



# Deleterious Effect of p-Cresol on Human Colonic Epithelial Cells Prevented by Proanthocyanidin-Containing Polyphenol Extracts from Fruits and Proanthocyanidin Bacterial Metabolites

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# Deleterious Effect of *p*-Cresol on Human Colonic Epithelial Cells Prevented by Proanthocyanidin-Containing Polyphenol Extracts from Fruits and Proanthocyanidin Bacterial Metabolites

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**ABSTRACT:** The protective effect of proanthocyanidin-containing polyphenol extracts from apples, avocados, cranberries, grapes, or proanthocyanidin microbial metabolites was evaluated in colonic epithelial cells exposed to *p*-cresol, a deleterious compound produced by the colonic microbiota from L-tyrosine. In HT29  $\text{Gle}^+$  cells, *p*-cresol significantly increased LDH leakage and decreased ATP contents, whereas in Caco-2 cell monolayers, it significantly decreased the transepithelial electrical resistance and increased the paracellular transport of FITC-dextran. The alterations induced by *p*-cresol in HT29  $\text{Gle}^+$  cells were prevented by the extracts from cranberries and avocados, whereas they became worse by extracts from apples and grapes. The proanthocyanidin bacterial metabolites decreased LDH leakage, ameliorating cell viability without improving intracellular ATP. All of the polyphenol extracts and proanthocyanidin bacterial metabolites prevented the *p*-cresol-induced alterations of barrier function. These results suggest that proanthocyanidin-containing polyphenol extracts and proanthocyanidin metabolites likely contribute to the protection of the colonic mucosa against the deleterious effects of *p*-cresol.

**KEYWORDS:** *p*-cresol, mitochondria, proanthocyanidins, colonic cells, microbiota, avocado, apple, cranberry, grapes, gut barrier function

## INTRODUCTION

Polyphenols are secondary metabolites including phenolic acids and flavonoid compounds (flavanols, flavonols, and anthocyanins) that are implicated in the protection of plants against predators, infection, and diverse types of stress. Because of their chemical structure, polyphenols exhibit a great number of health-promoting properties, such as antioxidant, antibacterial, anti-inflammatory, antihypertensive, antiproliferative, regulatory of mitochondrial function, and so forth.<sup>1</sup> For this reason, polyphenols are considered as beneficial dietary non-nutrients whose intake, in the form of fruits and vegetables, is recommended to decrease the risk of chronic noncommunicable diseases in the population. Among dietary polyphenols, proanthocyanidins (PACs), also known as condensed tannins, are oligomeric forms of flavan-3-ols abundant in plant-derived foodstuffs such as red wine, green tea, grape seeds and skin, cocoa, avocados, cranberries, peanut cuticles, cinnamon, and apples.<sup>2,3</sup> In the U.S. population, they are the second most abundant phenolic compounds in the diet after lignin with an estimated daily PAC intake of approximately 224 mg.<sup>2,3</sup> Dietary PACs with a degree of polymerization higher than 3 are in general poorly absorbed in the intestine, thus reaching the colon where they are metabolized by the colonic microbiota.<sup>4–7</sup> Studies in humans and animals consuming cocoa, red wine, apples, or green tea have shown that the PACs present in these foodstuffs stimulate the growth of bacterial populations from

the autochthonous microbiota in the colon, including *C. histolyticum*, *E. rectale* *Lactobacillus*, and *Bifidobacterium* spp.<sup>8–13</sup> It is therefore possible that this prebiotic-like effect explains some of their health-promoting properties. The bacterial metabolism of dietary PACs generates aromatic acids such as 3-hydroxyphenyl acetic acid (3-HPAA), 4-hydroxyphenyl valeric acid (4-HPVA), 4-hydroxyphenyl acetic acid (4-HPAA), 3,4-dihydroxyphenyl propionic acid (3,4-DHPPA), 3-(3-hydroxyphenyl) propionic acid (3-HPPA), and 3-phenylpropionic acid (3-PPA) that may be detected in high concentrations in the stool or fecal water of healthy subjects.<sup>6,8,13,14</sup> These compounds may be absorbed in the colon, and eventually, they may be detected in the bloodstream at micromolar concentrations (2–50  $\mu\text{M}$ ) and exert systemic activities. For example, 3,3-DHPPA has been shown to act as a potent vasodilator in rats, whereas 3,4-DHPPA exhibits cytotoxic and anti-inflammatory properties.<sup>15–17</sup>

Constrastingly, the colonic microbiota also expresses proteases and peptidases capable of partially degrading dietary and endogenous proteins that remain in the small intestine and reach the colon. The phenylalanine, tyrosine, and tryptophan

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released through this process are subsequently fermented, resulting in the formation of phenolic metabolites including *p*-cresol (4-methylphenol).<sup>18,19</sup> This compound is specifically produced from L-tyrosine by anaerobic bacteria, a process that occurs preferentially in the distal colon where protein breakdown is more intense, and then, the concentrations of *p*-cresol are more elevated.<sup>20</sup> *p*-Cresol is absorbed by the epithelial colonic cells, transferred into the bloodstream, metabolized in the liver, and excreted in the urine where it represents more than 90% of the phenolic compounds recovered.<sup>21</sup> Interestingly, higher urinary concentrations of *p*-cresol have been described in patients with colorectal cancer.<sup>22</sup> In this respect, we recently showed that *p*-cresol negatively affects human colonic epithelial cells (HT-29  $\text{Gle}^{\pm}$ ) in vitro, inhibiting their proliferation and respiration, decreasing their ATP content, and increasing DNA damage.<sup>23</sup> Additionally, *p*-cresol is also recognized as a uremic toxin, and in patients with chronic kidney disease, its accumulation or that of its conjugate, *p*-cresyl-sulfate, contributes to the development of renal and cardiovascular complications.<sup>24–26</sup> It is noteworthy that the intracolonic concentrations of *p*-cresol, and eventually its subsequent deleterious effects in the epithelium, increased in individuals consuming high protein diets,<sup>27</sup> whereas they decreased in those consuming diets rich in resistant starch or pre- and probiotics.<sup>28,29</sup> Interestingly, a decrease of *p*-cresol has also been reported in the colon of rats fed a diet supplemented with epigallocatechin gallate (EGCG), the main phenolic compound present in green tea.<sup>30</sup>

In this context, the aim of the present study was to determine whether polyphenol extracts containing PACs from apples, cranberries, grapes, avocados, or PAC microbial metabolites may protect colonic epithelial cells from the deleterious effects of *p*-cresol in human colonic cell lines in terms of cell viability, mitochondrial function, and epithelial integrity.

## MATERIALS AND METHODS

**Chemicals.** CytoTox-ONE homogeneous membrane integrity assay kits and CellTiter-Glo luminescent cell viability assay kits were from Promega (Madison, USA). Flavonol glycosides quercetin 3-O-galactoside (hyperoside), quercetin 3-O-glucoside (isoquercitrin), and quercetin 3-O-rhamnoside (quercitrin) were from Roth (Karlsruhe, Germany). 5-Caffeoyl quinic acid and 4-caffeoyl quinic acid were obtained from Phytolab (Vestenbergsgreuth, Germany). All solvents (HPLC-grade acetonitrile, methanol, and TFA) were purchased from Merck (Darmstadt, Germany). *p*-Cresol, 4-hydroxyphenylacetic acid (4-HPAA), 3,4-dihydroxyphenyl propionic acid (3,4-DHPPA), 3-phenylpropionic acid (3-PPA), fluorescein isothiocyanate-dextran (FD-4), rhodamine 123, quercetin, rutin, apigenin, kaempferol, myricetin, isorhamnetin, epicatechin, catechin, epicatechin gallate, catechin gallate, phloretin-2'-xyloglucoside, phloretin-2'-glucoside (phloridzin), cyanidin-3-O-glucoside, cyanidin-O-arabinoside, peonidin-O-arabinoside, gallic acid, syringic acid, vanillic acid, chlorogenic acid, caffeic acid, ellagic acid, sinapic acid, ferulic acid, anisic acid, *p*-coumaric acid, cinnamic acid, protocatechuic acid, *p*-hydroxybenzoic acid, *m*-hydroxybenzoic acid, caffeoyl glucoside, feruloyl glucoside, coumaroyl glucoside, and the Folin–Ciocalteu reagent were purchased from Sigma-Aldrich (St. Louis, MO, USA).

