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Modulation of (–)-Epicatechin Metabolism by Coadministration with Other Polyphenols in Caco-2 Cell Model[S]

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ABSTRACT

Widely consumed beverages such as red wine, tea, and cocoa-derived products are a great source of flavanols. Epidemiologic and interventional studies suggest that cocoa flavanols such as (–)-epicatechin may reduce the risk of cardiovascular diseases. The interaction of (–)-epicatechin with food components including other polyphenols could modify its absorption, metabolism, and finally its bioactivity. In the present study we investigate (–)-epicatechin absorption and metabolism when coexposed with other polyphenols in the intestinal absorptive Caco-2 cell model. Depending on the type of polyphenols coadministered, the total amount of 3′-*O*-methyl-epicatechin and 3′-*O*-sulfate-epicatechin conjugates found both in apical and basal compartments ranged from 19 to 801 nM and from 6 to 432 nM, respectively. The coincubation of (–)-epicatechin with flavanols, chlorogenic acid, and umbelliferone resulted in similar amounts of 3′-*O*-methyl-epicatechin effluxed into the apical compartment relative to control. Coincubation with isorhamnetin, kaempferol, diosmetin, nevadensin, chrysin, equol, genistein, and hesperitin promoted the transport of 3′-*O*-methyl-epicatechin toward the basolateral side and decreased the apical efflux. Quercetin and luteolin considerably inhibited the appearance of this (–)-epicatechin conjugate both in the apical and basolateral compartments. In conclusion, we could demonstrate that the efflux of (–)-epicatechin conjugates to the apical or basal compartments of Caco-2 cells is modulated by certain classes of polyphenols and their amount. Ingesting (–)-epicatechin with specific polyphenols could be a strategy to increase the bioavailability of (–)-epicatechin and to modulate its metabolic profile.

Introduction

Among the different classes of polyphenols, flavanols represent one of the most abundant compounds found in human diets (de Pascual-Teresa et al., 2000; Scalbert and Williamson, 2000). Flavanols are present in large amounts in beverages such as red wine, tea, and cocoa-derived products, which are widely consumed throughout the world (Scalbert and Williamson, 2000). A number of epidemiologic and interventional studies have suggested an inverse relationship between chronic consumption of flavanol-rich foods and the risk of cardiovascular diseases (Hertog et al., 1993, 1997; Arts et al., 2001; Mink et al., 2007; Shrime et al., 2011; Hooper et al., 2012). (–)-Epicatechin, the major flavanol in cocoa extracts, has been identified as one of the bioactive compounds (Schroeter et al., 2006; Loke et al., 2008).

Cocoa extracts are typically consumed in form of beverages and confectionery products. Several studies have shown that less than 30% of the (–)-epicatechin dose consumed is absorbed (Manach et al., 2005; Borges et al., 2010; Actis-Goretta et al., 2012, 2013). Following absorption in the intestinal tract, (–)-epicatechin is rapidly metabolized into glucuronide, sulfate, and/or methyl conjugates (Harada et al., 1999; Donovan et al., 2001; Actis-Goretta et al., 2012, 2013) (Fig. 1). These metabolites, chemically different from (–)-epicatechin, are the compounds circulating in the bloodstream and reaching target organs (Heiss et al., 2005; Schroeter et al., 2006; Tinahones et al., 2008; Borges et al., 2010).

As the (–)-epicatechin benefits are dependent of the amount of its metabolites appearing in the bloodstream, factors affecting the absorption and modulation of metabolites represent important considerations for increasing its bioefficacy.

Recently, we demonstrated that the plasma metabolic profile was different when (–)-epicatechin was consumed as chocolate or perfused as purified compound in the intestine (Actis-Goretta et al., 2012, 2013). Therefore, it could be suggested that other food ingredients modify the metabolism of (–)-epicatechin in vivo (Schramm et al., 2003; Neilson and Ferruzzi, 2011). Previous reports showed that coexposure of hesperetin with specific compounds modulates the amounts of hesperetin metabolites both in apical and basolateral chambers in a Caco-2 cell in vitro model (Brand et al., 2010). Other structure-related polyphenols consumed with foods have been shown to be metabolized by the same enzymes as (–)-epicatechin (Morimitsu et al., 2004). Likewise, the coadministration of polyphenols with (–)-epicatechin could modify the metabolic profile.

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ABBREVIATIONS: COMT, catechol *O*-methyl-transferase; DCNP, 2,6 dichloro-4-nitrophenol; P_{app}, apparent permeability coefficients; UPLC, Ultra-high-performance liquid chromatography.

