Tissue Distribution of Quercetin in Rats and Pigs

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ABSTRACT Quercetin is a dietary polyphenolic compound with potentially beneficial effects on health. Claims that quercetin has biological effects are based mainly on in vitro studies with quercetin aglycone. However, quercetin is rapidly metabolized, and we have little knowledge of its availability to tissues. To assess the long-term tissue distribution of quercetin, 2 groups of rats were given a 0.1 or 1% quercetin diet [~50 or 500 mg/kg body weight (wt)] for 11 wk. In addition, a 3-d study was done with pigs fed a diet containing 500 mg quercetin/kg body wt. Tissue concentrations of quercetin and quercetin metabolites were analyzed with an optimized extraction method. Quercetin and quercetin metabolites were widely distributed in rat tissues, with the highest concentrations in lungs (3.98 and 15.3 nmol/g tissue for the 0.1 and 1% quercetin diet, respectively) and the lowest in brain, white fat, and spleen. In the short-term pig study, liver (5.87 nmol/g tissue) and kidney (2.51 nmol/g tissue) contained high concentrations of quercetin and quercetin metabolites, whereas brain, heart, and spleen had low concentrations. These studies have for the first time identified target tissues of quercetin, which may help to understand its mechanisms of action in vivo. J. Nutr. 135: 1718–1725, 2005.

KEY WORDS: quercetin, glucuronides, tissue distribution, flavonoids, polyphenols

Quercetin is a plant-derived, dietary compound with potentially beneficial effects on cardiovascular diseases (1,2). It belongs to a group of polyphenolic compounds termed flavonoids. This is a large group of secondary metabolites from plants consisting of ~6000 different compounds (3). Vegetables and fruits are rich sources of flavonoids in our diet, but tea and red wines also contain flavonoids. The main dietary sources of quercetin are onions, apples, and tea (4). Furthermore, the intake of food supplements containing quercetin is becoming increasingly common (5).

Although quercetin is not the most predominant flavonoid in our diet (6), it is one of the most studied. Most research has focused on the antioxidant properties of quercetin, its effects on several enzyme systems, and effects on biological pathways involved in carcinogenesis, inflammation, and cardiovascular diseases (7–12). However, most of these studies were done in vitro with quercetin aglycone (the unconjugated molecule), which is not present in the plasma of humans due to its very efficient phase II metabolism (13). Because the properties of quercetin aglycone and quercetin metabolites differ, studies with the aglycone have limited value. For example, the effects of quercetin aglycone on neuronal apoptosis mediated through the mitogen-activated protein kinase pathway do not occur when quercetin is conjugated to a glucuronic acid (14).

Upon absorption in the small intestine, quercetin is metabolized immediately by enzymes in the epithelial cells and further metabolized by the liver. The catechol group of quercetin is methylated at the 3’ or 4’ position by catechol-O-methyl transferase (COMT),3 resulting in the formation of isorhamnetin (3’OCH3-quercetin) or tamarixetin (4’OCH3-quercetin). Both of these metabolites and quercetin can be conjugated at several hydroxyl groups with glucuronic acid or sulfate by UDP-glucuronosyltransferase or sulfotransferase, respectively (15). The plasma half-life of quercetin in humans is between 17 and 28 h (16,17).

The tissue distribution of quercetin and its metabolites has been assessed by a number of authors. Ueno et al. (18) analyzed the tissue distribution of a single oral dose of 14C-labeled quercetin aglycone in rats 6 h after administration. The highest radioactivity levels were found in the gastrointestinal tract, liver, and kidneys with much lower levels in other organs. A similar distribution was seen in rats 1 h after administration of an oral dose of radiolabeled quercetin 4’-O-glucoside, with

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3 Abbreviations used: COMT, catechol-O-methyl transferase; Hb, hemoglobin; LOD, limit of detection; QSG, quercetin 3-O-j-glucoside; wt, weight.
high levels in the intestines and the major metabolizing organs (19). Analysis of the quercetin metabolites with LC-MS-MS resolved 18 different metabolites in plasma and tissues. Free quercetin aglycone could be detected only in low levels in liver, but not in plasma and kidneys (19). These studies with radioactively labeled quercetin were done with a single oral dose and rats were killed shortly after ingestion. In a longer study with rats fed a 0.25% quercetin diet for 2 wk, the livers contained quercetin and/or quercetin metabolites (20). Furthermore, in a different study with rats fed a 0.5% quercetin diet for 2 wk, in addition to liver and kidney, heart tissue also appeared to contain detectable concentrations of quercetin and/or quercetin metabolites. The presence of quercetin and/or quercetin metabolites in other organs is not known because liver, kidney, and heart tissues were the only organs analyzed (21).

