

Pharmacokinetics and Bioavailability of Quercetin Glycosides in Humans

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Due to its potentially beneficial impact on human health, the polyphenol quercetin has come into the focus of medicinal interest. However, data on the bioavailability of quercetin after oral intake are scarce and contradictory. Previous investigations indicate that the disposition of quercetin may depend on the sugar moiety of the glycoside or the plant matrix. To determine the influence of the sugar moiety or matrix on the absorption of quercetin, two isolated quercetin glycosides and two plant extracts were administered to 12 healthy volunteers in a four-way crossover study. Each subject received an onion supplement or quercetin-4'-O-glucoside (both equivalent to 100 mg quercetin), as well as quercetin-3-O-rutinoside and buckwheat tea (both equivalent to 200 mg quercetin). Samples were analyzed by HPLC with a 12-channel coulometric array detector. In human plasma, only quercetin glucuronides, but no free quercetin, could be detected. There was no significant difference in the bioavailability and pharmacokinetic parameters between the onion supplement and quercetin-4'-O-glucoside. Peak plasma concentrations were $2.3 \pm 1.5 \mu\text{g}\cdot\text{mL}^{-1}$ and 2.1 ± 1.6

$\mu\text{g}\cdot\text{mL}^{-1}$ (mean \pm SD) and were reached after 0.7 ± 0.2 hours and 0.7 ± 0.3 hours, respectively. After administration of buckwheat tea and rutin, however, peak plasma levels were—despite the higher dose—only $0.6 \pm 0.7 \mu\text{g}\cdot\text{mL}^{-1}$ and $0.3 \pm 0.3 \mu\text{g}\cdot\text{mL}^{-1}$, respectively. Peak concentrations were reached 4.3 ± 1.8 hours after administration of buckwheat tea and 7.0 ± 2.9 hours after ingestion of rutin. The terminal elimination half-life was about 11 hours for all treatments. Thus, the disposition of quercetin in humans primarily depends on the sugar moiety. To a minor extent, the plant matrix influences both the rate and extent of absorption in the case of buckwheat tea administration compared with the isolated compound. The site of absorption seems to be different for quercetin-4'-O-glucoside and quercetin-3-O-rutinoside. The significance of specific carriers on the absorption of quercetin glycosides, as well as specific intestinal *b*-glucosidases, needs to be further evaluated.

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The flavonol quercetin (5,7,3',4'-hydroxyflavanol) (Figure 1) is a polyphenolic compound widely distributed in higher plants and thus constitutes a part of our daily diet with an average daily intake of 16 mg (expressed as aglycone).¹ In food, quercetin is usually glycosidated—mainly bound to glucose and rutinose (a 6-O-rhamnosyl-glucose)—with high concentrations found in onions, tea, and apples.^{1,2} Some epidemiological studies point to potential beneficial effects of a high intake of dietary flavonoids on the prevention of cardiovascular disease.^{3,4} Flavonoid glycosides are also considered to be the efficacious compounds of phytomedicines (e.g., buckwheat tea) used in the treatment of chronic venous insufficiency in certain countries.⁵⁻⁷ Rutin (quercetin-3-O-rutinoside) as well as quercetin can be found on the U.S. market as dietary

supplements. It is assumed that the observed protective effects derive from the antioxidative and radical-scavenging capacity that quercetin shows in many *in vitro* experiments.^{8,9} The bioavailability of the aglycone quercetin is, however, very poor. No free quercetin could be detected in human plasma after oral intake of high amounts of quercetin.^{10,11} Quercetin circulates in plasma only in conjugated form, and the antioxidant capacity of the detected quercetin metabolites is considerably lower compared with quercetin.⁸ Furthermore, after oral intake of the aglycone, even the total quercetin plasma concentrations measured after hydrolysis of quercetin conjugates were very low.¹⁰ However, total quercetin concentrations in plasma increased considerably when quercetin glycosides were administered instead of the aglycone.¹² Yet it is still poorly understood whether the nature of the glycoside (e.g., glucose or rutinose); its binding site to quercetin at the 3, 5, 7, or 4' position; or the plant matrix might account for the enhanced absorption of quercetin. Also, little is known about the sites of absorption and the mechanisms involved. It has been suggested that the intestinal sodium-glucose cotransporter might be involved in the absorption of quercetin glycosides, which has a higher affinity for quercetin glucosides than other glycosides.^{13,14} Recently, a human β -glucosidase was found in the liver, kidney, and intestinal wall with a high affinity for (iso)flavonoid glycosides when a glucose residue was bound in the 7 or 4' position, while the 3-glycosides of flavonols were not substrates possibly due to steric hindrance.¹⁵ Furthermore, various intestinal bacteria are known for both hydrolysis of quercetin glycosides and cleavage of quercetin to phenolic acids.¹⁶⁻¹⁹

