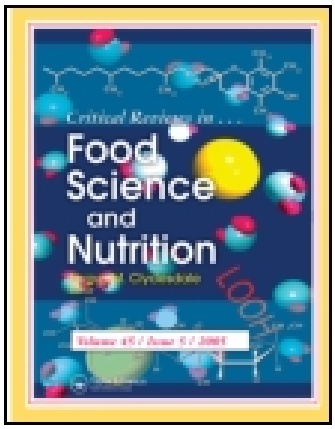

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Metabolic Fate of Ellagitannins: Implications for Health, and Research Perspectives for Innovative Functional Foods

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Metabolic Fate of Ellagitannins: Implications for Health, and Research Perspectives for Innovative Functional Foods

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Consumption of dietary ellagitannins (ETs) has been associated with different health benefits. Nonetheless, ETs are not bioavailable as such and are metabolized in vivo. They are partially converted into ellagic acid (EA) in the upper gastrointestinal (GI) tract, but this first metabolite is also poorly bioavailable. In the lower GI tract, EA and residual ETs are metabolized by gut microbiota to produce urolithins, which, together with their conjugate relatives, persist at relatively high concentrations in plasma and urine for days after ingestion of dietary ETs. Thus, ETs and EA may exert local health benefits on the GI tract but systemic health benefits are more likely to result from urolithins. Cellular models suggest that, at physiological concentration, urolithins are active against chronic degenerative diseases. Health benefits have been proven in animal models and during clinical studies. Even so, the crucial involvement of gut microbiota in ET bioconversion induces important variability of physiological response among humans, giving rise to the concept of high and low urolithin producers. This variability among consumers in obtaining potential health benefits from dietary ETs raises new challenges for the functional food industry. Different research perspectives are discussed to tackle this significant issue for nutritionists, food technologists, and consumers.

Keywords Urolithins, ellagic acid, bioavailability, gut microbiota, gastrointestinal tract

INTRODUCTION

Fruits and vegetables form an important component of a healthy diet. Numerous epidemiological studies have shown that regular consumption of this food group helps reduce the risk of major chronic and degenerative diseases, such as cardiovascular diseases and certain types of cancer (FAO/WHO, 2004). According to the World Health Organization (WHO, 2003), about 14% of gastrointestinal cancer deaths, 11% of ischemic heart deaths, and 9% of stroke deaths are caused by low intake of fruits and vegetables.

Fruits, vegetables, and nuts are rich sources of vitamins, minerals, dietary fiber, and many other phytochemicals. Most show biological activity, following different mechanisms of action such as antioxidant activity, modulation of detoxification enzymes, stimulation of the immune system, reduction of platelet aggregation, alteration of cholesterol metabolism, modulation of steroid hormone concentrations and hormone metabolism, blood pressure reduction, and antibacterial and antiviral activity (Tanaka and Sugie, 2007). Despite numerous epidemiological studies on health benefits associated with consumption of fruits, nuts, and vegetables, many gaps exist in identifying the bioactive compounds. Among such compounds are the ellagitannins, found in some fruits and nuts. This formerly underestimated class of phenolic compounds is attracting particular interest among scientists for its high potential in biological activity.

Ellagitannins (ETs) are a group of hydrolyzable tannins. They comprise hexahydroxydiphenoyl (HHDP) moieties esterified to a polyol (usually α-D-glucopyranose) and a galloyl residue (Niemetz and Gross, 2006) (Fig. 1). They form the largest group of tannins because of diverse possibilities of bonding the

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common monomeric moiety, HHDP residue, as either oligomeric or polymeric derivatives (Bakkalbai et al., 2009).

Certain fruits and nuts are reported as the main source of ETs, but most are not part of the common Western daily diet, as their occurrence is limited to wood-aged wine, berries, pomegranates, pecans, walnuts, and derived processed foodstuffs (Zafrilla et al., 2001; Lee et al., 2005; Espín et al., 2007; Hager et al., 2008; Bakkalbai et al., 2009; Gancel et al., 2010; Gasperotti et al., 2010). Although no reliable information is available, the daily intake of ellagitannins in the Western diet is generally extremely low, being no more than 5 mg day^{-1}. Probably, the source most regularly consumed comprises strawberries, both the fresh fruit and derived products (Clifford and Scalbert, 2000).

Compositional data on ellagitannins suggest that the dietary intake in northern Europe has been more recently reappraised to an average of 12 mg day^{-1}, mainly because of increased consumption of seasonal berries in these countries (Ovaskainen et al., 2008). Also, recent consumer interest worldwide in pomegranates, wild berry juices, and nuts is expected to increase the daily proportion of ETs in coming years. In tropical countries, although estimates are not available, the daily consumption of ellagitannins may be higher because some fruits, rich in ETs, such as pomegranates, guavas, and tropical highland blackberries (Clifford and Scalbert, 2000; Acosta et al., 2010), are heavily consumed in certain regions.

Ellagitannins exhibit important structural diversity according to food source. Punicalagins ($C_{48}H_{28}O_{30}$) and punicalins ($C_{34}H_{22}O_{22}$) are the main ETs found in pomegranates (Heber, 2008) and are the most studied so far. Sanguiin H6 ($C_{82}H_{54}O_{52}$), sanguiin H10 ($C_{68}H_{48}O_{44}$), and lambertianin C ($C_{123}H_{80}O_{78}$) are the major ETs found in berries (Mullen et al., 2003; Bakkalbai et al., 2009; Borges et al., 2010; Gasperotti et al., 2010). Pedunculagin ($C_{34}H_{24}O_{22}$) is the major ellagitanin found in walnuts, while the isomers vescalagin ($C_{41}H_{26}O_{26}$) and castalagin ($C_{41}H_{26}O_{26}$) predominate in oak-aged wine (Hourdes et al., 2009; Torronen, 2009). Whatever the structural complexity of ETs, they all share a common core—the HHDP—even if the number of monomer residues varies according to ET structure. Additionally, ellagic acid (EA) and direct derivatives are also found in free form in most ET-rich food products (Zafrilla et al., 2001: Mullen et al., 2002; Venketeshwer and Snyder, 2010), especially berries and nuts (Bakkalbai et al., 2009).

Interest in ellagitannins is still growing. The increasing number of studies on the ETs' metabolic fate and their potential health effects deserve a review. Here, we describe the most recent achievements to shed the light on this subject, which is of interest to nutritionists, food technologists, and consumers.

