Chlorogenic Acid Is Absorbed in Its Intact Form in the Stomach of Rats

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ABSTRACT The bioavailability of chlorogenic acid, a major polyphenol of the human diet that is particularly abundant in coffee and various fruits, was explored in rats. To identify the form under which it is absorbed through the gut mucosa and the site of absorption along the gastrointestinal tract, rats were fed a diet supplemented with chlorogenic acid (0.25%, wt:wt). Chlorogenic acid and its metabolites were estimated in the stomach, small intestine and cecal contents as well as in bladder urine and plasma by HPLC with coulometric detection at several time points (1.5, 3, 4.5, and 7 h) after the beginning of the meal. Minor hydrolysis of chlorogenic acid (<1%) occurred in the stomach and small intestine contents, whereas 15–32% of ingested chlorogenic acid was hydrolyzed into caffeic acid in the cecum. Chlorogenic acid and caffeic acid appeared early (at 1.5 h) in plasma and urine, suggesting an absorption of chlorogenic acid into the upper part of the gastrointestinal tract. Gastric absorption of chlorogenic acid was further examined by infusing chlorogenic acid in the ligated stomach of food-deprived rats. After 30 min of infusion, intact chlorogenic acid was found in the gastric vein and aorta. No other metabolites could be detected by HPLC-electrospray ionization-MS-MS. These results show for the first time that chlorogenic acid is quickly absorbed in the rat stomach in its intact form.


KEY WORDS: • chlorogenic acid • absorption • gastrointestinal tract • stomach • rats

Chlorogenic acid, an ester of caffeic acid, is found with quinic acid in a wide range of fruits and vegetables and is particularly abundant in coffee (1). Both chlorogenic and caffeic acid possess antioxidant properties in vivo (2–4). Chlorogenic acid and caffeic acid were reported to prevent different cancers and cardiovascular diseases in several experimental studies in animal models (5–9). The biological properties of hydroxycinamic acids depend on their absorption in the gut and on their metabolism. The intestinal absorption of caffeic acid is well characterized in both experimental animals and human subjects (10–13). The absorption and metabolism of chlorogenic acid are less studied. Caffeic acid appeared rapidly in plasma after chlorogenic acid ingestion in both rats and human subjects, suggesting that chlorogenic acid is hydrolyzed in the upper part of the gastrointestinal tract (13,14). Using an in situ intestinal perfusion model, we could confirm that chlorogenic acid was effectively absorbed in the small intestine of rats, hydrolyzed in the mucosa, and recovered as free phenolic acid in the plasma (15). However, other authors also identified intact chlorogenic acid in human urine after ingestion of chlorogenic acid, chlorogenic acid-containing coffee, or prune, with recovery yields varying from 0.3 to 2.3%, suggesting that chlorogenic acid is also absorbed without hydrolysis (12,16–18). More recently, chlorogenic acid was also identified in rabbit plasma shortly after oral administration of a honeysuckle extract (19).

The aim of this work was to explore the stability of chlorogenic acid in the gut, the form under which it is absorbed through the gut mucosa and the site of absorption along the gastrointestinal tract. Rats were fed a diet supplemented with chlorogenic acid, and phenolic acids were estimated in the stomach, small intestine, and cecal contents at different time points during the meal as well as in bladder urine and plasma. Absorption of chlorogenic acid through the stomach mucosa was also examined by infusing chlorogenic acid into the stomach of food-deprived rats.

MATERIALS AND METHODS

Chemicals. Chlorogenic acid (5-caffoylquinic acid according to the IUPAC numbering system), caffeic acid, ferulic acid, sinapic acid and β-glucuronidase from Escherichia coli type XA were purchased from Sigma. Isoferulic acid was purchased from Extrasyntèse. The sulfate ester of ferulic acid and p-coumaric acid, glucuronide of ferulic and p-coumaric acid were donated by O. Dangles and S. Galland, INRA Avignon, France.

Animals and diets. Male Wistar rats, weighing ~180 g were caged singly in temperature-controlled rooms (22°C), with a dark period from 0800 to 2000 and with access to food from 0800 to 1600. They were fed a semipurified control diet for 7 d (Table 1). Rats were...
TABLE 1
Composition of semipurified diet supplemented with chlorogenic acid

