



HAL
open science

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

Ximena Wong, Catalina Carrasco-Pozo, Elizabeth Escobar, Paola Navarrete, Francois Blachier, Mireille Andriamihaja, Annaig Lang, Daniel Tomé, Maria Jose Cires, Edgar Pastene, et al.

► To cite this version:

Ximena Wong, Catalina Carrasco-Pozo, Elizabeth Escobar, Paola Navarrete, Francois Blachier, et al.. Deleterious Effect of p-Cresol on Human Colonic Epithelial Cells Prevented by Proanthocyanidin-Containing Polyphenol Extracts from Fruits and Proanthocyanidin Bacterial Metabolites. *Journal of Agricultural and Food Chemistry*, 2016, 64 (18), pp.3574–3583. 10.1021/acs.jafc.6b00656 . hal-01568614

HAL Id: hal-01568614

<https://agroparistech.hal.science/hal-01568614>

Submitted on 8 Mar 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

Author

Wong, Ximena, Carrasco-Pozo, Catalina, Escobar, Elizabeth, Navarrete, Paola, Blachier, Francois, Andriamihaja, Mireille, Lang, Annaig, Tome, Daniel, Jose Cires, Maria, Pastene, Edgar, Gotteland, Martin

Published

2016

Journal Title

Journal of Agricultural and Food Chemistry

Version

Accepted Manuscript (AM)

DOI

<https://doi.org/10.1021/acs.jafc.6b00656>

Copyright Statement

This document is the Accepted Manuscript version of a Published Work that appeared in final form in Journal of Agricultural and Food Chemistry, copyright 2016 American Chemical Society after peer review and technical editing by the publisher. To access the final edited and published work see <https://doi.org/10.1021/acs.jafc.6b00656>

Downloaded from

<http://hdl.handle.net/10072/389509>

Griffith Research Online

<https://research-repository.griffith.edu.au>

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/299654337>

The deleterious effect of p-cresol on human colonic epithelial cells is prevented by proanthocyanidin-containing polyphenol extracts from fruits and proanthocyanidin bacterial meta...

Article in *Journal of Agricultural and Food Chemistry* · April 2016

DOI: 10.1021/acs.jafc.6b00656

CITATIONS

16

READS

225

11 authors, including:



Catalina Carrasco-Pozo

University of Chile

53 PUBLICATIONS 817 CITATIONS

SEE PROFILE



Elizabeth Escobar

University of Concepción

6 PUBLICATIONS 94 CITATIONS

SEE PROFILE



Paola Navarrete

University of Chile

99 PUBLICATIONS 1,370 CITATIONS

SEE PROFILE



François Blachier

French National Institute for Agricultural Research

211 PUBLICATIONS 5,164 CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:



Milk protein versus bone metabolism [View project](#)



Protein metabolism by the humang gut microbiota [View project](#)

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

Ximena Wong,[†] Catalina Carrasco-Pozo,[†] Elizabeth Escobar,[†] Paola Navarrete,[‡] François Blachier,[§] Mireille Andriamihaja,[§] Annaig Lan,[§] Daniel Tomé,[§] Maria-Jose Cires,[†] Edgar Pastene,^{||} and Martin Gotteland^{*,†,‡}

[†]Department of Nutrition, Faculty of Medicine, University of Chile, Independencia 1027, Independencia, Santiago, Chile

[‡]Institute of Nutrition and Food Technology (INTA), University of Chile, Santiago, Chile

[§]INRA/AGROPARISTECH, UMR 914 Nutrition Physiology and Ingestive Behavior, Paris, France

^{||}Laboratory of Pharmacognosy, Faculty of Pharmacy, University of Concepción, Concepción, Chile

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 Glc^{\pm} 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 Glc^{\pm} 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.¹ 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.^{2,3} 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.^{2,3} 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.^{4–7} 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.^{8–13} 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.^{6,8,13,14} These compounds may be absorbed in the colon, and eventually, they may be detected in the bloodstream at micromolar concentrations (2–50 μM) 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.^{15–17}

