Intestinal Transport of Quercetin Glycosides in Rats Involves Both Deglycosylation and Interaction with the Hexose Transport Pathway[1]

Jennifer M. Gee,[2] M. Susan DuPont, Andrea J. Day, Geoffrey W. Plumb, Gary Williamson and Ian T. Johnson

Diet, Health and Consumer Sciences Division, Institute of Food Research, Norwich Research Park, Colney, Norwich, NR4 7UA, UK

ABSTRACT Flavonoids are polyphenolic plant secondary metabolites with antioxidant and other biological activities potentially beneficial to health. Food-borne flavonoids occur mainly as glycosides, some of which can be absorbed in the human small intestine; however, the mechanism of uptake is uncertain. We used isolated preparations of rat small intestine to compare the uptake of the quercetin aglycone with that of some quercetin glucosides commonly found in foods, and investigated interactions between quercetin-3-glucoside and the intestinal hexose transport pathway. The nature of any metabolism of quercetin and its glucosides during small intestinal transport in vitro was determined by HPLC. The presence of quercetin-3-glucoside in the mucosal medium suppressed the uptake of labeled galactose by competitive inhibition and stimulated the efflux of preloaded galactose. Quercetin-3-glucoside and quercetin-4′-glucoside, but not quercetin-3,4′-diglucoside, were transported into everted sacs significantly more quickly than quercetin aglycone. Intact quercetin glucosides were not detected in mucosal tissue or within the serosal compartment, but both free quercetin and its metabolites were present, mainly as quercetin-3-glucuronide and quercetin-7-glucuronide. Evidently, quercetin derived from quercetin-3-glucoside passes across the small intestinal epithelium more rapidly than free quercetin aglycone. Monoglucosides of quercetin interact with the sodium-dependent glucose transporter. During passage across the epithelium, quercetin-3-glucoside is rapidly deglycosylated and then glucuronidated. J. Nutr. 130: 2765–2771, 2000.

KEY WORDS: • *quercetin glycosides* • *rat small intestine* • *deglycosylation* • *sodium-dependent glucose transporter 1* • *flavonol uptake*

Flavonoids are polyphenolic plant secondary metabolites providing much of the color and flavor of plant foods. Many of these compounds play a protective role in the plant and are biochemically active. Flavonoids have been regarded both as putative vitamins (Rusznyàk and Szent-Györgi 1936) and as potential carcinogens (Pabukeu et al. 1980), but they are now widely considered to be potentially beneficial to health by virtue of their antioxidant activities (Hollman and Katan 1998) and their capacity to inhibit enzymes such as cyclooxygenase and protein kinases involved in cell proliferation and apoptosis (Formica and Regelson 1995). In this context, the mechanism of uptake of flavonoids into intestinal epithelial cells and their transfer into the circulation are of great interest.

The flavonol quercetin occurs in plants predominantly in the form of glycosides, which are water soluble and chemically stable (Formica and Regelson 1995). It has long been assumed that quercetin glycosides are poorly absorbed in the small bowel and that the most likely route of intestinal transport is by passive uptake of the relatively lipophilic aglycone in the colon after hydrolysis of the glycosides by the large intestinal microflora (Manach et al. 1995 and 1997). However, Hollman and colleagues (1995 and 1996) showed that in humans, quercetin glucosides were absorbed within 30 min of ingestion, apparently in preference to quercetin aglycone. To explain this, they proposed that the glucose moiety may enable flavonoid glycosides to be transported by the sodium-dependent glucose transporter 1 (SGLT1)[3]. Several examples of glycoside transfer mediated by Na^+-D-glucose cotransporters expressed in *Xenopus* oocytes have been described, including transport of the cycad toxin cycasin (a β-D-glucoside of methylazoxymethanol) by SGLT1 (Hirayama et al. 1994), the alkylating drug β-D-glucosylisophosphoramide by the low affinity Na^+-D-glucose cotransporter sodium-dependent amino acid transporter (SAAT1) (Veyhl et al. 1998) and several phenyl glycosides by SGLT1 (Lostao et al. 1994). However, quercetin glycosides are relatively large molecules compared with β-napthyl glucoside, which is reported to be the largest substrate transported by SGLT1 (Panayotova-Heiermann et al. 1996). Phlorizin, which is structurally related to the flavonoids

[1] Supported by the BBSRC through a Core Strategic grant and by the EU (FAIR CT950953).

[2] To whom correspondence should be addressed.

[3] Abbreviations used: LPH, lactase phloridzin hydrolase; PEG, polyethylene glycol; PTFE, polytetrafluoro-ethylene; SAAT1, sodium-dependent amino acid transporter; SGLT1 and 2, sodium-dependent glucose transporters 1 and 2; TFA, triflouroacetic acid; UDPG, uridine-diphospho-D-glucose.

Manuscript received 25 April 2000. Initial review completed 25 May 2000. Revision accepted 24 July 2000.

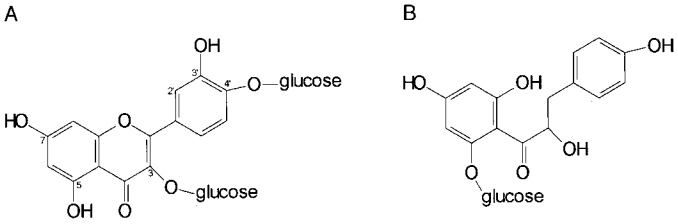

FIGURE 1 The structures of quercetin-3,4′-diglucoside (*A*) and phlorizin (*B*). Quercetin-3-glucoside differs from structure (*A*) in having a hydroxyl group in the 4′ position, whereas quercetin aglycone has a hydroxyl group at both positions.

(**Fig. 1**), is a highly efficient inhibitor of SGLT1 and is not transported across the cell membrane (Hirayama et al. 1996). Furthermore, like phlorizin, flavonoid glucosides are substrates for hydrolysis by lactase phlorizin hydrolase (Day et al. 2000b), an enzyme localized on the outer surface of the small intestinal brush border membrane (Leese and Semenza 1973). In an earlier study, we described the countertransport of sugars elicited by quercetin glycosides in rat small intestine, but we were unable to determine whether the glycosides were themselves transported (Gee et al. 1998). In this study, we sought further evidence that quercetin glucosides interact with SGLT1 and compared transport of quercetin-3-glucoside with that of the free quercetin aglycone.

