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Transport of Quercetin and Its Glucosides across Human Intestinal Epithelial Caco-2 Cells

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ABSTRACT. There is mounting evidence from human epidemiological, animal *in vivo*, and *in vitro* studies to suggest beneficial effects related to the consumption of quercetin and its glucosides. However, there is limited knowledge on the oral bioavailability of these natural products. This study examined the intestinal epithelial membrane transport of quercetin, quercetin 4′-glucoside, and quercetin 3,4′-diglucoside, using the Caco-2 human colonic cell line, a model of human intestinal absorption. The apparent permeability (P_{app}) of each agent was measured in both apical to basal and basal to apical directions. The apical to basolateral flux of quercetin, P_{app} 5.8 ± 1.1 × 10^{-6} cm · sec^{-1} (mean ± SEM), was more than 10-fold higher than for the paracellular transport marker mannitol, 0.48 ± 0.09 × 10^{-6} cm · sec^{-1} ($P < 0.01$). Under identical conditions, the P_{app} for the transcellular marker propranolol was about 5-fold higher than for quercetin ($P < 0.001$). Interestingly, the reverse, basolateral to apical, flux of quercetin (P_{app} 11.1 ± 1.2 × 10^{-6} cm · sec^{-1}) was almost 2-fold higher than the apical to basolateral flux ($P < 0.001$). In similar experiments, quercetin 4′-glucoside demonstrated no absorption, $P_{app} < 0.02 \times 10^{-6}$ cm · sec^{-1} in the apical to basal direction, but did demonstrate basal to apical flux, P_{app} 1.6 ± 0.2 × 10^{-6} cm · sec^{-1}. Quercetin 3,4′-diglucoside showed a low apical to basolateral transport (P_{app} 0.09 ± 0.03 × 10^{-6} cm · sec^{-1}); its reverse, basolateral to apical, transport was, however, 4-fold higher ($P < 0.05$). In these cells, glucose was actively transported with an apical to basolateral P_{app} of 36.8 ± 1.1 × 10^{-6} cm · sec^{-1}. These observations suggest facile absorption of quercetin through the human intestinal epithelium, but contrary to a previous proposal, they do not support an active transport process for quercetin glucosides. BIOCHEM PHARMACOL **55**;10:1721–1727, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. quercetin; bioflavonoids; Caco-2 cells; glucosides; intestinal absorption; apparent permeability

Quercetin and its glucosides (Fig. 1) are major flavonoids present in vegetables, fruits, and beverages [1, 2], in particular in fresh onions [3]. Epidemiological evidence suggests that a diet rich in flavonoids has a protective effect against coronary heart disease and stroke [4, 5]. Proposed mechanisms include inhibition of low density lipoprotein oxidation [6] and platelet aggregation [7], as well as antagonist activity at the A_3 adenosine receptor [8]. Animal studies as well as *in vitro* findings also suggest that flavonoids exert preventive effects in cancer [9–12]. Proposed mechanisms include effects on signal transduction pathways involved in cell proliferation [13, 14] and angiogenesis [15], as well as inhibition of cytochromes P450 [16] and sulfotransferases [17] involved in procarcinogen bioactivation.

Although there is strong evidence to suggest beneficial effects of quercetin and its glucosides in human health, there is very limited knowledge on the bioavailability of these natural products. The oral absorption of quercetin in both humans and rats is low and variable (0–24%) [18–20], possibly due, in part, to degradation in the intestine. Quercetin glucosides are most likely hydrolyzed to quercetin by the intestinal microflora [21]. An interesting hypothesis that quercetin glucosides may be actively absorbed [22] has not been substantiated.

The present study focused on the transepithelial flux of quercetin and its two major glucosides in the human colonic cell line Caco-2, a model of human intestinal absorption [23–25]. Permeability coefficients determined for the human Caco-2 monolayer have been shown to correlate highly with human absorption *in vivo* [26, 27]. The transport rates for quercetin and its glucosides were compared with those of markers for paracellular, transcellular, and active transport.