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

Received: February 7, 2016

Revised: April 1, 2016

Accepted: April 4, 2016



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

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

104 ■ MATERIALS AND METHODS

105 **Chemicals.** CytoTox-ONE homogeneous membrane integrity
106 assay kits and CellTiter-Glo luminescent cell viability assay kits were
107 from Promega (Madison, USA). Flavonol glycosides quercetin 3-*O*-
108 galactoside (hyperoside), quercetin 3-*O*-glucoside (isoquercitrin), and
109 quercetin 3-*O*-rhamnoside (quercitrin) were from Roth (Karlsruhe,
110 Germany). 5-Caffeoyl quinic acid and 4-caffeoyl quinic acid were
111 obtained from Phytolab (Vestenbergsgreuth, Germany). All solvents
112 (HPLC-grade acetonitrile, methanol, and TFA) were purchased from
113 Merck (Darmstadt, Germany). *p*-Cresol, 4-hydroxyphenylacetic acid
114 (4-HPAA), 3,4-dihydroxyphenyl propionic acid (3,4-DHPPA), 3-
115 phenylpropionic acid (3-PPA), fluorescein isothiocyanate-dextran
116 (FD-4), rhodamine 123 quercetin, rutin, apigenin, kaempferol,
117 myricetin, isorhamnetin, epicatechin, catechin, epicatechin gallate,
118 catechin gallate, phloretin-2'-xyloglucoside, phloretin-2'-glucoside
119 (phloridzin), cyanidin-3-*O*-glucoside, cyanidin-*O*-arabinoside, peoni-
120 din-*O*-arabinoside, gallic acid, syringic acid, vanillic acid, chlorogenic
121 acid, caffeic acid, ellagic acid, sinapic acid, ferulic acid, anisic acid, *p*-
122 coumaric acid, cinnamic acid, protocatechuic acid, *p*-hydroxybenzoic
123 acid, *m*-hydroxybenzoic acid, caffeoyl glucoside, feruloyl glucoside,
124 coumaroyl glucoside, and the Folin–Ciocalteu reagent were purchased
125 from Sigma-Aldrich (St. Louis, MO, USA).

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

100 μm). Marcs were homogenized for one min with an Ultraturrax 135
and extracted again with water at $65\text{ }^{\circ}\text{C}$. After 60 min of maceration, 136
the pH of the pooled extracts was adjusted to 2.5 with formic acid, and 137
these were poured on a glass column (150 mm i.d. \times 300 mm) packed 138
with Sepabeads SP-850 (Supelco, Bellefonte, USA) preconditioned 139
with acidic water. Sugars and other water-soluble compounds were 140
removed by washing with 5 L of distilled water, and the polyphenol 141
extracts were obtained after elution with absolute ethanol.²⁶ Cranberry 142
extract was obtained from concentrated juice (500 mL) by 5-fold 143
dilution with acidic water (pH 2.5) and adsorption on an Amberlite 144
XAD7HP column. The ethanolic fractions were evaporated under 145
vacuum ($<40\text{ }^{\circ}\text{C}$) followed by freeze-drying of the aqueous remnants. 146
Dried polyphenol-rich extracts were stored at $-70\text{ }^{\circ}\text{C}$ until use. 147

High-Performance Liquid Chromatography. The identification 148
and quantification of the polyphenols present in the four extracts were 149
carried out as previously described by Pastene et al.^{31,32} Polyphenols 150
were analyzed by RP-HPLC using a Waters Alliance 2695 system 151
equipped with a C18 column (Kromasil 250 \times 4.6 mm, 5 μm) and a 152
C18 precolumn (Nova-Pak Waters, 22 \times 3.9 mm, 4 μm) (Milford, 153
MA) at $30\text{ }^{\circ}\text{C}$. An elution gradient was performed by varying the 154
mobile phase from 0 to 25 min, 10–30% B; 25–30 min, 30–75% B; 155
30–35 min, 75–10% B at a rate flow of 0.8 mL/min with a final 156
stabilization period of 10 min. Known compounds were identified by 157
matching their retention times (t_{R}) and online UV spectra with those 158
of reference compounds. Quantification was performed by using a 159
UV–vis chromatogram acquired at 350 nm for flavonols, 320 nm for 160
phenolic acids, 280 nm for flavan-3-ols, and 520 nm for anthocyanins. 161
When available, standard compounds were used to construct the 162
respective external calibration curves. Rutin, quercetin, myricetin, 163
isorhamnetin, kaempferol, cyanidin, malvidin, delphinidin, peonidin, 164
and pelargonidin were used when authentic standards were not 165
available. All results were expressed as grams per 100 g of polyphenols. 166
Total polyphenolic contents (TPC) were determined with Folin– 167
Ciocalteu reagent and expressed as mg of gallic acid equivalents 168
(GAE) per gram of dried extract. 169