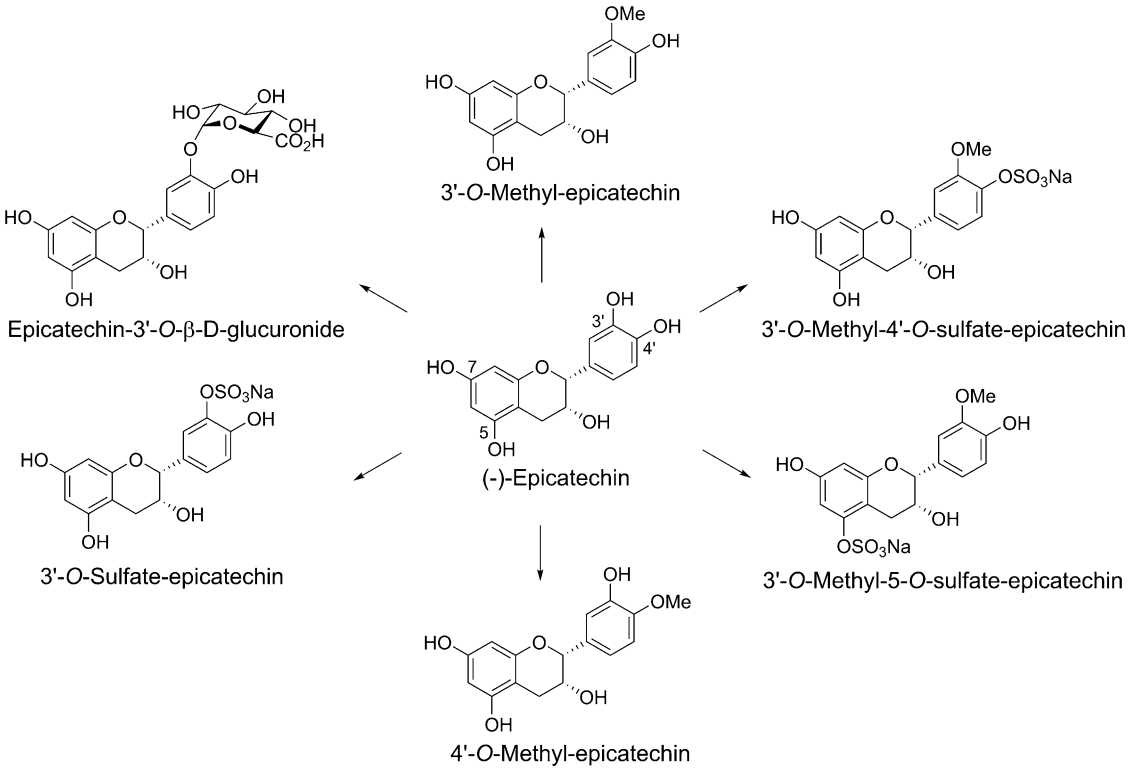


Fig. 1. Chemical structure of (–)-epicatechin and epicatechin metabolites.

To our knowledge, data about the effect of other polyphenols on the transport and generation of (–)-epicatechin metabolites has not been reported previously. Therefore, we aimed at investigating the modulation of (–)-epicatechin conjugates in Caco-2 cell monolayers, a well-recognized model for studying the intestinal transport of nutrients and drugs (Hidalgo et al., 1989; Yamashita et al., 2000), by coincubation with different dietary polyphenols. Altogether, understanding the interaction of polyphenols with the profile of (–)-epicatechin metabolites could provide future insight for increasing its bioavailability by addition or elimination of other polyphenols in the food products.

Materials and Methods

Chemicals. (–)-Epicatechin, quercetin, isoharmetin, kaempferol, lutein, diosmetin, chrysin, genistein, catechin, (–)-epigallocatechin, (–)-epigallocatechin-3-gallate, and chlorogenic acid were purchased from Extrasynthèse (Genay, France). Nevadensin was purchased from Apin Chemicals (Abingdon, Oxon, United Kingdom). Equol and umbelliferone, umbelliferone glucuronide, and umbelliferone sulfate were purchased from Toronto Research Chemicals (Toronto, Ontario, Canada), procyanidin B2, 3′-*O*-methyl-epicatechin, 4′-*O*-methyl-epicatechin, 4′-*O*-methyl-catechin, epicatechin-3′-*β*-D-glucuronide, epicatechin-4′-*β*-D-glucuronide, 3′-O-methyl-4′-sulfate-epicatechin, and hesperitin-7-glucoside were produced by complete chemical synthesis at the Nestlé Research Center (Lausanne, Switzerland). 3′-Sulfate-epicatechin and 4′-sulfate-epicatechin were synthesized as a mixture of compounds and then separated according to the method of Gonzalez-Manzano et al. (2009). Acetonitrile ultra-gradient high-performance liquid chromatography (HPLC) grade was purchased from J. T. Baker Europe (Deventer, The Netherlands). HPLC-grade water was prepared using a Millipore Milli-Q purification system (EMD Millipore, Billerica, MA). All other HPLC-grade solvents and reagents were purchased from Merck (Darmstadt, Germany). 2,6 Dichloro-4-nitrophenol was purchased from Sigma-Aldrich (Basel, Switzerland).

Cell Culture Experiments. Caco-2 cell lines are immortalized cells of human malignant colon cancer origin. Caco-2 cells were obtained from America Type Culture Collection (ATCC; under perpetual license agreement between NaviCyte Scientific and Nestec Ltd.). For maintenance, Caco-2 cells were seeded at a density of 40×10^3 cells/cm^2, and cultured in a humidified incubator at 5% CO_2 and 37°C in Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g glucose/l, 20% heat-inactivated fetal bovine serum (FBS), 1% nonessential amino acids, 2 mM L-glutamine, 1 μg/ml amphotericin B, 100 IU/ml penicillin, and 100 μg/ml streptomycin. Medium was changed every 2 days.

To obtain differentiated monolayers, Caco-2 cells were seeded at a density of 20×10^3 cells/cm^2 in 12-well inserts and cultured for 21 days. Medium was changed every 2 days. Cell culture media without phenol red was used during the differentiation period.

On the experiment day, medium was removed and replaced by Hanks' balanced salt solution supplemented with 25 mM glucose, 10 mM HEPES, and 1.8 mM $CaCl_2$. Catalase (189 IU/ml), and 0.5 mM ascorbic acid, referred to as exposure medium below. (–)-Epicatechin (100 μM) was placed in the apical compartment of the cell monolayers and incubated for 2 hours. Aglycone and conjugated compounds were detected in the cell culture media in the apical and basolateral sides using the analytical conditions described below. Transepithelial electrical resistance (TEER), measured before and after the transport study, indicated that the integrity of the monolayer was not affected by the transport experiment. Coincubation experiments were performed by adding (–)-epicatechin (100 μM) in the presence or absence of other polyphenols (50 μM, 10 μM, 5 μM, or 1 μM) to the apical side of the Caco-2 cell monolayer. The compounds were stocked in DMSO and diluted to the right concentration into the exposure medium. The concentration of DMSO at the apical side was kept at 0.05% in each experiment.

