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Microbial Metabolites of Ingested Caffeic Acid Are Absorbed by the Monocarboxylic Acid Transporter (MCT) in Intestinal Caco-2 Cell Monolayers

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It was previously reported that *m*-coumaric acid, *m*-hydroxyphenylpropionic acid (*m*HPP), and 3,4-dihydroxyphenylpropionic acid (DHPP) are major metabolites of ingested caffeic acid formed by gut microflora and would be transported by the monocarboxylic acid transporter (MCT). We have directly measured their absorption characteristics in Caco-2 cells using a coulometric detection method involving HPLC-ECD. The proton-coupled directional transport of *m*-coumaric acid, *m*HPP, and DHPP was observed, and the transport was inhibited by an MCT substrate. The permeation of *m*-coumaric acid and *m*HPP was concentration-dependent and saturable: The Michaelis constant for *m*-coumaric acid and *m*HPP was 32.5 and 12.9 mM, respectively, and the maximum velocity for *m*-coumaric acid and *m*HPP was 204.3 and 91.2 nmol $(min)^{-1}$ $(mg\ protein)^{-1}$, respectively. By contrast, the permeation of DHPP was nonsaturable even at 30 mM and was inversely correlated with the paracellular permeability of Caco-2 cells. Our results demonstrate that these compounds are absorbed by the MCT, although DHPP is mainly permeated across Caco-2 cells via the paracellular pathway. MCT-mediated absorption of phenolic compounds per se and their colonic metabolites would exert significant impact on human health.

KEYWORDS: Caffeic acid; monocarboxylic acid transporter; microbial metabolite; Caco-2

INTRODUCTION

Epidemiological studies indicate an association between consumption of whole grain products and prevention of chronic diseases such as coronary diseases (*1*) and certain forms of cancer (*2*). Various nutrients in whole grain cereals, such as fiber, vitamins, minerals, and phenolic compounds, have been assumed to exert gastrointestinal effects and antioxidant protection, although the exact mechanism is still unclear (*3*). The hydroxycinnamic acids are a major class of phenolic compounds in cereal bran, mainly present as esters linked to polymers in the cell wall (*4*, *5*). Caffeic acid is the main representative of the hydroxycinnamic acids and 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; other dietary sources include fruit, such as apples, pears, and berries (*6*).

Caffeic acid has vicinal hydroxyl groups on an aromatic residue and exhibits antioxidant activities and antimutagenic and carcinogenic effects in vitro (*7*, *8*). This is consistent with the reported inverse correlation between coffee intake and colon cancer in some epidemiological studies (*9*−*11*). The physiological importance of caffeic acid is dependent upon its availability for intestinal absorption and subsequent interaction with target tissues. However, the microbial metabolites of the ingested parent compound and their biological properties must be fully investigated to understand the health benefits of dietary polyphenols. *m*-Coumaric acid, *m*-hydroxyphenylpropionic acid (*m*HPP), and 3,4-dihydroxyphenylpropionic acid (DHPP) are major metabolites of ingested caffeic acid, formed by gut microflora (**Figure 1**), and have been considered to exert physiological effects through the prevention of antioxidant stress

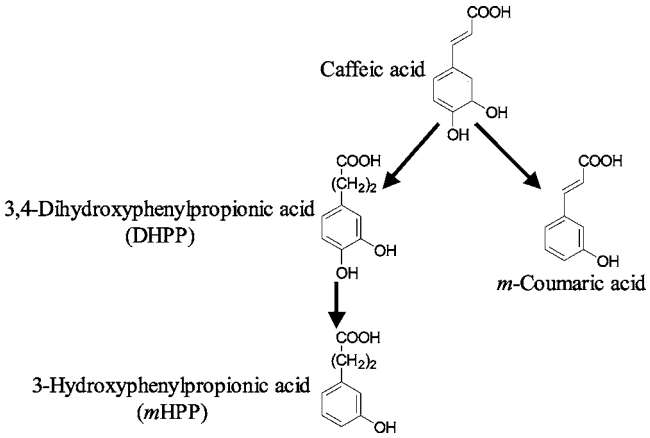

Figure 1. Metabolic pathway of caffeic acid metabolized by gut microflora.

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(*12*, *13*). However, the absorption characteristics of caffeic acid per se and their colonic metabolites are still obscure.

Recently, we established that measuring the competitive effect of dietary substances on the transport of fluorescein, a substrate of the monocarboxylic acid transporter (MCT), is a useful means of identifying compounds that can be transported by the MCT (*14*). MCT-mediated transport of dietary phenolic acids, such as ferulic and *p*-coumaric acids, was demonstrated using this method (*15*, *16*). Furthermore, we previously reported that caffeic acid is mainly absorbed via the paracellular pathway, whereas *m*-coumaric acid and *m*HPP seem to be absorbed by the MCT since they showed competitive inhibition of fluorescein transport (*17*). This study was designed to reveal the absorption mechanisms of *m*-coumaric acid, *m*HPP, and DHPP by directly measuring their transepithelial transport across Caco-2 cells as an in vitro model of intestinal absorption and metabolism (*18*−*20*).