Determination of the Mean Degree of Polymerization of 170
Proanthocyanidins. Phloroglucinolysis was used for estimating 171
proanthocyanidin contents and their mean degree of polymerization.³² 172
Briefly, 10 mg of the extracts were dissolved in 2 mL of a solution of 173
0.1 M HCl in methanol containing 50 mg/mL of phloroglucinol and 174
10 mg/mL of ascorbic acid. The reaction mixture was incubated at 50 175
 $^{\circ}\text{C}$ for 20 min, and 10 mL of 40 mM aqueous sodium acetate was 176
added to stop the reaction. The formed phloroglucinol adducts were 177
analyzed by the above-described method of RP-HPLC. Quantitative 178
determination of (–)-epicatechin, (+)-catechin, and phloroglucinol 179
adduct products was performed using external standards. Phloroglu- 180
cinol-adducts were prepared from *Peumus boldus* and apple peel 181
extracts as a source of catechin and epicatechin polymeric proanthocyanidins 182
according to Köhler and Winterhalter as well as Pastene et al.^{33,34} The 183
proanthocyanidin contents were calculated by summing the masses of all 184
subunits (excluding the phloroglucinol portion of the phloroglucinol 185
adducts). The mean degree of polymerization was obtained summing 186
the extension subunits and then dividing it by the sum of terminal 187
subunits. Native (–)-epicatechin and (+)-catechin in the samples were 188
quantified in extracts not treated with the nucleophile, and the 189
corresponding values were subtracted for calculations. 190

Cells and Cell Culture Conditions. The human colonic 191
adenocarcinoma cell line HT29 Gle^{\pm} (passages 46–56) was used in 192
the studies of cell viability and mitochondrial function, and the human 193
colonic adenocarcinoma cell line Caco-2 (passages 6–20) (ATCC, 194
USA) was used in the studies of barrier function. Both cell lines were 195
cultured in Dulbecco's modified Eagle's medium (DMEM) 196
supplemented with 4.5 g/L of glucose and L-glutamine (Corning), 197
10% heat inactivated fetal calf serum (Gibco, USA), and 1% penicillin/ 198
streptomycin (Hyclone). Caco-2 cells were also supplemented with a 199
1% nonessential amino acids solution (Gibco). Cell cultures were 200
grown at $37\text{ }^{\circ}\text{C}$ in a 5% CO_2 humidified atmosphere. On day 0, HT29 201
 Gle^{\pm} cells were seeded at density of 2×10^4 cells/cm² in 96-well plates. 202
The culture medium was changed on day 3, and each test was started 203
on day 4. Cells were coincubated with 0.8 mM *p*-cresol (freshly 204

Table 1. Phenolic Composition of the Fruit Extracts

compound (%)	apple peel ^a	cranberry ^a	grape ^a	avocado peel ^a
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) ^b	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 ^c	ND	1.43 ± 0.01	2.03 ± 0.05	ND
myricetin	ND	1.78 ± 0.03	ND	ND
myricetin hexosides ^d	ND	5.19 ± 0.09	ND	ND
isorhamnetin	ND	0.68 ± 0.36	ND	ND
isoramnetin derivatives ^e	ND	ND	3.75 ± 0.05	ND
∑ 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
∑ flavan-3-ol monomers	6.11 ± 0.50	2.46 ± 0.02	8.33 ± 0.11	14.01 ± 0.20
∑ total procyanidins ^f	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
∑ dihydrochalcones	10.56 ± 2.00	0.18 ± 0.01	ND	ND
Anthocyanins				
cyanidin- <i>O</i> -hexosides ^g	ND	5.23 ± 0.03	1.23 ± 0.02	ND
cyanidin- <i>O</i> -arabinoside ^g	ND	5.01 ± 0.03	ND	ND
cyanidin-3- <i>O</i> -glucoside ^g	ND	ND	ND	3.00 ± 0.03
peonidin- <i>O</i> -hexosides ^h	ND	8.46 ± 0.01	1.01 ± 0.01	ND
peonidin- <i>O</i> -arabinoside ^h	ND	4.97 ± 0.06	ND	ND
malvidin- <i>O</i> -hexosides ⁱ	ND	ND	12.05 ± 0.01	ND
delphinidin- <i>O</i> -hexosides ^j	ND	ND	4.53 ± 0.08	ND
petunidin- <i>O</i> -hexosides ^k	ND	ND	3.01 ± 0.13	ND
∑ 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
∑ 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

^aData 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. ^bCalculated on the basis of rutin measured by RP-HPLC. ^cCalculated on the basis of kaempferol measured by RP-HPLC. ^dCalculated on the basis of myricetin measured by RP-HPLC. ^eCalculated on the basis of isorhamnetin measured by RP-HPLC. ^fEstimated

