Chlorogenic Acid, Quercetin-3-Rutinoside and Black Tea Phenols Are Extensively Metabolized in Humans[1]

Margreet R. Olthof,*[2] Peter C. H. Hollman,† Michel N.C.P. Buijsman,† Johan M. M. van Amelsvoort** and Martijn B. Katan*

*Division of Human Nutrition and Epidemiology, Wageningen University, Wageningen, The Netherlands; †RIKILT, Wageningen, The Netherlands; and **Unilever Health Institute, Unilever Research Vlaardingen, The Netherlands

ABSTRACT Dietary phenols are antioxidants, and their consumption might contribute to the prevention of cardiovascular disease. Coffee and tea are major dietary sources of phenols. Dietary phenols are metabolized extensively in the body. Lack of quantitative data on their metabolites hinders a proper evaluation of the potential biological effects of dietary phenols in vivo. The aim of this study was to identify and quantify the phenolic acid metabolites of chlorogenic acid (major phenol in coffee), quercetin-3-rutinoside (major flavonol in tea) and black tea phenols in humans, and determine the site of metabolism. Healthy humans (n = 20) with an intact colon participated in a dietary controlled crossover study, and we identified and quantified ~60 potential phenolic acid metabolites in urine. Half of the ingested chlorogenic acid and 43% of the tea phenols were metabolized to hippuric acid. Quercetin-3-rutinoside was metabolized mainly to phenylacetic acids, i.e., 3-hydroxyphenylacetic acid (36%), 3-methoxy-4-hydroxyphenylacetic acid (8%) and 3,4-dihydroxyphenylacetic acid (5%). In contrast, in seven humans without a colon, we found only traces of phenolic acid metabolites in urine after they had ingested chlorogenic acid and quercetin-3-rutinoside. This implies that the colonic microflora convert most of these dietary phenols into metabolites that then reach the circulation. Metabolites of dietary phenols have lower antioxidant activity than their parent compounds; therefore, the contribution of dietary phenols to antioxidant activity in vivo might be lower than expected from in vitro tests. J. Nutr. 133: 1806–1814, 2003.

KEY WORDS: • *chlorogenic acid* • *quercetin* • *black tea* • *metabolism* • *humans*

Dietary phenols are antioxidants in vitro and might therefore contribute to the prevention of cardiovascular disease (1,2). Two important groups of phenols in foods are flavonoids and cinnamic acids. Major flavonoids in foods are flavonols and catechins. Tea is an important dietary source of flavonoids, i.e., 1 L of strong tea can provide ~0.5 g or more of phenols, consisting in large part of catechins and their condensed polymers such as theaflavins and thearubigins (3).

The major representative of dietary cinnamic acids is caffeic acid. In foods, caffeic acid is conjugated mainly with quinic acid, which yields chlorogenic acid (5-caffeoylquinic acid). Coffee is the major source of dietary chlorogenic acid, i.e., 1 L of coffee provides 500–800 mg of chlorogenic acid, which corresponds to ~250–400 mg of caffeic acid (4). Dietary phenols are antioxidants, and they can protect LDL particles from oxidation in vitro (5,6). Oxidized LDL might play a role in the pathogenesis of atherosclerosis and might therefore be associated with an increased risk for cardiovascular disease (7). Indeed, some, but not all epidemiologic studies show an inverse association between intake of flavonols and cardiovascular disease (8).

Thus, dietary phenols are strong antioxidants in vitro (9), but their contribution to the antioxidant defense in vivo is uncertain. One reason for this uncertainty is that dietary phenols are metabolized extensively in the body into partly unknown compounds. If metabolism of phenols occurs before they can reach the circulation and act as antioxidants there, then in vitro tests of antioxidant activity of the parent phenols might be less relevant to the in vivo situation. Metabolites might actually have antioxidant activity in vivo, although most of the metabolic reactions reduce the antioxidant activity of the parent phenol (9–12). Therefore, it is important to identify the circulating metabolites of phenols and the site at which they are produced. Data on the type and quantity of the metabolites of dietary phenols in humans are scarce. Therefore, we designed this dietary controlled trial in humans to identify and quantify a comprehensive range of phenolic metabolites of three major dietary phenols, i.e., chlorogenic acid, quercetin-3-rutinoside and black tea phenols. These phenols are important representatives of the group of dietary phenols and are stronger antioxidants in vitro than antioxidant vitamins (9,13). For this purpose, we developed a method to measure ~60 phenolic acids in urine (**Table 1**). Furthermore we investigated the site of metabolism of dietary phenols by

[1] Supported by the foundation for Nutrition and Health Research, The Netherlands.
[2] To whom correspondence should be addressed.
E-mail: margreet.olthof@wur.nl.

0022-3166/03 $3.00
Manuscript received 30 September 2002. Initial review completed 18 October 2002. Revision accepted 18 February 2003.

TABLE 1

Mass of target ion and qualifier ions (high response; low response) used to identify the major phenolic acid metabolites[1]

Metabolite	Target ion	Qualifier ions
	m/z	
Phenyl-C1 metabolites		
Benzoic acid (2)	179	105; 135
3-OH-benzoic acid (15)	267	223; 282
4-OH benzoic acid (22)	267	223; 193
2,4-diOH-benzoic acid (44)	355	281; 223
3,4-diOH-benzoic acid (protocatechuic acid) (47)	193	165; 223
3,5-diOH-benzoic acid (46)	370	170; 103
3,4,5-triOH-benzoic acid (gallic acid) (58)	458	281; 443
3-OCH3-4-OH-benzoic acid (vanillic acid) (32)	297	267; 312
3,5-diOCH_3-4-OH-benzoic acid (syringic acid) (52)	327	342; 312
N-benzoylglycine (hippuric acid, tautomeric form) (38)	105	206; 308
N-benzoylglycine (hippuric acid) (42)	105	206; 236
N-(2-OH-benzoyl)glycine (salicyluric acid) (60)	324	206; 193
Phenyl-C2 metabolites		
Phenylacetic acid (3)	164	193; 137
2-OH-phenylacetic acid (16)	253	164; 296
3-OH-phenylacetic acid (19)	164	281; 296
4-OH-phenylacetic acid (23)	296	281; 252
3,4-diOH-phenylacetic acid (48)	179	267; 384
3-OCH_3-4-OH-phenylacetic acid (homovanillic acid) (34)	209	326; 267
Phenylhydroxyacetic acid (mandelic acid) (7)	179	147; 253
Phenyl-C3 metabolites		
3-OH-cinnamic acid (51)	203	293; 308
4-OH-cinnamic acid (54)	219	293; 308
3,4-diOH-cinnamic acid (caffeic acid) (62)	396	381; 307
3-OCH_3-4-OH-cinnamic acid (ferulic acid) (61)	338	249; 323
3,5-diOCH3-4-OH-cinnamic acid (sinapinic acid) (63)	368	338; 353
5-caffeoylquinic acid (chlorogenic acid) (64)	345	255; 307
3-(3,4-diOH-phenyl)propionic acid (56)	179	398; 267

[1] The numbers in parentheses are for the metabolites as designated in Figures 1 and 2.

comparing the metabolites formed in volunteers with an intact colon with those formed in volunteers who lack a colon (ileostomy subjects) (14).

SUBJECTS AND METHODS

Subjects. Ten women and ten men with a mean (±SD) age of 24 ± 8 y and a mean body mass index of 22.2 ± 2.5 kg/m^2 participated. They were healthy as judged by a medical questionnaire, with normal blood values for hemoglobin, hematocrit and white blood cell counts and the absence of glucose and protein in urine. They were not allowed to take any drugs or other supplements during the study except for acetaminophen (paracetamol) and oral contraceptives. The study was fully explained to the subjects and they gave their written informed consent.

Details on the study with the subjects without a colon are described elsewhere (14). That study was designed to investigate the absorption of chlorogenic acid and caffeic acid in humans, but we were able to measure phenolic acid metabolites in unneeded 24-h urine samples of the participants of that study.

