Transepithelial Transport of Microbial Metabolites of Quercetin in Intestinal Caco-2 Cell Monolayers

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m-Hydroxyphenylacetic acid (mHPA), 3,4-dihydroxyphenylacetic acid (DHPA), and 4-hydroxy-3-methoxyphenylacetic acid (HMPA) are major microbial metabolites of quercetin. After administration of quercetin to human subjects, these metabolites are readily detected in blood and urine. mHPA, DHPA, and HMPA are thought to exert protective biological activity within the body due to their antioxidant properties. However, very little work has been published concerning their absorption. I have examined the absorption characteristics of the quercetin metabolites in Caco-2 cells by a coulometric detection method using HPLC-ECD. All of them exhibited nonsaturable transport in Caco-2 cells up to 30 mM, whereas HMPA and mHPA also showed proton-coupled polarized absorption. The proton-coupled directional transport of HMPA and mHPA was inhibited by the substrate of the monocarboxylic acid transporter (MCT). A considerable amount of apically loaded HMPA and mHPA was taken up and transported through to the basolateral side, while almost all of the apically loaded DHPA was retained on the apical side. Furthermore, the transepithelial flux of DHPA was inversely correlated with the paracellular permeability of Caco-2 cells, although those of HMPA and mHPA were almost constant. These results indicate that transport of DHPA was mainly via paracellular diffusion, although HMPA and mHPA were absorbed to some extent by the MCT.

KEYWORDS: Quercetin; monocarboxylic acid transporter; microbial metabolite; hydroxylated phenylacetic acids; Caco-2

INTRODUCTION

Dietary polyphenols found in fruit and vegetables are natural antioxidants that are thought to contribute to the prevention of cardiovascular diseases (*1*, *2*). The most abundant types of polyphenol in the human diet are the flavonoids and cinnamic acids. Flavonoids are divided into six main classes based on the degree of oxidation of the C-ring, the hydroxylation pattern of the ring-structure, and the substitution in the 3-position: flavanols, flavonols, flavones, flavanones, isoflavones, and anthocyanidins (*3*). Catechin and quercetin are the major representative flavanols and flavonols, respectively. The potential health benefit through regular consumption of these compounds has been extensively studied. Catechin and quercetin are powerful antioxidants capable of efficiently scavenging reactive oxygen or nitrogen species in vitro and have been reported to act as antitumor, antimutagenic, and antihypertensive agents (*4*–*6*). However, the potential antioxidant activity of catechin and quercetin in vivo is dependent on the intestinal absorption and subsequent interaction with target tissues. The absorption, metabolism, distribution, and excretion of these compounds have been extensively studied. However, their absorption efficiency was found to be low (*7*–*15*).

The major representative of dietary cinnamic acids is caffeic acid, which occurs in foods mainly as chlorogenic acid (5-caffeoylquinic acid), an ester of quinic acid. Coffee is a major source of chlorogenic acid in the diet, the daily intake of chlorogenic acid in coffee drinkers being ~1 g (*16*). Chlorogenic acid and caffeic acid also exhibit antioxidant activities, antimutagenic, and carcinogenic effects in vitro (*3*, *10*). Indeed, this is consistent with the reported inverse correlation between coffee intake and colon cancer in some epidemiological studies (*17*–*19*). Thus, the consumption of these compounds is thought to prevent diseases associated with oxidative stress. However, the bioactive forms of these compounds in vivo have not been elucidated.

Recent studies have suggested that to fully assess the health benefits of dietary polyphenols, the biological properties of both the ingested parent compound and its microbial metabolites must be investigated (*20*–*24*). *m*-Coumaric acid, *m*-hydroxyphenylpropionic acid (mHPP), and 3,4-dihydroxyphenylpropionic acid (DHPP) are major microbial metabolites of caffeic acid or chlorogenic acid, which possess significant antioxidant properties (*20*, *21*, *24*). Previously, we reported that these metabolites are absorbed by the monocarboxylic acid transporter (MCT) (*25*). The intestinal absorption of catechin was quite low (*7*, *8*) because catechin is mainly absorbed via paracellular pathways where it is then restricted by the epithelial tight junction (*26*).

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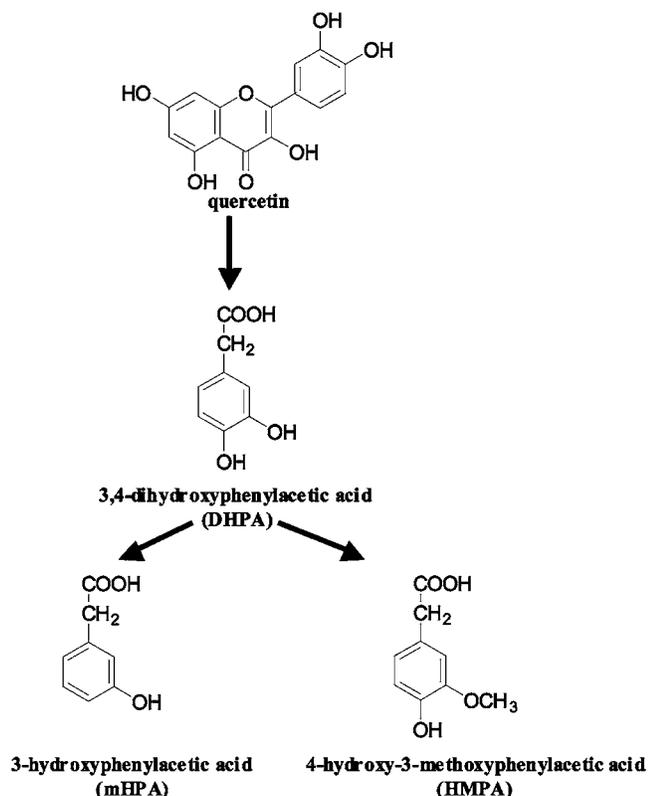**602** *J. Agric. Food Chem.,* Vol. 53, No. 3, 2005

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Figure 1. Metabolic pathway of quercetin by the gut microflora.

