Chlorogenic Acid and Caffeic Acid Are Absorbed in Humans

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ABSTRACT Chlorogenic acid, an ester of caffeic acid and quinic acid, is a major phenolic compound in coffee; daily intake in coffee drinkers is 0.5–1 g. Chlorogenic acid and caffeic acid are antioxidants in vitro and might therefore contribute to the prevention of cardiovascular disease. However, data on the absorption of chlorogenic acid and caffeic acid in humans are lacking. We determined the absorption of chlorogenic acid and caffeic acid in a cross-over study with 4 female and 3 male healthy ileostomy subjects. In such subjects, degradation by the colonic microflora is minimal and absorption can be calculated as the amount ingested minus the amount excreted in ileostomy effluent. The ileostomy subjects ingested 2.8 mmol chlorogenic acid and 2.8 mmol caffeic acid on separate days in random order and subsequently collected ileostomy fluid and urine for 24 h. Absorption of chlorogenic acid was 33 ± 17% (mean ± sd) and of caffeic acid 95 ± 4%. Traces of the ingested chlorogenic acid and 11% of the ingested caffeic acid were excreted in urine. Thus, one third of chlorogenic acid and almost all of the caffeic acid were absorbed in the small intestine of humans. This implies that part of chlorogenic acid from foods will enter into the blood circulation, but most will reach the colon.

KEY WORDS: phenolic compounds, chlorogenic acid, caffeic acid, absorption, ileostomy, humans

Phenolic compounds form a substantial part of plant foods. Most of these phenolic compounds are antioxidants in vitro and antioxidants might protect against cardiovascular disease.

A major class of phenolic compounds are hydroxycinnamic acids, which are found in almost every plant (2,3). The major representative of hydroxycinnamic acids is caffeic acid, which occurs in foods mainly as an ester with quinic acid called chlorogenic acid (5-caffeoylquinic acid) (Fig. 1). Coffee is a major source of chlorogenic acid in the human diet; daily intake in coffee drinkers is 0.5–1 g; coffee abstainers will usually ingest < 100 mg/d. Other dietary sources of chlorogenic acid include apples, pears, berries, artichoke and aubergines (4). Knowledge concerning the absorption of chlorogenic acid in humans is essential to evaluate possible health effects in vivo because the absorbed fraction of chlorogenic acid will enter into the blood circulation and thus can induce biological effects in the blood circulation. Furthermore, the fraction that is not absorbed will enter into the colon where it might have biological effects. Chlorogenic acid and caffeic acid are antioxidants and they might inhibit the formation of mutagenic and carcinogenic N-nitroso compounds because they are inhibitors of the N-nitrosation reaction in vitro (6). Further, chlorogenic acid can inhibit DNA damage in vitro (7,8). Therefore, the inverse association between coffee intake and colon cancer in some epidemiologic studies (9–13) might be explained in part by the chlorogenic acid present in coffee. However, there are no data on absorption of chlorogenic acid or caffeic acid in humans. The major problem in measuring the absorption of chlorogenic acid and caffeic acid in humans is their bacterial degradation in the colon (14). Thus, measurement of fecal excretion of chlorogenic acid and caffeic acid would lead to an overestimation of the amount absorbed. Therefore, we determined the absorption of chlorogenic acid and caffeic acid in healthy ileostomy subjects, who lack a colon. Ileostomy subjects were successfully employed previously to determine the absorption of flavonoids (15), coffee diterpenes (16) and dietary polysaccharides (17).

SUBJECTS AND METHODS

Subjects. The study was approved by the Ethical committee of the Division of Human Nutrition and Epidemiology. We recruited subjects by approaching volunteers who had participated in previous studies at our division (15,16). Exclusion criteria were as follows: signs of diseases related to the gastrointestinal tract; resection of > 50 cm of the terminal ileum; an ileostomy that did not function properly; use of drugs that influenced gastrointestinal transit; present illness; pregnancy or lactation. Four women and three men, with a mean age of 63 y (range: 46–74 y), and a mean body mass index of 27.1 kg/m² (range 23.3–34.9 kg/m²) were admitted to participate and signed an informed consent form. All subject had had a total colectomy between 7 and 27 y ago for ulcerative colitis or polyposis coli. The subjects were healthy, based on a medical questionnaire, normal blood values for hemoglobin, hematocrit and white blood cell counts, and absence of glucose and protein in urine.

