). In SDS micelles, incubation with the flavonoids dose-dependently (0–100 μM) reduced the concentrations of α-T$^{\bullet}$ generated by UV irradiation (**Fig. 2**).

None of the test compounds affected tocopherol-ω-hydroxylase activity in HepG2 cultures. The concentrations of δ-T metabolites (sum of 3′- and 5′-δ-carboxychromanols) in the 48 h cell culture medium were (means ± SD, n = 3) 171 ± 19, 173 ± 10, 200 ± 20, 198 ± 16, and 53 ± 3 nmol/l for control, EC, C, Q, and sesamin, respectively. Sesamin, which was used as a positive control to verify the functioning of the assay, inhibited tocopherol-ω-hydroxylase activity as expected. The uptake of δ-T into HepG2 cells was not affected by the test compounds (data not shown).

DISCUSSION

To identify dietary phenolic compounds capable of improving vitamin E status in vivo, we chose the structurally related catechol-type flavonoids Q, EC, and C because of their abundance in the diet (13), their documented bioavailability in rats and humans (30), their reported sparing of α-T in various in vitro systems (20–22, 31–33), and because structure-activity studies suggested that the catechol group is a vital structural feature for the regeneration of vitamin E from its radical (18). In a previous study, C was found to substantially increase α-T concentrations in the plasma, liver, and lungs of Sprague-Dawley rats (23). Here, we aimed to assess whether analogous flavonoids, namely Q and EC, might also increase α-T levels in vivo and to study the underlying mechanisms using an integrated ex vivo/in vitro approach. The flavonoids were established as the only variables in the diets of rats, and cholesterol was added to study whether potential effects on vitamin E status would be accompanied by similar effects on other dietary lipids. The test substances were given at concentrations between 350 and 165 mg/kg body weight (because of the disproportionate increases in body weight and feed consumption of the growing rats) that exceed the expected dietary intake of these flavonoids in humans by far (∼0.4–1.8 mg/kg body weight for a person weighing 70 kg) (34). These high doses were chosen to induce significant changes in the parameters of interest that would be clearly detectable against the biological background variation. Hence, the rat experiment should be considered a "proof of principle" rather than a model for human nutrition.

Consumption of the flavonoids at the doses (mean intake, 25 mg/day) used in this study was without negative effects on animal performance (Table 1), in accordance with results published by other researchers, some of which used even higher doses (23, 35–38). In this investigation,

TABLE 3. Plasma and liver cholesterol concentrations of rats fed control or flavonoid-supplemented (2 g/kg) diets for 4 weeks

Variable	Control	Quercetin	(−)-Epicatechin	(+)-Catechin
Plasma (mmol/l)	n = 7	n = 8	n = 8	n = 8
Total cholesterol	1.7 ± 0.4	1.7 ± 0.4	1.9 ± 0.3	1.6 ± 0.5
HDL-cholesterol	0.7 ± 0.1	0.7 ± 0.1	0.9 ± 0.2	0.7 ± 0.2
VLDL+LDL-cholesterol	0.5 ± 0.4	0.6 ± 0.4	0.6 ± 0.2	0.6 ± 0.5
HDL-cholesterol/total cholesterol	0.5 ± 0.2	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.2
Triacylglycerol	1.1 ± 0.4	1.0 ± 0.3	0.9 ± 0.3	0.8 ± 0.2
Liver	n = 8	n = 8	n = 8	n = 8
Total cholesterol (μmol/g)	49.3 ± 8.8	41.6 ± 3.8	47.3 ± 8.3	45.2 ± 11.0
Total cholesterol in liver lipids (%)	18.8 ± 4.0	17.7 ± 2.2	19.0 ± 3.2	17.5 ± 3.7

Values represent means ± SD. No statistically significant differences were observed.