MATERIALS AND METHODS

Synthesis of labeled quercetin glycosides. Radioactively labeled quercetin glucosides were synthesized from specifically labeled [4-^{14}C]quercetin (a gift from Unilever Research, Vlaadingen, The Netherlands) and uridine-diphospho-D-glucose (UDPG) in the presence of enzymes extracted from onion or leek. To prepare enzyme extracts, 100 g of plant tissue was homogenized in an extraction mixture containing polyvinylpyrrolidine (10 g) and β-mercaptoethanol (10 mmol/L) in 300 mL of McIlvaine buffer (pH 7.0), consisting of citric acid (100 mmol/L; 52.9 mL) and disodium hydrogen orthophosphate (200 mmol/L; 247.15 mL). The homogenate was centrifuged (10,000 × *g*) for 15 min at 4°C. The supernatant was filtered under vacuum through Whatman 541 filter paper (Maidstone, UK) before further centrifugation (10,000 × *g*) for 10 min at 4°C. Proteins were precipitated with ammonium sulfate and the mixture centrifuged again (15,000 × *g*) for 30 min at 4°C. Pellets were redissolved in buffer (4 mL) and applied in four portions to a PD-10 desalting column (Amersham Pharmacia Biotech, Little Chalfont, UK). The eluate was made up to 6 mL. In the case of the onion tissue, this extract was concentrated threefold by evaporation.

Quercetin-4′-glucoside and quercetin-3,4′-diglucoside were produced in roughly equal amounts by the incubation of 43.2 μL [^{14}C]quercetin (80 kBq), 50 μL of 2 mmol/L UDPG, 50 μL 10X PBS, 300 μL concentrated onion enzyme extract and 56.7 μL water at 37°C for 4 h. This procedure was repeated to yield ~50 kBq of each glucoside. Quercetin-3-glucoside was produced by the incubation of 43.2 μL labeled quercetin (80 kBq), 50 μL of 2 mmol/L UDPG, 50 μL 10X PBS, 100 μL leek enzyme extract and 256.7 μL water at 37°C for 2 h. This procedure yielded ~50 kBq of quercetin-3-glucoside. The quercetin glycosides were purified by preparative HPLC, using a Prodigy 5-μm ODS3 reversed-phase silica (250 mm × 21.2 mm i.d. (Phenomenex, Macclesfield, UK) column with an isocratic solvent [(8:2) acetonitrile/0.1% triflouroacetic acid (TFA)] at a flow rate of 5 mL/min. The column effluent was monitored at 270 and 370 nm, and the fractions were collected using a Gilson fraction collector (Anachem, Luton, UK).

Animals. Male Wistar rats (~200 g) were obtained from a licensed animal supplier (R. Tuck and Sons, Huntington, UK) and housed in an environmentally controlled animal facility before use. Rats consumed water and a standard nonpurified diet [proximate composition (g/kg): available carbohydrate, 523; dietary fiber, 14; protein, 147; lipid, 26; ash, 59; and moisture, 100; RMI, Special Diet Services, Witham, UK] ad libitum until their body mass reached ~300 g. All aspects of animal care complied with the ethical guidelines and technical requirements of the UK Home Office.

Intestinal preparations. Each rat was deeply anesthetized with sodium barbiturate (Euthetal, Rhone Merieux, Harlow, UK) and killed by cervical dislocation immediately before removal of the small intestine via an abdominal incision. The jejunum was identified (10–50% length), rinsed with 5 mL Krebs bicarbonate buffer [pH 7.4, containing (mmol/L): NaCl, 118.4; KCl, 4.8; $NaHCO_3$, 19.8; $MgSO_4 \cdot 7H_2O$, 1.2; KH_2PO_4, 1.2; and $CaCl_2 \cdot 6H_2O$, 2.5] and everted. Rings (~2 mm; 40/rat; 5 rats/experiment) or segments (5 cm; 4–6/rat; 5 rats/experiment) were cut from the proximal portion and randomized by swirling in oxygenated buffer. Everted rings were distributed among incubation flasks (4–6/flask) containing gassed incubation media as described below. Cannulated everted sacs were prepared by ligaturing everted segments at one end and tying the other end to tapered disposable syringes (1 mL) precharged with 0.5 mL Krebs buffer, filled by depressing the syringe plunger and emptied without contamination of the serosal solutions by withdrawing the plunger at the end of the experiment. The sacs were suspended in organ baths (37°C) containing incubation media (8 mL) as described below and gassed continuously with 5% carbon dioxide in oxygen for the times indicated.

Monosaccharide transport. The functional integrity of the everted sac preparation was assessed by its ability to accumulate radiolabeled D-galactose in the tissue and transfer it to the serosal solution more quickly than L-glucose, and by a sodium-dependent mechanism. Everted jejunal sacs were incubated in Krebs buffer (8 mL) containing D-glucose (1 mmol/L) and either [^{14}C] D-galactose (Amersham Pharmacia Biotech, Little Chalfont, UK; 1.2 MBq/L; 100 μmol/L), [^{14}C] D-galactose (100 μmol/L) in the absence of sodium, [^{14}C] L-glucose (100 μmol/L) or mannitol (100 μmol/L) plus [^{14}C] polyethylene glycol (PEG; Amersham Pharmacia Biotech UK; 1.2 MBq/L). The PEG functioned as an extracellular space marker and did not contribute appreciably to the osmolarity of the solution. Media were gassed continuously with 95% O_2: 5% CO_2. For the sodium-free incubation, sodium salts in the Krebs buffer were replaced with salts of potassium, which causes a particularly effective inhibition of active monosaccharide absorption in the small bowel (Bihler and Crane 1962). Incubations were carried out for 15 min at 37°C, after which serosal solutions were recovered; the rinsed sacs were cut from their cannulae and transferred to preweighed glass vials. The tissue was dried (18 h; 95°C), weighed and subjected to acid hydrolysis (0.4 mL 11 mol/L HCl, 70°C, 15 min) followed by the addition of 3.6 mL of 0.75 mol/L Trizma base (Sigma, Poole, UK). After mixing, a sample (0.5 mL) was diluted to 1 mL with distilled water and 9 mL cocktail (Zinsser Quicksafe A; Zinsser, Maidenhead, UK) added before scintillation counting using an automatic liquid-scintillation spectrometer (Packard, Pangbourne, UK). Serosal solutions were weighed to assess volume and diluted to ~1 mL with distilled water for scintillation counting.