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Study design and supplements. Subjects followed a diet that was low in chlorogenic acid and quercetin from d 1 to 14. The diet was low in quercetin because one of the supplements we tested was quercetin-3-rutinoside. To ensure adherence to the dietary guidelines, we gave the subjects a list of forbidden foods and beverages. Foods were prohibited if they contained > 15 mg/kg of quercetin or chlorogenic acid. Beverages were prohibited if they contained > 4 mg/L of quercetin or chlorogenic acid (4,18,19). Because subjects were not allowed to drink coffee and tea during the study, we supplied coffee and tea substitutes. The coffee substitute was an extract made of chicory, rye and barley (“Swiss coffee-like,” Tayala AG, Birsfelden, Switzerland) and the tea substitute was an extract of a mixture of herbs (“Droommix,” Piramide, Veenendaal, The Netherlands). We analyzed the coffee substitute and tea substitute for quercetin and chlorogenic acid, and the amounts were within the range of the dietary guidelines (results not shown). Compliance with the dietary guidelines was good. None of the subjects reported consumption of any foods or beverages that were on the list of forbidden foods and beverages during the study. Furthermore, the low chlorogenic acid and caffeic acid excretion in presupplement ileostomy effluent confirmed that subjects adhered to the dietary guidelines (Table 1).

On d 5, all subjects consumed a placebo supplement, which was 200 mL of water. On d 6, 10 and 14, all subjects consumed one of the following supplements in random order: 1000 mg (2.8 mmol) chlorogenic acid (Fluka Chemie, Buchs, Switzerland) or 500 mg (2.8 mmol) caffeic acid (Fluka Chemie) or 220 mg (0.3 mmol) quercetine-3-rutinoside (Rutosidum DAB; BUFA B.V., Uitgeest, The Netherlands). The placebo and the quercetin-3-rutinoside supplements were part of another study; these results will be reported elsewhere. Subjects received the supplements as a powder and were instructed to add 200 mL hot water and to consume the beverage within 5 min after preparation. Subjects ingested the supplements between 0700 and 0900 h at home, together with a light breakfast that we provided. The breakfast consisted of wheat bread, cheese, strawberry jam, milk and the coffee and tea substitutes; subjects had a free choice. After this breakfast with supplements, subjects were allowed only to drink water for 3 h.

Collection of ileostomy effluent and urine. On d 5, 6, 10 and 14, subjects collected one sample of ileostomy effluent and urine just before ingestion of the supplements. After ingestion of the supplements, they collected ileostomy effluent and urine during 24 h. During the daytime, they changed the ileostomy bags every 2 h and immediately stored the bags on dry ice to minimize degradation of the contents by residual bacterial flora. At night, subjects had to change the bags as often as possible. Subjects collected urine in 0.5 mL plastic bottles, with 0.13 thymol (# 8167; Merck, Amsterdam, The Netherlands) as a preservative and stored the bottles with urine on dry ice immediately after voiding. We checked the completeness of the urine collection by assessment of recovery of 270 μmol lithium in urine. It was ingested daily by the subjects as lithium chloride dissolved in 10 mL of tap water from 7 d before the first urine collection. Lithium chloride is completely absorbed and 95% is excreted in urine (20,21). Urinary recovery of lithium was 94 ± 12% (mean ± SD), indicating good compliance in collecting urine.

Sample preparation. The ileostomy bags were kept frozen with liquid nitrogen during separation of the plastic bags from the contents. The frozen contents were freeze-dried, ground to pass through an 0.5-mm sieve and stored at −20°C until analysis. We thawed the urine bottles in a water bath of ~40°C, pooled and mixed urine per subject and per supplement day, froze aliquots of urine in liquid nitrogen and stored the urine samples at −80°C until analysis. We prepared the samples collected before breakfast (presupplement sample) and the final collection at the end of the 24-h collection period (final sample) separately.

Incubation of chlorogenic acid and caffeic acid with gastrointestinal fluids. To check for degradation of chlorogenic acid and caffeic acid in gastrointestinal fluids, we incubated them in vitro in gastric juice and duodenal fluid, and ex vivo in ileostomy fluid. We incubated 30 mg of chlorogenic acid and 15 mg of caffeic acid with 3 mL human gastric juice and 9 mL of water at 37°C for 0.5 and 2 h (22,23). Similar amounts were incubated with 3 mL human duodenal fluid and 9 mL water at 37°C for 1 and 4 h, corresponding to the average and maximal transit time in the small intestine (24,25).