MATERIALS AND METHODS

Chemicals

D-[1-^{14}C]Mannitol (57.0 mCi/mmol), D-[U-^{14}C]glucose (291 mCi/mmol), and *rac*-[4′-^{3}H]propranolol hydrochloride (14.4 Ci/mmol) were purchased from Amersham Life Science. Quercetin dihydrate was purchased from the Sigma Chemical Co.

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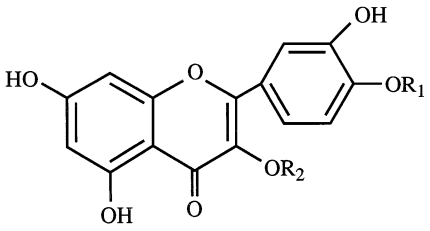

	R_1	R_2
Quercetin	H	H
Quercetin 4'-glucoside	D-glucose	H
Quercetin 3,4'-diglucoside	D-glucose	D-glucose

FIG. 1. Chemical structures of quercetin and its main glucosides in the onion.

Isolation of Quercetin Glucosides

Quercetin glucosides were extracted and isolated by HPLC from the red onion as previously described [3, 28], with certain modifications. Briefly, about 5 g of the edible portion of the onion was frozen in liquid nitrogen, pulverized, and extracted three times with 50 mL of methanol. After centrifugation, the extracts were combined, filtered, and evaporated to dryness under a stream of nitrogen. The residue was reconstituted in mobile phase and subjected to semipreparative HPLC. A SymmetryPrep C18 column, 7 μm, 7.8 × 300 mm (Waters Corp.) was used. The mobile phase, 35% methanol in 1% acetic acid, was used at a 4 mL/min flow rate, and detection was by UV at 254 nm. The two major HPLC peaks were collected and lyophilized. The quercetin glucosides were identified by mass spectrometry, using a Finnigan LCQ HPLC-ion trap mass spectrometer. Aqueous stock solutions of the isolated glucosides were quantified by HPLC assuming the same molar extinction coefficient as for the aglycone.

Cell Culture

Caco-2 cells obtained from the American Type Culture Collection were cultured in Eagle's Minimum Essential Medium (Cellgro, Mediatech) supplemented with 1% MEM nonessential amino acids (Mediatech), 10% fetal bovine serum (Sigma), 100 units/mL of penicillin, and 0.1 mg/mL of streptomycin (Sigma) and were grown in a humidified atmosphere of 5% CO_2 at 37°. Cells were subcultured at 80% confluency.

For all transport studies, Caco-2 cells were seeded in 12 mm i.d. Transwell® inserts (polycarbonate membrane, 0.4 μm pore size, Corning Costar Corp.) in 12-well plates at a density of 1.0×10^5 cells/cm^2. The basolateral (serosal) and apical (mucosal) compartments contained 1.5 and 0.5 mL of culture medium, respectively. Culture medium was replaced three times a week for 14 days and daily thereafter.

Transepithelial Permeability Experiments

Caco-2 cells in Transwells at passage 47–79 were used for transport experiments 20–35 days postseeding. TEER* values across the cell monolayers were measured using a Millicell-ERS voltohmmeter (Millipore Corp.). Inserts with TEER values >350 Ω cm^2 in culture medium were selected for transport experiments. The inserts were washed twice for 30 min with warm transport medium, Hanks' Balanced Salt Solution containing 25 mM of HEPES, pH 7.4. TEER values were also obtained after completion of transport experiments.