Therefore, most ingested catechin is likely to reach the colon intact where it is metabolized into hydroxylated phenylpropionic acids, which are absorbed and metabolized in a manner similar to that of caffeic or chlorogenic acids (*25, 27*). It was reported that quercetin was metabolized further by colonic microflora to give mainly 3,4-dihydroxyphenylacetic acid (DHPA), 3-hydroxyphenylacetic acid (mHPA), and 4-hydroxy-3-methoxyphenylacetic acid (HMPA) (**Figure 1**) (*21, 23, 24, 28*). These metabolites of quercetin also possess antioxidant activities and exhibit physiological effects such as inhibition of platelet aggregation (*29*). However, their absorption characteristics are still obscure. This study was designed to reveal the absorption mechanisms of the hydroxylated phenylacetic acids, such as HMPA, mHPA, and DHPA, by directly measuring their transepithelial transport across Caco-2 cells as an in vitro model of intestinal absorption and metabolism (*30−32*).

MATERIALS AND METHODS

Materials. The human colon adenocarcinoma cell line Caco-2 was obtained from the American Type Culture Collection (Rockville, MD). Dulbecco's modified Eagle's medium (DMEM) was purchased from Nissui Pharmaceuticals (Tokyo, Japan). Fetal calf serum, glutamine, nonessential amino acids, penicillin (10 000 units/mL in 0.9% NaCl), streptomycin (10 mg/mL in 0.9% NaCl), phosphate-buffered saline, and Hank's balanced salt solution (HBSS) were all purchased from Invitrogen Corp. (Carlsbad, CA). Type I collagen was purchased from Nitta Gelatin Inc. (Osaka, Japan). Plastic dishes, plates, and Transwell inserts with 0.4-μm polycarbonate membranes (12 mm in diameter) were obtained from Corning (Corning, NY). HMPA, mHPA, and DHPA were from Wako Pure Chemicals Inc., Ltd. (Osaka, Japan). All other chemicals used in this study were of analytical grade.

Cell Culture. Caco-2 cells were cultured in DMEM containing 10% fetal calf serum, 1% nonessential amino acids, 4 mM L-glutamine, 50 U/mL penicillin, and 50 μg/mL streptomycin (pH 7.4). Cells were grown in a humidified atmosphere of 5% CO_2 at 37 °C. All cells used were between passages 57 and 68.

Transepithelial Transport Experiments. Cells were grown in Transwell inserts with the semipermeable membrane first coated with type I collagen (12-mm diameter and 0.4-μm pore size, Corning Costar, Ithaca, NY). The cells were seeded at a density of $1 \times 10^5/\text{cm}^2$, and the monolayers were formed after culturing for 2 weeks. The integrity of the cell layer was evaluated by measurement of transepithelial electrical resistance (TER) with Millicell-ERS equipment (Millipore, Bedford, MA). Monolayers with a TER of more than 250 Ω cm^2 were used for transepithelial transport experiments.

To measure the apical-to-basolateral permeability, 1.5 mL of HBSS (pH 7.4, 37 °C) was added to the basal chamber of the Transwell insert, and 0.5 mL of the test solution (pH 6.0 or 7.4, 37 °C) containing HMPA (1mM), mHPA (1mM), or DHPA (5 mM) was added to the apical side. After the desired incubation time at 37 °C, the basal solution was collected and then replaced with an equal volume of HBSS. The amount of DHPA, mHPA, or HMPA transported by the Caco-2 cells was determined using a HPLC-electrochemical detector (ECD) with an ESA coulometric detection system (ESA Inc., Boston, MA). The results were expressed in terms of specific permeability (μL/cm^2), which was calculated as the amount transported divided by the initial concentration in the donor compartment.

To examine the basolateral-to-apical transport, HBSS (pH 6.0 or 7.4, 37 °C) was added to the apical side, and 1.5 mL of the test solution (pH 7.4, 37 °C) was added to the basolateral side.

Chromatographic Conditions. HPLC-ECD fitted with a coulometric detection system was used for analysis as previously reported (*25−27*). Chromatographic separation was performed on a C18 column (ODS150, MC Medical, Inc., Tokyo, Japan). The mobile phase A (solvent A) was 50 mM sodium acetate containing 5% methanol (pH 3.0), while mobile phase B (solvent B) was 50 mM sodium acetate containing 40% acetonitrile and 20% methanol (pH 3.5). The elution profile (0.6 mL/min) was as follows: 0−28.5 min, linear gradient from 85% solvent A/15% solvent B to 20% solvent A/80% solvent B; 28.5−31 min, isocratic elution 0% solvent A/100% solvent B; 31−35 min, isocratic elution 85% solvent A/15% solvent B. Eight electrode detector potentials (0−700 mV in increments of 100 mV) were used to measure the amount of HMPA. Similarly, eight electrode detector potentials (100−800 mV in increments of 100 mV) were used to measure the amount of mHPA and DHPA.

Distribution of HMPA, mHPA, and DHPA after Transport Experiments. At the end of the transport experiments, the level of HMPA, mHPA, or DHPA in the apical and basolateral solutions was measured. The monolayer cells were rinsed with HBSS (pH 6.0 or 7.4) and extracted with methanol/solvent A (10:1, v/v) for 30 min. HMPA, mHPA, or DHPA in this extract was measured and used as an index of the intracellular fractions taken up by the Caco-2 cells.
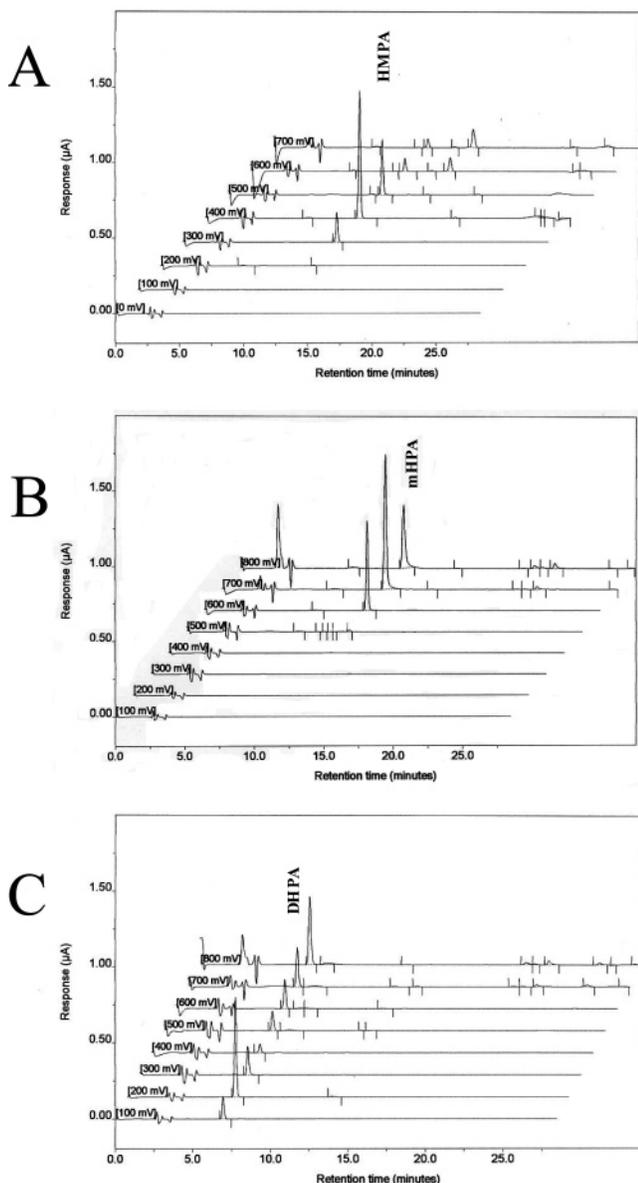
Data Analysis. The permeation rate [nmol min^{-1} (mg protein)$^{-1}$], *J*, was evaluated from the slope of the initial linear part of the plot of the amount transported [nmol min^{-1} (mg protein)$^{-1}$] against time (in minutes), calculated by linear regression analysis. The kinetic parameters for saturable transport across Caco-2 cells were estimated by the fitting of eq 1 with use of the nonlinear least-squares regression analysis program, MULTI (*33*):

