Bioavailability of Quercetin in Pigs Is Influenced by the Dietary Fat Content1,2

Stephanie Lesser, Rainer Cermak,3 and Siegfried Wolffram

Institute of Animal Nutrition, Physiology and Metabolism, Christian-Albrechts-University Kiel, D-24098 Kiel, Germany

ABSTRACT The flavonol quercetin is one of the most prevalent flavonoids found in edible plants. In this study, the influence of dietary fat on oral bioavailability of quercetin was investigated. Quercetin (30 μmol/kg body weight) was administered either as the lipophilic aglycone or as the more hydrophilic quercetin-3-O-glucoside in test meals differing in fat content (3, 17, or 32 g fat/100 g diet) to growing pigs. Blood samples were drawn repeatedly over a 24-h period and analyzed by HPLC. The main metabolite found in plasma was always conjugated quercetin. Quercetin bioavailability from each diet was always higher from the glucoside than from the aglycone. Irrespective of the chemical form applied, the bioavailability of quercetin was higher in the 17% fat diet compared with the 3% fat diet (P < 0.05). No further effect on bioavailability was observed when the flavonols were administered with diets containing 32% fat. The elimination of quercetin was significantly delayed after its application with fat-enriched diets (P < 0.05). Thus, in addition to the chemical form of the flavonol, the fat content of the diet influences oral bioavailability of quercetin. J. Nutr. 134: 1508–1511, 2004.

KEY WORDS: • flavonoids • bioavailability • quercetin • fat • pigs

The flavonol quercetin is one of the most prevalent flavonoids found in edible plants. In plants and subsequently in plant-derived foods, quercetin is present mainly as glycosides (1,2). It has been shown repeatedly that the sugar moiety is a major determinant governing the intestinal absorption of quercetin (3–6). In addition to the chemical form of the flavonol, the applied vehicle or the composition of the diet seems to have a substantial effect on quercetin bioavailability (7–10). In a previous study by our group, the bioavailability of quercetin was investigated. Quercetin (30 μmol/kg body weight) was administered either as the lipophilic aglycone or as the more hydrophilic quercetin-3-O-glucoside in test meals differing in fat content (3, 17, or 32 g fat/100 g diet) to growing pigs. Blood samples were drawn repeatedly over a 24-h period and analyzed by HPLC. The main metabolite found in plasma was always conjugated quercetin. Quercetin bioavailability from each diet was always higher from the glucoside than from the aglycone. Irrespective of the chemical form applied, the bioavailability of quercetin was higher in the 17% fat diet compared with the 3% fat diet (P < 0.05). No further effect on bioavailability was observed when the flavonols were administered with diets containing 32% fat. The elimination of quercetin was significantly delayed after its application with fat-enriched diets (P < 0.05). Thus, in addition to the chemical form of the flavonol, the fat content of the diet influences oral bioavailability of quercetin.

MATERIALS AND METHODS

Animals, diets, and experimental procedure. Cross-bred growing male castrated pigs (n = 7) with a body weight (BW) of 30–35 kg were purchased from a local farmer. The pigs were surgically equipped with permanent catheters (Cook Deutschland GmbH) placed in the jugular vein. They were restrictively fed (80% of voluntary feed intake) a commercial pig diet composed of barley, wheat, and defatted soybean meal (Plambeck Kraftfutter). The composition of this diet (designated as 3% fat diet) is shown in Table 1. Vitamins and minerals were supplemented according to the recommendations of the German Society of Nutritional Physiology (11). Water was freely available by nipple drinkers.

Each pig received in consecutive experiments either quercetin aglycone or Q3G (30 μmol/kg BW) mixed into a test meal directly before administration. The test meals consisted either of the regular pig diet (3% fat diet) or of an isoenergetic amount of the same diet enriched with either 15 or 30 g lard/100 g (wt/wt) (designated as the 17 and 32% fat diets, respectively) (Table 1). Lard was obtained from Fischermanns GmbH. Blood samples (8 mL each) were collected over a period of 24 h. After each experiment, there was a wash-out period of 24 h.

Processing of plasma samples and HPLC analysis. Blood samples were drawn into heparinized containers, immediately centrifuged (1500 × g, 10 min, 4°C) and stored at −70°C until analysis by HPLC as described previously (3,12,13). All samples were treated enzymatically with β-glucuronidase/sulfatase before the extraction of flavonol compounds. All flavonols were obtained from Carl Roth GmbH. β-Glucuronidase/sulfatase type H-2 (crude enzyme extract from Helix pomatia) was purchased from Sigma-Aldrich AG.

