Carbon Dioxide Is the Major Metabolite of Quercetin in Humans¹,²

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ABSTRACT A previous study in ileostomy patients indicated that dietary glucosides of the flavonoid quercetin are hydrolyzed efficiently in the intestinal lumen, followed by absorption of a large fraction of the quercetin aglycone. To determine the fate of quercetin, we administered 1.85 MBq (50 μCi) of ¹⁴C-quercetin both orally (100 mg, 330 μmol) and intravenously (iv; 0.3 mg, 1 μmol) to healthy volunteers. Serial plasma samples, urines and stools were collected for 72 h. Total radioactivity was determined by liquid scintillation spectrometry directly in plasma and urine and after repeated methanol extraction of stool homogenate samples. The oral absorption, based on total radioactivity, was surprisingly high, ranging from 36.4 to 53.0%. The biological half-life was very long, ranging from 20 to 72 h. The urinary recovery of total radioactivity ranged from 18.4 to 26.8% after the iv dose and from 3.3 to 5.7% after the oral dose. The corresponding fecal recoveries were only 1.5–5.0% and 1.6–4.6%, respectively. Thus, the total recovery of the ¹⁴C-quercetin doses, in particular after oral administration, was very low. In search for the unaccounted for fraction of the ¹⁴C-quercetin dose, we performed ¹⁴CO₂ recovery studies in three volunteers (3 iv and 3 oral doses). At timed intervals, ¹⁴CO₂ in expired air was trapped in hyamine hydroxide/thymolphthalein and analyzed for radioactivity. As much as 23.0–81.1% of the quercetin dose was recovered as ¹⁴CO₂ in the expired air from these volunteers, after both oral and iv doses. The disposition of quercetin in humans is thus highly complex, requiring further studies. J. Nutr. 131: 2648–2652, 2001.

KEY WORDS: • quercetin • intestinal absorption • enterohepatic recirculation • carbon dioxide formation • flavonoids • humans

Quercetin is one of the most prevalent as well as thoroughly studied dietary flavonoids. It is present in fruits, vegetables and beverages mainly as glucosides, with the highest content in onions, apples and red wine (1–3). Epidemiologic studies suggest that flavonoids are protective against coronary heart disease and stroke (4–6) as well as in certain cancers (7–9). Large numbers of in vitro studies suggest a variety of molecular targets for these effects (10). The numerous reports on quercetin’s potential beneficial effects on human health have more recently led to a proliferation of high dose quercetin nutraceutical preparations. However, a major concern has been the poor oral bioavailability of quercetin and most other flavonoids (11).

The original model of flavonoid absorption assumed that flavonoid glucosides were too polar to be absorbed from the small intestine and that absorption was dependent on a proliferation of high dose quercetin nutraceutical preparations. However, a major concern has been the poor oral bioavailability of quercetin and most other flavonoids (11).

Fig. 1


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The original model of flavonoid absorption assumed that flavonoid glucosides were too polar to be absorbed from the small intestine and that absorption was dependent on the cleavage of the β-glucoside linkage by the colonic microflora (12). In 1995, Hollman et al. (13) indirectly calculated the absorption of quercetin glucosides from an onion meal in ileostomy patients. The authors proposed that these glucosides were actively absorbed via the intestinal glucose transporter. However, there were no direct measurements of the quercetin glucosides in the ileostomy fluid or plasma to support such a conclusion. In contrast, in vitro studies using human intestinal Caco-2 cell monolayers as a model of human intestinal absorption showed complete lack of absorption of the glucosides, mainly due to effective efflux by the multidrug resistance protein 2 transporter (MRP2) (14,15), whereas quercetin itself was easily absorbed (16). In a subsequent reinvestigation of the absorption of the quercetin glucosides in ileostomy patients, we found that the glucosides were efficiently hydrolyzed in the small intestine, potentially independently of bacterial enzymes (17). This conclusion is supported by in vitro studies (16,18–20). After hydrolysis of the glucosides in the ileostomy patients, it was calculated that the absorption of the quercetin aglycone may be as high as 65–81% (17).

