Bioactivation of phytoestrogens: intestinal bacteria and health

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Abstract

Phytoestrogens are polyphenols similar to human estrogens found in plants or derived from plant precursors. Phytoestrogens are found in high concentration in soya, flaxseed and other seeds, fruits, vegetables, cereals, tea, chocolate, etc. They comprise several classes of chemical compounds (stilbenes, coumestans, isoflavones, ellagitannins and lignans) which are structurally similar to endogenous estrogens but which can have both estrogenic and antiestrogenic effects.

Although epidemiological and experimental evidence indicates that intake of phytoestrogens in foods may be protective against certain chronic diseases, discrepancies have been observed between *in vivo* and *in vitro* experiments.

The microbial transformations have not been reported so far in stilbenes and coumestans. However, isoflavones, ellagitanins and lignans are metabolised by intestinal bacteria to produce equol, urolithins and enterolignans, respectively. Equol, urolithin and enterolignans are more bioavailable, and them have more estrogenic/antiestrogenic and antioxidant activity than their precursors. Moreover, equol, urolithins and enterolignans have antiinflammatory effects and induce antiproliferative and apoptosis-inducing activities. The transformation of isoflavones, ellagitanins and lignans by intestinal microbiota is essential to be protective against certain chronic diseases, as cancer, cardiovascular disease, osteoporosis and menopausal symptoms.

Bioavailability, bioactivity and health effects of dietary phytoestrogens are strongly determined by the intestinal bacteria of each individual.

Keywords: Phytoestrogens, microbiota, equol, urolithin, enterodiol, enterolactone

INTRODUCTION

In the 1940s it was first realized that some plant derived compounds could cause an estrogenic effect (Bennetts et al., 1946). Sheep that were grazing on pastures containing red clover had multiple fertility problems. Immature animals were showing signs of estrus, ewes were unable to get pregnant and those that were pregnant often miscarried. The clover in these pastures had high amounts of the isoflavones (Rossiter and Beck, 1966) which were among the first phytoestrogens discovered.

The name phytoestrogens comes from the Greek phyto = plant and estrogen, the hormone which giving fertility to the female mammals. Also called "dietary estrogens", they are a diverse group of polyphenolic compounds found in plants that, because of their structural similarity with estradiol (17-ϐ-estradiol), have the ability to cause estrogenic or/and antiestrogenic effects different from those of estrogen (Kurzer and Xu, 1997). Working as estrogen mimics, the phytoestrogen can act like estrogen at low doses but block estrogen at high doses (Muller et al., 2004). Estrogen's effects are carried out by a family of proteins called estrogen receptors, these proteins are activated by their association with estrogen. On the other hand, the antioxidant activity showed by phytoestrogens is independent from their estrogenic properties (Moosmann and Behl, 1999).

Isoflavones remain the subject of many scientific studies, as illustrated by the more than 3000 scientific publications mentioning isoflavones in their title or abstract. Most of these studies

show that isoflavones may have some health benefit (Messina et al., 2006a; Xiao, 2008; Aso, 2010). In general, western populations consume low levels of isoflavones because few foods included in the typical western diet contain soya protein, the fraction with which isoflavones are associated. Setchell and Cassidy (1999) estimated the daily dietary intake of isoflavones in minus of 1 mg/day, whereas the Asian population consumes 25-100 mg/day (Messina et al., 2006b). The minor dietary intake is viewed as one explanation for the disparity in disease incidence rates between western and Asian populations. Soya has been a traditional staple in far eastern countries for generations, and the lower incidences of osteoporosis, breast cancer, and menopausal symptoms among women who consume soya as a dietary staple have been suggested in part to be due to the high intake of isoflavones (Hooper and Cassidy, 2006).

Although isoflavones are the phytoestrogens more studied and known, others four different families of phenolic compounds produced by plants are considered as phytoestrogens: stilbenes, lignans, ellagitannins and coumestans (Figure 1).

Although phytoestrogens cannot be considered as nutrients, given that the lack of these in diet does not produce any characteristic deficiency syndrome (Cornwell et al., 2004). Research into the role of phytoestrogens in health has generally shown they exert beneficial effects, although there are also some inconclusive studies or others that report negative effects (Sathyapalan et al., 2011; Haines et al., 2012). Intestinal bacteria may influence bioavailability and physiological activity of dietary phytoestrognes and strong interindividual differences in the microbial conversion of some dietary polyphenols have been reported (Cerdá et al., 2005; Setchell et al., 2005; Clavel et al., 2006a).

TYPES OF PHYTOESTROGENS

Five different families of phenolic compounds are considered as phytoestrogens, which we classify in two groups. Isoflavonoids, lignans and ellagitannins which are metabolised by the microbiota, and stilbenes and coumestans of which microbial transformations have not been reported so far (Figure 1).

Stilbenes

Stilbenes, like the flavonoids, are produced through the phenylpropanoid-acetate pathway. The main dietary source of phytoestrogenic stilbenes is resveratrol (Figure 2) from red wine and peanuts (Casiddy et al., 2000). Although there are two isomers of resveratrol, cis and trans, only the trans form has been reported to be estrogenic (Gehm et al., 1997).

Coumestans

Although there are a large number of coumestans, only a small number have shown estrogenic activity, predominantly coumestrol (Figure 3) and methoxycoumestrol (Ndebele et al.,

2010). The main dietary source of coumestrol is legumes. Clover and soybean sprouts are reported to have the highest concentrations (Wang et al., 1990; Franke et al., 1994).

Isoflavonoids

Twelve different soya bean isoflavone isomers have been identified. Most dietary sources contain a mixture of derivatives based on the isoflavone aglycones, daidzein, genistein and glycitein. As well as the aglycone form, isoflavones may be present in soya foods as glucosides, acetyl glucosides or malonyl glucosides (Coward et al., 1993; Jackson et al., 2002). Equol, an intestinal metabolite of daidzein, has the strongest binding affinities and estrogenic activities (Hwanga et al., 2006).

Analysis of levels in various species has found that the highest levels of genistein and daidzein in psoralea. Various legumes including soy, kudzu, lupine, fava and bean contained substantial amounts of isoflavones (D`Agostina et al., 2008). Highly processed foods made from legumes, such as tofu, retain most of their isoflavone content, with the exception of fermented miso, which has increased levels. Other dietary sources of isoflavones include chick pea (biochanin A), alfalfa (formononetin) and peanut (genistein).

Ellagitannins

Ellagitannins are bioactive polyphenols that are abundant in some fruits, nuts and seeds such as pomegranates, black raspberries, raspberries, strawberries, walnuts and almonds (Amakura et al., 2000; Clifford and Scalbert, 2000; Landete, 2011). Ellagitaninns, which belong to the hydrolysable tannin class of polyphenols, are complex derivatives of ellagic acid (Quideau and Feldman, 1996).

Pomegranate is a rich source in the ellagitannin punicalagin, raspberry and strawberry extracts mainly contain the ellagitannin sanguiin H-6, as well as various ellagic acid derivatives. Walnut extract contained, among others, pedunculagin, valoneic acid dilactone and casuarictin, sanguiin H-5 from muscadine grapes and vescalagin, castalagin and roburin E from oak wood (and oak-aged wines and spirits (Landete, 2011). The gut microbiota molecules urolithin A (3,8-dihydroxy-6H-dibenzopyran- 6-one) and its monohydroxylated analogue known as urolithin B are metabolites derived from ellagic acid and the related molecules ellagitannins and they showed weaker estrogenic activity than the other phytoestrogens (Larrosa et al., 2006a).

Lignans

Lignans include a number of diphenolic compounds such as secoisolariciresinol (Seco), matairesinol, pinoresinol, medioresinol, lariciresinol, syringaresinol, sesamin, 7Ǹj hydroxymatairesinol and isolariciresinol. Lignans are found in high concentration in flaxseed

and other seeds, as well as in fruits and vegetables, but also beverages such as coffee, tea and wine contain plant lignans, and are therefore present in substantial concentrations in Western diets (Valsta et al., 2003; Milder et al., 2005; Thompson et al., 2006; Landete, 2012). Lignans are metabolized in the mammalian gut to produce the most estrogenic enterolignans, enterodiol and enterolactone (Carreau et al., 2008; Landete, 2012).