Statistical analysis. The area under the plasma concentration-time curve (AUC) was determined according to the linear trapezoidal rule. For each pig and treatment, total bioavailability (AUCtotal) was calculated by adding up the AUC values of quercetin and its metabolites with an intact flavonol structure (isorhamnetin and tamarixetin). AUC data, maximal plasma concentrations (cmax), those at 480 (c480) and 720 min (c720) after ingestion of the test meal, and time at maximal plasma concentration (tmax) values were analyzed with the MIXED model procedure from SAS (Version 8.2, SAS Institute) based on the model: Yijk = μ + Di + Fj + (D × F)k + Ai + eijk, where μ = mean, Di = diet (i = 3, 17, or 32% fat diet), Fj = administered flavonol (j = quercetin aglycone or Q3G), (D × F) = diet × administered flavonol interaction, Ai = animal, and eijk = lactase-phlorizin hydrolase; LSM, least-squares means; Q3G, quercetin-3-O-glucoside; SGLT1, sodium-dependent glucose transporter; tmax, time at maximal plasma concentration.
TABLE 1
Composition of the diets1

<table>
<thead>
<tr>
<th>Component</th>
<th>3</th>
<th>17</th>
<th>32</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/kg diet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry matter</td>
<td>869.2</td>
<td>888.8</td>
<td>914.2</td>
</tr>
<tr>
<td>Crude fat</td>
<td>32.9</td>
<td>174.1</td>
<td>318.2</td>
</tr>
<tr>
<td>Crude protein</td>
<td>161.8</td>
<td>142.4</td>
<td>115.6</td>
</tr>
<tr>
<td>Ash-free NDF2</td>
<td>120.5</td>
<td>109.6</td>
<td>86.6</td>
</tr>
<tr>
<td>NFC3</td>
<td>500.1</td>
<td>419.0</td>
<td>353.2</td>
</tr>
<tr>
<td>Starch</td>
<td>399.1</td>
<td>347.3</td>
<td>292.0</td>
</tr>
<tr>
<td>MJ/kg diet</td>
<td>16.2</td>
<td>19.6</td>
<td>22.7</td>
</tr>
</tbody>
</table>

1 Isoenergetic amounts of each diet were fed: 200.0 g of the 3% fat diet, 162.0 g of the 17% fat diet, and 136.2 g of the 32% fat diet.
2 NDF, neutral detergent fiber.
3 NFC, nonfiber carbohydrates.

= residual error. The individual pig was treated as a random factor. AUC data, $c_{max}$, $c_{ave}$, $c_{24}$, and $t_{max}$ values are presented as least-squares means (LSM) ± SEM. A P-value < 0.05 was considered significant.

RESULTS

Irrespective of the flavonol administered or the diet fed, the main metabolite in plasma after β-glucuronidase/sulfatase treatment of the samples was always quercetin (78.8 ± 0.8%, n = 38). In addition to quercetin, the monomethylated derivatives isorhamnetin (3'-O-methyl quercetin, 11.0 ± 0.5%) and tamarixetin (4'-O-methyl quercetin, 10.3 ± 0.4%) were found. No differences in the relative abundance of these metabolites were observed after intake of the aglycone or of the glucoside.

After the intake of quercetin aglycone with the 3% fat diet, the mean peak plasma concentration ($c_{max}$) was reached ~100 min ($t_{max}$) after intake (Table 2). When the flavonol was administered together with the 17 or 32% fat diets, plasma concentrations of quercetin rose more sharply (Fig. 1). The elimination of quercetin was clearly delayed after administration of the 17 or 32% fat diets, and the quercetin plasma concentration was reached after ~24 h (Fig. 1). After the intake of Q3G with the 3% fat diet, the mean peak plasma concentration of quercetin was reached after ~70 min (Table 2). When the flavonol glucoside was administered together with fat-enriched diets, the plasma concentrations of the aglycone group or within the quercetin-3-glucoside group differ, P < 0.05.

After intake of QAG with the 3% fat diet, the mean peak plasma concentration of QAG was reached after ~70 min (Table 2). When the flavonol glucoside was administered together with fat-enriched diets, the plasma concentrations of QAG significantly increased with the fat-enriched diets, with no significant difference between the 17 and 32% fat diets.