**Preparation of the Polyphenol Extracts from Fruits.** The polyphenol extracts from cranberries, grapes, and apples, and avocados were prepared from concentrated cranberry juice (Agricola Cran Chile Ltd., Lanco, Chile), grape skin and seeds (Cabernet Sauvignon variety, De Neira Vinyard, Guarilhue, Chile), apple peels (Granny Smith variety, Surfrut Ltd., Chile), and avocado peels (Hass variety, purchased at a local market), respectively.<sup>31</sup> First, 500 g of frozen samples ( $-20^{\circ}\text{C}$ ) of grape, apple, and avocado peels were mixed with hot water (5 min,  $90^{\circ}\text{C}$ ) and filtered on a sintered glass funnel (70–

100  $\mu\text{m}$ ). Marcs were homogenized for one min with an Ultraturrax and extracted again with water at  $65^{\circ}\text{C}$ . After 60 min of maceration, the pH of the pooled extracts was adjusted to 2.5 with formic acid, and these were poured on a glass column (150 mm i.d.  $\times$  300 mm) packed with Sepabeads SP-850 (Supelco, Bellefonte, USA) preconditioned with acidic water. Sugars and other water-soluble compounds were removed by washing with 5 L of distilled water, and the polyphenol extracts were obtained after elution with absolute ethanol.<sup>26</sup> Cranberry extract was obtained from concentrated juice (500 mL) by 5-fold dilution with acidic water (pH 2.5) and adsorption on an Amberlite XAD7HP column. The ethanolic fractions were evaporated under vacuum ( $<40^{\circ}\text{C}$ ) followed by freeze-drying of the aqueous remnants. Dried polyphenol-rich extracts were stored at  $-70^{\circ}\text{C}$  until use.

**High-Performance Liquid Chromatography.** The identification and quantification of the polyphenols present in the four extracts were carried out as previously described by Pastene et al.<sup>31,32</sup> Polyphenols were analyzed by RP-HPLC using a Waters Alliance 2695 system equipped with a C18 column (Kromasil 250  $\times$  4.6 mm, 5  $\mu\text{m}$ ) and a C18 precolumn (Nova-Pak Waters, 22  $\times$  3.9 mm, 4  $\mu\text{m}$ ) (Milford, MA) at  $30^{\circ}\text{C}$ . An elution gradient was performed by varying the mobile phase from 0 to 25 min, 10–30% B; 25–30 min, 30–75% B; 30–35 min, 75–10% B at a rate flow of 0.8 mL/min with a final stabilization period of 10 min. Known compounds were identified by matching their retention times ( $t_R$ ) and online UV spectra with those of reference compounds. Quantification was performed by using a UV–vis chromatogram acquired at 350 nm for flavonols, 320 nm for phenolic acids, 280 nm for flavan-3-ols, and 520 nm for anthocyanins. When available, standard compounds were used to construct the respective external calibration curves. Rutin, quercetin, myricetin, isorhamnetin, kaempferol, cyanidin, malvidin, delphinidin, peonidin, and pelargonidin were used when authentic standards were not available. All results were expressed as grams per 100 g of polyphenols. Total polyphenolic contents (TPC) were determined with Folin–Ciocalteu reagent and expressed as mg of gallic acid equivalents (GAE) per gram of dried extract.

**Determination of the Mean Degree of Polymerization of Proanthocyanidins.** Phloroglucinolysis was used for estimating procyanidin contents and their mean degree of polymerization.<sup>32</sup> Briefly, 10 mg of the extracts were dissolved in 2 mL of a solution of 0.1 M HCl in methanol containing 50 mg/mL of phloroglucinol and 10 mg/mL of ascorbic acid. The reaction mixture was incubated at  $50^{\circ}\text{C}$  for 20 min, and 10 mL of 40 mM aqueous sodium acetate was added to stop the reaction. The formed phloroglucinol adducts were analyzed by the above-described method of RP-HPLC. Quantitative determination of (–)-epicatechin, (+)-catechin, and phloroglucinol adduct products was performed using external standards. Phloroglucinol-adducts were prepared from *Peumus boldus* and apple peel extracts as a source of catechin and epicatechin polymeric procyanidins according to Köhler and Winterhalter as well as Pastene et al.<sup>33,34</sup> The procyanidin contents were calculated by summing the masses of all subunits (excluding the phloroglucinol portion of the phloroglucinol adducts). The mean degree of polymerization was obtained summing the extension subunits and then dividing it by the sum of terminal subunits. Native (–)-epicatechin and (+)-catechin in the samples were quantified in extracts not treated with the nucleophile, and the corresponding values were subtracted for calculations.

**Cells and Cell Culture Conditions.** The human colonic adenocarcinoma cell line HT29  $\text{Gle}^{\pm}$  (passages 46–56) was used in the studies of cell viability and mitochondrial function, and the human colonic adenocarcinoma cell line Caco-2 (passages 6–20) (ATCC, USA) was used in the studies of barrier function. Both cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 4.5 g/L of glucose and L-glutamine (Corning), 10% heat inactivated fetal calf serum (Gibco, USA), and 1% penicillin/streptomycin (Hyclone). Caco-2 cells were also supplemented with a 1% nonessential amino acids solution (Gibco). Cell cultures were grown at  $37^{\circ}\text{C}$  in a 5%  $\text{CO}_2$  humidified atmosphere. On day 0, HT29  $\text{Gle}^{\pm}$  cells were seeded at density of  $2 \times 10^4$  cells/cm<sup>2</sup> in 96-well plates. The culture medium was changed on day 3, and each test was started on day 4. Cells were coincubated with 0.8 mM *p*-cresol (freshly

Table 1. Phenolic Composition of the Fruit Extracts

compound (%)	apple peel <sup>a</sup>	cranberry <sup>a</sup>	grape <sup>a</sup>	avocado peel <sup>a</sup>
Flavonoids				
hyperoside	22.12 ± 1.23	2.96 ± 0.06	ND	ND
isoquercitrin	6.58 ± 0.98	3.88 ± 0.13	ND	ND
quercitrin	8.23 ± 1.02	9.07 ± 0.11	ND	ND
quercetin	0.09 ± 0.01	4.02 ± 0.18	1.77 ± 0.07	1.23 ± 0.02
quercetin- <i>O</i> -pentosides (≠ of rutin) <sup>b</sup>	19.21 ± 2.66	1.03 ± 0.09	4.79 ± 0.05	ND
rutin	4.01 ± 0.12	ND	0.96 ± 0.01	1.09 ± 0.04
apigenin	ND	ND	ND	12.80 ± 0.12
kaempferol	ND	ND	1.81 ± 0.09	ND
kaempferol derivatives <sup>c</sup>	ND	1.43 ± 0.01	2.03 ± 0.05	ND
myricetin	ND	1.78 ± 0.03	ND	ND
myricetin hexosides <sup>d</sup>	ND	5.19 ± 0.09	ND	ND
isorhamnetin	ND	0.68 ± 0.36	ND	ND
isorhamnetin derivatives <sup>e</sup>	ND	ND	3.75 ± 0.05	ND
Σ flavonoids	60.24 ± 1.00	30.04 ± 0.12	15.11 ± 0.05	15.12 ± 0.06
Flavan-3-ol Monomers				
epicatechin	6.08 ± 0.98	1.02 ± 0.01	3.66 ± 0.20	12.09 ± 0.38
catechin	0.03 ± 0.01	1.44 ± 0.03	2.04 ± 0.13	1.92 ± 0.01
epicatechin gallate	ND	ND	1.74 ± 0.09	ND
catechin gallate	ND	ND	0.89 ± 0.02	ND
Σ flavan-3-ol monomers	6.11 ± 0.50	2.46 ± 0.02	8.33 ± 0.11	14.01 ± 0.20
Σ total procyanidins <sup>f</sup>	22.01 ± 2.88	32.01 ± 0.36	41.01 ± 0.36	29.08 ± 1.01
Dihydrochalcones				
phloretin-2'-glucoside	10.34 ± 3.88	0.18 ± 0.01	ND	ND
phloretin-2'-xyloglucoside	0.22 ± 0.11	ND	ND	ND
Σ dihydrochalcones	10.56 ± 2.00	0.18 ± 0.01	ND	ND
Anthocyanins				
cyanidin- <i>O</i> -hexosides <sup>g</sup>	ND	5.23 ± 0.03	1.23 ± 0.02	ND
cyanidin- <i>O</i> -arabinoside <sup>g</sup>	ND	5.01 ± 0.03	ND	ND
cyanidin-3- <i>O</i> -glucoside <sup>g</sup>	ND	ND	ND	3.00 ± 0.03
peonidin- <i>O</i> -hexosides <sup>h</sup>	ND	8.46 ± 0.01	1.01 ± 0.01	ND
peonidin- <i>O</i> -arabinoside <sup>h</sup>	ND	4.97 ± 0.06	ND	ND
malvidin- <i>O</i> -hexosides <sup>i</sup>	ND	ND	12.05 ± 0.01	ND
delphinidin- <i>O</i> -hexosides <sup>j</sup>	ND	ND	4.53 ± 0.08	ND
petunidin- <i>O</i> -hexosides <sup>k</sup>	ND	ND	3.01 ± 0.13	ND
Σ total anthocyanins	ND	23.67 ± 0.03	20.83 ± 0.03	3.00 ± 0.03
Phenolic Acids				
gallic acid	ND	ND	4.11 ± 0.01	ND
syringic acid	ND	ND	0.63 ± 0.03	ND
vanillic acid	ND	ND	3.94 ± 0.13	3.16 ± 0.01
chlorogenic acid	1.02 ± 0.05	ND	ND	1.00 ± 0.02
caffeic acid	0.06 ± 0.01	0.07 ± 0.01	1.96 ± 0.01	8.01 ± 0.01
ellagic acid	ND	1.94 ± 0.11	ND	ND
sinapic acid	ND	0.28 ± 0.09	ND	0.25 ± 0.02
ferulic acid	ND	0.09 ± 0.01	0.72 ± 0.06	6.54 ± 0.02
anisic acid	ND	ND	ND	2.28 ± 0.02
<i>p</i> -coumaric acid	ND	1.52 ± 0.18	1.16 ± 0.03	ND
cinnamic acid	ND	0.38 ± 0.02	ND	ND
5-caffeoyl quinic acid	ND	3.83 ± 0.12	ND	ND
4-caffeoyl quinic acid	ND	0.23 ± 0.01	ND	ND
protocatechuic acid	ND	0.78 ± 0.03	2.20 ± 0.02	0.98 ± 0.09
<i>p</i> -hydroxybenzoic acid	ND	0.98 ± 0.02	ND	9.92 ± 0.08
<i>m</i> -hydroxybenzoic acid	ND	ND	ND	6.65 ± 0.10
caffeoyl glucoside	ND	0.89 ± 0.01	ND	ND
feruloyl glucoside	ND	0.19 ± 0.01	ND	ND
coumaroyl glucoside	ND	0.46 ± 0.02	ND	ND
Σ phenolic acids	1.08 ± 0.03	11.64 ± 0.05	14.72 ± 0.04	38.79 ± 0.04
total	100 ± 1.28	100 ± 0.10	100 ± 0.12	100 ± 0.27