Methods. The study in subjects with an intact colon was approved by the Medical Ethical Committee (Wageningen University). Throughout the 4-wk crossover study, subjects consumed a controlled diet low in phenols, which was largely provided by the study group. In addition to the diet, subjects ingested four supplements in random order, each for 1 wk. There was a 7-d menu cycle, so that the menu on the day that blood and urine were collected in each supplement week (i.e., d 7) was always the same, as were the other days (i.e., d 1–6) in each of the four supplement weeks. To achieve the diet low in phenols, we supplied the subjects daily with foods low in phenols, which provided 90% of the energy required to maintain body weight. The remaining 10% of energy was chosen by the subjects from a list of food items low in phenols. Foods were considered low in phenols if they contained <15 mg quercetin or chlorogenic acid/kg, and beverages if they contained <4 mg quercetin or chlorogenic acid/L (4,15,16). Because coffee and tea consumption were not allowed, we provided the volunteers with the following substitutes: for coffee, an extract made of chicory, rye and barley (Swiss coffee-like; Tayala AG, Birsfelden, Switzerland) and for tea, tea bags containing a mix of herbs (droommix; Piramide, Veenendaal, the Netherlands) or tea bags containing stinging nettle (Jacob Hooy, Limmen, The Netherlands). The coffee substitute was not used by the subjects during the study. Chemical analyses of these coffee and tea substitutes showed that the amounts of chlorogenic acid and flavonols such as quercetin were within the allowed ranges, as described. We did not determine other phenolic acids in our coffee and tea substitutes.

In addition to the diet, subjects ingested daily one of the following supplements: 2 g (5.5 mmol) of chlorogenic acid (Fluka Chemie AG, Buchs, Switzerland); or 4 g of black tea solids (LN-0173–02, kindly provided by Unilever Research Vlaardingen, The Netherlands), in which the phenol fraction consisted mainly of catechins and their condensed polymers (theaflavins and thearubugins); or 440 mg (660 μmol) of quercetin-3-rutinoside (Rutosidum DAB, BUFA, Uitgeest, The Netherlands); or 0.5 g of citric acid as placebo (AC Citricum; Fagron, Nieuwerkerk a/d IJssel, The Netherlands). The 2 g of chlorogenic acid corresponds to the amount of chlorogenic acid present in ~1.5–4 L of coffee, depending on the strength of the coffee. The 4 g of black tea solids corresponds to ~2 L of strong black tea. Black tea solids contain 30–40 g phenols/100 g; thus, 4 g of black tea solids provided ~4.3 mmol phenols if expressed as monomeric equivalents, i.e., including catechins, theaflavins and thearubigins (3). The amount of quercetin in 440 mg quercetin-3-rutinoside corresponds to the amount in ~13 L of black tea (16). The chlorogenic acid, quercetin-3-rutinoside and citric acid and half of the black tea extract (2 g) were dissolved in hot water before ingestion. Subjects took the supplements under our supervision, just before the hot meal at noon. The other half of the black tea extract was used for tea preparation and consumption at home as follows: 1 g between 0800 and 1000 h (on Saturdays and Sundays, between 0800 and 1100 h), and 1 g between 1800 and 2000 h. The volunteers were urged to maintain their usual pattern of physical activity during the study.

Collection of urine and blood. On d 7 of each of the four supplement weeks, subjects collected urine for 24 h; urine was collected in 0.5-L plastic bottles with 0.13 g thymol (# 8167; Merck, Amsterdam, Netherlands) as a preservative and stored on dry ice immediately after voiding. The recovery of 277 μmol lithium in the 24-h urine of d 7 was measured to verify the completeness of urine collection. Lithium was ingested daily during the study by the subjects as lithium chloride dissolved in 10 mL of tap water. Lithium chloride is completely absorbed, and 95% is excreted in urine (17,18). Lithium was measured in undiluted, acidified urine by atomic absorption spectrophotometry (19). Urinary recovery of lithium was 105.0 ± 9.0% (mean ± SD), which indicated good compliance.

Analyses of phenolic acids in urine. Analytical grade reagents and solvents were used, except for ethyl acetate which was HPLC grade (LiChrosolv, Merck, Darmstadt, Germany). Reference compounds of phenolic acids were purchased from Fluka Chemie (Sigma-Aldrich Chemie Zwijndrecht, The Netherlands), except for *N*-phe-

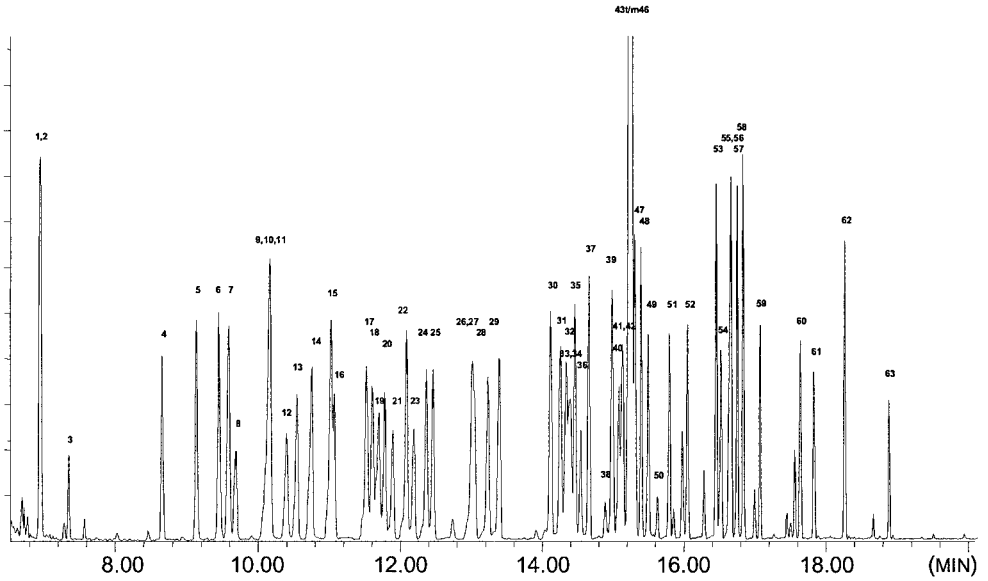

FIGURE 1 Chromatogram (total ion current) of all 60 reference phenolic acids (20 μg/L each); the peak number of the phenolic acid metabolite is in parentheses: benzoic acid d5(**1** or **2**); benzoic acid (**1** or **2**); phenylacetic acid (**3**); 3-(phenyl)propionic acid (**4**); 2OCH_3 benzoic acid (**5**); 3-OCH_3-benzoic acid (**6**); phenylhydroxyacetic acid (mandelic acid) (**7**); 2-OCH_3-phenylacetic acid (**8**); 3-OCH_3-phenylacetic acid (**9**); 4-OCH_3-benzoic acid (**10**); 2-OH-benzoic acid (**11**); 4-OCH_3-phenylacetic acid (**12**); cinnamic acid (**13**); MCPA-methylester (internal standard, IS) (**14**); 3-OH-benzoic acid (**15**); 2-OH-phenylacetic acid (**16**); 2,3-diOCH_3-benzoic acid (**17**); 3-(2-OCH_3-phenyl)propionic acid (**18**); 3-OH-phenylacetic acid (**19**); 2,6-di-OCH_3-benzoic acid (**20**); 5-phenyl-n-valeric acid (**21**); 4-OH-benzoic acid (**22**); 4-OH-phenylacetic acid (**23**); 3-(4-OCH_3-phenyl)propionic acid (**24**); 1,3,5,-triOH-benzene (phloroglucinol) (**25**); 3-(2-OH-phenyl)propionic acid (**26**); 3,5-diOCH_3-benzoic acid (**27**); 3,4-diOCH_3-benzoic acid (**28**); 2,4-diOCH_3-benzoic acid (**29**); 2,3-diOH-benzoic acid (**30**); 3-(4-OH-phenyl)-propionic acid (**31**); 3-OCH_3-4-OH-benzoic acid (vanillic acid) (**32**); 2-OCH_3-cinnamic acid (**33**); 3-OCH_3-4-OH-phenylacetic acid (homovanillic acid) (**34**); 2,6-diOH-benzoic acid (**35**); 3-OCH_3-cinnamic acid (**36**); 2,5-diOH-benzoic acid (**37**); *N*-benzoylglycine (hippuric acid, tautomeric form) (**38**); 2-OH-cinnamic acid (**39**); 4-OCH_3-cinnamic acid (**40**); 3-(3,4-diOCH_3-phenyl)propionic acid (**41**); *N*-benzoylglycine (hippuric acid) (**42**); 3,4,5-triOCH_3-benzoic acid (**43**); 2,4-diOH-benzoic acid (**44**); 3,4,5-triOCH_3-phenylacetic acid (**45**); 3,5-diOH-benzoic acid (**46**); 3,4-diOH-benzoic acid (protocatechuic acid) (**47**); 3,4-diOH-phenylacetic acid (**48**); 2,5-diOH-phenylacetic acid (**49**); *N*-phenylacetylglycine (phenylaceturic) acid (**50**); 3-OH-cinnamic acid (**51**); 3,5-diOCH_3-4-OH-benzoic acid (syringic acid) (**52**); 2,3,4-triOH-benzoic acid (**53**); 4-OH-cinnamic acid (**54**); 3-(3,4,5-triOCH_3-phenyl)propionic acid (**55**); 3-(3,4-diOH-phenyl)propionic acid (**56**); 3-(2,4-diOH-phenyl)propionic acid (**57**); 3,4,5-triOH-benzoic acid (gallic acid) (**58**); 2,4,6-triOH-benzoic acid (**59**); *N*-(2-OH-benzoyl)glycine (salicyluric acid) (**60**); 3-OCH_3-4-OH-cinnamic acid (ferulic acid) (**61**); 3,4-diOH-cinnamic acid (caffeic acid) (**62**); 3,5-diOCH_3-4OH-cinnamic acid (sinapinic acid) (**63**); 5-caffeoylquinic acid (chlorogenic acid) (**64**) not shown, elutes at 24.8 min. Abbreviations:OH, hydroxy; OCH_3, methoxy; MCPA, methyl (4-chloro-2-methylphenoxy) acetate.