| Diet       | $c_{max}$ | $t_{max}$ | AUCtotal | Relative bioavailability%
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmol/L min</td>
<td>min × μmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quercetin aglycone</td>
<td>3.0 0.518 ± 0.056</td>
<td>102.9 ± 8.0</td>
<td>117.3 ± 18.5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>17.0 0.583 ± 0.060</td>
<td>70.0 ± 8.6</td>
<td>184.5 ± 19.8</td>
<td>157</td>
</tr>
<tr>
<td></td>
<td>32.0 0.563 ± 0.056</td>
<td>51.4 ± 8.0</td>
<td>176.0 ± 18.5</td>
<td>150</td>
</tr>
<tr>
<td>Quercetin-3-O-glucoside</td>
<td>3.0 0.906 ± 0.089a</td>
<td>70.0 ± 7.9</td>
<td>205.5 ± 18.9</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>17.0 0.895 ± 0.089b</td>
<td>50.0 ± 7.9</td>
<td>270.9 ± 18.9</td>
<td>132</td>
</tr>
<tr>
<td></td>
<td>32.0 0.642 ± 0.089b</td>
<td>45.0 ± 7.9</td>
<td>249.7 ± 18.9</td>
<td>122</td>
</tr>
</tbody>
</table>

1 For composition of the diets, see Table 1; values are LSM ± SEM, n = 6; for quercetin aglycone in the 3 and 32% fat diets, n = 7. Means in a column not sharing a superscript letter within the quercetin aglycone group or within the quercetin-3-O-glucoside group differ, P < 0.05.
2 $c_{max}$, maximum plasma concentration of quercetin.
3 $t_{max}$, time between administration of test meal and the appearance of $c_{max}$.
4 AUCtotal, area under the plasma concentration-time curve from 0 to 24 h for the sum of quercetin and its metabolites (isorhamnetin and tamarixetin).
5 Relative bioavailability within the quercetin aglycone group or within the quercetin-3-O-glucoside group.

FIGURE 1 Plasma concentration-time curves of quercetin after oral administration of quercetin aglycone or of quercetin-3-O-glucoside (inset) to pigs (30 μmol/kg BW each) in test meals differing in their fat content. Filled circles represent values for 3% fat diet, open triangles for the 17% fat diet, and open squares for the 32% fat diet (w/w). Data are means ± SEM (n = 6; for quercetin aglycone in the 3 and 32% fat diets, n = 7).
quercetin reached their peak levels earlier, which was significant only for the 32% fat diet. However, the \( c_{\text{max}} \) value of plasma quercetin was lowest after intake with 32% fat (Table 2). Similar to what was observed with the diets containing the aglycone, the elimination of quercetin was delayed with the fat-enriched diets (inset Fig. 1). Plasma concentrations after 480 min differed significantly among all 3 diets (0.053 ± 0.020 \( \mu \)mol/L in the 3% fat diet, 0.123 ± 0.020 \( \mu \)mol/L in the 17% fat diet, \( P < 0.05 \) vs. 3% fat diet, and 0.174 ± 0.020 \( \mu \)mol/L in the 32% fat diet, \( P < 0.05 \) vs. 3% and 17% fat diet, \( n = 6 \)). The \( C_{\text{trough}} \) values from both the 17 and 32% fat diets (0.071 ± 0.015 \( \mu \)mol/L and 0.090 ± 0.020 \( \mu \)mol/L, respectively) were also significantly higher than the plasma concentration after intake with the 3% fat diet (0.026 ± 0.015 \( \mu \)mol/L, \( P < 0.05 \)). Hence, the fat content of the diet had a significant effect on the AUC\(_{\text{total}}\) from Q3G (Table 2). In the case of Q3G intake, the significantly increased AUC\(_{\text{total}}\) values obtained with the fat-enriched diets were due solely to the delayed elimination of quercetin aglycone. The amount of quercetin and methylated quercetin metabolites in the systemic circulation was also significantly influenced by the chemical form of quercetin administered. With quercetin aglycone, the mean total bioavailability from any of the diets was always significantly lower than from the respective diets containing the quercetin glucoside (\( P < 0.05 \)) (Table 2). The fat content of the diet and the chemical form of the flavonol, however, did not have a significant interaction.

**DISCUSSION**

In a recent experiment, total oral bioavailability of quercetin in pigs was increased by 140% when Q3G was administered with meat compared with a standard pig diet (3). One possible explanation for this finding could have been the higher fat concentration of meat. We therefore conducted the present study to investigate the influence of the dietary fat content on the oral bioavailability of quercetin from both quercetin aglycone and Q3G.

In pig plasma, quercetin and its metabolites with a flavonol structure are present in substantial amounts only as conjugates (3,12). Therefore, we treated all plasma samples with \( \beta \)-glucuronidase/sulfatase to release the flavonol aglycones. In all experiments, quercetin was found in plasma within 30 min after ingestion. This suggests that absorption had already occurred in the upper small intestine, irrespective of the diet and of the flavonol administered.