Cell Culture Media Preparation. After 2 hours incubation, the whole media in both chambers was collected and centrifuged for 5 minutes. An aliquot of 400 μl of supernatant was transferred into a 96-well plate, and 800 μl of 2% formic acid in H_2O added. Afterwards, 10 μl of 16 μM internal standard mixture (4′-O-methyl-catechin, umbelliferone sulfate, and umbelliferone glucuronide) was added to each well. Salt contained in the exposure medium

significantly influenced the shape of the peaks of some targeted compounds. To avoid this interference, the cell culture samples were cleaned up and concentrated using a solid-phase extraction (SPE) step (Strata-X; Phenomenex, Torrance, CA). The mixture was transferred into a preconditioned (1 ml methanol, followed by 1 ml water) 96-well SPE plate and allowed to pass through. The wells were washed with 1 ml water and epicatechin metabolites were eluted with 1 ml methanol/pyridine (1:1) into a 96-well collection plate. The eluates were dried under a flow of nitrogen gas at room temperature. Fifty microliters of solution of 8% acetonitrile and 0.1% CH_3COOH in H_2O was added to each well to reconstitute the sample. Ten μl was injected into ultra-performance liquid chromatography–mass spectroscopy (UPLC-MS) for analysis.

Analytical Methodology. The analytical assessment of (–)-epicatechin metabolites was conducted with an ACQUITY UPLC–MS System (Waters Corporation, Milford, MA). Separations were performed on a ACQUITY UPLC HSS C18 2.1 × 100 mm column (Waters Corporation).

Mobile phases A and B consisted of 0.1% (v/v) acetic acid in water and 0.1% (v/v) acetic acid in acetonitrile, respectively. Analysis was completed within 14 minutes with a flow rate of 0.5 ml/min. The following gradient was applied: 0–1 minute isocratic at 8% B; 1–10 minutes linear gradient to 16% B; followed by a 1 minute washing step (100% B) and 2 minutes re-equilibration step in initial conditions (92% A, 8% B).

Column effluent was perfused into a Quattro micro bench-top triple quadrupole mass spectrometer (Micromass/Waters Corporation) through its electrospray ionization (ESI) source working in negative mode. The source temperature, capillary voltage, desolvation gas flow, and temperature were set up as described elsewhere (Actis-Goretta et al., 2012, 2013). It allowed us to detect and quantify the epicatechin metabolites following their *m/z* transitions with specific cone voltage and collision energy in multiple-reaction monitoring (MRM) mode. The molecular transitions were *m/z* 289–245 for (–)-epicatechin aglycone, *m/z* 303–244 for 3′-*O*-methyl-(–)-epicatechin, *m/z* 303–137 for 4′-*O*-methyl-(–)-epicatechin and 4′-*O*-methyl-(+)-catechin (IS), *m/z* 465–289 for glucuronidated (–)-epicatechin metabolites, *m/z* 369–289 for sulfated (–)-epicatechin metabolites, *m/z* 479–303 for glucuronidated O-methyl-(–)-epicatechin metabolites, *m/z* 383–303 for sulfated *O*-methyl-(–)-epicatechin metabolites, *m/z* 241–161 for umbelliferone sulfate (IS) and *m/z* 337–161 for umbelliferone glucuronide (IS). MassLynx software (v 4.0; Micromass/Waters Corporation) was used to control the instruments and for data acquisition and processing.

Data Analysis. The apparent permeability coefficients (P_{app}) were calculated using the following equation $P_{app} = [(\Delta Q/\Delta T)/A \times C\underline{0}]$, where, $\Delta Q/\Delta t$ is the linear appearance rate of the compound on the receiver side (in mM/s), A is the membrane surface area (cm^2), and $C\underline{0}$ is the initial concentration in the donor (apical) compartment (in mM/cm^3).

All samples were analyzed at least in triplicate. Statistical analyses were conducted with GraphPad Prism version 6.04 (GraphPad Software, La Jolla, CA). Data are shown as mean ± S.D. Statistical differences were determined by one-way analysis of variance (ANOVA). A P value < 0.05 was considered to be statistically significant.

Results

Transport of (–)-Epicatechin in Caco-2 Cell Model. Although some in vitro studies have described a passive-diffusion transport (Vaidyanathan and Walle, 2001), others reported the relevance of the facilitated mechanism for flavanol absorption (Vaidyanathan and Walle, 2001; Zhang et al., 2004; Chan et al., 2007). With the objective of investigating its transport across Caco-2 cells, (–)-epicatechin was placed either in the apical or basal compartments. Results showed a similar linear correlation between the amount of (–)-epicatechin transported from apical-to-basal than basal-to-apical at different times, suggesting a passive diffusion or paracellular mechanism of transport (Supplemental Fig. 1A). Moreover, it was not possible to saturate the (–)-epicatechin transport by increasing the concentration up to 35 mM (Supplemental Fig. 1B).

In addition, P_{app} was calculated under our experimental conditions, where $\Delta Q = 1.25$ μM, $\Delta t = 7200$s, $A = 1.12$ cm^2, and $C\underline{0} = 250$ μM; therefore, $P_{app} = 0.6 \times 10^{-06}$ cm/s. The calculated value was similar to the one reported by Tian et al. (2009) ($0.60 \pm 0.05 \times 10^{-06}$ cm/s) confirming the hypothesis of passive diffusion or paracellular transport of (–)-epicatechin.