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). Fetal calf serum, glutamine, nonessential amino acids, penicillin, streptomycin (10 000 units/mL and 10 mg/mL in 0.9% sodium chloride, respectively), 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). *m*-Coumaric acid, *m*HPP, and DHPP 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 IU/mL penicillin, and 50 μg/mL streptomycin, together with sodium bicarbonate to adjust the pH to 7.4. The cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air. The monolayers became confluent 6 to 7 days after seeding with 1×10^5 cells per 100-mm dish. Cells were passaged at a split ratio of 4 to 8 by treatment with 0.1% trypsin and 0.02% EDTA acid in HBSS. All cells used were between passages 56 and 70.

Measurement of Transepithelial Electrical Resistance (TER). Cells were grown for TER measurement in Transwell inserts with the semipermeable membrane first coated with type I collagen (12-mm diameter and 0.4-μm pore size, Corning Costar, NY). The cells were seeded at a density of 1×10^5/cm^2, and the medium was changed every 1 or 2 days. Monolayers were formed after culturing for 2 weeks. The integrity of the cell layer was evaluated by measurement of TER with Millicell-ERS equipment (Millipore, MA). Monolayers with TER of more than 250 $\Omega \cdot$cm^2 were used for the experiments. The TER of the monolayer was measured before and after an assay sample was added to the insert.

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 *m*-coumaric acid (1 mM), *m*HPP (1 mM), or DHPP (5 mM) was added to the apical side. At a designated time after incubation at 37 °C, the basal solution was collected and replaced by an equal volume of HBSS. The amount of *m*-coumaric acid, *m*HPP, or DHPP transported by Caco-2 cells was estimated using an HPLC-electrochemical detector (ECD) with an ESA coulometric detection system (ESA Inc., Boston). 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. An HPLC-ECD fitted with a coulometric detection system was used in the analysis, as previously reported (*17*). 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 adjusted with phosphoric acid), while mobile phase B (Solvent B) was 50 mM sodium acetate containing 40% acetonitrile and 20% methanol (pH 3.5 adjusted with phosphoric acid). 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. To measure the amount of *m*-coumaric acid, the eight electrode detector potentials measured at 100−800 mV in increments of 100 mV and were from 0 to 700 mV in increments of 100 mV to measure the amount of *m*HPP and DHPP.

Distribution of *m*-Coumaric Acid, *m*HPP, and DHPP after Transport Experiments. At the end of the transport experiments, the level of *m*-coumaric acid, *m*HPP, or DHPP in the apical and basolateral solutions was measured. 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. *m*-Coumaric acid, *m*HPP, or DHPP in this extract was measured and used as an index of the intracellular fractions taken up by the Caco-2 cells.

Data Analysis. The amount transported [nmol (mg protein)$^{-1}$] against time (in minutes) was plotted and the permeation rate [nmol min^{-1} (mg protein)$^{-1}$], J, was evaluated from the slope of the initial linear part of the plot, calculated by linear regression analysis. The kinetic parameters for saturable transport across Caco-2 cells were estimated by fitting eq 1 using the nonlinear least-squares regression analysis program, MULTI (*21*):

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

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 performed using Student's two-tailed t-test, and differences with $P < 0.01$ were considered significant.

RESULTS

HPLC Analysis of *m*-Coumaric Acid, *m*HPP, and DHPP Transported across Caco-2 Cell Monolayers. Representative chromatograms of *m*-coumaric acid, *m*HPP, or DHPP transported (**Figure 2**) into the basolateral solution are presented in **Figure 2**. *m*-Coumaric acid, *m*HPP, or DHPP were determined at a detection limit of <0.5 pmol on the column: reproducibility was good and did not require any sample pretreatment. Peak purity was assessed using the peak area ratio accuracies from oxidation channels (lower or upper) adjacent to the dominant oxidation channel. The voltametric response of analyte across these channels was unique for each compound, and more than 70% ratio accuracy was considered to support the peak purity (*22*). The retention time (RT) and dominant oxidation potential for *m*-coumaric acid, *m*HPP, and DHPP are 17.3 min, 700mV; 15.9 min, 600mV; and 10.2 min, 200mV, respectively.

Characteristics of Transepithelial Transport of *m*-Coumaric Acid, *m*HPP, and DHPP. The bidirectional permeation of *m*-coumaric acid (1 mM), *m*HPP (1 mM), or DHPP (5 mM) across Caco-2 cell monolayers was examined in the presence and absence of an inwardly directed proton gradient (**Figure 3**). *m*-Coumaric acid, *m*HPP, and DHPP exhibited pH-dependent directional transport from the apical to the basolateral side ($J_{ap\rightarrow bl}$ and $J_{bl\rightarrow ap}$ being 7.23 and 0.44 nmol/min/mg protein, respectively, for *m*-coumaric acid; 7.47 and 0.34 nmol/min/mg protein, respectively, for *m*HPP; 2.27 and 0.33 nmol/min/mg protein, respectively, for DHPP: apical pH, 6.0; basolateral pH, 7.4). In the absence of a proton gradient (apical pH, 7.4; basolateral pH, 7.4), $J_{ap\rightarrow bl}$ of *m*-coumaric acid (0.48 nmol/min/mg protein)