To determine the effect of unlabeled quercetin-3-glucoside on the net uptake of galactose by mucosal tissue, everted rings from single rats were incubated in Krebs bicarbonate buffer (10 mL) containing [^{3}H] galactose (Amersham Pharmacia Biotech UK; 1 mmol/L; 7.3 MBq/L) without or with quercetin 3-glucoside (0.2, 0.5, 1 or 2 mmol/L) or phlorizin (0.1 or 1 mmol/L; prepared under sonication), and mannitol (to balance osmolarity), for 4 min at 37°C, and gassed continuously with 95% oxygen and 5% carbon dioxide. A separate flask in each series was incubated with [^{14}C] PEG 4000 (Amersham Pharmacia Biotech UK; 3.6 MBq/L) for extracellular space correction. At the end of the incubation period, the rings were harvested on a Buchner filter (Fisher Scientific, Loughborough, UK), rinsed with ice-cold saline (9 g/L; 50 mL) and transferred to preweighed vials. The tissue was digested and counted as described above. Net galactose uptake rate was calculated as nmol/(g · min). To determine the effect of quercetin-3-glucoside on net transfer of galactose across the mucosa, four sacs from individual rats were incubated in individual jacketed organ baths (37°C) loaded with 8 mL Krebs bicarbonate buffer containing [^{14}C] galactose (Amersham Pharmacia Biotech UK; 1 mmol/L; 0.86 MBq/L) without or with mannitol (1 mmol/L),

mannitol (1 mmol/L) and phlorizin (10 μmol/L) or quercetin-3-glucoside (1 mmol/L) for 20 min, after which samples (100 μL) of serosal solutions were prepared for scintillation counting as described above.

Galactose countertransport. Quercetin-3-glucoside (Extrasynethese, Genay, France) was solubilized with sonication in Krebs bicarbonate buffer (pH 7.2–7.4). Four everted jejunal sacs, prepared as described above, were first preloaded with radiolabeled galactose by incubating them in Krebs bicarbonate buffer containing [^{14}C] D-galactose (Amersham Pharmacia Biotech UK; final specific activity 5.44 MBq/L; 11.1 GBq/mmol) for 10 min; the solution was then drained from the baths and the sacs rinsed. The tissue was replaced in the rinsed organ baths containing Krebs buffer, with or without quercetin-3-glucoside or phlorizin (0.05–1.0 mmol/L). Samples of the mucosal solution (100 μL) were removed from each organ bath before introduction of the sacs and at 2.5-min intervals over the next 20 min. Scintillation cocktail (10 mL) was added to each sample in a 20-mL vial before liquid scintillation counting. The rates of stimulated efflux for each compound were determined by linear regression of the first four time points after correction for efflux from the control sac. The concentration of labeled galactose in the mucosal medium at the end of the incubation period, after similar correction for control conditions, was taken as the cumulative efflux.

Flavonol transport. Four sacs from each rat were incubated in Krebs buffer (8 mL) containing glucose (1.0 mmol/L) and [^{14}C]quercetin, [^{14}C]quercetin-3-glucoside, [^{14}C]quercetin-4′-glucoside or [^{14}C]quercetin-3,4′-diglucoside. The concentration of quercetin and quercetin glucosides was 100 μmol/L, and the specific radioactivity was 1.19 MBq/L. Because of the low solubility of quercetin in aqueous media at neutral pH, all of the flavonoids were dissolved in ethanol before addition to the incubation medium. The compounds were dissolved in 42 μL absolute ethanol; this solution was slowly added to Krebs-glucose maintained at 37°C. To assess the volume of entrapped liquid in the extracellular space, a fifth sac was incubated in the presence of high specific activity [^{14}C]PEG, together with mannitol (100 μmol/L) to control for the osmolarity of the flavonols. Incubations were carried out for 15 min; the serosal solutions were then recovered and the rinsed sacs cut from their cannulae and slit open. The mucosal tissue was scraped off each sac using two microscope slides. The sample was weighed, dried, reweighed and subjected to acid hydrolysis before scintillation counting as described above. Serosal solutions were weighed to assess volume and a sample of each (300 μL) taken for counting as before.

Identification of quercetin metabolites. In a separate experiment, six sacs were prepared from each of five rats as described previously. For each rat, pairs of sacs were incubated in Krebs-glucose (8 mL) containing quercetin, quercetin-3-glucoside or mannitol (100 mmol/L) for 15 min. At the end of the incubation, the sacs were rinsed and the mucosal tissue was scraped off each sac with a microscope slide onto a glass plate. The tissue and the collected serosal and mucosal solutions were then immediately frozen and stored. Before analysis, the thawed tissue was homogenized twice with methanol (2 × 0.75 mL) containing ascorbic acid (1 mmol/L; to stabilize the samples during analysis) followed by centrifugation at 13,600 × *g* for 10 min at 4°C. The supernatants were combined, acetic acid (glacial; 50 μL) was added and the sample was dried under vacuum by rotary evaporation to ~300 μL. Methanol was added to give a final volume of 400 μL. After a further centrifugation (9000 × *g*, 4°C, 2 min), the supernatant was filtered through 0.22 μm polytetrafluoroethylene (PTFE) filter units (HPLC Technology, Macclesfield, UK) and analyzed by HPLC as described below. Serosal solutions were diluted with an equal volume of methanol containing ascorbic acid, centrifuged (13,600 × *g*, 4°C, 10 min) and filtered as above for direct analysis by HPLC. A sample of mucosal solution (0.5 mL) was treated similarly to serosal solutions. In control studies, the recovery of unlabeled quercetin and quercetin glycosides, added to mucosal solutions or homogenized with mucosal tissues and treated as above, was >95% when analyzed by HPLC.

