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Influence of Dietary Antioxidants on Polyphenol Intestinal Absorption and Metabolism in Rats

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The ingestion of complex foodstuff implicates the simultaneous presence along the digestive tract of several antioxidants micronutrients. This work aimed to determine if the interactions that may occur at the digestive level between polyphenols and other antioxidant micronutrients may modulate the fate of polyphenols in the splanchnic area. This study was conducted in a rat in situ intestinal perfusion model. Polyphenols (gallic and caffeic acids, catechin, and naringenin) were perfused with or without an antioxidant cocktail. For gallic acid, a significant reduction (−20%) of its net transfer through the brush border associated with a drastic decrease of its intestinal secretion of conjugates (−90%) was observed. By contrast, the transfer of catechin through the brush border increased (18%) and the secretion of its conjugates was twice in the presence of the antioxidants. Perfused polyphenols largely differed in their respective biliary secretion, but these fluxes were not modified by the presence of the antioxidant cocktail. Finally, the simultaneous presence in the small intestine of polyphenols and other dietary antioxidants never affects polyphenol splanchnic metabolism but may modify their intestinal transport without noticeable consequence on their final availability for peripheral tissues.

KEYWORDS: Antioxidants; polyphenols; association; absorption; splanchnic metabolism

INTRODUCTION

Plant phenolics refer to one of the numerous and widely distributed groups of molecules in the plant kingdom. On the basis of their structure, they are classified into different families: phenolic acids flavonoids or less common lignans and stilbenes. These plant phenolics have gained increasing interest because of their numerous properties and biological effects such as free radical scavenging, modulation of enzymatic activities, and inhibition of cellular proliferation, as well as their potential utility as antibiotic, antiallergic, and antiinflammatory agents (*1*). They may participate in the prevention of cardiovascular diseases, cancers, and other degenerative diseases (*2*). Because they are widely distributed in foods and beverages of plant origin (such as fruit, vegetables, cereals, tea, coffee, cocoa, wine, and fruit juice), phenolics are common bioactive compounds in the human diet. The total polyphenol intake may commonly reach 1 g/day and is mainly constituted of phenolic acids and flavonoids (*3*).

The physiological interest of dietary polyphenols depends on their intestinal absorption and their subsequent interactions with target tissues. In the past 20 years, many studies investigated the bioavailability and metabolism of numerous polyphenols in rats or in humans. Thus, the mechanisms involved in the gastrointestinal absorption of different polyphenols are now well-detailed. However, scarce data are available on polyphenol absorption and metabolism when these compounds are present in the intestine, together with other dietary constituents. Therefore, in a complex diet, polyphenols are most of the time associated with many constituents that could influence their absorption.

Concerning macronutrients, for example, it has already been shown that some dietary proteins can exert a chelating activity toward polyphenols (*4*) even if milk proteins have not been shown to inhibit polyphenol absorption in human (*5*). Fat may also influence quercetin absorption in pigs as well as in rats (*6*, *7*).

Possible interactions of polyphenols with other dietary micronutrients may also exist. For this purpose, few data concerning vitamin C or minerals absorption are available. A human study has shown that citrus polyphenols slow the intestinal absorption of vitamin C. This effect may be linked to the ability of polyphenols to block sodium-dependent vitamin C transporter (SVCT1) (*8*). In the same way, it has already been shown that polyphenols can directly interact with minerals and thus limit their absorption, as shown for iron or aluminum (*9*, *10*). Some flavonoids, like quercetin, phloretin, and luteolin, have also been reported to inhibit the key enzyme involved in the conversion of β-carotene to vitamin A in intestinal cells (*11*). Finally, quercetin, (±)-catechin, and lignans have been shown to maintain the plasma vitamin E concentration in rats by inhibiting its degradation (*12*).

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Table 1. Supplementation of the Rat Perfusion Buffer in Antioxidants[a]

	groups		
	P	PM	PMA
micelles		X	X
antioxidant cocktail		(μM)	
caroteneoids			
β-carotene			1
lutein			1
vitamins			
ascorbic acid			75
α-tocopherol			10
polyphenols			
caffeic acid	50	50	50
gallic acid	50	50	50
catechin	25	25	25
naringenin	25	25	25

[a] This table details the conditions in which the perfusion buffer was supplemented according to the groups. For group P (n = 6), the perfusion buffer was supplemented with the polyphenol cocktail just before perfusion. For group PM (n = 6), the perfusion buffer was supplemented with the micelles and the polyphenols were added just before perfusion. For group PMA (n = 6), the perfusion buffer was supplemented with the lipophillic antioxidants solubilized within the micelles, with vitamin C and with the mix of polyphenols.