nylacetylglycine (phenylaceturic acid), purchased from Trade-TCI-max (Tokyo, Japan) and *N*-(2-OH-benzoyl)glycine (salicyluric acid), purchased from Merck (VWR International, Amsterdam, the Netherlands). For extraction, urine, previously stored at −80°C, was brought to room temperature.

Free, unconjugated phenolic acids. To a 10-mL test tube 1.00 mL of urine and 4.00 mL 0.2 mol/L hydrochloric acid (HCl) were added followed by mixing; a pH of ~1 is optimal.

Free plus conjugated phenolic acids. To a 4-mL vial (#WAT022468, Waters, Milford, MA) 1.3 mL of urine, 65 μL 3.0 mol/L acetate buffer, pH 4.8, and 260 μL β-glucuronidase/arylsulfatase from Helix Pomatia (104114, Merck) were added. The vial was sealed tightly with a septum (#73008, Waters) and cap (#72711, Waters), mixed, inserted into a preheated aluminum block (Reacti-Block C-1, Pierce Europe, Oud-Beijerland, The Netherlands) and heated in an oven at 50°C for 2 h. After cooling, 65 μL of 4 mol/L HCl was added followed by mixing and centrifuging at 1000 × *g* for 10 min. Of the supernatant, 1.3 mL was added to 3.7 mL of 0.2 mol/L HCl in a 10-mL tube.

A styrene divinyl benzene (SDB) solid phase extraction cartridge [Bakerbond SPE SDB, 200 mg/6 mL (33.3 g/L), J.T. Baker, Phillipsburg, NJ] was preconditioned sequentially with 5.0 mL ethyl acetate, 5.0 mL methanol and 5.0 mL of 0.1 mol/L HCl, at a flow rate of 4 mL/min. After release of the vacuum, the cartridge was loaded with the acidified urine sample at a flow rate of 1 mL/min. The cartridge was washed 2 times with 2.5 mL of 0.1 mol/L HCl (flow rate 4 mL/min), dried by aspirating nitrogen gas for 20 min and then eluted 3 times with 1.5 mL ethyl acetate into a silanized 10-mL test tube. Ethyl acetate was removed using a heating block at 50°C (Reacti-Block C-1, Pierce Europe) under a stream of nitrogen gas until ~100–200 μL ethyl acetate remained. To the residue, 0.50 mL dichloromethane (dried over molecular sieve 1- to 3-mm grains, 0.3 nm pores), 60 μL undecane (keeper or trapping agent) was added. The solvent was removed using the heating block until only the keeper remained.

The residue was silylated in the same tube by adding 300 μL bis(trimethylsilyl)trifluoroacetamide (Pierce Europe). The stoppered tube underwent vortex mixing, and was heated for 2 h at 60°C. The tube was mixed at least once during the heating process, to ensure complete silylation. After being cooled, 40 μL methyl (4-chloro-2-methylphenoxy) acetate (MCPA) (450 mg/L in heptane) was added as an internal standard, and after undergoing vortex mixing, 60 μL of the sample was added to a gas chromatography (GC) vial.

GC-MS determination. Derivatized samples were analyzed with a HP5971A GC/mass spectrometry (GC-MS) system. Metabolites were separated on a fused silica 25 m × 0.25 mm capillary column CP Sil 5B (Varian, Bergen op Zoom, The Netherlands), with helium as the carrier gas (constant flow). The sample (1 μL) was injected directly (splitless) into the injection port set at 250°C. The temperature program of the column oven from 80 to 290°C was as follows: 0–2 min at 0°C/min at 80°C; 2–7 min at 15°C/min to 155°C; 7–9 min at 0°C/min to 155°C; 9–14 min at 6°C/min to 185°C; 14–21 min at 15°C/min to 290°C; 21–35 min at 0°C/min to 290°C. The mass spectrometer was operated in electron impact mode at 280°C. Mass range was set to 100–460 *m*/*z*, and 2.9 scans/s were taken.

The total ion chromatograms of all reference phenolic acid standards (**Fig. 1**) shows a number of overlapping peaks, which could be resolved by selection of an appropriate target ion for each metabolite. Metabolites were identified by comparing their retention times, the *m*/*z* of their target ions and the *m*/*z* ratios (of target ions and two qualifier ions, see Table 1) in samples and standards. Criteria were selected based on a number of runs with standards and spiked urine samples. Variation of retention times had to be within 0.10 min, and of *m*/*z* ratios on average were within 20%.

Metabolites were quantified using the peak area of their target ions against an external calibration line of reference standards, allowing for the internal standard MCPA. Typical chromatograms of the 24-h urine of one of the volunteers after ingestion of each of the supplements are shown in **Figure 2**.

Recovery, precision and limit of detection. Recovery of phenolic acids added to a control urine low in phenolic acids, which increased their concentrations with 4 mg/L (25 mg/L for chlorogenic acid and hippuric acid), ranged from 70 to 105%. Relative SD of repeatability

