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Quercetin, but not Its Glycosides, Is Absorbed from the Rat Stomach

VANESSA CRESPY,* CHRISTINE MORAND, CATHERINE BESSON, CLAUDINE MANACH, CHRISTIAN DEMIGNE, AND CHRISTIAN REMESY

Laboratoire des Maladies Métaboliques et des Micronutriments, INRA de Clermont-Ferrand/Theix, 63122 Saint Genès Champanelle, France

Absorption and metabolism of quercetin, isoquercitrin (quercetin 3-*O*-glucose), and rutin (quercetin 3-*O*-glucose-rhamnose) were investigated in rats after in situ gastric administration (15 μmol/L) for 30 min. At the end of the experiment, 38% of the initial dose of quercetin had disappeared. Quercetin was rapidly absorbed by the stomach, and was recovered in the bile 20 min after infusion (4.07 $\pm$ 0.10 μmol/L). The administration of rutin and isoquercitrin indicated that these glycosides were not hydrolyzed nor absorbed by this tissue. In conclusion, when flavonols are present in the diet as aglycons, they could be partly absorbed in the stomach, in contrast to their glycosidic forms which are not absorbed.

KEYWORDS: Quercetin; flavonoid glucosides; stomach; absorption; rat

INTRODUCTION

Quercetin is one of the most abundant flavonoids in the human diet (*1*). Many studies have been focused on the beneficial properties of quercetin, namely its antibacterial, antiviral, antioxidant, antiproliferative, antiinflammatory, and anticarcinogenic effects (*2*). Therefore, it is of great interest to evaluate its bioavailability to fully appreciate its real physiological impact. In food, quercetin is present as glycosylated forms, mainly as β-glycosides. The nature of glycosylation is known to markedly influence the efficiency of quercetin absorption. In rats, as in humans, the absorption of rutin (quercetin-3-*O*-glucose-rhamnose) is delayed compared to that of quercetin, because rutin must be hydrolyzed by the microflora in the distal intestine prior to absorption of the aglycon (*3*, *4*). As the microflora also degrade the aglycon to phenolic acids, the absorption of rutin is less efficient than that of quercetin (*5*, *6*).

In contrast, the glucosylated forms of quercetin can be absorbed in the small intestine, and their absorption is even more efficient than that of the aglycon form (*7*, *8*). Thus, the absorption of glucosides could occur in a completely different way than that known for rutin. Hollman et al. have suggested that the active Na+/glucose cotransporter (SGLT1) could be involved in the transport of flavonol glucosides (*7*). Another study showed that the lactase phloridzin hydrolase present on the brush border, has an affinity for flavonol glucosides in vitro (*9*). These authors suggest that, if in vivo this enzyme was responsible for the hydrolysis of flavonol glucosides, the close proximity of the released aglycon with the membrane may increase the ability of the flavonol to passively diffuse into the enterocytes.

Although specific systems could favor the absorption of quercetin glucosides in the small intestine, the possibility of their absorption or hydrolysis in the stomach has been poorly investigated.

To clarify the fate of flavonols in stomach, we have compared the absorption and the metabolism of quercetin, rutin (quercetin 3-*O*-glucose-rhamnose), and isoquercitrin (quercetin 3-*O*-glucose) in the stomach using an in situ gastric administration.

MATERIALS AND METHODS

Chemicals. Diosmetin, isoquercitrin, and isorhamnetin were purchased from Extrasynthese (Genay, France). Quercetin, rutin, and β-glucuronidase/sulfatase from Helix Pomatia were purchased from Sigma (L'Isle D'Abeau, Chesnes, France).

Animals and Diets. Wistar rats, weighing about 150 g each, were housed, two per cage, in temperature-controlled rooms (22 °C), with a dark period from 8:00 to 20:00 h and access to food from 8:00 to 16:00 h. They were fed a standard semi-purified diet (73% wheat starch, 15% casein, 5% corn oil, 6% mineral mixture, 1% vitamin mixture) during 2 weeks.