To determine the influence of the sugar moiety or plant matrix on the absorption of quercetin, two isolated quercetin glycosides (quercetin-4'-*O*-glucoside and quercetin-3-*O*-rutinoside) (Figure 1) and the two corresponding plant extracts containing high amounts of these glycosides (onions and buckwheat tea) were administered to 12 healthy volunteers in a four-way crossover study.

SUBJECTS AND METHODS

Subjects

The study was carried out at the Institute of Experimental and Clinical Pharmacology and Toxicology, Department of Clinical Pharmacology, University of Rostock, Germany. Twelve healthy volunteers, 9 men and 3 women, were recruited for the study after full clinical examination. Routine blood and urine laboratory tests

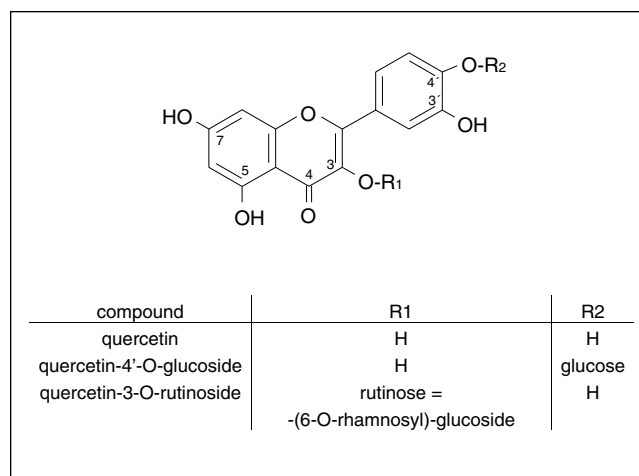


Figure 1. Structure of quercetin, quercetin-4'-*O*-glucoside, and quercetin-3-*O*-(6-*O*-rhamnosyl)-glucoside (= rutin).

were performed. Mean age was 24.3 ± 1.4 years (mean \pm SD), and mean body mass index was $21.7 \pm 2.4 \text{ kg}\cdot\text{m}^{-2}$. Subjects were not allowed to use any medicine during the study, except for oral contraceptives. All subjects signed an informed consent form according to the provisions of the Helsinki and good clinical practice (GCP) guidelines. The protocol was approved by the ethics committee of the University of Rostock, Germany.

Study Design and Supplements

Each subject received, according to a randomized four-way crossover design, the following treatments separated by a 24-hour washout interval: an onion supplement consisting of 160 g stewed and homogenized onions, which provided 331 μMol quercetin glucosides—mainly, quercetin-3,4'-*O*-diglucoside and quercetin-4'-*O*-glucoside (equivalent to 100 mg quercetin); 331 μMol quercetin-4'-*O*-glucoside isolated from onions (equivalent to 100 mg quercetin); buckwheat tea powder (SmithKline Beecham, Herrenberg, Germany, CH 980800010), providing 662 μMol quercetin rutinoside (equivalent to 200 mg quercetin); and 662 μMol quercetin-3-*O*-rutinoside (grounded Rutinon[®] forte tablets, biomo Natur-Medizin, Siegburg, Germany) (equivalent to 200 mg quercetin). Except for the onion supplement, each supplement was suspended in a formulation of a 0.5% hydroxypropylmethylcellulose gel (Methocel[®]K4M Premium EP, Dow Germany, Inc., Schwalbach, Germany) containing 15% ethanol (w/w). The preparation and administration of the formulations were carried out according to the European Pharmacopoeia and GCP guidelines. Subjects were supplied with a flavonoid-

free diet while being hospitalized, which was achieved by avoiding all fruit and vegetables from days 1 to 11 of the study. After a 3-day run-in phase, subjects received one of the four supplements on days 4, 6, 8, and 10. During the first 2 hours after administration, subjects were allowed to drink water only.