METABOLISM OF PHYTOESTROGENS: ENZYMATIC REACTIONS AND MICROORGANISMS IMPLICATED

Although the maximum concentration in plasma rarely exceeds 1 mM after the consumption of 10ï100 mg of a single phenolic compound, the total plasma phenol concentration is probably higher due to the presence of metabolites formed in the bodyôs tissues or by the colonic microbiota (Scalbert and Williamson, 2000). The absorption of food phenolics is determined primarily by their chemical structure, which depends on factors such as the degree of glycosylation, hydroxylation, acylation, conjugation with other phenolics, molecular size, degree of polymerization and solubility (Karakaya, 2004). Polyphenols are extensively metabolised either in tissues, once they are absorbed through the gut barrier, or, for the non-absorbed fraction and the fraction re-excreted in the bile, by the colonic microbiota (Figure 4). In general, dietary polyphenols are substrates for enzymes located in the small intestine, colon and

liver (hydrolyzing and conjugating enzymes) (Németh et al., 2003; Spencer, 2003; Rechner et al., 2004) (Figure 4). All polyphenols are conjugated to form *O*-glucuronides, sulphate esters and *O*-methyl ether. Virtually, no free aglycones are found in plasma (Manach et al., 1998; Bell et al., 2000), except for particular flavonoids such as phloretin (Crespy et al., 2001). This conjugation first occurs in the gut barrier and then reaches the liver, where it is further metabolised (Donovan et al., 2001) (Figure 4). The formation of anionic derivatives by conjugation with glucuronides and sulphate groups facilitates their urinary and biliary excretion and explains their rapid elimination.

Metabolism of stilbenes

No conclusive data regarding the microbial transformation of stilbenes have been reported so far. The only previously reported tentative gut microbiota derived was dihydroresveratrol (Walle et al., 2004) and *Slackia equolifaciens* and *Adlercreutzia equolifaciens* have been recently reported as dihydroresveratrol producers (Bode et al., 2013). This metabolite can be formed by the catalytic hydrogenation of trans-resveratrol (Stivala et al., 2001), and it was found in the human urine as glucuronide and sulfate conjugates upon oral ingestion of resveratrol (Walle et al., 2004). The presence of resveratrol in the diet is scarce and its bioavailability is very low (Walle et al., 2004; Asensi et al., 2002). It is rapidly conjugated to yield mainly glucuronide and sulfate derivatives through an active enterohepatic circulation (Marier et al., 2002). This means that resveratrol could not reach colon distal portions in which the microbial metabolism is higher. Although, strong interindividual differences in the trans-resveratrol metabolism were

observed by Bode et al. (2013). In this context, the metabolism of stilbenes by gut microbiota, especially that of resveratrol, deserves further research as it is largely unexplored (Selma et al., 2009).

Metabolism of isoflavones

Enzymatic reactions

Almost all soy isoflavones exist as glycosides, which are less estrogenic than their respective aglycones, in soy and unfermented soy foods. Isoflavone glycosides are not absorbed intact across the enterocyte of healthy adults because of their higher hydrophilicity and molecular weights (Hur et al., 2000). Their bioavailability requires the conversion of glycosides to aglycones via the action of ɓ-glycosidase from tissue or bacteria that colonize the small intestine for uptake to the peripheral circulation (Setchell et al., 2002a; Setchell et al., 2002b). After ingestion, soy isoflavones are partially hydrolyzed in the small intestine (Richelle et al., 2002), mostly in the jejunum (Zubik and Medyani, 2003) to release the aglycones, daidzein, genistein, and glycitein followed by absorption through the gut epithelium (Decroos et al., 2005). A considerable fraction of isoflavones, which is neither hydrolyzed nor absorbed in the small intestine, reaches the colon, together with an amount that is excreted into the small intestine through enterohepatic circulation. In the colon, the glycosylated, sulfated, and glucuronidated forms of daidzein are deconjugated by bacterial enzymes, and then absorbed or subjected to further metabolism by the intestinal microbiota (Setchell and Cassidy, 1999; Decroos et al., 2005; Zubik and Medyani, 2003).

Then, in the equol-producer individuals, daidzein is converted via dihydrodaidzein to *O*-desmethylangolensin or equol by enzymes of intestinal bacteria (Figure 5). Shimada et al. (2010) assumed that equol was produced from daidzein in *Lactococcus* strain 20-92 following three stages of enzymatic reactions, those stages were the conversion of daidzein to dihydrodaidzein, of dihydrodaidzein to tetrahydrodaidzein and of tetrahydrodaidzein to equol (Figure 5). The conversion from daidzein to dihydrodaidzein is a reducing reaction and this NADP(H)-dependent daidzein reductase was cloned and purified by Shimada et al. (2010). In the second stage, the keto group of dihydrodaidzein at C-4 position is converted to the alcohol group to yield tetrahydrodaidzein. Then it is converted to equol by removal of the alcohol group at the C-4 position. However, the tetrahydrodaidzein could not be detected in the chromatogram. Perhaps, this compound is not stable and rapidly changed to equol or was high in the turnover number of the enzyme that changes tetrahydrodaidzein to equol. The stereochemical course of (3*R*)-dihydrodaidzein reduction into (3*S*)-equol via (3*R*, 4*S*)-tetrahydrodaidzein by the human intestinal bacterium *Eggerthella* strain Julong 732 was determined by Kim et al. (2009). The experiment showed by Kim et al. (2009) firmly established the sole intermediary of tetrahydrodaidzein in the dihydrodaidzein reduction into equol, at least by Julong 732. Moreover, equol can be metabolized to 6-hydroxy-equol and 3´ hydroxy-equol by means of hydroxylation reactions (Rüfer et al., 2006).

Equol is absorbed more efficiently through the colon wall than daidzein (Decroos et al., 2005), and appears in plasma after intake of daidzein and remains in plasma for a relatively longer period of time than do genistein and daidzein (Zubik and Medyani, 2003). Equol concentration in plasma is negligible until 4 h and reaches a maximum concentration 24 h after

the ingestion of the isoflavones; thereafter, equol concentration in plasma decreases but remains higher than the baseline concentrations 48 h after ingestion. There were significantly higher plasma equol concentrations in subjects after ingestion of glucoside than after ingestion of aglycone over the 48 h period (Zubik and Medyani, 2003).

Microbiota and equol

Equol, an intestinal metabolite of daidzein, is produced exclusively by intestinal bacteria, but only approximately one-third of human individuals are estimated to harbour intestinal microorganisms capable of transforming daidzein into equol (Morton et al., 2002; Hedlund et al., 2006). Similar to that in humans, variation in the metabolism of daidzein by fecal bacteria from rhesus monkeys was evident; only five cultures of fecal bacteria from nine monkeys were able to produce equol (Raffi et al., 2003). Daidzein-metabolizing phenotypes are stable in individuals over time (Frankenfeld et al., 2005) because the intestinal microbiota of such individuals is stable (Frankenfeld et al., 2004).

Microorganisms capable of producing daidzein, dihydrodaidzein and/or *O*-desmethylangolensin have been isolated and identified. *Escherichia coli* HGH21 and *Clostridium* sp. HGH136 isolated in feces from a healthy individual convert isoflavone glucosides (daidzin) to aglycones (daidzein) (Hur et al. 2000). Moreover, *Clostridium*-like bacterium TM-40 (Tamura et al., 2007), which transform daidzein to dihydrodaidzein, and *Clostridium* sp. HGH136 (Hur et al., 2002), and *Eubacterium ramulus* (Schoefer et al., 2002), which transform daidzein to *O*-desmethylangolensin, were isolated from human feces. An

anaerobic bacterium, Niu-O16, capable of converting daidzein to dihydrodaidzein was isolated from bovine rumen contents by Wang et al. (2005b). Later this strain was referred as *Lactobacillus* sp. Niu-O16 (Wang et al., 2007). The capabilities of converting daidzin to aglycones daidzein have been demonstrated in lactic acid bacteria isolated from human as *Lactobacillus paraplantarum* KM, *Enterococcus durans* KH and *Weissella confusa* (Chun et al., 2007), as well as in lactic acid bacteria from culture collection (Marazza et al., 2009).

The first demonstration about equol production by intestinal bacteria was reported by Chang and Nair (1995), daidzein was fermented with human fecal bacteria under anaerobic conditions and dihydrodaidzein and equol were found. In some cases, although some authors have found mixtures of microorganisms as equol producer, equol producing individual isolates have not been identified in the mix, it suggesting that the different species are necessary for producing equol. It is possible that the different microorganisms present in the mix contribute to the different enzymatic reactions implicated in the formation of equol from daidzein. So, a mixture of three strains of *Bacteroides ovatus*, *Streptococcus intermedius* and *Ruminococcus productus*, identified by culturing the fecal flora from healthy Japanese adults, were able to convert daidzein to equol *in vitro* (Ueno and Uchiyama, 2001).

