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25 Abstract

Food matrix interactions with polyphenols can affect their bioavailability and as a consequence may modulate their biological effects. The aim of this study was to determine if the matrix and its processing modulate the bioavailability and the postprandial nutrigenomic response to a dietary inflammatory stress of apple flavan-3-ol monomers.

We carried out an acute randomized controlled study in minipigs challenged with a high fat meal (HFM) supplemented with raw fruit, puree, or apple phenolic extract with matched content in flavan-3-ol monomers. Fasting and postprandial blood samples were collected over 3h to quantify flavan-3-ol monomers in sera by UPLC-Q-TOF/MS and to isolate peripherical blood mononuclear cells (PBMCs) for assessing changes in gene expression profile using a microarray analysis.

36 When compared to the extract-supplemented meal, the peak of total flavan-3-ols concentrations 37 was reduced by half with both raw apple and puree. The apple matrices also affected gene 38 expression profile as revealed by the Principal Component Analysis of the microarray data from 39 PBMCs which discriminated supplementations of HFM with polyphenol extract from those 40 with raw apple or puree. A total of 309 genes were identified as differentially expressed by 41 apple-derived products compared to HFM, with 63% modulated only in the presence of the 42 food matrix (apple, puree). The number of differentially modulated genes was higher with the 43 puree (246) than with the unprocessed apple (182). Pathway enrichment analyses revealed that 44 genes affected by apple-derived products control inflammation and leukocyte transendothelial 45 migration both involved in the onset of atherosclerotic processes.

46 Overall, this study showed that the two apple matrices reduce the postprandial serum 47 concentration of flavon-3-ols whereas they increase the nutrigenomic response of PBMCs. The 48 biological processes identified as modulated by the apple products suggest an attenuation of the 49 transient pro-inflammatory response induced by a HFM. The differences observed between the 50 nutrigenomic responses support that apple matrix and its processing affect the nutrigenomic 51 response, probably by increasing the bioavailability of other apple phytochemicals. To 52 conclude, this study raises awareness for considering the impact of food matrix and processing 53 on the biological responsiveness of polyphenols in nutritional studies.

54

55 Introduction

56 Higher fruit intake is associated with lower risk of all-cause and disease-specific mortality (1). 57 Fruits are recognized as major contributors to polyphenol intake in humans, and several 58 prospective studies have reported positive associations between polyphenol-rich fruits and 59 lower incidence of cardiovascular diseases (CVD) (2). Apple fruits constitute one of the most popular and widespread polyphenol-rich fruits consumed in Europe due to their cultivation all 60 61 over the world and their availability on the market throughout the year (3). In a cohort of elderly 62 women, a 35% lower risk of all-cause mortality was reported in women who consumed >100 63 g/d of apple compared with those consuming <5 g/d (4).

64 The mean content of total polyphenols is highly variable between apple varieties, laying from 65 52 to 379 mg per 100g edible fresh weight (5). The predominant classes of polyphenols in apple 66 include flavan-3-ols (70-90%) and hydroxycinnamates (4-30%); the others, including 67 dihydrochalcones, flavonols and in red apple anthocyanins, are less abundant (6, 7). Flavan-3-68 ols are present as monomeric forms (epicatechin and catechin), but occur mostly as high 69 molecular weight polymers. Flavan-3-ol monomers are quickly absorbed in the upper part of 70 the intestine (8), whereas breakdown products from flavan-3-ol polymers may be absorbed only 71 after degradation by the microbiota in the colon (9, 10).

The cardiovascular protective effects of apple are primarily attributed to their flavonoid content
with most convincing evidences existing for their impact on vascular function and inflammation
(11, 12). Also the combination between flavonoids and pectins has been reported to be involved

in the positive effect of apple on lipid metabolism (12). In recent randomized clinical trials (RCT), flavonoid-rich apples have been shown to improve endothelial function in both healthy subjects and subjects at risk for cardiovascular diseases (13, 14). This improvement could notably be related to the apple content in flavan-3-ol monomers, considering that previously epicatechin has been causally linked with the vascular protective effects in human induced by the intake of flavan-3-ol-rich cocoa (15).

81 There is also a strong evidence base that flavonoids exert anti-inflammatory effects that could 82 be of interest in mediating their beneficial impact on cardiovascular health (16). In this respect, 83 in mice fed a pro-inflammatory diet, the supplementation with epicatechin attenuated 84 atherosclerosis and reduced inflammation by preventing the diet induced activation of the 85 nuclear factor kappa B (NF- κ B) (17). The capacity of epicatechin to regulate pro-inflammatory 86 cell signaling in physiological conditions was strengthened in a study demonstrating that flavan-87 3-ol metabolites at low concentrations can prevent the activation of the mitogen-activated 88 protein kinase (MAPK) and NF- κ B in endothelial cells (18).

A review of *in vitro* and *in vivo* studies examining through a nutrigenomic approach the mechanisms underlying the cardioprotective effects of fruit flavonoids clearly highlighted that these compounds are particularly efficient in modulating the expression of a number of genes involved in atherosclerosis development, especially those modulating inflammation, vascular homeostasis and cell adhesion (19, 20). However, the specific impact *in vivo* of apple flavonoids on the processes related to inflammation are still poorly documented.

As previously reported, the postprandial state which constitutes a dynamic period of metabolic trafficking, biosynthesis and oxidative metabolism is well suited to assess the ability of specific foods or food components to attenuate the negative effects induced by the consumption of proinflammatory or pro-oxidant meals (21). Especially, the consumption of a high fat meal is recognized to induce transient postprandial inflammation and endothelial dysfunction (22). The 100 postprandial period also coincides with the peak of flavan-3-ol monomers, only present 101 transiently in the circulation (nM to μ M range) as phase II metabolites (23).

Some of the factors that may affect the bioavailability of flavonoids are related to foods. They 102 103 include the form in which they are ingested and the food matrix in which they are found (24), 104 and both of them can be affected by food processing. The interactions of flavonoids with the 105 food matrix can modulate the bioaccessibility (25), uptake and metabolism of these compounds, 106 with as a consequence a likely impact on their health effects (26). Apart from one study 107 reporting that epicatechin administered from whole apple was less bioavailable than from apple 108 extract (27), the impact of apple matrix on the bioavailability of, or biological response to 109 epicatechin has been so far poorly studied.

110 The main aim of this acute study was to determine if the apple matrix and the processing can 111 affect the postprandial bioavailability of apple flavan-3-ol monomers and modulate the 112 postprandial nutrigenomic response to an inflammatory stress induced by a high fat meal. To 113 this end, we carried out a randomized crossover intervention study in minipigs and used 114 different apple products (apple polyphenol extract, raw fruit, puree) with matched content in 115 flavan-3-ol monomers. To assess the postprandial changes in the gene expression profile of 116 circulating peripheral blood mononuclear cells (PBMCs) after apple products intake, we used 117 a microarray approach.

