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The Dietary Hydroxycinnamate Caffeic Acid and Its Conjugate Chlorogenic Acid Increase Vitamin E and Cholesterol Concentrations in Sprague−Dawley Rats

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Vegetarian diets are correlated with a reduced risk of developing cardiovascular disease and comprise a great variety of bioactive compounds, including hydroxycinnamic acid derivatives. Therefore, this study aimed to identify dietary hydroxycinnamic acid derivatives that may alter two important factors related to the development of cardiovascular disease, namely, tocopherol (T) and cholesterol (C) concentrations in the body. The effects of caffeic acid (CA), chlorogenic acid (CGA), and ferulic acid (FA) on α-T, γ-T, and C levels in blood plasma, liver, and lungs were investigated after these compounds had been fed to rats for 4 weeks at concentrations of 2 g/kg in semisynthetic diets. None of the regimens affected weight gain, feed intake, or absolute weights of livers and lungs, although CA increased the liver weight relative to the body weight ($P < 0.05$). CA- and CGA-fed animals showed a tendency toward sparing vitamin E in all tissues, but statistical significance was obtained only for γ-T in the liver of CA-fed animals ($P < 0.005$) and for α-T in the lungs of CGA-treated rats ($P < 0.05$). CGA supplementation reduced concentrations of lipids in the lung tissue ($P < 0.05$). CA and CGA elevated the concentrations of C in liver tissue and lipids to a similar extent, but only CA decreased the ratio of high-density lipoprotein C to total C in blood plasma ($P < 0.05$ for all effects). Animals eating FA showed T and C values comparable to those in the control group. In conclusion, this study demonstrates that dietary caffeic and chlorogenic acid may elevate tocopherols and cholesterol in vivo.

KEYWORDS: Cholesterol; hydroxycinnamic acids; rats; tocopherols

INTRODUCTION

Evidence from epidemiological studies clearly indicates that diets with a high intake of whole grain cereals, vegetables, and fruits may reduce the incidence of degenerative diseases, such as cardiovascular disease and cancer (*1*−*4*). Because plant-derived foods and beverages are rich in phenolic antioxidants, these compounds may at least partly account for the observed protection from these conditions (*5*−*7*). Hydroxycinnamic acids, such as caffeic (CA) and ferulic acid (FA), are abundant in plant foods (especially coffee, whole grain cereals, citrus fruits, and juices made thereof), where they occur predominantly in conjugated forms, such as chlorogenic acid (CGA). The daily intake of hydroxycinnamic acid derivatives in humans may easily reach 500−1000 mg each (*8*, *9*).

The mechanisms of initiation and progression of cardiovascular disease and cancer appear to involve the formation of free radicals (*10*, *11*). Phenolic compounds from plant secondary metabolism, including hydroxycinnamates and their conjugates, have been shown to efficiently scavenge free radicals (*12*−*15*), to chelate metal ions (*16*, *17*), and/or to work synergistically with the antioxidant defense system by inducing antioxidant enzymes (*18*) and/or sparing vitamin E (*19*, *20*). Yet clinical trials with high doses of a single antioxidant revealed variable and sometimes contradicting results (*21*), suggesting the need for a broad spectrum of dietary antioxidants in combination with the right balance of other food constituents as the foundation of a healthy diet that may help to prevent the aforementioned conditions (*22*).

Regardless of plasma antioxidants, high blood cholesterol (C) concentrations, especially in low-density lipoproteins (LDL), are known to be major risk factors in the development of

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Table 1. Composition of the Control Diet[a]

ingredient	g/kg
maize starch	528
casein (vitamin-free)	200
rapeseed oil[b]	100
sucrose	80
cellulose powder	40
mineral and trace element premix[c]	40
vitamin premix (vitamin E-free)[c]	10
cholesterol	2