BIOAVAILABILITY OF ELLAGITANNINS AND ELLAGIC ACID

Of the phenolic compounds, ellagitannins possess some of the largest molecules and additionally they are characterized by their relatively high polarity. Consequently, their bioavailability is poor. Indeed, ETs have never been reported in the human systemic circulation system or in urine, even after consumption of high amounts of dietary ETs (Mertens-Talcott et al., 2006; Seeram et al., 2006c; Bialonska et al., 2009a; Borges et al., 2010). Ellagitannins have been detected in plasma in only one case study of laboratory rats subjected to a prolonged diet of ET-containing foods (Cerda et al., 2003). Although dietary ETs are not bioavailable, they are found in relatively low concentrations in the gastrointestinal (GI) tract, including feces (Losso et al., 2004; Gonzalez-Sarrias et al., 2010; Seeram et al., 2006b). In human subjects who have undergone ileostomy, only 23% of the ellagitannin sanguiin H6, ingested by consuming raspberries, reaches the ileum (final section of the small intestine), whereas recovery of free EA in ileum fluids increased 2.5 times (González-Barrio et al., 2010). This demonstrates that ETs are partially degraded into EA before reaching this region of the GI tract. Many studies have shown that ETs are partially hydrolyzed in the upper GI tract to EA (Cerda et al., 2003; Cerda et al., 2004; Cerda et al., 2005b; Larrosa et al., 2006b; Bialonska et al., 2009a; González-Barrio et al., 2010; Gonzalez-Sarrias et al., 2010; Sharma et al., 2010).

In vitro ET hydrolysis releases galloyl-glucose residues and the HHDP moiety, which undergoes lactonization and spontaneous rearrangement to the stable EA (Mullen et al., 2002; Mingshu et al., 2006; Aguilera-Carbo et al., 2007; Hager et al., 2008; Venketeshwer and Snyder, 2010) (Figure 1). The ET molecule is more stable under acidic conditions but unstable under alkaline and even neutral conditions (Larrosa et al., 2006b). Indeed, even at physiological pH, it has been suggested that most ETs from pomegranate released EA spontaneously into the medium, allowing uptake by cells (Larrosa et al., 2006a).

In vivo hydrolysis is more likely to occur in the small intestine rather than in the stomach (Figure 1). However, the ETs' susceptibility to acidic and basic hydrolysis depends probably on the origin of the source and on the food matrix, but little information has so far been reported on this topic. According to the sensitivity of ETs to the conditions of the upper GI tract, a heavier, or lesser, concentration of intact ETs enters the duodenum.

Unlike ETs, free EA released in the upper GI tract can be directly absorbed. Ellagic acid and direct derivatives, such as methyl and dimethyl ethers or glucuronic acid conjugates, have been found in human plasma and urine 1 to 5 hours after ingestion of dietary ETs, although at relatively low concentrations (Seeram et al., 2004; Mertens-Talcott et al., 2006; Seeram et al., 2006c). The rapid appearance of EA in the systemic recirculation system and its relatively short residence time indicates that absorption occurs mainly in the upper GI tract, that is, in the stomach and duodenum (Seeram et al., 2004; Stoner et al., 2006). A study of healthy human volunteers showed a maximum peak of 0.06 to 0.1 μmol l^{-1} at 1 hour after ingesting a standardized extract of pomegranate, with elimination half-time being less than 1 hour (Mertens-Talcott et al., 2006; Seeram et al., 2006c).

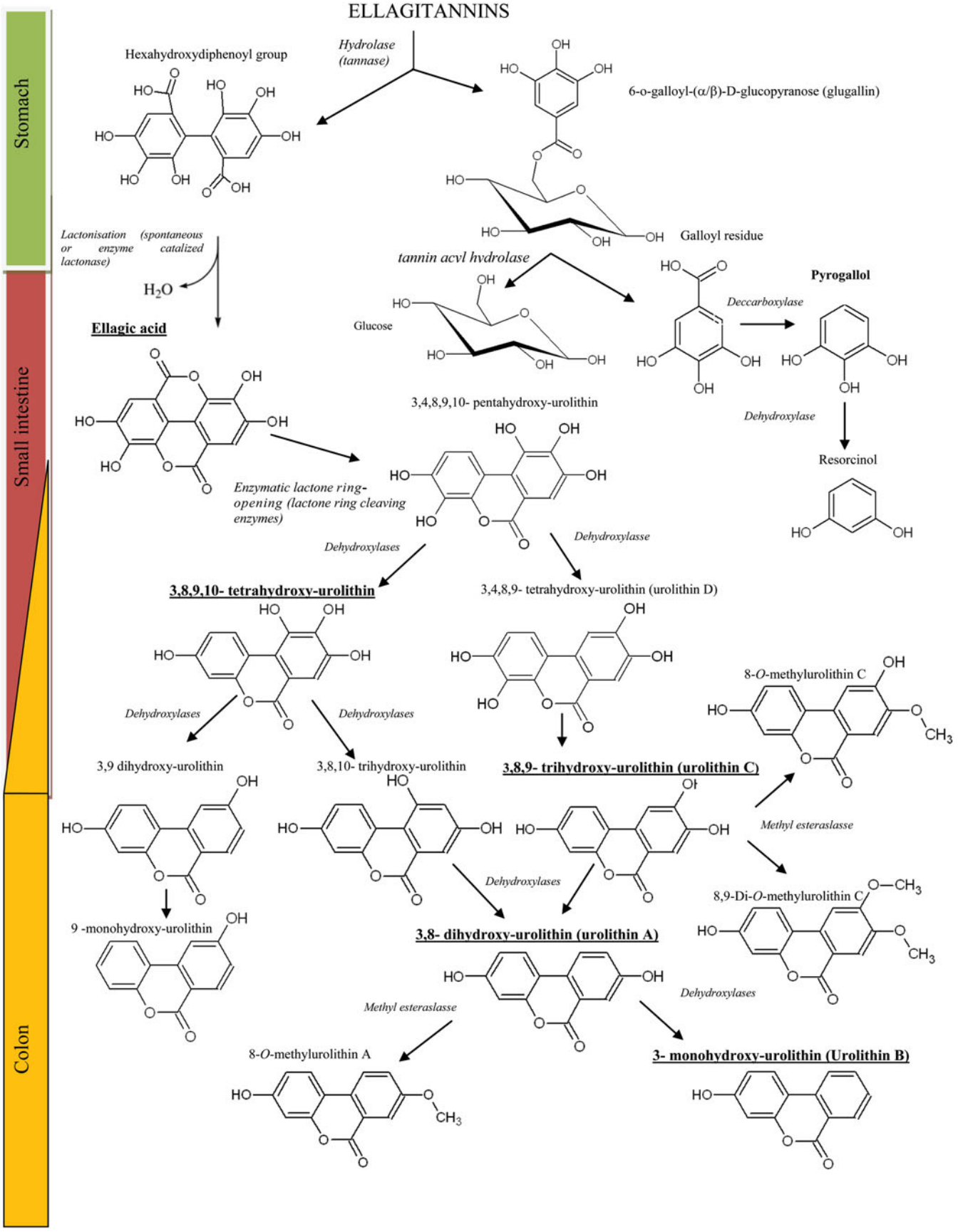

Figure 1 Tentative metabolic pathways of ellagitannins during passage through gastrointestinal tract (in bold and underlined: Major compounds found in human GI tract). (Color figure available online.)

When pure EA was orally administered, plasma concentration was relatively higher, reaching a peak of 0.66 μmol l^{-1} after 1 hour, with a serum elimination half-life of 8 hours (Hamad et al., 2009). However, the maximum concentration reached remained low, compared with the pharmacokinetic standard. Adsorption of EA presumably involves two main mechanisms based on the hydrolysis of glycosides. The first is related to a lactase phloridizin hydrolase (LPH), and the second involves cytosolic β-glucosidase (CBG), which yields lipophilic aglycones that are then absorbed by epithelial cells through passive diffusion (D'Archivio et al., 2010).