118 Materials and Methods

119 Standards and chemicals

(+)-catechin (C), (-)-epicatechin (EC), 3'-O-methyl epicatechin (3MEC), 4'-O-methyl 120 121 epicatechin (4MEC), 5-O-caffeoylquinic acid, *Helix pomatia H1* β-glucuronidase/arylsulfatase, as well as sucrose, glucose, fructose, ascorbic acid, dehydroascorbic acid, and toluene- α -thiol 122 123 were from Sigma-Aldrich (Saint Quentin-Fallavier, France). Phloretin, p-coumaric acid, and 124 quercetin were obtained from Extrasynthese (Lyon, France). Phloridzin was obtained from 125 Fluka (Buchs, Switzerland). Acetonitrile of HPLC grade and acetic acid were from Fischer 126 Scientific (Pittsburgh, PA, USA). Ethyl acetate, dichloromethane and hexane were obtained 127 from VWR International (Radnor, USA).

128

129 Apple test products

130 The test products, including raw apples, puree and phenolic extract, were obtained from two 131 apple varieties, including a cider apple (Bedan) and a dessert apple (Reinette des Flandres) 132 which composition is detailed in Supplemental Table 1. These varieties, chosen for their natural 133 richness in flavan-3-ols monomers (Suppl Table 2), were mixed and used in equal amounts 134 (1:1). Apple fruits (Malus domestica Borkh) of the Bedan cultivar at technological maturity 135 were obtained from the experimental orchard of Institut Français des Productions Cidricoles 136 (IFPC, Sées, France) in December 2013. Cultivar Reinette de Flandre was organically produced 137 by Bio-verger (Mrs Christine Boutin, Ambricourt, France) in October 2013. Apples were stored 138 at +4 °C until use. Three replicates were constituted by 10 apples of each cultivar. Each fruit 139 with peel and core was prepared as described in Le Bourvellec et al. (2011) (28). Apple pieces 140 (with peel and core) were freeze-dried and used for characterization in phenolic compounds, 141 vitamin C, organic acids and sugars as described in Supplemental methods. For extraction and purification of phenolic compounds, 8.5 kg of fruits of each cultivar without stalks were cutinto pieces and freeze-dried.

Apple puree processing was carried out by the Technical Center for the Conservation of Agricultural Products (Centre Technique de la Conservation des Produits Agricoles (CTCPA), Avignon, France) as detailed in the Supplemental methods. Apple puree (composition detailed in supplemental tables 1, 2) were stored at 4°C until use.

148 Polyphenols were extracted from the freeze-dried apple powder (150 g, mixture of 75 g of 149 Bedan and 75 g Reinette de Flandre) by stirring with 1 L of a water: acetone mixture (40:60 v:v) 150 (three times) during 15 min. Extracts were pooled and concentrated on a rotary evaporator prior 151 to freeze-drying. The extraction was repeated 7 times. The freeze-dried extracts were dissolved in acidified water (acetic acid 25 mL. L⁻¹) and filtered on a 3 µm filter (Cellulose, Merck 152 153 Millipore, Darmstadt, Germany). They were injected on a 20×5 cm column of LiChrospher 100 154 RP-18 (12 µm) (Merck, Darmstadt, Germany). The column was first washed by acidified water 155 until absence of sugars in the eluate, then a gradient of acetonitrile was applied. The eluate was 156 monitored by absorbance at 280 nm for the presence of polyphenols. Peaks were collected and 157 concentrated on a rotary evaporator prior to freeze-drying. The content of the phenolic fraction 158 in the different categories of polyphenols was analysed (supplemental table 2) and the extract 159 was stored under vaccum at -80 °C before use.

160

161

Polyphenol analysis in apple products

Polyphenols were measured by HPLC-DAD, with and without thioacidolysis, using a method described by Guyot et al. (29). Analyses were performed using an Ultra Fast Liquid Chromatography Shimadzu Prominence system (Kyoto, Japan) controlled by a LC Solution software (Shimadzu, Kyoto, Japan). Separation conditions were as in Le Bourvellec et al. (2011) (28). Individual compounds were quantified by comparison with external standards at 167 280 nm for C, EC, phloretin xyloglucoside (quantified as phloretin), phloridzin, (-)-epicatechin 168 benzylthioether (quantified as EC); at 320 nm for 5-caffeoylquinic acid, 4-*p*-coumaroylquinic 169 acid (quantified as *p*-coumaric acid) and their methylated derivatives obtained during 170 thioacidolysis reaction quantified as their respective non-methylated equivalents, and at 350 nm 171 for quercetin glycosides (quantified as quercetin).

172

173 Animal intervention

174 All procedures were conducted in accordance with the guidelines formulated by the European 175 Community for the use of experimental animals (L358-86/609/EEC), and the study was 176 approved by the Local Committee for Ethics in Animal Experimentation (Comité d'Ethique en 177 Matière d'Expérimentation Animale d'Auvergne, Aubière, France). Five adult male Yucatan 178 minipigs $(24.9 \pm 1.0 \text{ kg body weight})$ were used. They were housed in individual pens $(1 \times 1.5 \times$ 179 m) with Plexiglass walls in a ventilated room with controlled temperature (20-23 °C). Apart 180 from sampling days, they were fed with 400 g of a commercial feed (Porcyprima, Sanders 181 Nutrition Animale, France). Minipigs were surgically fitted with a permanent catheter 182 (polyvinyl chloride; 1.1-mm i.d., 1.9-mm o.d.) in the aorta allowing repeated blood samplings 183 (30).

184 After at least 3 recovery weeks, animals entered in a cross over design in which they were 185 randomly given once a week a non supplemented high-fat meal (HFM) or one of the HFM 186 supplemented with the different apple products (raw apples, puree or phenolic extract). A wash-187 out period of 1 week separated the intake of each 4 test meals. The HFM used to induced postprandial inflammation provided 50 g saturated fat/ m^2 body surface (31) and also contained 188 proteins and carbohydrates. The HFM included 100 g fresh cream (Crème d'Isigny 40 %, 189 190 Auchan, France), 120 g raw ground beef, 120 g wheat starch, 20 g sucrose and 15 g cellulose, 191 and provided an energy supply of 1160 kcal. The three supplemented HFM were prepared just before use by adding (1) 250 g of raw apples, peeled, cored and cut into 1 cm square (HFM+raw
apple), (2) 250 g of apple puree obtained (HFM + Apple Puree) and (3) 1.4 g of apple
polyphenol extract (HFM + PP extract). The vitamin C supply was adjusted between the four
test meals, based on the highest level of Vitamin C that was found in apple puree (suppl. table
1), namely 0.29 g Vitamin C/meal.

On sampling days, blood was collected in cold syringes (S-Monovettes, Starstedt, France) at fasting and 1, 2 and 3 hours after meal intake and immediately divided in two parts, 10 mL in dry tubes for serum separation and 20 mL in tubes containing sodium citrate (BD vacutainer CPT, Becton Dickinson, Le pont de Claix, France) for peripheral blood mononuclear cells (PBMCs) separation.

202

203 Blood sampling

PBMCs were immediately isolated from BD vacutainer CPT tubes according to the manifacturer's instructions. Briefly, after a 20 min centrifugation at 1650 g at room temperature, the cell ring was harvested, collected in a 15 mL tube, rinsed with 1X phosphate buffer saline solution, and then pelleted by centrifugation at 300 g for 15 min at room temperature. A second wash step was performed and PBMCs were pelleted by centrifugation at 300 g for 10 min. The supernatant was carefully discarded and the dried pellet of cells immediately stored at -80 °C until RNA extraction.

Serum was isolated from blood collected in dry tubes 30 min after clotting by centrifugation at 4 °C for 15 min at 2500 g. The serum fraction was acidified by adding 10 μ L of acetic acid 0.8 M per mL and then aliquoted and stored at -80 °C. These samples were used for quantitive analysis of flavan-3-ols.