Collection of Blood and Urine Samples

Venous blood samples (9 mL per blood sample) were collected into EDTA tubes once before subjects ingested the supplements; at 15, 30, 45, and 60 minutes after administration; and at 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, and 48 hours after administration. Blood was centrifuged for 10 minutes at $4700 \times g$ at 4°C . The supernatant plasma was transferred into reaction cups in aliquots of 0.5 mL, and 20 μL acetic acid 0.58 M was added to each aliquot for stabilization of phenols. The plasma was stored at -80°C until analysis. Urine was collected in plastic bottles from days 4 to 11 in intervals of 0 to 3, 3 to 6, 6 to 9, 9 to 12, 12 to 24, 24 to 36, and 36 to 48 hours. An aliquot of 50 mL of each sample was mixed with 1 g ascorbic acid as antioxidant and stored at -80°C until analysis.

Analytical Methods

For the determination of both conjugated metabolites and the total concentration of quercetin and its metabolites, each sample was extracted twice with and without enzymatic hydrolysis of conjugated metabolites. In detail, 10 μL of ascorbic acid 0.5% (w/v) was added to 500 μL of thawed plasma or urine, and the pH adjusted to 5.0 with approximately 50 μL 0.58 M acetic acid. For hydrolysis of conjugated metabolites, 30 μL β -glucuronidase/arylsulfatase (HP-2S ex *Helix pomatia*, Sigma, Deisenhofen, Germany) was added, vortexed, and incubated at 37°C for 60 minutes. The reaction was stopped by adding 600 μL acetone and 50 μL naringenin ($34.2 \mu\text{g}\cdot\text{mL}^{-1}$ in acetone for plasma samples and $146 \mu\text{g}\cdot\text{mL}^{-1}$ for urine samples) used as internal standard. After vortexing for 1 minute, the mixture was shaken for 10 minutes and centrifuged for 10 minutes at $7830 \times g$. The supernatant was transferred into a cup containing 10 μL ascorbic acid 0.5% (m/v) and evaporated in a vacuum centrifuge until almost dry. The residue was redissolved in 150 μL dimethylformamide/water 2:1 (v/v) and centrifuged for 10 minutes at $7830 \times g$, and the supernatant was used for HPLC analysis. Validation of the method was performed according to "FDA Draft Guidance for Industry No. 2578" (Bioanalytical Methods/Validation for Human Studies). The intra- and interday coefficient of variation of the assay

was below 3% and 5%, respectively. Stability of the samples was given over 18 weeks at -20°C and -80°C . Analysis of plasma samples was carried out by the German Institute of Nutrition (Bergholz-Rehbruecke, Germany) using HPLC coupled with a 12-channel coulometric array detector (ESA, Inc., Bedford, MA). Quercetin, isorhamnetin, and their conjugates were detected at 150 mV. The lower limit of quantification was $2 \text{ ng}\cdot\text{mL}^{-1}$. Analysis of urine samples was performed by HPLC with photodiodearray detection with a lower limit of quantification of $20 \text{ ng}\cdot\text{mL}^{-1}$, as described previously.²⁰ Linearity, range, and calibration curves were determined after spiking blank plasma and urine samples with reference compounds at eight different concentrations, followed by extraction and HPLC analysis. The correlation coefficient for all calibration curves was above 0.99, proving repeatability and intermediate precision of the assays over the concentration range. The stability of the assays was surveyed by both the peak area of the internal standard in each sample and by external quality control samples.

Data Analysis

Pharmacokinetic parameters were determined by noncompartmental analysis using Microsoft Excel based on the equations given by Gibaldi and Perrier.²¹ The maximum observed plasma concentration C_{max} and the time to reach C_{max} (t_{max}) were determined directly from the data. The $\text{AUC}_{0 \rightarrow 24\text{h}}$ was calculated using the linear trapezoidal rule. The apparent terminal elimination half-life was determined by linear regression of the terminal portion of the semilogarithmic plasma concentration-time profiles.