Transepithelial fluxes were measured for quercetin (50 μM), quercetin 4′-glucoside (50 μM), quercetin 3,4′-diglucoside (50 μM), and three transport marker substrates: [^{3}H]propranolol (100 μM), [^{14}C]glucose (3.4 μM), and [^{14}C]mannitol (1.1 μM) [23, 29]. A 10-mM stock solution of quercetin in ethanol was diluted to 50 μM in transport buffer. The resulting final concentration of ethanol, 0.5%, did not affect TEER values or the transport of mannitol. All other compounds were dissolved in transport buffer. Transport medium containing quercetin or its glucoside derivatives was added on either the apical (0.6 mL) or basolateral (1.65 mL) side of the inserts, while the receiving chamber contained the corresponding volume of transport medium. [^{14}C]Mannitol was added to the apical side of all inserts for the assessment of monolayer integrity. Upon termination of the 1-hr incubation at 37°, samples were collected from both sides of the cell monolayer and immediately frozen for assay of flavonoid content. Aliquots of basolateral medium were also assayed for transport of [^{14}C]mannitol.

For experiments involving radiolabeled propranolol and glucose, matched inserts were incubated with [^{14}C]mannitol. After 30 min (glucose) or 1 hr (propranolol and mannitol), duplicate samples were collected from the receiving side for analysis.

Sample Analysis

The amount of flavonoids transported was determined by reversed-phase HPLC of 100-μL samples on a Millennium HPLC system (Waters Corp.) with a Symmetry C18 column, 3.9 × 150 mm, and a model 996 photodiode array detector. The mobile phase consisted of 35% methanol in 5% acetic acid with a flow rate of 0.9 mL/min. Peak areas were measured at 370 nm for quercetin and 254 nm for the glucosides. UV spectra of sample peaks were compared with those of standards for positive identification of drug transport.

All radiolabeled samples were quantified on a Beckman LS 6000SC liquid scintillation system after the addition of Biodegradable Counting Scintillant (Amersham).

* *Abbreviation:* TEER, transepithelial electrical resistance.