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control diet</th>
<th>Chlorogenic diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat starch</td>
<td>749.5 g/kg dry feed</td>
<td>747 g/kg dry feed</td>
</tr>
<tr>
<td>Casein</td>
<td>150 g/kg dry feed</td>
<td>150 g/kg dry feed</td>
</tr>
<tr>
<td>Peanut oil</td>
<td>50 g/kg dry feed</td>
<td>50 g/kg dry feed</td>
</tr>
<tr>
<td>Mineral mixture†</td>
<td>35 g/kg dry feed</td>
<td>35 g/kg dry feed</td>
</tr>
<tr>
<td>Vitamin mixture†</td>
<td>10 g/kg dry feed</td>
<td>10 g/kg dry feed</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3 g/kg dry feed</td>
<td>3 g/kg dry feed</td>
</tr>
<tr>
<td>Choline</td>
<td>2.5 g/kg dry feed</td>
<td>2.5 g/kg dry feed</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>— g/kg dry feed</td>
<td>2.5 g/kg dry feed</td>
</tr>
</tbody>
</table>

† Mineral mixture AIN-93-M and Vitamin mixture AIN-93-VX described in (34) and (35).
RESULTS

Dietary supplementation and chlorogenic acid intake. Chlorogenic acid was administered with the diet (0.25%) from the beginning of the dark period and its consumption was determined for the next 7 h. The total amounts of chlorogenic acid consumed were as follows: 145.4 ± 16.7 μmol from 0 to 1.5 h, 37.3 ± 7.4 μmol from 1.5 to 3 h, 25.6 ± 5.6 μmol from 3 to 4.5 h, and 31.4 ± 7.5 μmol from 4.5 to 7 h.

The major part of the meal and of chlorogenic acid (60.5%) was ingested during the first 90 min of this period. Rats ingested lower amounts of chlorogenic acid during the next 3 periods (15.6, 10.7 and 13.1%, respectively). The amount of chlorogenic acid ingested during the first 90 min was greater than during the other periods (P < 0.001).

Chlorogenic and caffeic acid concentrations in gastrointestinal contents. In the stomach, similar quantities of chlorogenic acid were found at the end of each time period. In the small intestine, the chlorogenic acid content increased significantly after 3 h. The contents at 4.5 and 7 h did not differ from that at 3 h (Fig. 1). Only traces of caffeic acid (1.0 ± 0.1% of total phenolic acids) could be detected in either the stomach or the small intestine.

The contents of chlorogenic acid were much lower in the cecum. The maximal value was observed 4.5 h after the beginning of the meal. It was significantly higher than the contents found at the other time points (0.13 ± 0.02 μmol). There was also a significant amount of caffeic acid in the cecum, accounting for 15, 32, 21, and 26%, respectively, of total phenolic acids measured at each time point.

Plasma kinetics of chlorogenic acid and its metabolites. No chlorogenic acid or other phenolic acids were detected in the aortic plasma of the control group. In the supplemented group, both chlorogenic and caffeic acids were detected in similar concentrations early (1.5 h) after the beginning of the meal (Table 2). The concentrations did not differ during the meal. No ferulic or isoflavonuric acid could be detected.

Concentrations of phenolic acids in urine taken directly into the bladder and repartition of excreted metabolites. No chlorogenic acid or other phenolic acids were detected in urine of the control group. In the supplemented group, chlorogenic acid was detected in urine from 1.5 h after the beginning of the meal together with caffeic, ferulic, and isoflavonuric acids (Fig. 2). Chlorogenic acid excretion tends to decrease over time in proportion to the amount ingested. Caffeic acid was also excreted early after the beginning of the meal; its relative excretion over other phenolic acids increased at 3 h compared with 1.5 h (P < 0.001). The early appearance of chlorogenic acid in plasma and urine suggested a possible absorption in the stomach.

In situ gastric infusion of chlorogenic acid in rats. A significant proportion of chlorogenic acid was absorbed as indicated by its disappearance in the stomach after a 30-min infusion (Table 3). There were only traces of caffeic acid in the stomach contents after incubation. The plasma collected from the gastric vein and aorta by LC-ESI-MS/MS after the 30-min infusion period was examined for the presence of phenolic acids and their metabolites. There were 2 peaks with the same m/z transition as chlorogenic acid (Fig. 3). They were absent in plasma when rats were infused with control buffer. One of the 2 peaks has a retention time identical to that of the...
Phenolic acid concentrations in bladder urine of rats fed a diet supplemented with chlorogenic acid

<table>
<thead>
<tr>
<th>Time after the beginning of the meal, h</th>
<th>1.5</th>
<th>3</th>
<th>4.5</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic acids</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Caffeic acid</td>
<td>0.31 ± 0.15</td>
<td>0.50 ± 0.21</td>
<td>0.19 ± 0.02</td>
<td>0.29 ± 0.04</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>0.33 ± 0.07</td>
<td>0.53 ± 0.21</td>
<td>0.39 ± 0.11</td>
<td>0.50 ± 0.11</td>
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</tbody>
</table>