$$J = J_{\max}[C]/(K_t + [C]) \qquad (1)$$

where *C* is the initial concentration of the substrate, $J_{\max}$ is the maximum permeation rate, and K_t is the Michaelis−Menten constant. Results are expressed as the means ± SD. Statistical analysis was done with student's two-tailed *t*-test, and differences with $P < 0.01$ were considered significant.

RESULTS

HPLC Analysis of HMPA, mHPA, and DHPA Transported across Caco-2 Cell Monolayers. Representative chromatograms of HMPA, mHPA, and DHPA transported into the basolateral solution are presented in **Figure 2**. HMPA, mHPA, and DHPA were determined at a detection limit <0.5 pmol on the column. The results were reproducible without requiring any sample pretreatment. Purity of the peaks was assessed using

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Figure 2. Chromatograms of HMPA (**A**), mHPA (**B**), and DHPA (**C**) transported across Caco-2 cell monolayers.

Table 1. Distribution of HMPA, mHPA, and DHPA after Transepithelial Transport Experiments in the Presence and Absence of a Proton Gradient[a]

sample pH gradient	% compound recovered from		
	apical side	basolateral side	cells
HMPA			
6.0/7.4	94.22 ± 0.11	5.51 ± 0.11	0.27 ± 0.01
7.4/7.4	99.04 ± 0.11	0.83 ± 0.13	0.13 ± 0.03
mHPA			
6.0/7.4	96.93 ± 0.67	2.67 ± 0.56	0.40 ± 0.18
7.4/7.4	99.40 ± 0.14	0.49 ± 0.13	0.11 ± 0.06
DHPA			
6.0/7.4	99.25 ± 0.04	0.31 ± 0.04	0.43 ± 0.07
7.4/7.4	99.65 ± 0.05	0.22 ± 0.06	0.13 ± 0.05

[a] Transepithelial transport experiments were done as described in the Materials and Methods in the presence and absence of a proton gradient (apical pH, 6.0 or 7.4; basolateral pH, 7.4). Values are the mean ± SD of three or more experiments.

Table 2. Effects of Various Compounds on HMPA and mHPA Transport across Caco-2 Cell Monolayers in the Presence of a Proton Gradient[a]

|	relative permeation (% of control)	
	HMPA	mHPA
NaN$_3$, 10 mM	16.8 ± 2.1[b]	13.6 ± 1.1[b]
benzoic acid, 10 mM	18.8 ± 2.2[b]	20.6 ± 2.8[b]
lactic acid, 10 mM	113.0 ± 9.5	88.9 ± 9.1
acetic acid, 10 mM	38.4 ± 2.4[b]	50.0 ± 4.7[b]

[a] The amount of HMPA or mHPA transported was measured at 37 °C for 40 min by incubating Caco-2 cells in the absence or presence of each compound at the concentration indicated (apical pH, 6.0; basolateral pH, 7.4). Each value represents the mean ± SD of three or more experiments. [b] $P < 0.01$.

peak area ratio accuracies for the adjacent oxidation channels (lower or upper) to the dominant oxidation channel. The voltammetric response of the analyte across these channels was unique for each compound. Greater than 70% ratio accuracy was considered to support the peak purity (*34*). The retention time (RT) and dominant oxidation potential for HMPA, mHPA, or DHPA were 11.9 min, 400 mV, 12.0 min, 700 mV, and 7.3 min, 200 mV, respectively.

Characteristics of Transepithelial Transport of HMPA, mHPA, and DHPA. Bi-directional permeation of HMPA (1 mM), mHPA (1 mM), and DHPA (5 mM) across Caco-2 cell monolayers was examined in the presence and absence of an inwardly directed proton gradient (**Figure 3**). HMPA and mHPA exhibited pH-dependent directional transport from the apical to the basolateral side ($J_{ap→bl}$ and $J_{bl→ap}$ being 1.83 and 0.12 nmol/min/mg protein for HMPA, 1.17 and 0.09 nmol/min/mg protein for mHPA, with an apical pH, 6.0; basolateral pH, 7.4). However, in the absence of a proton gradient (apical pH, 7.4; basolateral pH, 7.4), $J_{ap→bl}$ of HMPA (0.17 nmol/min/mg protein) was almost the same as that of $J_{bl→ap}$ (0.11 nmol/min/mg protein), whereas $J_{ap→bl}$ of mHPA and DHPA (0.24 or 0.74

nmol/min/mg protein) was slightly greater than that of $J_{bl→ap}$ (0.08 or 0.29 nmol/min/mg protein). This is characteristic of polarized transport.

