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Commentary

Non-extractable polyphenols produce gut microbiota metabolites that persist in circulation and show anti-inflammatory and free radical-scavenging effects

Antonio González-Sarrías, Juan Carlos Espín, Francisco A. Tomás-Barberán*

Food & Health Laboratory, Research Group on Quality, Safety, and Bioactivity of Plant Foods, CEBAS-CSIC, P.O. Box 164, 30100 Campus de Espinardo, Murcia, Spain

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ABSTRACT

Recent studies demonstrate that fruits are rich in non-extractable polyphenols, macro-antioxidants, which have been underestimated. These are not absorbed and reach the colon where are catabolized by human gut microbiota releasing low molecular weight phenolics that are then absorbed efficiently. These metabolites persist in human plasma for extended times up to 3–4 days after the intake with significant concentrations. Preclinical studies with these metabolites at the concentrations that can be reached in plasma have reported anti-inflammatory and anti-oxidant effects that could be related to health benefits observed *in vivo* after the intake of the non-extractable macro-antioxidants.

1. Introduction

A recent re-evaluation of the polyphenol content of different food products (Pérez-Jiménez, Diaz-Rubio, & Saura-Calixto, 2015) has demonstrated that a significant part of these compounds had been underestimated due to difficulties for their extraction and analysis (Arranz, Saura-Calixto, Shaha, & Kroon, 2009). Thus, non-extractable phenolics have been evaluated in different food products, which are especially relevant in proanthocyanidin-containing foods (fruits, and juices and beverages obtained from them, and chocolate). These have also been recently described as macro-antioxidants (Pérez-Jiménez et al., 2015), as they can release antioxidant metabolites after catabolism by the human gut microbiota. In the present review, the absorption and excretion of metabolites obtained *in vivo* from non-extractable polyphenols in humans have been studied as well as the potential health effects evaluated using preclinical models that assay the physiologically relevant metabolites and at the concentrations that can be reached in human plasma.

2. Definition of non-extractable polyphenols

The occurrence of non-extractable polyphenols is known for a long time and was first studied in bark samples using thiolysis degradation methods (Matthews, Mila, Scalbert, & Donnely, 1997). Non-extractable polyphenols include all those phenolic compounds that are not extracted with the solvents of choice, and therefore are not assessed in most polyphenol analyses. The solvents of choice for extraction of polyphenols are methanol-water (8:2 vol:vol for fresh plant materials and 7:3 vol:vol for dried materials) and acetone-water mixtures. These extracts, however, do not allow the complete characterization and strong solvents like dimethylsulfoxide (DMSO) are needed for a complete solution of some polyphenols.

This approach has been recently applied to food products to conclude that the content of polyphenols, and particularly proanthocyanidins, has been widely underestimated (Arranz et al., 2009; Pérez-Jiménez et al., 2015). These antioxidant polyphenols have also been named as macromolecular antioxidants or macro-antioxidants (Pérez-Jiménez et al., 2015).

The non-extractable polyphenols concept can also be extended to other large molecular weight polyphenols as is the case of ellagitannins. These polymeric molecules can be difficult

* Corresponding author.
E-mail address: fatomas@cebas.csic.es (F.A. Tomás-Barberán).

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to quantify in the native form due to their structural complexity and to limitations in the resolution of the chromatographic methods used. Ellagitannin composition is better assessed after acid hydrolysis of food products without a previous extraction. For pomegranate ellagitannins the content after hydrolysis without extraction was two-fold higher than the content evaluated after extraction showing that degradation methods without a previous extraction provide more accurate results (García-Villalba et al., 2015).

In addition, this concept can also be applied to other polyphenols that are not chemically complex or oligomeric in nature such as the orange hesperidin. This flavanone shows solubility problems and precipitates to give an insoluble cloud in juices that make difficult their extraction and accurate quantification in foods (Tomás-Barberán & Clifford, 2000). This is also the case for ellagic acid, present in pomegranates, berries, and nuts, which often produces a haze due to self-association of such a planar molecule. Ellagic acid shows limited bioavailability (González-Sarrías et al., 2015) and has been reported to bind irreversibly to proteins and DNA (Whitley, Stoner, Darby, & Walle, 2003). As in the case of hesperidin, ellagic acid needs to be re-dissolved with strong solvents such as dimethylsulphoxide in order to be quantified in food products (González-Sarrías et al., 2015) (Fig. 1).

In this review paper we focus on three important groups of non-extractable polyphenols, flavanones (citrus fruits), ellagitannins and ellagic acid (pomegranate, walnuts, strawberries, other berries), and proanthocyanidins [many fruits (persimmons, apples, grapes), beverages (wine, tea) and cocoa]. All these polyphenols can be considered non-extractable antioxidants or macro antioxidants as they are not soluble in the food products and gastrointestinal content, thus limiting their absorption in the proximal gastrointestinal tract, and reach the colon where they are metabolized by the gut microbiota to release catabolites that are then absorbed.