Statistical Analysis

Data are presented as mean and standard deviation. Differences among the results after administration of the four treatments were tested for significance by a two-way analysis of variance, followed by Tukey's test with a significance level of $p < 0.05$ using Sigma Stat[®] for Windows, version 2.0 (Jandel Scientific Software, SPSS, Chicago).

RESULTS

Quercetin-4'-*O*-glucoside or quercetin-3-*O*-rutinoside was not present in human plasma or urine. Also, no free quercetin could be detected prior to enzymatic hydrolysis of the conjugates (Figure 2). Instead, four to five quercetin glucuronides were identified as quercetin metabolites by LC-MS/MS, with one major

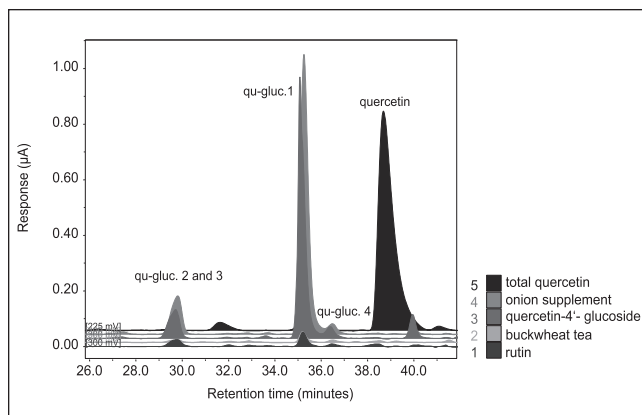


Figure 2. HPLC chromatograms of plasma samples before (1-4) and after (5) enzymatic hydrolysis of quercetin conjugates. Four quercetin glucuronides could be detected by LC-ECD with one major (qu-gluc. 1 at 35.2 min) and three minor metabolites (qu-gluc. 2, 3, and 4 at 29.3 min, 29.8 min, and 36.5 min, respectively). The metabolic pattern was identical for all treatments (1-4). Row 5 represents the corresponding total quercetin concentration of the plasma sample shown in row 4 after intake of the onion supplement.

and three to four minor metabolites.²² The metabolic pattern was identical for all four treatments (Figure 2). Furthermore, the plasma concentration versus time curves of the glucuronides showed parallel absorption and elimination profiles (data not shown), which suggests that there is no transformation from one metabolite into another. Hence, the quantification of quercetin

is based on the total quercetin concentration after hydrolysis of conjugates, which mainly represents the characteristics of one major metabolite.

The time course of the total quercetin concentrations in human plasma after administration of onions, quercetin-4'-*O*-glucoside, buckwheat tea, and quercetin-3-*O*-rutinoside is shown in Figure 3. The plasma concentration versus time profile of total quercetin after intake of onions or quercetin-4'-*O*-glucoside did not differ significantly, nor did the pharmacokinetic parameters and the bioavailability as given in Table I. Quercetin was rapidly absorbed from onions as well as quercetin-4'-*O*-glucoside, and peak plasma concentrations of $2.3 \pm 1.5 \mu\text{g}\cdot\text{mL}^{-1}$ and $2.1 \pm 1.6 \mu\text{g}\cdot\text{mL}^{-1}$ (mean \pm SD) were reached after 0.7 ± 0.2 hours and 0.7 ± 0.3 hours, respectively. The plasma concentration profile was biphasic, with the terminal phase beginning at 9 to 12 hours after administration.