Distribution of HMPA, mHPA, and DHPA. After the transport experiments in the absence of a proton gradient (apical pH, 7.4; basolateral pH, 7.4), >99% of apically loaded HMPA, mHPA, or DHPA was retained on the apical side, suggesting they were restricted by the tight junctions (**Table 1**). In the presence of a proton gradient (apical pH, 6.0; basolateral pH, 7.4), a considerable amount of HMPA and mHPA was transported into the basolateral side, although transported DHPA was much less than that of HMPA and mHPA. HMPA and mHPA are likely to be taken up and transported into the basolateral side according to a pH gradient, whereas DHPA seems to be restricted by the tight junction.

Concentration Dependence of HMPA, mHPA, and DHPA Transport. Figure 4 shows the relationship between the initial permeation rate of HMPA, mHPA, and DHPA, and their concentrations (apical pH, 6.0; basolateral pH, 7.4). The permeation rate of HMPA, mHPA, and DHPA increased linearly with concentration (~30 mM), indicating nonsaturable transport.

Inhibition of Directional Transport of HMPA and mHPA. To investigate the transport characteristics responsible for proton-coupled polarized transport of HMPA or mHPA (apical pH, 6.0; basolateral pH, 7.4), I added 0.5 mM HMPA and 1 mM mHPA to the apical chamber, and the effects of various compounds on permeation were examined (**Table 2**). A metabolic inhibitor, NaN$_3$ (10 mM), strongly reduced the permeation of HMPA and mHPA. The substrates for MCTs (10 mM), such as benzoic acid and acetic acid, significantly inhibited

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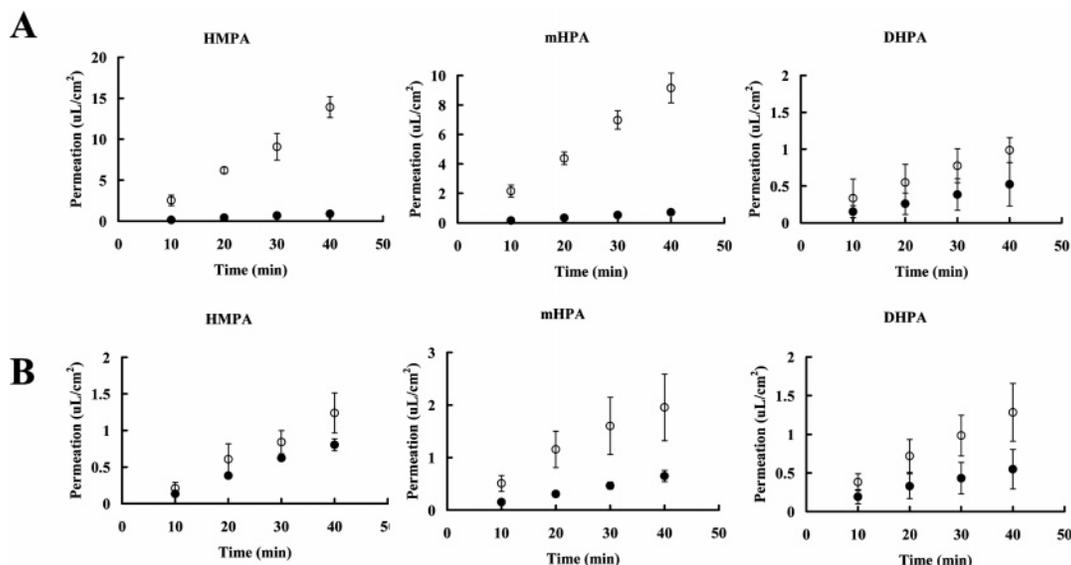
Figure 3. Characteristics of transepithelial transport of HMPA, mHPA, and DHPA across Caco-2 cell monolayers in the presence (**A**) or absence (**B**) of a proton gradient. Permeation of HMPA (1 mM), mHPA (1 mM), and DHPA (5 mM) from the apical to the basolateral side (○) and from the basolateral to the apical side (●) was measured at 37 °C in the presence (**A**) and absence (**B**) of a proton gradient (apical pH, 6.0 or 7.4; basolateral pH, 7.4). Each point is the mean ± SD of three experiments.
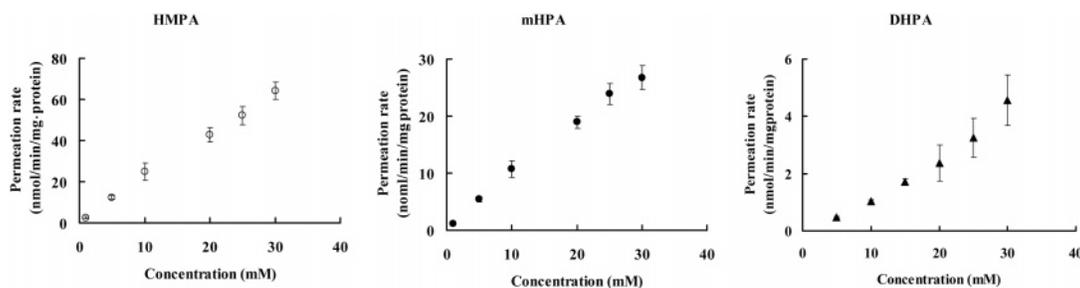
Figure 4. Concentration dependence of HMPA (○), mHPA (●), and DHPA (▲) transport across Caco-2 cell monolayers in the presence of a proton gradient. The initial permeation rate is shown. Values are the mean ± SD of three or more experiments.
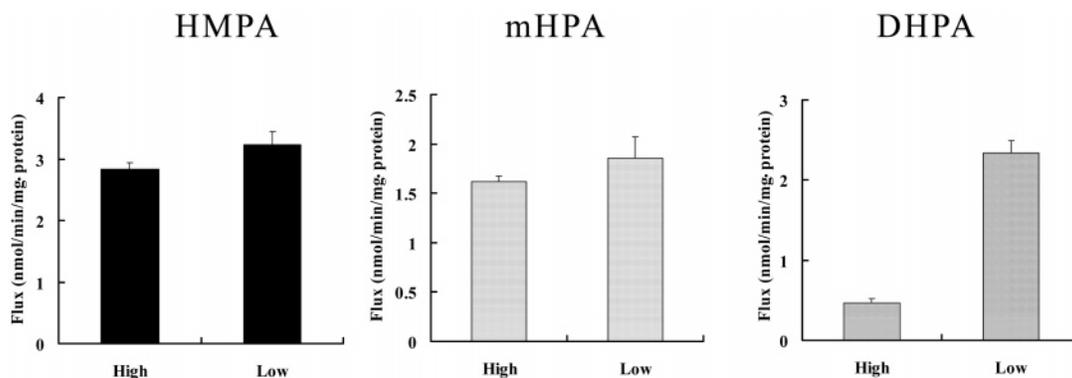
Figure 5. Correlation between TER and the transepithelial flux of HMPA, mHPA, and DHPA. HMPA (1 mM), mHPA (1 mM), or DHPA (5 mM) was loaded in the apical side, and the flux from the apical side to the basolateral side was measured at 37 °C (apical pH, 6.0; basolateral pH, 7.4). TER values are indicated as follows: (HMPA) high, 962 ± 44; low, 246 ± 42; (mHPA) high, 975 ± 17; low, 266 ± 31; (DHPA) high, 1046 ± 41; low, 285 ± 25. Each point is the mean ± SD of three experiments.