Table 1. continued

by RP-HPLC method after phloroglucinolysis. ^gCalculated on the basis of cyanidin measured by RP-HPLC. ^hCalculated on the basis of peonidin measured by RP-HPLC. ⁱCalculated on the basis of malvidin measured by RP-HPLC. ^jCalculated on the basis of delphinidin measured by RP-HPLC. ^kCalculated 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⁺ Cells Cultured without or with *p*-Cresol^a

	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 ^c	100	87 ± 13 ^c
cranberry (μg/mL)	12.5	94 ± 4 ^b	103 ± 6 ^d	135 ± 19 ^c	125 ± 13 ^{c,e}
	25	92 ± 6 ^c	96 ± 6 ^e	135 ± 14 ^c	127 ± 14 ^e
	50	91 ± 4 ^c	97 ± 5 ^e	133 ± 19 ^c	122 ± 11 ^{b,e}
avocado (μg/mL)	12.5	93 ± 4 ^c	100 ± 5 ^e	144 ± 15 ^c	125 ± 13 ^{c,e}
	25	91 ± 6 ^c	99 ± 5 ^e	144 ± 17 ^c	127 ± 14 ^{c,e}
	50	88 ± 4 ^c	96 ± 5	132 ± 14 ^c	122 ± 11 ^{c,e}
grape (μg/mL)	12.5	101 ± 5	174 ± 10 ^{b,e}	98 ± 7	84 ± 3 ^c
	25	107 ± 11	185 ± 13 ^{c,e}	103 ± 8	81 ± 5 ^c
	50	117 ± 8 ^c	225 ± 18 ^{c,e}	102 ± 7	53 ± 9 ^{c,e}
apple (μg/mL)	12.5	101 ± 10	170 ± 14 ^c	100 ± 10	82 ± 3 ^c
	25	105 ± 8	175 ± 12 ^c	103 ± 6	84 ± 2 ^c
	50	105 ± 7	176 ± 22 ^b	101 ± 10	82 ± 4 ^c
3-PPA (μM)	50	108 ± 3 ^c	105 ± 4	79 ± 9 ^c	81 ± 3 ^c
	200	107 ± 3 ^c	105 ± 9	95 ± 12	92 ± 9
3,4-DHPPA (μM)	30	112 ± 4 ^c	104 ± 6	81 ± 7 ^c	92 ± 6
	100	108 ± 3 ^c	108 ± 3	79 ± 5 ^c	92 ± 12
4-HPPA (μM)	10	107 ± 4 ^b	107 ± 3	77 ± 13 ^c	95 ± 8 ^b
	50	107 ± 4 ^b	108 ± 6	81 ± 7 ^c	89 ± 7 ^b

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

205 dissolved in culture medium) and the PAC-containing polyphenol
206 extracts (12.5, 25, and 50 μg/mL, i.e., in concentrations achievable in
207 the gut lumen after the intake of a standard diet) or the PAC bacterial
208 metabolites 3-PPA (50 and 200 μM), 3,4-DHPPA (30 and 100 μM),
209 4-HPPA (10 and 50 μM), i.e., in concentrations similar to those
210 reported by Jenner et al.⁸ in the fecal water of human subjects. The
211 culture medium was changed daily from days 4 to 7. Caco-2 cells were
212 cultured at a density of 10⁵ cells/cm² on Transwell polycarbonate
213 filters (12 mm diameter; 0.4 μm pore size) (Corning-Costar, USA)
214 previously treated with 0.044 μg/μL of collagen (Thermo) in 0.01 M
215 HCL. The basolateral and apical compartments of the transwell filters
216 were filled with 1.5 and 0.5 mL of culture medium, respectively. The
217 culture medium was changed three times per week; the cell
218 monolayers were used when their transepithelial electrical resistance
219 was stabilized, usually 10–15 days after reaching confluence.