[a] Antioxidants were added to the control diet at a concentration of 2 g/kg to make the experimental diets. [b] All vitamin E in the diet originated from the rapeseed oil. Tocopherol and tocotrienol concentrations were determined according to IUPAC 2.432 and were as follows: α-tocopherol, 190 ppm; γ-tocopherol, 345 ppm; δ-tocopherol, 7 ppm; α-, γ-, and δ-tocotrienol were present at concentrations of <5 ppm. The fatty acid composition of the rapeseed oil, determined according to IUPAC 2.302, was 16:0 (4.2%), 18:0 (1.6%), 18:1 (60.8%), 18:2 (20.3%), 18:3 (10.1%), 20:0 (0.6%), 20:1 (1.1%), 22:0 (0.3%), and 22:1 (0.4%). [c] The mineral and trace element premix and the vitamin premix were formulated to meet the nutritional requirements of laboratory rats with the exception of vitamin E and purchased from Lactamin (Lidköping, Sweden).

atherosclerosis and coronary heart disease (*23*, *24*). Various phytochemicals have been reported to lower C levels in blood plasma and liver tissue (*19* and references cited therein) and consequently to reduce the risk of cardiovascular disease (*2*, *5*).

Phytochemicals also influence many other physiological processes by modulating enzymes (*25*, *26*), affecting hormones (*27*), and acting as antimutagens (*16*, *28*). Nevertheless, little is known about the interactions of phytochemicals with the antioxidant defense system in the body. Our previous work showed that some phenolic substances, namely, sesamin, butylated hydroxytoluene, curcumin, and cyanidin-3-*O*-glucoside, elevated tocopherol (T) levels in rats (*19*, *20*). In our effort to screen a range of common dietary phenolic compounds for their influence on T and C levels in vivo, we report here the effects of the hydroxycinnamate derivatives caffeic acid (CA), chlorogenic acid (CGA) (5-*O*-caffeoylquinic acid, an ester of caffeic acid with quinic acid), and ferulic acid (FA) fed to male rats of the Sprague−Dawley strain at concentrations of 0.2% in their diets.

MATERIALS AND METHODS

Experimental Animals and Diets. Thirty-two 21-day-old Sprague−Dawley rats with a mean body weight of 58 g (B&K Universal AB, Sollentuna, Sweden) were used in this study. The animals were housed individually in Macrolon IV cages (Ehret GmbH und Co., Emmendingen, Germany) with aspen wood bedding (Beekay bedding, B&K Universal AB) in a conditioned room at 25 °C and 60% relative humidity with 12 h of light (7:00 a.m. to 7:00 p.m.) and 12 h of darkness. Each cage was equipped with a water bottle with a 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 composition of the control diet is given in **Table 1**. Rapeseed oil was a gift from Karlshamns AB (Karlshamn, Sweden). Cholesterol (C) and the phenolic compounds CA (CAS Registry No. 331-39-5), CGA (CAS Registry No. 327-97-9), and FA (CAS Registry No. 537-98-4) were purchased from Sigma Chemical Co. (St. Louis, MO). The phenolics CA, CGA, and FA were added to the control diet at concentrations of 2 g/kg to make the experimental feed.

Study Design and Sample Collection. The 32 rats were divided into groups of eight animals with similar mean body weights, kept on the basal diet for a 5-day accommodation period, and then fed their respective diets for 28 days. Body weights were measured weekly. At the end of the experiment, the rats were fasted for 12 h before intraperitoneal injection of an overdose of sodium pentobarbital and killed by exsanguination. Blood samples were withdrawn from the heart and collected in tubes containing EDTA as anticoagulant and centrifuged (1000*g*, 10 min), and the blood plasma was transferred to test tubes with screw caps and stored at −20 °C until analyzed. Liver and lung tissues were excised, weighed, and stored in 2-propanol at −80 °C until analyzed.