To improve our understanding of the mechanism of action of quercetin, it is necessary to know which metabolites are present in the body and in which tissues and organs they are found. Because only limited information on the tissue distribution is available at present, we conducted feeding studies in 2 species and determined quercetin and quercetin metabolites in tissues using an optimized extraction method. We analyzed the tissue distribution of quercetin and quercetin metabolites in rats after an 11-wk exposure to a 0.1 or 1% quercetin diet (~50 or 500 mg/[kg body weight (wt) d]) and in pigs after a 3-d exposure to a high-quercetin diet [500 mg/(kg body wt d)].

MATERIALS AND METHODS

Chemicals

Quercetin and β-glucuronidase/sulfatase (Helix pomatia, G1512) were obtained from Sigma-Aldrich. Isoharmetin (3’OCH₃-querce- tin) and quercetin-3-O-β-glucoside (Q3G) were obtained from Roth. Tamarixetin (4’OCH₃-querce- tin) was obtained from Extrasynthese. Quercetin 3-O-β-glucuronide was purchased from Apin Chemicals and verified by NMR. All other chemicals used were of analytical grade.

Animals and diets

Rats. The experimental protocol was approved by the Animal Welfare Committee of Wageningen University, Wageningen, The Netherlands. Inbred male Fisher 344 rats [age 7 wk (4 wk of age at arrival followed by 3 wk acclimatization), body wt 134 ± 5 g, mean ± SD; Charles River Laboratories] were housed in pairs in cages in a room with controlled temperature (20–22°C), controlled relative humidity (50–70%), and a 12-h light:dark cycle (lights on at 0600 h). The rat diet was a RM3|EFG SQC breeding diet (Special Diets Services) and did not contain any detectable concentrations of quercetin. The diet was supplemented with 0.1 or 1% quercetin (6 rats/group) for 11 wk. Rats consumed their food ad libitum and had unlimited access to water; their weight and food consumption were measured weekly. Calculated quercetin intake for rats fed a 1% quercetin diet during the 11 wk ranged from ~800 mg/[kg body wt d] in wk 1 to ~500 mg/[kg body wt d] during wk 11. The quercetin intake of the 0.1% quercetin group was 10% that of the 1% quercetin group. In addition to the rats fed a quercetin diet, 6 control rats were given the same diet without the addition of quercetin; their weight and food intake did not differ from the quercetin intake groups.

After consuming the quercetin diet for 11 wk, rats were anesthetized in the morning, without overnight fasting, by inhalation of isoflurane, using NO₂O₂ (1:1, v:v) as a carrier. Rats were fully bled via the abdominal aorta. Blood (8–12 mL) was collected in EDTA-tubes and plasma was subsequently prepared in Leucosep Centrifuge tubes (Greiner Bio-one) by centrifuging for 20 min at 1000 × g and 4°C. Plasma samples were stabilized with ascorbic acid (5.6 mmol/L final concentration) before storage at ~80°C. After blood collection, the following tissues were dissected, weighed, and immediately frozen in liquid nitrogen: testes (3.14 ± 0.14 g wt mean, ± SD, n = 12), spleen (0.74 ± 0.04 g), kidneys (1.92 ± 0.10 g), abdominal white adipose tissue (4.16 ± 0.74 g), liver (10.41 ± 0.57 g), bone (sternum + bone marrow) (2.11 ± 0.36 g), thymus (0.26 ± 0.03 g), heart (0.87 ± 0.07 g), lungs (1.34 ± 0.10 g), muscle (quadriceps) (4.39 ± 0.79 g), brown adipose tissue (0.28 ± 0.07 g), and brain (1.73 ± 0.24 g). The emphasis in this study was on nongastrointestinal tract organs, because gastrointestinal organs are predominantly exposed directly to quercetin from the diet; therefore, gastrointestinal organs were not collected.

Pigs. Two cross-bred castrated male pigs (PIC) weighing 122.4 and 138.9 kg, were restrictively fed (80% of the demand for maintenance) a high-quercetin diet [500 mg/[kg body wt d]], divided into 3 meals a day for 3 consecutive days. The diet consisted mainly of wheat and defatted soybean meal. After 3 d, the pigs were food deprived for ~8 h before killing. Then tissues (liver, kidney, spleen, brain, heart) and blood were collected. Tissue samples were collected by taking 3 pieces of the organ, each piece weighing ~100 g. Whole blood was collected in heparinized tubes and plasma was prepared by centrifuging for 10 min at 2000 × g.