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

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

Mitochondrial Membrane Potential. The mitochondrial mem- 237
brane potential (MMP) was quantified using Rhodamine-123 (R-123), 238
which diffuses easily across membranes due to its lipophilic nature, and
239 accumulates in the negatively charged mitochondrial matrix. HT29
240 Glc⁺ cells were incubated with the polyphenol extracts or PAC
241 metabolites in the presence or absence of 0.8 mM *p*-cresol until day 7.
242 For the experiment, cells were exposed to 1 μg/mL of R-123 for 30
243 min to allow its loading within the cells; these were subsequently
244 washed twice with PBS to remove the R-123 excess and lysed with 2%
245 Triton-X100. Finally, MMP was determined with a multimode
246 microplate reader (λ_{ex/em} = 500/530 nm). Results were expressed as
247 percentage of MMP relative to that of the control untreated cells. 248

Intestinal Barrier Function. Changes in the intestinal barrier 249
function were determined only in Caco-2 cells as HT29 Glc⁺ cells 250
develop a low transepithelial electrical resistance (TEER). TEER, as a
251 reflection of the integrity of the epithelial paracellular pathway, was
252 measured in the Caco-2 cell monolayers grown in a Transwell filter
253 with an ohm/voltmeter (EVOM, WPI, USA) as previously
254 described.³⁵ Cell monolayers were used after confluence when their
255 TEER was stabilized. Measurements were carried out at 24 h of
256 coincubation with the different concentrations of the polyphenol
257 extracts or PAC metabolites and 3.2 mM *p*-cresol. Results were
258 expressed as percentage of the TEER value obtained at time 0. 259

Changes in the barrier function of the Caco-2 cell monolayers were 260
also determined by measuring the paracellular passage of fluorescein
261 isothiocyanate-conjugated dextran (FD-4) from the apical to the
262 serosal side of the monolayer. FD-4 (4.4 kD MW, 0.8 mg/mL
263 dissolved in culture medium) was added to the apical compartment in
264 the absence or presence of the polyphenol extracts or PAC metabolites
265 at different concentrations with or without the addition of 3.2 mM *p*-
266 cresol. The amount of FD-4 transferred to the basolateral side after 24
267 h exposure was determined in the multimode microplate reader (λ_{ex/em} 268

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
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⁻ 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 μ 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⁻ 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 μ M.

312 Table 2 also shows that exposure of HT29 Glc⁻ 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⁻ 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

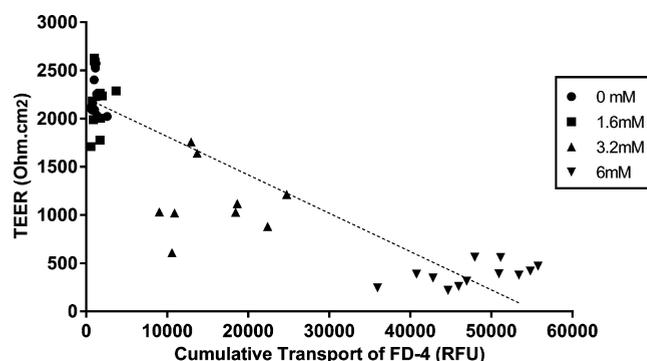


Figure 1. Correlation between transepithelial electrical resistance (TEER) and paracellular permeability to FD-4 in Caco-2 cell monolayers exposed for 24 h to different concentrations of *p*-cresol (Pearson $r = -0.9018$; $p < 0.0001$).

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 μ 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 μ M, 3-
PPA at 50 and 200 μ M, and 4-HPPA at 10 μ M.

■ DISCUSSION

p-Cresol is an aromatic compound produced by the
fermentation of tyrosine by anaerobic and aerobic bacteria
from the human gut microbiota.^{18,19} 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.²⁷ 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