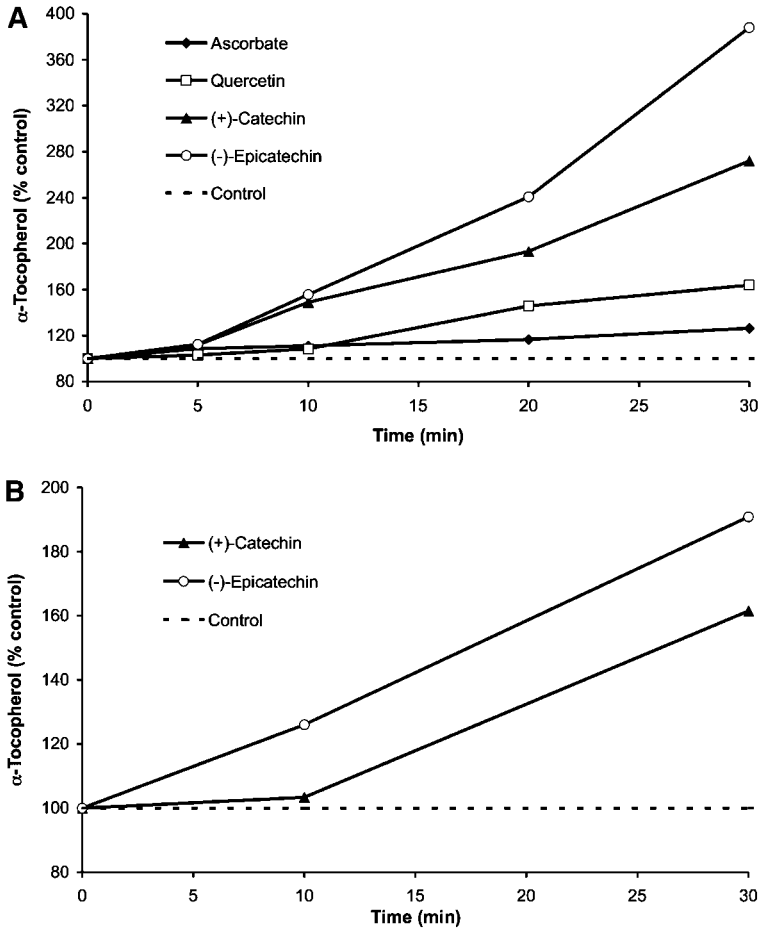

Fig. 1. α-Tocopherol concentrations (percentage of control) over time upon exposure to a constant flux of peroxyl radicals in LDL incubated with flavonoids (2 μM; A) and LDL isolated from human plasma preincubated with flavonoids (50 μM; B).

all three dietary flavonoids substantially increased the blood and liver concentrations of α-T (Table 2). Even when given at doses as low as 2 mg/day for 4 weeks, Q significantly increased serum and liver α-T levels in male Sprague-Dawley rats (39). These findings are supported by other studies that used mixtures of flavonoids and a different strain of rats. For example, male Wistar rats had increased α-T concentrations in their plasma and liver microsomes when fed 0.8% Q and C (2:1, w/w) for 4 weeks in their diets (36) and in plasma and erythrocytes after consuming a mixture of tea catechins containing EC and C (580 and 140 mg/kg diet, respectively) (38). On the other hand, no increase in α-T concentrations was reported in male Sprague-Dawley rats after C or Q consumption at 0.3% (w/w) in the diet (37). This was probably attributable to saturation of the absorptive and metabolic processes resulting from the high level of α-T in their diet [90 mg/kg plus ~60–136 mg/kg from the corn oil (37), compared

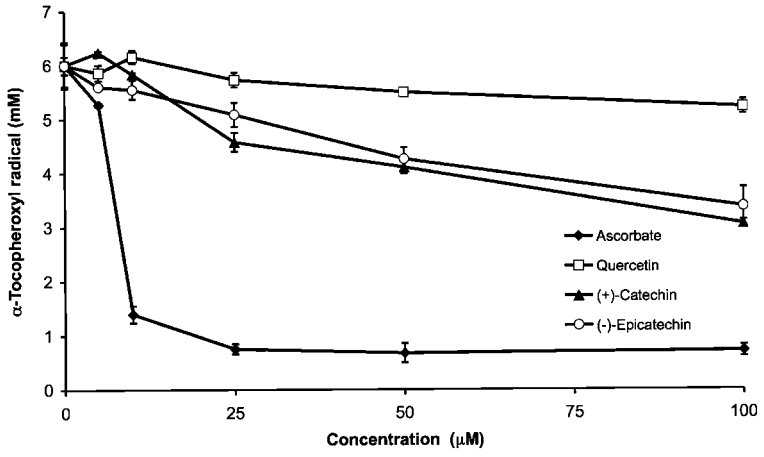

Fig. 2. Concentrations of α-tocopheroxyl radicals in micellar solutions of sodium dodecyl sulfate upon incubation with increasing concentrations of the antioxidants ascorbate, quercetin, (+)-catechin, and (−)-epicatechin. Error bars represent SEM.