Preparation of samples

All tissues were lyophilized before further processing. Subsequently, all pig tissues were chopped into small pieces in liquid nitrogen and milled using a Janke & Kunkel Analysemühle A10 (IKA labortechnik). Rat tissues were pooled per intake group (6 rats/group) and ground and homogenized with a mortar and pestle. Liver, bone, kidney, lung, muscle, and brain tissues required additional homogenization in the mill. Samples were stored in airtight containers at ~20°C.

Extraction: procedure

For extraction, 0.5 ± 0.02 g pig tissue or 0.1 ± 0.01 g of rat tissue except for spleen (0.06 g), brown adipose tissue (0.04 g), and thymus (0.04 g) were weighed and extracted in 50 mL tubes for pig tissues or 15-mL tubes for rat tissues. The dry samples were homogenized in 14 mL of 0.5 mol/L sodium acetate buffer (NaAc, pH 5.0, with 28 mmol/L ascorbic acid)/g tissue with a vortex and sonicated for 10 min. Each sample was either hydrolyzed with 3 mL Helix pomatia enzyme mix (7500 U β-glucuronidase and ~750 U sulfatase activity in 0.5 mol/L NaAc, pH 5.0, with 28 mmol/L ascorbic acid)/g tissue for 2 h in a water bath at 37°C, or not hydrolyzed but processed immediately with the addition of the same volume of NaAc buffer without enzyme mix. Subsequently, all samples were deproteinized with 2 volumes acetonitrile and 1 volume of 20% H₃PO₄ (with 17 mmol/L ascorbic acid) and centrifuged for 10 min at 2300 × g and 10°C. After centrifugation, 1 mL supernatant was filtered through a 0.22-μm acrodisc filter ( Pall Corporation) and injected into the HPLC system. Plasma samples were analyzed by a similar method as described previously (22).

Extraction: optimization

Extraction of quercetin, isoharmetin, tamarixetin, and their metabolites from tissues was optimized with 2 different methods. Method 1 (described in detail above) was based on the extraction of quercetin metabolites from plasma (22); method 2 was based on the extraction of quercetin metabolites from rat tissues by Mullen et al. (19). Method 2 consisted of the following steps: samples were extracted for 1 h in 60% methanol (with 5.6 mmol/L ascorbic acid) at 60°C in a shaking water bath. The extract was transferred to a 15-mL tube and centrifuged for 10 min at 2300 × g and 10°C. The supernatant was transferred to a clean tube and the residue was extracted 2 more times. The organic solvent from the supernatant was evaporated with a Zymark Turbo vap LV Evaporator (Zymark) at 60°C, under a mild
nitrogen flow (~35 kPa). Tubes were weighed before and after evaporation of extraction solvent. Each sample (350 μL) of the remaining water phase was hydrolyzed enzymatically for 2 h in an incubator at 37°C with 150 μL Helix pomatia enzyme mixture in 0.5 mol/L NaAc (pH 5, with 28 mmol/L ascorbic acid). After hydrolysis, the samples were deproteinized with 2 vol acetonitrile and 1 vol of 20% H3PO4 (with 17 mmol/L ascorbic acid), centrifuged for 10 min at 10,000 × g and 10°C, and injected into the HPLC.

All optimizations were performed with pig liver tissue samples. Method 1 had already been optimized for plasma samples (22). Further optimizations for freeze-dried tissue samples included variation of enzyme incubation time (0–6 h) and homogenization volume (3.5, 7, and 10.5 mL). For method 2, the following extraction conditions were optimized: type of extraction solvent (methanol or acetonitrile), volume fraction of extraction solvent (20–80% (v:v) solvent in water), extraction temperature (room temperature and 60°C), extraction time (a single time for 1 h or 3 times in 1 h), and extraction pH (2 and 7). Recovery for both methods was calculated by spiking ground chicken liver (from a local grocery store), pig liver, or rat liver samples with a known amount of quercetin, isorhamnetin, and tamarixetin, or Q3G standard. Standards were added before the addition of extraction solvent, at 50, 100, and 200% of the original concentration in the tissue sample.