Identification of (–)-Epicatechin Metabolites in Caco-2 Cell Model. (–)-Epicatechin metabolites were identified in the exposure media both at the apical and basolateral compartments. The concentration of conjugates measured in the apical compartment augmented with increasing concentrations of (–)-epicatechin (10, 50, and 100 μM) incubated with Caco-2 cells (Fig. 2A). 3′-*O*-Methyl-epicatechin, 3′-*O*-sulfate-epicatechin, and 3′-*O*-methyl-5-*O*-sulfate-epicatechin were identified as the main metabolites of (–)-epicatechin both at the apical and basolateral compartments after incubation of this compound with Caco-2 cells for 2 hours, (Fig. 2B). The 3′-*O*-methyl-4′-*O*-sulfate-epicatechin and 4′-*O*-methyl-epicatechin conjugate were quantified solely in the apical compartment, whereas other (–)-epicatechin metabolites such as 3′-*O*-methyl-7-*O*-sulfate-epicatechin and 4′-*O*-methyl-5-*O*-sulfate-epicatechin were identified at concentrations lower than the limit of quantification of 5 nM and are not reported in the figure.

In our previous results Caco-2 cells generated relevant amounts of umbelliferone glucuronide indicating the ability to glucuronidate other

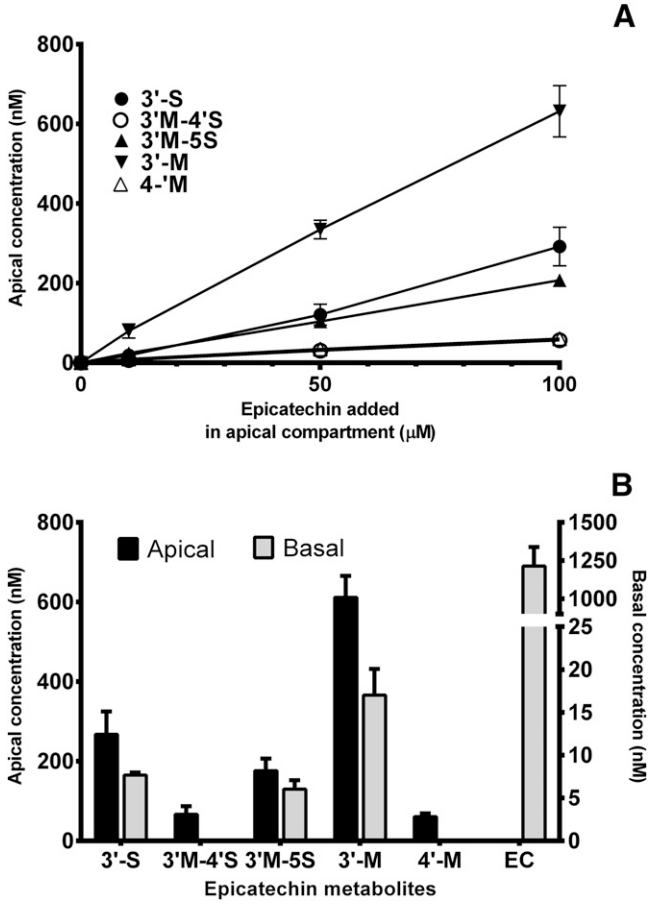


Fig. 2. (A) Concentration of (–)-epicatechin metabolites in apical compartment of Caco-2 cells after 2 hours incubation with different concentrations of (–)-epicatechin. (B) Concentration of (–)-epicatechin metabolites in apical and basal compartment of Caco-2 cells after incubation with 100 μM of (–)-epicatechin in apical compartment for 2 hours. Data are presented as mean ± S.D. ($n = 3$).

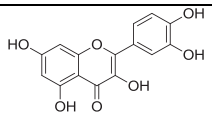
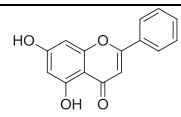
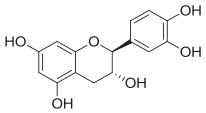
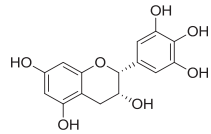
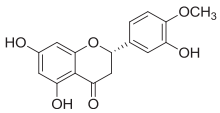
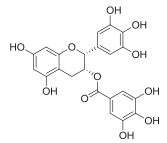
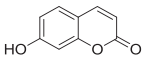
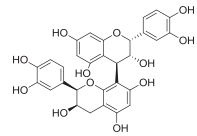
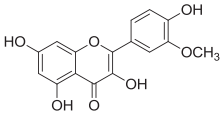
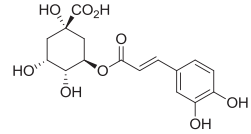
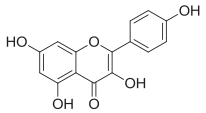
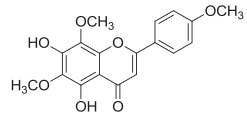
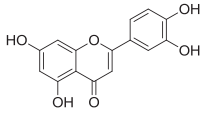
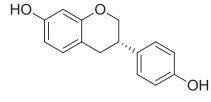
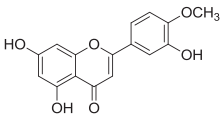
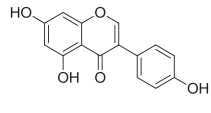
compounds but the lack of a specific isoform able to glucuronidate (–)-epicatechin (Actis-Goretta et al., 2013).

Modulation of the Metabolic Profile of (–)-Epicatechin by Other Polyphenols. To evaluate the effect of other food components on the modulation of (–)-epicatechin metabolism, 16 polyphenols were chosen for coincubation experiments (Table 1). These compounds were selected because they are present in dietary foods and belong to different polyphenol groups. The initial working concentration for these polyphenols was set at 50 μM (1:2 ratio to (–)-epicatechin). In the presence of other polyphenols, the conjugates of (–)-epicatechin identified were as before, i.e., 3′-methyl-epicatechin, 3′-*O*-sulfate-epicatechin, 3′-*O*-methyl-5-*O*-sulfate-epicatechin, and 4′-*O*-methyl-epicatechin, but their amount and distribution varied according to the polyphenol/(–)-epicatechin combination tested.