The increase in the dietary fat content from 3 to 17% crude fat enhanced the total bioavailability of quercetin (quercetin and its metabolites, isorhamnetin and tamarixetin) from the quercetin aglycone containing diet by ~50%. This effect was already maximal because a further increase in dietary fat had no additional effect. It is generally believed that the rather lipophilic quercetin aglycone diffuses passively through the brush border membrane (BBM) of enterocytes (14,15). Azuma et al. (8) observed that coadministration of lipids (soybean oil or lecithin) and emulsifiers such as the bile constituent taurocholate was able to enhance and accelerate the intestinal absorption of quercetin in rats. In another rat study, the absorption of the flavanol catechin was enhanced when green tea catechins were administered as a phospholipid complex rather than as free catechins (16). Thus, it is likely that in the presence of dietary fat, quercetin was partly incorporated into mixed bile salt micelles in the lumen of the duodenum. This could have promoted its solubility and transport through the unstirred water layer and passive diffusion through the BBM.

Interestingly, an enhancing effect of dietary fat on total bioavailability of quercetin was also found after administration of Q3G-containing diets. At first glance, this was an unexpected observation, because the more hydrophilic glucoside of quercetin should not directly interact with the absorption of lipids. In agreement with this finding, Azuma et al. (17) recently observed an enhancement of quercetin bioavailability from quercetin glucosides in onions by administration with at least 4.6% fat. In the present study, the effect of dietary fat on quercetin bioavailability from Q3G was due exclusively to a delayed elimination of quercetin from plasma. In contrast, the addition of fat to the quercetin aglycone–containing diets also enhanced the absorption of the flavonol. Several studies pointed to the involvement of \( \beta \)-glycosidases such as the BBM enzyme, lactase-phlorizin hydrolase (LPH, EC 3.2.1.62), in the intestinal absorption of Q3G (18–20). According to those studies, Q3G is hydrolyzed by LPH in the small intestine and the liberated aglycone diffuses passively across the BBM into the enterocyte. Because the liberation of quercetin from Q3G by LPH occurs after the BBB absorption already because a further increase in dietary fat had no additional effect. This could explain the higher plasma concentrations of quercetin several hours after ingestion with the fat-enriched diets.

The earlier appearance of maximal quercetin plasma concentrations after ingestion with fat could be explained by a partial incorporation of the quercetin conjugates formed in the enterocyte into chylomicrons with consecutive export into the peripheral blood via lymph. Thus, part of the quercetin would bypass the liver and, consequently, increase the plasma concentration. However, because no data are available concerning the incorporation of quercetin conjugates into lipoproteins during absorption, this remains speculative at present.

In our previous study, administration of Q3G together with meat enhanced total quercetin bioavailability by 140% (3), whereas the addition of fat in the present study enhanced bioavailability of Q3G by only ~30%. This suggests that other dietary factors in addition to fat influence the bioavailability of quercetin. It is conceivable that the lack of carbohydrates in the meat could have favored carrier-mediated uptake of Q3G from the test meal used compared with the meat meal (21). In another study, Prieur et al. (22) observed that the incorporation of quercetin conjugates into lipoproteins during absorption, this remains speculative at present.

In the present study, all test meals were isoenergetic to avoid differences in the rate of stomach emptying, which in turn would influence absorption kinetics. Hence, the test meals differed inevitably in protein (32, 23, and 16 g crude protein) and carbohydrate content (100, 68, and 48 g nonfiber carbohydrates) in the 3, 17, and 32% fat test meals, respectively. These differences in protein and carbohydrate content were rather small, however, compared with the relative differences in fat content (7, 28, and 43 g crude fat in the 3, 17, and 32% fat test meals, respectively). Thus, in our opinion, it is most likely that the differences in the fat content were responsible for the observed effects on quercetin bioavailability.
Regardless of these latter considerations, Q3G was always more bioavailable than quercetin aglycone with each of the different diets. This observation agrees with our previous study using pigs (3) and is also known from studies of humans (7) and rats (24). Most authors explain the higher bioavailability of Q3G compared with the aglycone by the occurrence of higher local quercetin concentrations adjacent to the BBM due to the Q3G-hydrolyzing activity of LPH (see above). In addition, transport of Q3G by SGLT1 could also contribute to the higher bioavailability of quercetin from Q3G compared with quercetin aglycone. Several studies showed an interaction of Q3G with SGLT1 (5,25,26). A recent study demonstrated transport of a quercetin glucoside by SGLT1 (27). In experiments with pig small intestine BBM vesicles, we also found evidence for transport of Q3G by SGLT1 (28).

In summary, we showed that the dietary fat content influences the bioavailability of quercetin. Bioavailability from both quercetin aglycone and quercetin-3-O-glucoside is enhanced in a diet enriched with fat compared with a low-fat diet. This could be explained by an improved solubility and an accelerated absorption of the lipophilic quercetin aglycone via lipid micelles and a prolonged enterohepatic circulation.

LITERATURE CITED