Decroos et al. (2005) obtained a mixed bacterial culture, also from human feces, that could transform daidzein into equol. The microbial community was composed of four different species, three of which could be cultured and identified as *Lactobacillus mucosae* EPI2, *Enterococcus faecium* EPI1 and *Finegoldia magna* EPI3. The fourth species was tentatively identified as *Veillonella* sp. strain EP. Later, Decroos et al. (2006) administered this mixed

microbial culture to the Simulator of the Human Intestinal Microbial Ecosystem and the equol production was induced in the distal colon compartment 5ï 6 days after the start of the treatment.

So, an anaerobic incubation mixture of two bacterial strains *Eggerthella* sp. Julong 732, which was previously isolated from a human fecal sample (Wang et al. 2005a), and *Lactobacillus* sp. Niu-O16 have been known to transform dihydrodaidzein to *S*-equol and daidzein to dihydrodaidzein respectively, produced *S*-equol from daidzein through dihydrodaidzein (Wang et al., 2007). The biotransformation kinetics of daidzein by the mixed cultures showed that the production of *S*-equol from daidzein was significantly enhanced, as compared to the production of *S*-equol from dihydrodaidzein by *Eggerthella* sp. Julong 732 alone.

Jin et al. (2008) isolated from human feces two intestinal bacteria capable of metabolizing puerarin. One of them, strain PUE, converted puerarin to daidzein by cleaving a C-glucosyl bond, whereas the other, strain DZE, converted daidzein to equol by reducing a double bond in ring C followed by elimination of an oxo group. Based on the 16S ribosomal RNA gene sequence, strain DZE showed 85% similarity with *Eggerthella lenta*.

Different soy milks were fermented with a mix of selected starters consisting of *Lactobacillus plantarum* DPPMA24W and DPPMASL33, *Lactobacillus fermentum* DPPMA114, and *Lactobacillus rhamnosus* DPPMAAZ1 observed as the concentration of equol increased in several soy milk preparations after incubation with this mix of selected starters (Di Cagno et al., 2010).

Minamida et al. (2006) reported an anaerobic gram-positive strain isolated from rat intestine capable of producing equol from daidzein. The strain was referred as do03 and had 99%

16S rRNA gene sequence homology to the human intestinal bacterium SNU-Julong 732 (AY310748). Then, Wang et al. (2005a) and Minamida et al. (2006) had isolated the same specie equol producer from different source.

Uchiyama et al. (2007) tried to isolate human intestinal bacteria capable of metabolizing daidzein to equol using the feces of healthy humans. Only one strain, *Lactococcus* 20-92, was equol producer. It was identified as *Lactococcus garvieae* by the 16S rDNA homology. *Lactococcus* 20-92 is the first reported lactic acid bacteria capable of metabolizing daidzein to equol directly. More recently, *Bifidobacterium breve* 15700 and *Bifidobacterium longum* BB536 out of twenty two strains of bifidobacterias tested produced equol from daidzein (Elghali et al., 2012). *Lactococcus* 20-92 and bifididobacteria are expected in functional foods. So far, *Lactococcus* 20-92 and these strains of bifidobacteria are the only euquol-producing microorganisms no belonging to the *Coriobacteriaceae* family.

Two equol-producing strains D1 and D2 were isolated by Yu et al. (2008), initially referred as *Eubacterium* sp. and with close relationships with two isolates, *Eggerthella* sp. Julong 732 and *Lactobacillus* sp. Niu-O16 above mentioned. A new equol-producing bacterium was isolated from the feces of healthy humans by Yokohama and Suzuki (2008). It was most closely related to *Eggerthella hongkongensis* HKU10, with 93.3% 16S rDNA nucleotide sequence homology. The isolate was identified as a novel species of the genus *Eggerthella* and it was named *Eggerthella sp*. YY7918. The strain YY7918 converted substrates daidzein and dihydrodaidzein into *S*-equol, but was not able to convert daidzin to daidzein. The genomic sequence of the equol-producing bacterium *Eggerthella* sp. strain YY7918 was completed recently by Yokohama et al. (2011).

Matthies et al. (2008) isolated a strictly anaerobic bacterium (Mt1B8) from the mouse intestine which converted daidzein via dihydrodaidzein to equol. The strain Mt1B8 was identified as a member of the *Coriobacteriaceae* family. Later, Matthies et al. (2009) isolated from human feces an anaerobic bacterium (HE8) able to convert the isoflavones daidzein and genistein to equol and 5-hydroxy-equol, respectively. Based on phenotypic and phylogenetic analyses, strain HE8 was described as a new species, *Slackia isoflavoniconvertens*. The genus *Slackia* belongs to the family *Coriobacteriaceae* and its members are Gram-positive, non-motile, obligate anaerobes (Wade et al., 1999).

Nine strains capable of metabolizing isoflavones to equol were isolated from human feces by Maruo et al. (2008). Four of them were characterized by determining phenotypic and biochemical features and their phylogenetic position based on 16S rRNA gene sequence analysis. These strains were related to *Eggerthella sinensis* HKU14T with about 93% 16S rRNA gene sequence similarity. The differences in the major menaquinone and cell-wall peptidoglycan type of these strains with respect to members of the genus *Eggerthella* led to Maruo et al. (2008) to propose a new genus, *Adlercreutzia,* with one species, *Adlercreutzia equolifaciens sp*.

An obligately anaerobic and equol-producing bacterium, designated strain do03T, was isolated from the caecal content of a rat by Minamida et al. (2008). The data presented in this work show that strain do03T differs from members of the related recognized genera *Eggerthella* and *Denitrobacterium* at both the phylogenetic and phenotypic level. Therefore, the strain constitutes a novel genus and species, for which the name *Asaccharobacter celatus* gen. nov., sp. nov. is proposed by Minamida et al. (2008). Also, a microorganism of the sames species and from the same source was isolated by Thawornkuno et al. (2009). *Asaccharobacter* is also a

genus of Actinobacteria in the family *Coriobacteriaceae*. Tsuji et al. (2010) found a non-spore-forming rod bacterium with high ability to convert daidzein to equol. The strain was found to belong to the genus *Slackia* family *Coriobacteriaceae* by 16S rRNA sequence-based analysis. The prevalence of the *Slackia sp*. was examined in Japanese adults, which was found to be 40% at a mean population level of 10^6 cells per gram of feces.

An equol-producing bacterium, strain DZE^T, which was isolated from human feces by Jin et al. (2010), showed 92.8, 91.0, 91.1 and 90.6 % similarities in the 16S rRNA gene sequence analysis with *Slackia faecicanis*, *Slackia exigua*, *Slackia heliotrinireducens* and *Slackia isoflavoniconvertens*, respectively. Based on these data, Jin et al. (2010) proposed a novel species of the genus *Slackia*, *Slackia equolifaciens* sp.

Finally, Matthies et al. (2011) demonstrated as a human intestinal *Slackia isoflavoniconvertens* converted daidzein and genistein to equol and 5-hydroxy-equol respectively in gnotobiotic rat. Equol and 5-hydroxy-equol were found in intestinal contents, feces, and urine of rats but not in the corresponding samples of the control rats. 5-Hydroxy-equol was present at much lower concentrations than equol and the main metabolite produced from genistein was the intermediate dihydrogenistein. The plasma of rats contained equol but no 5-hydroxy-equol (Matthies et al., 2011).

So far, and with the exception of bifidobacterias and *Lactococcus* 20-92, the equol-producing bacteria belong to the *Coriobacteriaceae* family. The fact that only a limited number of bacterial strains capable of degrading daidzein and producing equol have been isolated and identified may be due to the need for anaerobic culture conditions and specific medium constituents. Moreover, a large percentage of intestinal bacteria are non culturable.

Metabolism of ellagitannins, ellagic acid and urolithins

Seeram et al. (2004) conducted *in vivo* studies whereby a human subject consumed pomegranate juice; ellagic acid was detected in human plasma at a maximum concentration after 1 h postingestion but was rapidly eliminated by 4 h. The presence of free ellagic acid in human plasma could be due to its release from the hydrolysis of ellagitaninns, facilitated by physiological pH and/or gut microbiota (Figure 6). Ellagitaninns are not usually absorbed and must be metabolized prior to absorption (Mertens-Talcott et al., 2006; Borges et al., 2007).

Ellagitaninns and ellagic acid consumption is associated with the urinary excretion of dibenzopyran-6-one metabolites, mainly urolithin A and urolithin B, which are also observed in plasma as conjugates after consumption of ellagic acid derivatives (Cerdá et al., 2005). The large inter-individual variability observed in the production and excretion of these metabolites, and the fact that urolithins are excreted independently of the ellagitaninns consumed, would suggest microbial involvement and their production in the colon of those individuals with the microbiota able to achieve ellagitaninns degradation and transformation into dibenzopyran-6-one metabolites (Cerdá et al., 2003; Cerdá et al., 2004; Cerdá et al., 2005).