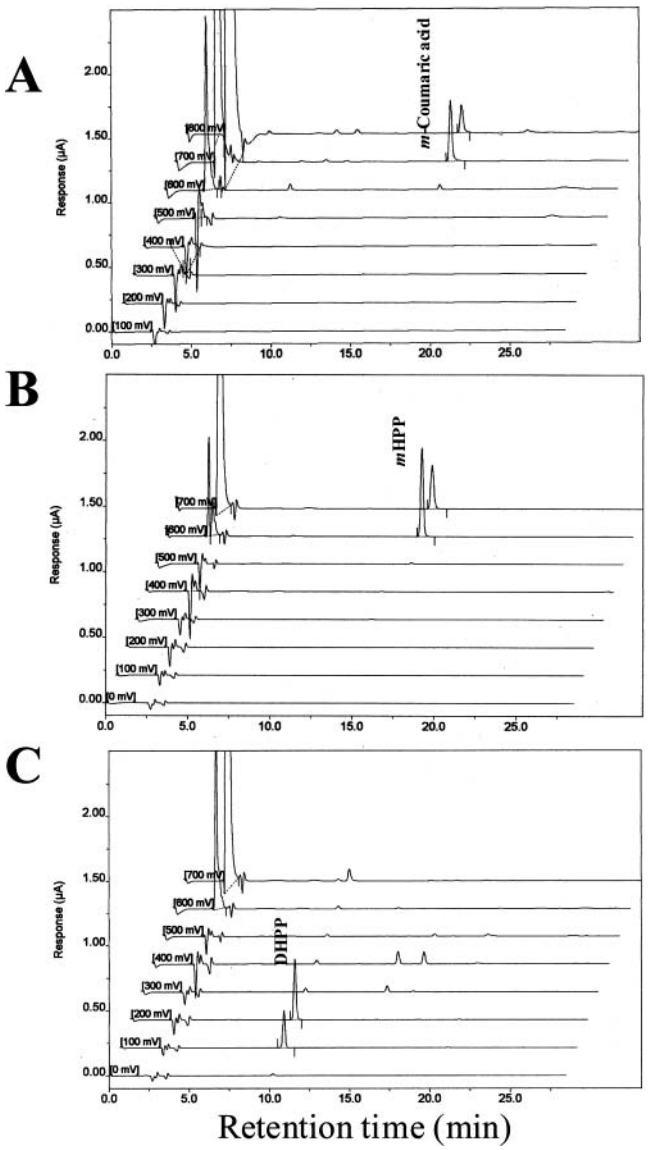

Figure 2. Chromatograms of *m*-coumaric acid (**A**), *m*HPP (**B**), and DHPP (**C**) transported across Caco-2 cell monolayers.

was almost the same as that of $J_{bl \rightarrow ap}$ (0.54 nmol/min/mg protein), whereas the $J_{ap \rightarrow bl}$ of *m*HPP and DHPP (0.57 or 0.43 nmol/min/mg protein) was greater than the $J_{bl \rightarrow ap}$ (0.36 or 0.24 nmol/min/mg protein), which is characteristic of polarized transport.

Distribution of *m*-Coumaric Acid, *m*HPP, and DHPP. After transport experiments in the absence of a proton gradient (apical pH, 7.4; basolateral pH, 7.4), >98% of apically loaded *m*-coumaric acid, *m*HPP, or DHPP 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 *m*-coumaric acid, *m*HPP, and DHPP was transported to the basolateral side, although there was greater transport of *m*-coumaric acid and *m*HPP compared with DHPP. Transport to the basolateral side is likely to be dependent on the pH gradient; however, DHPP was mainly restricted by the tight junction, similar to caffeic acid (*17*).

Concentration Dependence of *m*-Coumaric Acid, *m*HPP, and DHPP Transport. Figure 4 shows the relationship between the initial permeation rate of *m*-coumaric acid, *m*HPP, and DHPP and their concentration (apical pH, 6.0; basolateral pH, 7.4).

Table 1. Distribution of *m*-Coumaric Acid, *m*HPP, and DHPP after Transepithelial Transport Experiments in the Presence and Absence of a Proton Gradient[a]

	% compound recovered from		
pH gradient	apical side	basolateral side	cells
	m-Coumaric Acid		
6.0/7.4	85.84 ± 0.39	13.74 ± 0.39	0.42 ± 0.01
7.4/7.4	98.55 ± 0.05	1.34 ± 0.04	0.12 ± 0.09
	*m*HPP		
6.0/7.4	82.38 ± 0.51	16.90 ± 0.58	0.72 ± 0.07
7.4/7.4	98.49 ± 0.07	1.29 ± 0.08	0.22 ± 0.01
	DHPP		
6.0/7.4	97.75 ± 0.55	1.71 ± 0.06	0.54 ± 0.12
7.4/7.4	99.41 ± 0.06	0.40 ± 0.10	0.20 ± 0.04

[a] Transepithelial transport experiments were performed as described in Materials and Methods both 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. Effect of Various Compounds on *m*-Coumaric Acid, *m*HPP, and DHPP Transport across Caco-2 Cell Monolayers in the Presence of a Proton Gradient[a]

	relative permeation (% of control)		
	m-coumaric acid	*m*HPP	DHPP
NaN_3 10 mM	10.2 ± 2.1[b]	12.4 ± 2.1[b]	18.2 ± 2.6[b]
benzoic acid 20 mM	13.8 ± 1.2[b]	20.3 ± 2.8[b]	26.1 ± 3.2[b]
lactic acid 20 mM	104.8 ± 8.9	89.1 ± 9.1	107.2 ± 7.8
acetic acid 20 mM	51.8 ± 4.1[b]	48.7 ± 3.7[b]	33.7 ± 4.8[b]

[a] The amount of transported *m*-coumaric acid, *m*HPP, or DHPP 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] $P < 0.01$.