<sup>a</sup>Data are average (±SD) of triplicates determined by the HPLC method and expressed as the percentage of the total polyphenol content measured by the Folin–Ciocalteu method. <sup>b</sup>Calculated on the basis of rutin measured by RP-HPLC. <sup>c</sup>Calculated on the basis of kaempferol measured by RP-HPLC. <sup>d</sup>Calculated on the basis of myricetin measured by RP-HPLC. <sup>e</sup>Calculated on the basis of isorhamnetin measured by RP-HPLC. <sup>f</sup>Estimated



Table 1. continued

by RP-HPLC method after phloroglucinolysis. <sup>g</sup>Calculated on the basis of cyanidin measured by RP-HPLC. <sup>h</sup>Calculated on the basis of peonidin measured by RP-HPLC. <sup>i</sup>Calculated on the basis of malvidin measured by RP-HPLC. <sup>j</sup>Calculated on the basis of delphinidin measured by RP-HPLC. <sup>k</sup>Calculated on the basis of petunidin measured by RP-HPLC. ND = not detected.

Table 2. Effect of PAC-Containing Polyphenol Extracts and PAC Metabolites on Cell Integrity (LDH Leakage) and Mitochondrial Function (Cellular ATP Levels) in HT29 Glc<sup>+</sup> Cells Cultured without or with *p*-Cresol<sup>a</sup>

	concentration	LDH leakage (% of control without <i>p</i> -cresol)		ATP level (% of control without <i>p</i> -cresol)	
		without <i>p</i> -cresol	0.8 mM <i>p</i> -cresol	without <i>p</i> -cresol	0.8 mM <i>p</i> -cresol
control cells		100	128 ± 21 <sup>c</sup>	100	87 ± 13 <sup>c</sup>
cranberry (μg/mL)	12.5	94 ± 4 <sup>b</sup>	103 ± 6 <sup>d</sup>	135 ± 19 <sup>c</sup>	125 ± 13 <sup>c,e</sup>
	25	92 ± 6 <sup>c</sup>	96 ± 6 <sup>e</sup>	135 ± 14 <sup>c</sup>	127 ± 14 <sup>e</sup>
	50	91 ± 4 <sup>c</sup>	97 ± 5 <sup>e</sup>	133 ± 19 <sup>c</sup>	122 ± 11 <sup>b,e</sup>
avocado (μg/mL)	12.5	93 ± 4 <sup>c</sup>	100 ± 5 <sup>e</sup>	144 ± 15 <sup>c</sup>	125 ± 13 <sup>c,e</sup>
	25	91 ± 6 <sup>c</sup>	99 ± 5 <sup>e</sup>	144 ± 17 <sup>c</sup>	127 ± 14 <sup>c,e</sup>
	50	88 ± 4 <sup>c</sup>	96 ± 5	132 ± 14 <sup>c</sup>	122 ± 11 <sup>c,e</sup>
grape (μg/mL)	12.5	101 ± 5	174 ± 10 <sup>b,e</sup>	98 ± 7	84 ± 3 <sup>c</sup>
	25	107 ± 11	185 ± 13 <sup>c,e</sup>	103 ± 8	81 ± 5 <sup>c</sup>
	50	117 ± 8 <sup>c</sup>	225 ± 18 <sup>c,e</sup>	102 ± 7	53 ± 9 <sup>c,e</sup>
apple (μg/mL)	12.5	101 ± 10	170 ± 14 <sup>c</sup>	100 ± 10	82 ± 3 <sup>c</sup>
	25	105 ± 8	175 ± 12 <sup>c</sup>	103 ± 6	84 ± 2 <sup>c</sup>
	50	105 ± 7	176 ± 22 <sup>b</sup>	101 ± 10	82 ± 4 <sup>c</sup>
3-PPA (μM)	50	108 ± 3 <sup>c</sup>	105 ± 4	79 ± 9 <sup>c</sup>	81 ± 3 <sup>c</sup>
	200	107 ± 3 <sup>c</sup>	105 ± 9	95 ± 12	92 ± 9
3,4-DHPPA (μM)	30	112 ± 4 <sup>c</sup>	104 ± 6	81 ± 7 <sup>c</sup>	92 ± 6
	100	108 ± 3 <sup>c</sup>	108 ± 3	79 ± 5 <sup>c</sup>	92 ± 12
4-HPPA (μM)	10	107 ± 4 <sup>b</sup>	107 ± 3	77 ± 13 <sup>c</sup>	95 ± 8 <sup>b</sup>
	50	107 ± 4 <sup>b</sup>	108 ± 6	81 ± 7 <sup>c</sup>	89 ± 7 <sup>b</sup>

<sup>a</sup>Values represent the mean ± SD of 3 independent experiments. <sup>b</sup>*p* < 0.05 compared with untreated control cells. <sup>c</sup>*p* < 0.01 compared with untreated control cells. <sup>d</sup>*p* < 0.05 compared with cells treated only with 0.8 mM *p*-cresol. <sup>e</sup>*p* < 0.01 compared with cells treated only with 0.8 mM *p*-cresol.

dissolved in culture medium) and the PAC-containing polyphenol extracts (12.5, 25, and 50 μg/mL, i.e., in concentrations achievable in the gut lumen after the intake of a standard diet) or the PAC bacterial metabolites 3-PPA (50 and 200 μM), 3,4-DHPPA (30 and 100 μM), 4-HPPA (10 and 50 μM), i.e., in concentrations similar to those reported by Jenner et al.<sup>8</sup> in the fecal water of human subjects. The culture medium was changed daily from days 4 to 7. Caco-2 cells were cultured at a density of 10<sup>5</sup> cells/cm<sup>2</sup> on Transwell polycarbonate filters (12 mm diameter; 0.4 μm pore size) (Corning-Costar, USA) previously treated with 0.044 μg/μL of collagen (Thermo) in 0.01 M HCl. The basolateral and apical compartments of the transwell filters were filled with 1.5 and 0.5 mL of culture medium, respectively. The culture medium was changed three times per week; the cell monolayers were used when their transepithelial electrical resistance was stabilized, usually 10–15 days after reaching confluence.

**Cell Viability.** The cell viability of HT29 Glc<sup>+</sup> cells was measured using the CytoTox-One kit. Cells were incubated with the polyphenol extracts or PAC metabolites in the presence or absence of 0.8 mM *p*-cresol until day 7. LDH released into the culture medium (in relative fluorescence units, RFU) was determined with a multimode microplate reader (Synergy HT, Biotek) (λ<sub>ex/em</sub> = 560/590 nm). Results were expressed as percentage of LDH leakage in comparison to that of the untreated control cells.