Animals were maintained and handled according to the recommendations of the Ethics Committee of the Institut National de la Recherche Agronomique, in accordance with decree n*o*. 87-848.

Flavonol Administration. Rats were anesthetized with sodium pentobarbital (40 mg/kg body weight) 18 h after the beginning of the meal and were kept alive throughout the experiments. After cannulation of the biliary duct, the pylorus was ligated and a physiological buffer was injected into the stomach across the cardia. To prevent any gastro-esophagus reflux, this sphincter was ligated. The stomach was filled in situ with 6 mL of buffer containing KH_2PO_4 (5 mmol/L), K_2HPO_4 (2.5 mmol/L), pH 4.5, NaCl (50 mmol/L), KCl (30 mmol/L), $CaCl_2$ (2 mmol/L), $MgCl_2$ (1 mmol/L), acetic acid (20 mmol/L), $C_6H_5O_7K_3$ (10

* Correspondence should be sent to this author at the address above. Tel: 04 73 62 42 33. Fax: 04 73 62 46 38. E-mail: crespy@clermont.inra.fr.

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Table 1. Concentration and Fluxes of Quercetin and Its Glycosides after Their Administration in Gastric Lumen[a]

	gastric lumen			bile	
	initial concentration μmol/L	final concentration μmol/L	net transfer into the gastric wall nmol/min	quercetin nmol/min	isorhamnetin nmol/min
quercetin	14.78 ± 0.13	9.21 ± 0.40*	1.11 ± 0.08	0.013 ± 0.001	0.043 ± 0.001
rutin	15.59 ± 0.10	15.12 ± 0.70	ND	ND	ND
isoquercitrin	15.74 ± 0.20	15.51 ± 0.50	ND	ND	ND

[a] The presence of an asterisk (*) indicates significantly different from the initial concentration. ND indicates not detected. The biliary fluxes of quercetin and isorhamnetin were calculated from the bile concentration of these compounds and the biliary flow rate (13.83 μL/min).