Although EA is insoluble in aqueous solution, cells have been shown in vitro that they can take up EA from the nutritive liquid and metabolize it to produce methyl or dimethyl EA derivatives. These are released to the surrounding medium (Larrosa et al., 2006b), appearing as dimethyl-EA conjugates in human plasma and urine. Even so, concentrations reached in the plasma are relatively low, suggesting that most EA is not directly absorbed within the upper GI tract and may pass to the lower GI tract. Poor bioavailability can be explained by EA's low water solubility, its ability to bind to intestinal epithelial cells and proteins, and tendency to form an insoluble complex with calcium and magnesium ions that precipitates out at the stomach's physiological pH (Machado et al., 2002; Seeram et al., 2004; Seeram et al., 2006b; González-Barrio et al., 2010).

METABOLISM OF ETS AND EA BY GUT MICROBIOTA

Dietary ellagitannins are partially degraded into EA by the time they reach the duodenum but, as EA is poorly absorbed, EA and residual ETs may also enter the rest of the small intestine. However, in the colon and feces, ETs and EA are found only at relatively low concentrations, suggesting microbial degradation during intestinal transit (Cerda et al., 2003; Seeram et al., 2004; Bialonska et al., 2010; González-Barrio et al., 2010).

Ellagitannins and EA are degraded into urolithins, a subfamily of metabolites from the dibenzopyranone family. In slaughtered Iberian pigs fed on acorns, urolithins start to appear in the jejunum (middle section of the small intestine) (Espín et al., 2007). In the case of humans, however, urolithins are probably synthesized lower down in the GI tract, in the large intestine, as healthy volunteers with ileostomies do not produce urolithins (González-Barrio et al., 2010; Gonzalez-Barrio et al., 2011).

Residual ellagitannins are metabolized first into EA by microbial enzymes—tannin-hydrolase and lactonase—which, respectively, cleave the galloyl-glucose residue from the HHDP group and induce enzyme-catalyzed lactonization (Figure 1). Afterwards, farther down the GI tract, other microbial derivatives—the polyhydroxylated dibenzopyranones—are synthesized from EA. Microbial enzymes catalyze the opening of the EA lactone ring and progressive dehydroxylation to yield urolithins (Figure 1). Dehydroxylation of the polyhydroxylated dibenzopyranones by microbial hydrolases first produces urolithin D (3,4,8,9-tetrahydroxy-dibenzopyranone), which, in the case of the slaughtered Iberian pigs (Espín et al., 2007), was found mainly in the lumen of the jejunum. Urolithin C (3,8,9-trihydroxy-dibenzopyranone) is then produced, followed by urolithin A (3,8-dihydroxy-dibenzopyranone) and urolithin B (3-monohydroxy-dibenzopyranone), which are found mainly in the colon and feces (Figure 1). Other microbial derivatives may also be metabolized such as 3,8,9,10-tetrahydroxy-dibenzopyranone, isourolithins, and methyl derivatives (Figure 1).

Moreover, hydrolysis of ellagitannins yields not only EA and urolithins, but also galloyl-glucose residues, which are eventually transformed by gut microbiota to gallic acid, pyrogallol, and resorcinol (Mingshu et al., 2006; Aguilera-Carbo et al., 2007; Gonzalez-Barrio et al., 2011) (Figure 1). In fact, both pyrogallol and resorcinol were found to increase significantly during in vitro human fecal incubation with raspberry extract (Gonzalez-Barrio et al., 2011). Indeed, pyrogallol sulfate, together with urolithins, has been selected as a biomarker in the urine of healthy subjects who have consumed mixed nuts (61).

The extent of hydroxyl removal from urolithins and the variety of metabolites produced depend on the time of exposure to gut microbiota, the composition of microbiota, and, eventually, the type of dehydroxylase enzyme involved (González-Barrio et al., 2011). The progressive dehydroxylation from penta- to mono-hydroxy-dibenzopyranone corresponds to an increase in lipophilicity and also adsorption ability. After dietary ingestion of ETs, the prevalent ET-derived metabolites detected in human plasma and urine correspond to urolithins A and B, conjugated with either glucuronic acid or sulfates (Cerda et al., 2005b; Mertens-Talcott et al., 2006). Indeed, most of the absorbed metabolites are conjugated in the liver, giving rise to different derivatives.

In the Iberian pigs mentioned above, as many as 31 microbial ET-derived metabolites were identified in the plasma, urine, and bile, corresponding mostly to glycosylated and sulfated methyl glucuronides, glucuronidated conjugates of urolithins, and EA methyl ether glucuronides. In human plasma, conjugated derivatives of urolithins A and B are generally reported 24 hours after the ETs are first ingested until the fifth day (Cerda et al., 2004). In healthy humans, maximum concentration is reached between 48 and 72 hours, after ingestion of dietary ETs. In addition to the constant excretion of urolithins due to microbial bioconversion of ETs and EA during intestinal transit, the persistence of urolithin derivatives in plasma and urine has been also attributed to enterohepatic recirculation (Espín et al., 2007; Sharma et al., 2010).

However, urolithin concentration in both plasma and urine after ingestion of dietary ETs varies considerably between individuals and depends on the assay's conditions (i.e., amount of ET-rich food ingested, concentration and type of ETs, and composition of food matrix) (Leser and Mølbak, 2009). In some healthy humans, urolithin A was found in plasma at concentrations as high as 18.6 μmol l^{-1} (Cerda et al., 2004), while other studies reported an average of 1 μmol l^{-1} (Seeram et al., 2006c; Seeram et al., 2008). In all cases, the order of magnitude

is higher than the maximum concentration of EA reported immediately after dietary intake of ET-rich food. Also, in contrast with the EA's very short residence time in the peripheral system, the concentrations of urolithin derivatives remain high for days after ingestion of ET-rich food.

Nevertheless, some human subjects cannot produce significant levels of urolithins, while others can excrete high plasma concentrations of urolithin metabolites. In a study of six healthy subjects who consumed 1 liter of pomegranate juice daily for 5 days (corresponding to a daily ingestion of about 4.4 g of ETs), two subjects could not produce urolithins. In contrast, the other subjects, between the third and fourth day of juice consumption, presented high plasma concentrations of ET-derived metabolites (from 1.1 to 18.6 μmol l^{-1} of urolithin A) (Cerda et al., 2004).

In another study, where urine was collected 7 to 48 hours after ET ingestion, 5 of the 10 subjects were found to excrete relatively high concentrations of urolithins, while the other 5 excreted very low amounts. One subject did not produce any urolithins over the study period (González-Barrio et al., 2010). These observations led to the concept of "high-urolithin producer" and "low-urolithin producer." On the basis of subjects who participated in previous cohort studies, about 50% were "low-urolithin producers." More than genetic factors, the high variability of physiological responses to dietary ETs was attributed to differences in gut microbiota ecology (Aura, 2005; Cerda et al., 2005a; Selma et al., 2009). Indeed, in vitro anaerobic incubations of EA with fecal suspensions showed that up to 80% of EA was converted into urolithins in some cases, while other fecal suspensions either did not produce urolithins or produced only traces after 72-hour incubations (Gonzalez-Barrio et al., 2011). In addition, variations between fecal suspensions were not only quantitative but also qualitative, as the profiles of conversion of EA to urolithins D, C, A, and B and other derivatives were markedly different (Bialonska et al., 2010; Gonzalez-Barrio et al., 2011).