**Intracellular ATP Contents.** The intracellular concentrations of ATP of HT29 Glc<sup>+</sup> cells were measured using a CellTiter-Glo kit. Cells were incubated with the polyphenol extracts or PAC metabolites in the presence or absence of 0.8 mM *p*-cresol until day 7. ATP levels were assayed according to the manufacturer's instructions, and luminescence was expressed as relative luminescence units (RLU) measured with the multimode microplate reader. Results were expressed as the percentage of ATP levels relative to that of the untreated control cells.

**Mitochondrial Membrane Potential.** The mitochondrial membrane potential (MMP) was quantified using Rhodamine-123 (R-123), which diffuses easily across membranes due to its lipophilic nature, and accumulates in the negatively charged mitochondrial matrix. HT29 Glc<sup>+</sup> cells were incubated with the polyphenol extracts or PAC metabolites in the presence or absence of 0.8 mM *p*-cresol until day 7. For the experiment, cells were exposed to 1 μg/mL of R-123 for 30 min to allow its loading within the cells; these were subsequently washed twice with PBS to remove the R-123 excess and lysed with 2% Triton-X100. Finally, MMP was determined with a multimode microplate reader (λ<sub>ex/em</sub> = 500/530 nm). Results were expressed as percentage of MMP relative to that of the control untreated cells.

**Intestinal Barrier Function.** Changes in the intestinal barrier function were determined only in Caco-2 cells as HT29 Glc<sup>+</sup> cells develop a low transepithelial electrical resistance (TEER). TEER, as a reflection of the integrity of the epithelial paracellular pathway, was measured in the Caco-2 cell monolayers grown in a Transwell filter with an ohm/voltmeter (EVOM, WPI, USA) as previously described.<sup>35</sup> Cell monolayers were used after confluence when their TEER was stabilized. Measurements were carried out at 24 h of incubation with the different concentrations of the polyphenol extracts or PAC metabolites and 3.2 mM *p*-cresol. Results were expressed as percentage of the TEER value obtained at time 0.

Changes in the barrier function of the Caco-2 cell monolayers were also determined by measuring the paracellular passage of fluorescein isothiocyanate-conjugated dextran (FD-4) from the apical to the serosal side of the monolayer. FD-4 (4.4 kD MW, 0.8 mg/mL dissolved in culture medium) was added to the apical compartment in the absence or presence of the polyphenol extracts or PAC metabolites at different concentrations with or without the addition of 3.2 mM *p*-cresol. The amount of FD-4 transferred to the basolateral side after 24 h exposure was determined in the multimode microplate reader (λ<sub>ex/em</sub>

269 = 490/520 nm). Results were expressed as percentage of the basal  
270 value.

271 **Statistical Analysis.** Data were analyzed using GraphPad Prism 6  
272 statistical software. Values represent the means of three independent  
273 experiments each conducted in triplicate. Data distribution of the  
274 variables was first analyzed by the Shapiro–Wilk test and parametric  
275 (ANOVA) or nonparametric analysis of variance (Kruskal–Wallis)  
276 was used according to the results of the test. When the result of the  
277 analysis of variances was significant ( $p < 0.05$ ), a posthoc Tukey test  
278 or a Dunn's test was carried out.

## 279 ■ RESULTS

280 The polyphenolic composition of the fruit polyphenol extracts  
281 is described in Table 1. The grape extract had the highest PAC  
282 content (41.0%), followed by that from cranberry, avocado, and  
283 apple (32.0, 29.1, and 22.0%, respectively). The mean degree of  
284 polymerization of the grape PACs was also higher (9.8) than  
285 that of cranberry (7.3), avocado (6.1), and apple (3.2). In  
286 addition, other types of polyphenol were also present in the  
287 extracts: the apple extract was characterized by its high content  
288 of flavonoids and dihydrochalcones (60.2 and 10.6%,  
289 respectively) and the cranberry extract by its high content of  
290 anthocyanins (23.7%), and the avocado extract was rich in  
291 monomeric flavan-3-ols and phenolic acids (14 and 38.8%,  
292 respectively).

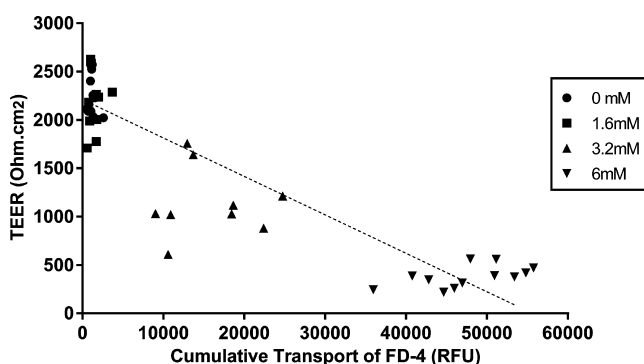
293 The effects of the polyphenol extracts and their bacterial  
294 metabolites on cell viability and mitochondrial function was  
295 first evaluated in HT29 Glc<sup>+</sup> cells. As shown in Table 2, extracts  
296 from cranberries, avocados, and apples did not increase LDH  
297 leakage used as a marker of membrane integrity and cell  
298 viability; on the contrary, they slightly decreased the basal  
299 release of LDH. Although this parameter was not affected by  
300 the grape extract at the lowest concentrations, it was  
301 significantly increased at the higher concentration (50  $\mu$ g/  
302 mL) ( $p < 0.01$ ). When the impact on the mitochondrial  
303 function of the polyphenol extracts was evaluated by measuring  
304 intracellular ATP contents, a significant increase was observed  
305 with cranberry and avocado extracts ( $p < 0.01$ ), whereas no  
306 change was observed with grape and apple extracts. At all of the  
307 concentrations tested, PAC metabolites induced a small  
308 (between 7 and 12%) but significant increase of LDH release  
309 by HT29 Glc<sup>+</sup> cells and a decrease (between 16 and 21%) of  
310 their cellular ATP content; however, this latter parameter was  
311 not affected by the addition of 3-PPA at 200  $\mu$ M.

312 Table 2 also shows that exposure of HT29 Glc<sup>+</sup> cells to *p*-  
313 cresol significantly increased LDH release ( $p < 0.01$ ) while at  
314 the same time it decreased their ATP content ( $p < 0.01$ ). The  
315 deleterious effect of *p*-cresol on LDH leakage was prevented by  
316 polyphenol extracts from cranberries and avocados. However,  
317 no protective effects were observed with the extracts from  
318 apples or grapes; on the contrary, they increase even more the  
319 *p*-cresol-induced LDH release; in the case of grape PACs, this  
320 deleterious effect was concentration-dependent. PACs from  
321 cranberries and avocados not only prevented the decrease of  
322 ATP content induced by *p*-cresol but also significantly  
323 increased ATP levels compared with cells not treated with *p*-  
324 cresol. Polyphenol extracts from apples and grapes did not  
325 exhibit any protective effect on the ATP content of the cells  
326 treated with *p*-cresol. In addition, the cellular ATP content  
327 decreased concentration-dependently with PACs from grapes,  
328 such that at the higher concentration of this extract the ATP  
329 values were lower compared with those of the control cells  
330 treated with *p*-cresol. The bacterial metabolites of PACs  
331 prevented the increase of LDH release induced by *p*-cresol.

However, these compounds did not exhibit effects on the  
cellular ATP content of the *p*-cresol-treated cells.

Changes in the mitochondrial function of HT29 Glc<sup>+</sup> cells  
were also evaluated through the determination of MMP; the  
results indicate that neither *p*-cresol nor the polyphenol extracts  
and PAC metabolites affect this parameter (data not shown).

For determining their effect on gut barrier function, extracts  
were placed on the apical side of Caco-2 cell monolayers and  
incubated for 24 h in the presence or absence of *p*-cresol. TEER  
and FD-4 transport across the epithelial monolayer were  
regularly measured. None of the polyphenol extracts affected  
the TEER and FD-4 transport across Caco-2 cell monolayers  
after 24 h of incubation regardless of the concentration tested  
(data not shown). As shown in Figure 1, cell exposure to