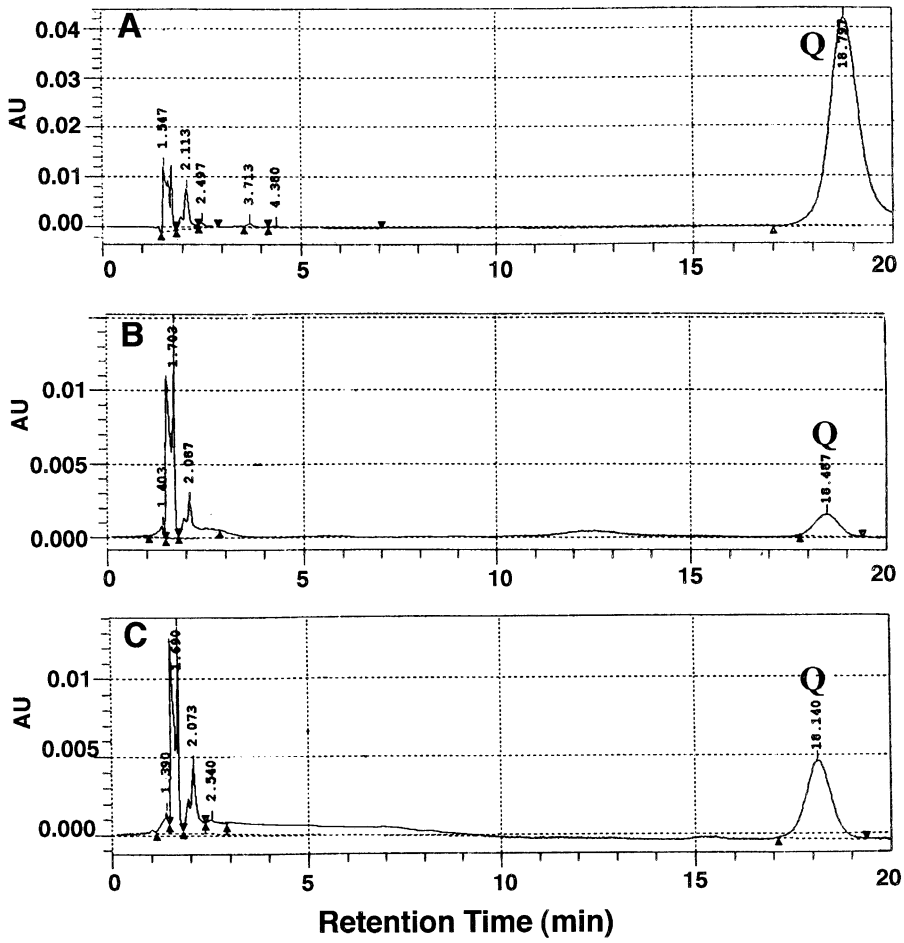

FIG. 2. HPLC of quercetin (Q) in Caco-2 transport experiments. (A) Loading solution (50 μM). (B) Basolateral side 60 min after loading apical side with quercetin. (C) From a separate experiment, apical side 60 min after loading basolateral side with 50 μM of quercetin. The same volume (100 μL) was injected in all tracings. AU is absorption units.

Calculations and Statistics

Apparent permeability coefficients (P_{app}) were calculated using the following equation:

$$P_{app} = \frac{V}{AC_0}\frac{dC}{dt} = \text{cm} \cdot \text{sec}^{-1}$$

where, V = the volume of the solution in the receiving compartment, A = the membrane surface area, C_0 = the initial concentration in the donor compartment, and dC/dT = the change in drug concentration in the receiver solution over time, [23, 30].

Data were expressed as the means ± SEM of six or more determinations. Differences in P_{app} were evaluated using Student's *t*-test. A P value < 0.05 was considered significant.

RESULTS

The integrity of Caco-2 cell monolayers has, in general, been assessed by measuring TEER values. However, during the initiation of these studies, mannitol permeability appeared to be a more reliable measure, and such determinations were adopted as a routine for all Caco-2 cell experiments. The cell monolayers were considered tight when the P_{app} for mannitol was less than 0.5×10^{-6} cm · sec^{-1}.

Recovery

Because of the instability of quercetin when exposed to cell culture conditions, great caution had to be exercised in the design of the experiments. Preliminary observations indicated that incubations for up to 60 min were acceptable and gave linear transport of quercetin. Representative HPLC tracings are shown in Fig. 2. The appearance of quercetin on both the basolateral and the apical side was easily detected at 370 nm. The chromatograms show very few interferences.

For quercetin, average recoveries of 67 and 84% were obtained for experiments with apical and basolateral loading, respectively. Recoveries were based on the amount of drug recovered in both apical and basolateral chambers at 60 min. Preliminary observations indicate that the unaccounted-for quercetin could be found in the cell monolayer. Recoveries were greater for the glucosides. Recovery of quercetin 3,4′-diglucoside in both apical and basolateral chambers was 87% for apical loaded experiments and 95% with basolateral loading. Recovery for experiments with quercetin 4′-glucoside was > 95%.

All experiments were run under sink conditions, defined as a final receiving compartment concentration which is less than 10% of the final donor compartment concentration.