The role of intestinal microbiota in the fate of dietary ETs parallels the fate of daidzein, a soybean isoflavone, which is transformed into equol by gut bacteria. Similarly, some subjects could not produce sufficient urolithin and equol to receive potential health benefits from these bioactive molecules. In both cases, the role of colonic flora predominates. Differences among human subjects in the excretion of urolithins are a consequence of diverse aptitudes to harbor colonic bacteria able to metabolize ETs and EA.

To date, the bacteria able to perform these tasks have not yet been characterized. The human gut ecosystem is extremely complex and the diversity of bacterial genera and species encountered is huge. Up to 1000 bacterial species have been found in humans, although most people harbor fewer than 200 (Qin et al., 2010), most of which belong to the phylotypes *Firmicutes* and *Bacteroidetes* (Leser and Mølbak, 2009). The strictly anaerobic species outnumber facultative anaerobes by about 1000-fold (Davis and Milner, 2009; Ventura et al., 2009). Most are not cultivable, requiring, for their identification, innovative culture-independent approaches such as metagenomics-based techniques.

During incubation of fecal suspension with punicalagins and EA, this substrate, by lowering pH, significantly inhibits the growth of pathogenic bacteria, such as *Clostridia* spp., *Staphylococcus aureus*, *Escherichia coli, Pseudomonas aeruginosa, Candida* spp., and *Mycobacterium* spp. (Machado et al., 2002; Bialonska et al., 2009b). These ETs also enhance the growth of some beneficial probiotic bacteria (Bialonska et al., 2010), although, so far, none of these bacteria has been shown to produce urolithins.

The fundamental importance of gut bacteria in the metabolic fate of ETs can also explain the large inter-individual variability observed in some clinical studies involving consumption of ET-rich food ingestion. Systemic biological effects (Cerda et al., 2005b; Davis and Milner, 2009) may be more likely attributed, not to poorly bioavailable EA, but to ET-microbial derivatives. These latter are readily absorbed by the organism, resulting in relatively high concentrations that remain for days in the plasma. Hence, separating low from high producers of urolithins would be prudent before developing conclusions on the potential health benefits of ETs at the clinical level.

MAIN BIOLOGICAL ACTIVITIES OF ETS AND THEIR METABOLITES

Effects of ETs and the EA Molecule in the GI Tract

Antioxidant Effect

An important quality of ETs and EA is their high antioxidant activity, as evidenced during in vitro chemical determination (Zhang et al., 2011). Large numbers of research studies have pointed out this specific quality and most, even recently, have enthusiastically extrapolated it to potential systemic health benefits. However, such an assumption is misleading, because native dietary ETs are not absorbed as such, but converted first into EA and then into successive microbial metabolite derivatives. The high antioxidant capacity exerted in vitro by ETs may be important locally in the GI tract, but is irrelevant for possible systemic biological effects. In addition, the bioavailability of EA is poor, with concentrations not exceeding tens of nanograms within the bloodstream, where it remains for a brief time only. Nor does it accumulate in any targeted organ, except the epithelial layer of the digestive tract (Espín et al., 2007).

In strictly chemical assays, ETs and EA showed relatively high antioxidant activity but, in cellular bioassays, only the antioxidant score of EA was considerably enhanced. This suggests a higher affinity with cells than with the chemical compounds used in antioxidant assays (Bialonska et al., 2009a). For instance, EA provides about three times more protection for the human erythrocyte cells used in the ERYCA assay than for fluorescein, the chemical compound used in the ORAC assay (González et al., 2010). However, the potential impact of the

high antioxidant capacity of dietary ETs and EA may be restricted mostly to local action in the GI tract, where organs and cells come in direct contact with ETs and EA during their transition through the intestine.

Chemopreventive Effect on Cancers of the GI Tract

The potential chemopreventive potential of dietary ETs and EA assessed during in vitro studies should be, therefore, limited to cells that represent the GI tract. Other results should be interpreted with great caution because the regulation of apoptotic pathways might be achieved only through intraperitoneal administration. Consequently, as shown in Table 1, potential chemopreventive benefits of dietary ETs and EA have been studied mostly for cancer cell lines from colon (Narayanan and Re, 2001; Losso et al., 2004; Seeram et al., 2005; Seeram et al., 2006a; Larrosa et al., 2006b; Zhang et al., 2008; Gonzalez-Sarrias et al., 2009a; Gonzalez-Sarrias et al., 2009b; Kasimsetty et al., 2010; Sharma et al., 2010), oral mucosa (Seeram et al., 2006a; Zhang et al., 2008), and, in intervention studies with animals, from the esophagus (Kresty et al., 2001; Stoner et al., 2006; Stoner et al., 2007) (Table 2).

Ellagitannins and EA were found to reduce, in a dose-dependent manner, carcinogenesis with the half maximal inhibitory concentration (IC_{50}) being of the same order (1–30 μM) of, for instance, the concentration levels found in vivo in ileal fluids (13 and 16 μM for EA and ET respectively) (González-Barrio et al., 2010). Ellagic acid was also shown to accumulate in colonic epithelial cells where concentration could remain even higher during intestinal transit (Landete, 2011). Because most ETs are converted into EA, even at physiological pH, the effects of ETs' intact molecules on cells should also be regarded with caution, with observed effects being more logically attributed to the EA released in the medium (Seeram et al., 2004). The EA's main mechanism of action is suggested through the modulation of the Wnt signaling pathway, which is known to play a pivotal role in human colon carcinogenesis (Sharma et al., 2010). Furthermore, a collateral effect of ETs and EA is their potent anti-inflammatory actions, which have been shown in cell models (Narayanana et al., 1998; Adams et al., 2006; Larrosa et al., 2006a; Heber, 2008; Larrosa et al., 2009) and through peritoneal injection on animals (Corbett et al., 2010).

Ellagitannins and EA were formerly thought to be responsible for most of the health benefits of ET-rich foods. However, as their low bioavailability became apparent, interest shifted to their microbial derivatives, the urolithins.

Effect of Urolithins

Chemoprevention of Prostate and Breast Cancers

Cell models have shown urolithins as having mainly cancer chemoprevention effects, more specifically on prostate, breast, and colon cancers (Table 1).

A major property of urolithins comprises their estrogenic and antiestrogenic activities in binding estrogenic receptors. This may be due to the type and number of biophores in their molecular structures (Zhang et al., 2011), giving them a structural analogy to estrogens (Larrosa et al., 2006a). Urolithin A, the main urolithin found in the circulatory system, has a significant affinity for estrogen receptors, especially for ER_a. Urolithin B, the second metabolite of importance, shows a lower but relatively high affinity for the estrogen receptors ER_b and ER_a (Larrosa et al., 2006a; Gonzalez-Sarrias et al., 2010). Thus, urolithins strongly inhibit the growth of both androgen-dependent and androgen-independent, human, prostate-cancer cell lines (CaP) at relatively low doses (IC_{50} <30 μM)(Kasimsetty et al., 2010). Urolithins appear to act by inhibiting the activation of the nuclear factor kappa B in CaP (Heber, 2008) (Table 3).