The mucosal solutions were also subjected to separation on a polyamide column as described by DuPont et al. (2000). Briefly, a polyamide column (1 g polyamide CC6; Macherey-Nagel, Düren, Germany) was packed into a 6-mL disposable filtration column (HPLC Technology) with a 20 μm frit at either end, conditioned with methanol (20 mL) and water (60 mL) before direct loading of a sample (5 mL) of the mucosal solution followed by 20 mL water. The neutral fraction, containing only the quercetin glycosides and aglycone (efficiency of separation ~99%), was eluted with methanol (40 mL), and the acidic fraction containing glucuronides (efficiency of separation > 90%) was eluted with methanol/ammonia (99.5:0.5 v/v). Each fraction was dried under vacuum by rotary evaporation before being redissolved in methanol/water (50:50 v/v). After a final centrifugation (9000 × *g*, 4°C, 2 min), the supernatant was filtered for analysis by HPLC.

HPLC was carried out using a modified version of the method of Price et al. (1998). Solvents A (water/tetrahydrofuran/TFA, 98:2:0.1) and B (acetonitrile) were run at a flow rate of 1 mL/min, using a gradient of 17% B (2 min), increasing to 25% B (5 min), 35% B (8 min), 50% B (5 min) and then to 100% B (5 min). A column clean-up stage maintained B at 100% (5 min) followed by a reequilibration at 17% B (15 min). The column was packed with Prodigy 5-μm ODS3 reverse-phase silica, 250 mm × 4.6 mm i.d., (Phenomenex), and the eluent was monitored with diode array detection at 270 and 370 nm. Quercetin and quercetin-3-glucoside were used as external standards at concentrations ranging from 0 to 100 μmol/L. Then identities of quercetin, quercetin-3-glucoside, quercetin-4′-glucoside, quercetin-3-glucuronide and quercetin-7-glucuronide were assessed by coelution of peaks with standard compounds or by matching UV spectra. Quercetin-3-glucoside and quercetin-3-glucuronide were resolved with a separation of ~0.3 min. Quercetin, quercetin-3-glucoside and quercetin-4′-glucoside standards were HPLC grade purchased from Extrasynthese. Quercetin-3-glucuronide was purified from green beans and the identity was confirmed by mass spectrometry and nuclear magnetic resonance as described by Price et al. (1998). Quercetin-7-glucuronide was identified by the effect of shift reagent on its UV absorption spectrum (Day et al., unpublished data).

Statistical methods. All numerical data are expressed as means ± SEM. The significance of differences were assessed by one-way ANOVA with Tukey's test for comparison of individual means. *P*-values < 0.05 were regarded as significant. All calculations were carried out using Minitab (State College, PA).

RESULTS

Galactose transport. The ability of the everted sac preparation to transport D-galactose by a sodium-dependent mechanism is illustrated in **Figure 2**. Exclusion of sodium from the mucosal solution reduced net serosal transfer and net tissue uptake of D-galactose by ~87%. Transport of L-glucose in the sodium-replete medium was ~15% of D-galactose transport under the same conditions (Fig. 2).

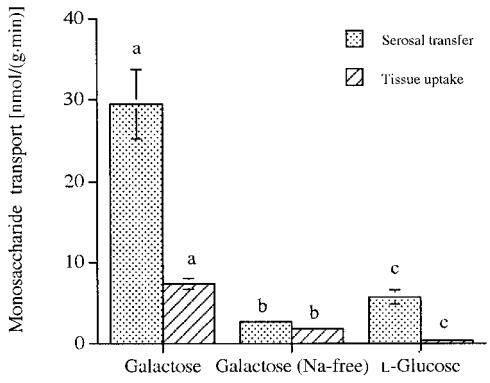

FIGURE 2 Serosal transfer and tissue uptake of ^{14}C-labeled D-galactose (100 μmol/L) by everted sacs of rat jejunum, in the presence and absence of Na^+ ions, and of ^{14}C-labeled L-glucose (100 μmol/L) in the presence of sodium, over a 15-min period. Individual points are means ± SEM, $n = 4$ or 5. Columns with identical shading but different index letters differ significantly, $P < 0.05$.

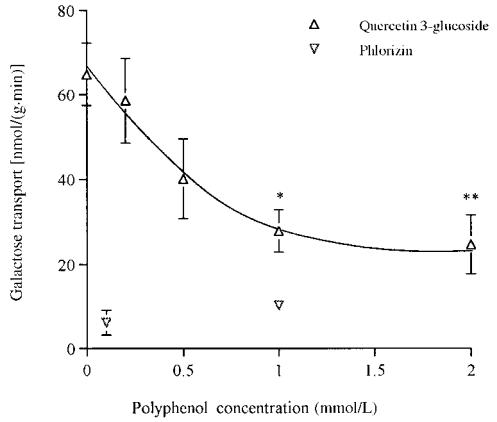

FIGURE 3 Uptake of ^{3}H-labeled D-galactose (1 mmol/L) into rat everted jejunal rings in the absence and presence of quercetin 3-glucoside (0.2, 0.5, 1.0 and 2.0 mmol/L) or phlorizin (0.1 and 1 mmol/L) over a 4-min period. Points are means ± SEM, $n = 5$ rats. Points with asterisks differ significantly from the control incubation free of quercetin-3-glucoside, $^*P < 0.05$, $^{**}P < 0.01$.

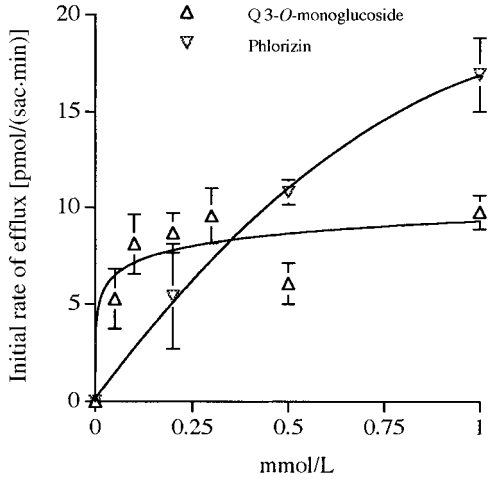

FIGURE 5 Rates of efflux of ^{14}C-labeled D-galactose from preloaded rat everted jejunal sacs stimulated by quercetin-3-glucoside ($n = 5$) and phlorizin ($n = 4$) over a range of substrate concentrations. Data points are means ± SEM, $n = 5$ rats.