The permeation rates of *m*-coumaric acid and *m*HPP were concentration dependent and saturable. The Michaelis constant for *m*-coumaric acid and *m*HPP was 32.5 ± 2.8 and 12.9 ± 3.5 mM, respectively, and the maximum velocity for *m*-coumaric acid and *m*HPP was 204.3 ± 10.4 nmol and 91.2 ± 13.9 nmol $(min)^{-1}$ (mg protein)$^{-1}$, respectively. In contrast, the permeation of DHPP was not saturable even at 30 mM, suggesting passive diffusion might be involved. These results are in good agreement with a previous distribution study which suggested that DHPP is mainly permeated via the paracellular diffusion. Lucifer Yellow, a marker compound for paracellular transport, and caffeic acid also showed this trend (*14*, *17*).

Inhibition of Directional Transport of *m*-Coumaric Acid, *m*HPP, and DHPP. To investigate the transport characteristics responsible for proton-coupled polarized transport of *m*-coumaric acid, *m*HPP, or DHPP (apical pH, 6.0; basolateral pH, 7.4), we added 0.1 mM *m*-coumaric acid, 0.1 mM *m*HPP, or 1 mM DHPP to the apical chamber and the effects of various compounds on the permeation were examined (**Table 2**). A metabolic inhibitor, NaN_3, (10 mM) strongly reduced the permeation of *m*-coumaric acid, *m*HPP, and DHPP. Substrates for the MCT (20 mM), such as benzoic acid and acetic acid, significantly inhibited permeation, although lactic acid, a good substrate for MCT1-MCT4 (*23*), had 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 acid transport, clearly demonstrating that they are transported at least in part by the MCT (*15*, *16*).

Paracellular Transport of DHPP across the Caco-2 Cell Monolayers. Caco-2 cell monolayers exhibiting different TER values were prepared by treating the cells with cytochalasin D

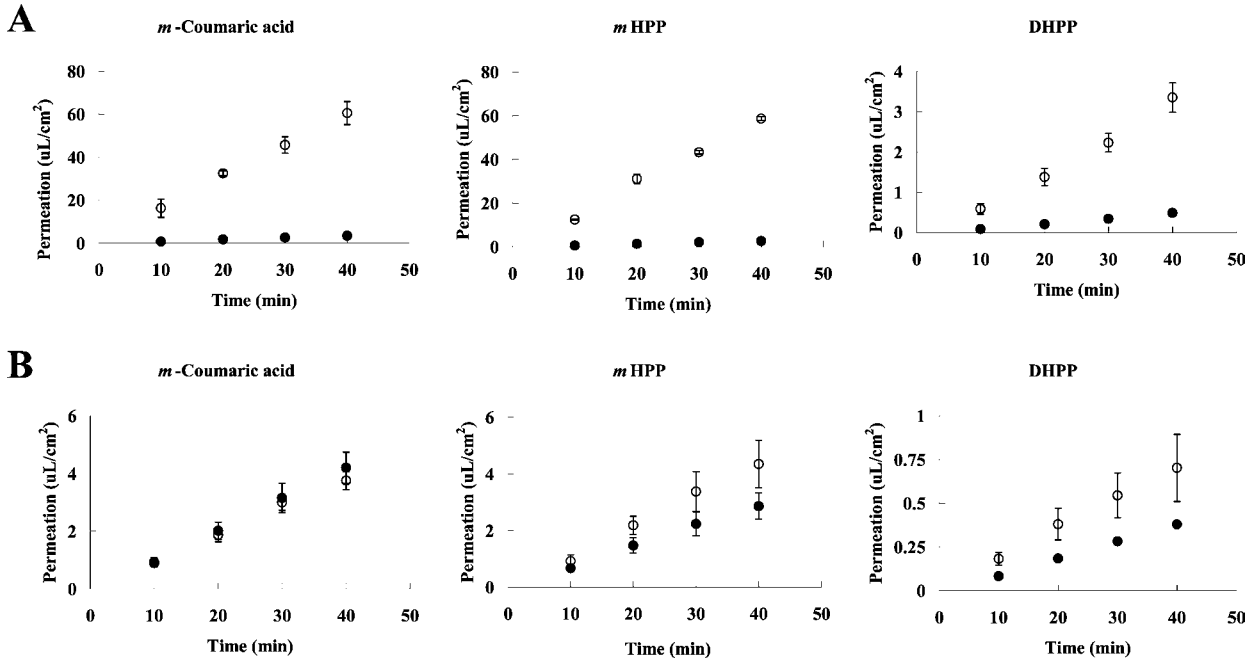

Figure 3. Characteristics of the transepithelial transport of *m*-coumaric acid, *m*HPP, and DHPP across Caco-2 cell monolayers in the presence (**A**) and the absence (**B**) of a proton gradient. Permeation of *m*-coumaric acid (1 mM), *m*HPP (1 mM), and DHPP (5 mM) from the apical side to the basolateral side (○) and from the basolateral side to the apical side (●) was measured at 37 °C both 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.