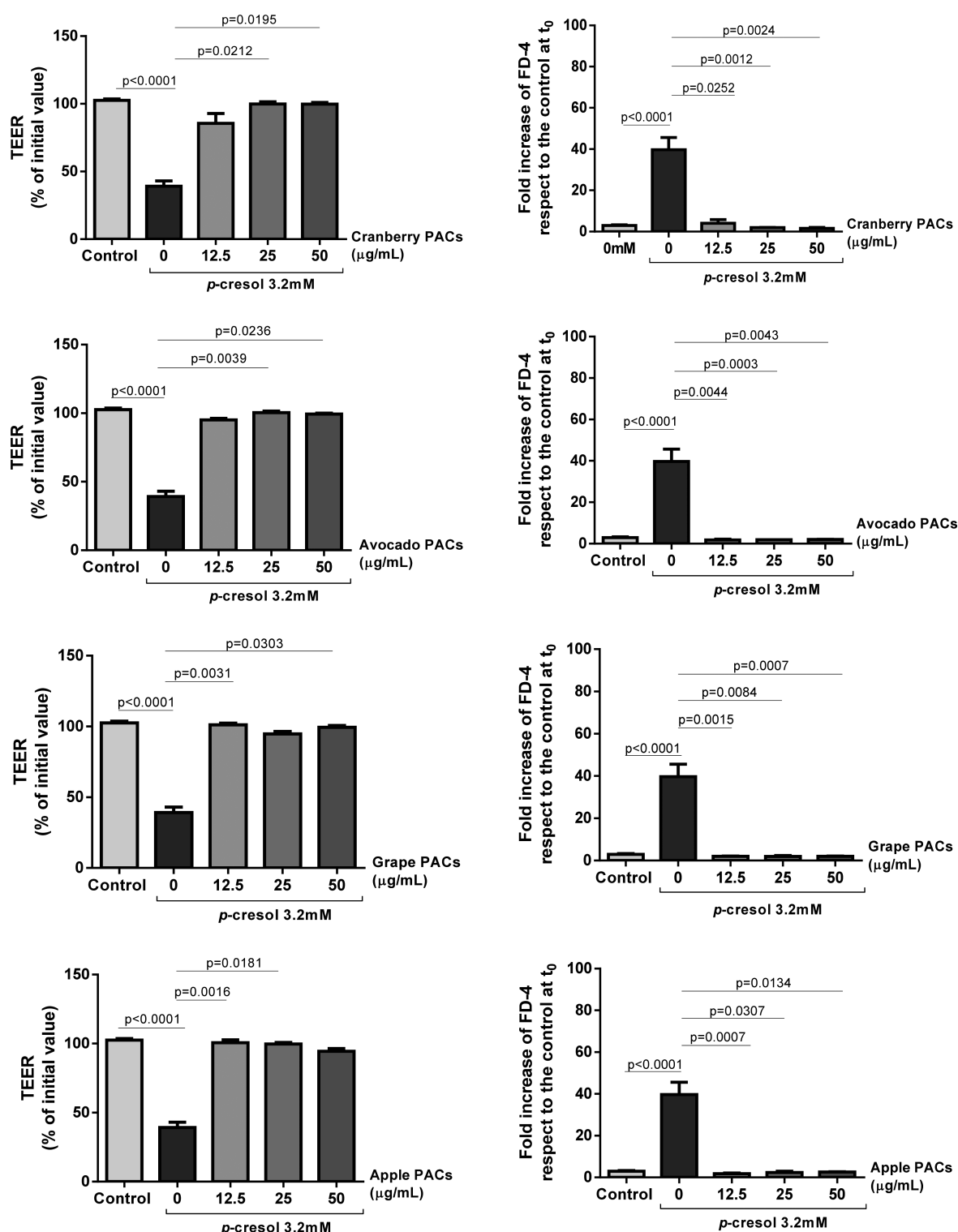
**Figure 1.** Correlation between transepithelial electrical resistance (TEER) and paracellular permeability to FD-4 in Caco-2 cell monolayers exposed for 24 h to different concentrations of *p*-cresol (Pearson  $r = -0.9018$ ;  $p < 0.0001$ ).

different *p*-cresol concentrations significantly decreased TEER  
values while correlatively increasing FD-4 transport ( $r = -0.90$ ;  
 $p < 0.0001$ ). The subsequent experiments were carried out  
incubating Caco-2 cell monolayers with 3.2 mM *p*-cresol for 24  
h. Under these conditions, TEER decreased 2.6-fold while FD-  
4 transport was increased 40-fold compared with that of  
untreated control monolayers at time 0. The protective effects  
of polyphenol extracts are shown in Figure 2. Results indicate  
that the extracts fully prevented the decrease of TEER induced  
by *p*-cresol, usually at concentrations of 25 and 50  $\mu$ g/mL. In  
addition, PACs also protected cell monolayers against the  
increase of FD-4 transport at all of the concentrations tested.  
Results showing the protective effects of bacterial PAC  
metabolites are depicted in Figure 3. They indicate that all of  
the metabolites fully prevented the increased FD-4 transport  
induced by *p*-cresol after a 24 h incubation. *p*-Cresol-induced  
TEER decrease was prevented by 3,4-DHPPA at 100  $\mu$ M, 3-  
PPA at 50 and 200  $\mu$ M, and 4-HPPA at 10  $\mu$ M.

## ■ DISCUSSION

*p*-Cresol is an aromatic compound produced by the  
fermentation of tyrosine by anaerobic and aerobic bacteria  
from the human gut microbiota.<sup>18,19</sup> The concentration of free  
(unbound) *p*-cresol in the colonic lumen, although difficult to  
quantitate with exactitude, is more elevated in subjects  
consuming high-protein diets.<sup>27</sup> High intracolonic and plasma  
concentrations of *p*-cresol are considered risk factors for several  
pathological conditions. In fact, *p*-cresol is recognized as a  
uremic toxin contributing to the development of renal and  
cardiovascular complications in patients with chronic renal



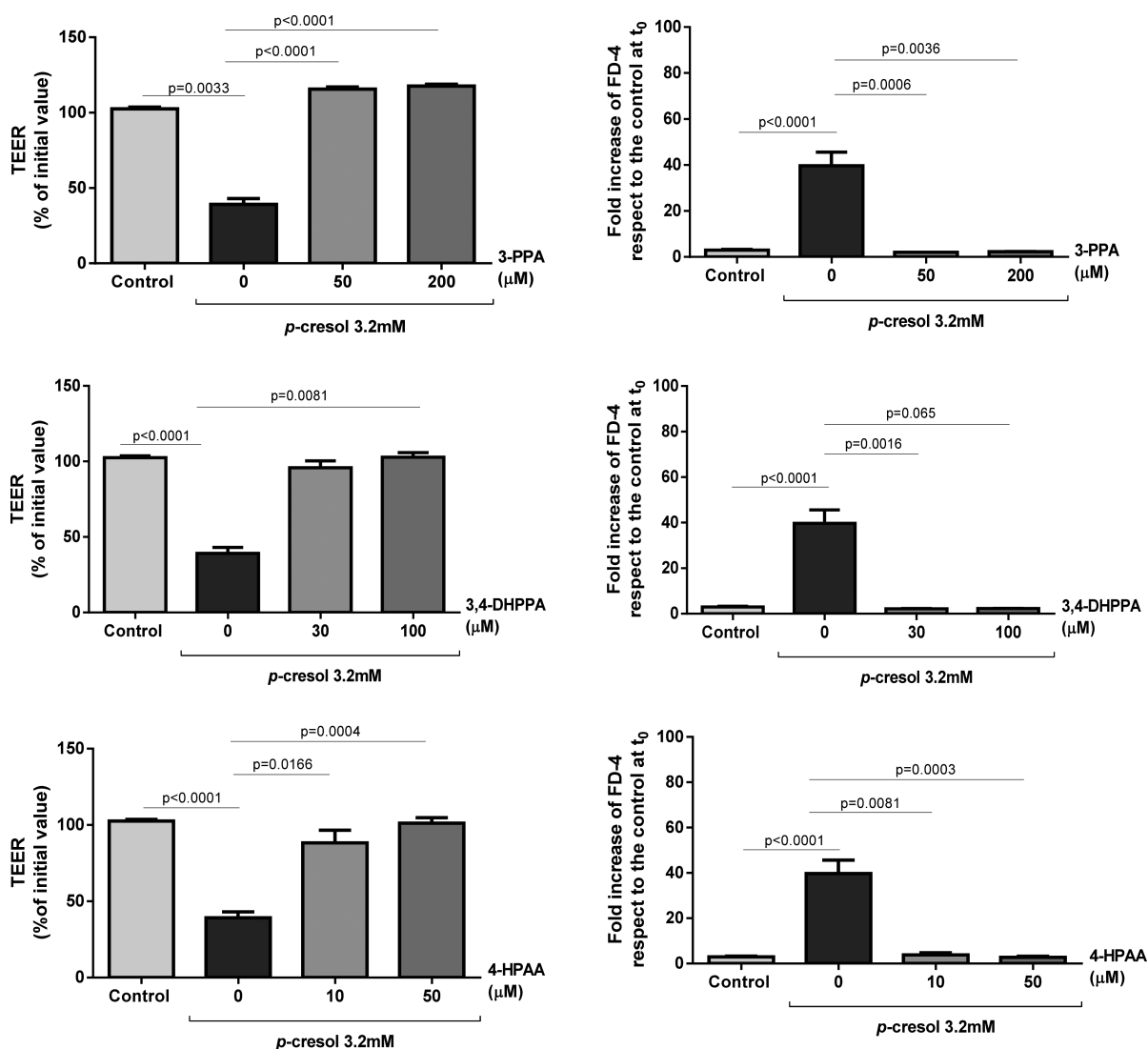


**Figure 2.** Protective effect of the different polyphenol extracts against the alterations of transepithelial electrical resistance (TEER) (left column) and paracellular permeability to FD-4 (right column) in Caco-2 cell monolayers exposed to 3.2 mM  $p$ -cresol for 24 h (mean  $\pm$  SEM).

diseases.<sup>24–26</sup> In addition, excess  $p$ -cresol has also been reported to be hepatotoxic because it induces mitochondrial dysfunction<sup>36</sup> and has been found to be associated with autism in young patients.<sup>37</sup> We recently reported that, in the colon,  $p$ -cresol affects cell proliferation and increases DNA damage in human colonic cells in *in vitro* experiments.<sup>23</sup> In the present study, we evaluated whether PAC-containing polyphenol extracts from different fruits as well as PAC bacterial

metabolites protect human colonic epithelial cell lines against the deleterious effects induced by  $p$ -cresol.