The inhibition of D-galactose transport into everted rings and everted sacs in the presence of quercetin-3-glucoside is illustrated in **Figures 3** and **4**, respectively. Net tissue uptake of galactose by rings was progressively suppressed by increasing concentrations of the flavonoid, with apparently saturable kinetics (Fig. 3). Addition of phlorizin to the galactose-mannitol bathing medium of everted sacs reduced galactose transport by ~80%, whereas the presence of quercetin-3-glucoside (1 mmol/L) in the mucosal solution resulted in a significant reduction in D-galactose transport of ~59%. In contrast, the inert sugar mannitol (1 mmol/L), which served as a control for any effects due to the osmolarity of the flavonol in the mucosal medium, had no effect on the transport of D-galactose (Fig. 4).

Galactose countertransport. When everted sacs preloaded with [^{14}C] D-galactose were incubated with increasing concentrations of quercetin-3-glucoside in the mucosal medium, an enhanced efflux of labeled substrate was observed (**Fig. 5**). This effect was saturable over the range 0–0.5 mmol/L. A similar stimulation of efflux was observed when sacs were exposed to phlorizin at 0.2, 0.5 and 1.0 mmol/L, although with less evidence of saturation over this concentration range (Fig. 5).

Flavonol transport. The net transport of radiolabeled quercetin was, in molar terms, quantitatively similar to the transport of galactose in sodium-free medium [4.9 ± 0.8 nmol/(g · min); **Fig. 6**]. Of the absorbed activity, ~85% was recovered from the serosal compartment. The transport and distribution of radioactivity derived from quercetin 3,4′diglucoside were quantitatively almost identical to those of quercetin, whereas in the case of 3-glucoside ($P < 0.01$) and 4′-glucoside ($P < 0.05$), the combined tissue uptake and serosal transfer were approximately twice that of the quercetin aglycone under the same conditions (Fig. 6).

Flavonol metabolites. Polyamide gave effective separation of quercetin-3-glucoside and quercetin aglycone from their metabolites in the acidic and neutral fractions of the tissue extracts. **Figure 7** shows representative chromatograms of mucosal tissue and serosal solutions extracted after incubation of everted sacs with either quercetin-3-glucoside or quercetin aglycone. The major metabolites in the serosal solution and the tissue are designated M_1 and M_2 in Figure 7, and correspond to quercetin-3-glucuronide and quercetin-7-glucuro-

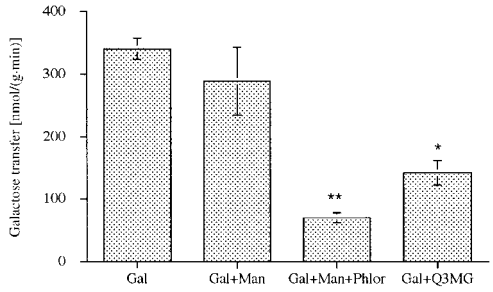

FIGURE 4 Serosal transfer of ^{14}C-labeled D-galactose (Gal; 1 mmol/L) into rat everted jejunal sacs in the absence and presence of mannitol (Man; 1 mmol/L), and in the presence of mannitol (1 mmol/L) and phlorizin (Phlor; 10 μmol/L) or quercetin-3-glucoside (Q3MG; 1 mmol/L) over a 20-min period. Individual points are means ± SEM, $n = 5$ rats. Points with asterisks differ significantly from the transport of galactose alone, $^*P < 0.01$, $^{**}P < 0.001$).

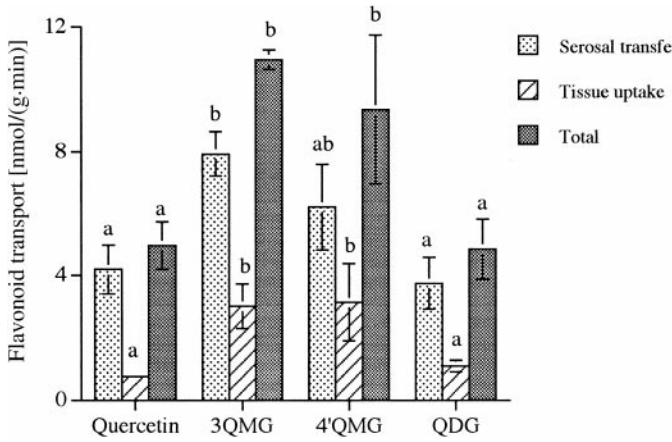

FIGURE 6 Serosal transfer, tissue uptake and total net transport of 100 μmol/L ^{14}C-labeled quercetin, quercetin-3-glucoside (3QMG), quercetin-4′-monoglucoside (4′QMG) and quercetin 3,4′ diglucoside (QDG) by rat everted jejeunal sacs over a 15-min period. Individual points are means ± SEM, $n = 4$ rats for quercetin-4′-monoglucoside, 8 rats for quercetin and 5 rats for the remaining groups. Columns with identical shading but different index letters differ significantly, $P < 0.05$.