215

216 Quantitative analysis of flavan-3-ols in serum

217 Serum flavan-3-ol analysis was performed according to a method based on Ottaviani et al. with 218 some modifications. Samples were thawed on ice and centrifuged (15 000 g) at 4 °C during 15 219 min. The *Helix pomatia H1* β-glucuronidase/arylsulfatase enzyme solution was carefully 220 prepared in 0.2 M sodium acetate (pH 5) to a final concentration of 50 mg/mL at 4 °C to yield 221 a final specific activity of 81,700 IU of β -glucuronidase per mL and 700 IU of arylsulfatase per 222 mL. A total of 200 µL of serum was transferred with 2.6 µL of 13.7 µM taxifolin (internal 223 standard), 20 µL acetic acid (1.2 M) and 40 µL of the enzyme. The samples were then incubated 224 at 37 °C for 40 min followed by centrifugation (15 000 g) at 4 °C during 15 min. Solid phase 225 extraction and further UPLC-Q-TOF MS analysis was performed on the hydrolyzed serum 226 using the validated method by Feliciano et al. (32) to quantify C, EC and methylated derivatives.

227

228 Microarray analysis

229 RNA was extracted from PBMCs using the RNeasyMini Kit (Qiagen, Hilden, Germany). Fifty 230 ng of total RNA extracted were amplified and fluorescently labelled RNA amplification was 231 performed using Low Input Quick Amp Labelling Agilent Kit (Agilent, Santa Clara, CA, USA) 232 according to the manufacturers' instructions. Labelled RNA were purified using RNeasyMini 233 Kit (Qiagen, Hilden, Germany), samples were vacuum dried at 55 °C and resuspended in the 234 hybridization mixture containing hybridization buffer and blocking reagent. Hybridization was 235 carried out on the S. scrofa V2 4x44k microarray (Agilent) in Agilent hybridization oven at 65 236 °C for 17 h according to the manufacturer's instructions. Following the hybridization, 237 microarrays were washed with GE Wash Buffer 1 and GE Wash Buffer 2 and scanned with 238 Agilent G2505 microarray scanner (Agilent Technologies, Santa Clara, CA, USA). The results 239 were extracted using Feature Extraction software version 11.0 and analyzed using GeneSpring GX software version 14.5 (Agilent Technologies, Santa Clara, CA, USA). Data were 240

normalized using quantile shift and reduced with fold-change (FC) cut-off set to 1.25 and then
analyzed with Friedman and a posteriori Wilcoxon tests.

243

244 **Bioinformatics analyses**

Bioinformatics analyses with differentially expressed genes (significant FC>1.25) in response
to the consumption of the flavanol-supplemented HFM were performed using GeneTrail2.0
(https://genetrail2.bioinf.uni-sb.de) to identify significantly over-represented KEGG pathways.
Only pathways with a number of hits superior of 3 and with a p-value < 0.01 were considered.
A network based on these enriched terms with an enrichment factor > 1.5 and a similarity > 0.3,
have been rendered using Metascape [http://metascape.org] (33).

251

252 Statistical Analyses

253 Prism software, version 6.0.c (GraphPad, La Jolla, CA), was used for the statistical analysis of 254 data. Data were analyzed using two-way analysis of variance (test meals, kinetic time) and 255 Tukey's multiple comparison test. Gene expression data were analyzed with Principal 256 Component Analysis and Friedman's test using R version 3.5.0. A value of p < 0.05 was 257 considered significant.

258 **Results**

259 Characterization of the Polyphenol content in administered apple products

Table 1 provides the administered doses of the different categories of polyphenols depending 260 261 on the apple-derived products consumed, with a size serving adapted for matching flavan-3-ol 262 monomers content as requested by the protocol. From table 1, a 250 g serving of an apple 263 mixture of Bedan and Reinette des Flandres varieties (1:1) contained 1248 mg of total 264 polyphenols, 77% being constituted by flavan-3-ols, including 154 mg of monomeric forms 265 (2/3 as (-)-epicatechin) and 1/3 as (+)-catechin). The remaining polyphenolic fractions in the apple mix was constituted for 4.5% by flavonoids (dihydrochalcones (DHC) and flavonols) and 266 267 for 18.5% by hydroxyinnamic acids. After processing apples into puree, the levels of EC and C was unchanged (152 mg EC+C/250 g), whereas the total polyphenol content was slightly 268 269 reduced (1012 mg/250 g), mainly reflecting its lower content in procyanidins. The puree 270 contained a higher level in total flavonoid monomers other than flavonols (with 80 mg/250 g, 271 including 66 mg DHC and 14 mg flavonols) compared to raw apple (58 mg/250 g, with 45 mg 272 DHC and 13 mg flavonols). Table 1 also showed that 1.4 g of the phenolic extract obtained 273 from the apple mix and used to supplement the test meals provided similar amounts of flavan-274 3-ol monomers and DHC than a serving of raw apple, whereas the dosage for flavonols was 275 half (8 mg versus 13 mg) and that of hydroxycinnamic acids 10% lower.

276

277 Effect of apple matrices on the serum distribution of flavan-3-ols

The distribution of serum flavan-3-ol metabolites was analyzed with UPLC-Q-TOF MS using authentic standards. Flavan-3-ol metabolites were quantified in the low nM range upon HFM that did not include apple products (Figure 1A). The consumption of the HFM containing raw apple, apple puree or the phenolic extract generated a rapid and significant increase of total flavan-3-ol monomers in serum as compared to the non-supplemented HFM. Despite the administered dose of flavan-3-ols was similar between the three apple products tested, the intake of the apple phenolic extract generated a significant higher concentration of flavan-3-ol metabolites compared to the raw apple or puree. The concentration of flavan-3-ol metabolites in serum 3 hours after the consumption of apple phenolic extract reached 996 \pm 359 nM, whereas a 55 % and 60 % lower concentration was reached after the intake of raw apple and puree, respectively (Figure 1A).

A significant higher concentration in all flavan-3-ol metabolites was observed 3 hours after consumption of the apple-based meals with respect to 1 and 2h post-consumption (Figure 1B-E). (+)-Catechin were quantified in the very low nM range (6-7% of total flavan-3-ol metabolites) whereas (-)-epicatechin, 3-O- and 4-O-methylepicatechins were more abundant (24-28% EC; 65-70% MEC) upon apple-based meals providing similar ratio of (+)-catechin and (-)-epicatechin (Figure 2).