3. Gut microbiota metabolism of non-extractable polyphenols

Non-extractable and high molecular weight polyphenols are not absorbed in the stomach or small intestine (proximal gastrointestinal tract) and reach the colon almost unaltered together with dietary fiber and other indigestible food constituents. In the gut, these polyphenols are not absorbed and display a two-way interaction with the resident microbiota. They modulate the gut microbial composition while the gut microbes catabolize these polyphenols leading to small size molecules that are much better

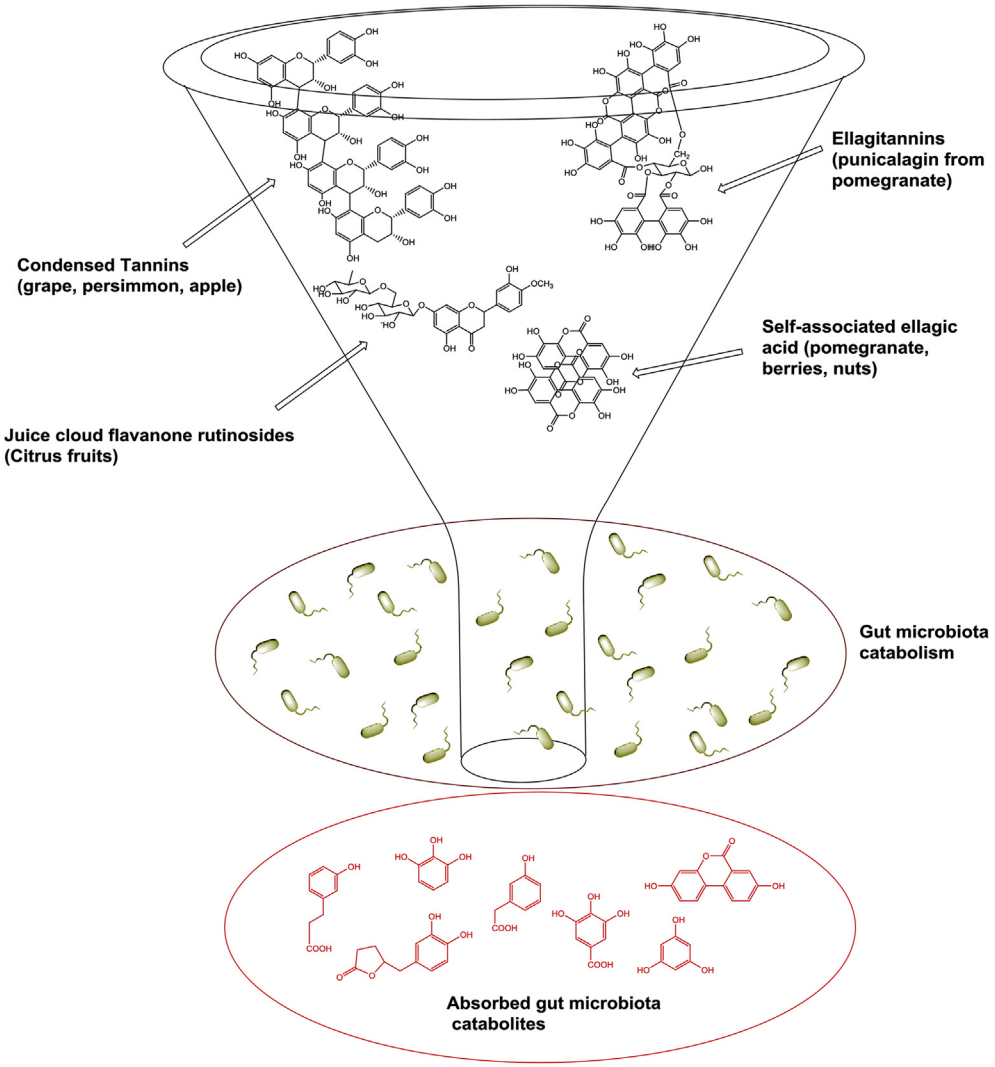

Fig. 1. Gut microbiota catabolism of non-extractable polyphenols leads to bioavailable low molecular weight phenolic metabolites.

absorbed, and that often show relevant biological effects (Selma, Espín, & Tomás-Barberán, 2009; Williamson & Clifford, 2010). In general, non-extractable polyphenols (proanthocyanidins and hydrolysable tannins) show a 'prebiotic-like' effect, promoting the growth of lactobacilli and bifidobacteria, and a positive modulation of the anti-obesity bacteria *Akkermansia muciniphila* and *Faecalibacterium prausnitzii.* They also decrease the Firmicutes/Bacteriodetes ratio which is also considered a beneficial effect (Tomás-Barberán, Selma, & Espín, 2016).