The total amount of 3′-*O*-methyl-epicatechin, 3′-*O*-sulfate-epicatechin, and 3′-*O*-methyl-5-*O*-sulfate-epicatechin conjugates found both in apical and basal compartments ranged from 19 to 801 nM, from 6 to 432 nM, and from lower than 5 (limit of detection) to 176 nM, respectively (Fig. 3). The coincubation of (–)-epicatechin with flavanols, chlorogenic acid, and umbelliferone resulted in similar amounts of 3′-*O*-methyl-epicatechin effluxed into the apical compartment relative to the control (Fig. 4). Coincubation with isorhamnetin, kaempferol, diosmetin, nevadensin, chrysin, equol, genistein, and hesperitin promoted the transport of 3′-*O*-methyl-epicatechin toward the basolateral side and decreased the apical efflux. Quercetin and luteolin considerably inhibited the appearance of this (–)-epicatechin conjugate both in the apical and basolateral compartments. These findings suggest that certain polyphenol families could compete

TABLE 1

Polyphenols chosen for coincubation experiments with epicatechin in Caco-2 cell model

Compound	Family	Structure	Compound	Family	Structure
Quercetin	Flavonol		Chrysin	Flavone	
Catechin	Flavanol		Epigallocatechin	Flavanol	
Hesperitin	Flavanone		Epigallocatechin gallate	Flavanol	
Umbelliferone	Coumarin		Procyanidin B2	Flavanol	
Isorhamnetin	Flavonol		Chlorogenic acid	Phenolic acids	
Kaempferol	Flavonol		Nevadensin	Flavone	
Luteolin	Flavone		Equol	Isoflavone	
Diosmetin	Flavone		Genistein	Isoflavone	

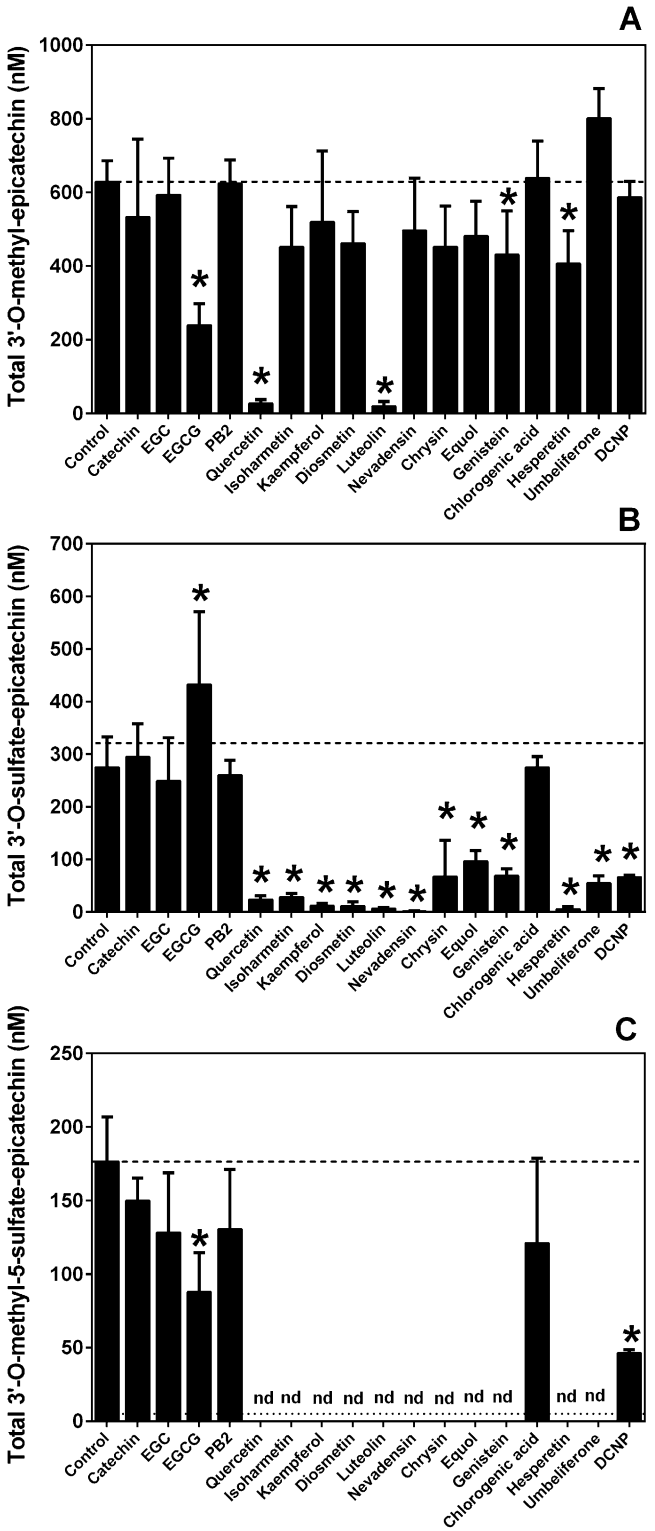


Fig. 3. Total amount of 3′-*O*-methyl-epicatechin (A), 3′-*O*-sulfate-epicatechin (B), and 3′-*O*-methyl-5-sulfate-epicatechin (C) in the apical and basolateral compartments of Caco-2 cell monolayers following coincubation of (–)-epicatechin (100 μM) with different polyphenol compounds (50 μM) at the apical side. Values are shown as mean ± S.D. (n = 6). *$P < 0.05$. nd, Not detected.

for or inhibit the basolateral transporter of the (–)-epicatechin metabolites.

