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Soy isoflavones and their relationship with microflora: beneficial effects on human health in equol producers

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Abstract The bioavailability of soy isoflavones depends on the composition of the microflora for each subject. Bacteria act on different isoflavones with increased or reduced absorption and cause biotransformation of these compounds into metabolites with higher biological activity. *S*-equol is the most important metabolite and only 25–65 % of the population have the microflora that produces this compound. The presence of equol-producing bacteria in soy product consumers means that the consumption of such products for prolonged periods leads to lower cardiovascular risk, reduced incidence of prostate and breast cancer, and greater relief from symptoms related to the menopause such as hot flushes and osteoporosis.

Keywords Aglycones · Glucosides · Daidzein · Equol-producer · Microflora

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Introduction

Isoflavones belong to a group of compounds known as flavonoids and these share a basic structure that consists of two benzene rings (A and B) linked through a heterocyclic pyrone C ring. The benzenoid B ring of isoflavones is in the 3-position whereas it is in the 2-position in flavones (Franke et al. 2008) (Fig. 1).

Isoflavones are phytoestrogens that are present almost exclusively in leguminous plants such as *Glycine max*, *Pueraria mirifica* and *Trifolium pretense*, amongst others (Kolodziejczyk-Czepas 2012; Malaiv-ijitnond 2012; Masilamani et al. 2012; Vitale et al. 2012). *Glycine max* is the most important legume plant to be used as a food (Young and Bharti 2012) and it also has the highest concentrations of isoflavones (Crozier et al. 2009). These compounds are associated with several health-enhancing properties, such as easing the symptoms of postmenopausal women, preventing cardiovascular disease, reducing the risk of osteoporosis, and antimutagenic effects (Chen et al. 2012). These activities are mainly attributed to the aglycone forms of isoflavones, which include daidzein, genistein and glycitein, because it has been demonstrated that isoflavone aglycones are absorbed more rapidly and in larger quantities than their glucosides and acetyl and malonyl-glucoside forms in human intestines (Barnes et al. 2011). However, isoflavones are present in soybeans and most soy foods primarily in the glucoside form (Okabe et al. 2011). Glucoside forms, along with acetyl and malonyl-glucoside forms, must be

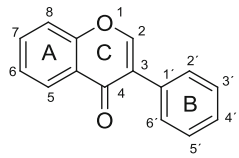

Fig. 1 Structure of isoflavones

hydrolyzed by β-glucosidase to produce absorbable aglycone in the intestinal microflora (Setchell et al. 2002b). Subsequently, some isoflavones are metabolized by the microflora and this results in new metabolites that have beneficial effects on human health (Yuan et al. 2007). The composition of each microflora is very important as the degree of activity of the resulting compounds depends on this factor.

The human body is a super-organism that contains 10 times more microbial cells than body cells. The metagenomic study of the human microbiome has demonstrated that there are 3.3 million unique genes in the human gut, i.e., 150 times more genes than our own genome, and bacterial diversity analysis has shown that around 1,000 bacterial species are living in our gut and the majority of them belong to the divisions of *Firmicutes* and *Bacteroidetes* (Zhu et al. 2010). The human gastrointestinal tract harbors the most complex human microbial ecosystem (Maccaferri et al. 2011). Depending on the composition of the core of the gut microflora, Arumugan et al. (2011) revealed that the 33 samples formed three distinct clusters that were designated as enterotypes by multidimensional cluster analysis and principal component analysis. These three enterotypes can be identified by variations in the levels of one of three genera: *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2) and *Ruminococcus* (enterotype 3). A controlled-feeding study (Wu et al. 2011) associated the *Prevotella* enterotype with a high-carbohydrate diet and *Bacteroides* with high animal fat and protein consumption. Short-term enforced dietary shifts from high-fat and high-protein to high-carbohydrate, and vice versa, somewhat modified organismal abundances but rarely changed the presence or absence of specific gut microbes in particular subjects. Bacteria that form part of these enterotypes could act in different ways on components of soy products.