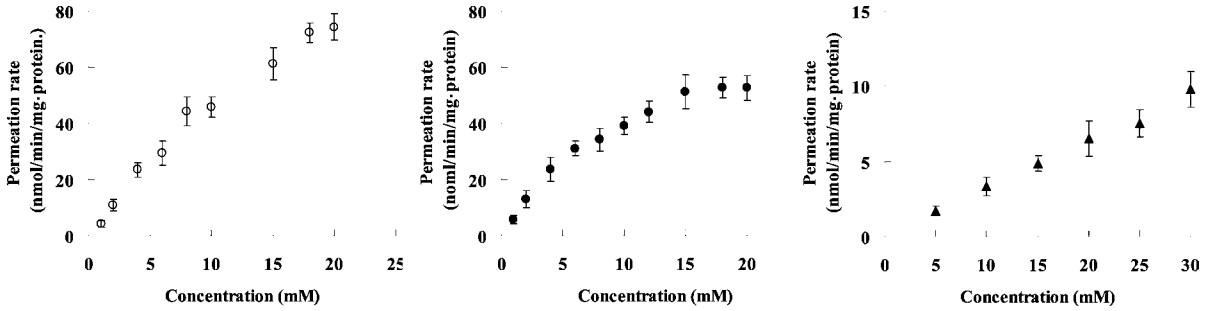

Figure 4. Concentration dependence of *m*-coumaric acid (○), *m*HPP (●), and DHPP (▲) transport across Caco-2 cell monolayers in the presence of a proton gradient. The initial permeation rate is shown. Values are the mean ± SD of three or more experiments.

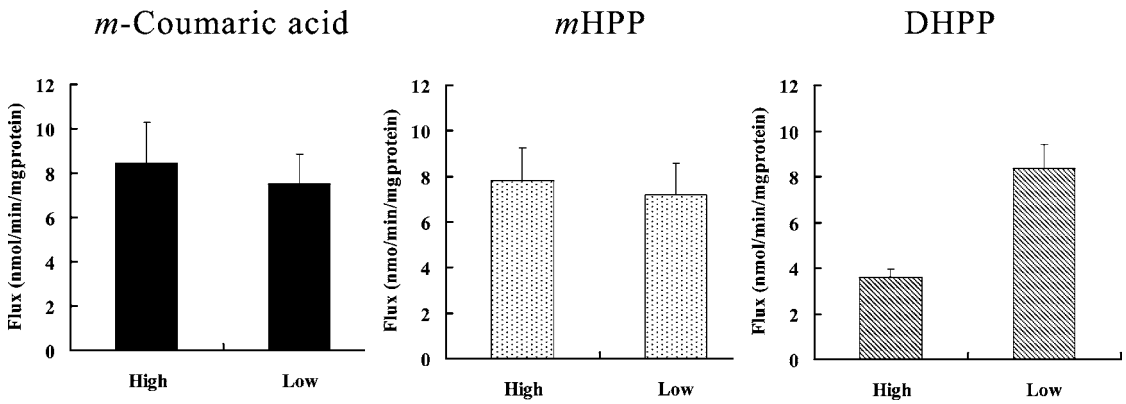

Figure 5. Correlation between TER and the transepithelial flux of *m*-coumaric acid, *m*HPP, and DHPP. *m*-Coumaric acid (1 mM), *m*HPP (1 mM), or DHPP (5 mM) were loaded in the apical side, and the flux from the apical side to the basolateral side was measured at 37 °C (apical pH, 6.0; basolateral pH, 7.4). TER values are indicated as follows: (*m*-coumaric acid), high: 1001 ± 20, low: 319 ± 30; (*m*HPP), high: 940 ± 12, low: 316 ± 28; (DHPP), high: 884 ± 60, low: 294 ± 15. Each point is the mean ± SD of three experiments.

(*14*). The apical-to-basolateral transport of DHPP was then characterized in comparison with that of *m*-coumaric acid and *m*HPP using these monolayers (apical pH, 6.0; basolateral pH, 7.4). As illustrated in **Figure 5**, the transepithelial flux of DHPP was inversely correlated with the TER, whereas the transepithelial flux of *m*-coumaric acid and *m*HPP was almost constant irrespective of the TER, suggesting that DHPP permeates across Caco-2 cells via the paracellular pathways. This result also agreed with the MCT-mediated transport of *m*-coumaric acid and *m*HPP, mentioned above.

DISCUSSION

In our previous study, we reported that *m*-coumaric acid and *m*HPP competitively inhibited fluorescein transport across Caco-2 cells, suggesting they were absorbed by the MCT (*17*). The results were in agreement with the structure of these compounds: both possess a monoanionic carboxyl group and a nonpolar side chain or aromatic hydrophobic moiety, which are thought to be necessary components of a substrate for a MCT (*24*). However, it was not known whether MCT could recognize and transport *m*-coumaric acid and *m*HPP in Caco-2 cells. Hence, the mechanism of intestinal transport of *m*-coumaric acid and *m*HPP together with DHPP, a precursor of *m*HPP, was investigated (**Figure 3**).