The appearance of 3′-*O*-sulfate-epicatechin in the apical and basolateral compartments is illustrated in Fig. 5. DCNP, a high-affinity substrate for sulfation, (Fayz et al., 1984; Morimitsu et al., 2004), inhibited the production of 3′-*O*-sulfate-epicatechin to a fourth of the control in the apical compartment and completely in the basolateral compartment. As described for 3′-*O*-methyl-epicatechin, flavanols and chlorogenic acid do not modify the efflux of 3′-*O*-sulfate-epicatechin to the apical compartment. The coincubation of (–)-epicatechin with flavonols (quercetin, isorhamnetin, and kaempferol), flavones (diosmetin, luteolin, nevadensin, and chrysin), hesperetin, and umbelliferone significantly inhibited the apical distribution of 3′-*O*-sulfate-epicatechin. Isoflavones, like equol and genistein, significantly diminished the efflux of 3′-*O*-sulfate-epicatechin to the apical side and increased its basolateral transport. Basolateral concentrations of 3-*O*-sulfate-epicatechin were close to the limit of detection of 5 nM.

Increasing Doses of Polyphenols Influence the Metabolism of (–)-Epicatechin. The results described above suggest that certain compounds may exert specific modulation effects on the metabolism of (–)-epicatechin in either apical or basolateral compartment of the Caco-2 cell model. Genistein, hesperitin, nevadensin, and chrysin were some of the compounds found to increase the concentration of 3′-*O*-methyl-epicatechin in the basal compartment. We performed coincubation experiments with (–)-epicatechin (100 μM) and the above-cited compounds at different concentration (1, 5, 10, and 50 μM) (Fig. 6). The efflux of 3′-*O*-methyl-epicatechin to the apical side was reduced and transport to the basolateral compartment was the same or slightly higher than the control when (–)-epicatechin was coincubated with 1 μM of these polyphenols (ratio 1:100). The transport of 3′-*O*-methyl-epicatechin to the basolateral compartment

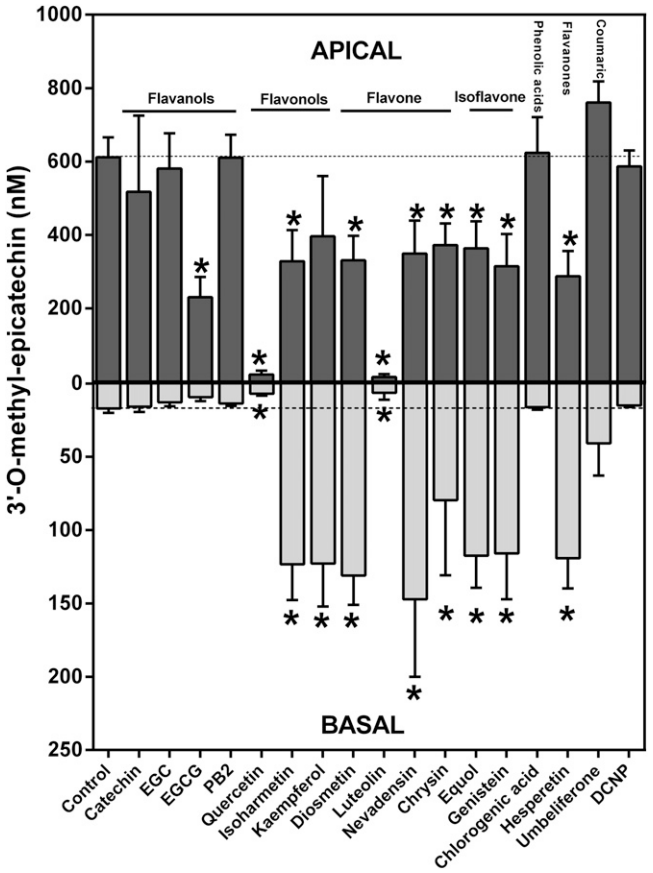


Fig. 4. Apical efflux and basolateral transport of 3′-*O*-methyl-epicatechin in Caco-2 cell monolayers following coincubation of (–)-epicatechin (100 μM) with different compounds (50 μM) at the apical side. Values are shown as mean ± S.D. (n = 6). *$P < 0.05$.