TABLE 1

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Intake</th>
<th>Presupplement sample2</th>
<th>24-h excretion3</th>
<th>Final sample4</th>
<th>Absorption % of intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorogenic acid5</td>
<td>1000</td>
<td>2.3 ± 2.5</td>
<td>667 ± 165</td>
<td>2.6 ± 3.8</td>
<td>33 ± 17</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>500</td>
<td>0.3 ± 0.6</td>
<td>27 ± 18</td>
<td>0.1 ± 0.1</td>
<td>95 ± 4</td>
</tr>
</tbody>
</table>

1 Values are means ± sd.
2 Ileostomy effluent sample collected before ingestion of the supplements; amounts represent the mean of 6 subjects because 1 subject did not collect the presupplement ileostomy effluent.
3 Includes the final but not the presupplement sample.
4 Ileostomy effluent sample collected at the end of the 24-h collection period.
5 Excretion of chlorogenic acid was calculated as the sum of the excretion of 3-cafeoylquinic acid, 4-cafeoylquinic acid and 5-cafeoylquinic acid in ileostomy effluent.
Gastric juice and duodenal fluid were obtained from two fasted healthy volunteers with a colon and stored at −20°C.

We also studied the stability of chlorogenic acid and caffeic acid ex vivo during collection of ileostomy fluid and during sample preparation in the laboratory. For this purpose, two ileostomy subjects, who also participated in this study, followed a diet low in chlorogenic acid and quercetin for 4 d. On d 4, they applied three ileostomy bags in total, one bag with 300 mg chlorogenic acid mixed with ~5 g of strawberry jam, one with 150 mg caffeic acid mixed with 5 g of strawberry jam and one with 5 g of strawberry jam only. Strawberry jam was used as a vehicle for chlorogenic acid and caffeic acid powder. Strawberry jam itself does not contain chlorogenic acid or caffeic acid. The subjects allowed ileostomy fluid to drain into the bag for ~2 h and kneaded the contents regularly. The ileostomy fluids were stored and analyzed as described.

Analysis of chlorogenic acid isomers and of caffeic acid in ileostomy effluent and urine. Chlorogenic acid and caffeic acid in ileostomy effluent were extracted simultaneously by mixing 0.500 g freeze-dried effluent with 25 mL 40% (v/v) aqueous methanol containing 2 g tert-butylhydroxyquinone/L. The effluent extract was refluxed at 90°C for 1 h with regular swirling, allowed to cool down and subsequently brought to 50 mL with methanol. The effluent extract was then sonicated for 5 min and filtered through a 0.45-μm filter for organic solvents (Acrodisc CR PTFE; German Sciences, Ann Arbor, MI) before HPLC injection. For HPLC analysis, we injected 20 μL of the effluent extract onto an Inertsil ODS-2 (GL Sciences, Tokyo, Japan) column (4.6 × 150 mm, 5 μm particle size) protected by an MPLC Newguard RP-18 (Brownlee; Applied Biosystems, San Jose, CA) column (3.2 × 15 mm, 7 μm particle size) using acetonitrile/0.025 mol/L phosphate buffer, pH 2.4 (8:92) as the mobile phase, at a flow rate of 1 mL/min. The columns were placed in a column oven set at 40°C. Ultraviolet absorption was measured at 325 nm. Excretion of chlorogenic acid was calculated as the sum of the excretion of 3-caffeoylquinic acid or 4-caffeoylquinic acid; Peak 2: 5-caffeoylquinic acid; Peak 3: 3-caffeoylquinic acid or 4-caffeoylquinic acid; Peak 4: caffeic acid

The HPLC method used for the determination of chlorogenic acid and caffeic acid in ileostomy fluid showed well-resolved isomer peaks. Quantification was not hampered by potential interferences from the sample matrix (Fig. 2). Of the ingested chlorogenic acid, 67% was excreted in ileostomy fluid, whereas only 5% of the caffeic acid was excreted (Table 1). Traces of the ingested chlorogenic acid and 11% of the ingested caffeic acid were excreted in urine (Table 2).

Chlorogenic acid and caffeic acid were recovered almost completely after in vitro incubation in gastric juice and duodenal fluid and after ex vivo incubation in ileostomy fluid (Table 3). Thus, the amounts not excreted in ileostomy effluent were likely absorbed rather than degraded in the gut or in the ileostomy bag. The absorption of chlorogenic acid therefore equaled 100% − 67% = 33% and that of caffeic acid 95%.