Hydrolysis of glucoside forms

Isoflavones in soybeans are found in four chemical forms: aglycone, glucoside, acetylglucoside and malonylglucoside. Examples of compounds that have been reported include the aglycones daidzein (4′,7-dihydroxyisoflavone), genistein (4′,5,7-trihydroxyisoflavone) and glycitein (4′,7-dihydroxy-6-ethoxyisoflavone) and the glucosides daidzin (daidzein 7-glucoside), genistin (genistein 7-glucoside) and glycitin (glycitein 7-glucoside) and their corresponding acetylglucoside and malonylglucosides: 6″-*O*-acetyl-7-*O*-β-D-glucosides (acetyl daidzin, acetyl genistin and acetyl glycitin), and 6″-*O*-malonyl-7-*O*-β-D-glucosides (malonyl daidzin, malonyl genistin, and malonyl glycitin) (Peñalvo et al. 2004).

Isoflavone aglycones are the bioactive forms, whereas the β-glucoside forms are predominant in the soybean. The latter compounds must be hydrolyzed to be absorbed across the enterocyte of healthy adults because of their higher molecular weight and hydrophilicity (Izumi et al. 2000). The β-glucosidase present in microflora partially hydrolyzes the glucoside forms to aglycones in the small intestine, and to a greater extent in the jejunum, to release the isoflavone aglycones, which can be absorbed through the gut epithelium (Setchell et al. 2002b). A proportion of the isoflavones, which are 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 glucuronidated and sulfated isoflavones, along with the glucoside forms, are hydrolyzed by bacterial enzymes (β-glucosidase and β-glucuronidase) and then absorbed or subjected to further metabolism by the intestinal microflora (Doerge et al. 2000; Zhang et al. 2003) (Fig. 2).

β-glucosidases (β-D-glucoside glucohydrolase, EC 3.2.1.21), which catalyze the hydrolysis of β-glucosidic linkages of various oligosaccharides and glucosides to form glucose and a shorter/debranched oligosaccharide, have attracted considerable interest in recent years due to their important roles in various biological processes, such as the hydrolysis of isoflavone glucosides (Matsuura and Obata 1993). These enzymes are found both free and in bacteria present in the intestinal microflora. In the intestinal tract the bacterial metabolism is higher than that of nonbacterial enzymes. Additionally, the hydrolysis rate varies through the gastrointestinal tract due to changes in the microflora composition (Turner et al. 2003). The microorganisms that have strong β-glucosidase activity are lactic acid bacteria, obligate anaerobes and