**HPLC analysis**

The HPLC system consisted of 2 Merck Hitachi LaChrom Elite L2130 pumps (Hitachi) and a Merck Hitachi LaChrom Elite L2200 injector. Separation was achieved by injecting 50 μL sample onto a Chromolith RP-18e column (100.0 × 4.6 mm, Merck), protected by a NewGuard RP18 guard column (15.0 mm × 3.2 mm, 5 μm, Perkin Elmer). Columns were housed in a column heater (ESA) at 30°C. The solvents for elution were 5% acetonitrile in 25 mmol/L citrate buffer, pH 3.7 (solvent A) and 70% acetonitrile in 25 mmol/L citrate buffer, pH 3.7 (solvent B). The elution program at a flow rate of 2.5 mL/min was as follows: 0–4 min, linear gradient from 0 to 28% B; 4–16 min, isocratic at 28% B; 16–17 min, linear from 28 to 100% B; 17–18.5 min, isocratic at 100% B; 18.5–19 min, linear return to 0% B. Total run time was 22 min. Peaks were detected with a photometric array detector (Coularray detector model 6210; ESA) set at 75, 250, and 500 mV (Pd as reference). Quercetin was quantified at the lowest potential (75 mV). Because baseline separation of isorhamnetin and tamarixetin could not be achieved, the 3 different oxidation potentials were used to quantitate isorhamnetin and tamarixetin. Tamarixetin did not oxidize at 75 mV; therefore isorhamnetin was quantitated at this potential. Tamarixetin was quantitated at 500 mV after subtracting the area of the small isorhamnetin peak at 500 mV. The latter was calculated from the isorhamnetin peak at 75 mV, using the ratio of the peak height of isorhamnetin at 75 mV and the peak height at 500 mV of an isorhamnetin standard.

The injection peak was discarded to the waste by using a Multiport Streamswitching system (model 790 MUST, Spark Holland). At 4 min after injection, the mobile phase was redirected to the detector and at 17 min after injection, the port was switched back to the waste, resulting in the lowest possible contamination of the detector cell. To prolong the lifetime of the Coularray detector cell, eluent was continually pumped through the cell with a Merck Hitachi L6000 pump when the detector was out of use.

**Correction for residual blood**

Because tissues of rats and pigs had not been perfused after excision, they contained residual blood containing quercetin, isorhamnetin, and their metabolites. Correction for residual blood was done by comparing the hemoglobin (Hb) content of freeze-dried tissues to the Hb content of freeze-dried whole blood determined with a spectrophotometric method (23). Whole-blood samples from pigs were collected from the same pigs as the tissues. Rat whole blood was taken from a 6-mo-old Wistar rat. Freeze-dried tissues, ~0.01 g for pooled rat tissues and ~0.1 g for pig tissues, were homogenized in 14 mL Tris-HCl pH 7.4, with 50 mmol/L EDTA/g tissue and centrifuged at 10,000 × g for 10 min at 10°C. Then, 2 μL of the supernatant was used for spectrophotometric determination of the hemoglobin content with a Nanodrop (ND-1000 UV-Vis Spectrophotometer). An absorbance spectrum was taken from 220 to 750 nm and all samples were analyzed in duplicate. The fraction of residual blood (fbl) in each tissue was calculated by dividing the peak maximum at 540 nm of each tissue extract (Hb tissue) with the peak maximum at 540 nm of a whole blood extract (Hb whole) (Eq. 1).

\[
f_{\text{bl}} = \frac{\text{Hb}_{\text{tissue}}}{\text{Hb}_{\text{whole}}}
\]

The linearity of the method was verified by spiking freeze-dried pig liver samples with increasing concentrations of freeze-dried whole blood from pig. The quercetin or isorhamnetin concentrations that were found in each tissue extract (Corg) were corrected for blood contamination by subtracting the quercetin or isorhamnetin concentrations that were found in plasma samples (Cpl) multiplied by the fraction of residual blood in that tissue (fbl) (Eq. 2).

\[
f_{\text{bl}} = C_{\text{org}} - f_{\text{bl}} \cdot C_{\text{pl}}
\]

where \(C_{\text{org}}\) is the corrected concentration for quercetin or isorhamnetin. Plasma concentrations of quercetin and isorhamnetin were assumed to be equal to whole-blood concentrations. Tamarixetin could not be detected in plasma samples; therefore, tamarixetin concentrations in tissue extracts were not corrected.

**Statistical methods**

All samples were extracted in duplicate. Quercetin, isorhamnetin, and tamarixetin concentrations were expressed in nmol/g wet wt (nmol/g tissue), and represent total concentrations of aglycones plus hydrolyzed conjugates, unless indicated otherwise. Tissues of 6 rats were pooled before analysis. Tissues of pigs were analyzed separately and individual values for both pigs are given.