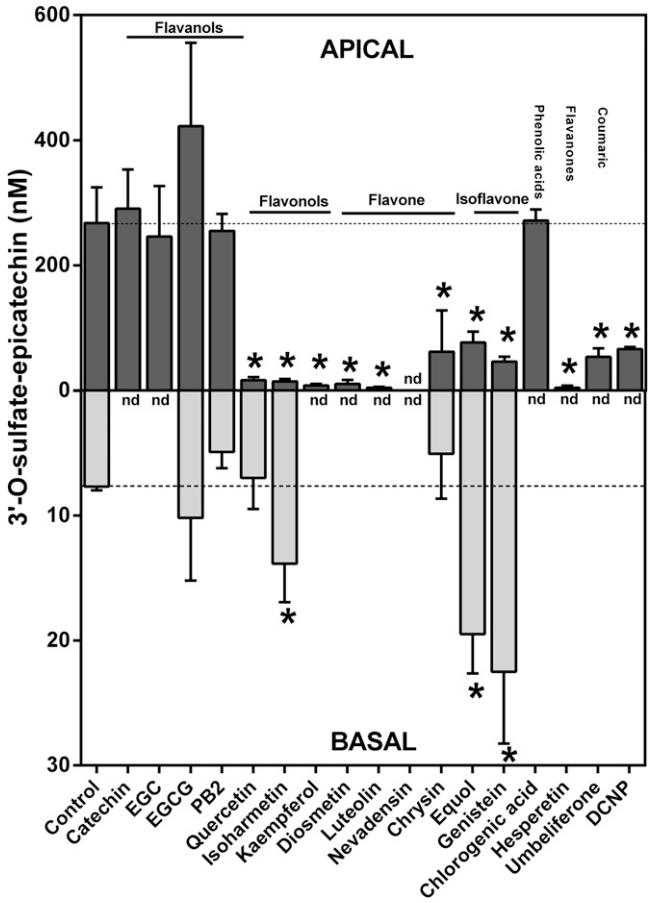


Fig. 5. Apical efflux and basolateral transport of 3′-*O*-sulfate-epicatechin in Caco-2 cell monolayers following coincubation of (–)-epicatechin (100 μM) with different compounds (50 μM) at the apical side. Values are shown as mean ± S.D. (n = 6). *$P < 0.05$. nd, Not detected.

increased with increasing doses of genistein, hesperitin, nevadensin, and chrysin. The efflux mechanism seemed to be less sensitive to the concentration change and remained constant with increasing concentrations of polyphenols but was still lower compared with the control experiment.

Discussion

Bioavailability of polyphenols is subject to transport and metabolism by phase II enzymes in intestinal cells (Scalbert and Williamson, 2000; Manach et al., 2005). With the use of a multilumen perfusion catheter in humans, it was recently demonstrated that nature and substitution position of (–)-epicatechin conjugation are major determinants of the metabolic fate in the body, influencing whether the compound is effluxed into the lumen or absorbed into the blood (Actis-Goretta et al., 2013). Previous in vitro studies using Caco-2 cell monolayers as intestinal barrier model highlighted the effect of coadministration of different flavonoids on the apical and basolateral efflux of hesperetin and hydroxycinnamic acid (Brand et al., 2010; Wong and Williamson, 2013). The objective of our study was to investigate the extent of competition of (–)-epicatechin with other polyphenols to better understand its absorption and metabolism.

The results obtained in this study suggest a passive diffusion of (–)-epicatechin to the basolateral side of the Caco-2 cell monolayer and imply that coadministration of other polyphenols should not compete for the transport of (–)-epicatechin.

In Caco-2 cells (–)-epicatechin was metabolized into 3′-*O*-methyl-epicatechin, 3′-*O*-sulfate-epicatechin, 3′-*O*-methyl-5-*O*-sulfate-epicatechin, 4′-*O*-methyl-epicatechin, and 3′-*O*-methyl-4′-*O*-sulfate-epicatechin, which were mainly effluxed to the apical compartment of the cellular model. In vivo, the main metabolites effluxed into the lumen were 3′-*O*-sulfate-epicatechin, epicatechin-3′-*O*-glucuronide, 3′-*O*-methyl-5-*O*-sulfate-epicatechin, and 7-*O*-sulfate-epicatechin (Actis-Goretta et al., 2013). The nature of the substitution of (–)-epicatechin was different in the two models: whereas sulfation was favored in vivo with 73% of metabolites sulfated, methylation was the main conjugation in vitro with 77.4% of metabolites methylated. Fifteen percent of metabolites were glucuronidated in vivo, whereas such substitution could not be detected in the Caco-2 cell model, suggesting the absence of some specific uridine 5′-diphospho-glucuronosyltransferase (UGT) isoforms in this model as anticipated by Vaidyanathan and Walle (2001) and Wong and Williamson (2013).

Under our experimental conditions, the influence of other polyphenols over the (–)-epicatechin metabolism was confirmed. Coadministration of (–)-epicatechin with flavonols, flavones and isoflavones reversed the transport of (–)-epicatechin metabolites to the basolateral side with the exception of quercetin and luteolin, which nearly abolished the metabolism and/or transport of (–)-epicatechin either by inhibition of the transporters and metabolic enzymes or by competition with these latter. In contrast, coincubation of (–)-epicatechin with other flavanols such as (+)-catechin, and (–)-epigallocatechin did not influence the conjugation profile or the apical and basolateral distribution of (–)-epicatechin metabolites.

Brand et al. (2010) previously suggested that several classes of polyphenols effectively inhibited the breast cancer resistance protein

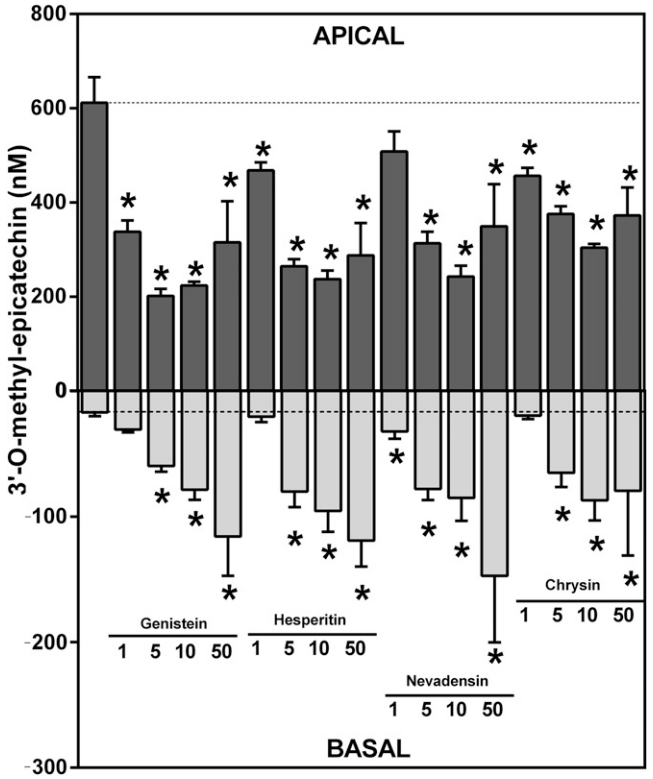
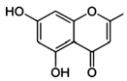


Fig. 6. Apical efflux and basolateral transport of 3′-*O*-methyl-epicatechin in Caco-2 cell monolayers following coincubation of (–)-epicatechin (100 μM) with genistein, hesperitin, nevadensin, and chrysin at 1, 5, 10, and 50 μM at the apical side for 2 hours. Values are shown as mean ± S.D. (n = 3). *$P < 0.05$.