Our results showed that the exposure of HT29 cells to  $p$ -cresol resulted in the alteration of their cell membrane integrity (evaluated through the LDH assay) and decreases of ATP synthesis. This latter event suggests mitochondrial dysfunction; however, no changes in MMP were detected under our experimental conditions. This confirms the results



**Figure 3.** Protective effect of bacterial PAC metabolites against the alterations of transepithelial electrical resistance (TEER) (left column) and paracellular permeability to FD-4 (right column) in Caco-2 cell monolayers exposed to 3.2 mM  $p$ -cresol for 24 h (mean  $\pm$  SEM).

from our previous study indicating that the decreased ATP synthesis was probably, at least in part, the underlying mechanism explaining the inhibitory effect of  $p$ -cresol on cellular proliferation.<sup>23</sup> In addition, our current results also indicate that the barrier function of Caco-2 cell monolayers was strongly altered after a 24 h incubation with  $p$ -cresol as reflected by the decrease of TEER and the correlative increase of the paracellular permeability of FD-4. In humans as well as in laboratory animals, such alterations of the gut barrier function are generally considered as an initial step previous to the development of local and systemic pathological events. For example, increased gut permeability is associated with the formation of gastrointestinal ulcers in rats treated with indomethacin, a nonsteroidal anti-inflammatory drug that induces mitochondrial dysfunction in intestinal epithelial cells<sup>38,39</sup> and in animal models of colorectal cancer. Increased gut permeability is accompanied by higher plasma endotoxin concentrations, mesenteric lymph node hypertrophy, and the progression of cachexia.<sup>40</sup>

After further characterization of the deleterious impact of  $p$ -cresol on the cultured intestinal cells, we evaluated whether this might be prevented by our characterized PAC-containing

polyphenol extracts. Relative to the changes in cell membrane integrity and ATP contents of HT29 cells, our results were relatively variable according to the fruit source of the extracts. Those from cranberries and avocados did not affect LDH release and increased the intracellular ATP content, whereas the extracts from apples and grapes had no effect on the cellular ATP content but tended to increment LDH release. Such differences between extracts were also observed when they were incubated with the cells in the presence of  $p$ -cresol. Avocado and cranberry extracts prevented the LDH release induced by  $p$ -cresol whereas the apple and grapes extracts magnified it. Similar results were observed on the cellular ATP content. The reason for these differences in the activity of the extracts remains unclear. However, it is interesting to note that apples and grapes mainly contain type-B PACs whereas avocados and cranberries mostly contain type-A PACs, whose constituent monomers are also linked through an additional ether bond between C2 and C7.<sup>1-3</sup> It has been shown that this chemical structure confers specific biological activities to type-A PACs that are not observed with the type-B PACs. This is the case, for example, of the potent antiadhesive activity exhibited by cranberry PACs against pathogens.<sup>41</sup> Therefore, it is possible

that this molecular feature may explain the better protective effect of the cranberry and avocado extracts on the membrane integrity and ATP content of *p*-cresol-exposed cells. It must also be stated that cytotoxicity induced by grape PACs has already been reported by Shao et al., who observed an increase of LDH release in cardiomyocytes exposed to grape seed PACs accompanied by increased free radical production, higher levels of nitric oxide, and intracellular glutathione depletion.<sup>42,43</sup> However, we did not find any data related to the differential toxicity of type-A and type-B PACs.

No differences between extracts were detected in their ability to prevent the decrease of TEER and the increased FD-4 transport induced by *p*-cresol in Caco-2 monolayers; the disturbance of the barrier function observed after 24 h of incubation with *p*-cresol was totally prevented by all of the extracts. These results confirm our previous studies and those of other authors reporting that gut barrier alterations in animals and intestinal cell lines are prevented by apple peel polyphenol extract, cocoa PACs, grape seed PACs, and epigallocatechin-gallate.<sup>35,38,44–48</sup> Such a protective effect could be related to the ability of some PACs to bind lipid rafts in the apical membrane of intestinal cells, a phenomenon associated with the regulation of some cell signaling cascade events.<sup>46</sup>

Finally, we also evaluated in *p*-cresol-treated cells the protective effect of three metabolites, 4-HPAA, 3,4-DHPPA, and 3-PPA, originating from PAC degradation by the microbiota. It is worth noting that one of these (4-HPPA) may also be generated by the microbiota through protein fermentation.<sup>49</sup> Bacterial metabolites of polyphenols, and more specifically proanthocyanidins, may be detected in urine of human subjects after the intake of polyphenol-rich foodstuffs, suggesting that these compounds are absorbed in the colon and that they could exert biological activities in colonocytes as well as in a number of other tissues and organs.<sup>50,51</sup> Various studies have shown that these metabolites may be found in high concentrations in the colonic lumen,<sup>8</sup> confirming that they are poorly absorbed in the small intestine. Accordingly, they could directly act on epithelial cells, modulating signaling events and contributing to the homeostasis of the colonic mucosa. We observed that, when incubated with HT29 *Gle+* cells in the absence of *p*-cresol, these compounds slightly increased LDH release and decreased cellular ATP content. Such effects suggest that these metabolites might eventually induce cell toxicity. This could be due to the fact that HT-29 *Gle+* cells, being derived from a human colonic adenocarcinoma, may be more sensitive to these compounds compared with non-transformed cells.<sup>52</sup> In the presence of *p*-cresol, the bacterial PAC metabolites prevented the increase of LDH release by HT29 *Gle+* cells without affecting the cellular ATP content, and when incubated with Caco-2 cell monolayers, they totally prevented the alterations of barrier function induced by *p*-cresol. Although studies exploring the biological activities of PAC metabolites are scarce, it has been shown that 3,4-DHPPA upregulates the expression of glutathione S-transferase T2 (GSTT2) and downregulates that of cyclooxygenase-2 (COX-2) in LT97 colonic cells.<sup>53</sup> COX-2 inhibition in HT29 *Gle+* cells has also been reported after incubation with human fecal water with high levels of phenolic compounds.<sup>52</sup> The inhibition of cyclooxygenase-2 may explain the protective effect against gut barrier alterations we observed in Caco-2 cell monolayers, as previously described by Gentile et al.<sup>54</sup>

In conclusion, our present study indicates that exposure of colonic epithelial cells to high and therefore abnormal *p*-cresol

concentrations affects cell integrity, the intestinal barrier, and mitochondrial function. Our data indicate that PAC-containing polyphenol extracts and PAC bacterial metabolites prevent to a considerable extent some of these alterations with those from cranberries and avocados (characterized by the presence of type-A PACs) apparently being more effective. These *in vitro* results need further assessment in *in vivo* conditions to explain the presumed beneficial effect of diets rich in fruits and vegetables against colorectal cancer in humans.

## AUTHOR INFORMATION

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### Notes

The authors declare no competing financial interest.