Urolithins also inhibit the activity of the enzyme CYP1B1, which affects the three stages of prostate cancer development, and encourages the initiation, progress, and development of drug resistance. Urolithins, therefore, appear to display a dual mode of action—inhibiting the activity and/or expression of the enzyme—and thus decreasing the incidence of prostate cancer (Kasimsetty et al., 2009). In addition, if used as an adjuvant during chemotherapy, these microbial metabolites appear to decrease the incidence of drug inactivation mediated by CYP1B1.

Urolithin A glucuronide and, in a much lesser amount, dimethyl ellagic acid were reported as accumulating in human prostate tissues after subjects had consumed pomegranate juice (Gonzalez-Sarrias et al., 2010) which could make relevant the effects observed in cells model using high doses and therefore, results of test obtained on cells model using high doses of these compounds may be relevant (Table 1). Subjects who had received the primary treatment of surgery or radiation, also demonstrated significant decrease in prostate-specific antigens (PSA) after consuming pomegranate juice (Pantuck et al., 2006; Heber, 2008).

All these findings, together with animal studies (Seeram et al., 2007; Sartippour et al., 2008) (Table 2), tend to justify high interest in ET-rich diets as a means of reducing risks of prostate cancer. Less information has been published to date on the urolithins' role in reducing risks of breast cancer, although they have been shown in vitro to enhance the apoptosis of breast-cancer cell lines even at low concentration (<10 μM) (Adams et al., 2006; Larrosa et al., 2006a; Adams et al., 2010) (Table 1).

Chemoprevention of Colorectal Cancer

Cell models have shown that urolithins induce dose-dependent apoptosis of colon-cancer cells at doses between 1 and 100 μM (Table 1). As urolithins were also shown to accumulate in colonic epithelial cells (Espín et al., 2007), these doses may be physiologically relevant. Urolithins A and B appear to modulate phases I and II in detoxifying enzymes in Caco-2 cells (Gonzalez-Sarrias et al., 2009a). Both phase enzymes enhance the detoxification of carcinogenic compounds by blocking DNA damage and reducing cell injury. Another

Table 1 Summary of some recent results obtained on cell models by ETs, EA, and urolithins

Product tested	Cell lines	Doses	Results	Reference
Punicalagin, EA	Colon cancer cell lines (HT-29 and HCT116)	100 μM	Potent antiproliferative activity	(Seeram et al., 2005)
Pomegranate juice (PJ) total pomegranate tannins TPN punicalagin (P)	Colon cancer cell line (HT-29)	50 μM punicalagin	Suppressed COX-2 expression by 97% (PJ), 55% (TPN), 48% (P), respectively. TPT and P at 100 mg/L suppressed TNkF binding 10-fold and 3,6-fold, respectively	(Adams et al., 2006)
Pomegranate juice extract	Prostate cancer		Regulation of androgen-independent prostate cancer cells	(Hong et al., 2008)
Raspberry extracts	Human cervical cancer (HeLa)	EC50 = 10 μM GAE	Inhibition of the proliferation of cancer cells	(Ross et al., 2007)
Berry extracts	Human oral (KB, CAL-27), breast (MCF-7), colon (HT-29, HCT116), and prostate (LNCaP), Caco-2	25 to 200 μg.mL^{-1}	Dose-dependant inhibition in all cell lines	(Seeram et al., 2006a)
Crude strawberry extract	Human oral (CAL-27, KB), colon (HT29, HCT-116), prostate (LNCaP, DU145)	100 μg.mL^{-1}	Cell growth inhibition	(Zhang et al., 2008)
EA				
ETs, EA	Caco-2 cells colon cancer	1–30 μM 1–100 μM	Apoptosis of cancer cells Mitochondrial pathway, release of cytochrome C	(Larrosa et al., 2006b)
EA	Colon, breast, and prostatic cancer	1–100 μM	Induce dose dependant apoptosis	(Losso et al., 2004)
EA	Colon cancer cells (SW480)	10 M	Induce dose dependant apoptosis	(Narayanan and Re, 2001)
EA	Human neuroblastoma (SH-SY5Y)	1–100 μM	Growth inhibition, cell detachment, and apoptosis	(Nanberg and Fjaeraa, 2009)
Punicalagin	PMA- cells (normal cells)	Punicalagin (IC_{50} = 1.4 μM)	Cellular antioxidant capacity	(Bialonska et al., 2009a)
EA		EA(IC_{50} = 1.1 μM)		
UA		UA (IC_{50} = 13.6 μM)		
UB		UB(IC_{50} = 0.33 μM)		
UC		UC (IC_{50} = 0.16 μM)		
EA	Colon cancer (293T)	ETs (IC_{50} = 30.0 μg.mL^{-1})	Inhibited Wnt signaling	(Sharma et al., 2010)
ETs		EA (IC_{50} = 63 μM)	Reduce colon carcinogenesis	
UA		UA (IC_{50} = 39 μM)		
UA	Prostate cancer	after 24 hours	Inhibition of CYP1B1protein	(Kasimsetty et al., 2010)
UB		UA (IC_{50} = 13 μM)	Expression	
UC		UB(IC_{50} = 17 μM) UC (IC_{50} = 27 μM)		
EA, UA, UB	Breast cancer	Significant inhibition when UB = 5 μM	Chemoprevention of breast cancer Inhibition aromatase activity	(Adams et al., 2010)
EA	Pre-B acute lymphoblastic leukemia (ALL) cell lines	33 μM	Induce apoptosis in high-risk leukemia cells	(Zuninoa et al., 2009)
EA, UA	Colon cancer Caco-2	40 μM	Chemopreventive effects, modulate phase I and phase II detoxifying enzymes	(Gonzalez-Sarrias et al., 2009a; Gonzalez-Sarrias et al., 2009b)
UB				
UA, UB	Breast cancer (MCF-7)	IC50 ERα and ERβ binding assays were: 0.4 and 0.75 μM for UA; 20 and 11 μM for UB	Estrogenic antiestrogenic activity	(Larrosa et al., 2006a)
UA, UB, MUA, MUB, UBS, acetylated-UB	Breast cancer	UB highest at 2.35:4.7 μM	UB inhibit significantly aromatase activity and testosterone-induced MCF-7aro cell proliferation	(Adams et al., 2010)
EA	Cervical cancer	10 μM	Inhibit cell growth and induce apoptosis	(Narayanana et al., 1998)

Table 2 Summary of intervention studies with animals to evidence health benefits of dietary ETs