Extraction of Tissue Lipids. For the extraction of plasma tocopherol (T), blood plasma (500 μL) was mixed with ethanol containing 0.005% BHT (500 μL) and extracted with hexane (2 mL) after manual shaking for 3 min. The lipids from livers and lungs were extracted according to the method developed by Hara and Radin (*29*). The liver tissue was homogenized in 40 mL of hexane/2-propanol (HIP) (v/v) with a Diax 600 homogenizer (Heidolph Elektro GmbH und Co., Kelheim, Germany) and centrifuged (4000*g*, 0 °C) for 10 min, and the lipid extract was collected in a separatory funnel. The extraction was repeated twice, and the extracts were pooled and mixed with 75 mL of aqueous sodium sulfate (prepared from 120 g of anhydrous sodium sulfate and 1800 mL of water) for 1 min. After 30 min, the lower layer was discarded and the supernatant filtered through anhydrous sodium sulfate. The solvent was evaporated on a rotary vacuum evaporator with the water bath set to 30 °C, and the lipids were then dried under a stream of nitrogen gas. The lipid content was determined gravimetrically. The lipids were dissolved in 7 mL of HIP, transferred to a 10 mL volumetric flask, and made up to volume. The extract was then poured into a sealed test tube with screw cap and stored at −20 °C until analyzed. Lung lipids were generally extracted as described above but, because of the smaller size of the lungs in comparison to the livers, the method was slightly modified as follows. The lungs were homogenized in 20 mL of HIP and centrifuged at 1400*g* (10 min, room temperature), and the pooled extracts were mixed with 40 mL of aqueous sodium sulfate (prepared as described above).

Tocopherol Analyses. Plasma T were analyzed by HPLC using a Merck-Hitachi (Hitachi, Ltd., Tokyo, Japan) system (pump L-6000, autosampler AS-4000, and detector D-2500). The separation of plasma T was performed on a LiChrospher 100 NH_2 column (250 × 4 mm, E. Merck, Darmstadt, Germany) using isooctane/methyl *tert*-butyl ether/methanol (75:25:0.035, by volume) as mobile phase. Liver and lung T were separated on an Alltima SI 5U silica column (Alltech Associates Inc., Deerfield, IL) with hexane/1,4-dioxane (96:4, v/v) as mobile phase. For the analyses of liver lipid extracts, the HPLC system consisted of an autosampler Midas (type 380, Spark Holland BV, Emmen, The Netherlands), an HPLC pump model 2250 (Bischoff Analysentechnik und -geräte GmbH, Leonberg, Germany), and a Merck Hitachi L-7480 fluorescence detector (Hitachi, Ltd.). For the determination of T in the lung lipid extracts, the HPLC system consisted of an AS3000 autosampler (Spectra-Physics Analytical, Fremont, CA), a Bischoff HPLC pump 2250, and an Agilent 1100 series fluorescence detector (Agilent Technologies, Waldbronn, Germany). In all systems the fluorescence detector was operated at an excitation wavelength of 296 nm and an emission wavelength of 324 nm, and the peaks were recorded and integrated using the chromatography software Chromeleon (version 4.12, Softron GmbH, Germering, Germany). The concentrations of α-T and γ-T were quantified against authentic T used as external standards (T standards, article no. 15496 from E. Merck).

Cholesterol and Triacylglycerol Analyses. C was quantified in the plasma and in the isolated lipoprotein fractions according to the IL test cholesterol Trinder's method 181618-80 and IL test enzymatic−colorimetric method 181709-00; triacylglycerols (TAG) were quantified according to the IL test triglyceride enzymatic−colorimetric method 181610-60 employing a Monarch apparatus (Instrumentation Laboratories, Lexington, MA). Serum high-density lipoproteins (HDL) were separated as reported by Seigler and Wu (*30*) using sodium phosphotungstate and magnesium chloride with the only modification of leaving the sample to precipitate at 4 °C for 20 min. Levels of very low-density lipoprotein plus low-density lipoprotein cholesterol (VLDL + LDL-C) were calculated by subtraction of HDL-C values from those of plasma total C. For C analyses in the liver lipid extracts, 493 μg of