The apical-to-basolateral flux ($J_{ap\rightarrow bl}$) of *m*-coumaric acid and *m*HPP in the presence of the proton gradient (7.23 nmol/min/mg·protein for *m*-coumaric acid and 7.47 nmol/min/mg·protein for *m*HPP) was similar to that of ferulic acid (9.79 nmol/min/mg·protein) and greater than that of *p*-coumaric acid (3.73 nmol/min/mg·protein) (*15*, *16*). The $J_{ap\rightarrow bl}$ of *m*-coumaric acid and *m*HPP in the presence of the proton gradient was 13∼15-fold higher than the $J_{ap\rightarrow bl}$ of each in the absence of a proton gradient (0.48 nmol/min/mg·protein for *m*-coumaric acid and 0.57 nmol/min/mg·protein for *m*HPP). The $J_{ap\rightarrow bl}$ of *m*-coumaric acid and *m*HPP was 16∼22-fold higher than the basolateral-to-apical flux ($J_{bl\rightarrow ap}$) of each in the presence of the proton gradient (0.44 nmol/min/mg·protein for m-coumaric acid, 0.34 nmol/min/mg·protein for *m*HPP). The proton-coupled polarized transport of *m*-coumaric acid and *m*HPP is similar to other MCT substrates, such as ferulic acid, *p*-coumaric acid, and fluorescein (*14*−*16*). Considering the results of the distribution study (**Table 1**), and the specific inhibition of this polarized transport by MCT substrate (**Table 2**), the polarized transport seen in this study is presumably mediated by the MCT, as is the case for ferulic and *p*-coumaric acid (*15*, *16*). The concentration-dependent saturable transport of *m*-coumaric acid and *m*HPP (**Figure 4**) also supports this conclusion.

In addition, proton-coupled directional transport of DHPP (5 mM) from the apical to the basolateral side was observed ($J_{ap\rightarrow bl}$; 2.27 nmol/min/mg·protein, $J_{bl\rightarrow ap}$, 0.34 nmol/min/mg·protein: apical pH, 6.0; basolateral pH, 7.4), although the $J_{ap\rightarrow bl}$ was much lower than that of 1 mM *m*-coumaric acid and *m*HPP. The transport of DHPP increased linearly, even at 30 mM (**Figure 4**), and the permeation was inversely correlated with the TER, that is, paracellular permeability of Caco-2 cells (**Figure 5**). This findings were similar to that of caffeic acid at the concentration of 5 mM ($J_{ap\rightarrow bl}$; 2.69 nmol/min/mg·protein, $J_{bl\rightarrow ap}$, 0.59 nmol/min/mg·protein: apical pH, 6.0; basolateral pH, 7.4), indicating that the permeation of DHPP was mainly via paracellular diffusion across Caco-2 cells: DHPP may be absorbed by the MCT, but to a lesser extent (*17*). The result of the distribution study indicated that DHPP was restricted by a tight junction, irrespective of the proton gradient, which is in agreement with the measurements above (**Table 1**). Hydroxylation of MCT substrates, such as benzoic and cinnamic acids, would be predicted to decrease affinity for MCT (*25*). DHPP, a dihydroxy derivative of phenylpropionic acid, may still have some affinity for MCT, though the affinity would be expected to be lesser than that of *m*-coumaric acid and *m*HPP, monohydroxy derivatives of cinnamic or phenylpropionic acids.

We observed directional transport of *m*HPP and DHPP in the apical-to-basolateral direction in the absence of an inwardly directed proton gradient (**Figure 3**); however, this was not true for *m*-coumaric acid. The reason for the polarized transport is not clear; however, the characteristics of the two directional transport systems appear to differ. NaN_3 (10 mM) did not inhibit the directional transport of DHPP, indicating that transport was not energy-dependent, although DIDS (2 mM) inhibited the transport to some extent. On the contrary, HCO_3^- (25 mM) and benzoic acid (20 mM) seemed to increase the permeation of DHPP and decrease the TER of the Caco-2 cells (**Table 3**). Basolateral-loaded DHPP caused an increase in the TER of the Caco-2 monolayer, which would agree with the greater $J_{ap\rightarrow bl}$ over $J_{bl\rightarrow ap}$ (apical pH, 7.4; basolateral pH, 7.4). The inhibitory effect of DIDS is still unknown, but interaction of DIDS with DHPP may occur. In contrast, the permeation of *m*HPP was inhibited by NaN_3 (10 mM), HCO_3^- (25 mM), benzoic acid (20 mM), and, to a lesser extent, DIDS (2 mM). In the absence of a proton gradient, monocarboxylic acids such as lactic acid and benzoic acid are transported by an anion exchanger (*26*). Our results suggest the directional transport of *m*HPP would be via an anion exchanger.

Table 3. Transport Characteristics of *m*-Coumaric Acid, *m*HPP, and DHPP across Caco-2 Cell Monolayers in the Absence of a Proton Gradient[a]

	*m*HPP	DHPP
relative permeation (% of control)		
NaN_3 10 mM	62.4 ± 2.4[b]	93.9 ± 7.0
DIDS 2 mM	80.5 ± 6.5[b]	60.1 ± 7.3[b]
benzoic acid 20 mM	51.5 ± 7.9[b]	133.7 ± 10.4[b]
HCO_3^- 25 mM	45.9 ± 6.9[b]	150.3 ± 13.2[b]
relative TER		
$_{ap\rightarrow bl}$ control	0.80 ± 0.21	0.69 ± 0.16
$_{ap\rightarrow bl}$ NaN_3 10 mM	0.87 ± 0.20	0.65 ± 0.15
$_{ap\rightarrow bl}$ DIDS 2 mM	1.04 ± 0.22	0.70 ± 0.17
$_{ap\rightarrow bl}$ benzoic acid 20 mM	0.77 ± 0.15	0.55 ± 0.11
$_{ap\rightarrow bl}$ HCO_3^- 25 mM	0.73 ± 0.22	0.44 ± 0.03
$_{bl\rightarrow ap}$ control	0.96 ± 0.27	1.41 ± 0.12[b]

[a] The amount of *m*HPP or DHPP transported in the apical-to-basolateral direction 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, 7.4; basolateral pH, 7.4). TER values are expressed as relative to the initial value before the assay. Values are the mean ± SD of three or more experiments. [b] $P < 0.01$.