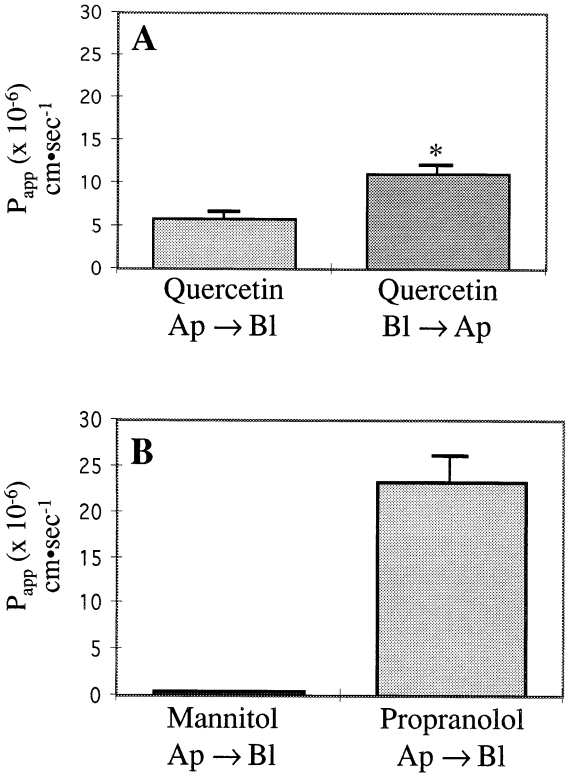

FIG. 3. Caco-2 transport of (A) quercetin (50 μM) and (B) paracellular transport marker [^{14}C]mannitol and transcellular transport marker [^{3}H]propranolol. Ap → Bl denotes apical to basolateral transport, and Bl → Ap denotes basolateral to apical transport. Each set of data represents the mean ± SEM of 8–10 experiments. *Significantly greater than quercetin Ap → Bl ($P < 0.001$).

Transport of Quercetin

The P_{app} values following either apical or basolateral loading with 50 μM of quercetin are summarized in Fig. 3A. These values should be compared with those for the paracellular transport marker mannitol and the transcellular transport marker propranolol in Fig. 3B. The P_{app} for the apical to basolateral flux of quercetin of 5.8 ± 1.1 × 10^{-6} cm · sec^{-1} ($N = 9$) was more than 10-fold higher than the P_{app} of mannitol of 0.48 ± 0.09 × 10^{-6} cm · sec^{-1} ($P < 0.01$), obtained simultaneously in the same inserts. Interestingly, the reverse, basolateral to apical flux of quercetin was almost 2-fold higher ($P < 0.001$), P_{app} of 11.1 ± 1.2 × 10^{-6} cm · sec^{-1} ($N = 10$). For comparison, the transcellular flux rates of propranolol for apical to basolateral transport, P_{app} of 23.4 ± 2.8 × 10^{-6} cm · sec^{-1} ($N = 8$), and for basolateral to apical transport, 28.0 ± 1.8 × 10^{-6} cm · sec^{-1} ($N = 7$), were similar to each other but significantly higher than for quercetin absorption ($P < 0.001$).

Transport of Quercetin Glucosides

The earliest eluting peak from the preparative HPLC of the onion extract was identified as quercetin 3,4′-diglucoside, whereas the late eluting peak was quercetin 4′-glucoside based on the molecular ions, $(M + H)^+$, of m/z 627 and 465, respectively, as well as the subsequent loss of two versus one glucose residues during MS/MS conditions. The positions of the glucose residues according to Fig. 1 had been assigned previously by NMR [3, 28]. The purity of the glucosides isolated this way was greater than 98%. The UV absorption spectra were essentially identical to that of quercetin.

QUERCETIN 4′-GLUCOSIDE. In Caco-2 cell transport experiments identical to those for quercetin described above, loading 50 μM of glucoside on the apical side, no transport was detectable. These experiments were carried out for up to 180 min without any change in the concentration on the loading side, i.e. the glucoside was quite stable, in contrast to quercetin. [^{14}C]Mannitol in the same inserts gave a P_{app} of 0.41 ± 0.02 × 10^{-6} cm · sec^{-1} for apical loading ($N = 7$), i.e. typical of the very low paracellular transport by these cells. It was estimated that a P_{app} for quercetin 4′-glucoside as low as 0.02 × 10^{-6} cm · sec^{-1} would have been detectable. In contrast, transport of quercetin 4′-glucoside in the basal to apical direction was observed, 1.6 ± 0.2 × 10^{-6} cm · sec^{-1} ($N = 8$), and was significantly greater than the P_{app} for mannitol in the same inserts, 0.36 ± 0.08 × 10^{-6} cm · sec^{-1} ($P < 0.01$) (Fig. 4).