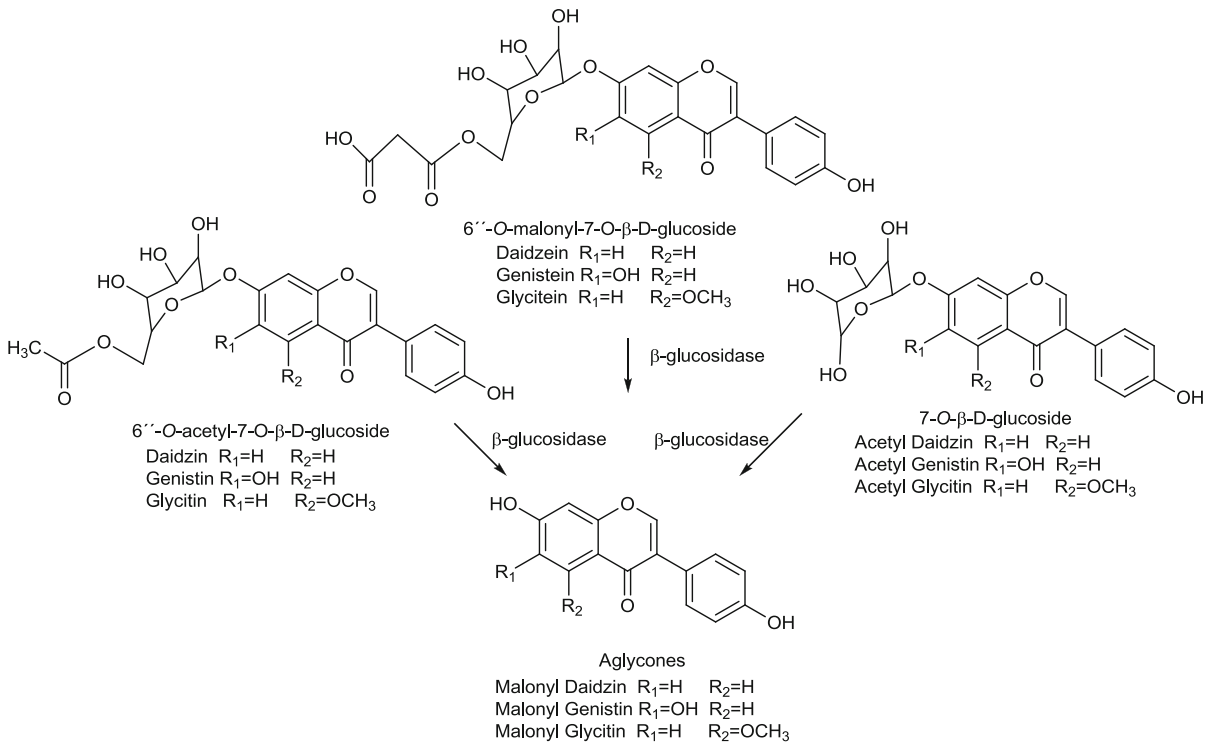

Fig. 2 Structures of isoflavone aglycones and their glucoside derivatives

Escherichia coli, all of which colonize the small intestine.

In several studies lactic acid bacteria such as *Lactobacillus* have proven to be excellent probiotics since they have β-glucosidase activity. Species such as *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Lactobacillus plantarum* and *Lactobacillus fermentum* were found to have the ability to hydrolyze isoflavone glucosides to aglycones to a significant extent (>95 %) when the conversions of daidzin and genistin were quantified (Otieno and Shah 2007; Rekha and Vijayalakshmi 2011; Marazza et al. 2012). Malashree et al. (2012) isolated five *L. rhamnosus* from soymilk and these have different isoflavone biotransformation potentials, with a two to threefold increase in genistein and a 6- to 14-fold increase in daidzein. This finding confirms that the degree of biotransformation of isoflavones is characteristic of an individual strain. In one study it was found that *L. rhamnosus* JCM 2771, in addition to the conversion of daidzin into daidzein, also produced genistein from daidzin (Tamura et al. 2011).

One of the major lactic acid bacterial groups of the gut microflora in humans is *Bifidobacteria.* These bacteria are among the first colonizers of the sterile gastrointestinal tract of newborns. In a similar way to *Lactobacillus*, they have beneficial effects on their host as they act as probiotics. Almost all strains of *Bifidobacteria* have these enzymes, although the hydrolysis rate is very variable. Raimondi et al. (2009) studied 22 strains and most of them released aglycone from daidzin and 12 strains gave yields higher than 90 %. The enzyme activity of these bacteria can vary depending on the glucoside forms. Thus, *Bifidobacterium animalis*, one of the most widely studied strains, hydrolyzes isoflavone glucosides and this biotransformation is higher in daidzin (Tsangalis et al. 2005; Chien et al. 2006; Otieno and Shah 2007; Pham and Shah 2007; Youn 2012). On the other hand, a study involving five strains of microorganisms (*Lactobacillus paracasei*, *L. acidophilus* and *Bifidobacterium longun*) showed a significant increase in the range 62–96 % in bioactive isoflavone aglycones in fermented soymilk with the highest value for genistin (Wei et al. 2007).

Other lactic acid bacteria, namely *Enterococcus faecalis* and *Streptococcus bovis*, also metabolized isoflavone glucosides to their aglycones (daidzein and genistein) (Tsuchihashi et al. 2008).

The obligate anaerobes *Bacteroides distasonis*, *Bacteroides ovatus*, *Bacteroides fragilis*, *Clostridium ramosum*, *Peptostreptococcus productus* and *Coprobacillus* sp., showed high production rates for daidzein and genistein, while *Bacteroides vulgatus*, *Clostridium spiroforme*, *Fusobacterium nucleatum* and *Peptostreptococcus hydrogenalis* did not metabolize soy isoflavone glucosides at all. The bacteria *Mitsuokella multacida*, *Prevotella veroralis* and *Clostridium celatum* could not appreciably convert soy isoflavone into aglycones (Tamura et al. 2007; Tsuchihashi et al. 2008).