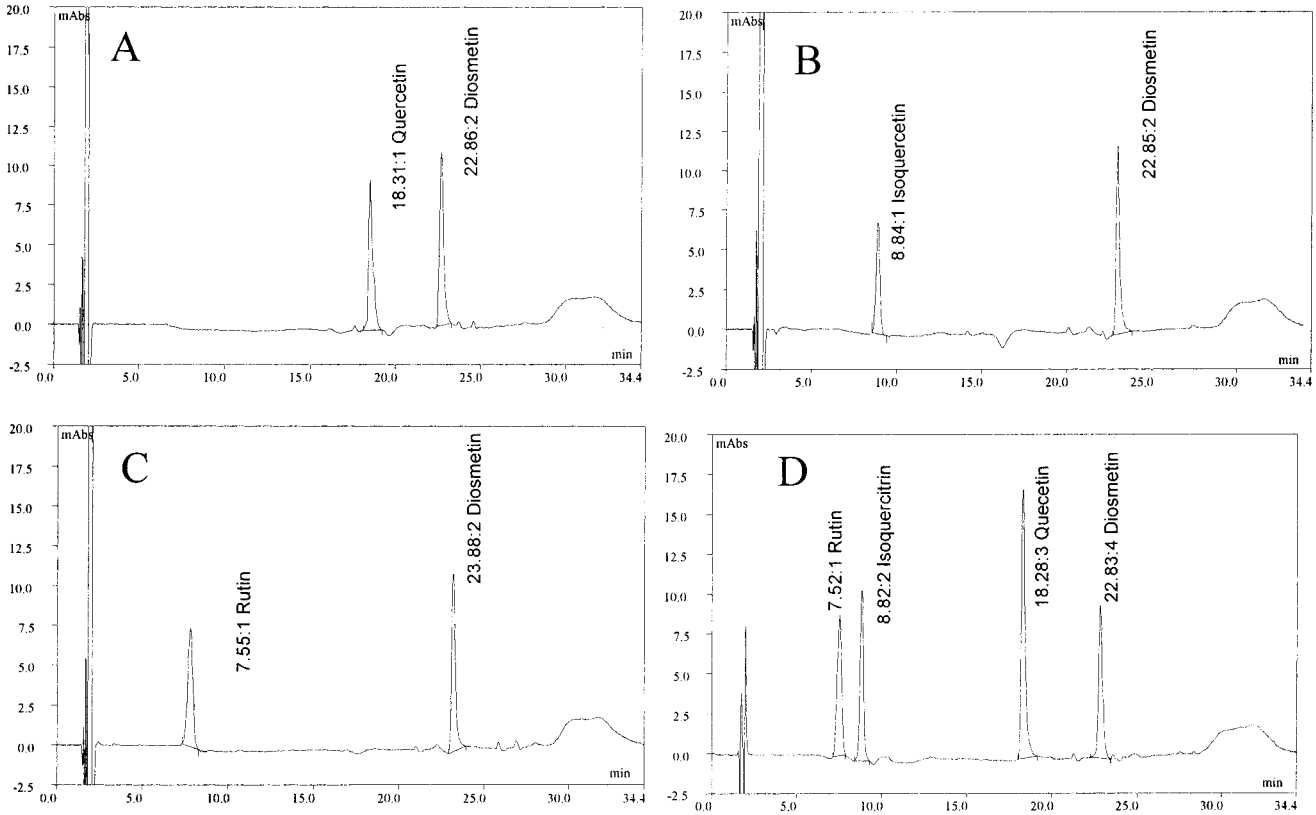

Figure 1. Representative HPLC–UV (370 nm) chromatograms of nonhydrolyzed stomach content after a 30-min incubation of quercetin (A), isoquercitrin (B), and rutin (C). A typical chromatogram of a mix of standards at 5 μM (rutin, isoquercitrin, quercetin, and diosmetin (internal standard)) was given in D.

mmol/L), and PEG (5 g/l), at 37 °C and supplemented with (i) 15 μmol/L rutin, (ii) 15 μmol/L isoquercitrin, or (iii) 15 μmol/L quercetin. This buffer was elaborated to mimic the osmotic and pH conditions found in the stomach during a meal.

At 30 min after administration, stomach contents and blood samples (aorta) were withdrawn and bile was collected in two fractions at 0 to 20 min and 20 to 30 min. Samples were acidified with 10 mmol/L acetic acid and then stored at −20 °C.

HPLC Analysis. *Sample Treatment.* Plasma, bile, and content-of-stomach samples were spiked with diosmetin as an internal standard (5 μM). Samples were acidified with 0.1 vol of acetic acid 0.58 mol/L, except for stomach content, and then incubated for 30 min at 37 °C in the absence (unconjugated forms) or in the presence (total forms) of 5×10^6 units/L β-glucuronidase and 2.5×10^5 units/L sulfatase. Proteins were eliminated by adding 2.85 vol of methanol/HCl 200 mmol/L, and centrifugation was performed for 4 min at 14 000*g*. The supernatant (20 μL) was injected and analyzed by HPLC.

Absorption by the gastric wall was estimated by the difference between the concentrations recovered at the beginning and at the end of the incubation.

Chromatographic Conditions. The HPLC system used consisted of an UV detector (set at 370 nm for flavonols). The system was fitted with a 5-μm C-18 Hypersil BDS analytical column (150 × 4.6 mm; Life Sciences International, Cergy, France). The mobile phase consisted of H_2O/H_3PO_4 (99.5:0.5) (solvent A) and acetonitrile (solvent B). The chromatographic conditions were as follows (flow rate 1 mL/min): 0−2 min, 15% B; 2−22 min, linear gradient from 15% B to 37% B; 22−24 min, 37% B. This gradient has been used for the analysis for supernatant of stomach content.

Bile and plasma samples were analyzed in isocratic conditions (73% of solvent A and 27% of solvent B) with a flow rate set at 1.5 mL/min during 15 min.