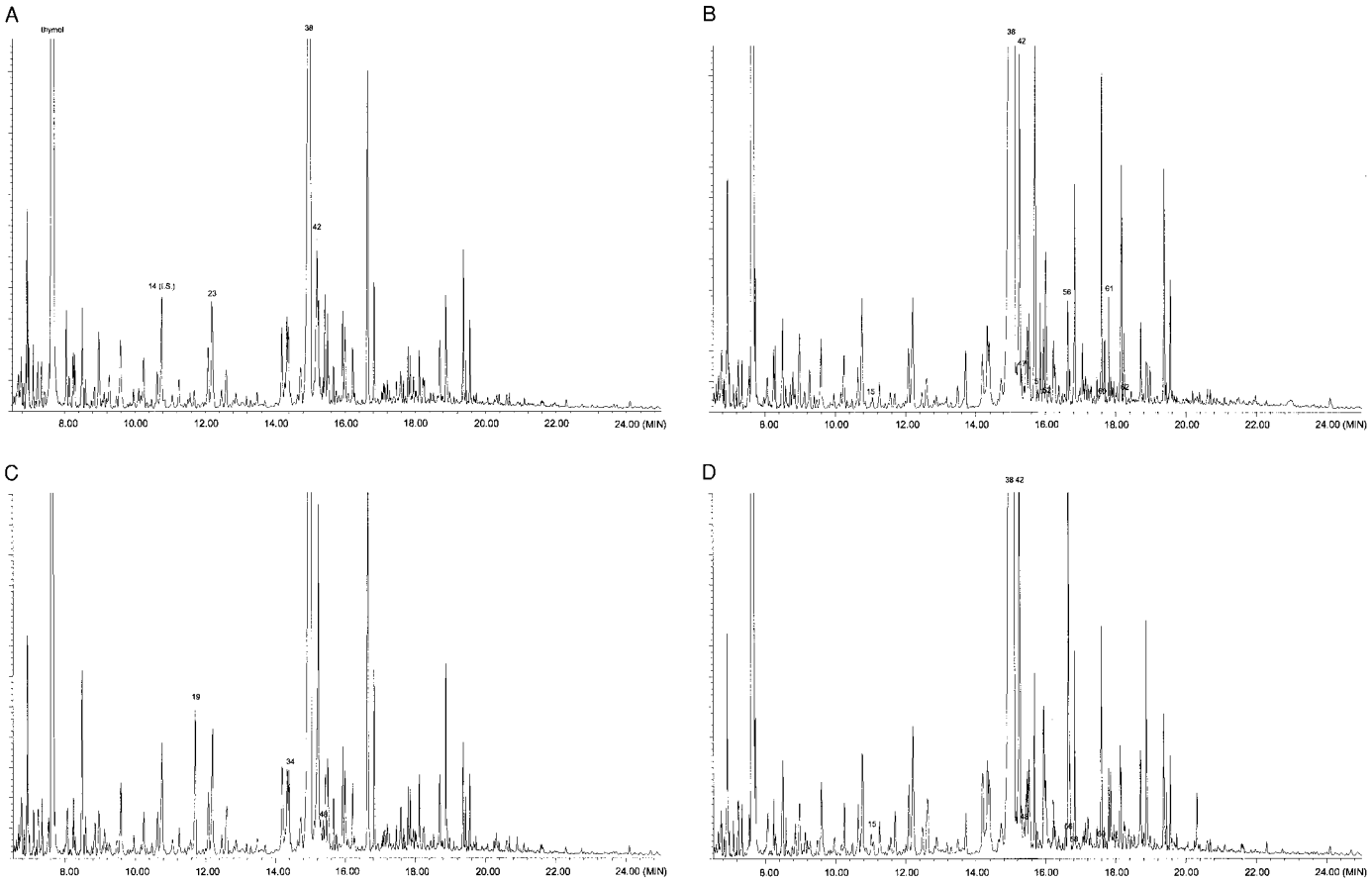

FIGURE 2 Typical chromatogram (total ion current) of the 24-h urine sample (after deconjugation) of one of the volunteers with a colon after ingestion of placebo (*A*), 2 g (5.5 mmol) chlorogenic acid (*B*), 440 mg (660 μmol) quercetin-3-rutinoside (*C*) and 4 g black tea solids (~4.3 mmol phenol monomeric equivalents) (*D*). For peak identification, see Figure 1.

ranged from 4 to 12%, depending on the type of phenolic acid. Relative SD of reproducibility ($n = 7$) ranged from 6 to 20% for various phenolic acids. The detection limit (three times SD of baseline noise) in urine was 4 mg/L urine for chlorogenic acid, 5 mg/L for hippuric acid and 0.4 mg/L for other phenolic acids.

Of 60 reference standards of different phenolic acids, we detected 27 in the urine samples of volunteers with a colon. Two phenolic acids were excluded because more than half of the samples had concentrations of these acids that were below the detection limit in all supplement periods. Thus, 25 phenolic acids were regarded as potential metabolites of the supplements in volunteers with a colon (**Table 2**). Of 60 reference standards of different phenolic acids in urine of volunteers without a colon, we detected 27. Ten phenolic acids were excluded because more than half of the samples had concentrations of these acids that were below the detection limit in all supplement periods. Thus, 17 phenolic acids were regarded as potential metabolites of the supplements in volunteers without a colon.

Statistical analyses. Statistical significance and 95% confidence intervals of the mean differences relative to placebo were calculated using a paired Student's *t* test. We chose to use a one-sided significance level because we reasoned that rises in the excretion of phenolic acids with consumption of placebo relative to the phenol supplements must be spurious. Further, because we tested several phenolic acids as potential metabolites (multiple testing) we decided to use an α of 0.001 for statistical testing to distinguish genuine metabolites of dietary phenols from compounds that were present by accident. To indicate possible other (quantitatively less important) metabolites, we reported *P*-values between 0.01 and 0.001 separately (one-sided) (Table 2). Statistical analyses were done using SAS (SAS Institute, Cary, NC).

RESULTS

Hippuric acid (*N*-benzoylglycine) was the major metabolite of chlorogenic acid and of phenols from tea, representing ~50% of the metabolites formed (**Tables** 2 and **3**). Additional phenyl-C_1 and phenyl-C_3 metabolites were formed after chlorogenic acid and after black tea supplementation, but their amounts were small (Fig. 2). We assume that each molecule of chlorogenic acid can yield two molecules of hippuric acid, because the caffeic acid moiety and the quinic acid moiety of chlorogenic acid are probably metabolized to hippuric acid in humans (20,21). Therefore, 49.5 mol/100 mol of the ingested chlorogenic acid was recovered in urine as hippuric acid (Table 3). In contrast, in volunteers without a colon, we found only trace amounts of caffeic acid (3,4-dihydroxycinnamic acid) and ferulic acid (3-methoxy-4-hydroxycinnamic acid) as metabolites of chlorogenic acid (Table 3).

Phenylacetic acids (phenyl-C_2 metabolites) were the major metabolites of quercetin-3-rutinoside (Tables 2 and 3) in subjects with an intact colon and accounted for about half of the ingested rutin. In subjects without a colon, we did not find any metabolites in urine (Table 3).

Of the ingested chlorogenic acid, 1.7% was recovered unchanged in urine. We did not assess the amount of quercetin-3-rutinoside or tea phenols in urine. However, in other human studies < 0.5% of ingested quercetin-3-rutinoside was recovered in urine as quercetin or its conjugates (22), and ~6% of

TABLE 2

Phenolic acid excretion in urine after ingestion of each of the supplements for 7 d by 20 subjects with an intact colon[1]