Escherichia coli is the most important *Enterobacteriaceae* and it is a commensal bacteria of colonic microflora (Parkar et al. 2008). Hur et al. (2000) isolated one strain of *E. coli* HGH21 from human intestine and this converted daidzin and genistin to their respective aglycones daidzein and genistein. The hydrolysis rate by β-glucosidase of *E. coli* also depends on the type of isoflavone, with the highest hydrolysis rate produced when β-glucosides are not bonded to malonyl or acetyl moieties. Additionally, the activity on daidzin and genistin is higher than on glycitin (Ismail and Hayes 2005). Other authors did not find activity on glucoside isoflavones (daidzin and genistin) in some strains of *E. coli* and in other Enterobacteria such as *Klebsiella pneumoniae* (Tsuchihashi et al. 2008).

A number of other microorganisms present in soy products, such as *Aspergillus oryzae*, *Aspergillus pulverulentus*, *Bacillus subtilis* and *Rhizopus oligosporus* (Chiou and Cheng 2001; Mase et al. 2004; Kaya et al. 2008; Lun-Cheng and Kung-Ta 2008; Horii et al. 2009; Cheng et al. 2010), have been proposed to be involved in the hydrolysis of isoflavones in the intestinal microflora.

In general, these enzymes have higher hydrolysis rates in glucosides rather than malonyl and acetyl glucosides. It has been suggested that the latter compounds might be hydrolyzed in distant regions of the gut where bacterial concentrations are higher (Barnes et al. 1996). It is likely that the malonyl and acetyl groups at C-6 of the glucose ring cause ionic or steric hindrance of the enzyme, thus preventing it from detaching the glucose from the isoflavone. The lower conversion of glycitein is probably due to the presence of the methoxy group at C-6 of the aglycone ring, which would also cause ionic or steric hindrance of the enzyme. Thus, the bacterial β-glucosidases present in the human gut could have variable activity toward the different forms of glycosides and this in turn would affect their rates of hydrolysis.

Changes in the intestinal microflora could also affect the hydrolysis of isoflavones and thus affect the bioavailability of these phytoestrogens. Therefore, diseases that produce dysbiosis of gut microflora, such as inflammatory bowel disease, irritable bowel syndrome, necrotizing enterocolitis, celiac disease, colorectal cancer, obesity, metabolic syndrome and diabetes (Marteau 2009; Walker et al. 2011; Chassard et al. 2012; Qin et al. 2012; Smith et al. 2012; Zupancic et al. 2012; Ohigashi et al. 2013; Sjoberg et al. 2013), could decrease the bioavailability of isoflavones.

Metabolism of aglycones

Gut microflora play an important role in the metabolism of daidzein, which is converted to *O*-desmethylangolensin (ODMA) and the highly active metabolite *S*-equol. In addition, dihydrodaidzein (DHD), tetrahydrodaidzein, 3′-hydroxydaidzein, 6-hydroxydaidzein, 8-hydroxydaidzein, benzopyran-4,7-diol, 3-(4-hydroxyphenyl) and 2-dehydro-ODMA have also been reported to be metabolites of daidzein (Rowland et al. 2000).

Once the daidzein has been absorbed, it is hydrogenated by bacteria of gut microflora to produce dihydrodaidzein. Subsequently, DHD can undergo two processes: reductive cleavage across the heterocycle to produce *O*-desmethylangolensin and a two-step deoxygenation to *S*-equol (Kelly et al. 1995) (Fig. 3).

Genistein can also be metabolized by gut microflora. Bacteria reduce the double bond between C-2 and C-3 of the C-ring of genistein to a single bond, a process that results in the formation of dihydrogenistein (Hur et al. 2000). Other bacteria are capable of biotransforming dihydrogenistein to 5-hydroxy-equol, a compound that shows a higher antioxidant activity than genistein (Arora et al. 1998; Matthies et al. 2009). On the other hand, genistein can be completely degraded by 6′-hydroxy-O-desmethylangolensin to 2-(4-hydroxyphenyl)propionic acid (Schoefer et al. 2002) (Fig. 4).