Table 2. Body Weights, Liver Weights, Lung Weights, and Lipid Contents of Liver and Lungs[a]

	diet				
	control	caffeic acid	chlorogenic acid	ferulic acid	$P <$[b]
	$n = 8$	$n = 8$	$n = 8$	$n = 8$	
body wt (g)	238.6 ± 10.1	242.9 ± 7.1	233.1 ± 4.9	233.6 ± 14.6	ns
liver wt (g)	10.5 ± 0.9ab	11.4 ± 0.5a	10.5 ± 0.7ab	10.4 ± 1.1b	0.05
relative liver wt (g/100 g of body wt)	4.38 ± 0.26a	4.68 ± 0.21b	4.53 ± 0.29ab	4.43 ± 0.24ab	0.05
lung wt (g)	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	ns
relative lung wt (g/100 g of body wt)	0.53 ± 0.03	0.55 ± 0.03	0.54 ± 0.03	0.55 ± 0.02	ns
	$n = 8$	$n = 7$	$n = 8$	$n = 8$	
liver lipids (mg/g of fresh wt)	172.5 ± 25.6	157.9 ± 20.9	160.2 ± 15.4	162.9 ± 23.1	ns
lung lipids (mg/g of fresh wt)	42.4 ± 8.0a	41.3 ± 5.3a	30.9 ± 4.6b[c]	na[d]	0.01

[a] Values represent means ± SD. Values within each row not sharing a common letter are statistically different at P. n = number of observations. [b] ns, not significant. [c] Different from control at $P < 0.005$. [d] na, not analyzed.

cholestane (Sigma) was added as internal standard to 200 μL of the lipid extracts in a test tube with a screw cap, and solvents were removed under nitrogen gas. KOH in ethanol (1 mL, 2 M) was added for saponification in a boiling water bath with intermittent shaking for 10 min. After the mixture had cooled to room temperature, water (1 mL) and hexane (2 mL) were added, and the test tubes were shaken and allowed to stand until the two layers separated. The hexane layer was transferred to another glass tube and dried under a stream of nitrogen. Trimethylsilyl (TMS) ether derivatives of C were prepared by incubation at 60 °C for 30 min with Tri-Sil reagent (reagent no. 48999, Pierce Chemical Co., Rockford, IL). Thereafter, the solvent was evaporated under nitrogen, and the TMS ether derivatives were dissolved in hexane, shaken, and centrifuged at 1000*g* for 3 min. TMS ether derivatives of C were analyzed, using pulsed split injection (split ratio 20:1), on an HP 6890 series gas chromatograph with a flame ionization detector equipped with an HP-5 nonpolar fused silica column (30 m × 0.32 mm i.d.; 0.25 μm film) (Hewlett-Packard, Avondale, PA). Helium was used as carrier gas at a flow rate of 1.5 mL/min and an inlet pressure of 1.03 bar, the injector temperature was set to 250 °C, the oven temperature was set to 280 °C, and the detector temperature was set to 300 °C. The peaks were recorded and integrated using Chromeleon chromatography software (version 4.3, Softron GmbH).

Statistical Analyses. Statistical analysis of the registered variables was performed by an analysis of variance procedure and the general linear model (GLM) supported by the Statistical Analysis System (*31*). Least significant differences from the *t*-test function of the SAS GLM procedure were used to make statistical comparisons, and effects were considered to be significant at $P < 0.05$.

RESULTS AND DISCUSSION

In an effort to screen a variety of dietary phytochemicals for their effects on tocopherol (T) and cholesterol (C) levels in vivo, we selected the natural plant phenolic compounds caffeic acid (CA), its ester with quinic acid, chlorogenic acid (CGA), and ferulic acid (FA) because of their abundance in the human diet. CA, CGA, and FA are absorbed and/or metabolized in humans (*32*−*34*) and rats (*35*−*37*). Especially high concentrations of CA, CGA, and FA are found in coffee, apples, citrus fruits and juices, and the bran of cereal grains (*9*). The amount of hydroxycinnamic acids ingested by humans has been estimated to reach as much as 1000 mg/day (*8*, *9*), which corresponds to 14 mg/kg/day for a human of 70 kg body weight. Because the increase in body weight during the course of this experiment was larger than the increase in feed consumption, our rats received megadoses ranging from 350 mg/kg/day at the beginning of the treatment period to 165 mg/kg/day prior to sacrifice. This high dosage was chosen to provoke and detect possible physiological reactions. We are aware of the limitations of this setup when it comes to applying the results to human nutrition and, therefore, consider it to be a first step in the identification of compounds with possible in vivo effects.