Gut microbes are also able to catabolize non-extractable polyphenols releasing small molecules that can exert additional health effects in the gut or be absorbed and often further metabolized by phase II metabolism leading the metabolites that circulate in plasma and are excreted in urine (Fig. 1). Bacterial species that deglycosylate dietary polyphenols in the gut include *Bacteroides*, *Enterococcus*, *Bifidobacterium*, *Blautia*, *Eubacterium*, and *Lactobacillus* (Braune & Blaut, 2016). The microbiota that cleave the polyphenol nucleus and reduce double bonds, dehydroxylate, and demethylate belong mainly to the families Coriobacteriaceae, including species of *Eggerthella*, *Paraeggerthella*, *Slackia*, *Adlercreutzia* and *Gordonibacter*, and Clostridiaceae (*Clostridium* and *Flavonifractor*). The family Coriobacteriaceae is particularly interesting as it has been associated with beneficial metabolic effects in obesity and diabetes (Clavel et al., 2014).

Non-extractable polyphenols can exert health benefits through modulation of microbes locally in the gut, thus indirectly showing health effects as gut microbiota correlates with health status (Tomás-Barberán et al., 2016). Some studies also show evidence of the causal effect of gut microbes in some ailments including obesity (Ridaura et al., 2013), cardiovascular disease (Koeth et al., 2013), and Parkinson's disease (Sampson et al., 2016), and non-extractable polyphenols can modulate gut microbiota composition and function and therefore have an indirect impact in these deseases.

There is a considerable inter-individual variability in the metabolism, absorption and excretion of the gut microbiota metabolites of non-extractable polyphenols (Manach et al., 2016). This is expected for gut microbiome metabolites due to the known significant variations in the gut microbiota composition as subjects can be stratified by their enterotypes (Arumugan et al., 2011), their microbiome diversity (Yatsunenko et al., 2012) and their metabotypes (Tomás-Barberán et al., 2016). Thus, gut microbiome composition and functionality can modulate the health effects of dietary polyphenols and explain, at least partly, the large inter-individual variability in health effects observed after non-extractable polyphenols interventions as recently reported for pomegranate ellagitannins (González-Sarrías et al., 2017).

4. Bioavailability of microbial catabolites of non-extractable polyphenols

Non-extractable polyphenols gut microbiota metabolites are known to be better absorbed than their precursors (Selma et al., 2009). Thus, proanthocyanidin oligomers are not absorbed, with the exception of dimers that are detected in plasma and urine although at very low concentrations (Baba, Osakabe, Natsume, & Terao, 2002). In the case of ellagitannins, there is no report showing their absorption in humans, although one study reported the presence of punicalagin in rat plasma after intake of large amounts of pomegranate ellagitannins (Cerdá, Llorach, Cerón, Espín, & Tomás-Barberán, 2003). Ellagitannins can release ellagic acid during food processing, and this can be slightly absorbed in the first portion of the gastrointestinal tract and detected unconjugated in plasma (González-Sarrías et al., 2015; Seeram et al., 2006). Citrus flavanone-rutinosides (hesperidin) are not absorbed as they cannot be hydrolysed by human intestinal enzymes. Thus, hesperidin has not been detected in plasma, and after hydrolysis by gut microbiota rhamnosidases to yield the aglycone hesperetin its phase II conjugates are detected in circulation (Vallejo et al., 2010).

The gut microbiota metabolites are however much better absorbed reaching significant concentrations in plasma (Table 1). Moreover, the presence of these gut microbiota metabolites persists for a long time in the human body, as it has been demonstrated for some of the metabolites. In the case of urolithins, the ellagitannin gut microbiota metabolites, they have been detected in human urine with a maximum excretion at 32 h but they persist up to 80 h after the intake of ellagitannin-containing food products (Fig. 2). This can be due to a combination of two different effects, the enterohepatic recirculation, and the moderate bioaccessibility of the ellagitannins and ellagic acid to the gut microbiota to enable their catabolic transformation into urolithins. In the case of the valerolactones produced by the gut microbiota from proanthocyanidins, a similar trend is observed with a maximum at 24 h and a significant excretion up to 48 h. This intervention, however, did not continue the sample collection beyond 48 h but the trend indicates that velerolactones could be detected in urine for longer times as for urolithins. In the case of 3-hydroxyphenyl acetic acid (3HPPA) produced from proanthocyanidins, the maximum excretion is observed after 48h, thus showing a long persistence of these microbiota metabolites in circulation in the human body as is also the case for 3,4-dihydroxyphenyl acetic acid (3DHPAA) (Fig. 2). The gut microbiota metabolites obtained from citrus flavanones (3-hydroxy-4'methoxyphenyl hydrocrylic acid), however, displayed a maximum excretion at around 10 h, being shorter that the maximum excretion times observed for the metabolites obtained from tannins. This could be related to the different ability of hesperidin and tannins to link to proteins or to intestinal tissues and mucin that delays the interaction of tannins with gut microbes while this interaction is faster in the case of citrus hesperidin.