After administration of buckwheat tea and rutin, peak plasma levels were—despite the twofold higher dose of quercetin equivalents—only $0.6 \pm 0.7 \mu\text{g}\cdot\text{mL}^{-1}$ and $0.3 \pm 0.3 \mu\text{g}\cdot\text{mL}^{-1}$, respectively. Peak concentrations were reached 4.3 ± 1.8 hours after administration of buckwheat tea and 7.0 ± 2.9 hours after ingestion of rutin. Correspondingly, the bioavailability as indicated by the $\text{AUC}_{0 \rightarrow 24\text{h}}$ was about half of that after intake of quercetin glucosides. C_{max} , t_{max} , and $\text{AUC}_{0 \rightarrow 24\text{h}}$ of buckwheat tea and rutin were significantly different from onions and quercetin-4'-glucoside. In addition, t_{max} of

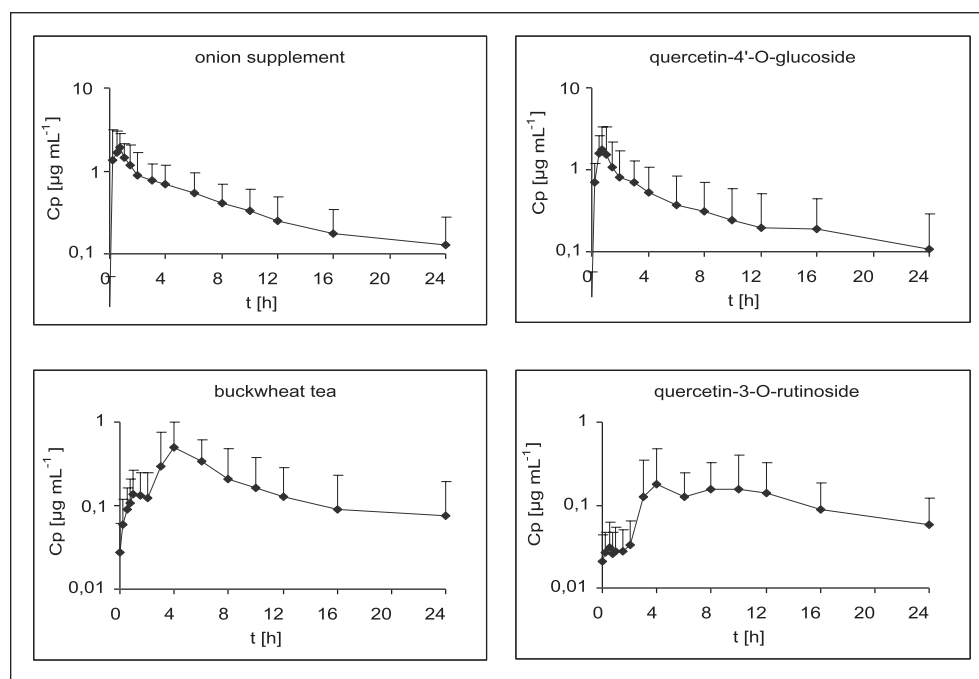


Figure 3. Total quercetin concentration in plasma (mean \pm SD) of 12 volunteers after administration of 100 mg (331 μMol) quercetin in the form of an onion supplement or quercetin-4'-*O*-glucoside or 200 mg (662 μMol) of quercetin in the form of buckwheat tea or quercetin-3-*O*-rutinoside (rutin) in a randomized crossover design.

Table I Pharmacokinetic Parameters of Total Quercetin Absorption and Elimination in Human Plasma after Single Oral Administration of Four Supplements in a Crossover Study