To study the effects of the aforementioned compounds, we established them as the only variables in the experimental diets (**Table 1**). Rats were chosen as model animals because their physiology and metabolism are well studied, organ samples are easily accessible, and experiments with rats are easy to reproduce. The liver, the central organ for lipid and vitamin E metabolism, the lung, as an example of a tissue under permanent oxidative challenge, and blood plasma were selected to detect changes in T and C levels.

The dietary supplementation of the cinnamates and the cinnamate conjugate in this experiment did not trigger any adverse effects on animal performance (**Table 2**) in agreement with previously published data (*19*, *38*−*40*).

In this study, CA feeding elevated only liver γ-T levels significantly ($P < 0.01$) (**Table 3**), whereas it elevated α-T values in plasma and VLDL + LDL fractions from Sprague−Dawley rats maintained on diets containing 0.2 or 0.8% CA for 6 weeks in a previous study (*40*). This difference may be due to the 2−4 times higher daily intake of CA of their rats as well as to the longer experimental period. The general idea that CA may be capable of sparing vitamin E is supported by numerous in vitro studies performed on human LDL, erythrocyte membrane ghosts, and monocytic cells showing that CA delayed lipid oxidation, spared α-T, and recycled α-T from its tocopheroxyl radical (*41*−*45*). Although we did not obtain any changes in plasma total C, HDL-C, or TAG in CA-fed animals, we observed adverse effects on other lipid parameters, namely, a nonsignificant increase in plasma VLDL + LDL-C and a significant decrease in the HDL-C/total C ratio ($P < 0.05$), as well as an elevation in liver C and the percentage of C in liver lipids ($P < 0.05$) (**Table 4**). These observations are in agreement with the results of Nardini et al. (*40*), who did not observe any alteration in C or TAG values in the analyzed VLDL + LDL fractions from their rats. In another study, CA had no effect on hydroxymethylglutaryl-coenzyme A reductase (HMG-CoA reductase), one of the rate-limiting enzymes in C biosynthesis, in vitro (*46*).

Even though reports on the antioxidant actions of CGA in vivo and in vitro have been published (*39*, *42*, *47*), no data seem to be available on the effects of this hydroxycinnamic acid conjugate on vitamin E in vivo. In this study on Sprague−Dawley rats, dietary CGA elevated α-T concentrations numerically, but not statistically, in plasma and livers and elevated them significantly in the lungs ($P < 0.05$) (**Table 3**). This elevation was more pronounced for α-T ($P < 0.001$) but statistically significant also for γ-T ($P < 0.05$), when the T

Table 3. Effects of Phenolic Compounds on Tocopherol Concentrations in Rat Plasma, Liver, and Lungs[a]

	diet				
	control	caffeic acid	chlorogenic acid	ferulic acid	$P <$
			Plasma (μg/mL)		
	$n = 8$	$n = 8$	$n = 8$	$n = 8$	
α-T	1.65 ± 0.20ab	1.98 ± 0.16a	1.97 ± 0.23a	1.46 ± 0.26b	0.05
γ-T	0.24 ± 0.10ab	0.30 ± 0.08a	0.25 ± 0.10ab	0.21 ± 0.05b	0.05
α-T + γ-T	1.89 ± 0.28ab	2.27 ± 0.21a	2.22 ± 0.25a	1.67 ± 0.28b	0.05
γ-T/α-T	0.14 ± 0.05	0.15 ± 0.03	0.13 ± 0.05	0.14 ± 0.03	ns[b]
			Liver (μg/g)		
	$n = 8$	$n = 6$	$n = 7$	$n = 7$	
α-T	8.08 ± 0.57ab	9.24 ± 1.42a	9.52 ± 2.43a	7.45 ± 0.45b	0.05
γ-T	1.82 ± 0.51a	2.87 ± 0.71b	1.82 ± 0.60a	1.89 ± 0.57a	0.005
α-T + γ-T	9.90 ± 0.60ac	12.10 ± 1.97b	11.34 ± 2.25bc	9.34 ± 0.62a	0.05
γ-T/α-T	0.23 ± 0.07a	0.31 ± 0.06b	0.21 ± 0.10a	0.26 ± 0.08ab	0.05
			Lung (μg/g)		
	$n = 8$	$n = 6$	$n = 7$	$n = 8$	
α-T	10.59 ± 1.06a	11.09 ± 1.32ab	12.53 ± 1.39b	na[c]	0.05
γ-T	3.58 ± 0.93	3.89 ± 0.19	3.80 ± 0.98	na	ns
α-T + γ-T	14.17 ± 1.73a	14.98 ± 1.35ab	16.34 ± 1.21b	na	0.05
γ-T/α-T	0.34 ± 0.07	0.36 ± 0.05	0.31 ± 0.10	na	ns