permeation, although lactic acid, a good substrate for MCT1-MCT4 (*35*), seemed to have no effect. The order of the inhibitory activity of the MCT substrates was benzoic acid > acetic acid > lactic acid. This order was the same as that for ferulic and *p*-coumaric acids, clearly demonstrating that they are transported, at least in part, by MCT (*36, 37*).

Paracellualr Transport of DHPA across the Caco-2 Cell Monolayers. Caco-2 cell monolayers exhibiting different TER values were prepared by treating the cells with cytochalasin D (*38*). Using these monolayers, the apical-to-basolateral transport

of DHPA was then characterized by comparison to that of HMPA and mHPA (apical pH, 6.0; basolateral pH, 7.4). As illustrated in **Figure 5**, the transepithelial flux of DHPA was inversely correlated with the TER, whereas the transepithelial flux of HMPA and mHPA was almost constant irrespective of the TER. These findings suggest that DHPA permeates across Caco-2 cells via the paracellular pathways, while HMPA and mHPA permeate across via the transcellular pathways. This result matched the MCT-mediated transport of HMPA and mHPA mentioned above.
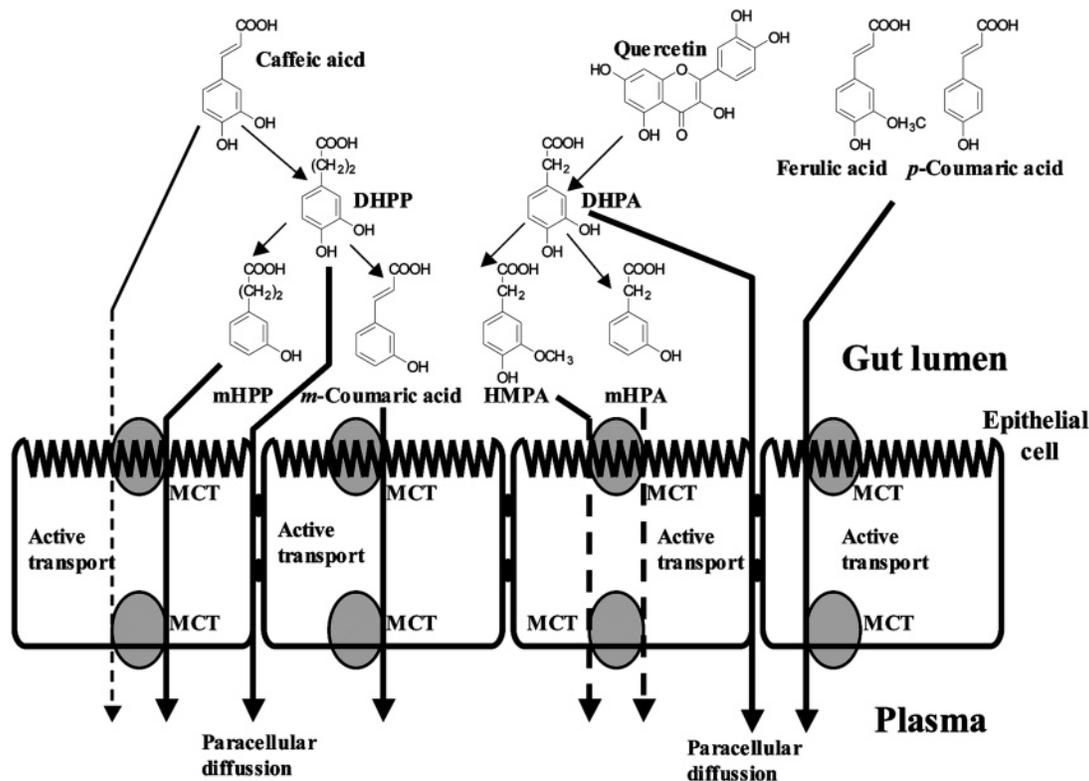
Figure 6. Proposed pathways for intestinal absorption of ingested polyphenols.

DISCUSSION

Recently, we developed a means of identifying compounds that can be transported by the MCT in Caco-2 cells (*38*). This technique measures the competitive effect of dietary substances on the transport of fluorescein, a known substrate of the MCT. MCT-mediated transport of dietary phenolic acids, such as ferulic acid and *p*-coumaric acid, was demonstrated using this method (*36*, *37*). Furthermore, we have reported that *m*-coumaric acid and mHPP, major microbial metabolites of caffeic acid, are also absorbed by the MCT in Caco-2 cells (*25*, *27*). The key components of a substrate for MCTs are thought to be monoanionic carboxyl group and a nonpolar side chain or aromatic hydrophobic moiety (*39*). The major metabolites of quercetin (HMPA, mHPA, and DHPA) fulfill the structural criteria for an MCT substrate, but it was not known whether MCT in Caco-2 cells recognizes and transports these compounds. Hence, the mechanism for their intestinal transport was investigated (**Figure 3**).