QUERCETIN 3,4′-DIGLUCOSIDE. In contrast to quercetin 4′-glucoside, quercetin 3,4′-diglucoside demonstrated transport in all experiments. The P_{app} for apical to basolateral transport, 0.08 ± 0.03 × 10^{-6} cm · sec^{-1} ($N = 6$), was, however, only one-fourth of the P_{app} for mannitol transport, 0.36 ± 0.08 × 10^{-6} cm · sec^{-1}, in the same inserts ($P < 0.05$) (Fig. 4). As for quercetin and quercetin 4′-glucoside, the basolateral to apical transport rate, 0.38 ± 0.01 × 10^{-6} cm · sec^{-1} ($N = 6$), exceeded that for the apical to basolateral transport ($P < 0.05$).

To be able to assess whether the Caco-2 cells had an active glucose transporter, transport experiments with [^{14}C]glucose were conducted. A high P_{app} of 36.8 ± 1.1 × 10^{-6} cm · sec^{-1} was obtained ($N = 7$) in the apical to basolateral direction, a value that was even greater than for the transcellular transport of propranolol (Fig. 3B) ($P < 0.01$).

DISCUSSION

Although the importance of the dietary flavonoids, including quercetin, in human health is finding increasing support in the literature, few studies have addressed the ability of flavonoids to reach proposed sites of action. The limited number of studies in humans [18] and rats [19, 20] indicate very poor and variable intestinal absorption. Studies *in vivo*, however, are highly complex and may have been confounded by the chemical instability of quercetin and its metabolic breakdown by intestinal microflora, as well as by inadequate analytical methodology, lacking molecular

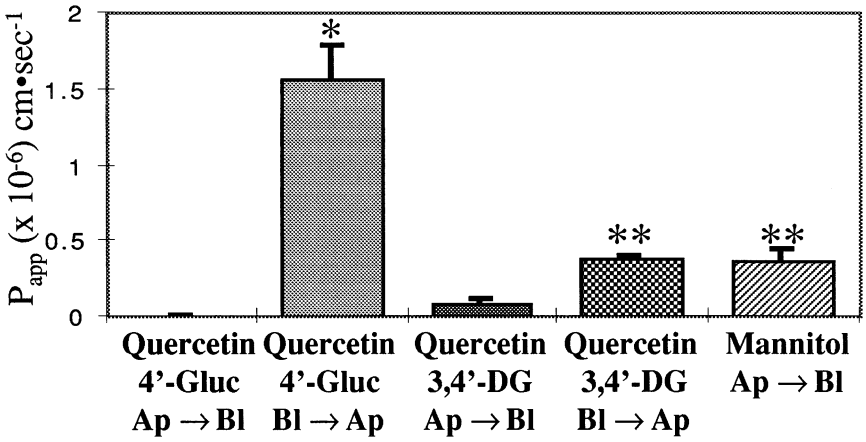

FIG. 4. Caco-2 transport of quercetin 4′-glucoside (50 μM), quercetin 3,4′-diglucoside (50 μM), and the paracellular transport marker [^{14}C]mannitol. Ap → Bl denotes apical to basolateral transport, and Bl → Ap denotes basolateral to apical transport. Each set of data represents the mean ± SEM of 6–8 experiments. *Significantly greater than mannitol ($P < 0.01$); and **significantly greater than quercetin 3,4′-diglucoside Ap → Bl ($P < 0.01$). Gluc = glucoside; DG = diglucoside.

specificity. Our approach in the present investigation was to use the Caco-2 cell monolayer, a model of human intestinal absorption [23–27], to understand the intestinal epithelial membrane transport of quercetin and its two major glucosides.

The concentration of quercetin employed in this study was based on the total daily intake of quercetin in the Zutphen epidemiological study, i.e. 16.3 mg daily [31]. Assuming a gastric fluid volume of 100–500 mL and near complete extraction of the flavonoids, the resulting concentration would be in the range of 100–500 μM. Thus, a 50-μM concentration is a realistic value when considering a divided daily intake of quercetin, variability in extraction, and variability in fluid volumes along the gastrointestinal tract.