## REFERENCES

- (1) Rodrigo, R.; Libuy, M.; Feliu, F.; Hasson, D. Polyphenols in disease: from diet to supplements. *Curr. Pharm. Biotechnol.* **2014**, *15*, 304–317.
- (2) Xu, Z.; Du, P.; Meiser, P.; Jacob, C. Proanthocyanidins: oligomeric structures with unique biochemical properties and great therapeutic promise. *Nat. Prod. Commun.* **2012**, *7*, 381–388.
- (3) Gu, L.; Kelm, M. A.; Hammerstone, J. F.; Beecher, G.; Holden, J.; Haytowitz, D.; Gebhardt, S.; Prior, R. L. Concentrations of proanthocyanidins in common foods and estimations of normal consumption. *J. Nutr.* **2004**, *134*, 613–617.
- (4) Deprez, S.; Mila, I.; Huneau, J. F.; Tomé, D.; Scalbert, A. Transport of proanthocyanidin dimer, trimer, and polymer across monolayers of human intestinal epithelial Caco-2 cells. *Antioxid. Redox Signaling* **2001**, *3*, 957–967.
- (5) Dueñas, M.; Muñoz-González, I.; Cueva, C.; Jiménez-Girón, A.; Sánchez-Patán, F.; Santos-Buelga, C.; Moreno-Arribas, M. V.; Bartolomé, B. A survey of modulation of gut microbiota by dietary polyphenols. *BioMed Res. Int.* **2015**, *2015*, 850902.
- (6) Appeldoorn, M. M.; Vincken, J. P.; Aura, A. M.; Hollman, P. C.; Gruppen, H. Procyanidin dimers are metabolized by human microbiota with 2-(3,4-dihydroxyphenyl)acetic acid and 5-(3,4-dihydroxyphenyl)-gamma-valerolactone as the major metabolites. *J. Agric. Food Chem.* **2009**, *57*, 1084–1092.
- (7) Marin, L.; Miguelez, E.; Villar, C.; Lombo, F. Bioavailability of Dietary Polyphenols and Gut Microbiota Metabolism: Antimicrobial Properties. *BioMed Res. Int.* **2015**, *2015*, 1.
- (8) Jenner, A. M.; Rafter, J.; Halliwell, B. Human fecal water content of phenolics: the extent of colonic exposure to aromatic compounds. *Free Radical Biol. Med.* **2005**, *38*, 763–772.
- (9) Etxeberria, U.; Fernández-Quintela, A.; Milagro, F. I.; Aguirre, L.; Martínez, J. A.; Portillo, M. P. Impact of polyphenols and polyphenol-rich dietary sources on gut microbiota composition. *J. Agric. Food Chem.* **2013**, *61*, 9517–9533.
- (10) Massot-Cladera, M.; Pérez-Berezo, T.; Franch, A.; Castell, M.; Pérez-Cano, F. J. Cocoa modulatory effect on rat faecal microbiota and colonic crosstalk. *Arch. Biochem. Biophys.* **2012**, *527*, 105–112.
- (11) Jin, J. S.; Touyama, M.; Hisada, T.; Benno, Y. Effects of green tea consumption on human fecal microbiota with special reference to *Bifidobacterium* species. *Microbiol. Immunol.* **2012**, *56*, 729–739.
- (12) Shinohara, K.; Ohashi, Y.; Kawasumi, K.; Terada, A.; Fujisawa, T. Effect of apple intake on fecal microbiota and metabolites in humans. *Anaerobe* **2010**, *16*, 510–515.
- (13) Sánchez-Patán, F.; Cueva, C.; Monagas, M.; Walton, G. E.; Gibson, G. R.; Quintanilla-López, J. E.; Lebrón-Aguilar, R.; Martín-Álvarez, P. J.; Moreno-Arribas, M. V.; Bartolomé, B. In vitro