These results show that microbiota metabolites persist in the human body, and therefore, they can exert biological affects for longer times than those polyphenols that are absorbed in the small intestine and reach pharmacokinetic peaks soon after the intake as is the case of flavan-3-ols (epicatechin), hesperetin-7-*O*-glucoside (Nielsen et al., 2006), or free ellagic acid (González-Sarrías et al., 2015; Seeram et al., 2006).

5. Systemic biological effects of the gut microbiota metabolites

The evaluation of dietary polyphenol gut microbiota metabolites health effects has been difficult as these metabolites cannot be used in human intervention studies as they are not food constituents and they should be considered under a pharmacological approach. Thus, a few studies have evaluated the systemic biological activity of microbial metabolites using animal models. However, most of the studies have evaluated *in vitro* the potential health effects of the metabolites using human cell lines. In addition, there are even less those studies that have evaluated changes in cell media metabolites in order to ascertain whether the metabolite triggers significant effects on the cells before its evolution to other compounds and/or these new compounds are also active. (Aragonès, Danesi, Del Rio, & Mena, 2017; González-Sarrías et al.,

Table 1
Bioavailability, metabolism, and excretion of polyphenol-derived gut-microbiota metabolites in human studies.

Food	Polyphenol	Gut-microbiota metabolites	Experimental	Results	Reference
Flavanones					
Orange juice pulp-enriched	Hesperidin (hesperetin 7-*O*-rutinoside)	Hesperetin and HPPA and HPAA derivatives	250 mL orange juice containing 537 μmol flavanones *vs.* placebo. Healthy volunteers (n = 12). Urine analysis.	Hesperetin conjugates excreted in 24 h urine = 16% of intake. 31 μmol 2–5 h; 38 μmol 5–10h; 13 μmol 10–24 h. Gut microbiota metabolites excreted in urine = 88% of intake[a]. HPPA, HPAA and phenyl hydracrylic derivatives 26 μmol 2–5 h; 62 μmol 5–10h; 50 μmol 10–24 h. Hyppuric acid derivatives. 65 μmol 2–5 h; 114 μmol 5–10h; 209 μmol 10–24 h.	[Pereira-Caro et al., 2014]
Orange juice; fresh squeezed and commercially processed.	Hesperidin (hesperetin 7-*O*-rutinoside)	Hesperetin	Crossover trial. Healthy subjects (n = 24). Consumed 11.5 mL/kg body weight = 800 mL for 70 kg bw.	Plasma hesperetin conjugates. T max 4.5–6.3 h. Urinary excretion = 4.13 −3.76% of intake.	[Silveira et al., 2014]
Orange juice, flavanone enriched	Hesperidin (hesperetin 7-*O*-rutinoside)	Hesperetin	Crossover study with healthy volunteers (n = 10). 400 mL orange juice (different hesperidin content)	Plasma hesperetin conjugates T max 4.5 −6.8 h. Relative urinary excretion in 48h (1 −8.9%). Large inter-individual variability.	[Vallejo et al., 2010]
Orange juice and orange fruit	Hesperidin (hesperetin 7-*O*-rutinoside)	Hesperetin	Randomized cross-over study (n = 20). 300g orange juice or 200 g orange fruit.	Plasma hesperetin conjugates Tmax 5.9 h. Urinary excretion in 48h 4.6–3.9% of intake. Large inter-individual variability.	[Brett et al., 2009]
Orange juice rich in flavanones. Orange juice treated with hesperidinase.	Hesperidin (hesperetin 7-*O*-rutinoside); Hesperidin 7-glucoside	Hesperetin	Healthy volunteers (n = 16). Double blind, randomized, placebo-controlled, crossover study. 300 and 900 mL orange juice.	Plasma hesperetin conjugates max. 7.0–7.4 h. Relative urinary excretion, % of intake 4.06–8.90%. Large inter-individual variability.	[Nielsen et al., 2006]
Ellagitannins & ellagic acid					
Pomegranate extract	Ellagitannins and ellagic acid	Urolithins and ellagic acid	Crossover pharmacokinetic study with healthy volunteers (n = 20). Pomegranate extracts (1.8 g) with different EA/punicalagin compositions.	Urinary excretion of Uros (Uro-A, IsoUro-A, Uro-B) continued up to 72h when the maximum excretion was found for the extract richer in free EA. Concentrations of EA in plasma (T max 1.42–1.98 h and C max 75 nM). Large inter-individual variability and some peaks in plasma EA at longer times indicate solubilization of the rather insoluble EA under specific gastrointestinal conditions.	[González-Sarrías et al., 2015]
Strawberry, fresh berries and processed puree	Ellagitanins and ellagic acid	Urolithins	Healthy volunteers (n = 20). 200g fresh strawberries or the equivalent puree.	Urinary excretion up to 80 h after intake. No differences between fresh or processed berries. Relative urinary recovery from 7.66 to 98.84% after fresh berry intake and from 14.46 to 80.43% after puree intake. Large inter-individual variability. Tmax for urinary excretion 28–32 h.	[Truchado et al., 2012]
Pomegranate extract	Ellagitannins	Urolithins	Healthy volunteers (n = 18) 180 mL pomegranate juice concentrate.	EA in plasma (max 0.06 μM) and a T max of 0.98 h. The urinary excretion of Uros started after 12 h and were also detected in the second 24 h after the intake.	[Seeram et al., 2006]
Strawberry, raspberry, walnuts, oak aged red wines	Ellagitannins and ellagic acid	Urolithins	Healthy volunteers (n = 40) single dose trial. Subjects randomly divided into 4 groups. Raspberry group consumed 225 g fresh fruit; strawberry 250 g; walnuts 35g, red wine 300 mL oak-aged red wine.	Metabolites not detected during the first 16 h. Main excretion in urine between 32 and 56 h. Relative urinary excretion up to 28% of intake. Dependent on food source and individuals.	[Cerdá, Tomás-Barberán, & Espín, 2005]
Proanthocyanidins					