The aim of the present study was to investigate the influence of some dietary antioxidants on the intestinal absorption and splanchnic metabolism of a mix of widely distributed plant phenolics (catechin, naringenin, gallic acid, and caffeic acid). These phenolics have been chosen according to their wide distribution, consumption, and biological interest (*3*, *13*). The antioxidant cocktail used in this study contains the major hydrophilic and lipophilic antioxidative micronutrients classically found in a human diet, namely, vitamin C, β-carotene, lutein, and α-tocopherol. The consequence of the presence of these antioxidants on polyphenol absorption and metabolism in the intestine has been studied in rats using an in situ intestinal perfusion model.

MATERIALS AND METHODS

Chemicals. Naringenin and catechin were purchased from Extrasynthese (Genay, France). Caffeic acid, gallic acid, phosphatidylcholine, lysophosphatidylcholine, monoolein, free cholesterol, sodium oleate, α-tocopherol, β-carotene, lutein, and β-glucuronidase/sulfatase from Helix Pomatia were purchased from Sigma (L'Isle D'Abeau, Chesnes, France).

Animals and Diets. Before perfusion experiments, male Wistar rats (about 150 g) were housed two per cage in temperature-controlled rooms (22 °C) with a dark period from 20:00 to 8:00 h and had access to food from 16:00 to 8:00 h. They received a standard semipurified diet [73% wheat starch, 15% casein, 6% mineral mixture (AIN 93M) (*14*), 1% vitamin mixture (93VX) (*15*), and 5% corn oil] for 2 weeks. Animals were maintained and handled according to the recommendations of the "Institut National de la Recherche Agronomique" Ethics Committee, as legally required.

Micelles Preparation for the Supplementation of the Perfusion Buffer with Lipophilic Antioxidants. Mixed micelles were prepared as described previously (*16*) in order to dissolve lipophilic antioxidants (α-tocopherol, β-carotene, and lutein) in the perfusion buffer. Briefly, the final concentrations for the micelles were as follows: 0.04 mM phosphatidylcholine, 0.16 mM lysophosphatidylcholine, 0.3 mM monoolein, 0.1 mM free cholesterol, and 0.5 mM oleic acid. For the micelles supplemented with antioxidants, 10 μM α-tocopherol, 1 μM β-carotene, and 1 μM lutein were added to the micelles during preparation (**Table 1**). Stock solution solvents were carefully evaporated under nitrogen. Dried residue was solubilized in the perfusion buffer containing 5 mM taurocholate and vigorously mixed by 25 W sonication (Branson 250 W sonifier, Osi) for 3 min. The mixtures obtained were sterilized and filtered by passing them through a sterilized 0.22 μm filter (Millipore), and the obtained solutions were optically clear and stored at −80 °C until use.

In Situ Intestinal Perfusion. Rats fasted for 24 h were anesthetized with sodium pentobarbital by intraperitoneal administration (40 mg/kg body weight) and maintained alive on a heating pad throughout the perfusion period. The biliary duct was first cannulated, and cannulas were introduced at the extremities of the jejunal + ileal segment (from 5 cm distal from the flexura duodenojejunualis to the valvula ileocoecalis). This segment was continuously perfused in situ for 45 min without recycling, with a physiological and thermostated (37 °C) buffer, pH 6.6, supplemented with the selected cocktail of antioxidants, just before use (**Table 1**). The composition of the basal perfused buffer was as follows: KH_2PO_4 (5 mM), K_2HPO_4 (2.5 mM), $NaHCO_3$ (5 mM), NaCl (50mM), KCl (40 mM), tripotassium citrate (10 mM), $CaCl_2$ (2 mM), $MgCl_2$ (2 mM), glucose (8 mM), and taurocholic acid (1 mM) at pH 6.6. Just before the perfusion, the addition of the selected polyphenols to the buffer was performed from a concentrated solution in DMSO (DMSO in the final perfusion buffer never exceeded 0.5‰) as for ascorbic acid that was diluted in the perfusion buffer. The flow rate was set at 0.8 mL/min. Caffeic and gallic acids were perfused at 50 μM whereas catechin and naringenin were perfused at 25 μM. The effluent at the exit of the ileum and the bile were collected throughout the perfusion and stored at −20 °C until analysis.