On the basis of these observations, we focused our attention on the absorption and the biological fate of the quercetin aglycone. ¹⁴C-Labeled quercetin (Fig. 1) was administered both orally and intravenously (iv)⁴ to normal, healthy volunteers, greatly facilitating estimates of recoveries and fraction absorbed. A large fraction of the oral dose of quercetin was

⁴ Abbreviations used: AUC, area under the curve; ERP, endogenous rate of production; iv, intravenous;
Subjects and study design. Six healthy subjects (23–44 y, 70–110 kg) participated in the study. Two subjects were female; one was Asian and five were Caucasian. Written informed consents were obtained. The study was approved by the Institutional Review Board for Human Research. The oral and iv radiation doses were estimated to be ~1% of the annual whole-body background radiation in the United States. All subjects were studied in a Clinical Research Unit.

The diet during and for 4 d before the study was low in flavonoids. Oral (6 subjects) and iv (4 subjects) quercetin doses, at least 10 d apart, were administered in the morning after an overnight fast. Breakfast was served 3 h later. Serial blood samples drawn at 0–72 h after the dose were centrifuged at 1000 g for 10 min to separate plasma; six 12-h urine samples were collected with thimerosal and collected (see below).

Oral doses. The oral dose was followed by 500 mL of water. Immediately before administration, 0.6 mL of this solution was added to 10 mL of 1% thymolphthalein. Aliquots (4.0 mL) of this blue solution were put into 20-mL glass scintillation vials and capped tightly. At timed intervals before and after the oral dose (Fig. 2), 100 mg of 14C-quercetin (330 µmol) was infused into the pretext in 10 mL of 1% thymolphthalein (21). Hyamine hydroxide (1 mol/L in methanol, J. T. Baker, Phillipsburg, NJ) was mixed with an equal volume of 100% ethanol and added to 50 mL of 1% thymolphthalein. Aliquots (4.0 mL) of this blue solution were put into 20-mL glass scintillation vials and capped tightly. At timed intervals before and after the oral dose, the subjects were instructed to blow bubbles through a pipet with a one-way valve into a collection vial until the solution turned colorless, at which point 2 mmol of CO₂ was trapped (~1 min). The vials were then tightly capped; after addition of Aquasol-2 and dark-adaptation, they were counted for radioactivity. The predose counts were ~10 dpm/sample, whereas the peak counts after both oral and iv doses were 10,000–40,000 dpm/sample.

Calculations. The areas under the plasma concentration vs. time curves (AUC) were calculated by the trapezoidal rule to the last time point, 72 h. The plasma, urine and exhaled carbon dioxide half-lives were calculated by least-squares linear regression. The fraction of the oral dose absorbed (in %) was calculated as (AUCoral ⋅ Doseoral) / (AUCiv ⋅ Doseiv) ⋅ 100. For calculations of total amount of 14CO₂ exhaled, the endogenous rate of production (ERP) of CO₂ was calculated as 5 mmol CO₂/m² body surface area ⋅ min (21). The dpm exhaled during a collection interval was calculated as dpm measured (corrected for background) ⋅ ERP/2 (mmol). This value could then be expressed as a percentage of the administered dose (110 × 10⁶ dpm) exhaled per hour. The AUC of the percentage of dose exhaled per hour vs. time was finally calculated by the trapezoidal rule to give the total fraction of the dose excreted as 14CO₂.

RESULTS

After an oral dose of 100 mg 14C-quercetin (330 µmol) into six human subjects, an early peak plasma concentration of 270 ng/mL (890 nmol/L) was reached as early as 30 min after the dose (Fig. 2). At 8 h after the dose, there was a second peak of 350 ng/mL (1160 nmol/L). The plasma concentrations then declined exponentially over the entire study period of 72 h. After an iv dose of 0.3 mg 14C-quercetin (1 µmol) in four subjects, there was a rapid fall of the plasma concentrations of total radioactivity over the first 4 h after the bolus injection, indicating the distribution phase (22). The plasma concentrations then fell in parallel with those after the oral dose for the remainder of the study period.

The terminal elimination half-lives were quite long, ranging from 20 to 72 h (Table 1). There was no statistical difference between the two routes of administration in the limited number of individuals studied. The interindividual variability in AUC values for the oral doses was less than twofold, with even less variability for the iv doses (Table 1). For the four individuals who received the iv doses, the absorption, ranging from 36.4 to 53% of the dose, could for the first time be directly determined for quercetin. It should be noted that measured and calculated kinetic parameters in the single obese female subject (110 kg) did not differ from those obtained with the five subjects of normal weight (70–89 kg).
The recoveries in urine and feces after the oral dose were surprisingly low, with 3.3–5.7% of the dose found in the urine and only 0.2–4.6% in the feces (Table 2). After the iv dose, the recoveries in urine were as high as 18.4–26.8% of the dose with only 1.5–5.0% found in the feces. Thus, the overall recoveries in urine and feces were 3.5–8.3% after the oral and 21.3–30.2% after the intravenous dose. Further studies attempting to increase the recoveries of radioactivity, in particular from feces, compared with the procedure described in Methods, gave no improvement.