	Placebo	Chlorogenic acid (5.5 mmol/d)	Supplement Quercetin-3-rutinoside (660 μmol/d)	Black tea solids[5] (~4.3 mmol phenol/d)
		μmol/24 h		
Phenolic acids in urine[2,3,4]				
Total phenolic acids	3300 ± 200	9300 ± 800	3800 ± 200	5300 ± 400
Phenyl-benzoic (C1) metabolites				
Benzoic acid	8 ± 2	12 ± 2**	9 ± 2	8 ± 1
3-OH-benzoic acid	ND	6.3 ± 0.8[6]**	ND	6.6 ± 1.3[6]**
4-OH-benzoic acid	29 ± 3[5]	29 ± 3	29 ± 4	27 ± 3
2,4-diOH-benzoic acid	15 ± 3	13 ± 2[5]	16 ± 2	15 ± 3[5]
3,4-diOH-benzoic acid (protocatechuic acid)	10 ± 1	27 ± 5[5]*	13 ± 1	12 ± 2
3,5-diOH-benzoic acid	45 ± 5	42 ± 4[5]	47 ± 4	48 ± 5
3,4,5-triOH-benzoic acid (gallic acid)	ND	ND	ND	7 ± 1[5]**
3-OCH_3-4-OH-benzoic acid (vanillic acid)	85 ± 16	102 ± 16	80 ± 10	69 ± 6
3,5-diOCH_3-4-OH-benzoic acid (syringic acid)	6.7 ± 0.6	13.6 ± 2.2[5]*	8.3 ± 1.9	6.2 ± 0.7[5]
N-Benzoylglycine (hippuric acid)	2500 ± 200	100 ± 800**	2700 ± 200	4400 ± 400**
N(2-OH-benzoyl)glycine (salicyluric acid)	5.5 ± 0.6[5]	9.2 ± 1.5[5]*	6.2 ± 1.2[5]	9.7 ± 1.4[5]*
Phenyl-acetic (C2) metabolites				
Phenylacetic acid	18 ± 4	20 ± 4	22 ± 4	21 ± 5
2-OH-phenylacetic acid	8.4 ± 0.5[5]	8.0 ± 0.6[5]	8.2 ± 0.6	6.5 ± 0.6[6]
3-OH-phenylacetic acid	21 ± 3	18 ± 2	259 ± 51**	68 ± 6**
4-OH-phenylacetic acid	160 ± 20[5]	160 ± 10	150 ± 20[5]	170 ± 20
3,4-diOH phenylacetic acid	19 ± 1	24 ± 2[5]*	52 ± 6**	26 ± 2**
3-OCH_3-4-OH-phenylacetic acid (homovanillic acid)	52 ± 3	41 ± 3	103 ± 15**	54 ± 4
Phenylhydroxyacetic acid (mandelic acid)	5 ± 1	6 ± 1	6 ± 1	7 ± 2
Phenyl-cinnamic (C3) metabolites				
3-OH-cinnamic acid	ND	20 ± 3[5]**	ND	ND
4-OH-cinnamic acid	3.5 ± 0.3[6]	4.2 ± 0.5[6]	ND	ND
3,4-diOH-cinnamic acid (caffeic acid)	ND	56 ± 8**	ND	ND
3-OCH_3-4-OH-cinnamic acid (ferulic acid)	26 ± 3	71 ± 6**	27 ± 3	34 ± 4
3,5-diOCH_3-4-OH-cinnamic acid (sinapinic acid)	2.8 ± 0.5[6]	ND	2.6 ± 0.5[6]	ND
3-(3,4-diOH-phenyl)propionic acid	ND	97 ± 16[5]**	ND	5 ± 1[6]*
5-Caffeoylquinic acid (chlorogenic acid)	ND	100 ± 20[5]**	ND	ND

[1] Values are mean ± SEM, $n = 20$. * Significantly different from placebo, $0.001 \leq P \leq 0.01$; ** significantly different from placebo, $P \leq 0.001$.

[2] Abbreviations: OH, hydroxy; OCH_3, methoxy; ND, nondetectable; peaks had to be above the detection limit in at least half of the subjects; otherwise the phenolic acid is not shown in the table.

[3] The following phenolic acids were absent in all subjects during all supplementation periods: 2,3-diOH-benzoic acid; 2,6-diOH-benzoic acid; 1,3,5-triOH-benzoic acid, 2,3,4-triOH-benzoic acid; 2,4,6-triOH-benzoic acid; 2-OCH_3-benzoic acid; 3-OCH_3-benzoic acid; 4-OCH_3-benzoic acid; 2,3-diOCH3-benzoic acid; 2,4-diOCH_3-benzoic acid; 2,6-diOCH_3-benzoic acid; 3,4-diOCH_3-benzoic acid; 3,5-diOCH_3-benzoic acid; 3,4,5-triOCH_3-benzoic acid; 2,5-diOH-phenylacetic acid (homogentisic acid); 2-OCH_3-phenylacetic acid; 3-OCH_3-phenylacetic acid; 4-OCH_3-phenylacetic acid; 3,4,5-triOCH_3-phenylacetic acid; cinnamic acid; 2-OH-cinnamic acid; 2-OCH_3-cinnamic acid; 3-OCH_3-cinnamic acid; 4-OCH_3-cinnamic acid; 3-(phenyl)propionic acid; 3-(2-OH-phenyl)propionic acid; 3-(4-OH-phenyl)propionic acid; 3-(2,4-diOHphenyl)propionic acid; 3-(2-OCH3-phenyl)propionic acid; 3-(4-OCH_3-phenyl)propionic acid; 3-(3,4-diOCH_3-phenyl)propionic acid; 3-(3,4,5-triOCH_3-phenyl)propionic acid; phenylaceturic acid. The peaks of 2-OH-benzoic acid, 2,5-OH-benzoic acid, were above the detection limit in less than half of the subjects after each supplement period and therefore they are not shown in the table.

[4] Amounts determined after deconjugation with β-glucuronidase/arylsulfatase.

[5] Peaks of 1–5 subjects were below the detection limit or missing.

[6] Peaks of 6–10 subjects were below the detection limit or missing.

monomeric catechins were recovered in urine of humans after ingestion of the pure compounds or of green tea (23,24).

Furthermore, the absolute excretion of 4-hydroxyphenylacetic acid in urine was in general greater than that of other phenolic acids after all treatments including placebo, but excretion was not influenced by the supplements ingested (Table 2). 4-Hydroxyphenylacetic acid is probably a metabolite of dietary phenylalanine and tyrosine (25,26). These

TABLE 3

Change in excretion of phenolic acid metabolites in urine relative to placebo after subjects ingested the supplements[1,2]

	Volunteers	
	With a colon[3]	Without a colon[4]
	mol/100 mol ingested supplement	
Chlorogenic acid supplement		
5-Caffeoylquinic acid (chlorogenic acid)	1.7 (1.0–2.4)	0.2 (0.1–0.4)
N-Benzoylglycine (hippuric acid)	49.5[5] (37.2–61.8)	NM[6]
3(3,4-Dihydroxyphenyl)propionic acid	1.7 (1.1–2.3)	ND[7]
3,4-Dihydroxycinnamic acid (caffeic acid)	1 (0.6–1.3)	0.3 (0.1–0.4)
3-Methoxy-4-hydroxycinnamic acid (ferulic acid)	0.8 (0.6–1.0)	0.5 (0.2–0.7)
3,4-Dihydroxybenzoic acid (protocatechuic acid)	0.3 (0.1–0.5)	NM
3-Hydroxycinnamic acid	0.3 (0.2–0.4)	ND
Benzoic acid	0.1 (0.0–0.1)	ND
3-Hydroxybenzoic acid	0.1 (0.1–0.1)	ND
3,5-Dimethoxy-4-hydroxybenzoic acid (syringic acid)	0.1 (0.0–0.2)	ND
3,4-Dihydroxyphenylacetic acid	0.1 (0.0–0.2)	NM
N(2-OH-benzoyl)glycine (salicyluric acid)	0.1 (0.0–0.1)	ND
Quercetin-3-rutinoside supplement		
3-Hydroxyphenylacetic acid	36.1 (19.9–52.2)	NM
3-Methoxy-4-hydroxyphenylacetic acid (homovanillic acid)	7.8 (3.6–11.9)	NM
3,4-dihydroxyphenylacetic acid	5.0 (3.1–6.9)	NM
Black tea supplement[8]		
N-Benzoylglycine (hippuric acid)	42.8 (29.6–55.9)	—
3-Hydroxyphenylacetic acid	1.1 (0.8–1.4)	—
3,4,5-Trihydroxybenzoic acid (gallic acid)	0.2 (0.1–0.2)	—
3,4-Dihydroxyphenylacetic acid	0.2 (0.1–0.3)	—
3-Hydroxybenzoic acid	0.1 (0.1–0.2)	—
N(2-OH-benzoyl)glycine (salicyluric acid)	0.1 (0.0–0.2)	—
3-(3,4-Dihydroxyphenyl)propionic acid	0.1 (0.0–0.1)	—

[1] Values are means (95% confidence interval); $n = 20$ with a colon; $n = 7$ without a colon.

[2] Amounts determined after deconjugation with β-glucuronidase/arylsulfatase.

[3] Results calculated from Table 2.

[4] Seven healthy volunteers without a colon ingested chlorogenic acid (2.8 mmol), caffeic acid (2.8 mmol) and quercetin-3-rutinoside (0.3 mmol) and placebo for 1 d each, in random order (14).

[5] Based on the assumption that each molecule of chlorogenic acid yields two molecules of hippuric acid. For the other phenolic acids we assumed that each molecule of chlorogenic acid yields one molecule of the phenolic acid metabolite.

[6] NM, not a metabolite of the ingested phenol.

[7] ND, not detectable.

[8] This supplement was not ingested by volunteers without a colon.

amino acids were present to an equal extent in the background diet of all supplement periods.