Parameter	Onion Supplement	Quercetin-4'-O-Glucoside	Buckwheat Tea	Quercetin-3-O-Rutinoside
Dose (mg)	100	100	200	200
C_{\max} ($\mu\text{g}\cdot\text{mL}^{-1}$)	2.31 \pm 1.46 (1.01-5.67)	2.12 \pm 1.63 (0.70-6.60)	0.64 \pm 0.67* (0.06-2.24)	0.32 \pm 0.34* (0.02-1.04)
t_{\max} (h)	0.68 \pm 0.22 (0.30-1.00)	0.70 \pm 0.31 (0.48-1.48)	4.32 \pm 1.83* (1.52-8.00)	6.98 \pm 2.94** (3.00-12.10)
$t_{1/2}$ (h)	10.9 \pm 4.1 (5.0-20.3)	11.9 \pm 4.0 (7.4-19.4)	10.3 \pm 3.5 (4.0-16.5)	11.8 \pm 3.1 (8.4-18.5)
$\text{AUC}_{0\rightarrow 24\text{h}}$ ($\text{h}\cdot\mu\text{g}\cdot\text{mL}^{-1}$)	9.7 \pm 6.9 (2.7-26.9)	8.4 \pm 9.1 (2.6-34.9)	3.8 \pm 3.9* (0.4-11.0)	2.5 \pm 2.2* (0.2-7.3)
MRT_{abs} (h)	11.8 \pm 5.1 (4.2-20.7)	11.9 \pm 5.4 (6.3-24.6)	14.0 \pm 5.4 (6.9-25.5)	19.5 \pm 5.4** (11.3-27.3)
MAT (h)	0.15 \pm 0.07 (0.05-0.30)	0.16 \pm 0.10 (0.08-0.40)	1.41 \pm 0.72* (0.4-2.9)	1.83 \pm 1.87* (0.2-5.6)
Cl_{tot}/f ($\text{L}\cdot\text{h}^{-1}$)	13.3 \pm 8.9 (2.6-34.6)	17.4 \pm 9.8 (2.0-35.9)	131 \pm 134* (11-430)	159 \pm 184* (20-553)
Vd_{ss}/f (L)	128 \pm 66 (48-241)	181 \pm 119 (38-499)	1653 \pm 1887 (190-5000)	2905 \pm 3745* (238-13,258)
$\text{Vd}_{\text{area}}/f$ (L)	189 \pm 121 (54-432)	273 \pm 144 (43-570)	1951 \pm 2065 (214-5641)	2790 \pm 3692* (305-12,168)

C_{\max} , peak plasma concentration; t_{\max} , time to reach C_{\max} ; $t_{1/2}$, elimination half-life; $\text{AUC}_{0\rightarrow 24\text{h}}$, area under the concentration-time curve from 0 to 24 hours; MRT_{abs} , mean residence time after extravascular administration; MAT, mean absorption time; Cl_{tot}/f , total body clearance with respect to unknown bioavailability f ; Vd_{ss}/f , volume of distribution at steady state with respect to unknown bioavailability f ; $\text{Vd}_{\text{area}}/f$, volume of distribution during the elimination phase with respect to unknown bioavailability f .

Data are expressed as mean \pm SD; the range (minimum-maximum) is given in parentheses ($n = 12$).

* Significant difference ($p < 0.05$) between the two glucoside groups. ** Significant difference within one group (buckwheat tea vs. rutin).

rutin was also significantly different from buckwheat tea due to a profound lag time of 3.1 ± 1.6 hours, which was observed after administration of rutin. Average peak plasma concentrations after intake of rutin were only about half of those after intake of buckwheat tea. However, due to a high variability of the data, the difference is not statistically significant (Table I).

The elimination half-life was 10 to 11 hours after administration of all four treatments. Thus, differences could only be observed with respect to the rate and extent of absorption.

Isorhamnetin (3'-O-methylquercetin) was the only detectable phase I metabolite with an intact flavonoid moiety. Again, only conjugated forms of isorhamnetin were present in human plasma and urine. The time course of isorhamnetin measured after hydrolysis of conjugates was parallel to that of quercetin with regard to both absorption and elimination phases. In plasma, the total concentrations of isorhamnetin conjugates were about one-tenth of total quercetin concentrations.

In urine, the elimination of both quercetin and isorhamnetin glucuronides was completed within the 48-hour intervals. The amount of quercetin and

isorhamnetin conjugates excreted in 48-hour urine after intake of onions was $6.4\% \pm 2.5\%$ of quercetin glucosides intake and significantly higher than after intake of the pure compound ($4.5\% \pm 1.7\%$) (Table II). Only $1.0\% \pm 0.8\%$ of the dose was recovered in urine as quercetin and isorhamnetin conjugates after administration of buckwheat tea and $0.9\% \pm 0.9\%$ after intake of rutin. The renal clearance, however, did not differ among the four treatments and was consistently $0.7 \text{ L}\cdot\text{h}^{-1}$, which is in accordance with the assumption that identical quercetin metabolites are formed and eliminated regardless of the quercetin glycosides applied.