At the end of the experiment, blood samples were withdrawn from the mesenteric vein and sampled into heparinized tubes. Then, plasma was collected by centrifugation and acidified with acetic acid (10 mM final) and stored at −20 °C until analysis.

HPLC Analysis. *Sample Treatment*. Bile, plasma, and perfusate samples were acidified (to pH 4.9) with 0.1 volume of 0.58 M acetic acid and incubated for 30 min (overnight for naringenin analysis) at 37 °C with (for total forms) or without (for unconjugated forms) 5×10^6 units/L β-glucuronidase and 2.5×10^5 units/L sulfatase. After the addition of 2.85 volumes of methanol/200 mM HCl and centrifugation at 14000g for 4 min, the resulting supernatants were analyzed by HPLC−UV as described below. Calibration curves were prepared in the perfusion buffer, plasma, or bile by spiking control pools with known concentrations of standards (from 5 to 100 μM in the perfusion buffer, from 1 to 10 μM in the plasma, and from 2.5 to 25 μM in the bile). These standards were then treated exactly as the samples. Quality control samples (triplicates of two different concentrations) were added in each batch of analyses to control the accuracy of the quantification. Differences between the measured value and the actual value never exceeded 5% for all batches.

Chromatographic Conditions. The HPLC system consisted of an autosampler (Kontron 360), fitted with a 5 μm C-18 Hypersil BDS analytical column (150 mm × 4.6 mm; Life Sciences International, Cergy, France). Mobile phase A consisted of 15% acetonitrile, and mobile phase B consisted of 30% acetonitrile; for each phase, the water fraction contained 0.5% H_3PO_4. The flow rate was set at 1 mL/min, and the gradient conditions were as follows: 0−5 min, 100% A; 5−35 min, linear gradient from 100% A to 100% B; 35−40 min, 100% B; 40−50 min, 100% A. The UV detector was set at 320 nm. Retention times for gallic acid, catechin, and caffeic acid were 2.9, 8.6, and 11.5 min, respectively. For the detection of naringenin, chromatographic conditions were isocratic during 10 min, and mobile phase A consisted of 27% acetonitrile in 0.5% H_3PO_4 in water. The UV detector was set at 320 nm, and the naringenin retention time was 6.5 min.

Determination of the Intestinal and Biliary Fluxes. All of the calculated fluxes were expressed in nmol/min. The fluxes in the effluent were calculated by taking into account the intestinal absorption of water as previously described (*17*). The net transfer in the enterocyte was measured by the difference between the perfused flux and the flux in the nonhydrolyzed effluent at the end of the perfusion. The secretion of conjugates back in the lumen resulted from the difference between the fluxes measured in the hydrolyzed and nonhydrolyzed effluent. The net absorption was obtained by the difference between the perfused flux and the flux measured in the hydrolyzed effluent. The biliary secretion of conjugates resulted from the product between the biliary

Table 2. Intestinal and Biliary Fluxes of Perfused Polyphenols[a]