Treatment of the urine with β-glucuronidase and arylsulfatase enzymes before GC-MS analysis markedly increased the number and amount of phenolic acids detected in urine (data not shown). This indicates that most phenolic acids were conjugated with glucuronic acid or sulfates.

DISCUSSION

We investigated a broad range of potential metabolites in urine; under strictly controlled dietary conditions, chlorogenic acid, tea phenols and quercetin-3-rutinoside are metabolized extensively to specific (groups of) phenolic acid metabolites in humans. To our knowledge, this is the first dietary controlled study in humans that identified and quantified a complete profile of all phenolic acid metabolites of chlorogenic acid, black tea phenols and quercetin-3-rutinoside. The fraction that we did not recover in urine was likely metabolized to nonphenolic compounds that we could not measure, or it was excreted with the feces or degraded to carbon dioxide (27). In subjects with a colon, we recovered in urine about half of the ingested chlorogenic acid and tea phenols as hippuric acid, and about half of the quercetin-3-rutinoside as phenylacetic acids. Other studies also found hippuric acid in urine as a major metabolite of ingested phenols (28,29). In contrast, in subjects without a colon, we recovered only minor amounts of metabolites after they had ingested chlorogenic acid or quercetin-3-rutinoside. This difference can be explained by the fact that chlorogenic acid and quercetin-3-rutinoside are not well absorbed in the small intestine and thus become available for metabolism by the colonic microflora, which are absent in subjects without a colon (14). Thus, the colon plays an important role in the metabolism of phenols. Evidently, the dietary phenols that reach the colon are degraded there into metabolites. These are then absorbed from the colon and after circulation and further metabolism in tissues such as liver and kidneys, they are excreted in the urine. Therefore, a large part of the ingested phenols will probably never enter the peripheral circulation as such.

Metabolism of chlorogenic acid. The most pronounced difference in the metabolic profile of chlorogenic acid between volunteers with and without a colon was the absence of hippuric acid as a metabolite of chlorogenic acid in those without a colon (Table 3). We previously found in subjects without a colon that about one third of the chlorogenic acid ingested appeared to be absorbed from the small intestine (14). Our results therefore suggest that hippuric acid arises from metabolism by microorganisms in the colon. In volunteers with a colon, two thirds of the ingested chlorogenic acid reaches the colon, where the colonic microflora probably first hydrolyze chlorogenic acid into caffeic acid and quinic acid (14,30). Subsequently, the caffeic acid moiety is dehydroxylated by bacteria in the colon; after absorption, it is β-oxidized to a large extent into benzoic acid (**Fig. 3**). The quinic acid moiety is dehydroxylated into cyclohexane carboxylic acid and then aromatized into benzoic acid by the colonic microflora (31,32) or after absorption in body tissues (33). The benzoic acid formed is conjugated with glycine and excreted in urine as hippuric acid (Table 3, Fig. 3).

Another explanation for the fact that we did not find hippuric acid as a metabolite of chlorogenic acid in subjects without a colon might be that those subjects received a single dose of the phenols, whereas subjects with a colon received the phenols for 7 d. It is possible that a single dose was insufficient to produce hippuric acid. However, others (34,35) did find metabolites after a single dose of chlorogenic acid and caffeic acid, which makes this explanation less likely.

Metabolism of tea phenols. The phenols in black tea were metabolized mainly to hippuric acid (Tables 2 and 3), which agrees with results of Clifford et al. (28). Hippuric acid probably originates in part from the catechins and their condensed polymers such as theaflavins and thearubigins, which are an important group of phenols in tea (3,28). Part of the catechins

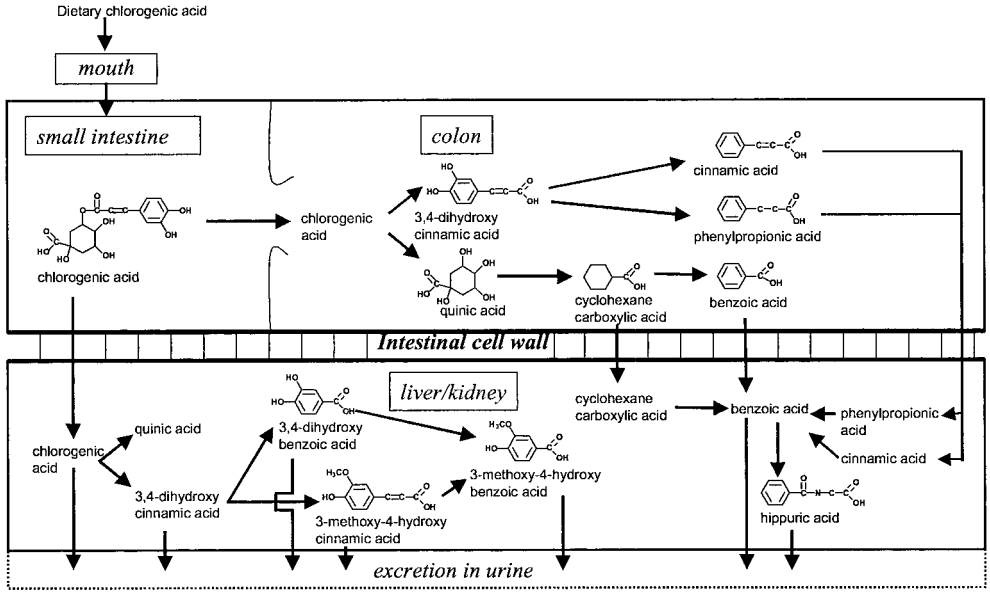

FIGURE 3 Proposed metabolic pathway of chlorogenic acid in humans [based on the results in this paper and reference (45)]. Conjugation reactions are not indicated.

are absorbed and excreted in urine as catechin conjugates and as 3′-methoxycatechin (23,36). Catechins and their condensed polymers that reach the colon will undergo cleavage of the catechin ring into valerolactones by microorganisms in the colon (**Fig. 4**). Valerolactones are then metabolized to phenylpropionic acids in the colon, which are further metabolized to benzoic acids and excreted in urine as hippuric acid (37). We did not measure the valerolactones in urine, but others have found them in urine (36).

Gallic acid (3,4,5-trihydroxybenzoic acid) was present in urine only in the tea period. It probably originated from gallic acid present in tea, or from its esters with catechins (epigallocatechin gallate, epicatechin gallate) (38). We also found a small amount of 3-hydroxyphenylacetic acid as a metabolite in urine after intake of tea. This could be a metabolite of quercetin-3-rutinoside, a flavonoid that is present in tea (16).

Metabolism of quercetin-3-rutinoside. Quercetin-3-rutinoside is not well absorbed in the small intestine of humans, and ~83% will be transported into the colon and metabolized there (14). Therefore, it was not surprising that we did not find metabolites of quercetin-3-rutinoside in humans without a colon. In volunteers with a colon, we recovered about half of the ingested quercetin-3-rutinoside as phenylacetic acids (phenyl-C_2 acids). Other studies also found phenylacetic acids as metabolites of quercetin-3-rutinoside, but they did not quantify the amount (39–41). Our results indicate that quercetin-3-rutinoside is probably first deglycosylated to quercetin aglycone (42) and then ring cleavage of the quercetin moiety into phenolic acids occurs by the colonic microflora (**Fig. 5**). In contrast to humans, the metabolites of quercetin-3-rutinoside in rats also include phenylpropionic acid (phenyl-C_3 acids) in addition to phenylacetic acids (39,43). This might be explained by differences in the colonic microflora between rats and humans and thereby in the sites of ring cleavage (44). Our data suggest that phenylacetic acids are subsequently absorbed from the colon and further metabolized in the liver and kidneys. Methylation is an important metabolic reaction that occurs after absorption in the liver, as indicated by the pres-