**RESULTS**

**Extraction method and residual blood.** Two different extraction methods were tested and optimized. Optimization of method 1 for tissue samples resulted in an optimal homogenization volume of 7 mL buffer for a 0.5-g sample and a 2-h incubation period with Helix pomatia β-glucuronidase/sulfatase at 37°C. These conditions gave the highest yield for quercetin with the least variation: 14 ± 1.35 nmol/g dry wt (mean ± SD, n = 8 different extractions on 4 different days). The recoveries for quercetin aglycone, isorhamnetin aglycone, tamarixetin aglycone, and Q3G were determined by addition of standard compounds and ranged from 72 to 91% (Table 1).

Optimization of method 2 resulted in large differences in yield under different extraction conditions. The optimal solvent concentration was 60% (v:v) methanol in water. Extraction with 70% (v:v) acetonitrile gave similar quercetin concentrations. When the pH of the extraction solution during methanolic extraction was lowered to pH 2, the quercetin concentrations decreased by 50% compared with methanolic extraction at pH 7. Extracting at room temperature resulted in concentrations that were 20% lower than extracting at 60°C. The optimized method 2 gave ~20% lower quercetin concentrations than method 1. The recoveries of quercetin aglycone and Q3G for method 2 were 86 ± 4 and 103 ± 3% (mean ± SD, n = 2 different extractions), respectively. Because method 1 gave higher quercetin values than method 2 and was less time-consuming, method 1 was chosen for analysis of the tissues from pigs and rats. The analytical characteristics for method 1 are shown in Table 1.

To verify the validity of the blood correction method, pig liver tissue was spiked with freeze-dried whole blood. No additional peaks appeared in the Hb absorbance spectrum, and the peak maximum at 540 nm increased linearly with in-
TABLE 1
Characteristics of the method used for analyzing pig and rat tissues (method 1)

<table>
<thead>
<tr>
<th>Component</th>
<th>Recovery, %</th>
<th>LOD, nmol/g tissue</th>
<th>Within-day CV, %</th>
<th>Between-day CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td>81 ± 9</td>
<td>0.12</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Isorhamnetin</td>
<td>85 ± 8</td>
<td>0.14</td>
<td>9</td>
<td>—</td>
</tr>
<tr>
<td>Tamarixetin</td>
<td>91 ± 22</td>
<td>0.19</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Q3G</td>
<td>72 ± 3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

1 Values are means ± SD. Recovery was determined at 3 levels of addition of the flavonoid (50, 100, and 200% of the original concentration in the sample).
2 The LOD is defined as the concentration producing a peak height 3 times the baseline noise and calculated for uncorrected tissue levels in nmol/g wet wt (nmol/g tissue).
3 The within-day CV was determined by comparing different injections (n = 3) of the same extract within the same run.
4 The between-day CV was determined by comparing different extractions of the same pig liver samples over a period of 3 mo of at least 5 different analyses.
5 (—) = not analyzed.

Increased spiked blood content (data not shown). The fraction of residual blood in the tissues ranged from 0.01 to 0.25 (Table 2).

Tissue distribution of quercetin, isorhamnetin, and tamarixetin in rats. Analysis of tissues and plasma of rats fed a 0.1 or 1% quercetin diet showed that the tissue and plasma concentrations of quercetin, isorhamnetin, and tamarixetin were ~4 times higher in rats fed the 1% quercetin diet than in those fed 0.1% (Tables 3 and 4). Thus, a 10-fold increase in the dose of dietary quercetin resulted in a 4-fold increase in concentrations of quercetin, isorhamnetin, and tamarixetin in plasma and tissues. In contrast to the difference in the concentrations of quercetin, isorhamnetin, and tamarixetin between the rats fed the 0.1 and 1% diets, the distribution of quercetin, isorhamnetin, and tamarixetin over the different tissues was similar for the 2 groups of rats. The highest concentration of the sum of quercetin, isorhamnetin, and tamarixetin was found in the lungs of both the 0.1% (3.98 nmol/g tissue) and 1% (15.3 nmol/g tissue) quercetin intake groups. The lowest concentrations were found in the brain, white fat, and spleen (Tables 3 and 4). The major metabolizing organs (liver and kidney) contained intermediate quercetin, isorhamnetin, and tamarixetin concentrations. For liver, this was 1.79 nmol/g tissue (0.1% group) and 6.34 nmol/g tissue (1% group) and for kidney it was 2.85 nmol/g tissue (0.1% group) and 11.6 nmol/g tissue (1% group) (Tables 3 and 4).