The apical-to-basolateral flux ($J_{ap\rightarrow bl}$) of 1 mM HMPA or mHPA in the presence of a proton gradient (1.83 nmol/min/mg·protein for HMPA, 1.17 nmol/min/mg·protein for mHPA) were lower than those of 1 mM *m*-coumaric acid, *m*HPP, ferulic acid, and *p*-coumaric acid (7.23 nmol/min/mg·protein for *m*-coumaric acid, 7.47 nmol/min/mg·protein for *m*HPP, 9.79 nmol/min/mg·protein for ferulic acid, 3.73 nmol/min/mg·protein for *p*-coumaric acid) (*25*, *36*, *37*). The $J_{ap\rightarrow bl}$ of HMPA and mHPA in the presence of the proton gradient was 5−11-fold $J_{ap\rightarrow bl}$ of each in the absence of the proton gradient (0.17 nmol/min/mg·protein for HMPA, 0.24 nmol/min/mg·protein for mHPA). The $J_{ap\rightarrow bl}$ of HMPA and mHPA was 13−15-fold the basolateral-to-apical flux ($J_{bl\rightarrow ap}$) of that of each in the presence of the proton gradient (0.12 nmol/min/mg·protein for HMPA, 0.09 nmol/min/mg·protein for mHPA). Thus, the proton-coupled polarized transport of HMPA and mHPA is consistent with MCT substrates, such as ferulic and *p*-coumaric acids, although their

affinity for MCT is likely to be lower. The results of the distribution study of HMPA and mHPA (**Table 1**) also indicate a lower absorption efficiency than those of other MCT substrate (e.g., ferulic and *p*-coumaric acids). Specific inhibition of polarized transport of HMPA and mHPA by the MCT substrate (**Table 2**) suggests that transport is mediated by the MCT, as is the case for ferulic and *p*-coumaric acids (*36*, *37*). Saturable transport of HMPA and mHPA was not observed at concentrations up to 30 mM (**Figure 4**), which might be due to the low affinity for MCT.

Directional transport of mHPA (1 mM) or DHPA (5 mM) in the apical-to-basolateral direction was observed in the absence of the inwardly directed proton gradient (**Figure 3**). The reason for this polarized transport is not clear. NaN_3 (10 mM), DIDS (2 mM), benzoic acid (10 mM), and HCO_3^- (10 mM) did not inhibit the transport of mHPA or DHPA, suggesting a specific transport system was not involved.

The potential benefit of flavonoids in nutrition has attracted huge research interest over the past decade. Many studies have focused on the absorption and metabolism to examine their bioactive compound in vivo and the mechanism by which they might exert physiological effects. Unlike flavonoids, phenolic acids have not been extensively studied and are not considered to be of great nutritional interest. Phenolic acids are present in many foods including grains, vegetables, and fruits (*40*). It has been shown that certain phenolic acids, such as ferulic or *p*-coumaric acids, are absorbed by MCT in Caco-2 cells (*36*, *37*). However, dihydroxy and trihydroxy derivatives of benzoic or cinnamic acids, such as caffeic and gallic acids, have a lower affinity for MCT and are mainly absorbed via paracellular diffusion (*27*, *37*). Indeed, the relative oral bioavailability of *p*-coumaric acid against gallic acid is ∼70 in rats after oral administration. This significant difference in bioavailability illustrates the high absorption efficiency of MCT-mediated transport in vivo (*41*). The finding that phenolic acids are rapidly

absorbed and distributed intact within the body has focused significant research effort toward understanding the nutritional value of these compounds.

Researchers are interested in studying the biological effect of the microbial metabolites of ingested polyphenols, in particular those that are poorly absorbed in the small intestine (i.e., catechin, quercetin, and caffeic acid) (*20−23*). In general, the phenolic degradation products formed by gut microflora can be classified as hydroxylated phenylpropionic acids or hydroxylated phenylacetic acids, depending on the structural characteristics of the parent polyphenols (*23*). For instance, it has been reported that the hydroxylated phenylpropionic acids, such as mHPP and DHPP, can be formed from caffeic acid, catechin, or procyanidin and naringin, while quercetin was metabolized to give hydroxylated phenylacetic acids, such as HMPA, mHPA, and DHPA (*20−24, 28, 42*). These bioactive metabolites could be responsible for the health effects of dietary polyphenols. Currently, there is a paucity of data on their absorption and distribution within the body. The results obtained in this study, together with our previous work (*25, 27, 36, 37, 41*), give new insight into the absorption characteristics of dietary polyphenols and their microbial metabolites (**Figure 6**). This work also exemplifies the physiological significance of MCT-mediated absorption to give a fuller understanding of the health benefits of dietary polyphenols.

Currently, nine isoforms of MCT have been identified (*43*), but only MCT1−MCT4 are characterized in terms of their substrate and inhibitor kinetics. Each MCT isoform is likely to have a unique biological role, which is related to the different tissue distribution. MCT1 is the most well-studied isoform and is found in almost all tissues, that is, heart, skeletal muscle, small intestine, colon, liver, brain, spinal cord, testis, ovary, placenta, and adrenal gland (*44*), which would imply a physiological significance for humans. Short-chain fatty acids (SCFA), such as acetate, propionate, and butyrate, are the most abundant organic anions in the colonic lumen. SCFA are microbial metabolites of undigested carbohydrates and proteins, as well as poorly absorbed polyphenols. Butyrate is the principal energy source in colonic epithelial cells (*45*), exerting a number of biological effects to induce cell differentiation and regulate growth and proliferation of the colonic mucosal epithelia (*46, 47*). The mechanism of absorption of butyrate is still not fully resolved. MCT1 can recognize and transport butyrate (*48*), although the nonelectrogenic $SCFA^-/HCO_3^-$ antiporter is also implicated in its absorption (*49*). SCFA and hydroxylated-phenylpropionic or -phenylacetic acids are colonic microbial metabolites of poorly absorbed food materials that exert various biological activities. I therefore propose to call them "metabonutrients" because this nomenclature distinguishes them from classical nutrients, such as sugars, lipids, amino acids, minerals, and vitamins. Until recently, research concerning the physiological function of food has focused on the biologically active constituents of the food itself. In the future, the metabo-nutrients, which are generated by colonic microflora from ingested food materials, should also be investigated for their health benefits. It is possible that MCT-mediated absorption plays a major role in metabo-nutrient uptake.

In conclusion, I have demonstrated that MCT is involved in the absorption of the principal microbial metabolites of quercetin. HMPA and mHPA are absorbed by MCT to a lesser extent, although DHPA is mainly transported via the paracellular pathways. This highlights the physiological significance of the MCT-mediated absorption of dietary components. Further studies to investigate the MCT-mediated absorption and the distribution of dietary bioactive components will help to assess the health benefits of specific foods and beverages.