On the other hand, Ito et al. (2008) prepared a fecal suspension from rats and after centrifugation the supernatant was used as a microbiota suspension. An aliquot of the suspension was incubated with ellagitaninns and after incubation for 96 h, the reaction mixture contained different urolithins demonstranted the microbial origin of urolithins.

The microbially mediated origin of some phytoestrogens, such as equol, enterodiol and enterolactone has been suggested and demonstrated; in fact microorganisms that produce them have even been isolated and identified (Uchiyama et al., 2007; Maruo et al., 2008; Clavel et al., 2006b). Furthermore, the microbially mediated origin of urolithin has been demonstrated (Cerdá et al., 2005; Ito et al., 2008); however, urolithin-producing microorganisms have not been isolated so far. Both ellagitaninns and ellagic acid are largely metabolized by the colon microbiota of different mammals, including rats (Cerdá et al. 2003), pigs (Espín et al., 2007) and humans (Cerda et al. 2004; Larrosa et al., 2006a). In all these cases, both ellagic acid and ellagitaninns produce dibenzopyranones known as urolithin A and urolithin B (Cerdá et al. 2004) (Figure 6). Therefore, in the intestine ellagic acid seems to be transformed by lactone-ring cleavage, decarboxylation and dehydroxylation reactions (Selma et al., 2009).

Cerdá et al. (2005) investigated the metabolism of different dietary ellagitaninns and ellagic acid derivatives in forty healthy volunteers, distributed into four groups. Each group consumed a single dose of a particular ellagitaninns-containing foodstuff. Neither ellagitaninns nor ellagic acid were detected in urine. However, urolithin B conjugated with glucuronic acid was detected in all the subjects, independently of the consumed foodstuff. Considerable inter-individual differences were resgistered, identifying ñhigh and low metabolite excretersò in each group. According to the results obtained by Cerdá et al. (2005), urolithin B derivatives were excreted independently of the ellagitaninns consumed. Recently, González-Barrio et al. (2010) also observed how ellagitaninns were catabolized with the appearance of urolithin A-*O*-glucuronide, two of its isomers, and urolithin B-*O*-glucuronide in urine collected 7-48 h after raspberry consumption.

Further studies show that urolithins appear in human systemic circulation within a few hours pomegranate product consumption, reaching maximum concentrations at between 24 h and 48 h, and being present in plasma and urine for up to 72 h, in free and conjugated forms (Seeram et al., 2006; Mertens-Talcott et al. 2006, Larrosa et al., 2006a).

Espin et al. (2007) showed in Iberian pig as a model animal that ellagitaninns release ellagic acid under physiological conditions *in vivo* and that ellagic acid is then gradually metabolized in the intestine, starting in the jejunum, to produce urolithin D, urolithin C and finally urolithin A and urolithin B (Figure 6). Urolithin B is mainly produced at the distal parts of the intestine. In addition, analyses of the intestinal tissues show that the metabolites are absorbed preferentially when their lipophilicity increases. Glucuronides and methyl glucuronides of ellagic acid and particularly urolithin A, C, and D derivatives were detected in bile, confirming active enterohepatic circulation. Urolithins A, urolithin B and dimethyl-ellagic acid-glucuronide were also detected in peripheral plasma by Espin et al. (2007). The presence of ellagic acid metabolites in bile and urine and its absence in intestinal tissues suggests its absorption takes place in the stomach. Urolithin A is the main ellagitaninns-derived metabolite detected in feces in both pigs and humans (Cerdá et al., 2005), whereas urolithin B is not detected in either feces or bile. Following metabolism of ellagitannins, urolithins A and B are formed and conjugated in the liver prior to their excretion in urine after a single administration of food containing ellagitaninns and ellagic acid. Therefore, these urolithins circulate in the blood and must also reach the target organs, where the effects are noticeable.

The distribution of ellagitannin metabolites in muscle, adipose (subepidermal and visceral), lung, liver, heart and kidney was analysed by Espin et al. (2007). None of the

metabolites was detected in the crude extracts, showing that these tissues are not targets for ellagitaninns metabolite accumulation (Espin et al., 2007). Conversely, traces of punicalagin metabolites were detected in liver and kidney of rats (Cerda et al., 2003) while tissue disposition studies reveal that urolithins are enriched in prostate, intestinal, and colon tissues in mice (Seeram et al. 2007). Moreover, regarding tissue distribution of urolithins and their conjugates, González-Sarrias et al. (2010) recently found that these molecules reach and enter the human prostate after consumption of ellagitaninns-rich foods. The main metabolite detected was urolithin A glucuronide (up to 2 ng/g tissue) together with traces of urolithin B-glucuronide and ellagic acid-dimethyl ether. These metabolites were present only in a small number of the 63 patients studied, which may be explained by rapid clearance of the compounds during pre-surgery fasting. This was confirmed in a parallel study with rats (González-Sarrias et al., 2010).

A large person-to-person variation in the timing, quantity and types of urolithins excreted in urine was observed by González-Barrio et al. (2010). These interindividual variations in urinary excretion of urolithins are almost certainly due to ellagitannins and ellagic acid degradation being dependent on intestinal bacteria composition. Similar variability was also observed in others studies with raspberries and pomegranate juice (Cerdá et al., 2004; Cerdá et al., 2006; Seeram et al., 2006).

Although, the microbially mediated origin of urolithins is proposed, specific bacteria involved in the metabolic urolithin-producing processes are, as yet, unknown. Environmental conditions in the colon involved in the production of urolithins have yet to be discovered. Although urolithin production has been established *in vitro*, the need for anaerobic culture

conditions and specific medium constituents can be the reason for failure to identify or isolate bacteria.

Metabolism of plant lignans and mammalian lignans

Studies in which flaxseed were fed to rats, monkeys, or humans have found that the urinary excretion of the lignans enterodiol and enterolactone significantly increased (Axelson et al., 1982; Saarinen et al., 2010). Excretion of enterodiol and enterolactone increased 3- to 285-times after flaxseed consumption (5 to 10 g daily for 6 weeks) in the urine of 18 healthy young women, 31 healthy postmenopausal women and six healthy young men (Lampe et al., 1994; Hutchins et al., 2000).

Mazur et al. (2000) confirmed that enterolignans are primarily produced and absorbed in the colon from lignans intake in the diet. Several authors have described how a bacterial community transforms plant lignans into enterodiol and enterolactone (Figure 7). Accordingly, Wang et al. (2010) obtained a bacterial consortium, designated as END-49, which transformed flaxseed to enterodiol. Based on analysis using pulsed field gel electrophoresis, END-49 was found to consist of five genomically distinct bacterial lineages. Woting et al. (2010) demonstrated how a defined bacterial community, *Clostridium saccharogumia*, *Eggerthella lenta*, *Blautia producta* and *Lactonifactor longoviformis* convert the plant lignan SDG via Seco into the bioactive enterolignans enterodiol and enterolactone. Previously, *Clostridium saccharogumia* and *Lactonifactor longoviformis* had been identified as two novel species,

involved in the conversion of the dietary phytoestrogen SDG to enterodiol and enterolactone (Clavel et al., 2007).

The metabolism of plant lignans by human fecal microbiota was investigated by Heinonen et al. (2001), enterodiol was the major compound identified after fecal incubation of matairesinol with 62% of the added matairesinol being recovered as enterolactone. Seco was converted to enterodiol and enterolactone, which accounted for 72% of the added Seco. Conversion of pinoresinol to enterolactone and enterodiol was almost as effective (55%) as that for the known plant precursors, Seco and matairesinol. Moreover, enterolactone and enterodiol were the only major metabolites detected during incubation, although a minor metabolite, enterofuran, was also detected in the incubation extract of pinoresinol. Syringaresinol was partly converted to enterodiol and enterolactone during incubation (4%). Lariciresinol incubated with a fecal suspension was completely converted to enterolactone and enterodiol (Heinonen et al., 2001). Enterofuran was also identified as a minor metabolite of lariciresinol. Four percent of the arctigenin was metabolized to enterolactone. Isolariciresinol remained mostly unchanged during incubation.

The transformation between different plant lignans could also be produced by intestinal microbiota, pinoresinol and lariciresinol are transformed to Seco by *Eggerthella lenta* (Clavel et al. 2006b) and pinoresinol to lariciresinol by *Enterococcus fecalis* strain PDG-1 (Xie et al., 2003b).