295

Effect of the apple matrices on the postprandial nutrigenomic response to flavan-3-ols in porcine PBMCs

298 To assess the ability of apple polyphenols in modulating postprandial changes in the 299 gene expression profile of circulating PBMCs, we performed microarray analysis on porcine 300 PBMCs isolated from blood 3h after consumption of the HFM supplemented or not with the 301 different flavanol-rich apple products. Principal Component Analysis (PCA) of the microarray 302 data showed gene expression patterns in response to the tested meals as three distinct clusters 303 (Figure 3A). The first cluster correspond to the gene expression profiles after consumption of 304 the HFM. This cluster was well separated from the two other clusters, one corresponding to 305 gene expression profile from animals receiving a HFM supplemented with raw apples or apple 306 puree, and a third cluster corresponding to gene expression profile of cells from animals 307 consuming the HFM supplemented with polyphenol extract. PCA also suggest that the gene

308 expression profile in PBMCs in response to the HFM + PP extract (*i.e.* food matrix free) was 309 different from those supplemented with raw apples and apple puree. Statistical analyses of the 310 obtained data identified 309 genes, as having a significant change in the expression with fold 311 change superior to 1.25 in at least one pairwise comparison of tested meals (Figure 3B, and 312 Suppl. Table 3). Hierarchical clustering of expression profile of these genes also showed that 313 the expression profile in the HFM + PP extract group is more distinct than the profiles from the 314 HFM + raw apples and HFM + apple puree groups. Moreover, we observed that the intake of 315 the HFM + PP extract affected the expression of 107 genes whereas the others meals, that results 316 in a 2-fold lower concentrations in circulating flavanols, modulated the expression of 182 and 317 246 genes for the HFM + raw apples and HFM + apple puree, respectively (Figure 3C). 318 Comparison of the nutrigenomic effect of the tested meals allowed the identification of 59 319 overlapping genes, *i.e.* modulated by the 3 flavanol-rich meals (Figure 3D). We also observed 320 that 63% of the genes were modulated exclusively in response to the consumption of the HFM 321 supplemented with raw apple and/or apple puree. Among the set of 93 genes modulated by 322 HFM + PP extract and HFM + raw apples and/or HFM + apple puree, 74 genes exhibited similar 323 changes with respect to HFM + PP extract (Suppl. Table 4) suggesting that the food matrix (raw 324 apple or puree) affects about 20% of the gene modulated by the HFM + PP extract.

Next, we focused on the 105 genes modulated by HFM+PP extract when compared to the unsupplemented HFM and determined how the food matrix affect the expression of this set of genes. We observed significant changes in the gene expression for 49 and 61 genes in presence of raw apple and apple puree respectively, whereas the other genes were unchanged.

329

Biological processes associated with the nutrigenomic response of PBMCs to
 flavan-3-ol supplemented HFM

332 Pathway enrichment analysis obtained by submitting the list of 182, 246 and 107 genes 333 with a significant FC>1.25 in response to the consumption of HFM supplemented with raw 334 apples, apple puree and PP extract respectively, is presented in Figure 4. This analysis showed 335 that the consumption of the HFM with a flavan-3-ol supplementation can modulate significantly 336 a set of 54 pathways (figure 4A). Particularly, we observed that whatever their sources (raw 337 apple, puree or PP extract) flavan-3-ols always affects the expression of genes involved in acute 338 inflammation (e.g., CCL4, CCL5, CXCR4, IL10RB, TNFSF13) and in the control of the 339 leukocyte transendothelial migration (e.g., CDC42, CLDN4, CLDN7, MMP2). We also 340 observed that HFM supplemented with food matrix-conveyed flavan-3-ols (raw apples and 341 apple puree) may impact more pathways than a free-food matrix supplementation of flavan-3-342 ols, notably other pathways contributing to inflammation (e.g., NF-KB signaling pathway), to 343 transendothelial migration (e.g., Rap1 signaling pathway, cell adhesion molecules, adherens 344 junction) or involved in other biological processes (e.g., complement and coagulation 345 cascades). To further capture the biological processes impacted by the flavan-3-ol 346 supplementation, enriched terms have been rendered as a network plot (figure 4B). In this 347 network, enriched terms were connected and clustered by similarities. Based on the top-5 348 percentage of expressed genes in response to the test meals found in the given ontology term, 349 this network revealed that the control of inflammation and leukocyte transendothelial migration 350 (cell-cell adhesions, immune cell regulation, cytoskeleton reorganization, protein localization) 351 and of the clotting cascade were the main biological processes affected in PBMCs by the 352 consumption of HFM supplemented with apple flavan-3-ols. Flavan-3-ols supplementation also 353 affects the peroxisome proliferator-activated receptor gamma (PPARy)-signaling pathway 354 activated in response to HFM.

355 **Discussion**

356 In the present study, we investigate the effect of the apple matrix and processing on the 357 absorption and the postprandial nutrigenomic response to an acute intake of flavan-3-ol 358 monomers. To address this question, we compared two usual modes of apple consumption, 359 including raw fruit and puree, to the intake of an apple phenolic extract in mini-pigs fed with a 360 high fat meal. The mini pig model was used because of its known relevance to mimick the 361 human digestive physiology (34, 35). Despite the absence of appendix, a more developed cecum 362 and the spiral arrangement of the colon in pig, there are many notable anatomical, histological 363 (e.-g. epithelial cell and enterocyte population) and functional (e.-g. amino-acids digestion and 364 absorption) similarities between the porcine and the human gastro-intestinal tracts which make 365 the porcine model a powerful tool for studying human nutrient digestion and absorption (35-366 37).

367 Previous controlled RCTs have consistently reported improvement in endothelial 368 function in response to an acute intake of flavan-3-ols (100-200 mg EC) with a maximum effect 369 coinciding with the peak of EC in plasma observed at 2-3h post-consumption (15, 38, 39). 370 Similarly, improvements in plasma cytokines involved in cellular adherence and inflammation 371 have been observed in subjects consuming from 100 to 350 mg EC (40). Based on these studies 372 and in agreement with the acceptable diet-supplementation limits for minipigs (up to 250 g), 373 we composed in this study a mix of dessert and cider apple cultivars (Reinette de Flandre and Bedan respectively) providing about 150 mg flavan-3-ol monomers per serving. The puree and 374 375 the phenolic extract produced from this apple mix allowed to supplement the experimental 376 meals with similar amounts of flavan-3-ols.

377 Generally, the food matrix is viewed as a physical domain that contains and/or interact 378 with specific food constituents (nutrients, micronutrients, fibers and phytochemicals) providing 379 functionalities and behaviors which are different from those exhibited by a given isolated 380 constituent (41). In case of fruits or vegetables, the matrix refers to the entrapment inside cell 381 walls of microstructural elements and cell vacuoles containing nutrients and functional 382 phytochemicals and this matrix can affect their digestibility (42). In this minipig study, the 383 assessment of the postprandial concentration of flavan-3-ol monomers did not reveal any impact 384 of the apple matrix on the distribution profile of flavan-3-ols in serum, with an unchanged ratio 385 between methylated (2/3) and unmethylated (1/3) forms whatever the apple product 386 administered. This similarity of the serum profiles regardless of the apple products indicates 387 that the phase II metabolism of flavan-3-ol monomers is not affected by the food matrix. By 388 contrast, our results showed that the mode of administration of apple flavan-3-ols can influence 389 their serum concentrations, as showed by the half reduction of their total circulating levels when 390 consumed as a meal supplemented with raw apple or pure instead of phenolic extract. This 391 result is in agreement with a previous RCTs reporting higher plasma concentrations of EC 392 (about +40%) after the ingestion of an EC-rich apple extract incorporated in a water-based 393 beverage compared to the same amount of epicatechin consumed from whole apple (27). In 394 addition to confirming that the bioavailability of apple flavan-3-ols is better without matrix, our 395 results also highlighted that raw apple or puree affected the flavan-3-ols bioavailability with the 396 same magnitude, indicating that the processing of apple into puree did not affect the 397 bioavailability of these compounds. It is well established that the bioavailability of polyphenols 398 depends on the quantity of molecules released from the solid food matrix during digestion that 399 may be able to cross the intestinal barrier and then become available for metabolism (24). 400 According to food composition databases, apples are mainly constituted by water (85%) and 401 carbohydrates (14%) including fibres and sugars. The reduced postprandial absorption of 402 flavan-3-ols we observed when consumed as raw fruit or puree may originate from apple fibres 403 which may hamper bioaccessibility, and from an increased viscosity of the food bolus due to 404 apple pectins. However, because the present study was carried out during the first 3 hours of the postprandial period only, it is not possible to determine if the reduced effect of the matrix
on the maximal serum flavan-3-ol concentrations that we observed, reflected only a slowdown
of the intestinal absorption or rather an actual decrease of flavan-3-ol bioavailability.