[a] Values represent means ± SD. Values within each row not sharing a common letter are statistically different at P. n = number of observations. [b] ns, not significant. [c] na, not analyzed.

Table 4. Effects of Phenolic Compounds on Plasma and Liver Cholesterol[a]

	diet				
	control	caffeic acid	chlorogenic acid	ferulic acid	$P <$
			Plasma (mg/dL)		
	$n = 8$	$n = 8$	$n = 8$	$n = 8$	
total C	68.4 ± 10.7	77.2 ± 8.9	74.7 ± 15.3	71.2 ± 11.9	ns[b]
HDL-C	35.0 ± 6.1	32.1 ± 4.9	34.3 ± 5.6	34.2 ± 4.2	ns
VLDL + LDL-C	33.4 ± 12.9	45.1 ± 9.7	40.4 ± 12.3	37.0 ± 12.9	ns
HDL-C/total C	0.53 ± 0.13a	0.42 ± 0.07b	0.47 ± 0.08ab	0.49 ± 0.10ab	0.05
TAG	123.2 ± 26.1	126.3 ± 36.1	103.5 ± 33.5	95.2 ± 44.2	ns
			Liver (mg/g)		
	$n = 8$	$n = 6$	$n = 7$	$n = 7$	
C	29.6 ± 4.0a	34.9 ± 5.1b	34.5 ± 2.6b	31.0 ± 2.7ab	0.05
			Lung (mg/g)		
	$n = 8$	$n = 8$	$n = 8$	$n = 8$	
% C in liver lipids	17.5 ± 3.6a	21.7 ± 2.6b	21.8 ± 4.1b	19.0 ± 3.5ab	0.05

[a] Values represent means ± SD. Values within each row not sharing a common letter are statistically different at P. n = number of observations. [b] ns, not significant.

were calculated as micrograms per gram of extracted lipids instead of micrograms per gram of organ fresh weight because of the lipid-lowering effect of CGA in the lung tissue ($P <$ 0.005) (**Table 2**). In accordance with the results of Tsuchiya and co-workers (*39*), we did not observe alterations in any of the measured plasma C and TAG levels (**Table 4**), whereas the C contents of liver tissue and lipids were increased ($P < 0.05$). Although CGA inhibited the activity of hepatic HMG-CoA reductase by 15% in hepatocytes in an in vitro study (*46*), it failed to exhibit a C-lowering effect in vivo, showing the difficulties to directly apply conclusions from in vitro experiments to the extremely complex situation in the living organism. The ineffectiveness of CGA to lower C in vivo may find its explanation in the very low absorption of intact CGA in rats and humans (*33*, *34*, *36*). Current thinking is that dietary CGA may undergo cleavage into CA and quinic acid by colonic microflora (*48*−*50*). In both species, an increase in CA was observed in plasma samples following CGA consumption, whereas CGA was not detectable, supporting the idea that CGA-derived CA may be responsible for the in vivo effects of CGA (*34*, *36*).