DISCUSSION

Quercetin is present in human plasma only in conjugated form. Our LC-MS/MS experiments gave no evidence for quercetin sulfates, which confirms previous observations made by Watson and Oliveira.²³ Glucuronidation seems to be the dominant metabolic pathway in humans, whereas sulfatation has also been observed

Table II Renal Clearance of Quercetin and Elimination of Total Quercetin and Isorhamnetin in Urine Expressed as Percentage of Quercetin Intake within 48 Hours after Single Oral Administration of the Four Supplements

Parameter	Onion Supplement	Quercetin-4'-O-Glucoside	Buckwheat Tea	Quercetin-3-O-Rutinoside
Quercetin intake (mg)	100	100	200	200
Quercetin (%)	4.4 ± 1.6	3.3 ± 1.3	0.8 ± 0.6*	0.7 ± 0.6*
Isorhamnetin (%)	2.0 ± 1.3**	1.1 ± 0.5**	0.2 ± 0.2*	0.2 ± 0.2*
Total (%)	6.4 ± 2.5**	4.5 ± 1.7**	1.0 ± 0.8*	0.9 ± 0.9*
Renal clearance (L•h ⁻¹)	0.7 ± 0.4	0.7 ± 0.4	0.7 ± 0.4	0.7 ± 0.5

* Significant difference ($p < 0.05$) between the two glycoside groups. ** Significant difference within one group (onions vs. quercetin-4'-O-glucoside).

in rats.^{22,24,25} Thus, the in vitro activities of free quercetin cannot be directly transferred to the conditions in vivo, and the observed protective effects of a high intake of quercetin might rather be due to bioactive metabolites. Quercetin conjugates also have antioxidant activity in vitro, although the antioxidant activity is only about half that of quercetin aglycone.⁸

So far, little has been known about the conjugated metabolites after intake of quercetin since in most studies, only the total quercetin concentration after hydrolysis of conjugates has been determined.²⁶⁻²⁹ The results of this study demonstrate that independent of the quercetin glycoside administered, one major metabolite, a quercetin glucuronide, is formed together with three to four minor conjugates. Thus, the compounds that circulate in human blood after administration of different quercetin glycosides are identical. This is also supported by the fact that the elimination half-life, as well as the renal clearance, is almost identical for all four treatments. However, the disposition of these compounds is highly dependent on the glucose moiety attached to quercetin. The relative bioavailability of these metabolites (calculated from total quercetin concentrations) after intake of rutin is only about 15% to 20% of that of quercetin-4'-glucoside based on AUC and renal excretion data.

After intake of quercetin-4'-O-glucoside, considerable plasma concentrations could already be detected after 15 minutes, and peak plasma concentrations of 2.1 $\mu\text{g}\cdot\text{mL}^{-1}$ were reached after 40 minutes. This corresponds well with the peak concentrations of 1.3 $\mu\text{g}\cdot\text{mL}^{-1}$ reached 30 minutes after intake of 100 mg quercetin-4'-O-glucoside as reported by Olthof et al.²⁹

The bioavailability and pharmacokinetics of total quercetin did not differ significantly after administration of onions and quercetin-4'-O-glucoside. Thus, the plant matrix of onions has no determinable impact on the absorption of quercetin glycosides. Total renal excretion of quercetin and isorhamnetin was, however,

significantly higher after the onion supplement. This might point to a higher bioavailability of quercetin from onions, which was not determinable from plasma data possibly due to large intersubject variability. The bioavailability of quercetin (conjugates) is higher when rutin is administered as a plant extract than as a pure compound. This is mainly due to a shorter lag time in the case of buckwheat tea, which resulted in higher plasma concentrations.