The bioactivity of lignans depends on their transformation by gut bacteria. Mammalian lignans are formed in the human body in the gastrointestinal tract, where gastrointestinal bacteria hydrolyze the sugar moiety of Secoisolariciresinol diglucoside (SDG) to release Seco (Ford et

al., 2001; Touré and Xueming, 2010). Strains of *Bacteroides distasonis*, *Bacteroides fragilis*, *Bacteroides ovatus* and *Clostridium cocleatum* and *Clostridium sp*. SDG-Mt85-3Db were found to be involved in the deglycosylation of SDG to Seco by Clavel et al. (2006a). Recently, Roncaglia et al. (2011) have demonstrated how bifidobacterium cultures hydrolyzed SDG to Seco.

The deglycosylation is followed by demethylation and dehydroxylation by the colonic microbiota to give the mammalian lignan enterodiol (Figure 7). Demethylation of Seco produces dihydroxyenterodiol (2,3-bis(3,4-dihydroxybenzyl)-1,4-butanediol) by intestinal bacteria such as *Butyribacterium methylotrophicum*, *Eubacterium callanderi*, *Eubacterium limosum*, *Ruminococcus productus* and *Peptostreptococcus productus* (Clavel et al., 2006a). Although these data were obtained with Seco alone, bacteria involved in enterolignan production from SDG also convert other plant lignans. For instance *R. productus* not only catalyses Seco demethylation, but also the demethylation of lariciresinol, mataresionol, pinoresinol and of a variety of other methylated aromatic compounds (Clavel et al., 2006a). Demethylation of arctigenin, an aglycone of arctiin, has been observed after incubation with *Eubacterium. sp*. ARC-2. Moreover, this bacterium can transform Seco into dihydroxyenterodiol by demethylation (Jin et al., 2007b).

Clostridium scindens and *Eggerthella lenta* produce the dehydroxylation of Seco (Clavel et al., 2005; Clavel et al., 2006a). *Eggerthella lenta* SDG-2 could transform (+)-dihydroxyenterodiol to (+)-enterodiol, but not (-)-dihydroxyenterodiol to (-)-enterodiol (Jin et al., 2007c). These authors isolated a different bacterium, strain ARC-1, capable of dehydroxylating (-)-dihydroxyenterodiol to (-)-enterodiol from human feces. Strain ARC-1 could transform not

only (-)-dihydroxyenterodiol to (-)-enterodiol, but also (+)-dihydroxyenterolactone to (+)-enterolactone. However, the bacterium could not transform (+)-dihydroxyenterodiol or (-)-dihydroxyenterolactone (Jin et al., 2007c).

Enterodiol is oxidized by the gastrointestinal microbiota to give enterolactone (Figure 7). Intestinal bacteria have also been isolated and identified in the transformation of enterodiol to enterolactone through of a dehydrogenation reaction. Jin and Hattori (2010) observed a human intestinal bacterium, strain END-2, which enantioselectively oxidizes (+)-enterodiol to (+)-enterolactone. The same authors had identified a strain of *Ruminococcus* sp. END-1 capable of oxidizing (-)-enterodiol to (-)-enterolactone (Jin et al., 2007a; Jin and Hattori, 2009), indicating enantioselective oxidation by intestinal bacteria. Previously, Clavel et al., (2006a) observed the dehydrogenation of enterodiol to enterolactone by the strains ED-Mt61/PYG-s6.

One or two additional reduction steps are involved in enterolignan production from lariciresinol and pinoresinol, respectively (Xie et al., 2003b). The structure of syringaresinol differs from pinoresinol in two additional methoxy groups at meta positions in both phenolic rings and this relatively complex substitution pattern can yield several metabolites during incubation. Most of the metabolites that were tentatively identified for syringaresinol share an oxydiarylbutane structure, suggesting that syringaresinol breakdown occurs similarly to that of pinoresinol, involving the corresponding lariciresinol and Seco intermediates. Two reactions of demethylation and dehydroxylation are needed to transform matairesinol into enterolactone, with dihydroxyenterolactone (2,3 bis (3,4 dihydroxybenzyl) butyrolactone) being found after demethylation reactions (Touré and Xueming, 2010). Borriello et al. (1985) showed how matairesinol is metabolised to enterolactone by human intestinal microbiota, while other authors

investigated how *Ruminococcus productus* catalyses the demethylation of matairesinol to 2,3-bis(3,4-dihydroxybenzyl) butyrolactone (Clavel et al., 2006a).

The transformation of arctiin, already bearing a butyrolactone structure, consists of the first three types of reactions as mentioned above for SDG, hydrolysis of lignan glucosides to their aglycones is the first transformation step. Second, demethylation of a methoxy group adjacent to a hydroxy group occurs. Third, dehydroxylation, fourth and fifth demethylation and the last dehydroxylation reactions produce enterolactone. Jin and Hattori (2009) showed as arctiin and Seco are converted to (-)-dihydroxyenterolactone and (+)-dihydroxyenterodiol by the human intestinal bacterium *Ruminococcus (R.) sp.* END-1; this bacterium showed oxidation, demethylation and deglucosylation activities for plant lignans. Moreover, by coincubation with *Eggerthella sp*. SDG-2, the bacterium transformed arctiin and SDG to (-) enterolactone and (+) enterodiol, respectively.

In the transformation of pinoresinol diglucoside (PDG), SDG and arctiin by human intestinal microbiota, the absolute configuration at the adjoining positions of two phenylpropanol moieties seems to be retained; PDG and arctiin give (-)enterolactone, while SDG gives (+) enterolactone (Landete, 2012). So far, conversion of medioresinol to enterolactone is unknown, although similarity with pinoresinol suggests additional demethylation and dehydroxylation steps (Landete, 2012).

Liu et al. (2006) observed how sesamin was converted into mammalian lignans when fermented *in vitro* with human fecal inoculums, although at a lower rate (1.1%) compared with SDG (57.2%). These authors surmise that sesamin is only partially metabolized to enterodiol and enterolactone in the colon; it is absorbed and metabolized in the liver to hydroxylated

metabolites, which are then excreted in bile. These metabolites, excreted in bile, undergo enterohepatic circulation and are further metabolized to mammalian lignans by the intestinal microbiota. Hence, the primary site of sesamin metabolism may differ from that of the more hydrophilic lignan precursors such as SDG, which is more readily metabolized to enterodiol and enterolactone by the intestinal microbiota before absorption (Nesbitt et al., 1999; Rickard and Thompson, 2000). Recently, asarinin, an epimer of sesamin, was also reported as mammalian lignan precursor (Jin and Hatorri, 2011).

Once produced by intestinal bacteria, enterolignans may be efficiently absorbed. Ingestion of 13.5 g of flaxseed per day for 6 weeks has been reported to lead to micromolar concentrations of enterolactone-sulphate, enterolactone-glucuronide and enterodiol-glucuronide in human plasma (Atkinson et al., 1993). This is 1000-10000 times the plasma level of the circulating endogenous steroidal estrogens (Rickard and Thompson, 1997). Conjugation of enterolignans with sulphate and glucuronic acid occurs in the intestinal wall and liver, with the predominant conjugates being glucuronides.

Controlled feeding studies have demonstrated dose-dependent urinary enterodiol and enterolactone in response to lignan consumption; however, even in the context of controlled studies, there is substantial inter-individual variation in plasma concentrations and urinary excretion of enterolignans. The complex interaction between the colonic environment and the external and internal factors modulating it, likely contribute to this variation. Factors controlling the bioactivation of lignans in the large intestine are diet, transit time, intestinal redox state and, most importantly, the composition and activity of the colonic microbiota (Rowland et al., 2000). Due to variations in these factors, large differences among individuals have been observed in

lignan bioactivation in urine, fecal and blood samples (Knust et al., 2006; Possemiers et al., 2007), leading to a subdivision of enterolignan producers in weak, moderate and strong phenotypes (Possemiers et al., 2007).

HEALTH AND PHYTOESTROGENS

Coumestrol has been shown to have both estrogenic and antiestrogenic effects on reproductive physiology including uterine proliferation in immature female rats (Whitten et al., 1992) and infertility in sheep (Adams, 1995), gene activation (Register et al., 1995), and behavior (Whitten et al., 1995). Studies indicate that coumestrol can cross the blood-brain barrier and affect estrogen-dependent gene expression (Jacob et al., 2001).

Resveratrol has chemopreventive activity against cardiovascular disease and a variety of cancers in model systems (Bhat and Pezzuto, 2002; Pervaiz, 2003). Based on *in vitro* studies resveratrol can inhibit cell proliferation, induce apoptosis, and block cell cycle progression in numerous types of human cancer cell lines, such as those of the colon, skin, breast, lung, prostate, and liver, as well as pancreas but it is not clear whether the resveratrol reaches the proposed sites of action *in vivo* after oral ingestion, especially in humans (Pervaiz, 2003).