		intestine			bile	peripheral tissues
group	perfused flux (nmol/min)	transfer through the brush border (nmol/min)	secretion of conjugates (nmol/min)	net absorption (nmol/min)	biliary excretion (nmol/min)	bioavailable fraction (nmol/min)
gallic acid						
P	31.06 ± 0.29	4.66 ± 0.21	1.68 ± 0.39	2.98 ± 0.36	ND	2.98 ± 0.36
PM	32.39 ± 0.47	4.46 ± 0.22	1.85 ± 0.28	2.61 ± 0.35	ND	2.61 ± 0.35
PMA	32.06 ± 0.90	3.54 ± 0.39 [c]	0.19 ± 0.09 [b]	3.35 ± 0.32	ND	3.35 ± 0.32
caffeic acid						
P	32.37 ± 0.32	8.58 ± 0.91	1.77 ± 0.21	6.81 ± 0.42	0.39 ± 0.09	6.42 ± 0.81
PM	32.25 ± 0.27	9.01 ± 0.80	1.80 ± 0.25	7.41 ± 0.36	0.50 ± 0.05	6.91 ± 0.33
PMA	31.53 ± 0.83	8.71 ± 0.37	2.13 ± 0.45	6.58 ± 0.26	0.44 ± 0.05	6.14 ± 0.31
catechin						
P	18.17 ± 0.75	5.54 ± 0.25	0.50 ± 0.24	5.04 ± 0.21	0.57 ± 0.08	4.47 ± 0.60
PM	17.96 ± 0.32	5.68 ± 0.41	0.58 ± 0.21	5.10 ± 0.19	0.56 ± 0.06	4.54 ± 0.32
PMA	18.29 ± 0.60	6.72 ± 0.37[b]	1.68 ± 0.31[b]	5.04 ± 0.20	0.54 ± 0.07	4.50 ± 0.44
naringenin						
P	19.31 ± 0.33	16.12 ± 0.47	3.46 ± 0.58	12.66 ± 0.78	9.64 ± 0.32	3.02 ± 0.55
PM	19.41 ± 0.25	16.24 ± 0.73	3.59 ± 0.39	12.65 ± 0.87	10.25 ± 0.62	2.40 ± 0.44
PMA	18.33 ± 0.66	15.98 ± 0.60	4.00 ± 0.31	11.98 ± 0.81	9.26 ± 0.30	2.72 ± 0.42

[a] For group P ($n = 6$), the perfusion buffer was supplemented with polyphenols (gallic acid, caffeic acid at 50 μM, naringenin, and catechin at 25 μM). For group PM ($n = 6$), the perfusion buffer was supplemented with the micelles and the polyphenols. For group PMA ($n = 6$), the perfusion buffer was supplemented with polyphenols and with α-tocopherol, β-carotene, and lutein within the micelles and with ascorbate. Values are means ± SEM. ND, not detected. [b] Statistically different from other groups with $p < 0.05$. [c] Statistically different from group P with $p < 0.05$.

flow rate (μL/mL) and the concentrations of the total forms present in the bile after enzymatic hydrolysis (μM).

Statistics. Values are means ± error type, and the differences between values were determined by one-way analysis of variance coupled with the Neumann−Keuls posthoc test. Values of $P < 0.05$ were considered significant.

RESULTS

Presentation of the Experimental Pattern. The mix of polyphenols, composed of gallic acid, caffeic acid, catechin, and naringenin, was perfused in the intestine (i) after its direct addition in the perfused buffer (group P), (ii) in combined addition with mixed micelles (group PM), or (iii) in association with mixed micelles enriched with the antioxidants cocktail (containing vitamin C, vitamin E, β-carotene, and lutein) (group PMA) (**Table 1**). The control group (group P) was included to evaluate possible modification in polyphenol absorption consecutive to the presence of mixed micelles alone.

No significant change in the perfused flux of each polyphenol was observed between the different groups. Thus, according to the perfusion conditions, when polyphenols were perfused at 50 (gallic and caffeic acid) and 25 μM (catechin and naringenin), the measured perfused fluxes were, respectively, about 31 and 18 nmol/min (**Table 2**).

As shown in **Table 2**, the simultaneous presence of micelles combined with the mix of polyphenols in the perfusion buffer did not induce a significant change in the fluxes measured at the intestinal and biliary levels (group P vs group PM). Consequently, the results obtained in the presence of micelles (group PM) could be safely and directly compared to those observed when polyphenols were associated with other antioxidants (group PMA).

Effect of the Antioxidant Cocktail on the Intestinal and Biliary Fluxes of Polyphenols. *Transfer through the Brush Border.* As shown in **Table 2**, the efficiency of this transfer largely differed according to the polyphenol, since this parameter reached 14, 28, 32, and 84% of the perfused flux for, respectively, gallic acid, caffeic acid, catechin, and naringenin in the PM group.

The presence of the antioxidant cocktail reduced the transfer through the brush border into the intestinal wall for gallic acid (−20%) and significantly increased that of catechin (+18%), whereas this parameter was unchanged for caffeic acid and naringenin.