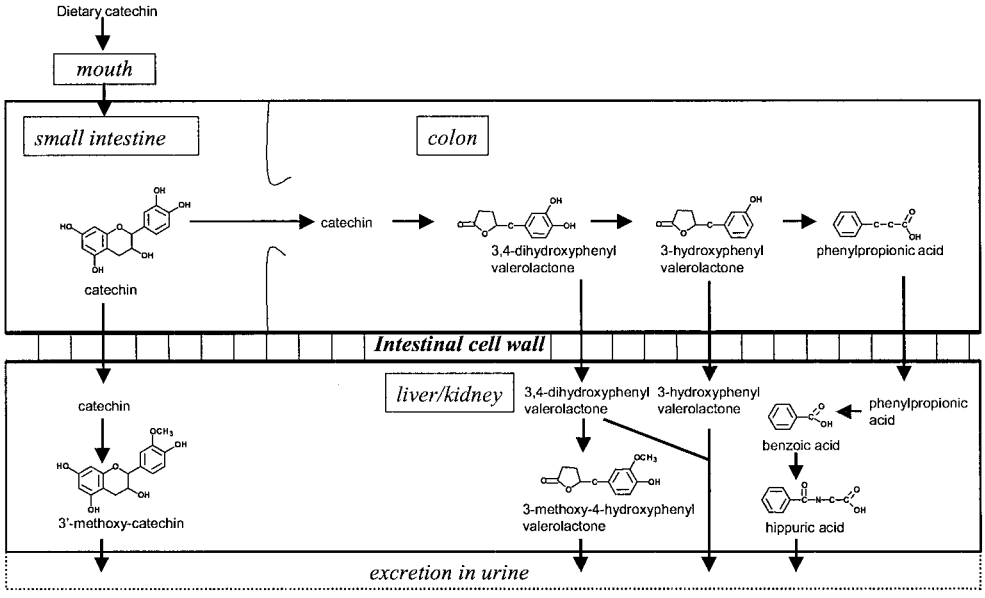

FIGURE 4 Proposed metabolic pathway of catechin in humans [based on the results in this paper and reference (45)]. Conjugation reactions are not indicated.

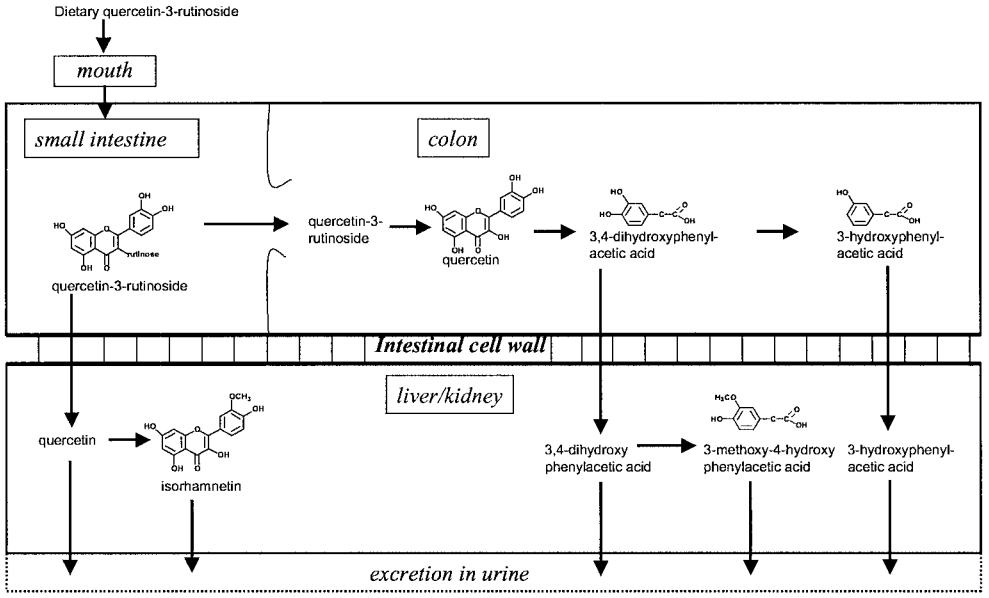

FIGURE 5 Proposed metabolic pathway of quercetin in humans [based on the results in this paper and reference (45)]. Conjugation reactions are not indicated.

ence of 3-methoxy-4-hydroxyphenylacetic acid in urine (Tables 2 and 3) (39,41).

Antioxidant activity. Dietary phenols are strong antioxidants in vitro (9), but our data show that their antioxidant action in vivo is uncertain because they are metabolized extensively. In general, the metabolites of dietary phenols that we found have much lower antioxidant activity than their parent compounds, or have no antioxidant activity at all. Hippuric acid, the most important metabolite of chlorogenic acid and of tea phenols has no antioxidant activity, because it has no hydroxyl group. Phenylacetic acids, the major metabolites of quercetin-3-rutinoside, have antioxidant activity in vitro that is similar to that of vitamin E, but lower than that of the parent compound quercetin (9,10).

Because we measured the metabolites of dietary phenols in urine, however, it is possible that intact phenols or intermediary metabolites actually circulate in blood and that these might act as antioxidants in vivo. Unfortunately, we could not measure metabolites in blood, because no analytical method is available. However, we hypothesize that the metabolic reactions that lower the antioxidant activity of the parent compound occur mainly before they can reach the circulation (Figs. 3–5). The breakdown of flavonoids and phenolic acids into smaller molecules through ring cleavage and β-oxidation in the colon and liver drastically lowers their antioxidant activity (9). Subsequently, phenols and their metabolites are conjugated with glucuronic acid, sulfates or glycine, which also lowers their antioxidant activity (11,12).

In conclusion, we identified and quantified a broad spectrum of potential phenolic acid metabolites in the urine of humans after they had ingested chlorogenic acid, tea phenols and quercetin-3-rutinoside. We found that about half of the chlorogenic acid and of the tea phenols is metabolized to hippuric acid, and half of the querctin-3-rutinoside is metabolized to phenyl-C_2 acids. Thus, a large proportion of dietary phenols is metabolized extensively in humans into compounds with potentially lower antioxidant activity, mainly before they enter the circulation. The antioxidant activity of dietary phenols in vivo might thus be lower than is expected on the basis of their in vitro antioxidant activity.

ACKNOWLEDGMENTS

We thank the volunteers for their participation; Els Siebelink, Marieke Spaan, Marjan van Hest and Sandra ten Bruggencate for their assistance during the study; Gerrit Bor, Wim Traag and Dini Venema for their excellent technical assistance during the analyses of the phenolic acids in urine; the laboratory staff of the Division of Human Nutrition and Epidemiology of the Wageningen University for their support and technical assistance; and Frans Russel and Peter Zock for valuable discussion during the preparation of this manuscript.

LITERATURE CITED

1. Hollman, P. C., Feskens, E. J. & Katan, M. B. (1999) Tea flavonols in cardiovascular disease and cancer epidemiology. Proc. Soc. Exp. Biol. Med. 220: 198–202.
2. Mukamal, K. J., Maclure, M., Muller, J. E., Sherwood, J. B. & Mittleman, M. A. (2002) Tea consumption and mortality after acute myocardial infarction. Circulation 105: 2476–2481.
3. Balentine, D. A., Wiseman, S. A. & Bouwens, L. C. (1997) The chemistry of tea flavonoids. Crit. Rev. Food Sci. Nutr. 37: 693–704.
4. Clifford, M. N. (1999) Chlorogenic acids and other cinnamates—nature, occurrence and dietary burden. J. Sci. Food Agric. 79: 362–372.
5. de Whalley, C. V., Rankin, S. M., Hoult, J. R., Jessup, W. & Leake, D. S. (1990) Flavonoids inhibit the oxidative modification of low density lipoproteins by macrophages. Biochem. Pharmacol. 39: 1743–1750.
6. Nardini, M., D'Aquino, M., Tomassi, G., Gentili, V., Di-Felice, M. & Scaccini, C. (1995) Inhibition of human low-density lipoprotein oxidation by caffeic acid and other hydroxycinnamic acid derivatives. Free Radic. Biol. Med. 19: 541–552.
7. Heinecke, J. W. (1998) Oxidants and antioxidants in the pathogenesis of atherosclerosis: implications for the oxidized low density lipoprotein hypothesis. Atherosclerosis 141: 1–15.
8. Hollman, P.C.H. (2001) Evidence for health benefits of plant phenols: local or systemic effects? J. Sci. Food Agric. 81: 842–852.
9. Rice-Evans, C. A., Miller, N. J. & Paganga, G. (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic. Biol. Med. 20: 933–956.
10. Pietta, P., Simonetti, P., Gardana, C. & Mauri, P. (2000) Trolox equivalent antioxidant capacity (TEAC) of *Ginkgo biloba* flavonol and *Camellia sinensis* catechin metabolites. J. Pharm. Biomed. Anal. 23: 223–226.
11. Manach, C., Morand, C., Crespy, V., Demigné, C., Texier, O., Regerat, F. & Rémésy, C. (1998) Quercetin is recovered in human plasma as conjugated derivatives which retain antioxidant properties. FEBS Lett. 426: 331–336.
12. Morand, C., Crespy, V., Manach, C., Besson, C., Demigné, C. & Rémésy, C. (1998) Plasma metabolites of quercetin and their antioxidant properties. Am. J. Physiol. 275: R212–R219.
13. Williamson, G., Plumb, G. W. & Garcia-Conesa, M. T. (1999) Glycosylation, esterification and polymerization of flavonoids and hydroxycinnamates: effects on antioxidant properties. Basic. Life Sci. 66: 483–494.