Cranberry juice	Oligomeric proanthocyanidins A type	HPPA and HPAA derivatives	Healthy volunteers (n = 10; > 50 y.o.), 24 h absorption and excretion. Single-dose pharmacokinetic trial. 237 mL double-strength (54% juice)	Large inter-individual variability. 4,3-methoxy-HPPA = Tmax, 7.1 h plasma. 3,4-DHPAA = Tmax 8.4h. Urinary at 24 h high excretion.	[McKay, Chen, Zampariello, & Blumberg, 2015]
Green tea	Flavan-3-ols and proanthocyanidins	HPPA and HPAA derivatives and valerolactone	5 healthy volunteers. 300 mL green tea.	Urinary excretion of different gut microbiota metabolites during 24 h (different collection times). Main excretion in the fraction 8–24 h. 4-HPPA, 4,3-methoxy-HPPA, hippuric, 3-(3hydroxyphenyl)-3-hydroxypropionic acid. 5-(3′,4′,5′-trihydroxyphenyl-γ-valerolactone.	[Roowi et al., 2010]
Almond skin extract	Oligomeric proanthocyanidins A type	HPPA and HPAA derivatives and valerolactones	Healthy volunteers (n = 2). Almond skin extract (884 mg polyphenols).	Urine 24 h 3.4 DHPPA 3.6 μM; 3,4DHPAA 3.1 μM; 3-HPAA 7 mM. 4,3-methoxy-HPPA 25 μM; valerolactones 23 μM.	[Urpi-Sarda et al., 2009]
Grape seed extract	Oligomeric proanthocyanidins	HPPA and HPAA derivatives	Hypertensive volunteers (n = 74) intake for 6 weeks. 1000 mg polyphenols extract.	3-HPPA and 3-HPAA wide variation among individuals	[Ward, Croft, Puddey, & Hodgson, 2004]
Chocolate (polyphenol-rich)	Oligomeric proanthocyanidins	HPPA and HPAA derivatives	Human healthy volunteers (n = 11) consumed 80 g chocolate (439 mg proanthocyanidins). Urine collected after 3, 6, 9, 24 and 48h.	Urinary excretion 3HPP 24–48h; 4HPAC 0–24 and 24–48h; 3HPAC 24–48h.	[Rios et al., 2003]

3,4-DHPPA, 3,4-dihydroxyphenylpropionic acid; 3-HPPA, 3-hydroxyphenylpropionic acid, 3,4-DHPAA, 3,4-dihydroxyphenylacetic acid; 3-HPAA, 3-hydroxyphenylacetic acid., 3-HPPA, 3-hydroxyphenylpropionic acid; EA, ellagic acid; Uro, urolithin.

[a] Values calculated subtracting the excretion of the same metabolites after the placebo drink.

Fig. 2. Urinary excretion of different gut microbiota metabolites after the intake of non-extractable polyphenols-rich food products. Values represent the percentage of excretion ate different times compared with the maximum excretion (100%).
Uro-A glur (urolithin A-glucuronide excreted after the intake of fresh strawberries and strawberry puree) (Truchado et al., 2012). DHPAA (3,4-dihydroxyphenyl acetic acid) and 3HPAA (3-hydroxyphenyl acetic acid) gut microbiota metabolites excreted after the intake of chocolate (Rios et al., 2003). Valerolactone 5-(3′,4′,5′-trihydroxyphenyl-γ-valerolactone) gut microbiota metabolites excreted after the intake of green tea proanthocyanidins (Roowi et al., 2010). 3H4MPH 3(3′-hydroxy-4′-methoxyphenyl) hydracrylic acid. Flavanone gut microbiota metabolites produced after the intake of pulp-enriched orange juice (Pereira-Caro et al., 2014).