Intestinal Secretion of Conjugates. By measuring the level of conjugated metabolites recovered in the effluent at the end of the perfusion, it was possible to determine the intestinal secretion of polyphenols conjugates. As shown in **Table 2**, in group PM, this flux was quite low and in a similar range for gallic acid, caffeic acid, and catechin (representing 6, 6, and 3% of the perfused flux, respectively), whereas for naringenin it reached up to 18% of the perfused flux.

The presence of the antioxidant cocktail induced a drastic drop in the secretion of gallic acid conjugates (−90%), which became quite negligible (0.19 nmol/min). By contrast, the intestinal secretion of catechin conjugates increased by almost 2-fold (+190%) when the antioxidant cocktail was present in the perfusion buffer. Considering caffeic acid and naringenin, their intestinal secretion was similar in both groups.

Net Absorption. By taking into account the efficiency of the transfer through the brush border and the intestinal secretion of conjugates, it is possible to determine the resulting net absorption. Considering the PM group, among perfused compounds, gallic acid and naringenin were distinguished from other polyphenols: gallic acid by its low net absorption (8% of the perfused flux) and naringenin by its high level of absorption (65% of the perfused flux) (**Table 2**). The net absorption of caffeic acid and catechin was intermediate; it represented 23 and 28% of their respective perfused fluxes.

The presence of the antioxidant cocktail did not induce significant change in the absorption efficiency for each perfused polyphenol. As shown above, for gallic acid and catechin, the magnitude of their transfer through the brush border as well as their intestinal secretion of conjugates were significantly dif-

Table 3. Polyphenols Concentrations in Rat Mesenteric Plasma[a]

	group			
	PM		PMA	
	total forms (μM)	conjugated forms (%)	total forms (μM)	conjugated forms (%)
gallic acid	0.76 ± 0.10	9	0.94 ± 0.13	0
caffeic acid				
caffeic acid	0.26 ± 0.11	0	0.32 ± 0.05	9
ferulic acid	1.47 ± 0.42	28	2.00 ± 0.30	32
isoferulic acid	0.15 ± 0.02	0	0.27 ± 0.01	7
sum	1.88 ± 0.54	15	2.59 ± 0.10	10
catechin	0.61 ± 0.09	56	0.51 ± 0.04	54
naringenin	7.35 ± 0.76	100	6.50 ± 0.50	100

[a] In each group (PM and PMA), the perfusion buffer contains micelles and the polyphenols mix composed of gallic acid and caffeic acid at 50 μM, naringenin, and catechin at 25 μM. In group PMA, the perfusion buffer also contains an antioxidant cocktail composed of α-tocopherol, β-carotene, lutein, and ascorbate as described in **Table 1**. Values are means ± SEM; $n = 6$. No statistical difference was observed between the groups.

ferent between groups PM and PMA. Both fluxes, which operate in opposite directions, increased for catechin or decreased for gallic acid. Finally, these variations canceled each other out leading to a similar net absorption for gallic acid as for catechin in PM and PMA groups (**Table 2**).

Biliary Secretion. First, it could be noted that all polyphenols detected in bile were present as conjugates (data not shown). In group PM, the rates of biliary secretion strongly differed among the perfused polyphenols. No significant secretion of gallic acid metabolites could have been detected in bile, and the biliary secretions of caffeic acid and catechin were moderate, representing about 2 and 3% of the perfused flux, respectively. By contrast, naringenin was efficiently secreted in this compartment (53% of the perfused flux). All of the biliary fluxes of polyphenols measured in group PMA were not significantly different from those determined in group PM, indicating that whatever the polyphenols, this parameter was not affected by the presence of antioxidants.

Bioavailability for Peripheral Tissues. Another interesting feature of this in situ experimental model is to evaluate the fraction of absorbed compounds, which could reach peripheral tissues. As far as the biliary duct was cannulated before the perfusion, the bioavailable fraction resulted from the difference between the net absorption and the biliary secretion (**Table 2**). The fraction of perfused caffeic acid available for peripheral tissues was twice that of gallic acid. This difference may be linked to the greater efficiency of caffeic acid transfer through the brush border. Even if the net absorption of naringenin was about 2.5-fold higher than that of catechin, its final bioavailability for peripheral tissues was lower than that of catechin due to the large difference in the levels of their biliary secretion (**Table 2**).