The consumption of soy products has many health benefits, including protection against breast cancer (Fournier et al., 1998), prostate cancer (Messina et al., 2006a), menopausal symptoms (Han et al., 2002), heart disease (Erdman, 2000) and osteoporosis (Messina et al., 2001). Many of the health benefits of soy are derived from its isoflavones. However, some critics

claim that isoflavones can increase the incidence of epithelial hyperplasia (Petrakis et al., 1996) and cause goitre and hyperthyroidism (Cerundolo et al., 2009).

Ellagitaninns, ellagic acid and urolithins exhibit anticancer properties *in vitro* and *in vivo* (Heber, 2008; Seeram 2008). Recent research *in vitro* has shown that pomegranate extracts selectively inhibit the growth of breast, prostate, colon and lung cancer cells in culture (Kim et al., 2002; Malik et al., 2005; Seeram et al., 2005; Larrosa et al., 2006b). Furthermore, in preclinical animal studies, oral consumption of pomegranate extract inhibited growth of lung, skin, colon and prostate tumors (Kohno et al., 2004; Pantuck et al., 2006; Khan et al., 2006; Stoner, 2009).

Research into the role of lignans in breast, colon and prostate cancer has generally shown they exert beneficial effects (Landete, 2012), although there are also some inconclusive studies or others that report negative effects. Lignans may be of use in ameliorating some menopausal symptoms. Flaxseed can protect against atherosclerotic plaque deposition in carotid arteries and shows antiatherosclerotic effects in the aorta (Dupasquier et al., 2006). Hepatoprotective effects have also been associated to lignans in a flaxseed supplemented diet (Hemmings and Barker, 2004).

Which phytoestrogens have benefitial effects on the health?, Why these phytoestrogens produce those effects?, How these phytoestrogens produce the effects on the health?.

The health benefits from phytoestrogens consumption should be attributed to their bioactive metabolites and also to the modulation of the intestinal bacterial population. Then, dietary phytoestrogens are often transformed before absorption and this transformation modulates their biological activity. So, daidzin and daidzein are transformed in equol,

ellagitaninns in urolithins and plant lignans in enterodiol and enterolactone (Decross et al., 2006; Espín et al., 2007; Touré and Xueming, 2010). Which equol, urolithin and enterolignans are more bioavailable, and have more estrogenic/antiestrogenic and antioxidant activity than their precursors could explain the beneficial effects of these phytoestrogens. Then, one cannot infer biological responses from phytoestrogens dietary intake without considering polyphenolï microbiota interactions (Bolca et al., 2012).

Although, the polyphenols usually have beneficial effects on the health, some harmful effects have been described and discrepancies are observed between *in vivo* and *in vitro* experiments (Keinan-Boker et al., 2004; Tempfer et al., 2007).

Health and Equol

The clinical effectiveness of soy isoflavones may be a function of the ability to biotransform soy isoflavones to the more potent estrogenic metabolite as the equol, which may enhance the actions of soy isoflavones, owing to its greater affinity for estrogen receptors, unique antiandrogenic properties, and superior antioxidant activity (Setchell, 2004).

Unlike daidzein and genistein, equol is unique in having a chiral center due to the lack of a double bond in the heterocyclic ring, and is a chiral molecule that can exist in two enantiomeric forms, *S*- and *R*-equol. Human intestinal bacteria can exclusively synthesize *S*-equol, the

naturally occurring enantiomer, from daidzein (Muthyala et al., 2004; Setchell, 2004; Setchell et al., 2005). The two equol enantiomers *R*- and *S*-equol show very different behavior in terms of their binding affinities with estrogen receptor Ŭand estrogen receptor ɓ (Muthyala et al., 2004). In binding assays, *S*-equol has a high binding affinity, preferential for estrogen receptor ɓ, that is comparable to that of genistein, whereas *R*-equol binds more weakly and with a preference for estrogen receptor Ŭ (Muthyala et al., 2004). All equol isomers have higher affinity for both estrogen receptors than does the biosynthetic precursor daidzein. Equol can be readily synthesized from daidzein by catalytic hydrogenation, but this yields the (±) equol form (Lamberton et al., 1978) and it is the form that has been commercially available and mostly utilized in studies of its biological potency and properties. Indeed, unless otherwise stated, it can be assumed that all previously reported experiments used (±) equol and not the individual enantiomers. Equol production may enhance the action of isoflavones as it has a lower affinity for serum proteins, greater affinity for estrogen receptors compared with its precursors, daidzein and dihydrodaidzein (Cassidy, 2005). So, for example, equol administration appears to have potential beneficial effects for prostate health and other 5Ŭ-dihydrotestosterone mediated disorders *in vitro* and *in vivo* (Lund et al., 2011).

The higher antioxidant activity of equol may be a result of its nonplanar structure that confers equol with a greater flexibility for conformational changes, which can enable it to penetrate more easily into the interior of the membrane and protein or lipid structures to prevent oxidative damage *in situ* than some of the other isoflavones that are more rigid in structure (Rüfer and Kulling, 2006).

Health and Urolithins

Epidemiological evidence indicates that intake of food rich in ellagitaninns and ellagic acid may be protective against chronic diseases, although some *in vitro* results often do not match the findings in the *in vivo* studies. This could be explained by the low bioavailability; ellagitaninns are not usually absorbed and must be metabolized to ellagic acid prior to absorption (Landete, 2011). As above mentioned, ellagic acid is gradually metabolized by the microbiota to produce urolithin D, urolithin C and finally urolithin A and urolithin B. The metabolites are absorbed preferentially when their lipophilicity increases (urolithin A and B) (Espín et al., 2007).

Urolithins have been reported as a less potent antioxidant compared to the ellagitaninns and ellagic acid. Urolithins could display estrogenic and/or antiestrogenic activity and tissue disposition studies reveal that urolithins are enriched in prostate, intestinal, and colon tissues in mouse, fact that explain that urolithins inhibit prostate and colon cancer cell growth (Seeram et al., 2007).

Larrosa et al. (2006a) evaluated the ability of urolithins to bind to Ŭ- and ɓ-estrogen receptors. Both urolithins A and B showed estrogenic activity in a dose-dependent manner even at high concentrations (40 µM), without antiproliferative or toxic effects, whereas the other phytoestrogens inhibited cell proliferation at high concentrations. Overall, urolithins showed weaker estrogenic activity than other phytoestrogens as daidzein, genistein and enterolactone (Larrosa et al., 2006a). However, both urolithins displayed slightly higher antiestrogenic activity

(antagonized the growth promotion effect of 17- ϐ-estradiol in a dose-dependent manner) than the other phytoestrogens.

Moreover, antiproliferative and apoptosis inducing activities of ellagic acid and urolithins have been demonstrated in the inhibition of cancer cell growth. Juranic et al. (2005) demonstrated how ellagic acid extracted from raspberries possesses the potential for antiproliferative action against human colon carcinoma cells *in vitro*. Kasimsetty et al. (2010) showed that the ellagitaninns and urolithins released in the colon upon consumption of pomegranate juice in considerable amounts could potentially curtail the risk of colon cancer development, by inhibiting cell proliferation and inducing apoptosis.

Urolithins inhibit the proliferation of colon cancer cells, induce cell cycle arrest, and modulate key cellular processes associated with colon cancer development, such as MAPK signaling *in vitro* (Larrosa et al., 2006b; Gonzalez-Sarrías et al., 2009). Furthermore, urolithin A decreases inflammatory markers including inducible nitric oxide synthase, cyclooxygenase-2 (COX-2), prostaglandin E synthase, and prostaglandin E2 in colonic mucosa in a rat colitis model (Larrosa et al., 2010).

Urolithins are also bioactive and inhibit prostate cancer cell growth. Inhibition of Nuclear Factor Kappa-B activation has been shown in prostate cancer cells and in human prostate cancer xenografts in mice by urolithins. Seeram et al. (2007) showed that urolithins are concentrated to a high degree in mouse prostate tissues. An initial phase II clinical trial of pomegranate juice in patients with prostate cancer reported significant prolongation of prostate specific antigen

doubling time (Pantuck et al. 2006) and inhibition of angiogenesis has also been demonstrated both *in vitro* and *in vivo* for prostate cancer (Albrecht et al., 2004).

Health and enterodiol and enterolactone

Plant lignans are not usually absorbed and must be metabolized to enterodiol and enterolactone prior to absorption. Mainly enterodiol and enterolactone are responsible for the beneficial effects on the health produced by lignans, thus the transformation of plant lignans by intestinal microbiota might be essential for these functions to be manifested (Landete, 2012).