2014). There are very few reports on the *in vivo* systemic activity of microbial metabolites. For example, the daidzein-derived metabolite equol has been reported to improve arterial stiffness in human subjects at moderate cardiovascular risk (Hazim et al., 2016) and the ellagic acid-derived urolithin A has shown anti-inflammatory and antioxidant activities in a rat model (Ishimoto et al., 2011). In the *in vitro* studies, the most relevant results were obtained when the metabolites that circulate in plasma were used and at the actual concentrations that can be reached in plasma. Studies using either unrealistic concentrations or inappropriate metabolites were not considered in this review.

Table 2 provides an updated overview of *in vitro* studies that reported systemic biological effects of the gut microbiota metabolites as well as their phase-II conjugates that can reach the blood circulation after consumption of flavanones, ellagitannins and ellagic acid, and proanthocyanidins with the main focus on antioxidant, anti-inflammatory and cardioprotective effects.

The citrus flavanone hesperetin is released by the gut microbiota from hesperidin and circulates in plasma as glucuronide and sulfate conjugates. These metabolites, at concentrations that can be found in plasma, attenuate TNF-α induced inflammation in human vascular cells, and modulate gene expression involved in anti- and pro-inflammatory effects. These metabolites also showed antioxidant effects including inhibition of ROS production, and decrease in iNOS and COX-2 expression. They also enhanced NO release and suppressed superoxide production.

The pomegranate ellagitannin-derived metabolites, urolithins, that circulate as Phase II conjugates in plasma, also show anti-inflammatory effects in human endothelial cells and cardiomyocytes and neutrophils stimulated by TNF-α of LPS and other inflammatory inducers. Only one of the studies, however, assayed the main urolithin glucuronide (Uro-A glucuronide) that circulates in plasma (Giménez-Bastida et al., 2012). Two studies showed antioxidant effects of urolithins by decreasing ROS generation and increasing SOD activity.

Regarding proanthocyanidin gut microbiota metabolites, both hydroxyphenyl acetic (HPAA) and hydroxyphenyl propionic acids

Table 2
Systemic biological effects of the gut-microbiota metabolites and their conjugates reported in systemic cell models.