For most of the tissues in both the 0.1 and 1% groups, the concentration of quercetin was ~30% (range 23–36%) of the total concentration of quercetin metabolites (Tables 3 and 4). However, in some tissues, the relative contribution of quercetin to the total concentration of quercetin metabolites was lower. In bone, muscle, thymus, and brown fat tissue of the 0.1% group (Table 3), the quercetin concentration ranged from 10 to 16% of the total concentration of quercetin metabolites, indicating that the level of methoxylation and/or tissue uptake of methylated metabolites differed between tissues. In addition, tamarixetin was not detected in the plasma of either group, whereas moderate concentrations of tamarixetin were detected in most of the tissues. Tamarixetin concentrations were always lower than quercetin or isorhamnetin concentrations.

Tissue distribution of quercetin, isorhamnetin, and tamarixetin in pigs. In pigs, the plasma concentration of quercetin was 1.1 nmol/mL and that of isorhamnetin 0.15 nmol/mL (Table 5). Tamarixetin was not detected in pig plasma. In contrast to what was observed in rats, the tissue concentrations in the liver and kidney of pigs were higher than the plasma concentrations. The quercetin concentrations in liver and kidney were 3.8 and 1.8 nmol/g tissue, respectively. The other tissues (brain, heart, and spleen) contained only low concentrations of quercetin; these were at the limit of detection (LOD), which is ~0.12 nmol/g tissue for quercetin. Furthermore, isorhamnetin, and tamarixetin could not be detected in brain, heart, and spleen.

**DISCUSSION**

This study shows for the first time that quercetin and its metabolites are widely distributed in rat tissues after long-term
oral exposure to quercetin. The highest concentrations of quercetin, isorhamnetin, and tamarixetin were found in the lungs and the lowest in brain and white fat. The tissue distribution of quercetin, isorhamnetin, and tamarixetin in both intake groups showed a comparable pattern, but the concentrations in the 0.1% group were about 4 times lower than those in the 1% group. The high concentrations of quercetin metabolites in lungs of rats may be a result of the 11-wk chronic exposure of the rats. In short-term studies in which rats were given quercetin (or its glycoside), no such marked distribution was seen. Mullen et al. (19) showed that 1 h after administration of a single oral dose of radioactively labeled quercetin 4-O-β-glucoside (~8 mg/kg body wt) most of the radioactivity was recovered, apart from the gastrointestinal tract, in the plasma, liver, and kidney of rats. Only low levels of radioactivity were found in lungs, heart, testes, spleen, and muscle, and the lowest levels of radioactivity were found in the brain. Ueno et al. (18) also did not find high concentrations of quercetin in

### Table 3

Quercetin, isorhamnetin, and tamarixetin concentrations in tissues of rats fed a 0.1% quercetin diet for 11 wk

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Quercetin</th>
<th>Isorhamnetin</th>
<th>Tamarixetin</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of sum</td>
<td>% of sum</td>
<td>% of sum</td>
<td>% of sum</td>
</tr>
<tr>
<td>Plasma, μmol/L</td>
<td>23.4</td>
<td>7.70</td>
<td>33</td>
</tr>
<tr>
<td>Lung</td>
<td>3.98</td>
<td>1.04</td>
<td>26</td>
</tr>
<tr>
<td>Testes</td>
<td>2.98</td>
<td>0.82</td>
<td>27</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.85</td>
<td>0.93</td>
<td>33</td>
</tr>
<tr>
<td>Heart</td>
<td>2.20</td>
<td>0.50</td>
<td>23</td>
</tr>
<tr>
<td>Liver</td>
<td>1.79</td>
<td>0.52</td>
<td>29</td>
</tr>
<tr>
<td>Brown fat</td>
<td>1.53</td>
<td>0.15</td>
<td>10</td>
</tr>
<tr>
<td>Thymus</td>
<td>1.37</td>
<td>0.15</td>
<td>11</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.10</td>
<td>0.12</td>
<td>11</td>
</tr>
<tr>
<td>Bone</td>
<td>0.92</td>
<td>0.14</td>
<td>16</td>
</tr>
<tr>
<td>Brain</td>
<td>0.33</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.02</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>White fat</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
</tr>
</tbody>
</table>

1 All quercetin, isorhamnetin, and tamarixetin concentrations represent total concentrations of aglycones plus hydrolyzed conjugates and are corrected for residual blood.
2 Tissues of 6 rats were pooled before analysis.
3 Data are expressed as nmol/g wet wt.
4 LODs are as follows: quercetin, 0.12 nmol/g tissue; isorhamnetin, 0.14 nmol/g tissue; and tamarixetin, 0.19 nmol/g tissue.
5 Values can be lower than the LOD because of the correction factor for residual blood.