The current investigation confirms our previously published findings (*19*) that FA does not significantly affect α-T, γ-T, and C levels in rats (see **Tables 3** and **4**) despite its distinct function as an antioxidant in various in vitro systems (*14*, *15*, *51*). This may be partly due to the fact that FA circulates predominantly as conjugated forms (mainly glucuronates and sulfates) and not as free acid in rats (*37*, *52*, *53*). Similar to CA and CGA, but to a lesser extent, FA tended to decrease the HDL-C/total C-ratio in plasma and to elevate liver C and the percentage of C in liver lipids.

The mechanisms responsible for the effects of dietary phenolic compounds found in this and the previous studies (*19*, *20*) are not known at present. Possible mechanisms for interactions with tocopherols might be related to (i) up- or down-regulation of different cytochrome P_{450} isozymes as was shown for BHT (*54*) and sesamin (*61*, *62*), (ii) sparing of tocopherols by redox cycling and regeneration from their tocopheroxyl radicals as suggested for vitamin C, different flavonoids, procyanidins, and phenolic acids (*45*, *55*−*57*), (iii) reaction with reactive oxygen species that consume T in vivo, such as peroxyl radicals (*58*), (iv) effects on enzyme systems involved in the production of such radicals, (v) inhibitory or stimulatory effects on antioxidant enzyme systems such as catalase and glutathione peroxidase (*18*), and (vi) alteration of the binding capacity and/or expression of the tocopherol transfer protein (*59*). All three

investigated compounds have been shown to selectively inhibit certain cytochrome P_{450} isoforms, but whereas CA and CGA inhibited the O-dealkylation of benzyloxyresorufin in hamster liver microsomes, FA did not (*60*). Sontag and Parker (*61*) recently reported an initial ω-hydroxylation catalyzed by the cytochrome P_{450} isoform 4F2 in the metabolism of α-T and γ-T. It may, thus, be hypothesized that CA and CGA possibly exert their vitamin E-sparing action by inhibition of cytochrome P_{450} isozymes involved in tocopherol metabolism (*61*), whereas FA is ineffective in this regard. An alternative or, rather, a complementary explanation may be derived from the chemical structure of the compounds. The *o*-dihydroxy substitution in CA and CGA gives rise to better chelation properties, higher antioxidant activities, and lower redox potentials compared to FA with a single hydroxy group. These properties would render CA and CGA much more effective than FA in reactions ii and iii above (*14*, *15*, *51*, *55*).

With increasing interest in the protective function of predominantly vegetarian diets (*2*), the quest for the responsible components and their mechanisms of action continues. The results obtained in the present investigation support the current idea that there is no such thing as a *magic bullet* and that the key to a healthy diet is to choose from a wide variety of foods. Our results demonstrate that caffeic acid, previously reported to have vitamin E-sparing activity in vitro (*41*−*45*), and its quinic acid ester, chlorogenic acid, are capable of elevating α- and γ-tocopherol concentrations in rats. Ferulic acid, on the other hand, despite being a potent antioxidant in vitro (*14*, *15*, *51*), did not show this effect. Furthermore, the studied compounds exhibited unfavorable effects on lipid and cholesterol levels. Caffeic acid and chlorogenic acid elevated cholesterol concentrations in the liver and displayed a tendency to do so in blood plasma. More studies are needed to establish the significance of these findings for human health and nutrition and to elucidate the mechanisms behind these observations.

ABBREVIATIONS USED

BHT, butylated hydroxytoluene; C, cholesterol; CA, caffeic acid; CGA, chlorogenic acid; FA, ferulic acid; GLM, general linear model; HDL, high-density lipoprotein; HIP, hexane/2-propanol; HMG-CoA, hydroxymethylglutaryl-coenzyme A; LDL, low-density lipoprotein; SD, standard deviation; T, tocopherol; TAG, triacylglycerols; TMS, trimethylsilyl; VLDL, very low-density lipoprotein.

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NOTE ADDED AFTER ASAP POSTING

Units for plasma tocopherol concentration in **Table 3** were incorrectly given in the original posting of March 29, 2003. The correct units are given in this version.

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