Product ingested	Objective	Doses	Results	Reference
Pomegranate extract	DU145, LNCaP, 22Rv1 LNCAP-AR cells Human prostate cancer cells (LNCaP) injected subcutaneously into mice	0.3 mg equ. punicalagin/mice	-Urolithins inhibited the growth of both androgen-dependent and androgen-independent prostate cancer cell lines, with IC50 values lower than EA.-EA and UA decreased prostate cancer xenograft size, tumor vessel density	(Sartippour et al., 2008)
Raspberry juice	Heart health	Equ. 275 mL by a 70 kg human	Inhibited cardiac and aortic production of superoxide anion and increased hepatic glutathione peroxidase; lower plasma triglyceride level	(Suh, 2011)
Blackberry extract	Antioxidant	2.68 mg EA eq kg^{-1} body weight Wistar rat	Reduced thiobarbituric acid reactive substance levels and increased glutathione levels in the liver, kidney, and brain	(Hassimotto and Lajolo, 2011)
Berry juice and tea berry	Atherosclerosis in hamsters	Equivalent to 275 mL by a 70 kg human	Inhibited aortic lipid deposition by 79–96% and triggered reduced activity of hepatic antioxidant enzymes after 12 weeks	(Rouanet et al., 2010)
Black raspberries extract	Anti-cancer		Inhibit esophageal tumorigenesis	(Seguin et al., 2011)
Freeze-dried berries	Esophagus and colon cancer	0.25–0.5 mg/kg body weight	Inhibition of rodent esophagus cancer by 30–60% and of the colon by up- to 80%; reduction of levels of carcinogen-induced DNA damage	(Stoner et al., 2007)
Purified Geranniin from *Geranuuim thunbergii*	Plasma antioxidant level	20 mg/rat	Increase plasma ORAC antioxidant value after 6 hours after ingestion	(Hideyuki, 2011)
Lyophylized black raspberries, LBRs	Inhibition of *N*- NMBA-induced esophageal tumorigenesis in the rat during initiation and postinitiation of carcinogenesis	0.25 mg/kg, weekly for 15 weeks and throughout a 30-week bioassay	Reduced tumor multiplicity (39 and 49%, respectively); inhibit adduct formation (64%) after NMBA administration at 0.50 mg/kg	(Kresty et al., 2001)
		0.25 mg/kg, three times per week for 5 weeks	At 25 weeks, both 5 and 10% LBRs significantly reduced tumor incidence (54 and 46%, respectively), tumor multiplicity (62 and 43%, respectively), proliferation rates, and preneoplastic lesion development	
EA	Mitochondrial damage in beta adrenergic agonist induced myocardial infarction	EA (7.5–15 mg/kg)/ 10 days	Protective effect of EA against mitochondrial damage in myocardial infarction	(Mari Kannan and Darlin Quine, 2012)
EA	Apoptosis	60 mg/kg body weight p.o. every day	Prevention of P13K-AKT activation in 15 weeks	(Sudhandiran and Umesalma, 2011)
EA	Anti-inflammatory properties	4 mg/kg rat	Significantly reduce paw edema	(Corbett et al., 2010)
EA, robinetin, myricetin	Inhibitory effect on tumorigenecity of B[a]P 7,8-diol-9,10-epoxide-2 on mouse skin	300 nmol	59-66% reduction in the number of skin tumors after 15 and 20 weeks of promotion with 12–0-tetradecanoylphorbol-13-acetate	(Chang et al., 1985)
	In the newborn mouse	45, 90, 180 nmol EA 200, 400, 800 nmol robinetin, myricetin	Avoid the formation of tumors in animals killed at 9–11 months later 44–75% inhibition in the number of diol-epoxide-induced pulmonary tumors per mouse	
Pomegranate extract and urolithin A	Colon inflammation	250 mg PE/Kg day 50 mg UA/Kg day	Both PE and UROA decreased inflammation markers (iNOS, cycloxygenase-2, PTGES and PGE2 in colonic mucosa) and modulated favorably the gutmicrobiota	(Larrosa et al., 2009)
Pomegranate extract Urolithin A	Inhibition of tumor xenograft (LAPC-4) grow in immune compromised SCID mice	Orally/intraperitoneal PG: 0.8 mg/mouse/ dose 5 days/week UA: 0.3 mg/ mouse/dose	Inhibition of 50% of tumor volume 2 weeks after	(Seeram et al., 2007)
Urolithin A	Anti-inflammatory effect carrageenan-induced paw edema in mice	5 mg/mice	Reduction of paw edema after one hour of oral administration, increase of ORAC plasma level	(Ishimoto et al., 2011)

Table 3 Recent prospective clinical studies of consumption of ellagitannins-rich food

ETs source	ETs intake	Subject number	Status	Duration intervention	Biomarker affected	Mains biomarker not affected	Reference
Pomegranate juice	Daily juice supplementation	10	Healthy	1 and 3 years	Decrease in arotid intima-media thickness (IMT)		(Aviram et al., 2004)
Pomegranate juice	50 mL daily	20	Healthy (10) noninsulin-dependent diabetes mellitus (10)	3 months	Reduction of cellular peroxides (by 71%), and increased glutathion levels (by 141%); decreased the extent of Ox-LDL cellular uptake (by 39%)		(Rosenblat et al., 2006)
POMx capsules	1 capsule/days (410–850 mg GAE)	64 32	Overweight	4 weeks	Increase in plasma antioxidant status	Glucose, BUN, creatinine, lipids, insulin, c-peptide, paraoxonase-1, or electrolytes or liver enzymes (AST or ALT)	(Heber et al., 2007)
Pomegranate juice	230 mL daily	24	Patients with prostate cancer	54 months	Significant reduction of prostate-specific antigen		(Pantuck et al., 2006)
Commercial pomegranate extract in capsule	$2 * 400$ mg in a single administration	11	Healthy	24 hours	+32% ORAC plasma antioxidant activity at 30 minutes	Generation of reactive oxygen species (ROS), inflammation marker interleukin-6 (IL-6)	(Mertens-Talcott et al., 2006)
Pomegranate juice	400 mL juice	30	Healthy	5 weeks	No benefit	Serobiochemical and haematological, urinary 8-iso-PGF(2 alpha), respiratory function variables and clinical symptoms of chronic obstructive pulmonary disease	(Cerda et al., 2006)
Pomegranate juice	240 mL/day	45	Patients with CHD and myocardial ischemia	3 months	Stress-induced ischemia decreased	No changes in cardiac medications, blood sugar, hemoglobin A1c, weight, or blood pressure	(Sumner et al., 2005)
Pomegranate juice	50 mL/day	8	Hypertensive patients	2 weeks	36% decrement in serum angiotensin converting enzyme (ACE) activity and a 5% reduction in systolic blood pressure		(Aviram and Dornfeld, 2001)
Mixed nuts	30g/day	42	subjects with metabolic syndrome	12 weeks	Increased excretion of serotonin metabolites		(Tulipani et al., 2011)
Strawberry	250 g daily	21	Healthy	3 weeks	Lipid peroxidation lag time increased by 20%	DNA strand breaks in lymphocytes, and activity of phase II enzymes.	(Henning et al., 2010)

study proposes an additional pathway that modulates the expression levels of multiple genes in the epithelial cells lining the colon. This pathway might be used by EA and urolithins A and B, which would therefore exert an effect toward reducing risks of colon cancer (Gonzalez-Sarrias et al., 2009b). In this study, the urolithins' antiproliferative activity may have been due to the modulation of gene expression, including the MAPK/ERK pathway signaling. Urolithin A can induce cell cycle arrest, and modulate key cellular processes associated with colon cancer development, such as MAPK signaling in vitro. Additionally, urolithin A was shown to inhibit Wnt signaling with IC_{50} at even lower concentrations than for EA (Sharma et al., 2010).