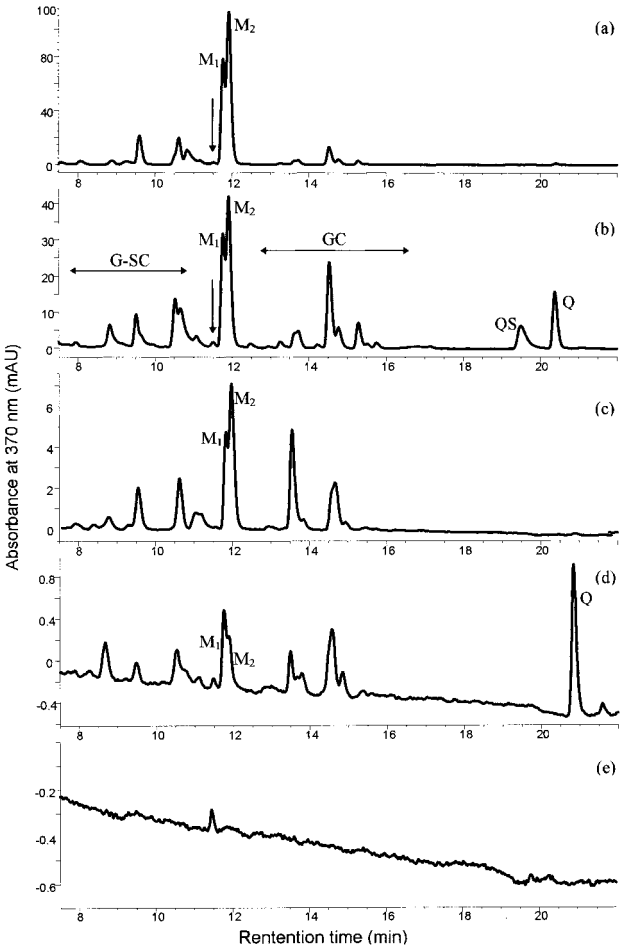

FIGURE 7 HPLC chromatograms showing the metabolism of quercetin-glucoside and quercetin after tissue uptake and serosal transfer by everted sacs of rat jejunum. *Traces a* and *c* were obtained from serosal solutions collected after incubation with quercetin-3-glucoside or quercetin aglycone. *Traces b* and *d* show mucosal tissue extracted after incubation with quercetin-3-glucoside or quercetin, respectively, and *trace e* shows tissue extracted after a control incubation with mannitol. M_1 and M_2 are the major metabolites appearing after incubation with either compound. Peaks in the region G-SC are mixed glucuronide-sulfate conjugates and those in GC are glucuronide conjugates. QS is quercetin sulfate and Q is quercetin. The vertical arrow indicates the elution position of quercetin-3-glucoside under these solvent gradient conditions. Note the differing absorbance scales for different traces.

nide, respectively. The resolution by HPLC of quercetin-3-glucoside and quercetin-3-glucuronide was adequate, and coelution of samples with quercetin-3-glucoside confirmed that this compound (position designated by vertical arrows in Fig. 7) was not present at significant levels in any of the serosal fluids or tissue samples. The peaks appearing in the regions delineated by G-SC and GC in Figure 7 are mixed glucuronide-sulfate conjugates and glucuronide conjugates, respectively (Day et al. 2000a). The aglycone (designated by Q in Fig. 7) was found after incubation with both quercetin and quercetin-3-glucoside, although only in the tissue extract and not in the serosal fluid. This was confirmed using polyamide separation as an additional step, as described previously. No peaks were observed in the control incubations with mannitol (*trace e* in Fig. 7). No hydrolysis of the glycoside occurred after incubation in the absence of tissue, but quercetin and its metabolites were detectable in the final mucosal solutions after incubation of the everted sacs with quercetin or quercetin-3-glucoside (data not shown), thus providing evidence for the efflux of quercetin metabolites from the mucosal surface of the enterocytes.

DISCUSSION

This study has demonstrated that quercetin-3-glucoside inhibits intestinal transport of D-galactose when both are present simultaneously in the mucosal medium, and that efflux of labeled D-galactose from preloaded mucosal tissue proceeds more quickly in the presence of quercetin-3-glucoside compared with a substrate-free medium. These observations suggest that the glucoside interacts with the Na^+-D-glucose cotransporter (SGLT1), which provides the main route of glucose and galactose absorption. Countertransport was defined by Robinson (1974) as the acceleration of the movement of a substance across a membrane by the presence of the substance or its homologue on the opposite side of the membrane. Several previous examples of stimulated efflux from preloaded intestinal epithelial cells have been reported for substrates, including amino acids (Hajjar et al. 1970), folic acid (Blair et al. 1976) and β-methylglucoside (Robinson 1974). Robinson considered two models for countertransport, an "accelerative" model in which the rate of translocation of an unloaded carrier is slower than that of the loaded form, and a "competitive" model in which the labeled and unlabeled substrates compete for the extracellular binding site of a transporter. On the basis of a kinetic analysis of the stimulated efflux data, Robinson concluded that the latter model was the more plausible, and this seems consistent with modern concepts of transporter function. Robinson also observed that exposure to phlorizin, which is generally thought not to be transported (Hirayama et al. 1996, Toggenburger et al. 1982), would stimulate the efflux of a glucose analog, and concluded that this was consistent with the competitive model for countertransport.

To address the issue of quercetin transport directly, we compared the uptake of radiolabeled quercetin aglycone with that of three similarly labeled quercetin glucosides in an everted sac preparation, which we had established was able to discriminate between D- and L-isomers of monosaccharides and transport galactose via a sodium-dependent pathway. Quercetin-3-glucoside and quercetin-4′-glucoside, but not quercetin-3,4′ diglucoside, were transported into everted sacs significantly more quickly than quercetin aglycone. Evidently, glycosylation can increase the transport of quercetin, but the number and positions of the glucose moieties are of critical importance. This finding is consistent with the work of Hollman and colleagues (1995 and 1996), who concluded that quercetin glucosides are absorbed preferentially in the human small intestine, but it does not establish the mechanism by which the glucose moiety facilitates transport. Panayotova-Heiermann et al. (1996) showed that although neither of the two Na^+/glucose cotransporters (SGLT1 and SGLT2) expressed in the mammalian proximal kidney tubule are themselves capable of transporting phlorizin, a chimeric protein, constructed from amino acids 1–380 from porcine SGLT2 and 381–362 from porcine SGLT1, does transport phlorizin and other inhibitors of glucose transport when expressed in *Xenopus* oocytes. The authors attributed this to a physical enlargement of the molecular pocket involved in organic substrate transport in the chimera, relative to those of the parent transporters. Quercetin-3-glucoside is of similar size to phlorizin but its evident ability to recognize and bind to the receptor

region of SGLT1 raises the possibility that it may cross the intestinal brush border by this route.

Spencer et al. (1999) reported that quercetin-3-glucoside was transported across a perfused rat gut model, intact and relatively unmetabolized, whereas Manach et al. (1999) reported preferential absorption of quercetin-3-glucoside from rat intestine in vivo but found only quercetin-conjugated metabolites in the serum. The current evidence for the presence of intact quercetin glucosides in human plasma remains inconclusive. The low sensitivity of detection for these compounds usually requires the acid or enzymic hydrolysis of plasma samples before analysis by HPLC, and those studies that have reported detection of quercetin glycosides in plasma have employed spectroscopic methods with a limited ability to distinguish between conjugated quercetin metabolites and glycosides (Paganga and Rice-Evans 1997).