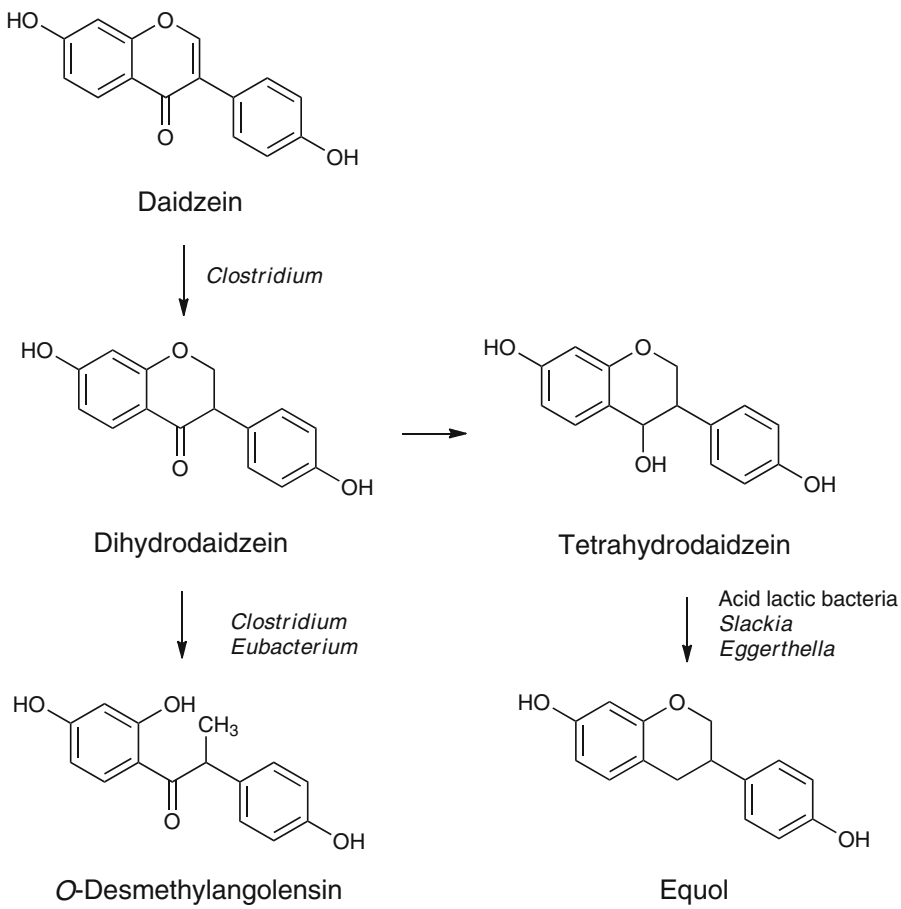

Fig. 3 Biotransformation of daidzein by gut microflora

Several bacteria have been implicated in the metabolic pathways of isoflavone aglycones. It is not completely clear if the conversion of daidzein to *S*-equol is performed by a single bacterium or whether there are a number of bacteria that execute these reactions. The large variability in the levels of DHD and *S*-equol in human urine seem to indicate that there is more than a single bacterium responsible for producing *S*-equol. Furthermore, the finding that certain antibiotics selectively inhibit the formation of equol but not dihydrodaidzein when human feces from equol-producers are incubated with daidzein also supports this contention (Atkinson et al. 2004). Thus, there are bacteria that can only convert daidzein to dihydrodaidzein, while other bacteria can biotransform daidzein to *S*-equol. Human intestinal bacteria that can metabolize daidzein are listed in Table 1.

Species that biotransform daidzein into dihydrodaidzein are diverse. For example, Park et al. (2011) found a Gram-negative anaerobic microorganism (MRG-1) that was identified as a new species with 91.0 % homology to *Coprobacillus* species isolated from the human intestine. This microorganism showed high activities in the deglycosylation and reduction of daidzin to DHD and stereoselective reductase activity on isoflavone, daidzein, genistein and 7-hydroxyisoflavone, producing the corresponding *R*-isoflavanone enantiomers. Under the reported conditions, the Gram-positive strains HGH6 and TM-40 converted daidzin to DHD incompletely, although strain HGH6 converted genistein to dihydrogenistein with a yield of 90 % (Hur et al. 2000; Tamura et al. 2007).