Cell model	Gut-microbiota metabolites and assay conditions	Biological activities and molecular associated events	Reference
Flavanones			
HAEC cells stimulated with TNF-α	Hesperetin 3′-*O*-glur, hesperetin 7-*O*-glur, hesperetin 3′-*O*-sulfate, hesperetin 7-*O*-sulfate and hesperetin (0.1, 1 and 10 μM)/16 h	Attenuated cell migration in the presence of TNF-α; decreased of thrombogenic PAI-1 levels (all compounds); no effect on production of pro-inflammatory IL-6 and IL-8.	[Giménez-Bastida, González-Sarrías, Vallejo, Espín, & Tomás-Barberán, 2016]
Human macrophages activated with LPS and IFNγ or IL-4	Naringenin, naringenin-7-*O*-glur and narigenin-4′-O-glur (0.6 μM)/6 h	Modulation of gene expression involved in anti and pro-inflammatory effects (i.e. Nrf2, SOCS1, SOD2, CD36 and TNFα).	[Dall'Asta et al., 2013]
Primary endothelial cells isolated from Sprague-Dawley rat aortas stimulated with H_2O_2	Hesperetin, hesperetin-7-*O*-glur and hesperetin-3′-*O*-glur (100 μM)/6 h	Decreased ICAM-1 and MCP-1expression (hesperetin-7-*O*-glur, but not hesperetin-3′-*O*-glur).	[Yamamoto et al., 2013]
HUVEC cells stimulated with TNF-α	Hesperetin-3′-*O*-sulfate, hesperetin-3′-glur and naringenin-4′-glur (0.5, 2 and 10 μM)/24 h	Attenuated monocyte adhesion (all compounds); modulation of the expression of genes involved in atherogenesis, such as those involved in inflammation, cell adhesion and cytoskeletal organisation.	[Chanet et al., 2013]
Murine RAW264.7 macrophages and A7r5 cells stimulated with LPS	Hesperidin, hesperetin and hesperetin metabolites (obtained from rat serum) (2.5–20 μM)/0.5–18 h	Inhibition of ROS production and iNOS and COX-2 protein expression (higher effect of hesperetin metabolites (sulfates and glucuronides) than hesperetin); higher antioxidant activity against various oxidative systems than hesperidin or hesperetin.	[Yang et al., 2012]
HUVEC cells	Hesperetin or hesperetin 7-*O*-glur (1, 5, 10, 25, and 50 μM)/6–24 h	Enhanced NO release by inhibiting nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase) at ≥ 25 μM (both compounds); suppressed $O2^{\cdot-}$ production (both compounds).	[Takumi et al., 2012]
BAEC cells in primary culture stimulated with TNF-α	Hesperetin (10 μM)/5 h	Increased phosphorylation of Src, Akt, AMP kinase, and eNOs; increased adhesion of monocytes and expression of VCAM-1.	[Rizza et al., 2011]
Ellagitannins & ellagic acid			
TNF-α inflamed HUVEC cells; THP-1; PMA challenged THP-1 (macrophages)	Pre-, co-treatments with Uro-A, Uro-B, Uro-C, Uro-D, EA, Uro-A + Uro-B, 10 μM each/6, 18, 24h	Inhibition of monocyte adhesion (only Uro-A + Uro-B 10 μM each); reduce cholesterol accumulation in HCS-exposed macrophages (Uro-C and EA at 10 and 5 μM, Uro-A + Uro-B 10 μM each); No effect on cholesterol efflux or cholesterol content; reduction of sVCAM-1 secretion (only Uro-A + Uro-B 10 μM each); reduction IL-6 levels (Uro-C and EA).	[Mele et al., 2016]
HAEC cells	Uro-A, Uro-B, Uro-B-glur; (15 μM) or mixture of 5 μM each/24 h	Increased nitrite/nitrate levels (only mixture of urolithins); Activation of eNOS expression (Mixture of urolithins at 5 μM and Uro-B-glur).	[Spigoni et al., 2016]
Ventricular cardiomyocytes and fibroblasts isolated from neonatal rats (inflammatory status by hyperglycemia)	Uro-A, Uro-B, Uro-B-glur, Uro-C, Uro-D (1 μM)/16 d	Reduction of CX3CL1, MCP-1 and VEGF (only Uro-B and Uro-B-glur); increased of MCP-1 and TIMP-1 (Uro-A, Uro-C and Uro-D) (in cardiomyocytes); reduction of CX3CL1, CINC2, CINC3, MCP-1, TIMP-1, and VEGF expression (all compounds except Uro-D) (in fibroblasts).	[Sala et al., 2015]
Human neutrophils stimulated with LPS or cytochalasin A/f-MLP	Uro-A, Uro-B, Uro-C, 8-methyl-*O*-Uro-A, 8,9-dimethyl-*O*-Uro-C; (1–20 μM)/1.5 and 24 h	Inhibition of IL-8 and MMP-9 production (Uro-A, Uro-B and Uro-C); prevention of cytochalasin A/f-MLP-triggered selectin CD62L shedding (Uro-B and methyl-*O*-Uro-A); reduction of elastase release (Uro-C); reduction of myeloperoxidase release (Uro-A and Uro-C); reduction of ROS and superoxide anion production.	[Piwowarski, Granica, & Kiss, 2014]
H_2O_2-treated T24 cells	Uro-A, Uro-B, 8-*O*-methyl-Uro-A; (IC_{50} = 43.9, 35.2 and 46.3 μM respectively)/3 h	Decreased of intracellular ROS and MDA levels; increased of SOD activity.	[Qiu et al., 2013]
HAEC/THP-1 cells stimulated with TNF-α	Uro-A, Uro-B, Uro-A-glur and Uro-B-glur (10–20 μM)/12 h	Inhibition of endothelial cell migration (only Uro-A and Uro-A-glur); inhibition of monocyte adhesion (only Uro-A-glur); decreased of CCL-2 and PAI-1 levels (only Uro-A-glur); decreased of CCL-2 and IL-8 (only Uro-A).	[Giménez-Bastida et al., 2012]
THP-1 cells stimulated with TNF-α	EA, Uro-A, Uro-B, Uro-C (5 μM)/24 h	No effect on TNF-α-mediated reduction cell viability; reverse TNF-α-mediated reduction HDAC activity (only EA); reverse TNF-α-mediated increase HAT activity (EA, Uro-B and Uro-C).	[Kiss, Granica, Stolarczyk, & Melzig, 2012]
PMA-induced HL-60 cells	Uro-A, Uro-B, Uro-C, Uro-D, 8-methyl-O-Uro-A, 8,9-di-*O*-methyl-Uro-C and 8,9-di-*O*-methyl-Uro-D (0.035–15 μM)/30 min	Decreased of intracellular generation of ROS (only Uro-C, Uro-D and Uro-A).	[Bialonska, Kasimsetty, Khan, & Ferreira, 2009]
HUVEC cells stimulated with IL-1β	EA (25–50 μM)/18 h	Decreased monocyte adhesion; decreased ROS production; inhibition of the expression of VCAM-1 and E-selectin; suppression of nuclear translocation of p65 and p50.	[Yu, Wang, Liu, & Chen, 2007]
Proanthocyanidins			
Immortalized murine brown pre-adipocyte cell line (C57 BAT)	Phenyl-γ-valerolactones and their sulphated forms (2 or 10 μM)/6–24 h	(R)-5-(3′,4′-dihydroxyphenyl)-γ-valerolactone and (R)-5-(3′-hydroxyphenyl)-γ-valerolactone-4′-O-sulphate protected brown adipocytes from increased reactive oxygen species production.	[Mele et al., 2017]