The time course of 14CO2 formation in three individuals receiving either an oral or an iv dose is shown in Figure 3. The curves for the two routes of administration were very similar except for higher peak levels after the oral dose. The large variability in the early part of the time courses is due mainly to the fact that in some individuals, 14CO2 started to appear in the expired air 4 h after the 14C-quercetin dose, whereas in others not until 8 h after the dose. The 14CO2 formation/exhalation accounted for 23.0–81.1% of the administered 14C-labeled quercetin doses, both oral and intravenous (Table 2).

### DISCUSSION

A large number of studies have been devoted to determining the biological fate of quercetin, mainly as glucosides, the major form in which it appears in the diet. Unfortunately, very little information has been gained from these studies, except that some form of acid- or enzyme-hydrolyzable conjugates does reach the systemic circulation (11,13,23,24) and that some biological activity may be associated with such conjugates (23). Of greater significance may be observations consistent with efficient hydrolysis of the glucoside conjugates in the intestinal lumen to the quercetin aglycone (17,20), which then appears to be efficiently absorbed. The biological fate of the aglycone was the focus of the present study.

In four subjects, who received both an oral and an iv dose of 14C-quercetin, we could for the first time establish the oral absorption of this flavonoid, taking into account a variety of metabolic and chemical breakdown products. The absorption was surprisingly high, ranging from 36 to 53%. This had previously been suggested on the basis of studies in a preclinical absorption model, the Caco-2 cell monolayer (16). After administration of the quercetin glucosides in ileostomy patients, the absorption of quercetin after enzymatic hydrolysis of the glucosides was calculated to be as high as 65–85% (17). The dietary quercetin glucosides may thus, as suggested, act as more soluble quercetin prodrugs with favorable absorption (17).

The terminal elimination half-life for the total quercetin radioactivity was quite long, i.e., 20–72 h. This should be compared with that of quercetin alone, which was only 0.7–2.4 h after iv administration in two previous studies (22,25). When quercetin or quercetin glycosides were administered orally (24,26), half-lives for quercetin of 15–28 h have been reported. However, this was after acid or enzymatic hydrolysis of the samples and would thus be expected to reflect quercetin conjugates. The very long half-life observed in our study could be due to multiple factors. A high volume of distribution does

### TABLE 1

Plasma total radioactivity after oral and intravenous (i.v.) 14C-quercetin doses of 100 mg (330 μmol) and 0.3 mg (1 μmol), respectively, in human subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>100 mg oral dose</th>
<th></th>
<th>0.3 mg i.v. dose</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t1/2, h</td>
<td>AUC, (μmol h)/L</td>
<td>t1/2, h</td>
<td>AUC, (μmol h)/L</td>
</tr>
<tr>
<td>1</td>
<td>50</td>
<td>37.7</td>
<td>20</td>
<td>0.30</td>
</tr>
<tr>
<td>2</td>
<td>64</td>
<td>65.5</td>
<td>33</td>
<td>0.37</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>68.0</td>
<td>36</td>
<td>0.39</td>
</tr>
<tr>
<td>4</td>
<td>31</td>
<td>37.0</td>
<td>72</td>
<td>0.30</td>
</tr>
<tr>
<td>5</td>
<td>39</td>
<td>44.3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>49</td>
<td>51.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>41 ± 8</td>
<td>50.6 ± 5.5</td>
<td>40 ± 11</td>
<td>0.34 ± 0.02</td>
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</tbody>
</table>

### TABLE 2

Recovery of total radioactivity in urine, feces and expired air in human subjects after oral and intravenous (i.v.) 14C-quercetin doses