For all perfused polyphenols, the bioavailable fraction was not slightly modified by the presence of the antioxidant cocktail. This result suggests that the simultaneous presence of polyphenols and other dietary antioxidants in the intestine did not lead to a significant change in polyphenol bioavailability.

Polyphenols Concentrations in Rat Mesenteric Plasma. The determination of polyphenols in the mesenteric vein before and after enzymatic deconjugation led to an estimate, for each compound, of the level of conjugates that had an intestinal origin (**Table 3**). Regarding caffeic acid, the plasma metabolites found in mesenteric vein were composed of caffeic, ferulic and isoferulic acid derivatives; the methylated forms were largely preponderant (representing more than 86% of its total forms). The proportion of conjugates was markedly higher for naringenin and catechin than for phenolic acids (gallic and caffeic acids) indicating that difference in the activity of intestinal metabolism between polyphenols. The presence of the antioxidant cocktail did not significantly modify either the total plasma concentration for each polyphenol or the proportion of their conjugates. This suggests that the intestinal metabolism of polyphenol was not affected by the antioxidants.

DISCUSSION

Vegetal foodstuff constitutes an important source of various dietary micronutrients and bioactive compounds (minerals, oligoelements, vitamins, caroteneoids, and polyphenols) that are simultaneously consumed during a complex meal. In that way, some scarce data showed that interactions between these compounds may influence their biological effects (*18*, *19*). At the intestinal level, it has been shown that polyphenols could interfere with minerals, vitamin C and E, or carotenoid, leading to modulation of the bioavailability of these micronutrient bioactive compounds (*11*, *12*, *20*, *21*). However, no data are available about the influence of micronutrients on polyphenol absorption and metabolism. Thus, the aim of the present study was to bring data in this field. To investigate this question, among the large variety of dietary antioxidants, the most abundant and most potent compounds supplied by a complex meal have been selected, namely, vitamins E and C, lutein, and β-carotene. To assess the impact of these antioxidants on polyphenol bioavailability, we have selected various polyphenols among the most abundant in vegetal foodstuffs, largely consumed and known for their biological interest (*2*, *3*). Besides, this mix of polyphenols contained representative compounds of the two major classes of polyphenols, namely, caffeic and gallic acid for phenolic acids and catechin and naringenin for flavonoids.

All of the present experiments were conducted in rats using an in situ intestinal perfusion model, particularly suitable to determine possible interactions between antioxidant micronutrients and polyphenols at the small intestine level (like physical interactions in the lumen, competition for the absorption, and transport sites, etc.). This study did not aim to precisely determine the nature of these putative interactions but rather to investigate their consequences on polyphenol bioavailability and metabolism. For the experimental design, most attention has been focused on polyphenol concentration with respect to the estimated mean dietary intake of these compounds (≈1 g/day) (*22*). Thus, the total perfused polyphenol concentration (150 μM) corresponded to a supply of about 900 mg in humans, which is close to the estimated daily intake in the human diet (*3*).

According to previous studies dealing with the fate in the splanchnic area of polyphenols from various origins (*23*, *24*), the present data showed large differences in the intestinal and biliary fluxes measured for each polyphenol present in the mix. The perfused compounds differed in the efficiency of transfer through the brush border since this parameter, expressed in percentage of the perfused flux, represented 14% for gallic acid, 28% for caffeic acid, 32% for catechin, and 84% for naringenin. Such differences may be linked to differences in the systems involved in their uptake by the intestinal cells. Some previous studies using Caco2 cells monolayers have evaluated the intestinal permeability of polyphenols. Numerous phenolic acids,

particularly hydroxycinamic acids, have been shown to be transported into the enterocyte via the monocarboxilic acid transporter (MCT) (*25*−*27*). At this purpose, caffeic acid was shown to have a better affinity for MCT than gallic acid (*28*). However, the paracellular pathway also appeared largely involved in the absorption of these compounds (*29*). In our model, the transfer through the brush border measured takes into account both paracellular and transcellular components. Because a substantial secretion of gallic acid conjugates in the lumen was observed, this indicates that a noticeable fraction of this compound entered the intestinal cells to be further conjugated. In consequence, this clearly showed that the paracellular pathway did not totally account for gallic acid absorption. Because a very limited amount of gallic acid conjugates was recovered in the mesenteric vein, it can be assumed that all gallic acid reaching the enterocyte was metabolized into conjugated forms and re-excreted in the lumen. In these conditions, the flux of the intestinal secretion of conjugates measured could reflect the minimum rate of gallic acid uptake by the enterocytes. According to the data presented in **Table 2**, this component may account for at least 40% of the gallic acid that crossed the brush border.