408 A RCT revealed that an acute consumption of flavonoid-rich apples, supplying 180 mg 409 quercetin and 180 mg epicatechin, lowered systolic blood pressure and improved endothelium-410 dependent brachial artery flow-mediated dilatation (FMD) in healthy volunteers (13). However, 411 other studies failed to reveal subtle changes in biomarkers of cardiovascular risk in response to 412 apples or apple-polyphenol extract in fasted conditions during which no metabolic challenges 413 occur (11, 43). During the postprandial state, the body is responding with compensatory and 414 adaptive mechanisms managing the short-term metabolic stress to restore 415 balance/homoeostasis. Particularly in light of Western eating patterns, the postprandial period 416 also coincides with the peak of plasma concentrations of many plant bioactives, especially 417 flavonoids (8). Therefore we examined in this study the potency of apple flavan-3-ols intake to 418 attenuate the transient inflammatory response induced by a postprandial metabolic stress (22, 419 31), which is known to promote interactions between activated leukocytes and activated 420 endothelium which are at the initiation of atherogenesis (44).

421 PBMCs are recognized as a valuable source of material to investigate changes in metabolic and 422 inflammatory processes by assessing whole genome transcriptional variations (45, 46). This 423 approach has been used previously in several clinical and preclinical dietary interventions with 424 polyphenol-rich foods or polyphenol extracts (47-50). Here, we reported that the consumption 425 of apple products rich in flavan-3-ols induced changes in the gene expression profile of PBMCs 426 collected after the HFM. Interestingly, the profile of the HFM+ phenolic extract group was 427 more distinct than those from the HFM+raw apple and HFM+puree groups with a lower number 428 of modulated genes (extract: 107; raw apple: 182; puree: 246). The higher number of genes 429 found as modulated by the puree when compared to raw apple also suggests that the processing 430 into puree increased the bioavailability of some compounds, that could contribute with flavan-431 3-ols in the postprandial nutrigenomic response. The composition of the apple products (Suppl.Table 1) pointed out two other flavonoid classes as potential contributors. These 432 433 flavonoids include flavonols (mainly identified as quercetin glycosides) and dihydrochalcones 434 (DHC) which were less abundant in the administered dose of extract (53 mg) than in the raw 435 apple (58 mg) and puree (80 mg). The plasma concentrations of quercetin have been previously 436 reported to peak within 100 to 220 min after the intake of an apple peel extract (51-54). In 437 addition, in human subjects the co-ingestion of quercetin and pectins, at levels comparable to 438 those present in apples, has been reported to improve the absorption of quercetin (55). 439 Regarding DHC, which is concentrated in apple seeds and peel (56), its high content in the 440 puree could originate from an increased extraction and solubilization occurring during the 441 crushing and heating phases of the puree processing. A rapid transient peak of phloridzin 442 metabolites in plasma at 0.7-1 hour has been previously reported after consumption of cider or 443 phloridzin extract respectively (57, 58). Because potentially better bioavailable from puree, 444 absorbed DHC may explain the difference in the nutrigenomic response between puree and raw 445 apple. Taken together, these bibliographic data and our results support the contribution of the 446 circulating flavonols and DHC in the modulation of gene expression profile of PBMC in 447 response to the intake of the flavan-3-ols rich apple products tested and in the differences we 448 observed between groups.

Enrichment analyses of differentially expressed genes reveal a core of biological processes commonly modulated in response to the consumption of apples, puree and polyphenol extract. These processes are involved in the control of inflammation and leukocyte transendothelial migration both contributing to the early stage of the atherogenesis. It is worth to note that the subtle changes in gene expression induced by apple matrix do not compromise the antiinflammatory effect of flavan-3-ols previously reported in PBMCs from male smokers exposed 455 to grape seed flavanols (57) and from healthy young adults who consumed 93 mg cocoa flavan-456 3-ols (mainly (-)-epicatechin) (59). Similar biological processes were modulated in blood cells from healthy volunteers upon orange juice intake or of its main flavonoid (50), blueberry intake 457 458 (61) and from healthy subjects and patients with metabolic syndrome after acute intake of extra 459 virgin olive oil (62). These results support a common response to polyphenols in blood cells 460 characterized by an anti-inflammatory feature contributing to attenuate the activation and 461 migration of immune cells to the endothelium which constitute early steps of vascular 462 dysfunction and atherosclerosis development.

463 In summary, the present study suggests that the apple matrix, in its original form or after 464 processing into puree, affects the bioaccessibility of apple flavan-3-ols resulting in a lower 465 postprandial serum concentration. However, this effect did not induced any negative impact in 466 the nutrigenomic response of PBMCs to apple polyphenosl as reflected by the higher number 467 of modulated genes in the presence of the apple matrix, probably due to the individual or 468 synergic nutrigenomic effect of other apple phytochemicals released and better bioavailable. 469 Interestingly, it can be highlihted that the processing of raw apple into puree amplified the 470 nutrigenomic response, probably by increasing the bioavailability of phytochemicals present in 471 the matrix that are less bioaccessible in the raw fruit. Overall, the observed changes in gene 472 expression profiles following apple product intake suggest modulation of a range of biological 473 processes which could counteract the pro-inflammatory response induced by a high fat meal. 474 In view of its improved nutrigenomic response, apple pure consumption could be particularly 475 recommended in seniors, all the more this population often suffers from mastication and 476 swallowing disturbances and need soft but cohesive food matrices.

477

478 **Statement of authorship**

The authors' responsibilities were as follows: LEM performed statistical and bioinformatics analyses, analyzed the data and wrote the paper; CLB and CD characterized and prepared the apple products, analyzed the data; CB and DR conducted the intervention study in minpigs and collected blood samples, and wrote related sections; SM, DB, and CB prepared the samples and carried out microarray analysis; GI and ARM analyzed circulating flavan-3-ols; DM, PB and CM carried out study design, data interpretation and manuscript preparation. All authors have carefully reviewed and then approved this manuscript.

486

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490

491 **Conflict of interest**

492 There are no conflicts of interest to declare.

493

494 Legends

495

Table 1: Phenolic composition and contents (mg) in raw apple (250 g), apple puree (250
g) and phenolic extract (1.4 g) administrated in the study.