with 21 mg/kg in our study]. High doses of C (3 g/day for 1 month followed by 1.5 g/day for 2 months) also increased blood concentrations of vitamin E in human subjects with chronic hepatitis (40). Interestingly, ingestion of green tea extracts [containing EC, EC gallate, (−)-epigallocatechin, and (−)-epigallocatechin gallate] dose-dependently reduced the lymphatic absorption of α-T and cholesterol in female ovariectomized Sprague-Dawley rats (41). However, the reduction in lymphatic absorption may not occur with the isolated compounds, or its extent may not be sufficient to affect vitamin E status.

When considering the mechanisms that may cause the observed increases in α-T levels in vivo, a possible explanation is redox interactions of the flavonoids with α-T. This concept has been shown to apply to phenolic acids with a catechol structure (similar to Q, EC, and C), resulting in a synergistic protection of LDL against oxidation (18). Therefore, we implemented an integrated approach using ex vivo (LDL) and in vitro (SDS micelles) model systems and a physical generation of α-T$^{\bullet}$ (UV irradiation) to compare the relative efficacies of these compounds to protect α-T and recycle α-T$^{\bullet}$. The generation of α-T$^{\bullet}$ by UV irradiation is favored over the use of chemical radical initiators because it avoids interaction of the test compounds with the (chemical) radical initiators, which could confound the results (42). The concentrations of the flavonoids used in these experiments most likely exceed the concentrations that are typically reached in LDL in vivo; however, after oral application of plant foods or individual flavonoids, plasma concentrations in excess of 2 μM have been reported (34).

In human LDL incubated with the flavonoids, we observed a protection of α-T from oxidative degradation (Fig. 1A). Consistent results were observed using LDL isolated from human plasma that was preincubated with the flavonoids (Fig. 1B), although the lipoprotein fraction did not contain detectable amounts of the flavonoids. We further studied the ability of Q, EC, or C to react with α-T$^{\bullet}$ generated in SDS micelles by UV irradiation; indeed, the flavonoids dose-dependently accelerated the decay of the radical (Fig. 2). EC and C were more efficient at regenerating α-T than was Q, but none of these compounds matched the efficacy of ascorbate (Fig. 2). These findings are in agreement with previous in vitro and ex vivo experiments using chemical radical initiators that showed vitamin E-sparing and/or α-T$^{\bullet}$-regenerating effects of Q, EC, and C (20–22, 31–33).

The lipophilic vitamin E is thought to take part in an antioxidant network in which vitamin E radicals are recycled at the lipid-water interface by the hydrophilic antioxidant ascorbic acid, which, in turn, is regenerated from its ascorbyl radical by thiol or polyphenol antioxidants, which eventually are recycled through the conversion of NAD(P)H+H^{+} to NAD(P)$^{+}$ (42, 43). Accordingly, Q and EC were shown to work synergistically with ascorbate to prevent the oxidation of cytochrome C in the presence of liposomes. Those authors suggested that the amphiphilic flavonoids may mediate electron transfer between the aqueous and lipid phases and thus regenerate oxidized cytochrome C while being reduced to their nonradical form by ascorbate (44). Another indicator for tocopherol-flavonoid interactions occurring at the lipid-water interface (rather than within the lipoprotein particle) was that neither Q, EC, or C nor their metabolites could be detected in LDL isolated from rats and mice fed these compounds (37, 45). Similarly, we did not detect EC or C in LDL isolated from human plasma incubated with the flavanols. In support of the above, we observed a reduction of the α-T$^{\bullet}$ generated in the lipid phase (SDS micelles) by the flavonoids present in the aqueous phase (Fig. 2). The observation that the protection of α-T from oxidative degradation by the flavonoids was more pronounced than their capacity to reduce α-T$^{\bullet}$ (Figs. 1, 2) suggests that direct free radical scavenging may be the predominant mechanism of action of their α-T-sparing activity.