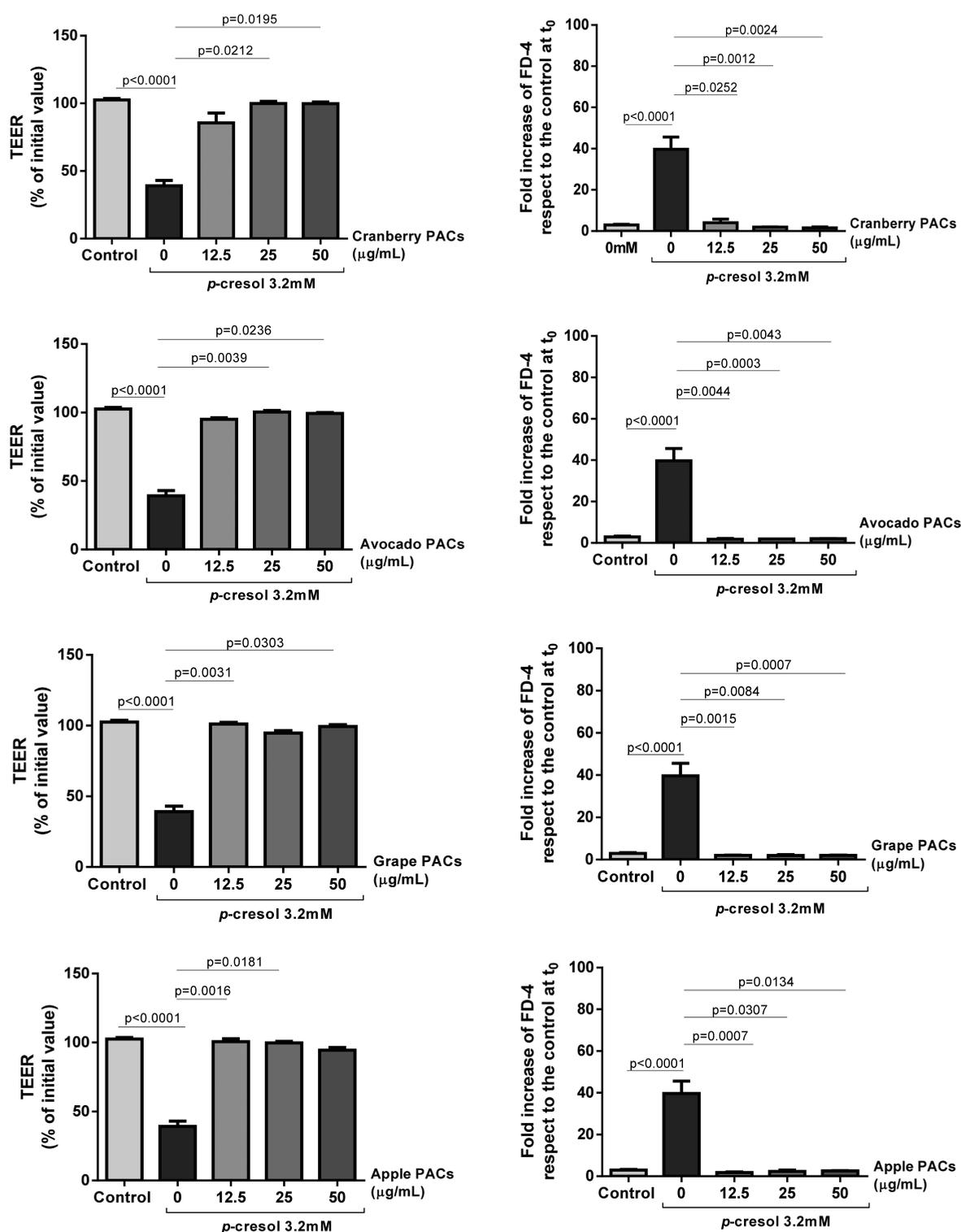


Figure 2. Protective effect of the different polyphenol extracts against the alterations of transepithelial electrical resistance (TEER) (left column) and paracellular permeability to FD-4 (right column) in Caco-2 cell monolayers exposed to 3.2 mM p -cresol for 24 h (mean \pm SEM).

375 diseases.^{24–26} In addition, excess p -cresol has also been
 376 reported to be hepatotoxic because it induces mitochondrial
 377 dysfunction³⁶ and has been found to be associated with autism
 378 in young patients.³⁷ We recently reported that, in the colon, p -
 379 cresol affects cell proliferation and increases DNA damage in
 380 human colonic cells in *in vitro* experiments.²³ In the present
 381 study, we evaluated whether PAC-containing polyphenol
 382 extracts from different fruits as well as PAC bacterial

metabolites protect human colonic epithelial cell lines against 383
 the deleterious effects induced by p -cresol. 384

Our results showed that the exposure of HT29 *Cle*⁺ cells to 385
 p -cresol resulted in the alteration of their cell membrane 386
 integrity (evaluated through the LDH assay) and decreases of 387
 ATP synthesis. This latter event suggests mitochondrial 388
 dysfunction; however, no changes in MMP were detected 389
 under our experimental conditions. This confirms the results 390