TABLE 2

Structure-relationship for the inhibition of 3′-*O*-methyl-epicatechin in the basolateral compartment of Caco-2 cell model

	 Catechol group	 Benzopyranone group	Ratio[a] of 3′M in basolateral compartment
Quercetin	Yes	Yes	<1
Luteolin	Yes	Yes	<1
Catechin	Yes	No	~1
Epigallocatechin	Yes	No	~1
Epigallocatechin gallate	Yes	No	~1
Procyanidin B2	Yes	No	~1
Chlorogenic acid	Yes	No	~1
Isorhamnetin	No		>1
Kaempferol	No		>1
Diosmetin	No		>1
Nevadensin	No		>1
Chrysin	No		>1
Equol	No		>1
Genistein	No		>1
Hesperitin	No		>1
Umbelliferone	No		>1

[a]Ratio between the level of 3′-*O*-methyl-epicatechin in the basolateral compartment obtained by coincubating (–)-epicatechin with the corresponding polyphenols and the level of 3′M in the basolateral compartment obtained by incubating only (–)-epicatechin (control treatment).

(BCRP) transporters responsible for the efflux of hesperetin. The affinity of polyphenols for the ATP-binding cassette (ABC) transmembrane transporters, specifically P-glycoprotein (Pgp) and multidrug resistance proteins (MRP1 and MRP2) is also a key factor limiting their intestinal transport (Feng, 2006; Takano et al., 2006). These transporters actively remove xenobiotics from the cell interior to the lumen (Feng, 2006). The affinity of (–)-epicatechin metabolites for these transport systems significantly limits the ability of these compounds to cross into the bloodstream. Therefore, we can suggest that the addition of other polyphenols can generate a competition for these efflux transporters. As a result, some compounds (such as hesperitin, isorhamnetin, kaempferol, diosmetin, chrysin, nevadensin, equol, and genistein) could have more affinity for the efflux transporters, increasing the level of (–)-epicatechin metabolites in the bloodstream as demonstrated with coadministration of (–)-epicatechin and increasing doses of genistein, hesperitin, nevadensin, and chrysin (Fig. 6).

As previously suggested by Brand et al. (2010) for hesperidin and other flavonoids, a structure-activity relationship could be suggested for the inhibition/competition mechanism leading to the presence or absence of 3′-*O*-methyl-epicatechin in the basolateral compartment of the cellular model. Among the polyphenols chosen for coadministration with (–)-epicatechin, three structural groups could be distinguished: 1) presence of catechol and benzopyranone group, inhibition of the production of the conjugate compared with the levels observed with (–)-epicatechin treatment; 2) presence of catechol and absence of benzopyranone group, no difference in production of conjugate relative to (–)-epicatechin control treatment; 3) absence of catechol and benzopyranone group, increase of 3′-*O*-methyl-epicatechin in basolateral compartment compared with the treatment with (–)-epicatechin (Table 2).

Within all the polyphenols tested in our experiments only quercetin and luteolin reduced the levels of 3′-*O*-methyl-epicatechin both in the apical and basal compartments. These findings suggest a higher affinity of the catechol *O*-methyl-transferases (COMT) for these compounds than (–)-epicatechin. Quercetin and luteolin were previously identified as high-affinity substrates for COMT (Zhu and Liehr, 1996; Chen et al., 2011). Quercetin already showed higher affinity to COMT than catecholestrogens (Zhu and Liehr, 1996), neurotransmitters (Singh et al., 2003), and other flavonoids (Wang et al., 2012).

Under our experimental conditions, the conjugates of (–)-epicatechin in apical and basal compartments represented 1–2% of its initial amount under the control conditions or when (–)-epicatechin was coadministered with other polyphenols. The metabolites produced by the enterocytes had a low impact on the total amount transport across the epithelium. However, the type and quantity of metabolites produced by the enterocytes was different and could promote either the absorption or the efflux of the conjugate, highlighting the importance of different metabolic profiles.

In conclusion, using the Caco-2 cell model we could demonstrate that (–)-the efflux of epicatechin conjugates into the apical or basal compartment is modulated by certain classes of polyphenols and their amount. In vivo, efflux into the apical or basolateral compartment really translates into excretion of metabolites back into the intestinal lumen or absorption and, consequently, bioavailability. Ingesting (–)-epicatechin with specific polyphenols, like genistein or nevadensin, could be a strategy to modulate the bioavailability of (–)-epicatechin or its metabolites. In addition, decreasing the amount of quercetin and luteolin in food recipes might also contribute to increase the bioavailability of (–)-epicatechin. However, additional in vivo clinical trials would be necessary to test this hypothesis and prove the benefit of the association of (–)-epicatechin and other polyphenols on human health.

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Authorship Contributions

Participated in research design: Actis-Goretta.

Conducted experiments: Sanchez-Bridge, Lévèques, Li, Bertschy, Patin

Contributed new reagents or analytic tools: Lévèques, Patin

Performed data analysis: Lévèques, Li, Actis-Goretta.

Wrote or contributed to the writing of the manuscript: Sanchez-Bridge, Actis-Goretta.

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