In this study, analysis of the mucosal tissue extracts and serosal solutions provided no evidence for the presence of intact quercetin-3-glucoside in either compartment. Furthermore, the profiles of the metabolites derived from incubation of everted sacs of small bowel with quercetin-3-glucoside or quercetin were virtually identical, suggesting that deglycosylation of the glycoside had occurred rapidly. Ioku et al. (1998) described β-glucosidase activity toward various β-glucosides, including quercetin-3-glucoside, along the entire rat small intestine, with highest levels in the jejunum. Day et al. (1998) also provided evidence for the involvement of broad-specificity cytosolic β-glucosidase in the deglycosylation of quercetin-4′-glucoside in human small intestine and liver cell-free extracts. However, quercetin-3-glucoside was deglycosylated only in the small intestine and at a lower rate than quercetin-4′-glucoside. The authors suggested that another enzyme, such as lactase phlorizin hydrolase (LPH), which is a brush border β-glucosidase, may be responsible for the activity toward quercetin-3-glucoside. Purified LPH from sheep small intestine has been shown subsequently to have activity toward quercetin-3-glucoside, quercetin-4′-glucoside and quercetin-3,4′-diglucoside (Day et al. 2000b).

Extracellular hydrolysis of quercetin-3-glucoside by LPH might facilitate the uptake of the reaction products independently of SGLT1. For example, LPH hydrolyzes phlorizin to release the aglycone, phloretin, and glucose. Hanke et al. (1980) used phlorizin labeled in the glucose moiety to study the transport of the liberated monosaccharide. They concluded that glucose derived from the hydrolysis of phlorizin has a kinetic advantage for transport compared with free glucose because the liberated sugar occupies a specialized extracellular compartment or microclimate in close proximity to the transporter (Warden et al. 1980). Similarly, the aglycone released into the microenvironment of the brush border by hydrolysis of quercetin glucosides would be favorably placed to diffuse passively into the enterocyte. Thus, any localized concentration of aglycone would result in a preferential rate of uptake compared with free quercetin in solution. Robinson (1974) postulated the existence of a poorly stirred mucosal compartment preventing back-diffusion of substrate to account for some of the kinetic phenomena associated with countertransport evoked by amino acids and β-methylglucoside, but the physical characteristics of this putative region have not been confirmed. Nonetheless, two potential mechanisms for the transport of quercetin glucosides by enterocytes can be envisaged, namely, transport of intact quercetin glucosides by SGLT1, and extracellular hydrolysis by LPH, followed by passive diffusion of the aglycone (**Fig. 8**).

To conclude, it is important to emphasize that plant foods contain a rich variety of flavonoids, most of which exist as

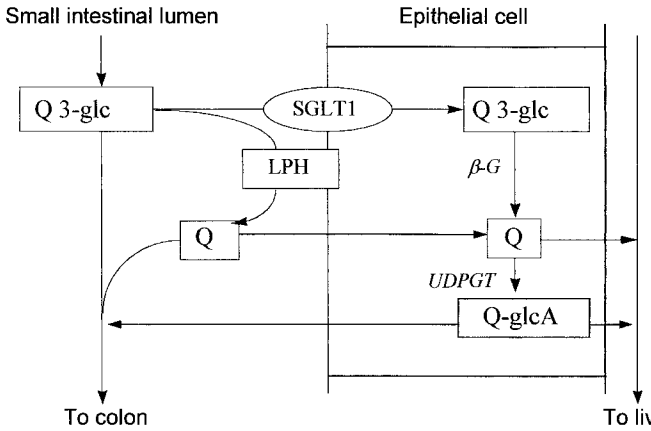

FIGURE 8 Possible mechanisms for the absorption of quercetin glycosides in the small intestine. Abbreviations: Q 3-glc, quercetin-3-glucoside; Q, quercetin; SGLT1, sodium-dependent glucose transporter; LPH, lactase phlorizin hydrolase; Q-glcA, quercetin glucuronides; *UDPGT*, uridine-diphospho-D-glucose glucuronosyltransferase; *β-G*, cytosolic β-glucosidase.

gycosides. We have shown that certain quercetin glucosides exhibit both competitive inhibition and countertransport phenomena associated with the carrier-mediated transport of D-galactose in rat small intestine. Although the quercetin aglycone is itself absorbed across the intestinal epithelium, at least two of the monoglucosides are transferred more rapidly. Rat intestine is evidently capable of hydrolyzing quercetin-3-glucoside to quercetin aglycone, and conjugation of quercetin, mainly to the 3- and 7-glucuronides, also occurs within the enterocyte. The flavonol glucosides may be deglycosylated in the cytosol, perhaps after uptake via the hexose transporter. Alternatively the deglycosylation may result from the extracellular activity of LPH in the brush border, followed by transport of the released aglycone by passive diffusion. In the case of compounds that escape both hydrolysis and absorption in the small intestine, deglycosylation by bacterial enzymes will release aglycones, which probably diffuse passively across the colonic mucosa (Manach et al. 1995). In all cases, deglycosylation of the flavonols is likely to be an important factor influencing the uptake of these potentially beneficial compounds.

ACKNOWLEDGMENTS

The authors thank Lillian Tijburg of Unilever Research, Vlaardingen, for generously providing the ^{14}C quercetin. We also wish to thank Simon Deakin for animal care and technical assistance.

LITERATURE CITED

Bihler, I. & Crane, R. K. (1962) Studies on the mechanism of intestinal absorption of sugars: the influence of several cations and anions on the active transport of sugars in vitro by various preparations of hamster small intestine. Biochim. Biophys. Acta 59: 78–93.

Blair, J. A., Johnson, I. T. & Matty, A. J. (1976) Aspects of intestinal folate transport in the rat. J. Physiol. (Lond.) 256: 197–208.

Day, A. J., Bao, Y., Morgan, M.R.A. & Williamson, G. (2000a) Conjugation position of quercetin glucuronides and effect on biological activity. Free Radic. Biol. Med. (in press).

Day, A. J., Cañada, F. J., Díaz, J. C., Kroon, P. A., Mclauchlan, R., Faulds, C. B., Plumb, G. W., Morgan, M.R.A. & Williamson, G. (2000b) Dietary flavonoid and isoflavone glycosides are hydrolysed by the lactase site of lactase phlorizin hydrolase. FEBS Lett. 468: 166–170.