### Table 4

Quercetin, isorhamnetin, and tamarixetin concentrations in tissues of rats fed a 1% quercetin diet for 11 wk

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Quercetin</th>
<th>Isorhamnetin</th>
<th>Tamarixetin</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of sum</td>
<td>% of sum</td>
<td>% of sum</td>
<td>% of sum</td>
</tr>
<tr>
<td>Plasma, μmol/L</td>
<td>107.5</td>
<td>40.4</td>
<td>38</td>
</tr>
<tr>
<td>Lung</td>
<td>15.3</td>
<td>5.02</td>
<td>33</td>
</tr>
<tr>
<td>Testes</td>
<td>14.4</td>
<td>4.37</td>
<td>30</td>
</tr>
<tr>
<td>Kidney</td>
<td>11.6</td>
<td>3.67</td>
<td>32</td>
</tr>
<tr>
<td>Thymus</td>
<td>10.4</td>
<td>2.66</td>
<td>26</td>
</tr>
<tr>
<td>Heart</td>
<td>7.94</td>
<td>2.46</td>
<td>31</td>
</tr>
<tr>
<td>Liver</td>
<td>6.34</td>
<td>2.25</td>
<td>36</td>
</tr>
<tr>
<td>Brown fat</td>
<td>5.55</td>
<td>1.53</td>
<td>28</td>
</tr>
<tr>
<td>Bone</td>
<td>5.36</td>
<td>1.83</td>
<td>34</td>
</tr>
<tr>
<td>Muscle</td>
<td>4.16</td>
<td>1.21</td>
<td>29</td>
</tr>
<tr>
<td>White fat</td>
<td>1.22</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
</tr>
<tr>
<td>Brain</td>
<td>0.68</td>
<td>0.06⁵</td>
<td>8</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.11</td>
<td>&lt;LOD</td>
<td>&lt;LOD</td>
</tr>
</tbody>
</table>

1 All quercetin, isorhamnetin, and tamarixetin concentrations represent total concentrations of aglycones plus hydrolyzed conjugates and are corrected for residual blood.
2 Tissues of 6 rats were pooled before analysis.
3 Data are expressed as nmol/g wet wt.
4 LODs are as follows: quercetin, 0.12 nmol/g tissue; isorhamnetin, 0.14 nmol/g tissue; and tamarixetin, 0.19 nmol/g tissue.
5 Values can be lower than the LOD because of the correction factor for residual blood.
Tissue Distribution of Quercetin

TABLE 5
Quercetin, isorhamnetin and tamarixetin concentrations in tissues of pigs fed a high-quercetin diet
[500 mg/(kg body wt · d)] for 3 d

<table>
<thead>
<tr>
<th>TISSUE DISTRIBUTION OF QUERCETIN</th>
<th>Quercetin</th>
<th>Isorhamnetin</th>
<th>Tamarixetin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± Δ</td>
<td>Mean ± Δ</td>
<td>% of sum</td>
</tr>
<tr>
<td>Plasma, µmol/L</td>
<td>1.25 ± 0.35</td>
<td>1.1 ± 0.30</td>
<td>88</td>
</tr>
<tr>
<td>Liver</td>
<td>5.87 ± 0.42</td>
<td>3.78 ± 0.07</td>
<td>64</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.51 ± 0.54</td>
<td>1.84 ± 0.07</td>
<td>73</td>
</tr>
<tr>
<td>Brain</td>
<td>0.22 ± 0.07</td>
<td>0.22 ± 0.07</td>
<td>100</td>
</tr>
<tr>
<td>Heart</td>
<td>0.11 ± 0.04</td>
<td>0.11 ± 0.04</td>
<td>100</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.07 ± 0.07</td>
<td>0.07 ± 0.07</td>
<td>100</td>
</tr>
</tbody>
</table>

¹ All quercetin, isorhamnetin, and tamarixetin concentrations represent total concentrations of aglycones plus hydrolyzed conjugates and are corrected for residual blood.
² Data are expressed as nmol/g wet wt.
³ LODs are as follows: quercetin, 0.12 nmol/g tissue; isorhamnetin, 0.14 nmol/g tissue; and tamarixetin, 0.19 nmol/g tissue.


tissues other than the gastrointestinal tract, liver, and kidney, after a single oral dose of 630 mg radioactively labeled quercetin/kg body wt. Lung, muscle, and heart levels of radioactive quercetin were significantly lower than those in kidney and liver. A 2-wk exposure to a 0.5% quercetin diet in rats resulted in high concentrations not only in kidney and liver, but also in heart (21), indicating that longer exposures cause quercetin and its metabolites to reach tissues other than liver and kidney.