ABBREVIATIONS USED

HBSS, Hanks' balanced salt solution; TER, transepithelial electrical resistance; MCT, monocarboxylic acid transporter; ECD, electrochemical detector; SCFA, short-chain fatty acid.

ACKNOWLEDGMENT

LITERATURE CITED

(1) Hollman, P. C.; Feskens, E. J.; Katan, M. B. Tea flavonols in cardiovascular diseases and cancer epidemiology. *Proc. Soc. Exp. Biol. Med.* **1999**, *220*, 198−202.

(2) Mukamal, K. J.; Maclure, M.; Muller, J. E.; Sherwood, J. B.; Mittleman, M. A. Tea consumption and mortality after acute myocardial infarction. *Circulation* **2002**, *105*, 2476−2481.

(3) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure-antioxidant activity relationship of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20*, 933−956.

(4) Okuda, T.; Mori, K.; Hayatsu, H. Inhibitory effect of tannins on direct-acting mutagens. *Chem. Pharm. Bull.* **1984**, *32*, 3755−3758.

(5) Henry, J. P.; Stephan-Larson, P. Reduction chronic psychosocial hypertension in mice by decaffeinated tea. *Hypertension* **1984**, *6*, 437−444.

(6) Formica, J. V.; Regelson, W. Whole grain intake and cancer: an expanded review and meta-analysis. *Food Chem. Toxicol.* **1995**, *33*, 1061−1080.

(7) Chen, L.; Lee, M. J.; Li, H.; Yang, C. S. Absorption, distribution, and elimination of tea polyphenols in rats. *Drug Metab. Dispos.* **1997**, *25*, 1045−1050.

(8) Chow, H. H.; Cai, Y.; Alberts, D. S.; Hakim, I.; Dorr, R.; Shahi, F.; Crowell, J. A.; Yang, C. S.; Hara, Y. Phase I pharmacokinetics study of tea polyphenols following single-dose administration of epigallocatechin gallate and polyphenone E. *Cancer Epidemiol., Biomarkers Prev.* **2001**, *10*, 53−58.

(9) Scalbert, A.; Williamson, G. Dietary intake and bioavailability of polyphenols. *J. Nutr.* **2000**, *130*, 2073S−2085S.

(10) Scalbert, A.; Morand, C.; Manach, C.; Remesy, C. Absorption and metabolism of polyphenols in the gut and impact on health. *Biomed. Pharmacother.* **2002**, *56*, 276−282.

(11) Lee, M. J.; Wang, Z. Y.; Li, H.; Chen, L.; Sun, Y.; Gobbo, S.; Balentine, D. A.; Yang, C. S. Analysis of plasma and urinary tea polyphenols in human subjects. *Cancer Epidemiol., Biomarkers Prev.* **1995**, *4*, 393−399.

(12) Nakagawa, K.; Okuda, S.; Miyazawa, T. Dose-dependent incorporation of tea catechins, (−)-epigallocatechin-3-gallate and (−)-epigallocatechin, into human plasma. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 1981−1985.

(13) Piskula, M. K.; Terao, J. Accumulation of (−)-epicatechin metabolites in rat plasma after oral administration and distribution of conjugation enzymes in rats tissues. *J. Nutr.* **1998**, *128*, 1172−1178.

(14) Hollman, P. C. H.; van Trijp, J. M. P.; Buysman, M. N. C. P.; van der Gaag, M. S.; Mengelers, M. J. B.; de Vries, J. H. M.; Katan, M. B. Relative bioavailability of the antioxidant flavonoid quercetin from various food in man. *FEBS Lett.* **1997**, *418*, 152−156.

(15) Shimoi, K.; Yoshizumi, K.; Kido, T.; Usui, Y.; Yumoto, T. Absorption and urinary excretion of quercetin, rutin, and αG-rutin, a water soluble flavonoid, in rats. *J. Agric. Food Chem.* **2003**, *51*, 2785−2789.

(16) Clifford, M. N. Chlorogenic acids and other cinnamates: nature, occurrence, and dietary burden. *J. Sci. Food Agric.* **1999**, *79*, 362−372.

(17) Giovannucci, E. Meta-analysis of coffee consumption and risk of colorectal cancer. *Am. J. Epidemiol.* **1998**, *147*, 1043−1052.

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(18) Favero, A.; Franceschi, S.; La Vecchia, C.; Negri, E.; Conti, E.; Montella, M. Meal frequency and coffee intake in colon cancer. *Nutr. Cancer* **1998**, *30*, 182−185.

(19) Tavani, A.; Pregnolato, A.; La Vecchia, C.; Negri, E.; Talamini, R.; Franceschi, S. Coffee and tea intake and risk of cancers of the colon and rectum: a study of 3,530 cases and 7,057 controls. *Int. J. Cancer* **1997**, *73*, 193−197.

(20) Gonthier, M. P.; Verny, M. A.; Besson, C.; Remesy, C.; Scalbert, A. Chlorogenic acid bioavailability largely depends on its metabolism by the gut microflora in rats. *J. Nutr.* **2003**, *133*, 1853−1859.

(21) Rechner, A. R.; Kuhnle, G.; Bremner, P.; Hubbard, G. P.; Moore, K. P.; Rice-Evans, C. A. The metabolic fate of dietary polyphenols in humans. *Free Radical Biol. Med.* **2002**, *33*, 220−235.

(22) Gonthier, M. P.; Cheynier, V.; Donovan, J. L.; Manach, C.; Morand, C.; Mila, I.; Lapierre, C.; Remesy, C.; Scalbert, A. Microbial aromatic acid metabolites formed in the gut account for a major fraction of the polyphenols excreted in urine of rats fed red wine polyphenols. *J. Nutr.* **2003**, *133*, 461−467.

(23) Rechner, A. R.; Smith, M. A.; Kuhnle, G.; Gibson, G. R.; Debnam, E. S.; Srai, S. K. S.; Moore, K. P.; Rice-Evans, C. A. Colonic metabolism of dietary polyphenols: Influence of structure on microbial fermentation products. *Free Radical Biol. Med.* **2004**, *36*, 212−225.

(24) Olthof, M. R.; Hollman, P. C.; Buijsman, M. N.; van Amelsvoort, J. M.; Katan, M. B. Chlorogenic acid, quercetin-3-rutinoside and black tea polyphenols are extensively metabolized in humans. *J. Nutr.* **2003**, *133*, 1806−1814.