498

499 Figure 1: Postprandial concentrations of flavan-3-ol monomers in minipig serum after 500 consumption of the different apple derived products. (A) total monomeric flavan-3-ols, (B) 501 (+)-catechin, (C) (-)-epicatechin, (D) 3-O-methylepicatehcin and (E) 4-O-methylepicatechin in 502 1h-, 2h and 3h postprandial sera. Graphs represent mean +/- SEM (n=5). * indicates a significant 503 difference (*:p<0.05; **: p<0.01; ***:p<0.001) in the kinetic curve in comparison with that 504 obtained after the intake of HFM alone. § indicates significant differences between 505 concentration values at a given time point when compared to the condition HFM+ PP extract. 506 The insert in A represents the area under the curve (AUC) of the total flavan-3-ol concentrations 507 measured from 0 to 3 h after intake of the different experimental meals. a: p < 0.01 and p < 0.001508 when compared to b,and c respectively. b: p < 0.05 whn compared to c.

509

Figure 2: Distribution of flavan-3-ol metabolites in sera at 3h after intake of the different
apple derived products.

512

Figure 3: Post-prandial nutrigenomic response in porcine PBMCs at 3 h after consumption of apple products. (A) The Principal Component Analysis (PCA) shows gene expression patterns in response to HFM without polyphenols, meals containing either apple polyphenols within their food matrix (raw apple and apple puree) or polyphenols extract (without matrix). (B) Hierarchical clustering dendrogram build on the Pearson distance and heat map of gene expression profile of 951 probes with a significant FC>1.25 in at least one experimental condition (Friedman with a posteriori Wilcoxon tests). The gene expression profiles are hierarchically clustered with Pearson Distance test using PermutMatrix Software. (C) Number of differentially expressed genes (with a FC>1.25 in comparison to the HFM) in response to the three test meals are graphically represented by function of the monomeric flavan-3-ols concentration detected in 3h-postprandial serum. (D) Vein diagram shows the number of common differentially expressed genes in response to the 3 test meals containing apple polyphenols.

526

527 Figure 4: Functional enrichment analysis of the differentially expressed genes in PBMCs.

528 (A) List of KEGG pathways provided by GeneTrail 2.0 with a number of hits superior of 3 and 529 with a p-value < 0.01 were presented. (B) Network of enriched terms have been carried out with 530 a p-value < 0.01, a minimum of count of 3 and an enrichment factor > 1.5, and then rendered 531 as a network plot using Metascape (http://metascape.org). Terms with a similarity > 0.3 have 532 been connected by edges. Nodes are represented as pie charts, where the size of pie is 533 proportional to the total number of genes that fall into that specific pathway for each test meal. 534 The coverage of modulated genes within enriched pathway clusters (expressed in percent) and 535 the enrichment p-value were noted in italics.

536

537

Table 1: Polyphenol content in the administered test meals

	Raw apples (mg/250g)	Apple Puree (mg/250g)	Phenolic extract (mg/1.4g)
Total Flavanols	957	717	891
Catechin	50	49	50
Epicatechin	104	104	106
Procyanidins	803	564	735
Total Dihydrochalcones	45	66	45
Total Flavonols ^a	13	14	8
Total Hydroxycinnamic acid	233	216	208
Total Polyphenols	1248	1013	1152

Above data were calculated from data presented in the supplemental table 2. ^aquantified as quercetin.





Figure 2









Figure 4A

KEGG -Pathways	HFM + Raw apples		HFM + Apple Puree		HFM + PP extract	
	Number of hits	p-value	Number of hi	its p-value	Number of hits	p-value
Cytokine-cytokine receptor interaction	6	4,1E-04	5	9,5E-03	6	2,0E-05
Leukocyte transendothelial migration	6	1,4E-05	6	7,7E-05	4	2,2E-04
Pathways in cancer	9	8,8E-06	11	2,6E-06	5	1,0E-03
Tight junction	4	2,2E-03	5	9,2E-04	3	3,8E-03
Herpes simplex infection			5	4,0E-03	3	9,9E-03
Hippo signaling pathway			4	7,6E-03	3	4,4E-03
HTLV-I infection			9	1,9E-05	6	3,9E-05
Chemokine signaling pathway	4	6,2E-03			4	8,6E-04
Dilated cardiomyopathy					3	1,3E-03
Jak-STAT signaling pathway					3	6,0E-03
RIG-I-like receptor signaling pathway					3	6,6E-04
Complement and coagulation cascades	3	3,8E-03	4	9,2E-04		
Glioma	3	2,3E-03	5	3,3E-05		
Huntington's disease	4	8,9E-03	5	5,1E-03		
Intestinal immune network for IgA production	3	9,9E-04	3	2,4E-03		
Melanoma	3	3,1E-03	3	7,3E-03		
NF-kappa B signaling pathway	3	6,5E-03	4	1,8E-03		
Non-small cell lung cancer	3	1,4E-03	4	2,5E-04		
Pancreatic cancer	3	2,7E-03	5	4,5E-05		
PPAR signaling pathway	3	3,3E-03	5	5,9E-05		
Prostate cancer	3	5,7E-03	4	1,5E-03		
Proteoglycans in cancer	6	2,9E-04	7	2,4E-04		
Rap1 signaling pathway	7	3,3E-05	9	4,0E-06		
Rheumatoid arthritis	4	5,5E-04	5	1,7E-04		
Spliceosome	4	2,6E-03	4	7,6E-03		
ErbB signaling pathway	3	5,9E-03				
Ribosome	5	3,2E-04				
Toll-like receptor signaling pathway	3	9,3E-03				
Adherens junction			3	8,5E-03		
Antigen processing and presentation			3	6,1E-03		
Bladder cancer			3	1,3E-03		
Cell adhesion molecules (CAMs)			4	8,2E-03		
Chronic myeloid leukemia			3	7,6E-03		
Colorectal cancer			3	8,2E-03		
Cytosolic DNA-sensing pathway			3	5,9E-03		
Dilated cardiomyopathy			4	1,7E-03		
Epstein-Barr virus infection			5	4,9E-03		
Fc gamma R-mediated phagocytosis			4	1,4E-03		
GABAergic synapse			4	1,7E-03		
Gap junction			4	1,8E-03		
Gastric acid secretion			3	8,5E-03		
GnRH signaling pathway			4	1,8E-03		
Hepatitis C			4	6,6E-03		
Long-term potentiation			3	7,9E-03		
Lysosome			4	4,1E-03		
Melanogenesis			4	2,3E-03		
Phagosome			6	1,9E-04		
Renal cell carcinoma			3	6,1E-03		
Salivary secretion			4	1,1E-03		
Thyroid hormone synthesis			3	8,8E-03		
Tuberculosis			5	3,2E-03		
Vascular smooth muscle contraction			4	5,0E-03		
Viral myocarditis			3	5,1E-03		
Wnt signaling pathway			4	6,8E-03		

Figure 4B



REFERENCES

1. Wang X, Ouyang Y, Liu J, Zhu M, Zhao G, Bao W, et al. Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: systematic review and dose-response meta-analysis of prospective cohort studies. BMJ. 2014;349:g4490.

2. Zhao CN, Meng X, Li Y, Li S, Liu Q, Tang GY, et al. Fruits for Prevention and Treatment of Cardiovascular Diseases. Nutrients. 2017;9(6).

3. European Statistics Handbook - Fruit Logistica. 2019.

4. Hodgson JM, Prince RL, Woodman RJ, Bondonno CP, Ivey KL, Bondonno N, et al. Apple intake is inversely associated with all-cause and disease-specific mortality in elderly women. Br J Nutr. 2016;115(5):860-7.