The lowest concentrations of quercetin, isorhamnetin, and tamarixetin were found in brain tissues of rats. Some flavonoids were shown to enter the brain after i.v. or oral exposure. Naringenin (24), puerarin (25), genistein (27,28), and epicatechin (29) were detected in brain tissues. However, as noted by Youdim et al. (30) most of these studies did not correct for residual blood in the brain. The importance of correcting for residual blood was described by several authors (23,31–35). Although for some tissues, like spleen, the residual blood concentrations are probably overestimated, because of its role in the breakdown of erythrocytes, the concentrations of residual blood reported in literature (31,32,35) agree with the concentrations of blood we found in tissues (Table 2). In our study, we corrected for residual blood; thus, the quercetin, isorhamnetin, and tamarixetin that we found in the brain were located intracellularly or interstitially and not intravasculary.

The difference between quercetin concentrations in the testes and brain is striking. Like the brain and placenta, the testes have a specific barrier (36), but the quercetin concentrations in the testes were among the highest of all organs. This indicates that the influx and efflux of quercetin conjugates is regulated differently in brain and testis tissue. Although most of the transporters in the brain are also expressed in the testis, the location of efflux transporters in the brain is mainly in the endothelium cell layer, whereas efflux pumps in the testis are expressed mainly in the sertoli cells (37). This may result in higher interstitial concentrations of quercetin in the testis than in brain.

The tissue and plasma concentrations of quercetin, isorhamnetin, and tamarixetin in pigs differed from the concentrations in tissues and plasma of rats. The plasma concentrations in pigs were lower than those in liver and kidney of pigs, and the concentrations in pig heart tissue were similar to those in pig brain and spleen (Table 5), whereas in rats, plasma concentrations were at least 6 times higher than tissue concentrations; heart tissue was similar to liver and kidney (Table 3 and 4). This may be explained by several factors that differ between the 2 feeding studies. The administration of the diet differed in the 2 feeding studies. Pigs were given the quercetin diet in 3 separate meals each day, whereas the rats consumed their food ad libitum. In addition, the pigs were food deprived for 8 h before they were killed, whereas the rats were not food deprived. Pigs and rats also differ in the way they eliminate quercetin from their body. Pigs are much less effective in converting quercetin to isorhamnetin. The activity of COMT in pigs is probably much lower than in rats; 1 h after a single dose of quercetin 4-O-β-glucoside more than half of the total quercetin metabolite concentrations consisted of methoxy derivatives in rats (19), whereas in pigs the concentrations of methoxy derivatives were always much lower than quercetin derivatives (38,39), which is in accordance with our data. The plasma half-life for quercetin, isorhamnetin, and tamarixetin in pigs is only 4 h (38), whereas the plasma half-life for quercetin, isorhamnetin, and tamarixetin in rats is much longer.
longer than 4 h [>8 h, as derived from (40)]. Thus, quercetin is eliminated at least twice as fast from the blood in pigs as in rats.

Although quercetin is present primarily in tissues conjugated to methoxy, sulfate, and glucuronic acid groups, it cannot be excluded that quercetin appears in tissues in the unmetabolised form. We found that especially lung, liver, and kidney of rats possess a high deconjugation activity, probably due to enzymes with β-glucuronidase activity. This high enzyme activity resulted in an ex vivo conversion of conjugated quercetin to free aglycone during the extraction procedure (see Fig. 1). However, this high β-glucoronidase activity of tissues may also cause in vivo conversion of conjugated quercetin to free aglycone. Enzymes with β-glucuronidase activity can either be released under certain physiologic conditions such as inflammation and neoplasm (41,42) or microsomal β-glucuronidase enzymes can convert glucuronides directly to the aglycone. O’Leary et al. (43) showed that quercetin 3-O-β-glucuronide and quercetin 7-O-β-glucuronide were deconjugated in hepatocytes and further metabolized to quercetin 3'-sulphate, indicating that quercetin glucuronides can enter tissue cells and that microsomal enzymes with β-glucuronidase activity are able to convert quercetin glucuronides intracellularly to the quercetin aglycone.

In conclusion, these studies demonstrated that long-term exposure to quercetin in rats results in a wide distribution of quercetin metabolites to most of the organs. A short exposure in pigs did not result in high concentrations of quercetin metabolites in tissues other than kidney and liver. In addition, this study does not exclude the presence of free aglycone in tissues, but when aglycone concentrations are analyzed, attention should be paid to deconjugation reactions during extraction. These experiments have identified target tissues of quercetin, which may help to understand the mechanisms of action of quercetin in vivo.

ACKNOWLEDGMENT

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LITERATURE CITED