(25) Konishi, Y.; Kobayashi, S. Microbial metabolites of ingested caffeic acid are absorbed by the monocarboxylic acid transporter. *J. Agric. Food Chem.* **2004**, *52*, 6418−6424.

(26) Konishi, Y.; Kobayashi, S.; Shimizu, M. Tea polyphenols inhibit the transport of dietary phenolic acids mediated by the monocarboxylic acid transporter (MCT) in intestinal Caco-2 cell monolayers. *J. Agric. Food Chem.* **2003**, *51*, 7296−7302.

(27) Konishi, Y.; Kobayashi, S. Transepithelial transport of chlorogenic acid, caffeic acid, and their colonic metabolites in intestinal Caco-2 cell monolayers. *J. Agric. Food Chem.* **2004**, *52*, 2518−2526.

(28) Aura, A. M.; O'Leary, K. A.; Williamson, G.; Ojala, M.; Bailey, M.; Puupponen-Pimia, R.; Nuutila, A. M.; Oksman-Caldentey, K. M.; Poutanen, K. Quercetin derivatives are deconjugated and converted to hydroxyphenylacetic acids but not methylated by human fecal flora in vitro. *J. Agric. Food Chem.* **2002**, *50*, 1725−1730.

(29) Kim, D. H.; Jung, E. A.; Sohng, I. S.; Han, J. A.; Kim, T. H.; Han, M. J. Intestinal bacterial metabolism of flavonoids and its relation to some biological activities. *Arch. Pharm. Res.* **1998**, *21*, 17−23.

(30) Hidalgo, I. J.; Raub, T. J.; Borchardt, R. T. Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. *Gastroenterology* **1989**, *96*, 736−749.

(31) Hilgers, A. R.; Conradi, R. A.; Burton, P. S. Caco-2 cell monolayers as a model for drug transport across the intestinal mucosa. *Pharm. Res.* **1990**, *7*, 902−910.

(32) Artursson, P.; Karlsson, J. Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. *Biochem. Biophys. Res. Commun.* **1991**, *175*, 880−885.

(33) Yamaoka, K.; Tanigawara, Y.; Nakagawa, T.; Uno, T. A pharmacokinetics analysis program (MULTI) for microcomputer. *J. Pharmacobio-Dyn.* **1981**, *4*, 879−885.

(34) Guo, C.; Cao, G.; Sofic, E.; Prior, R. L. High-performance liquid chromatography coupled with coulometric array detection of electroactive components in fruits and vegetables: relationship to oxygen radical absorbance capacity. *J. Agric. Food Chem.* **1997**, *45*, 1787−1796.

(35) Price, N. T.; Jackson, V. N.; Halestrap, A. P. Cloning and sequencing of four new mammalian monocarboxylate transporter (MCT) homologues confirms the existence of a transporter family with an ancient past. *Biochem. J.* **1999**, *329*, 321−329.

(36) Konishi, Y.; Shimizu, M. Transepithelial transport of ferulic acid by monocarboxylic acid transporter in Caco-2 cell monolayers. *Biosci., Biotechnol., Biochem.* **2003**, *67*, 856−862.

(37) Konishi, Y.; Kobayashi, S.; Shimizu, M. Transepithelial transport of *p*-coumaric acid and gallic acid by monocarboxylic acid transporter in Caco-2 cell monolayers. *Biosci., Biotechnol., Biochem.* **2003**, *67*, 2317−2324.

(38) Konishi, Y.; Hagiwara, K.; Shimizu, M. Transepithelial transport of fluorescein in Caco-2 cell monolayers and its use in *in vitro* evaluation of phenolic acids availability. *Biosci., Biotechnol., Biochem.* **2002**, *66*, 2449−2457.

(39) Rahman, B.; Schneider, H. P.; Broer, A.; Deitmer, J. W.; Broer, S. Helix 8 and Helix 10 are involved in substrate recognition in the rat monocarboxylate transporter MCT1. *Biochemistry* **1999**, *38*, 11577−11584.

(40) Manach, C.; Scalbert, A.; Morand, C.; Remesy, C.; Jimenez, L. Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.* **2004**, *79*, 727−747.

(41) Konishi, Y.; Hitomi, Y.; Yoshioka, E. Intestinal absorption of p-coumaric and gallic acids in rats after oral administration. *J. Agric. Food Chem.* **2004**, *52*, 2527−2532.

(42) Gonthier, M. P.; Donovan, J. L.; Texier, O.; Felgines, C.; Remesy, C.; Scalbert, A. Metabolism of dietary procyanidins in rats. *Free Radical Biol. Med.* **2003**, *35*, 837−844.

(43) Price, N. T.; Jackson, V. N.; Halestrap, A. P. Cloning and sequencing of four new mammalian monocarboxylate transporter (MCT) homologues confirms the existence of a transporter family with an ancient past. *Biochem. J.* **1999**, *329*, 321−329.

(44) Lin, R. Y.; Vera, J. C.; Chaganti, R. S. K.; Golde, D. W. Human monocarboxylate transporter 2 (MCT2) is a high affinity pyruvate transporter. *J. Biol. Chem.* **1998**, *273*, 28959−28965.

(45) Cummings J. H. The importance of SCFA in man. *Scand. J. Gastroenterol.* **1984**, *19*, 89−99.

(46) Roediger, W. E. W. Role of anaerobic bacteria in the metaolic welfare of the colonic mucosa in man. *Gut* **1980**, *21*, 793−798.

(47) Scheppach, W.; Bartram, H. P.; Richter, A.; Richter, F.; Liepold, H.; Dusel, G.; Hofstetter, G.; Ruthlein, J.; Kasper, H. Effect of short-chain fatty acid on the human colonic mucosa in vitro. *J. Parenter. Enteral Nutr.* **1992**, *16*, 43−48.

(48) Hadjiagapiou, C.; Schmidt, L.; Dudeja, P. K.; Layden, T. J.; Ramaswamy, K. Mechanism of butyrate transport in Caco-2 cells: role of monocarboxylate transporter 1. *Am. J. Physiol. Gastrointest. Liver Physiol*. **2000**, *279*, G775−780.

(49) Stein, J.; Schroder, O.; Milovic, V.; Caspary, W. F. Mercapto-propionate inhibits butyrate uptake in isolated apical membrane vesicles of the rat distal colon. *Gastroenterology* **1995**, *108*, 673−679.