5. Ceymann M, Arrigoni E, Schärer H, Bozzi Nising A, Hurrellb RF. Identification of apples rich in health-promoting flavan-3-ols and phenolic acids by measuring the polyphenol profile. Journal of Food Composition and Analysis. 2012;26(1-2):128-35.

6. Vrhovsek U, Rigo A, Tonon D, Mattivi F. Quantitation of polyphenols in different apple varieties. J Agric Food Chem. 2004;52(21):6532-8.

7. Wojdylo A, Oszmianski J, Laskowski P. Polyphenolic compounds and antioxidant activity of new and old apple varieties. J Agric Food Chem. 2008;56(15):6520-30.

8. Manach C, Williamson G, Morand C, Scalbert A, Remesy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. Am J Clin Nutr. 2005;81(1 Suppl):230S-42S.

9. Monagas M, Urpi-Sarda M, Sanchez-Patan F, Llorach R, Garrido I, Gomez-Cordoves C, et al. Insights into the metabolism and microbial biotransformation of dietary flavan-3-ols and the bioactivity of their metabolites. Food Funct. 2010;1(3):233-53.

10. Ottaviani JI, Borges G, Momma TY, Spencer JP, Keen CL, Crozier A, et al. The metabolome of [2-(14)C](-)-epicatechin in humans: implications for the assessment of efficacy, safety, and mechanisms of action of polyphenolic bioactives. Sci Rep. 2016;6:29034.

11. Auclair S, Milenkovic D, Besson C, Chauvet S, Gueux E, Morand C, et al. Catechin reduces atherosclerotic lesion development in apo E-deficient mice: a transcriptomic study. Atherosclerosis. 2009;204(2):e21-7.

12. Koutsos A, Lima M, Conterno L, Gasperotti M, Bianchi M, Fava F, et al. Effects of Commercial Apple Varieties on Human Gut Microbiota Composition and Metabolic Output Using an In Vitro Colonic Model. Nutrients. 2017;9(6).

13. Bondonno CP, Yang X, Croft KD, Considine MJ, Ward NC, Rich L, et al. Flavonoid-rich apples and nitrate-rich spinach augment nitric oxide status and improve endothelial function in healthy men and women: a randomized controlled trial. Free Radic Biol Med. 2012;52(1):95-102.

14. Bondonno NP, Bondonno CP, Blekkenhorst LC, Considine MJ, Maghzal G, Stocker R, et al. Flavonoid-Rich Apple Improves Endothelial Function in Individuals at Risk for Cardiovascular Disease: A Randomized Controlled Clinical Trial. Mol Nutr Food Res. 2018;62(3).

15. Schroeter H, Heiss C, Balzer J, Kleinbongard P, Keen CL, Hollenberg NK, et al. (-)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. Proc Natl Acad Sci U S A. 2006;103(4):1024-9.

16. Tangney CC, Rasmussen HE. Polyphenols, inflammation, and cardiovascular disease. Curr Atheroscler Rep. 2013;15(5):324.

17. Morrison M, van der Heijden R, Heeringa P, Kaijzel E, Verschuren L, Blomhoff R, et al. Epicatechin attenuates atherosclerosis and exerts anti-inflammatory effects on diet-induced human-CRP and NFkappaB in vivo. Atherosclerosis. 2014;233(1):149-56.

18. Claude S, Boby C, Rodriguez-Mateos A, Spencer JP, Gerard N, Morand C, et al. Flavanol metabolites reduce monocyte adhesion to endothelial cells through modulation of expression of genes via p38-MAPK and p65-Nf-kB pathways. Mol Nutr Food Res. 2014;58(5):1016-27.

19. Krga I, Milenkovic D, Morand C, Monfoulet LE. An update on the role of nutrigenomic modulations in mediating the cardiovascular protective effect of fruit polyphenols. Food Funct. 2016;7(9):3656-76.

20. Milenkovic D, Berghe WV, Morand C, Claude S, van de Sandt A, Gorressen S, et al. A systems biology network analysis of nutri(epi)genomic changes in endothelial cells exposed to epicatechin metabolites. Sci Rep. 2018;8(1):15487.

21. Burton-Freeman B. Postprandial metabolic events and fruit-derived phenolics: a review of the science. Br J Nutr. 2010;104 Suppl 3:S1-14.

22. Herieka M, Erridge C. High-fat meal induced postprandial inflammation. Mol Nutr Food Res. 2014;58(1):136-46.

23. Borges G, Ottaviani JI, van der Hooft JJJ, Schroeter H, Crozier A. Absorption, metabolism, distribution and excretion of (-)-epicatechin: A review of recent findings. Mol Aspects Med. 2018;61:18-30.

24. Ribas-Agusti A, Martin-Belloso O, Soliva-Fortuny R, Elez-Martinez P. Food processing strategies to enhance phenolic compounds bioaccessibility and bioavailability in plant-based foods. Crit Rev Food Sci Nutr. 2018;58(15):2531-48.

25. Dufour C, Loonis M, Delosiere M, Buffiere C, Hafnaoui N, Sante-Lhoutellier V, et al. The matrix of fruit & vegetables modulates the gastrointestinal bioaccessibility of polyphenols and their impact on dietary protein digestibility. Food Chem. 2018;240:314-22.

26. Bohn T. Dietary factors affecting polyphenol bioavailability. Nutr Rev. 2014;72(7):429-52. 27. Hollands WJ, Hart DJ, Dainty JR, Hasselwander O, Tiihonen K, Wood R, et al. Bioavailability of epicatechin and effects on nitric oxide metabolites of an apple flavanol-rich extract supplemented beverage compared to a whole apple puree: a randomized, placebo-controlled, crossover trial. Mol Nutr Food Res. 2013;57(7):1209-17.

28. Le Bourvellec C, Bouzerzour K, Ginies C, Regis S, Plé Y, Renard CMGC. Phenolic and polysaccharide composition of applesauce is close to that of apple flesh. Journal of Food Composition and Analysis. 2011;24:537-47.

29. Guyot S, Marnet N, Drilleau J. Thiolysis-HPLC characterization of apple procyanidins covering a large range of polymerization states. J Agric Food Chem. 2001;49(1):14-20.

30. Remond D, Buffiere C, Godin JP, Mirand PP, Obled C, Papet I, et al. Intestinal inflammation increases gastrointestinal threonine uptake and mucin synthesis in enterally fed minipigs. J Nutr. 2009;139(4):720-6.

31. van Oostrom AJ, Sijmonsma TP, Verseyden C, Jansen EH, de Koning EJ, Rabelink TJ, et al. Postprandial recruitment of neutrophils may contribute to endothelial dysfunction. J Lipid Res. 2003;44(3):576-83.

32. Feliciano RP, Mecha E, Bronze MR, Rodriguez-Mateos A. Development and validation of a high-throughput micro solid-phase extraction method coupled with ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry for rapid identification and quantification of phenolic metabolites in human plasma and urine. J Chromatogr A. 2016;1464:21-31.

33. Tripathi S, Pohl MO, Zhou Y, Rodriguez-Frandsen A, Wang G, Stein DA, et al. Meta- and Orthogonal Integration of Influenza "OMICs" Data Defines a Role for UBR4 in Virus Budding. Cell Host Microbe. 2015;18(6):723-35.