Recently, the biological effects of microbial metabolites of ingested polyphenols have been focused on, particularly for those poorly absorbed in the small intestine (*12*, *27*−*29*). The total microbial metabolites of ingested chlorogenic or caffeic acids accounted for 57.4% and 28.1%, respectively, of total intake: this includes *m*-coumaric acid, *m*HPP, DHPP, and other metabolites (*12*). Colonic metabolites formed by gut microflora in the gastrointestinal tract have also been reported from various dietary polyphenols. For instance, major phenolic metabolites identified were 3-(*p*)-hydroxyphenylpropionic and 3-phenylpropionic acid for naringin or naringenin and 3,4-dihydroxyphenylacetic acid, 3-(*m*)-hydroxyphenylpropionic acid, and 3-hydroxyphenylacetic acid for rutin or quercetin (*13*). The phenolic degradation products could be generally classified as hydroxylated 3-phenylpropionic acids and the hydroxylated phenylacetic acids, dependent on the structural characteristics of the parent polyphenol. Since these metabolites still possess a free phenolic group, they could have significant reducing and antioxidant properties and therefore protect against oxidative stress, although the monophenolic structure is generally a poorer antioxidant than ortho-diphenolic structures (*30*). Indeed, microbial metabolites such as 3,4-dihydroxyphenylacetic acid and 4-hydroxyphenylacetic acid were more effective than their rutin and quercetin precursors in inhibiting platelet aggregation in vitro (*31*). Considering the higher yield and biological properties of the microbial metabolites in vivo, these bioactive compounds

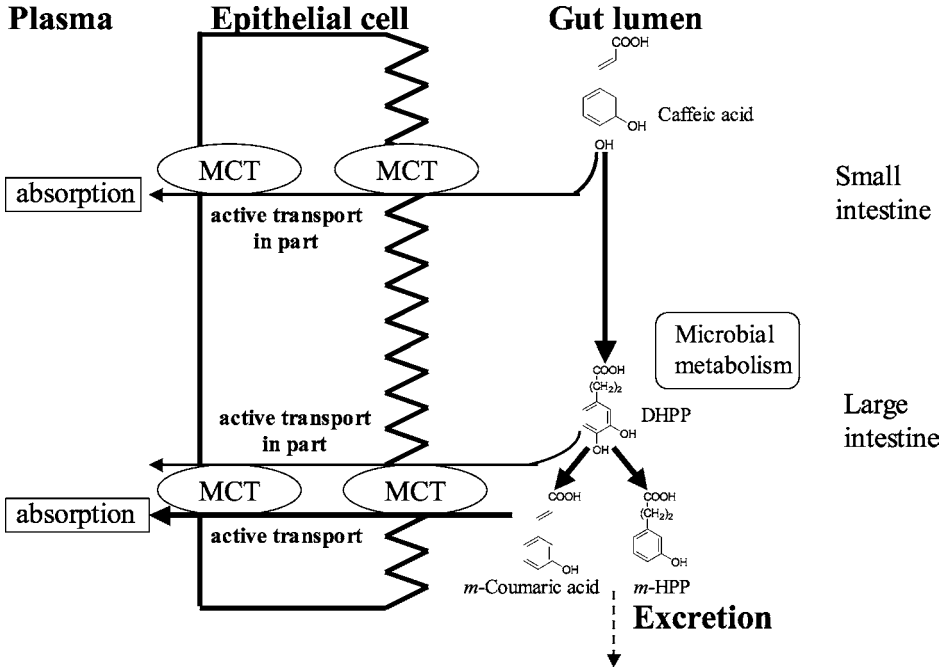

Figure 6. Possible routes for absorption of ingested caffeic acid.

might be responsible for the health effects of dietary polyphenols. However, there is too little information on their absorption and distribution within the body to investigate the health effects of these microbial metabolites. The results obtained in this study, together with data from the literature (*17*), suggest that caffeic acid is absorbed not only via paracellular diffusion, but it is actively absorbed in part by the MCT in the intestine. Furthermore, caffeic acid reaches the large intestine, is metabolized to DHPP, *m*-coumaric acid, and *m*HPP using gut microflora, which are then absorbed by the MCT (**Figure 6**). This clearly shows that the physiological significance of MCT-mediated absorption must be better understood to realize the full health benefits of dietary polyphenols. The high efficacy of MCT-mediated absorption was demonstrated in this study using hydroxylated 3-phenylpropionic acids, *m*HPP, and DHPP. Additional experiments are necessary to understand the absorption characteristics of the hydroxylated phenylacetic acids.