Anti-inflammatory Effect

The inhibitory effect of urolithins on cancer may also be a result of their anti-inflammatory activity, as, in a colitis rat model, urolithin A was found to decrease inflammatory biomarkers, including nitric oxide synthase, cyclooxygenase-2 (COX-2), prostaglandin E synthase, and prostaglandin E2 (Larrosa et al., 2009). Oral ingestion of EA and urolithin A has also been shown to quickly and significantly decrease inflammation in rat's-paw edema (Corbett et al., 2010; Ishimoto et al., 2011). This anti-inflammatory effect was also observed in a clinical study on both healthy and noninsulin-dependent diabetic patients consuming pomegranate juice, which greatly reduced cellular peroxides

and increased glutathione levels (Rosenblat et al., 2006) after 4 weeks of daily intake. However, another study using a much shorter period (24 hours) found that inflammation biomarkers, such as interleukin-6, remained unchanged (Mertens-Talcott et al., 2006).

Reducing Risks of Cardiovascular Disease (CVD)

Consumption of ET-rich food has also been associated with cardiovascular health. Clinical studies have demonstrated inhibited lipid peroxidation (Rosenblat et al., 2006: Henning et al., 2010), antiatherogenic activity (Aviram et al., 2008), reduced stress-induced ischemia (Sumner et al., 2005), reduced systolic blood pressure (Aviram and Dornfeld, 2001), reduced platelet aggregation (Mattiello et al., 2009), and decreased carotid intima-media thickness and angiotensin-converting enzyme, which are linked to artery distensibility (Aviram and Dornfeld, 2001; Aviram et al., 2004).

Antioxidant Activity of Urolithins

Contrary to what was previously shown by strictly chemical antioxidant assays (Cerda et al., 2004; Ito, 2011), cell-based assays show that the urolithins' antioxidant activity correlates with the number of hydroxyl groups and molecule lipophilicity. As determined by the 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) method (Reddy et al., 2007), using myelomonocytic HL-60 cells, the most potent antioxidant urolithins were C and D, with IC_{50} values, respectively, at 0.16 and 0.33 μM. These were followed by urolithin A with an IC_{50} value of 13.6 μM. Urolithins C and D presented lower activity than their parent molecules—respectively, EA and punicalagin—which had IC_{50} at 1.1 and 1.4 μM, respectively (Bialonska et al., 2009a). The monohydroxylated urolithin B and the derived methylated compounds did not show antioxidant activity.

Although urolithin A presented the weakest antioxidant activity, its IC_{50} remained at physiological concentration, at least for high-urolithin producers, and thus may potentially contribute to in vitro antioxidant capacity. However, antioxidant capacity is apparently not a major property of the most bioavailable ET metabolites (urolithins A and B), at least, not in chemical or in vitro cell-based tests, unlike their parental molecules ETs and EA. Clinical studies showed a slight increase in the antioxidant capacity of plasma after long-term consumption of ET-rich food (Mertens-Talcott et al., 2006; Heber et al., 2007; Henning et al., 2010), whereas another study failed to show this (Cerda et al., 2006). Again, the high variability of physiological responses of human subjects during clinical studies prevented results from being conclusive. Their nature as a "low-urolithin producer" or "high-urolithin producer" must be taken into account to better demonstrate the urolithins' in vivo antioxidant potential.

TOXICITY OF DIETARY ETS

Various pharmacokinetics and toxicity studies have proved that ET-rich foods are not toxic, even when consumed in large amounts (Cerda B. et al., 2003; Viuda-Martos et al., 2010). For example, a daily diet of 30% oak-flavored milk powder, containing large quantities of ellagitannins and fed to rats for almost 100 days, was not toxic. Urolithin A and its glucuronide were detected in feces and urine, but no accumulations were found in the liver or kidneys—findings that were corroborated by histopathological analyses. Nor were accumulations found in the uterus, neither did the diet change common biomarkers in the blood (Azorin-Ortuno et al., 2008). Another study, using high doses of pomegranate in the rats' diet, had similar results, except for urea and triglycerides, which remained low throughout the experiment, because the diet was of lower nutritional value than standard rat food (Cerda et al., 2003). Clinical trials on overweight human subjects indicated that ETs as a dietary supplement are safe for human up to 870 mg of gallic acid equivalents per day (i.e., equivalent to about 8 liters of pomegranate juice per day) over 1 month (Heber et al., 2007).

However, no information is available on the possible toxicity of microbial-derived urolithins at higher concentrations than those observed in vivo. Indeed, in vivo concentrations appeared limited, independently of the levels of dietary ETs consumed (Cerda et al., 2005b).

RESEARCH PERSPECTIVES FOR INNOVATIVE FUNCTIONAL FOODS

The significant role played by gut microbiota in the ETs' metabolic fate and the potentially important contributions of ET-microbial derivatives to health benefits are issues that must be taken into account when developing innovative functional foods. The high variability of gut bacteria ecosystems among humans (which results in different abilities to produce microbial derivatives) transposes into equally enormous inter-individual variability of physiological responses to dietary ETs.

In terms of dietary ETs, we are not equal. Some people can expect quick and effective systemic health benefits, but others will—eventually—receive only limited health benefits that would probably be confined to the GI tract. What, therefore, should be the recommended dietary strategy? How can the functional food industry tackle this challenge to provide food that will better benefit all consumers?

At least four strategies can be considered: (1) provide consumers with the exogenous and appropriate microorganisms they lack, that is, with a new generation of probiotic bacteria that will establish themselves, at least temporarily, in the large intestine and convert dietary ETs and EA as they pass through the GI tract; (2) modify gut microflora ecosystems by promoting endogenous beneficial microorganisms that can metabolize dietary ETs and EA into urolithins; (3) design a food matrix that more efficiently enhances urolithin production by providing

microbiota with optimal substrates for sufficient time; and (4) execute in vitro what some people cannot do efficiently in vivo, for example, provide food that already contains urolithins.

In all cases, the first step is to identify and characterize the microorganisms able to bioconvert ETs into urolithins. Because of the different enzyme families involved, probably at least a two-step microbial bioconversion should be adopted. The first step is to convert residual ETs to EA and then convert EA to urolithins.

For the first step, tannin-degrading bacteria *Lactobacillus plantarum*, *L. paraplantarum*, and *L. pentosus*, able to convert ETs to EA, have been already isolated from the gut (Mingshu et al., 2006; Osawa et al., 2000; Vaquero et al., 2004). These well-known probiotic bacteria could be eventually used in dietary strategies to enhance the release of EA from ETs in the colon.