Desmethylangolensin is one of the most frequently isolated metabolites because about 80–90 % of the human intake of isoflavones are desmethylangolensin producers (Frankenfeld 2011). Only three bacteria, *Eubacterium ramulus*, *Clostridium* sp. HGH 136 and *Clostridium* sp. SY8519, have been described as producing O-DMA from daidzein (Hur et al. 2002; Schoefer et al. 2002; Yokoyama et al. 2010), although

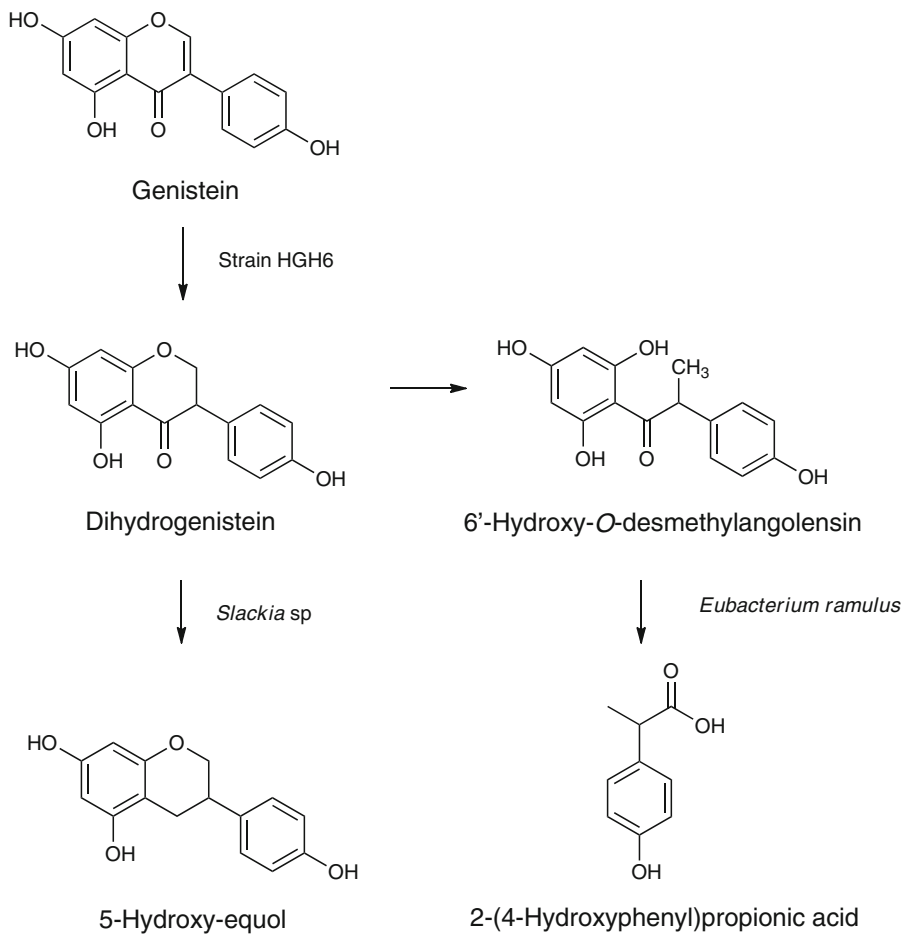

Fig. 4 Biotransformation of genistein by gut microflora

the high percentage of desmethylangolensin producers leads us to believe that the number should be higher. All of these strains are closely related or belong to *Clostridium* rRNA cluster XIVa, which is one of the most abundant phylum in the microflora of the human intestinal tract (Ringel-Kulka et al. 2013).

Some equol-producing bacteria have also been isolated. Both single strains, such as the lactic acid bacteria *Slackia* sp. and *Eggerthella* sp., and mixed cultures convert daidzein to equol (Uchiyama et al. 1999, 2005; Tsangalis et al. 2002; Decroos et al. 2005; Maruo et al. 2008; Yokoyama and Suzuki 2008; Tsuji et al. 2010; Tamura et al. 2011). Probiotic bacteria, e.g. *L. rhamnosus*, in fecal incubation with daidzein led to an increase in equol concentration on increasing the concentration of this microorganism (Tamura et al. 2011). Other lactic acid bacteria, such as *Bifidobacterium longum*, found in the human gastrointestinal tract might be involved in the process of transforming isoflavones to equol. The production of *S*-equol occurs predominantly during the exponential growth of this strain, over the period between 12 and 24 h incubation, with a subsequent reduction in its formation after 24 h (Tsangalis et al. 2002). In both cases a higher density of probiotic bacteria was necessary to produce a high equol concentration. Other bacteria, such as the genera *Slackia* and *Eggerthella*, belong to the family *Coriobacteriaceae* and these showed good equol-producing rates from daidzein (>80 %) (Matthies et al. 2009; Jin et al. 2010; Tsuji et al. 2010). Members of this family from human feces were capable of metabolizing DHD to *S*-equol, although this conversion was only possible with a specific strain. So, Mauro et al. demonstrated that only two of the nine strains of *Adlercreutzia equolifaciens* gen. nov., sp. nov. (FJC-A10 and FJC-A161) could metabolize DHD to *S*-equol, including