Short-chain fatty acids (SCFA), acetate, propionate, and butyrate, are the most abundant organic anions in the colonic lumen: they are the products of microbial fermentation of undigested carbohydrates and proteins, such as the poorly absorbed polyphenols. SCFA play a pivotal role in maintaining homeostasis in the colon. Butyrate is present in colonic epithelial cells at millimolar concentrations and is the principal energy source (*32*), exerting several biological effects to induce cell differentiation and regulate growth and proliferation of colonic mucosal epithelial cells (*33*, *34*). The mechanism of absorption of butyrate is still debated, although the nonelectrogenic $SCFA^-/HCO_3^-$ antiporter is at least in part involved in its absorption (*35*). Currently, nine isoforms of the MCT have been identified (*23*), 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 (*36*). It was reported that butyrate is transported by MCT1 (*37*, *38*). Recently, it was determined that butyrate induces an increase in the expression and activity of MCT1, which may serve as a physiological mechanism to maximize intracellular availability of butyrate (*39*). Furthermore, butyrate, taken up by the MCT, reduces colonic paracellular permeability by enhancing peroxisome proliferator-activated receptor γ (PPARγ), which is attributed to preventing inflammation of the colon (*40*). Further identification and characterization of MCT-mediated absorption of dietary components and their microbial metabolites is necessary to clarify the health benefits of whole grains ingested daily.

In conclusion, we have demonstrated that MCT is involved in the absorption of the major microbial metabolites of caffeic acid. *m*-Coumaric acid and *m*HPP are absorbed by the MCT, although DHPP is mainly transported via the paracellular pathways. This suggests a physiological significance of MCT-mediated absorption of dietary components. Further studies on MCT-mediated transport of dietary substances and their colonic metabolites are required to fully understand the health effects of dietary components.

ABBREVIATIONS USED

HBSS, Hanks' balanced salt solution; TER, transepithelial electrical resistance; MCT, monocarboxylic acid transporter; ECD, electrochemical detector, SCFA; short chain fatty acids; PPARγ, peroxisome proliferators-activated receptor γ; *m*HPP, *m*-hydroxyphenylpropionic acid; DHPP, 3,4-dihydroxyphenylpropionic acid

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LITERATURE CITED

(1) Jacobs, D.; Pereira, M.; Slavin, J.; Marquart, L. Defining the impact of whole-grain intake on chronic disease. *Cereal Foods World* **2000**, *45*, 51−53.

(2) Jacobs, D. R.; Marquart, L.; Slavin, J. L.; Kushi, L. H. Whole grain intake and cancer: an expanded review and meta-analysis. *Nutr. Cancer* **1998**, *30*, 85−96.

(3) Slavin, J. L.; Jacobs, D.; Marquart, L.; Wiemer, K. The role of whole grains in disease prevention. *J. Am. Diet. Assoc.* **2001**, *101*, 780−785.

(4) Garcia-Conesa, M. T.; Plumb, G. W.; Waldron, K. W.; Ralph, J.; Williamson, G. Ferulic acid dehydrodimers from wheat bran: Isolation, purification, and antioxidant properties of 8-*O*-4′-diferulic acid. *Redox Rep.* **1997**, *3*, 319−323.

(5) Andreasen, M. F.; Christensen, L. P.; Meyer, A. S.; Hansen, A. Ferulic acid dehydrodimers in rye (*Secale ceresle* L.). *J. Cereal Sci.* **2000**, *31*, 303−307.

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

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

(8) 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.

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

(10) 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.

(11) 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.

(12) 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.

(13) 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.

(14) 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.

(15) 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.

(16) 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.

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

(18) 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.

(19) 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.

(20) 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.

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

(22) 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.

(23) 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.

(24) 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.

(25) Konishi, Y.; Kubo, K.; Shimizu, M. Structural effects of phenolic acids on transepithelial transport of fluorescein in Caco-2 cell monolayers. *Biosci., Biotechnol., Biochem.* **2003**, *67*, 2014−2017.

(26) Ogihara, T.; Tamai, I.; Tsuji, A. Structural characterization of substrates for the anion exchange transporter in Caco-2 cells. *J. Pharm. Sci.* **1999**, *88*, 1217−1221.

(27) 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.

(28) Chesson, A.; Provan, G. J.; Russell, W. R.; Scobbie, L.; Richardson, A. J.; Stewart, C. Hydroxycinnamic acids in the digestive tract of livestock and humans. *J. Sci. Food Agric.* **1999**, *79*, 373−378.

(29) 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.

(30) Glasser, G.; Graefe, E. U.; Struck, F.; Veit, M.; Gebhardt, R. Comparison of antioxidative capacities and inhibitory effects on cholesterol biosynthesis of quercetin and potential metabolites. *Phytomedicine* **2002**, *9*, 33−40.

(31) 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.

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

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

(34) 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. Parent. Enter. Nutr.* **1992**, *16*, 43−48.

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

(36) 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.

(37) Stein, J.; Zores, M.; Schroder, O. Short-chain fatty acid (SCFA) uptake into Caco-2 cells by a pH-dependent and carrier mediated transport mechanism. *Eur. J. Nutr.* **2000**, *39*, 121−125.

(38) 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.

(39) Cuff, M. A.; Lambert, D. W.; Shirazi-Beechey, S. P. Substrate-induced regulation of the human colonic monocarboxylate transporter, MCT1. *J. Physiol.* **2002**, *539*, 361−371.

(40) Kinoshita, M.; Suzuki, Y.; Saito, Y. Butyrate redices colonic paracellular permeability by enhancing PPARγ activation. *Biochem. Biophys. Res. Commun.* **2002**, *293*, 827−831.

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