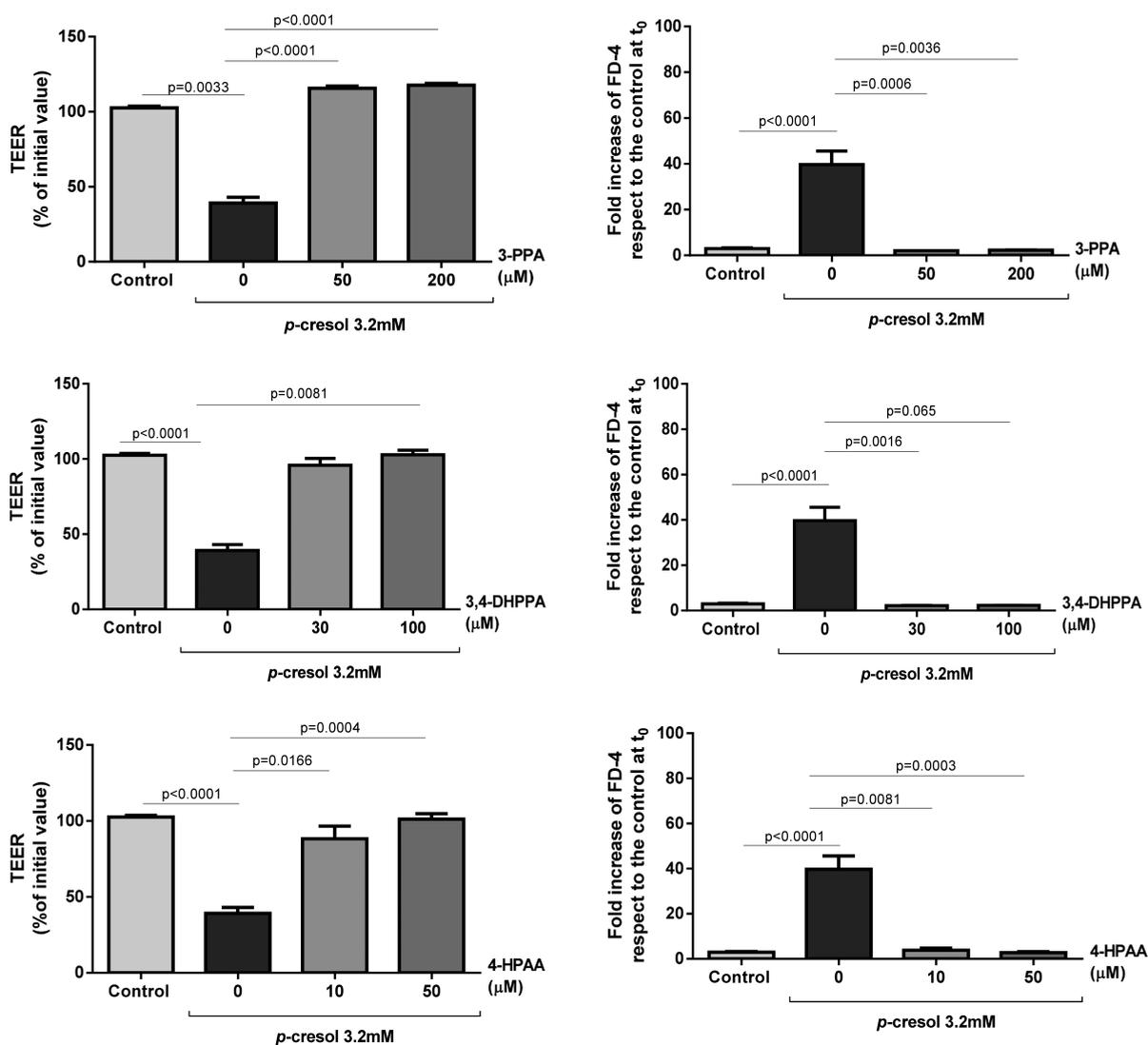


Figure 3. Protective effect of bacterial PAC metabolites against the alterations of transepithelial electrical resistance (TEER) (left column) and paracellular permeability to FD-4 (right column) in Caco-2 cell monolayers exposed to 3.2 mM *p*-cresol for 24 h (mean \pm SEM).

391 from our previous study indicating that the decreased ATP
 392 synthesis was probably, at least in part, the underlying
 393 mechanism explaining the inhibitory effect of *p*-cresol on
 394 cellular proliferation.²³ In addition, our current results also
 395 indicate that the barrier function of Caco-2 cell monolayers was
 396 strongly altered after a 24 h incubation with *p*-cresol as reflected
 397 by the decrease of TEER and the correlative increase of the
 398 paracellular permeability of FD-4. In humans as well as in
 399 laboratory animals, such alterations of the gut barrier function
 400 are generally considered as an initial step previous to the
 401 development of local and systemic pathological events. For
 402 example, increased gut permeability is associated with the
 403 formation of gastrointestinal ulcers in rats treated with
 404 indomethacin, a nonsteroidal anti-inflammatory drug that
 405 induces mitochondrial dysfunction in intestinal epithelial
 406 cells^{38,39} and in animal models of colorectal cancer. Increased
 407 gut permeability is accompanied by higher plasma endotoxin
 408 concentrations, mesenteric lymph node hypertrophy, and the
 409 progression of cachexia.⁴⁰
 410 After further characterization of the deleterious impact of *p*-
 411 cresol on the cultured intestinal cells, we evaluated whether this
 412 might be prevented by our characterized PAC-containing