- 562 fermentation of a red wine extract by human gut microbiota: changes  
563 in microbial groups and formation of phenolic metabolites. *J. Agric.*  
564 *Food Chem.* **2012**, *60*, 2136–2147.
- 565 (14) Loke, W. M.; Jenner, A. M.; Proudfoot, J. M.; McKinley, A. J.;  
566 Hodgson, J. M.; Halliwell, B.; Croft, K. D. A metabolite profiling  
567 approach to identify biomarkers of flavonoid intake in humans. *J. Nutr.*  
568 **2009**, *139*, 2309–2314.
- 569 (15) Najmanova, I.; Pourova, J.; Voprsalova, M.; Pilarova, V.;  
570 Semecky, V.; Novakova, L.; Mladenka, P. The flavonoid metabolite 3-  
571 (3-hydroxyphenyl) propionic acid formed by human microflora  
572 decreases arterial blood pressure in rat. *Mol. Nutr. Food Res.* **2016**,  
573 n/a.
- 574 (16) Waldecker, M.; Kautenburger, T.; Daumann, H.; Busch, C.;  
575 Schrenk, D. Inhibition of histone-deacetylase activity by short chain  
576 fatty acids and some polyphenol metabolites formed in the colon. *J.*  
577 *Nutr. Biochem.* **2008**, *19*, 587–593.
- 578 (17) Karlsson, P. C.; Huss, U.; Jenner, A.; Halliwell, B.; Bohlin, L.;  
579 Rafter, J. J. Human fecal water inhibits COX-2 in colonic HT-29 cells:  
580 role of phenolic compounds. *J. Nutr.* **2005**, *135*, 2343–2349.
- 581 (18) Davila, A. M.; Blachier, F.; Gotteland, M.; Andriamihaja, M.;  
582 Benetti, P. H.; Sanz, Y.; Tomé, D. Intestinal luminal nitrogen  
583 metabolism: role of the gut microbiota and consequences for the host.  
584 *Pharmacol. Res.* **2013**, *68*, 95–107.
- 585 (19) Smith, E. A.; Macfarlane, G. T. Formation of phenolic and  
586 indolic compounds by anaerobic bacteria in the human large intestine.  
587 *Microb. Ecol.* **1997**, *33*, 180–188.
- 588 (20) Macfarlane, G. T.; Macfarlane, S. Human colonic microbiota:  
589 ecology, physiology and metabolic potential of intestinal bacteria.  
590 *Scand. J. Gastroenterol.* **1997**, *222*, 3–9.
- 591 (21) Schmidt, E. G. Urinary phenols; the simultaneous determination  
592 of phenol and *p*-cresol in urine. *J. Biol. Chem.* **1949**, *179*, 211–215.
- 593 (22) Cheng, Y.; Xie, G.; Chen, T.; Qiu, Y.; Zou, X.; Zheng, M.; Tan,  
594 B.; Feng, B.; Dong, T.; He, P.; Zhao, L.; Zhao, A.; Xu, L. X.; Zhang, Y.;  
595 Jia, W. Distinct urinary metabolic profile of human colorectal cancer. *J.*  
596 *Proteome Res.* **2012**, *11*, 1354–1363.
- 597 (23) Andriamihaja, M.; Lan, A.; Beaumont, M.; Audebert, M.; Wong,  
598 X.; Yamada, K.; Yin, Y.; Tomé, D.; Carrasco-Pozo, C.; Gotteland, M.;  
599 Kong, X.; Blachier, F. The deleterious metabolic and genotoxic effects  
600 of the bacterial metabolite *p*-cresol on colonic epithelial cells. *Free*  
601 *Radical Biol. Med.* **2015**, *85*, 219–227.
- 602 (24) Vanholder, R.; Schepers, E.; Pletinck, A.; Nagler, E. V.; Glorieux,  
603 G. The uremic toxicity of indoxyl sulfate and *p*-cresyl sulfate: a  
604 systematic review. *J. Am. Soc. Nephrol.* **2014**, *25*, 1897–1907.
- 605 (25) Poesen, R.; Viaene, L.; Verbeke, K.; Augustijns, P.; Bammens,  
606 B.; Claes, K.; Kuypers, D.; Evenepoel, P.; Meijers, B. Cardiovascular  
607 disease relates to intestinal uptake of *p*-cresol in patients with chronic  
608 kidney disease. *BMC Nephrol.* **2014**, *15*, 87.
- 609 (26) Brocca, A.; Virzi, G. M.; de Cal, M.; Cantaluppi, V.; Ronco, C.  
610 Cytotoxic effects of *p*-cresol in renal epithelial tubular cells. *Blood Purif.*  
611 **2013**, *36*, 219–225.
- 612 (27) Geypens, B.; Claus, D.; Evenepoel, P.; Hiele, M.; Maes, B.;  
613 Peeters, M.; Rutgeerts, P.; Ghoo, Y. Influence of dietary protein  
614 supplements on the formation of bacterial metabolites in the colon.  
615 *Gut* **1997**, *41*, 70–76.
- 616 (28) Birkett, A.; Muir, J.; Phillips, J.; Jones, G.; O'Dea, K. Resistant  
617 starch lowers fecal concentrations of ammonia and phenols in humans.  
618 *Am. J. Clin. Nutr.* **1996**, *63*, 766–772.
- 619 (29) Miyazaki, K.; Masuoka, N.; Kano, M.; Iizuka, R. *Bifidobacterium*  
620 fermented milk and galacto-oligosaccharides lead to improved skin  
621 health by decreasing phenols production by gut microbiota. *Benefic.*  
622 *Microbes* **2014**, *5*, 121–128.
- 623 (30) Unno, T.; Sakuma, M.; Mitsuhashi, S. Effect of dietary  
624 supplementation of (–)-epigallocatechin gallate on gut microbiota  
625 and biomarkers of colonic fermentation in rats. *J. Nutr. Sci. Vitaminol.*  
626 **2014**, *60*, 213–219.
- 627 (31) Pastene, E.; Speisky, H.; Troncoso, M.; Alarcón, J.; Figueroa, G.  
628 In vitro inhibitory effect of apple peel extract on the growth of  
629 *Helicobacter pylori* and respiratory burst induced on human  
630 neutrophils. *J. Agric. Food Chem.* **2009**, *57*, 7743–7749.
- (32) Pastene, E.; Troncoso, M.; Figueroa, G.; Alarcón, J.; Speisky, H. 631  
Association between polymerization degree of apple peel polyphenols 632  
and inhibition of *Helicobacter pylori* urease. *J. Agric. Food Chem.* **2009**, 633  
*57*, 416–424. 634
- (33) Köhler, N.; Winterhalter, P. Large-scale isolation of flavan-3-ol 635  
phloroglucinol adducts by high-speed counter-current chromatography. 636  
*J. Chromatogr. A* **2005**, *1072*, 217–222. 637
- (34) Pastene, E.; Parada, V.; Avello, M.; Ruiz, A.; García, A. Catechin- 638  
based procyanidins from *Peumus boldus* Mol. aqueous extract inhibit 639  
*Helicobacter pylori* urease and adherence to gastric adenocarcinoma 640  
gastric cells. *Phytother. Res.* **2014**, *28*, 1637–1645. 641
- (35) Carrasco-Pozo, C.; Morales, P.; Gotteland, M. Polyphenols 642  
protect the epithelial barrier function of Caco-2 cells exposed to 643  
indomethacin through the modulation of occludin and zonula 644  
occludens-1 expression. *J. Agric. Food Chem.* **2013**, *61*, S291–S297. 645
- (36) Kitagawa, A. Effects of cresols (*o*-, *m*-, and *p*-isomers) on the 646  
bioenergetic system in isolated rat liver mitochondria. *Drug Chem.* 647  
*Toxicol.* **2001**, *24*, 39–47. 648
- (37) Gabriele, S.; Sacco, R.; Cerullo, S.; Neri, C.; Urbani, A.; Tripi, 649  
G.; Malvy, J.; Barthelemy, C.; Bonnet-Brihault, F.; Persico, A. M. 650  
Urinary *p*-cresol is elevated in young French children with autism 651  
spectrum disorder: a replication study. *Biomarkers* **2014**, *19*, 463–470. 652
- (38) Carrasco-Pozo, C.; Speisky, H.; Brunser, O.; Pastene, E.; 653  
Gotteland, M. Apple peel polyphenols protect against gastrointestinal 654  
mucosa alterations induced by indomethacin in rats. *J. Agric. Food* 655  
*Chem.* **2011**, *59*, 6459–6466. 656
- (39) Carrasco-Pozo, C.; Gotteland, M.; Speisky, H. Apple peel 657  
polyphenol extract protects against indomethacin-induced damage in 658  
Caco-2 cells by preventing mitochondrial complex I inhibition. *J. Agric.* 659  
*Food Chem.* **2011**, *59*, 11501–11508. 660
- (40) Puppa, M. J.; White, J. P.; Sato, S.; Cairns, M.; Baynes, J. W.; 661  
Carson, J. A. Gut barrier dysfunction in the Apc(Min/+) mouse model 662  
of colon cancer cachexia. *Biochim. Biophys. Acta, Mol. Basis Dis.* **2011**, 663  
*1812*, 1601–1606. 664
- (41) Serrano, J.; Puupponen-Pimiä, R.; Dauer, A.; Aura, A. M.; Saura- 665  
Calixto, F. Tannins: current knowledge of food sources, intake, 666  
bioavailability and biological effects. *Mol. Nutr. Food Res.* **2009**, *53* 667  
(Suppl 2), S310–S329. 668
- (42) Shao, Z. H.; Vanden Hoek, T. L.; Xie, J.; Wojcik, K.; Chan, K. 669  
C.; Li, C. Q.; Hamann, K.; Qin, Y.; Schumacker, P. T.; Becker, L. B.; 670  
Yuan, C. S. Grape seed proanthocyanidins induce pro-oxidant toxicity 671  
in cardiomyocytes. *Cardiovasc. Toxicol.* **2003**, *3*, 331–339. 672
- (43) Shao, Z. H.; Hsu, C. W.; Chang, W. T.; Waypa, G. B.; Li, J.; Li, 673  
D.; Li, C. Q.; Anderson, T.; Qin, Y.; Schumacker, P. T.; Becker, L. B.; 674  
Hoek, T. L. Cytotoxicity induced by grape seed proanthocyanidins: 675  
role of nitric oxide. *Cell Biol. Toxicol.* **2006**, *22*, 149–158. 676
- (44) Pierre, J. F.; Heneghan, A. F.; Feliciano, R. P.; 677  
Shanmuganayagam, D.; Roenneburg, D. A.; Krueger, C. G.; Reed, J. 678  
D.; Kudsk, K. A. Cranberry proanthocyanidins improve the gut 679  
mucous layer morphology and function in mice receiving elemental 680  
enteral nutrition. *JPEN, J. Parenter. Enteral Nutr.* **2013**, *37*, 401–409. 681
- (45) Gentile, C.; Perrone, A.; Attanzio, A.; Tesoriere, L.; Livrea, M. 682  
A. Sicilian pistachio (*Pistacia vera* L.) nut inhibits expression and 683  
release of inflammatory mediators and reverses the increase of 684  
paracellular permeability in IL-1 $\beta$ -exposed human intestinal epithelial 685  
cells. *Eur. J. Nutr.* **2015**, *54*, 811–821. 686
- (46) Verstraeten, S. V.; Jagers, G. K.; Fraga, C. G.; Oteiza, P. I. 687  
Procyanidins can interact with Caco-2 cell membrane lipid rafts: 688  
involvement of cholesterol. *Biochim. Biophys. Acta, Biomembr.* **2013**, 689  
*1828*, 2646–53. 690
- (47) Bitzer, Z. T.; Glisan, S.-L.; Dorenkott, M. R.; Goodrich, K. M.; 691  
Ye, L.; O'Keefe, S. F.; Lambert, J. D.; Neilson, A. P. Cocoa 692  
procyanidins with different degrees of polymerization possess distinct 693  
activities in models of colonic inflammation. *J. Nutr. Biochem.* **2015**, *26*, 694  
827–831. 695
- (48) Song, P.; Zhang, R.; Wang, X.; He, P.; Tan, L.; Ma, X. Dietary 696  
grape-seed procyanidins decreased postweaning diarrhea by modulat- 697  
ing intestinal permeability and suppressing oxidative stress in rats. *J.* 698  
*Agric. Food Chem.* **2011**, *59*, 6227–6232. 699



- (49) Russell, W. R.; Duncan, S. H.; Scobbie, L.; Duncan, G.; Cantlay, L.; Calder, A. G.; Anderson, S. E.; Flint, H. J. Major phenylpropanoid-derived metabolites in the human gut can arise from microbial fermentation of protein. *Mol. Nutr. Food Res.* **2013**, *57*, 523–535.
- (50) Henning, S. M.; Wang, P.; Abgaryan, N.; Vicinanza, R.; de Oliveira, D. M.; Zhang, Y.; Lee, R. P.; Carpenter, C. L.; Aronson, W. J.; Heber, D. Phenolic acid concentrations in plasma and urine from men consuming green or black tea and potential chemopreventive properties for colon cancer. *Mol. Nutr. Food Res.* **2013**, *57*, 483–493.
- (51) Carrasco-Pozo, C.; Gotteland, M.; Castillo, R.; Chen, C. High cholesterol induces apoptosis, mitochondrial dysfunction, oxidative stress and impairment of insulin secretion in pancreatic  $\beta$ -cells; protection by 3,4-dihydroxyphenylacetic acid, a microbiota-derived metabolite of quercetin. *Exp. Cell Res.* **2015**, *334*, 270–282.
- (52) Gao, K.; Xu, A.; Krul, C.; Venema, K.; Liu, Y.; Niu, Y.; Lu, J.; Bensoussan, L.; Seeram, N. P.; Heber, D.; Henning, S. M. Of the major phenolic acids formed during human microbial fermentation of tea, citrus, and soy flavonoid supplements, only 3,4-dihydroxyphenylacetic acid has antiproliferative activity. *J. Nutr.* **2006**, *136*, 52–57.
- (53) Miene, C.; Weise, A.; Glei, M. Impact of polyphenol metabolites produced by colonic microbiota on expression of COX-2 and GSTT2 in human colon cells (LT97). *Nutr. Cancer* **2011**, *63*, 653–662.
- (54) Karlsson, P. C.; Huss, U.; Jenner, A.; Halliwell, B.; Bohlin, L.; Rafter, J. J. Human fecal water inhibits COX-2 in colonic HT-29 cells: role of phenolic compounds. *J. Nutr.* **2005**, *135*, 2343–2349.