1 Values are means ± SEM, n = 4.
2 Ferulic and isoferulic acids were not detected at 0.06 and 0.08 μmol/L, respectively.

DISCUSSION

The aim of this study was to explore the fate and metabolism of chlorogenic acid in the gastrointestinal tract of rats over the postprandial period and to determine the form under which chlorogenic acid is absorbed through the different parts of the gut barrier. Chlorogenic acid and its metabolites were estimated in the gut contents, plasma, and urine of rats fed a diet supplemented with chlorogenic acid. Chlorogenic acid was the major phenolic acid present in the gut for the 7 h of the experiment. Only traces of caffeic acid were detected in the stomach and small intestine, showing the absence of significant esterase activity in the gut lumen in agreement with previous experiments in ileostomized patients or rats (12,13). The presence of esterase activity was reported in the small intestinal mucosa, but only toward methyl esters of hydroxycinnamic acids (21). We showed previously, using an in situ intestinal perfusion model, that chlorogenic acid is absorbed in the stomach and was identified in both the gastric vein and aorta in its intact form. Using a similar model, several polyphenols such as daidzein, genistein, quercetin, rutin, anthocyanins or ferulic acid were shown to be absorbed in the stomach (24–29). The direct absorption of chlorogenic acid shows that esterification with quinic acid does not prevent its absorption in the stomach. In contrast to our previous experiments with perfused small intestine, the absence of caffeic acid or of its conjugated forms in the gastric vein and aorta shows that the absorption of chlorogenic acid in the stomach follows different mechanisms. Chlorogenic acid could be absorbed through the bilitranslocase, which is involved in the transport of anthocyanins in the stomach (28,30) or through other unidentified organic anion transporters. Passive transport of chlorogenic acid was also described in Caco-2 cell cultures (31).

Caffeic acid also appears early in plasma and urine (Table 2, Figure 3). Its O-methylated derivatives, ferulic and isoferulic

![FIGURE 2](https://academic.oup.com/jn/article-abstract/136/5/1192/4669958)

**FIGURE 2** Phenolic acid concentrations in bladder urine of rats fed a diet supplemented with chlorogenic acid (0.25%). Values are means ± SEM; n = 4 per time point.

| Chlorogenic acid absorption and plasma concentrations after its infusion into the stomach of rats over a 30-min period
<table>
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<tbody>
<tr>
<td><strong>Chlorogenic acid</strong></td>
</tr>
<tr>
<td>Injected into the gastric lumen, <strong>μmol</strong></td>
</tr>
<tr>
<td>Recovery from the gastric lumen, <strong>μmol</strong></td>
</tr>
<tr>
<td>at the end of infusion, <strong>μmol</strong></td>
</tr>
<tr>
<td>Absorption from the gastric lumen, <strong>% of injected dose</strong></td>
</tr>
<tr>
<td>Gastric vein, <strong>μmol/L</strong></td>
</tr>
<tr>
<td>Aorta, <strong>μmol/L</strong></td>
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</table>

1 Values are mean ± SEM, n = 6. *Different from rats injected in the gastric lumen, P < 0.001.
acids, were also present in urine. They most likely arose from the absorption and hydrolysis of chlorogenic acid in the mucosa of the small intestine as suggested previously (15,26). Other authors reported that caffeic acid was detected early in plasma, 30 to 60 min after consumption of pure chlorogenic acid or coffee by rats or humans (13,14). These authors suggested that chlorogenic acid was hydrolyzed in the upper digestive tract because they could not detect any chlorogenic acid in plasma. The absence of chlorogenic acid would most likely be explained by a too rapid transit through the empty stomach in these 2 studies carried out with chlorogenic acid solutions or brewed coffee in food-deprived rats or fasting humans. In contrast, when volunteers consumed coffee with a whole breakfast, chlorogenic acid was present in urine (18).

The fraction of chlorogenic acid that is not absorbed in the upper intestinal tract reaches the cecum. The low amounts of chlorogenic acid estimated in the cecal content suggest an intense microbial metabolism. Chlorogenic acid is hydrolyzed into caffeic acid and further degraded to low-molecular-weight phenolic acids such as m-coumaric acid, 3-hydroxyphenylpropanoic acid, or 3-hydroxybenzoic acid (32,33). These metabolites were shown to be the main metabolites identified in rat urine, together with much lower amounts of intact caffeic acid and its O-methylated metabolites (33).

In conclusion, this study shows for the first time that chlorogenic acid is not hydrolyzed in the stomach and the small intestine, but absorbed in the stomach in its intact form and as hydrolyzed forms such as caffeic and (iso)ferulic acids in the small intestine. Once reaching the cecum, chlorogenic acid is hydrolyzed into caffeic acid and further metabolized into other aromatic acids. Further studies will be required to understand the exact mechanisms of absorption and identify the transporters involved in the different parts of the gastrointestinal tract. Moreover, the role of the stomach in polyphenol absorption and the influence of the food matrix on absorption should be investigated further.

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LITERATURE CITED