polyphenol extracts. Relative to the changes in cell membrane
 413 integrity and ATP contents of HT29 Glc^+ cells, our results
 414 were relatively variable according to the fruit source of the
 415 extracts. Those from cranberries and avocados did not affect
 416 LDH release and increased the intracellular ATP content,
 417 whereas the extracts from apples and grapes had no effect on
 418 the cellular ATP content but tended to increment LDH release.
 419 Such differences between extracts were also observed when
 420 they were incubated with the cells in the presence of *p*-cresol.
 421 Avocado and cranberry extracts prevented the LDH release
 422 induced by *p*-cresol whereas the apple and grapes extracts
 423 magnified it. Similar results were observed on the cellular ATP
 424 content. The reason for these differences in the activity of the
 425 extracts remains unclear. However, it is interesting to note that
 426 apples and grapes mainly contain type-B PACs whereas
 427 avocados and cranberries mostly contain type-A PACs, whose
 428 constituent monomers are also linked through an additional
 429 ether bond between C2 and C7.¹⁻³ It has been shown that this
 430 chemical structure confers specific biological activities to type-A
 431 PACs that are not observed with the type-B PACs. This is the
 432 case, for example, of the potent antiadhesive activity exhibited
 433 by cranberry PACs against pathogens.⁴¹ Therefore, it is possible
 434

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

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

458 Finally, we also evaluated in *p*-cresol-treated cells the
459 protective effect of three metabolites, 4-HPAA, 3,4-DHPPA,
460 and 3-PPA, originating from PAC degradation by the
461 microbiota. It is worth noting that one of these (4-HPAA)
462 may also be generated by the microbiota through protein
463 fermentation.⁴⁹ Bacterial metabolites of polyphenols, and more
464 specifically proanthocyanidins, may be detected in urine of
465 human subjects after the intake of polyphenol-rich foodstuffs,
466 suggesting that these compounds are absorbed in the colon and
467 that they could exert biological activities in colonocytes as well
468 as in a number of other tissues and organs.^{50,51} Various studies
469 have shown that these metabolites may be found in high
470 concentrations in the colonic lumen,⁸ confirming that they are
471 poorly absorbed in the small intestine. Accordingly, they could
472 directly act on epithelial cells, modulating signaling events and
473 contributing to the homeostasis of the colonic mucosa. We
474 observed that, when incubated with HT29 Glc^{\pm} cells in the
475 absence of *p*-cresol, these compounds slightly increased LDH
476 release and decreased cellular ATP content. Such effects
477 suggest that these metabolites might eventually induce cell
478 toxicity. This could be due to the fact that HT-29 Glc^{\pm} cells,
479 being derived from a human colonic adenocarcinoma, may be
480 more sensitive to these compounds compared with non-
481 transformed cells.⁵² In the presence of *p*-cresol, the bacterial
482 PAC metabolites prevented the increase of LDH release by
483 HT29 Glc^{\pm} cells without affecting the cellular ATP content,
484 and when incubated with Caco-2 cell monolayers, they totally
485 prevented the alterations of barrier function induced by *p*-
486 cresol. Although studies exploring the biological activities of
487 PAC metabolites are scarce, it has been shown that 3,4-DHPPA
488 upregulates the expression of glutathione S-transferase T2
489 (GSTT2) and downregulates that of cyclooxygenase-2 (COX-
490 2) in LT97 colonic cells.⁵³ COX-2 inhibition in HT29 Glc^{\pm}
491 cells has also been reported after incubation with human fecal
492 water with high levels of phenolic compounds.⁵² The inhibition
493 of cyclooxygenase-2 may explain the protective effect against
494 gut barrier alterations we observed in Caco-2 cell monolayers,
495 as previously described by Gentile et al.⁵⁴

496 In conclusion, our present study indicates that exposure of
497 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

Corresponding Author

*Phone: 56-2-29786977. E-mail: mgottela@med.uchile.cl.

Funding

This work was supported by ECOS C12S01 and Fondecyt
1120290 from CONICYT, Chile. M.J.C. was the recipient of a
doctoral fellowship (21120806) from CONICYT, Chile.

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. Procyranidin 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.; Miguez, 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.; Ghos, 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*, 5291–5297. 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), S310–329. 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 reverts the increase of 684
paracellular permeability in IL-1 β -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

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