The extent of the observed effects in the present in vivo, ex vivo, and in vitro experiments does not simply follow the order of magnitude of the antioxidant potentials of the test compounds. Trolox equivalent antioxidant capacity values against radicals generated in the aqueous phase have been reported to be 4.7, 2.5, and 2.4 nM for Q, EC, and C, respectively. Of the three flavonoids, Q was reported to be the most active scavenger of radicals generated in the lipid phase (46). However, the catechins were more potent than Q in protecting α-T from oxidation and reducing the α-T$^{\bullet}$ in our ex vivo and in vitro experiments. The differences in the antioxidant capacity of the flavonoids in these assays and ours may be attributable to differences in *i*) the partitioning of the antioxidants in the lipid and water phases and *ii*) the nature and rate of decay of the oxidizing radicals used. In vivo, C and Q increased α-T concentrations in plasma and liver to approximately the same extent, whereas EC was only ∼60–75% as effective (Table 2). These discrepancies between in vivo and in vitro results are likely caused by differences in *i*) the absorption of the compounds, *ii*) the concentrations of their circulating aglycones, and/or *iii*) the formation of metabolites, and they may also indicate the different biological activities and/or antioxidant capacities of the formed metabolites. Interestingly, in this study, EC, although being an almost identically efficient antioxidant compared with C (17), was the only compound to show at least a tendency toward increasing γ-T concentrations in blood plasma and liver (Table 2).

This observation encouraged us to investigate another potential mechanism that might relate to the observed effects. An important factor determining the blood and tissue concentrations of vitamin E is the degradation of the vitamin to its water-soluble metabolites and their excretion from the body. The shortening of the vitamin E side chain was suggested to involve the cytochrome P_{450} (CYP) isozymes 3A and 4F2 (2, 29). Tea catechins were reported to affect a range of CYPs, including CYP1A1, CYP1A2, CYP2B1, and CYP3A4 (47–49). In our previous experiments, an increase in γ-T levels in rats caused by dietary supplementation with sesamin or alkylresorcinols could be explained by the inhibition of vitamin E metabolism in HepG2 cells (29, 50, 51). To elucidate whether or not the

hepatic metabolism of the tocopherols to their corresponding carboxyethyl hydroxychroman metabolites was affected by Q, EC, or C, HepG2 cells were incubated with the test compounds in the presence of δ-T and the formation of carboxyethyl hydroxychromans was quantified. δ-T was previously shown to be the most sensitive of all vitamers in the tocopherol-ω-hydroxylase assay (2). We found that incubation of liver cells with Q, EC, or C did not affect the metabolism of vitamin E. This is supported by the results of our previous study, in which C failed to inhibit tocopherol-ω-hydroxylase activity (23), and by findings that the formation of CYP3A-produced metabolites of paclitaxel was not inhibited in human microsomes by incubation with Q, EC, or C (49).

Changes in tocopherol concentrations may also result from an altered availability of lipoproteins, which facilitate vitamin E transport, in the blood. If that were the case, one would expect similar changes in the cholesterol content of the lipoprotein fractions, in which cholesterol and tocopherol concentrations are closely correlated (52). However, the increases in vitamin E in this study were not accompanied by corresponding changes in cholesterol levels (Table 3).

In conclusion, we found that dietary supplementation with the flavonoids Q, EC, and C resulted in a substantial increase in α-T concentrations in blood plasma and liver tissue of male Sprague-Dawley rats. The mechanism(s) underlying this improvement of vitamin E status does not appear to involve the inhibition of vitamin E metabolism. Rather, our ex vivo and in vitro experiments suggest that the flavonoids directly scavenge free radicals and may also regenerate α-T from its radical form.

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ERRATA

In the article "Dietary flavonoids with a catechol structure increase α-tocopherol in rats and protect the vitamin from oxidation in vitro" by Frank et al., published in the December 2006 issue of the *Journal of Lipid Research* (Volume 47, pages 2718–2725), the text giving the concentrations of vitamins contained in the vitamin premix that was used to make the rat diets contained errors. In the Materials and Methods section, under the heading "Experimental animals and diets" (page 2719, right column, second paragraph, lines 5–10), the corrected text should read as follows:

The vitamin premix provided (mg/kg diet): retinol, 3.6; cholecalciferol, 0.04; thiamin, 4.0; riboflavin, 11.9; pyridoxine, 5.1; calcium pantothenate, 11.1; niacin, 40.0; cobalamin, 0.02; menadione, 0.8; biotin, 0.3; ascorbic acid, 500.2; inositol, 30.0; choline chloride, 1000.0; folic acid, 0.5; and cornstarch, 6385.9.