Day, A. J., DuPont, M. S., Ridley, S., Rhodes, M., Rhodes, M. J., Morgan, M. R. & Williamson, G. (1998) Deglycosylation of flavonoid and isoflavonoid glycosides by human small intestine and liver beta-glucosidase activity. FEBS Lett. 436: 71–75.

DuPont, M. S., Mondin, Z., Williamson, G. & Price, K. P. (2000) Effect of variety,

processing and storage on the flavonoid glycoside content and composition of lettuce and endive. J. Agric. Food Chem. (in press).
Formica, J. V. & Regelson, W. (1995) Review of the biology of quercetin and related bioflavonoids. Food Chem. Toxicol. 33: 1061–1080.
Gee, J. M., DuPont, M. S., Rhodes, M. J. & Johnson, I. T. (1998) Quercetin glucosides interact with the intestinal glucose transport pathway. Free Radic. Biol. Med. 25: 19–25.
Hajjar, J. J., Lamont, A. S. & Curran, P. F. (1970) The sodium-alanine interaction in rabbit ileum. Effect of sodium on alanine fluxes. J. Gen. Physiol. 55: 277–296.
Hanke, D. W., Warden, D. A., Evans, J. O., Fannin, F. F. & Diedrich, D. F. (1980) Kinetic advantage for transport into hamster intestine of glucose generated from phlorizin by brush border beta-glucosidase. Biochim. Biophys. Acta 599: 652–663.
Hirayama, B., Hazama, A., Loo, D. F., Wright, E. M. & Kisby, G. E. (1994) Transport of cycasin by the intestinal Na+/glucose cotransporter. Biochim. Biophys. Acta 1193: 151–154.
Hirayama, B. A., Lostao, M. P., Panayotova-Heiermann, M., Loo, D. D., Turk, E. & Wright, E. M. (1996) Kinetic and specificity differences between rat, human, and rabbit Na+-glucose cotransporters (SGLT-1). Am. J. Physiol. 270: G919–G926.
Hollman, P. C., de Vries, J. H., van Leeuwen, S. D., Mengelers, M. J. & Katan, M. B. (1995) Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. Am. J. Clin. Nutr. 62: 1276–1282.
Hollman, P. C. & Katan, M. B. (1998) Bioavailability and health effects of dietary flavonols in man. Arch. Toxicol. (suppl.) 20: 237–248.
Hollman, P. C., v.d. Gaag, M., Mengelers, M. J., van Trijp, J. M., de Vries, J. H. & Katan, M. B. (1996) Absorption and disposition kinetics of the dietary antioxidant quercetin in man. Free Radic. Biol. Med. 21: 703–707.
Ioku, K., Pongpiriyadacha, Y., Konishi, Y., Takei, Y., Nakatani, N. & Terao, J. (1998) β-Glucosidase activity in the rat small intestine toward quercetin monoglucosides. Biosci. Biotechnol. Biochem. 62: 1428–1431.
Leese, H. J. & Semenza, G. (1973) On the identity between the small intestinal enzymes phlorizin hydrolase and glycosylceramidase. J. Biol. Chem. 248: 8170–8173.
Lostao, M. P., Hirayama, B. A., Loo, D. D. & Wright, E. M. (1994) Phenylglucosides and the Na+/glucose cotransporter (SGLT1): analysis of interactions. J. Membr. Biol. 142: 161–170.
Manach, C., Morand, C., Crespy, V. & Rémésy, C. (1999) Influence of the nature of quercetin glycosylation on its bioavailability in rats. SFRR Europe Winter Meeting: Bio-Flavonoids and Polyphenols in Health and Disease, Dinard, France, OP17.
Manach, C., Morand, C., Demigné, C., Texier, O., Regerat, F. & Rémésy, C. (1997) Bioavailability of rutin and quercetin in rats. FEBS Lett. 409: 12–16.
Manach, C., Morand, C., Texier, O., Favier, M. L., Agullo, G., Demigné, C., Regerat, F. & Rémésy, C. (1995) Quercetin metabolites in plasma of rats fed diets containing rutin or quercetin. J. Nutr. 125: 1911–1922.
Pabukeu, A. M., Yalcener, S. & Hatcher, J. F. (1980) Quercetin as an intestinal and bladder carcinogen present in bracken fern. Teratog. Carcinog. Mutagen. 1: 213–221.
Paganga, G. & Rice-Evans, C. A. (1997) The identification of flavonoids as glycosides in human plasma. FEBS Lett. 401: 78–82.
Panayotova-Heiermann, M., Loo, D. D., Kong, C. T., Lever, J. E. & Wright, E. M. (1996) Sugar binding to Na+/glucose cotransporters is determined by the carboxyl-terminal half of the protein. J. Biol. Chem. 271: 10029–10034.
Price, K. R., Colquhoun, I. J., Barnes, K. A. & Rhodes, M.J.C. (1998) The composition and content of flavonol glycosides in green beans and their fate during processing. J. Food Chem. 46: 4898–4903.
Robinson, J.W.L. (1974) The question of counter-transport in the intestine. Biochim. Biophys. Acta 367: 88–101.
Rusznyàk, S. & Szent-Györgi, A. (1936) Vitamin nature of flavones. Nature (Lond.) 139: 798.
Spencer, J. P., Chowrimootoo, G., Choudhury, R., Debnam, E. S., Srai, S. K. & Rice-Evans, C. (1999) The small intestine can both absorb and glucuronidate luminal flavonoids. FEBS Lett. 458: 224–30.
Toggenburger, G., Kessler, M. & Semenza, G. (1982) Phlorizin as a probe of the small intestinal Na+, D-glucose cotransporter: a model. Biochim. Biophys. Acta 688: 557–571.
Veyhl, M., Wagner, K., Volk, C., Gorboulev, V., Baumgarten, K., Weber, W. M., Schaper, M., Bertram, B., Wiessler, M. & Koepsell, H. (1998) Transport of the new chemotherapeutic agent beta-D-glucosylisophosphoramide mustard (D-19575) into tumor cells is mediated by the Na+-D-glucose cotransporter SAAT1. Proc. Natl. Acad. Sci. U.S.A. 95: 2914–2919.
Warden, D. A., Fannin, F. F., Evans, J. O., Hanke, D. W. & Diedrich, D. F. (1980) A hydrolase-related transport system is not required to explain the intestinal uptake of glucose liberated from phlorizin. Biochim. Biophys. Acta 599: 664–672.