14. Olthof, M. R., Hollman, P.C.H. & Katan, M. B. (2001) Chlorogenic acid and caffeic acid are absorbed in humans. J. Nutr. 131: 66–71.

15. Hertog, M.G.L., Hollman, P.C.H. & Katan, M. B. (1992) Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. J. Agric. Food Chem. 40: 2379–2383.

16. Hertog, M.G.L., Hollman, P.C.H. & van de Putte, B. (1993) Content of potentially anticarcinogenic flavonoids of tea infusions, wines and fruit juices. J. Agric. Food Chem. 41: 1242–1246.

17. Sanchez-Castillo, C. P., Branch, W. J. & James, W.P.T. (1987) A test of the validity of the lithium-marker technique for monitoring dietary sources of salt in man. Clin. Sci. (Lond.)72: 87–94.

18. Sanchez-Castillo, C. P., Seidell, J. & James, W.P.T. (1987) The potential use of lithium as a marker for the assessment of the sources of dietary salt: cooking studies and physiological experiments in men. Clin. Sci. (Lond.)72: 81–86.

19. Anonymous (1976) Analytical Methods for Atomic Absorption Spectrophotometry. Perkin-Elmer, Norwalk, CT.

20. Quick, A. J. (1931) The conjugation of benzoic acid in man. J. Biol. Chem. 92: 65–85.

21. Adamson, R. H., Bridges, J. W., Evans, M. E. & Williams, R. T. (1970) Species differences in the aromatization of quinic acid in vivo and the role of gut bacteria. Biochem. J. 116: 437–443.

22. Hollman, P. C., van Trijp, J. M., Buysman, M.N.C.P., van der Gaag, M. S., Mengelers, M. J., de Vries, J. H. & Katan, M. B. (1997) Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. FEBS Lett. 418: 152–156.

23. Yang, C. S., Chen, L., Lee, M. J., Balentine, D., Kuo, M. C. & Schantz, S. P. (1998) Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. Cancer Epidemiol. Biomark. Prev. 7: 351–354.

24. Van Amelsvoort, J. M., Van Hof, K. H., Mathot, J. N., Mulder, T. P., Wiersma, A. & Tijburg, L. B. (2001) Plasma concentrations of individual tea catechins after a single oral dose in humans. Xenobiotica 31: 891–901.

25. Teuchy, H. & Van Sumere, C. F. (1971) The metabolism of [1-^{14}C] phenylalanine, [3-^{14}C] cinnamic acid and [2- ^{14}C] ferulic acid in the rat. Arch. Int. Physiol. Biochim. 79: 589–618.

26. Booth, A. N., Masri, M. S., Robbins, D. J., Emerson, O. H., Jones, F. T. & DeEds, F. (1960) Urinary phenolic acid metabolites of tyrosine. J. Biol. Chem. 235: 2649–2652.

27. Walle, T., Walle, U. K. & Halushka, P. V. (2001) Carbon dioxide is the major metabolite of quercetin in humans. J. Nutr. 131: 2648–2652.

28. Clifford, M. N., Copeland, E. L., Bloxsidge, J. P. & Mitchell, L. A. (2000) Hippuric acid as a major excretion product associated with black tea consumption. Xenobiotica 30: 317–326.

29. Rechner, A. R., Kuhnle, G., Bremner, P., Hubbard, G. P., Moore, K. P. & Rice-Evans, C. A. (2002) The metabolic fate of dietary polyphenols in humans. Free Radic. Biol. Med. 33: 220–235.

30. Plumb, G. W., Garcia-Conesa, M. T., Kroon, P. A., Rhodes, M. J., Ridley, S. & Williamson, G. (1999) Metabolism of chlorogenic acid by human plasma, liver, intestine and gut microflora. J. Sci. Food Agric. 79: 390–392.

31. Adamson, R. H., Bridges, J. W., Evans, M. E. & Williams, R. T. (1969) The role of gut bacteria in the aromatization of quinic acid in different species. Biochem. J. 112: 17P.

32. Cotran, R., Kendrick, M. I. & Kass, E. H. (1960) Role of intestinal bacteria in aromatization of quinic acid in man and guinea pig. Proc. Soc. Exp. Biol. Med. 104: 424–426.

33. Beer, C. T., Dickens, F. & Pearson, J. (1951) The aromatization of hydrogenated derivatives of benzoic acid in animal tissues. Biochem. J. 48: 222–237.

34. Booth, A. N., Emerson, O. H., Jones, F. T. & DeEds, F. (1957) Urinary metabolites of caffeic acid and chlorogenic acids. J. Biol. Chem. 229: 51–59.

35. Rechner, A. R., Spencer, J. P., Kuhnle, G., Hahn, U. & Rice-Evans, C. A. (2001) Novel biomarkers of the metabolism of caffeic acid derivatives in vivo. Free Radic. Biol. Med. 30: 1213–1222.

36. Hollman, P.C.H., Tijburg, L.B.M. & Yang, C. S. (1997) Bioavailability of flavonoids from tea. Crit. Rev. Food Sci. Nutr. 37: 719–738.

37. Das, N. P. (1971) Studies on flavonoid metabolism. Absorption and metabolism of (+)-catechin in man. Biochem. Pharmacol. 20: 3435–3445.

38. Hodgson, J. M., Morton, L. W., Puddey, I. B., Beilin, L. J. & Croft, K. D. (2000) Gallic acid metabolites are markers of black tea intake in humans. J. Agric. Food Chem. 48: 2276–2280.

39. Baba, S., Furuta, T., Fujioka, M. & Goromaru, T. (1983) Studies on drug metabolism by use of isotopes XXVII: urinary metabolites of rutin in rats and the role of intestinal microflora in the metabolism of rutin. J. Pharm. Sci. 72: 1155–1158.

40. Baba, S., Furuta, T., Horie, M. & Nakagawa, H. (1981) Studies on drug metabolism by use of isotopes XXVI: Determination of urinary metabolites of rutin in humans. J. Pharm. Sci. 70: 780–782.

41. Booth, A. N., Murray, C. W., Jones, F. T. & DeEds, F. (1956) The metabolic fate of rutin and quercetin in the animal body. J. Biol. Chem. 223: 251–257.

42. Bokkenheuser, V. D., Shackleton, C. H. & Winter, J. (1987) Hydrolysis of dietary flavonoid glycosides by strains of intestinal *Bacteroides* from humans. Biochem. J. 248: 953–956.

43. Nakagawa, Y., Shetlar, M. R. & Wender, S. H. (1965) Urinary products from quercetin in neomycin-treated rats. Biochim. Biophys. Acta 97: 233–241.

44. Schneider, H., Schwiertz, A., Collins, M. D. & Blaut, M. (1999) Anaerobic transformation of quercetin-3-glucoside by bacteria from the human intestinal tract. Arch. Microbiol. 171: 81–91.

45. Hollman, P.C.H. & Katan, M. B. (1998) Absorption, metabolism and bioavailability of flavonoids. In: Flavonoids in Health and Disease (Rice-Evans, C. A. & Packer, L., eds.), pp. 483–522. Marcel Dekker, New York, NY.