The observations for quercetin demonstrate an apical to basolateral transport with a P_{app} of 5.8×10^{-6} cm · sec^{-1}, which exceeds that for the paracellular transport marker mannitol by more than 10-fold. Based on previous observations by Artursson and Karlsson [26], examining the relationship between P_{app} values obtained from the Caco-2 cells and human *in vivo* oral absorption for a number of drugs, this value would suggest oral absorption of quercetin. Even though the P_{app} value for quercetin was only one-fifth of that for the highly lipophilic propranolol, it is likely that quercetin is also using the transcellular diffusional pathway, albeit less efficiently. The finding of a basolateral to apical efflux mechanism for quercetin, however, may lead to an underestimate of the passive diffusion. Previous studies have demonstrated interactions between flavonoids and P-glycoprotein [32]. However, the possibility that the efflux of quercetin is related to this transporter, expressed on the apical side of the Caco-2 cells [33], does not seem likely, as quercetin does not possess the structural features common to P-glycoprotein substrates [34]. Other possibilities could be the multidrug resistance protein (MRP) [35], or the multispecific organic anion transporter (MOAT) [36].

Hollman *et al.* [22] put forward the hypothesis that quercetin glucosides may be actively absorbed in the human intestine by hexose transporters. The Caco-2 cells do express the sodium-dependent glucose cotransporter, SGLT1, in the apical membrane and the GLUT2 facilitated hexose transporter in the basolateral membrane [37, 38]. In our hands, [^{14}C]glucose was indeed transported from the apical to the basolateral side at a high rate with a P_{app} of 36.8×10^{-6} cm · sec^{-1}. However, no apical to basolateral transport of quercetin 4′-glucoside was evident with the Caco-2 cell monolayer. A very preliminary study of rat jejunal segments suggests that some absorption of quercetin monoglucosides may occur in that system [39]. However, the functional integrity of this model system was not known.

Considering the observations with quercetin 4′-glucoside, it was surprising to see some, albeit rather low, apical to basolateral transport of quercetin 3,4′-diglucoside. It may be possible that a glucose moiety in the 3-position promotes absorption while one in the 4′-position prevents it. It would therefore be interesting to test the transport of the quercetin 3-glucoside, a minor component in the onion [3, 28]. Nevertheless, quercetin 3,4′-diglucoside had a P_{app} value that, according to the classification of Artursson and Karlsson [26], may lead to some oral absorption in humans. As with quercetin, both quercetin 3,4′-diglucoside and, in particular, quercetin 4′-glucoside showed prominent efflux in the Caco-2 cells. Although the efficiency of the efflux of quercetin 4′-glucoside is rather low, it is likely the reason why no absorption of this glucoside is observed. The nature of this potential transporter is under further investigation.

Following ingestion of the flavonoids, the lumenal concentration would be expected to be much higher than the basolateral concentration, thus promoting passive diffusion. This gradient is expected to be maintained by removal of the flavonoids from the basolateral side by blood flow and plasma protein binding. Superimposed on the passive absorption is the efflux of these agents, which would tend to reduce net absorption. In our experiments, carried out under initial velocity conditions, a high gradient for apical to basolateral transport was maintained throughout the 60-min time course. In the *in vivo* situation, inhibition of the outwardly directed transport by dietary components may lead to enhanced absorption of the flavonoids. The oral absorption of dietary flavonoids can be anticipated to

be further complicated by their binding to food constituents and often limited solubility. Dietary components, which bind the flavonoids in the lumen, will alter the equilibrium towards reduced transepithelial absorption. In contrast, ethanol from beverages such as wine may aid in extracting the flavonoids from foods and act as a permeation enhancer.

In conclusion, the data generated in this study, using the human Caco-2 cell line as a model, suggest facile absorption of quercetin through the human intestinal epithelial cell lining. In contrast, quercetin 4′-glucoside and quercetin 3,4′-diglucoside are not appreciably absorbed. A basolateral to apical efflux system was detected, the nature and function of which will be the subject of further investigations.

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