TABLE 2

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Intake</th>
<th>Caffeic acid mg</th>
<th>Chlorogenic acid mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorogenic acid</td>
<td>1000</td>
<td>1.7 ± 0.8</td>
<td>2.9 ± 1.5</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>500</td>
<td>53.4 ± 14.1</td>
<td>ND</td>
</tr>
</tbody>
</table>

1 Values are mean ± SD; ND, not detected.
2 Excretion of chlorogenic acid was calculated as the excretion of 5-caffeoylquinic acid. 3-Caffeoylquinic acid and 4-caffeoylquinic acid were not present in large amounts in urine.
A maximum of 33% of the ingested chlorogenic acid and 95% of the ingested caffeic acid was absorbed from the small intestine in humans. Traces of chlorogenic acid in urine were recovered after ingestion of chlorogenic acid, whereas 11% of caffeic acid in urine was recovered after ingestion of caffeic acid. This indicates that at least some of the chlorogenic acid was absorbed intact. Indications that caffeic acid and chlorogenic acid are absorbed in the small intestine are also found in a rat intestine perfusion model (28). However, because the recovery in urine was not nearly complete, chlorogenic acid and caffeic acid are probably metabolized extensively into other compounds after absorption. Unfortunately, data on metabolism of chlorogenic acid and caffeic acid in the human body are scarce.

Validity of ileostomy model. We measured absorption as the difference between the amount of supplement ingested and the amount excreted in ileostomy fluid in subjects without a colon. Absorption of nutrients in the small intestine of ileostomy subjects is probably not affected by the lack of the colon (29), as indicated by their normal serum cholesterol concentrations (30), normal absorption of para-aminobenzoic acid (15) and of lithium in this study (20,21).

It is unlikely that appreciable amounts of chlorogenic acid or caffeic acid disappeared through degradation in the gastric juice, duodenal fluid, in the ileostomy bag or during analysis on the laboratory because chlorogenic acid and caffeic acid were recovered completely after in vitro and ex vivo incubations in gastrointestinal fluids (Table 3). However, we cannot exclude that part of the supplements were lost somewhere in the gastrointestinal tract; therefore, the absorption values we found in this study should be regarded as maximum absorption values rather than as fixed absorption values. In subjects with a colon, absorption of dietary phenolic compounds and their metabolites in the colon is possible. Therefore, in subjects with a colon, caffeic acid in urine might originate from dietary chlorogenic acid.

Collection of ileostomy effluent. The amounts of caffeic acid and chlorogenic acid that were not recovered in ileostomy effluent cannot be explained by loss of ileostomy effluent. None of the subjects reported loss of ileostomy effluent during the 24-h collection periods, and the 9-11 ileostomy bags that subjects collected during 24 h also indicated that they had collected all ileostomy effluent. Therefore, we conclude that collection of ileostomy effluent was complete.

The subjects in this study collected ileostomy effluent for 24 h, which should be long enough to detect all nonabsorbed supplement in ileostomy effluent because the mean transit time through the stomach and small intestine is ~8–11 h (31,32). This was also supported by the fact that the amount of chlorogenic acid and caffeic acid excreted in the final collection of ileostomy effluent at the end of the 24-h period was similar to that in the presupplement collection (Table 1). Furthermore, the recovery of 84 ± 19% (mean ± sd) of quercetin in ileostomy effluent during 24 h after ingestion of quercetin-3-rutinoside in this study was similar to the recovery during the first 13 h in a previous study (15).

Comparison with previous studies. We found that the absorption of caffeic acid esterified with quinic acid (chlorogenic acid) is three times lower than that of caffeic acid itself. To our knowledge, there are no previous quantitative data on absorption of chlorogenic acid and caffeic acid in humans. The studies that were done on absorption of chlorogenic acid and caffeic acid measured the recoveries of these compounds and their metabolites in urine of rats (33,34).

We recovered 0.3% of chlorogenic acid in urine after ingestion. After ingestion of chlorogenic acid by rats, no chlorogenic acid was found in urine (35). We recovered 11% of caffeic acid in urine after ingestion, which was comparable to the recovery of 13% found by (34) after ingestion of caffeic acid in rats. After intravenous injection of chlorogenic acid and caffeic acid in rats, only 9% of chlorogenic acid and 26% of caffeic acid were recovered in urine (35). This indicates that the fraction of chlorogenic acid and of caffeic acid that is absorbed is metabolized extensively in the body and therefore only small amounts are recovered in urine.