34. Miller ER, Ullrey DE. The pig as a model for human nutrition. Ann Rev Nutr. 1987;7:361-82.

35. Rowan AM, Moughan PJ, Wilson MN, Maher K, Tasman-Jones C. Comparison of the ileal and faecal digestibility of dietary amino acids in adult humans and evaluation of the pig as a model animal for digestion studies in man. Br J Nutr. 1994;71(1):29-42.

36. Gandarillas M, Bas F. The domestic pig (*Sus scrofa domestica*) as a model for evaluating nutritional and metabolic consequences of bariatric surgery practiced on morbid obese humans. Cien Inv Agr. 2009;36(2):163-76.

37. Ziegler A, Gonzalez L, Blikslager A. Large Animal Models: The Key to Translational Discovery in Digestive Disease Research. Cell Mol Gastroenterol Hepatol. 2016;2(6):716-24.

38. Heiss C, Dejam A, Kleinbongard P, Schewe T, Sies H, Kelm M. Vascular effects of cocoa rich in flavan-3-ols. JAMA. 2003;290(8):1030-1.

39. Hooper L, Kay C, Abdelhamid A, Kroon PA, Cohn JS, Rimm EB, et al. Effects of chocolate, cocoa, and flavan-3-ols on cardiovascular health: a systematic review and meta-analysis of randomized trials. Am J Clin Nutr. 2012;95(3):740-51.

40. Esser D, Mars M, Oosterink E, Stalmach A, Muller M, Afman LA. Dark chocolate consumption improves leukocyte adhesion factors and vascular function in overweight men. FASEB J. 2014;28(3):1464-73.

41. Aguilera JM. The food matrix: implications in processing, nutrition and health. Crit Rev Food Sci Nutr. 2019;59(22):3612-29.

42. Ogawa Y, Donlao N, Thuengtung S, Tian J, Cai Y, Reginio Jr. FC, et al. Impact of food structure and cell matrix on digestibility of plant-based food. Current Opinion in Food Science. 2018;19:36-41.

43. Hollands WJ, Tapp H, Defernez M, Perez Moral N, Winterbone MS, Philo M, et al. Lack of acute or chronic effects of epicatechin-rich and procyanidin-rich apple extracts on blood pressure and cardiometabolic biomarkers in adults with moderately elevated blood pressure: a randomized, placebo-controlled crossover trial. Am J Clin Nutr. 2018;108(5):1006-14.

44. Alipour A, Elte JW, van Zaanen HC, Rietveld AP, Cabezas MC. Postprandial inflammation and endothelial dysfuction. Biochem Soc Trans. 2007;35(Pt 3):466-9.

45. Diaz-Rua R, Keijer J, Caimari A, van Schothorst EM, Palou A, Oliver P. Peripheral blood mononuclear cells as a source to detect markers of homeostatic alterations caused by the intake of diets with an unbalanced macronutrient composition. J Nutr Biochem. 2015;26(4):398-407.

46. Leonardson AS, Zhu J, Chen Y, Wang K, Lamb JR, Reitman M, et al. The effect of food intake on gene expression in human peripheral blood. Hum Mol Genet. 2010;19(1):159-69.

47. Afman L, Milenkovic D, Roche HM. Nutritional aspects of metabolic inflammation in relation to health--insights from transcriptomic biomarkers in PBMC of fatty acids and polyphenols. Mol Nutr Food Res. 2014;58(8):1708-20.

48. Barber-Chamoux N, Milenkovic D, Verny MA, Habauzit V, Pereira B, Lambert C, et al. Substantial Variability Across Individuals in the Vascular and Nutrigenomic Response to an Acute Intake of Curcumin: A Randomized Controlled Trial. Mol Nutr Food Res. 2018;62(5).

49. Lopez S, Bermudez B, Montserrat-de la Paz S, Abia R, Muriana FJG. A microRNA expression signature of the postprandial state in response to a high-saturated-fat challenge. J Nutr Biochem. 2018;57:45-55.

50. Milenkovic D, Deval C, Dubray C, Mazur A, Morand C. Hesperidin displays relevant role in the nutrigenomic effect of orange juice on blood leukocytes in human volunteers: a randomized controlled cross-over study. PLoS One. 2011;6(11):e26669.

51. Cermak R, Landgraf S, Wolffram S. The bioavailability of quercetin in pigs depends on the glycoside moiety and on dietary factors. J Nutr. 2003;133(9):2802-7.

52. Krogholm KS, Bredsdorff L, Knuthsen P, Haraldsdottir J, Rasmussen SE. Relative bioavailability of the flavonoids quercetin, hesperetin and naringenin given simultaneously through diet. Eur J Clin Nutr. 2010;64(4):432-5.

53. Lee J, Mitchell AE. Pharmacokinetics of quercetin absorption from apples and onions in healthy humans. J Agric Food Chem. 2012;60(15):3874-81.

54. Lesser S, Cermak R, Wolffram S. Bioavailability of quercetin in pigs is influenced by the dietary fat content. J Nutr. 2004;134(6):1508-11.

55. Nishijima T, Iwai K, Saito Y, Takida Y, Matsue H. Chronic ingestion of apple pectin can enhance the absorption of quercetin. J Agric Food Chem. 2009;57(6):2583-7.

56. Guyot S, Marnet N, Laraba D, Sanoner P, Drilleau J-F. Reversed-Phase HPLC following Thiolysis for Quantitative Estimation and Characterization of the Four Main Classes of Phenolic Compounds in Different Tissue Zones of a French Cider Apple Variety (Malus domestica Var. Kermerrien). Journal of Agricultural and Food Chemistry. 1998;46:1698-705.

57. Marks SC, Mullen W, Borges G, Crozier A. Absorption, metabolism, and excretion of cider dihydrochalcones in healthy humans and subjects with an ileostomy. J Agric Food Chem. 2009;57(5):2009-15.

58. Wang Z, Gao Z, Wang A, Jia L, Zhang X, Fang M, et al. Comparative oral and intravenous pharmacokinetics of phlorizin in rats having type 2 diabetes and in normal rats based on phase II metabolism. Food Funct. 2019;10(3):1582-94.

59. Barrera-Reyes PK, Hernandez-Ramirez N, Cortes J, Poquet L, Redeuil K, Rangel-Escareno C, et al. Gene expression changes by high-polyphenols cocoa powder intake: a randomized crossover clinical study. Eur J Nutr. 2019;58(5):1887-98.

60. Milenkovic D, Vanden Berghe W, Boby C, Leroux C, Declerck K, Szarc vel Szic K, et al. Dietary flavanols modulate the transcription of genes associated with cardiovascular pathology without changes in their DNA methylation state. PLoS One. 2014;9(4):e95527.

61. Rodriguez-Mateos A, Istas G, Boschek L, Feliciano RP, Mills CE, Boby C, et al. Circulating Anthocyanin Metabolites Mediate Vascular Benefits of Blueberries: Insights From Randomized Controlled Trials, Metabolomics, and Nutrigenomics. J Gerontol A Biol Sci Med Sci. 2019;74(7):967-76.

62. D'Amore S, Vacca M, Cariello M, Graziano G, D'Orazio A, Salvia R, et al. Genes and miRNA expression signatures in peripheral blood mononuclear cells in healthy subjects and patients with metabolic syndrome after acute intake of extra virgin olive oil. Biochim Biophys Acta. 2016;1861(11):1671-80.