In some cases, antioxidant activity appears to be responsible for the bioactivity of plant and mammalian lignans. The antioxidant activity of enterodiol and enterolactone is higher than SDG in both lipid and aqueous *in vitro* model systems (Kitts et al., 1999). Enterodiol and enterolactone act as antioxidants against DNA damage and lipid peroxidation. The antioxidant activities of Seco, enterodiol and enterolactone have also been suggested to contribute to the reduction of hypercholesterolemia, hyperglycemia and atherosclerosis (Prasad, 2000).

The mechanism of action of enterolignans on estrogen receptors has been studied and there is evidence from human observational studies that phytoestrogens may modulate hormone levels and estrogen receptors expression (Low et al., 2005; Touillaud et al., 2005). It is assumed that the biological action of phytoestrogen is mediated by the estrogen receptors Ŭand ϐ (Morito et al., 2001). Because of their structural similarity to 17ϐ-estradiol, enterolignans are natural ligands of estrogen receptors and are believed to be naturally-existing selective estrogen receptor

modulators. They might therefore act as anticarcinogens, either through antioestrogenic actions (e.g., by competing with oestradiol to bind estrogen receptors) or by initiating their own anticarcinogenic effects (e.g., by recruiting specific transcriptional co-regulators to phyto-oestrogen-activated estrogen receptors) (Landete, 2012).

The estrogenic effects of flaxseed lignans in postmenopausal women include decreased plasma levels of estrone sulphate and estradiol (Hutchins et al., 2001) and switching estrogen metabolism from 16Ŭ-hydroxylation to a less carcinogenic pathway (2-hydroxylation) (Haggans et al., 1999; Brooks et al., 2004).

CONCLUSIONS

Several recent dietary intervention studies examining the health effects of soy isoflavones allude to the potential importance of equol by establishing that maximal clinical responses to soy protein diets are observed in people who are good "equol-producers". Equol may enhance the actions of soy isoflavones, owing to its greater affinity for estrogen receptors, unique antiandrogenic properties, and superior antioxidant activity. It is now apparent that there are two distinct subpopulations of people and that ñbacterio-typingò individual for their ability to make equol may hold the clue to the effectiveness of soy protein diets in the treatment or prevention of hormone-dependent conditions.

The large inter-individual variability observed in the production of urolithins, and the fact that urolithins are excreted independently of the ellagitaninns consumed, would suggest

microbial involvement and their production in the colon of those individuals. Urolithins could display estrogenic and/or antiestrogenic activity and tissue disposition studies reveal that urolithins are enriched in prostate, intestinal, and colon tissues in mouse, which could explain why urolithins inhibit prostate and colon cancer cell growth. Moreover, antiproliferative and apoptosis-inducing activities of ellagic acid and urolithins have been demonstrated by the inhibition of cancer cell growth.

Enterolignans can be detected in the blood of most individuals due to the high prevalence of enterolignan-producing bacteria, and inter-individual differences in cell densities of enterolignan-producing bacteria may explain inter-individual differences in blood concentrations of enterolignans. Research into the role of lignans in breast, colon and prostate cancer has generally shown they exert beneficial effects, although there are also some inconclusive studies or others that report negative effects. There are several possible mechanistic explanations for the observed bioactivities, including involvement in hormonal metabolism or availability, angiogenesis, antioxidation and gene suppression. Moreover, it has been shown that physiologically relevant concentrations of enterolignan lead to *in vitro* and *in vivo* activation of estrogen receptors.

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Figure 1. Types of phytoestrogens.

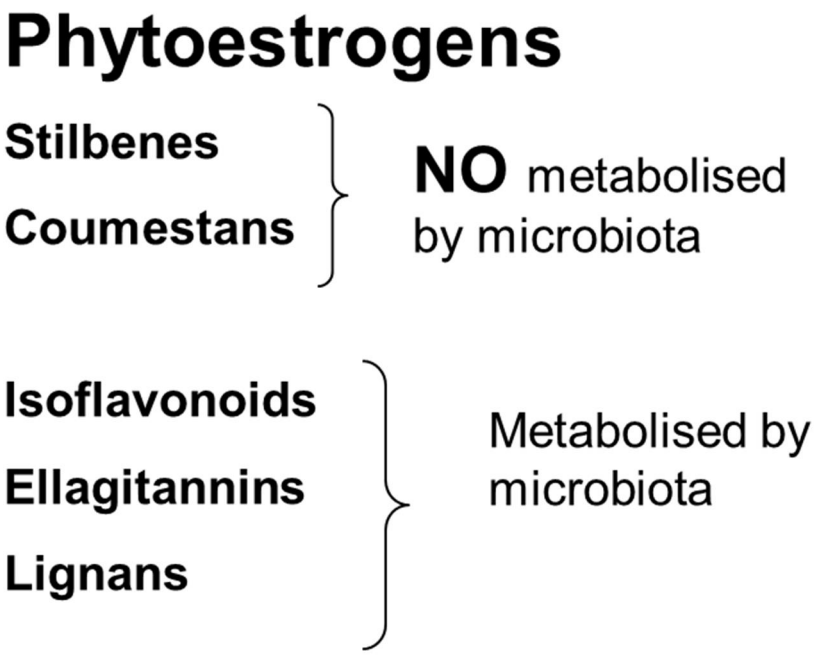

Figure 2. Resveratrol

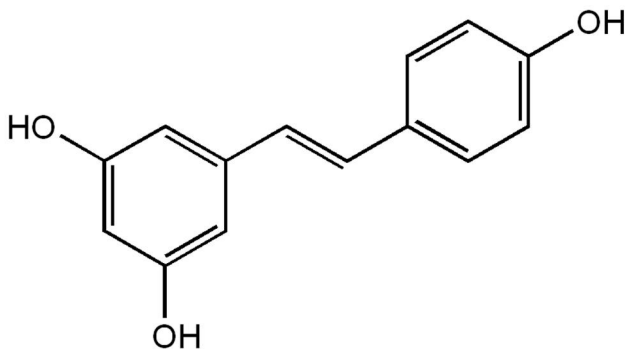

Figure 3. Coumestrol

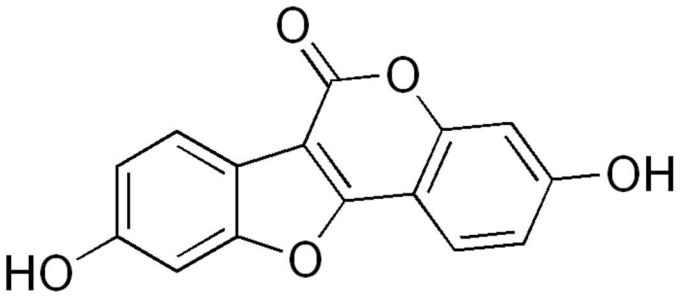

Figure 4. Polyphenols are extensively metabolised either in tissues, once they are absorbed through the gut barrier, or, for the non-absorbed fraction and the fraction re-excreted in the bile, by the colonic microbiota.

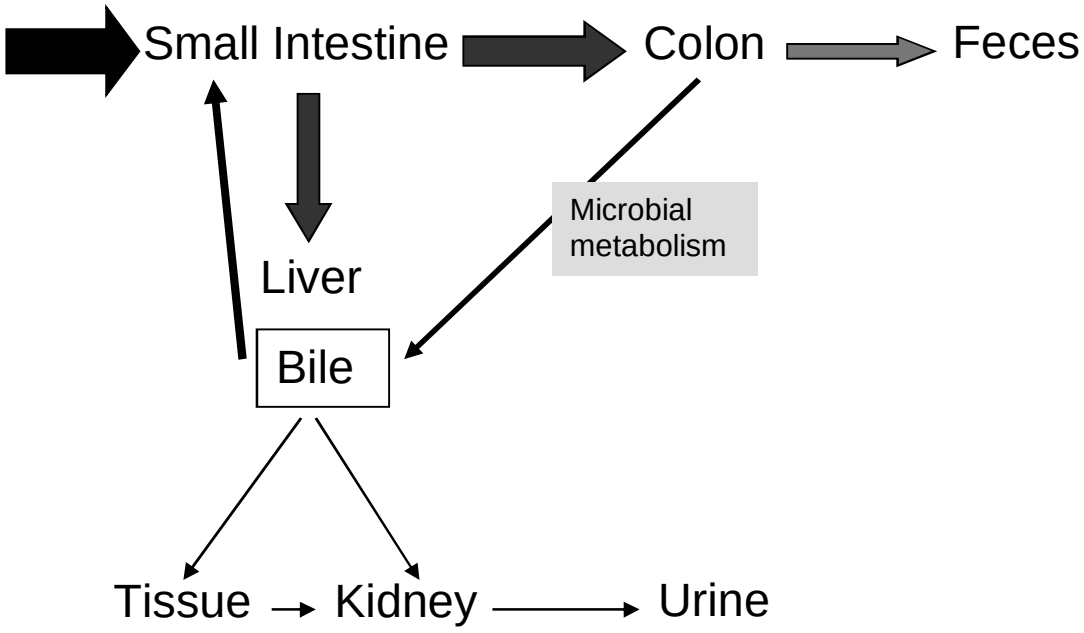

Figure 5. Metabolic pathway of equol.