Table 2 (*continued*)

Cell model	Gut-microbiota metabolites and assay conditions	Biological activities and molecular associated events	Reference
Beta cell line INS-1E and rat pancreatic islets	3,4-DHPAA, 2,3- DHBA and 3-HPPA (1, 5 and 10 μM)/20 h	Increased glucose-stimulated insulin secretion (DHPAA and HPPA); protection of against cytotoxicity induced by oxidative stress (DHPAA and HPPA).	[Fernández-Millán et al., 2014]
PBMC cells stimulated with LPS	3,4-DHPPA, 3-HPPA, 3,4-DHPAA, 3-HPAA (1 μM)/16 h (pretreatment)	Inhibition of the secretion of TNF-alpha, IL-1beta and IL-6 (only 3,4-DHPPA and 3,4-DHPAA).	[Monagas et al., 2009]
Human monocytes isolated from donors stimulated with LPS	δ-(3,4-dihydroxyphenyl)-γ-valerolactone and δ-(3-methoxy-4-hydroxyphenyl)-γ-valerolactone (0.5 μM)/48 h	Inhibition of total MMP-9 release.	[Grimm, Schäfer, & Högger, 2004]
Human EA.hy926 endothelial cells	3,4-DHPPA (20–200 μM)/16–24 h	Decreased intracellular oxidation; increased eNOS activity.	[Huang, de Paulis, & May, 2004]

A7r5, rat thoracic aorta cell line; BAEC, bovine aortic endothelial cells; EA.hy926, human umbilical vein cell line; HAEC, human aortic endothelial cell line; HL-60, human promyelocytic leukaemia cells; HUVEC, human umbilical vein endothelial cells; INS-1E, insulin secreting rat beta cell derived line; PBMC, peripheral blood mononuclear cells; RAW264.7, nouse leukemic monocyte-macrophage cell line; T24, human bladder cancer cell line. THP-1, human acute monocytic leukaemia cell line. 3,4-DHPPA, 3,4-dihydroxyphenylpropionic acid; 3-HPPA, 3-hydroxyphenylpropionic acid, 3,4-DHPAA, 3,4-dihydroxyphenylacetic acid; 3-HPAA, 3-hydroxyphenylacetic acid., 2,3-DHBA, 2,3-dihydroxybenzoic acid; 3-HPPA, 3-hydroxyphenylpropionic acid; A/f-MLP, A/formyl-met-leu-phenylalanine; Akt, protein kinase B; AMP, adenosine monophosphate; CCL-2, chemokine (C-C motif) ligand 2; CD62L, L-selectin; CD36, cluster of differentiation 36; CINC, cytokine-induced neutrophil chemoattractant; COX-2, cyclooxygenase 2; CX3CL1, Fractalkine or Chemokine (C-X3-C Motif) Ligand 1; EA, ellagic acid; eNOS, endothelial NO synthase; glur, glucuronide; H_2O_2, hydrogen peroxide; HAT, histone acetyltransferase; HDAC, histone deacetylase; ICAM, intracellular adhesion molecule-1; IFN-γ, interferon gamma; IL, interleukin; iNOS, nitric oxide synthase; LPS, lipopolysaccharide; μM, micromolar; MCP-1, monocyte chemoattractant protein 1; MDA, malonic aldehyde; MMP-9, matrix metalloproteinase; NADPH, nicotinamide adenine dinucleotide phosphate; Nrf2, nuclear factor (erythroid-derived 2)-like 2; $O2^{-}$, superoxide; PAI-1, plasminogen activator inhibitor-1; PMA, 4β-phorbol-12β-myristate-R13-acetate; ROS, reactive oxygen species; SOCS1, suppressor of cytokine signaling 1; SOD, superoxide dismutase; Src, proto-oncogene tyrosine-protein kinase Src; TIMP-1, tissue inhibitor metalloprotease 1; TNFα, tumour necrosis factor alpha; Uro, urolithin; VCAM-1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor.

(HPPA) showed protection against cytotoxicity induced by oxidative stress, intracellular oxidation, and inflammation. Finally, valerolactone metabolites showed an inhibition of MMP-9 release.

6. Conclusion

Dietary polyphenols can be considered non-extractable when they are not soluble in the food products and gastrointestinal content, which limits their absorption. These 'so-called' macro-antioxidants reach the colon where they can exert local health effects but are mainly metabolized by the gut microbiota to produce catabolites that are efficiently absorbed. The production of these metabolites can persist up to 3–4 days in the blood stream where they can exert mainly anti-inflammatory and antioxidant systemic effects. Instead of acute and short-time peaks of bioactive molecules in blood stream, the intake of food sources rich in non-extractable polyphenols such as hesperidin, ellagitannins and proanthocyanidins could be a strategy to reach a sustained systemic anti-inflammatory and antioxidant status in the human body thanks to the constant production of bioactive microbial metabolites.

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