The identification of the compounds present in samples was made by comparison of the HPLC profile with that of standards according to their respective retention times.

Poly(ethylene glycol) Measurements. Poly(ethylene glycol) (PEG), a compound which is not absorbed by the stomach, was added to the gastric buffer. Its concentration in the gastric buffer was determined by the method described by Powell and Malawer (*10*). The ratio between initial concentration and that measured at the end of the experiment reflects the intensity of the gastric secretion. This parameter must be taken into account to obtain the right concentration of quercetin and its glycosides recovered at the end of the experiment.

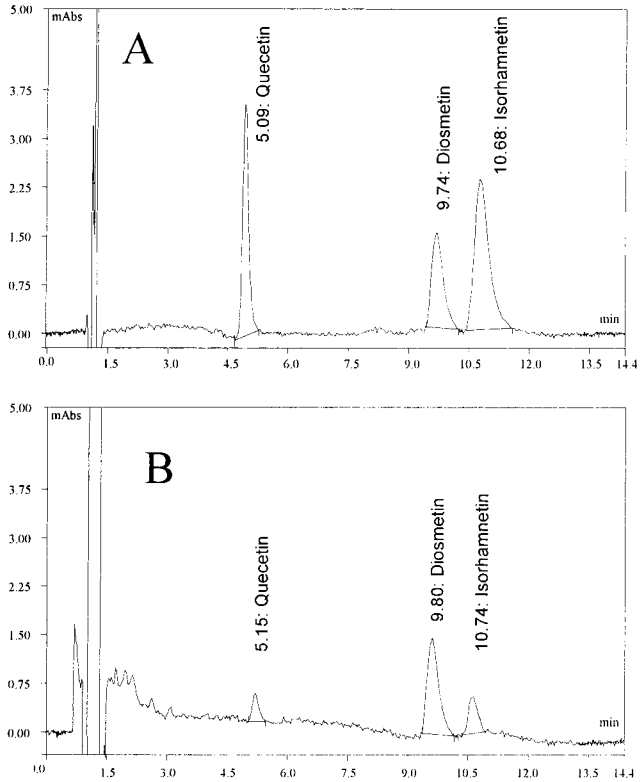

Figure 2. Representative HPLC–UV chromatograms of hydrolyzed bile resulting from a 30-min gastric incubation of quercetin (A). The chromatogram B corresponds to a mix of standards at 5 μM containing quercetin, diosmetin (internal standard), and isorhamnetin.

Statistics. Values are means ± SEM, and the differences between values were determined by one-way ANOVA coupled with the Student−Newman−Keuls multiple-comparison test. Values of $P < 0.05$ were considered significant.

RESULTS

First, it has been checked that aglycon and glycosides were not degraded into the gastric buffer. All the tested compounds were stable in the following conditions: 15 μmol/L incubated for 30 min in the defined gastric buffer pH 4.5.

At the end of the experiment, 62% of the administrated dose of quercetin was recovered in the gastric content (8.59 ± 0.40 μmol/L versus 14.78 ± 0.13 μmol/L) (**Table 1**; **Figure 1A and 1B**), and this rate was not affected by a β-glucuronidase/sulfatase hydrolysis. It corresponded to the absorption of quercetin, and its absorption flux by the gastric wall was to 1.11 ± 0.08 nmol/min. In our experimental conditions, no quercetin was detected in plasma before or after enzymatic hydrolysis. Nevertheless, quercetin and isorhamnetin (3′-*O*-methyl-quercetin) appeared in the bile samples during the 10 last min of the experiment. Their concentrations were 0.94 ± 0.01 μmol/L and 3.13 ± 0.11 μmol/L respectively (**Figure 2**). The biliary flux of these two compounds corresponded to 5% of the total absorption flux of quercetin by the gastric wall (**Table 1**).