Mechanisms of absorption. The absorption of caffeic acid esterified with quinic acid (chlorogenic acid) was less than that of caffeic acid itself. It is possible that chlorogenic acid and caffeic acid are absorbed through different absorption mechanisms. We can envisage two mechanisms for the absorption of chlorogenic acid in humans. The first mechanism might involve absorption of chlorogenic acid as an intact molecule as indicated by the presence of traces of chlorogenic acid in urine after ingestion of chlorogenic acid in our study. We probably found only traces of the absorbed chlorogenic acid in urine because chlorogenic acid is metabolized extensively after absorption (35). The second mechanism might involve hydrolysis of chlorogenic acid in the stomach and/or small intestine into caffeic acid and quinic acid before absorption. The caffeic acid moiety and the quinic acid moiety are subsequently absorbed (36,37). If this mechanism plays a role in the absorption of chlorogenic acid, we would expect to find caffeic acid in urine as we found after intake of the caffeic acid supplement. If we assume that the absorption of chlorogenic acid is 33% and the amount of caffeic acid in urine after ingestion of caffeic acid is 11%, then we would expect to recover ~4% of chlorogenic acid in urine as caffeic acid. However, we found only 0.3% of chlorogenic acid as caffeic acid in urine after ingestion of chlorogenic acid, which is ~10 times lower than we expected. This indicates that hydrolysis of chlorogenic acid in the stomach or small intestine is not very important. This is also supported by the fact that we found a large amount of the ingested chlorogenic acid unchanged in ileostomy effluent. Thus, this second mechanism likely does not play an important role in the absorption of chlorogenic acid. Therefore, we propose that in ileostomy subjects, most of the absorbed chlorogenic acid is absorbed intact and is metabolized extensively in the liver.
Caffeic acid is probably absorbed through different absorption mechanisms than chlorogenic acid. We can envisage two mechanisms for the absorption of caffeic acid in humans. The first mechanism for absorption of caffeic acid might involve passive absorption of caffeic acid in the stomach. This is supported by the fact that caffeic acid and the structurally related compound cinnamic acid were rapidly absorbed in rats (38). Further, in the acid environment of the stomach, caffeic acid will be primarily in the nonionic form, which can be absorbed by passive nonionic diffusion. Passive absorption in the small intestine is not very likely because at a pH of ~7 in the small intestine, caffeic acid will be mainly in the ionic form, which is difficult to absorb by passive diffusion (39). The second mechanism for absorption of caffeic acid might involve absorption by an active transport mechanism in the small intestine. Results from in vitro studies indicate that in the small intestine, an active Na\textsuperscript{+}-dependent transport mechanism might be involved in the absorption of cinnamic acids such as caffeic acid (39,40). Both mechanisms, passive absorption in the stomach and active absorption in the small intestine, might play a role in the absorption of caffeic acid in humans.

Chlorogenic acid, caffeic acid and health. The absorbed fraction of chlorogenic acid and caffeic acid and its metabolites might induce biological effects in the blood circulation. Chlorogenic acid and caffeic acid inhibit oxidation of LDL in vitro (41,42) and might therefore protect against cardiovascular disease. There are no in vivo data available that show that chlorogenic acid is present in the blood circulation after ingestion, but caffeic acid is present in blood after ingestion by rats (34). In our human study, we did find chlorogenic acid and caffeic acid in urine, which also suggests that a small part will be present as such in blood. We did not measure chlorogenic acid and caffeic acid in blood because we did not have an available method of analysis.

The fraction of chlorogenic acid that escapes absorption is present throughout the whole gastrointestinal tract, where it might induce biological effects. Chlorogenic acid and caffeic acid are antioxidants in vitro (1,5), and they might inhibit the formation of mutagenic and carcinogenic N-nitroso compounds (6). Further, chlorogenic acid can inhibit DNA damage in vitro (7,8). Therefore, chlorogenic acid, the major phenolic compound in coffee, might be involved in the inverse association between coffee consumption and colon cancer that was found by some epidemiologic studies (9–13), but not all (43). Thus the one third of ingested chlorogenic acid that is absorbed could have biological effects in the blood circulation, and the fraction of chlorogenic acid that is not absorbed could have biological effects in the colon in humans.

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LITERATURE CITED