<table>
<thead>
<tr>
<th>Subject</th>
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<th></th>
<th>0.3 mg (1 μmol) i.v. dose</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine</td>
<td>Feces</td>
<td>CO2</td>
<td>Urine</td>
</tr>
<tr>
<td>% administered dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3.7</td>
<td>4.6</td>
<td>—</td>
<td>18.4</td>
</tr>
<tr>
<td>2</td>
<td>5.4</td>
<td>2.4</td>
<td>—</td>
<td>20.1</td>
</tr>
<tr>
<td>3</td>
<td>5.7</td>
<td>1.6</td>
<td>—</td>
<td>19.7</td>
</tr>
<tr>
<td>4</td>
<td>4.9</td>
<td>1.7</td>
<td>50.7</td>
<td>26.8</td>
</tr>
<tr>
<td>5</td>
<td>3.3</td>
<td>0.2</td>
<td>41.8</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>4.6</td>
<td>0.6</td>
<td>63.9</td>
<td>—</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>4.6 ± 0.4</td>
<td>1.9 ± 0.6</td>
<td>52.1 ± 6.4</td>
<td>21.3 ± 1.9</td>
</tr>
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</table>
not seem to be a significant contributor. The volume of distribution for quercetin itself is thus <0.5 L/kg (22,25) and for the total radioactivity in this study, ~1.4 L/kg. A more likely explanation is enterohepatic recirculation, which may replenish the plasma concentrations over an extended time period. There was very strong evidence for such recirculation after the oral dose, with all subjects showing a distinct second peak of plasma radioactivity 6–12 h after the dose, although there was only weak evidence after the iv dose (Fig. 2). An additional factor, which is the subject of a separate investigation (27), is the potential for covalent binding of quercetin to plasma proteins, presumably after initial enzymatic bioactivation.

The most challenging part of the disposition of quercetin in humans was the metabolic fate, including the route(s) of elimination, which required the use of radioactively labeled compound. The recoveries in both urine and feces after the oral dose were very low, amounting to <10% of the dose. The much higher recovery (~20%) in the urine after the iv dose was interesting. This may be an effect of the much lower dose given iv (0.3 mg) compared with the oral dose (100 mg). Such an effect may be explained by saturation of an efflux transporter in the kidney after the higher oral dose. However, the major fraction of both doses was still unaccounted for, suggesting an alternative route of elimination.

This was explored by measuring potential exhalation of radioactivity in the form of 14CO2, after both oral and iv doses. It was fortuitous that we were able to detect this major metabolic route because the 14C-quercetin used has only one of its 14 carbon atoms labeled, i.e., the one in the 4-position (Fig. 1). The total recoveries of 14C-quercetin in urine, feces and exhaled air in the individuals in Table 2 amounted to 46.7–106.2% of the dose. The reason for this variability in the recoveries of the 14CO2 excretory route is not known. There has been one previous study with 14C-quercetin administration, in this case in rats, in a study previously receiving little attention (28). In that study, 14CO2 exhalation occurred as well. Also, when 14C-quercetin was incubated with rat gut contents, large amounts of 14CO2 were produced. Thus, we conclude that the 14CO2 exhalation in the subjects receiving 14C-quercetin most likely originated from the intestine. This is supported by the finding that in all subjects, negligible amounts of CO2 were formed before 4 h. The efficiency of this total metabolic breakdown of quercetin is likely enhanced by enterohpatic recirculation. The detailed pathway followed for the abundant CO2 formation from quercetin is not under-

stood. It may be a combination of bacterial enzyme-mediated and strictly chemical (nonenzymatic) reactions (29,30). This warrants further studies.

Although the use of 14C-quercetin with the labeled carbon atom in the 4-position of the C-ring (see Fig. 1) as the dosage form provided a number of critically important pieces of information regarding the biological fate of this flavonoid in humans, it also left some unanswered questions. The substantial loss of 14CO2, ranging from 23 to 81%, means that a large proportion of the dose not retaining the 14C-label was unaccounted for. This would be expected to include small phenolic carboxylic acids derived either from the A-ring or the B-ring (11,29,30), which would be expected to be excreted mainly in urine (30). Because the C-ring of quercetin is opened and cleaved in this process, complete recovery of the radioactive doses of quercetin would require specific labeling of both the A- and the B-ring.

Of key importance now is to attempt to define the nature of the radioactivity in plasma. Preliminary measurements support previous observations (17,23,24,31) that there is no unchanged quercetin in plasma after oral doses. The main metabolites may be glucuronide conjugates (17), recently confirmed by mass spectrometry (31). Such conjugates may have antioxidant activity (32). However, other metabolites with potential antioxidant activity (23), including glutathione conjugates (33) and small phenolic carboxylic acids (see above), cannot be excluded. Alternatively, it may consist of labile covalent adducts to plasma proteins, as recently suggested (27). These studies are currently in progress.

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LITERATURE CITED