When rutin or isoquercitrin (15 μmol/L) were injected into the gastric lumen, their concentrations measured at the end of the experiment were not significantly different from those found at the beginning of the incubation (**Table 1**; **Figure 1C and 1D**). In parallel, the pH of gastric lumen was slightly decreased at the end of the experiment (4.50 ± 0.02 versus 4.16 ± 0.08, $P < 0.05$). This pH lowering, which was also observed in the previous experiment using quercetin, did not promote glycosides hydrolysis and/or their absorption as we failed to detect any trace of glycosides, aglycon, or conjugated forms in the gastric lumen, bile, and plasma.

DISCUSSION

In contrast to other extrahepatic organs, such as the intestine and kidneys, the stomach has been widely ignored as a drug metabolizing organ although it has been identified as a site of absorption for different compounds. The present study clearly shows that quercetin may be absorbed by the stomach and then secreted in the bile. These results are in agreement with a previous study performed in rats, after ligation of pylorus and gavage with daidzein and genistein, and reporting that these compounds were rapidly absorbed by the stomach (*11*). Isoflavones metabolites were detected in the plasma only 3 min after administration, leading the authors to conclude that the absorption in the stomach was a rapid and efficient process. Although isoflavones metabolites were detected in the plasma from the jugular vein, the contribution of the gastric mucosa in the metabolism of flavonoids could not be ruled out because the stomach possesses conjugative enzyme activities (UDP-glucuronosyltransferase, sulfotransferase, and catechol-*O*-methyl transferase) (*12*−*14*). Besides, several in vitro studies showed that 1-naphthol is metabolized in glucuronidated and sulfated metabolites by the gastric wall (*15*, *16*). The mucous cells were identified as the predominant cell type active for sulfation and glucuronidation (*16*).

Recently, we have shown that the small intestine was able to glucuronide, sulfate, and methoxylate quercetin leading to the recovery of these metabolites in the intestinal lumen (*17*). The efflux of quercetin metabolites into the mucosal side could be due to a transporter, probably MRP2, as this has been shown for the conjugated forms of chrysin (*18*). In our present experiment, no conjugated forms of quercetin could be identified in the gastric lumen after quercetin administration. Such a result could be explained by the absence in gastric cells of the transporter involved in the efflux conjugates. However, this point needs further investigation.

The present results support the view that the structural integrity of rutin and isoquercitrin is not affected by pH conditions. This is in agreement with the data of Gee et al. (*19*) who failed to detect any trace of quercetin after incubation of isoquercitrin or rutin at pH 2 for 2 h at 37 °C. By contrast, another study reported a slight degree of deglycosylation in an incubation test simulating the physiological conditions of the stomach (pH 1.5; 37 °C; 2 h) (*20*).

In a previous study dealing with the absorption and metabolism of flavonoids glucosides by the stomach, Piskula et al. showed that no metabolites of isoflavone glucosides (daidzin and genistin) were recovered in plasma, when the site of absorption was restricted to the stomach (*11*). These data suggested that isoflavone glucosides were not metabolized and absorbed from this tissue and perfectly corroborates with our present findings on flavonol glycosides absorption in stomach. By contrast, only 2 h after gavage of rats with a persimmon extract, the presence of the flavonoids glucosides and of their corresponding aglycon in the gastric wall was found (*20*). These authors suggested that this tissue could possess a β-glucosidase liable to hydrolyze these glucosides. However, the HPLC−DAD profile of persimmon extract presented in this study was quite complex, due to the presence of numerous flavonoids. So the chromatographic conditions and the detection system used were not adapted to a perfect identification of all the present compounds, and particularly to differentiate glycosides from conjugates.

In conclusion, the present study does not support the hypothesis of a crucial role of the stomach in flavonoid glycosides metabolism, because quercetin glycosides were not hydrolyzed and absorbed, in contrast to the aglycon. Thus, because quercetin is chiefly present in the diet as glycosides, the contribution of the stomach to absorbtion of this compound is probably limited. However, some processes, such as fermentation of the grape in wine which liberates the aglycon from the glycosidic forms (*21*), could lead to an effective absorption of quercetin in the stomach.

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