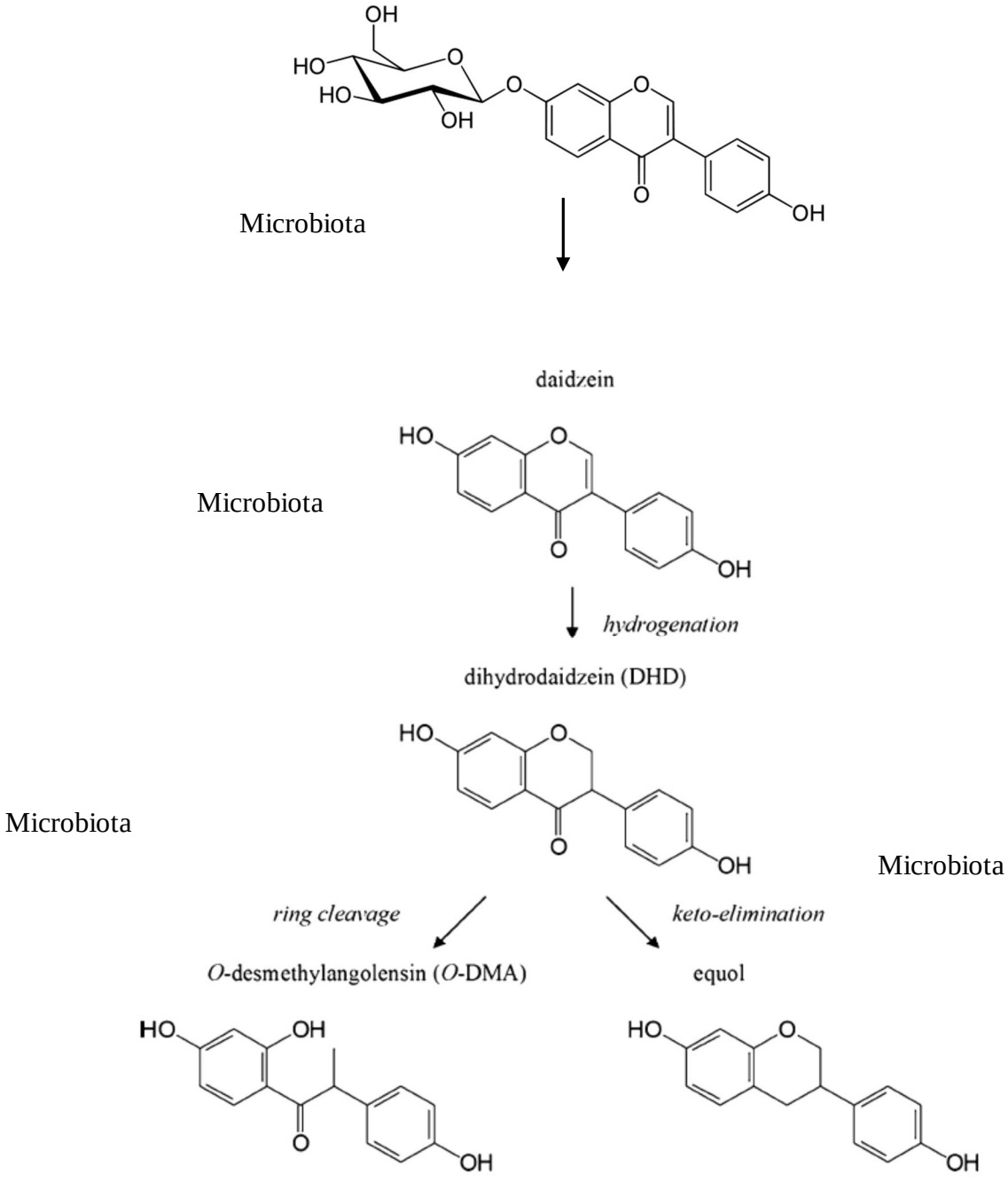

Figure 6. Metabolism of ellagitannins and ellagic acid

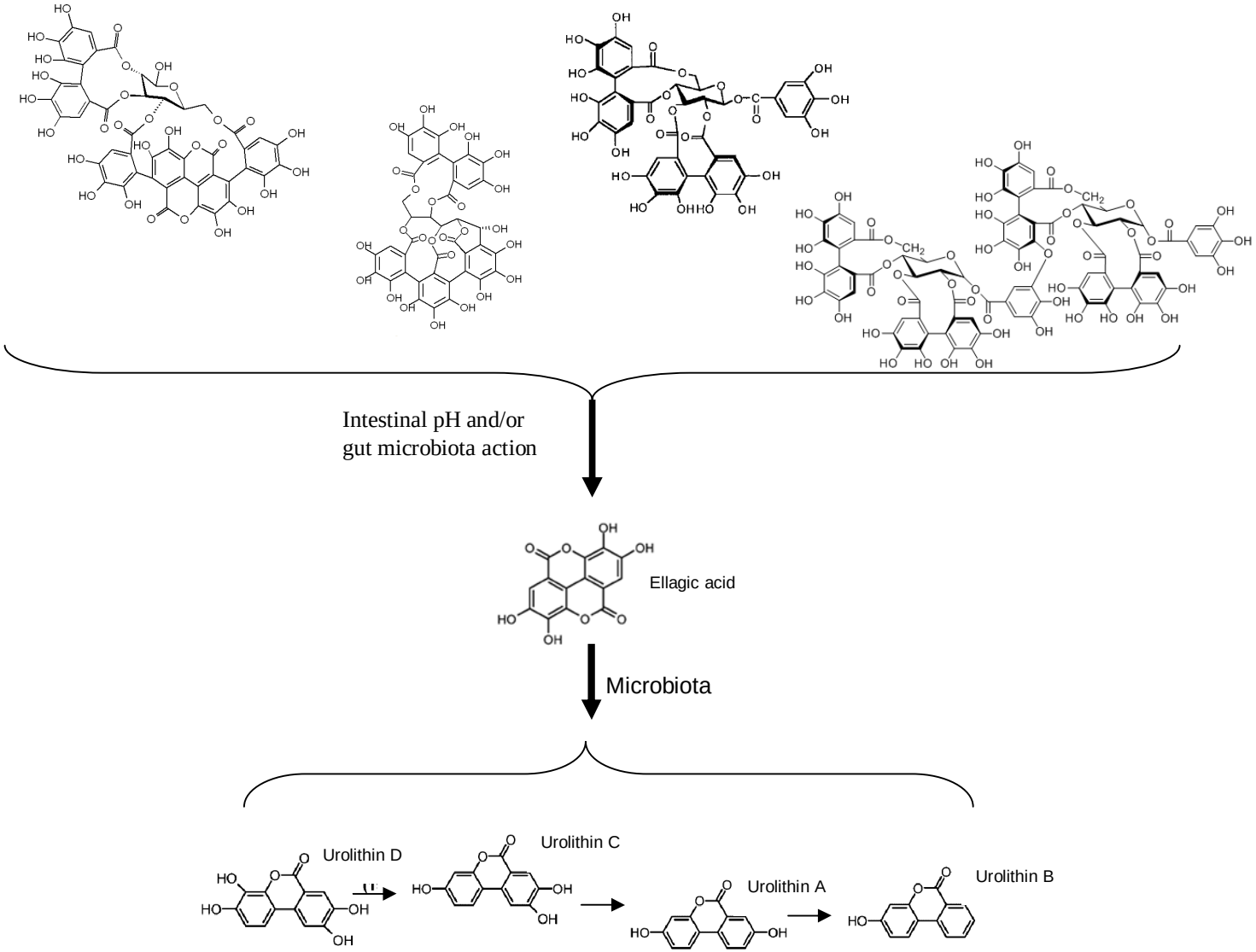

Figure 7. Conversion of plant lignan secoisolariciresinol diglucoside (SDG) and secoisolariciresinol (SECO) to mammalian lignan enterodiol (END) and enterolactone (END) by human intestinal bacteria.