The presence of the antioxidants in the lumen seems to alter the influx of gallic acid into the enterocyte (−20%) as well as the efflux of its conjugates produced by intestinal cells (−90%). The drastic decrease in the intestinal secretion of conjugates indicated that the transcellular pathway was strongly affected by the presence of the antioxidant cocktail. It is conceivable that the antioxidants may either physically interfere with gallic acid in the lumen or be competitors for intestinal transporters, leading to an impairment of gallic intestinal uptake. Among the antioxidants present in the cocktail, the hydrophilic one, namely, vitamin C, enters intestinal cells via a sodium-dependent system called SVCT (*30*). The existence of competitive interactions between gallic acid and vitamin C for their intestinal transport could not be excluded. Even if no study has been performed on this particular topic, a previous study has already reported that some polyphenols decreased ascorbate absorption by inhibiting its intestinal transport via SVCT (*8*). It is possible to suggest that such competition may occur for gallic acid active uptake by the enterocytes. From our data, caffeic acid did not appeared as such sensitive as gallic acid to the antioxidants; thus, their impact on gallic acid transport could not be generalized to all phenolic acids.

Concerning catechin, according to our data and as previously shown (*23*), this compound is characterized by a quite limited transfer through the gut as compared to that of other flavonoids. This lesser transfer efficiency could be linked to the low lipophilicity of catechin (*23*), which accounts for an active transport rather than for a passive transfer into the enterocyte. Until now, little has been known about the mechanisms involved in the transepithelial transport of catechin. It has been proposed that even if this compound could also interfere with MCT (*25*), its absorption essentially absorbed via a paracellular pathway. Whatever the mechanisms involved, they were affected by the presence of the antioxidant cocktail since an increase in both the transfer through the brush border and the intestinal secretion of conjugates was observed. When the antioxidant cocktail was perfused, the shift in the flux of transfer through the brush border was similar to that of the secretion of conjugates: at about +1 nmol/min. This change in fluxes operating in opposite directions did not significantly modify the final net absorption of catechin. The effect of the antioxidant cocktail concerned the fraction of catechin, which had entered the enterocyte, and in no way the part of catechin that had followed the paracellular route.

For each perfused polyphenol, the fraction finally available for peripheral tissues was not affected by the presence of the antioxidants. This is largely due to the absence of effect of the antioxidants on the hepatic metabolism as shown by the unchanged fluxes of biliary secretion. Thus, the influence of the antioxidants on the intestinal transport of gallic acid and catechin had no significant consequence on their final bioavailability. From our data, it appeared that the final bioavailability of caffeic acid was markedly higher than that of gallic acid due to the better efficiency (more than 2-fold) of its transfer through the brush border. By contrast, naringenin was the polyphenol best transferred through the brush border as its intestinal uptake was about 3-fold that of catechin and 4-fold that of gallic acid, but this flavanone did not appear to be the most bioavailable compound. Actually, the final bioavailability of naringenin for peripheral tissues was limited by the extensive secretion of its conjugates into the bile. These results showed that polyphenol bioavailability is not only governed by their intestinal uptake and secretion of conjugates but also by the level of their biliary secretion.

For all selected polyphenols, the level of their conjugated metabolites in the mesenteric vein (**Table 3**) was not modified by the presence of the antioxidant cocktail indicating that the intestinal metabolism of these compounds was not altered. This result is in agreement with a previous study, which compared the in vivo bioavailability of catechin and quercetin when administered simultaneously in the rat diet (*31*). This study reported a modification in the absorption kinetic and efficiency of both molecules without any change of their respective intestinal and hepatic metabolisms.

In conclusion, the simultaneous presence of polyphenols with other antioxidant micronutrients never affects their splanchnic metabolism but could modify the processes involved in their intestinal transport without leading to noticeable changes in their final bioavailability.

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