For the second step, no information is so far available. Although urolithins have been produced in vitro during the incubation of human fecal suspensions with ETs and EA, the bacterial strains able to produce urolithins have not yet been identified. As most gut bacteria species are not cultivable, recent developments in culture-independent technologies, based on metagenomics, would help elucidate this research gap. Promising fingerprinting techniques, such as DNA microarray or PCR coupled to denaturing gradient gel electrophoresis (DGGE), may help identify bacterial strains of interest by crossing gut-microbiota ecosystems, sourced from different individuals and differing in their ability to produce urolithins.

Once identified and their culture in bioreactors mastered, bacterial strains able to convert EA into urolithins would potentially be part of a new generation of probiotic bacteria that could be included in the formulation of novel functional foods. But, if they are not found to be "generally recognized as safe" (GRAS) and with their probiotic benefits well documented, their application in the food industry will not be possible, at least not in the short term.

The second strategy is to promote endogenous gut-microbial ecosystems that more efficiently convert ETs. It raises the question of possibly transforming low-urolithin producers into high-urolithin producers by changing dietary habits or including prebiotics or probiotics in the diet. As the notion of *urolithin-producer status* is relatively new, little information is available on how a human can change status by these means. We need to confirm if long exposure to ET-rich food could modify gut-microbiota ecology and induce the growth of urolithin-producing bacteria in human GI tracts, as was achieved for rats that were fed punicalagin for 20 days (Cerda et al., 2003). If so, then ET-rich food would be considered as a novel category of prebiotics. Nonetheless, a 4-day supplementation with raspberries did not produce statistically significant changes in the colonic bacteria profile in humans (Chris et al., 2010). The period of consumption may have been too short and the method of analysis not sufficiently accurate. For equol, a microbial derivative of daizein, which has been more intensively studied than urolithins, long exposure to isoflavone tends to increase equol production in "low-equol producer" subjects. Even so, production levels always remain much lower than for "high-equol producers," and physiological concentration of equol still remains below the threshold for potential health benefits (Yuan et al., 2007).

Little information is available on the impact of classical prebiotics and probiotics on urolithin production, although ETs and EA enhance the growth of certain *Bifidobacterium* species in liquid anaerobic culture (Bialonska et al., 2009b). But whether these probiotic strains could stimulate urolithin-producing bacterial species is not known. Specific probiotic bacteria (*L. plantarum*, *L. paraplantarum*, and *L. pentosus*), which can bioconvert ETs into EA, may also be able to enhance the in vivo growth of endogenous bacteria able to use EA. However, no information is available on their possible interaction with urolithin production. For example, soy isoflavone, consumed with common probiotic strains (*L. acidophilus* and *Bifidobacterium longum*) for 2 months, did not affect equol production (Yuan et al., 2007). Failure to select appropriate probiotics may explain this result, but gut-microbiota composition is difficult to change because of the relative predominance of the host's genotype over external factors such as short-term dietary shift (Ventura et al., 2009). However, promoting transitory change could constitute a promising research topic for enhancing urolithin production, not only by affecting microbiota ecosystems but also by modulating metabolic pathways.

The third approach is to formulate a food matrix that improves the bioavailability of urolithins. Little is known about the dietary ingredients that would act synergistically with ETs and enhance urolithin excretion (Aura, 2008; D'Archivio et al., 2010; Lansky and Newman, 2007). Fresh pomegranate juice or extract with purified phenolics from pomegranate, orally administered to volunteers, showed similar bioavailability for both EA and urolithin A (Seeram et al., 2008).

However, the presence of different compounds, such as anthocyanins, proteins, and calcium and magnesium ions, can bind to ETs and EA, sometimes inducing precipitation of the complex (Hourdes et al., 2009). Such precipitation may modulate the bioavailability of ETs and EA to gut microbiota, but this subject has not been studied so far.

In another study, phospholipids were added to EA, significantly enhancing health benefits in rats. The phospholipids appeared to increase the solubilization of EA in aqueous intestinal fluids (Venkatesh et al., 2009).

Indeed, the percentage of urolithin excretion with respect to the amount of ETs consumed seemed also to depend on the food matrix, as it was much higher for walnut, average for oak-aged red wine, and least for berries (Cerda et al., 2005b). This result may be related to structural differences of the ET molecules involved, and the presence of free EA or EA derivatives and other micronutrients. We point out that peduncalagin (MW 784 g mol^{-1}), the predominant ellagitannin in walnuts, has a simpler structure than vescalagin or castalagin (MW 934 g mol^{-1}) in oak-aged wine, and a much simpler one than sanguiin H6 or lambertianin C (MW 1870 and 2084 g mol^{-1}, respectively).

Hence, according to oligomeric structure, ETs could be more or less easily broken down by physiological conditions or microbial enzymes. Again, further information is needed on this particular issue.

During incubation with fecal suspension in vitro, the microflora was apparently more productive with EA as a substrate than with punicalagin, following the same incubation period (Gonzalez-Barrio et al., 2011). It means that conversion of ETs to EA could be a limiting pathway in some cases.

Modulation of intestinal transit time may also be an additional factor that influences urolithin production. Transit time commands the period of interaction between substrate and microflora. A study showed, for instance, that the bioavailability of total phenolics increases when these are associated with dietary fiber, which apparently delays their absorption (Perez-Jimenez et al., 2009). This factor may also benefit urolithin production. The influence of food matrix and ET structure on urolithin excretion deserves more attention from researchers. Better understanding of the mechanisms involved would be immediately useful for the food industry.

The fourth research approach concerns the in vitro production of urolithins, which could then be directly incorporated into food. This subject raises other issues such as the innocuity, pharmacokinetics, and stability of urolithins within the food matrix, and the in vitro transit in the upper GI tract. A recent study showed that urolithin A, orally administered to mice, was rapidly absorbed and had good bioavailability (Ishimoto et al., 2011).

For urolithin production, the first step is to convert ETs into EA. This can be done either chemically or by biotechnological methods (enzymatic or microbial). Even though the enzymatic pathways are not yet fully elucidated, commercial microbial strains and enzymes (tannase), able to degrade ETs into EA, already exist (Aguilera-Carbo et al., 2007; Huang et al., 2007). Bioconversion of EA into urolithins is a more complex step and so far has been performed only in vitro with fecal suspension from "high-urolithin producer" donors. Further research is needed to isolate urolithin-producing gut bacteria or to eventually elucidate the biomimetic pathways with enzymes. Lactone ring-cleaving enzymes and dehydroxylases appear to be the predominant catalysts in the bioconversion chain of EA to urolithins (Figure 1). Nonetheless, this strategy of providing food with urolithins should be more adapted to the development of dietary supplements and even drugs.

This description of the different research approaches to better exploit dietary ETs highlights the main gaps to be addressed in future research. Indeed, research on ellagitannins is unbalanced, with emphasis being given more to the biological activities of ETs and their metabolites than to their bioavailability and or to the possibilities of modulating it in the design of relevant dietary strategies. Such emphasis has even been counterproductive to the better understanding of the potential health benefits of ETs in clinical studies.

We, therefore, hope that this review will help focus research on this particularly promising and fascinating subject where results can be immediately used by food industries, bringing higher added value to end consumers.

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