The mechanism of absorption of quercetin or its glycosides is still unclear. The fast absorption in the case of quercetin-4'-O-glucoside suggests that absorption takes place in the upper part of the intestine, where hydrolysis of the glucoside by intestinal bacteria is negligible. Thus, the data indicate that active absorption mechanisms are involved. Considering both recent published data and our own observations, a possible approach has been postulated in the course of these investigations. Most likely, the cleavage of glycosides occurs at the luminal side of the intestine.³⁰ The enzymes involved seem to have a higher affinity for the glucosides than rutinoside. Recently, it has been reported that the lactase site of lactase phlorizin hydrolase, a membrane-bound β -glucosidase found on the brush border of the mammalian small intestine, effectively hydrolyzed quercetin-4'-glucoside and quercetin-3-glucoside.³¹ Apparently, the released quercetin aglycone diffuses passively across the brush border. However, administration of quercetin aglycone resulted in even lower plasma concentrations and renal excretion of quercetin glucuronides compared with rutin, as observed in a previous pilot study.¹⁰ Possibly the absorption of rutin as well as the administered aglycone is hindered by the intestinal mucus layer, which provides a barrier for lipophilic substances and compounds with a molecular weight of more than 600 to 700 $\text{g}\cdot\text{mol}^{-1}$.³² The transport through the mucus toward the intestinal wall is thus more difficult for the lipophilic quercetin aglycone and also for rutin (due to

its molecular weight) than for quercetin monoglucosides. Furthermore, the administered aglycone is chemically unstable in the pH and temperature conditions of the small intestine. The rutinose, however, may protect quercetin from chemical and/or bacterial degradation on its gut passage. In the lower intestine, the rutinose is cleaved under the influence of bacterial microflora.^{13,16} This may result in increased local quercetin concentrations, which stimulate diffusion through the mucus and across the brush border. The absorption from a buckwheat tea preparation compared with the pure compound may be increased due to cofactors in the plant matrix, which not only increase the solubility of rutin but also decrease the viscosity of the mucus layer.^{33,34} Additional research will be needed to support this approach.

After absorption, quercetin is quickly metabolized. The complete lack of free quercetin and the parallel concentration-time profiles of all metabolites suggest that the metabolism of quercetin to the corresponding glucuronides or methylation to isorhamnetin seems to occur primarily in the enterocyte. This assumption is supported by in situ experiments with rat intestine, in which quercetin was rapidly and extensively conjugated in the enterocytes.^{24,30}

The intersubject variability was very high despite the homogeneous study group, hospitalization, and identical food supply. However, when considering each subject individually, the order among the treatments remained generally the same (onions \geq quercetin-4'-glucoside \gg buckwheat tea $>$ rutin) (e.g., one subject who absorbed very little rutin also absorbed less quercetin-4'-glucoside). This demonstrates that for further studies on flavonoids and possibly other polyphenols, a crossover design is inevitable. Furthermore, the profound intersubject differences need to be taken into consideration when using quercetin glycosides for the prevention or the treatment of chronic diseases.

The pharmacodynamic effect of the detected quercetin metabolites is also still unclear. Depending on the binding site, quercetin glucuronides retained about half of the antioxidant activity compared with quercetin in the in vitro assays.^{8,35} However, this capacity was no longer observed in vivo, most likely due to high plasma protein binding.^{36,37} The intestinal metabolites also showed considerable antioxidant effects.^{17,38} Yet little is known about their potential effectiveness in vivo. Based on our knowledge today, the observed clinical efficacy cannot be related to specific (group of) compounds.

However, the pharmacodynamic effect is not necessarily exerted by those metabolites that can be detected

in plasma. It is presently under investigation whether quercetin glucuronides are deglucuronidated at the site of action (e.g., the endothelium).^{39,40} Various tissues are known for having profound β -glucuronidase activity.⁴¹ A turnover of the glucuronide to the aglycone at the site of action would provide a significant link between in vitro effects and in vivo studies. Further studies on the bioavailability and pharmacokinetics of plant phenolics should thus also include investigations on the mechanisms of absorption and pharmacodynamic effects.

The results of this study demonstrate clearly that the potentially active quercetin metabolites (glucuronides) in human blood are identical regardless of the quercetin glycosides administered. The bioavailability of these metabolites is about five times higher when quercetin is administered as bound to a glucose instead of a rutinose. The binding site does not influence absorption.²⁹ Particularly in the case of rutin administration, bioavailability of potentially bioactive quercetin conjugates after administration of the pure compound was only about two-thirds of that after intake of the corresponding plant extract. This seems to support the use of high-quality phytomedicines in rational phytotherapy as performed in many Western European countries.

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