Table 1 Human bacteria that biotransform isoflavone aglycones

Bacterium strain	Biotransformation	References
Lactobacillus rhamnosus JCM 2771	D → E	Tamura et al. (2011)
Coprobacillus sp. MRG-1	D → DHD	Park et al. (2011)
Clostridium sp. SY8519	D → ODMA	Yokoyama et al. (2010)
Slackia sp. NATTS	D → E	Tsuji et al. (2010)
Slackia equolifaciens sp. nov., DSM 22006	D → E	Jin et al. (2010)
Slackia isoflavoniconvertens HE8	D → E G → 5-OH E	Matthies et al. (2009)
Slackia equolifaciens sp. nov. DZE	D → E G → 5-OH E	Jin et al. (2008)
Adlercreutzia equolifaciens sp. nov.	D → E DHD → E	Maruo et al. (2008)
Eggerthella sp. YY7918	D → E	Yokoyama and Suzuki (2008)
Clostridium sp. TM-40	D → DHD	Tamura et al. (2007)
Mixed culture (EP4) with *Veillonella* sp. EP *Enterococcus faecium* EPI1 *Lactobacillus mucosae* EPI2 *Finegoldia magna* EPI3	D → E	Decroos et al. (2005)
Eggerthella sp. Julong 732	DHD → E	Wang et al. (2005)
Lactococcus garvieae 20–92	D → E	Uchiyama et al. (2005)
Eubacterium ramulus Julong 601	D → ODMA G → 2-HPPA	Schoefer et al. (2002)
Bifidobacterium longum-a	D → E	Tsangalis et al. (2002)
Clostridium sp. HGH 136	D → ODMA	Hur et al. (2002)
Gram-positive HGH6	D → DHD G → DHG	Hur et al. (2000)
Group consisting of *Bacteroides ovatus* E-23-15 *Streptococcus intermedius* E-23-17 *Streptococcus constellatus* Abg225	D → E	Uchiyama et al. (1999)

D Daidzein, *E* Equol, *DHD* dihydrodaidzein, *ODMA* O-desmethylangolensin, *G* Genistein; *5-OH E* 5, hydroxyequol; *2-HPPA* 2-(4-hydroxyphenyl) propionic acid, *DHG* dihydrogenistein

Eggerthella sp. Julong732, an equol-producing bacterium isolated from human feces (Wang et al. 2005). The other seven isolates could metabolize daidzein to equol via dihydrodaidzein (Maruo et al. 2008). A stable mixed microbial culture, consisting of four associated bacteria that were identified as *Enterococcus faecium* strain EPI1, *Lactobacillus mucosae* strain EPI2, *Finegoldia magna* strain EPI3 and an undescribed species related to *Veillonella* sp., was capable of transforming daidzein into equol. Only the first three bacteria could be brought in culture individually, but they did not produce equol or DHD from daidzein. The related *Veillonella* sp. bacterium could not be isolated through conventional plating techniques. When the three isolated bacteria and the purchased *Veillonella* sp. strains were combined, equol production was again not observed. This could indicate that replacement of the poorly identified species by the different *Veillonella* strains did not mimic the equol-producing mixed culture. It is possible that one or more species, which could be present in very small numbers and whose presence was not detected, contributed to equol production (Decroos et al. 2005). This possibility is consistent with another study involving a group of bacteria that converted daidzein to equol only when all were present (Uchiyama et al. 1999).

While the formation of equol from daidzein via dihydrodaidzein is catalyzed by several bacterial species, the corresponding conversion of genistein to 5-hydroxy-equol has been demonstrated for only two strains isolated from human feces, HE8 and DZE (Jin et al. 2008; Matthies et al. 2009). The enrichment of the intermediate dihydrogenistein in the course of genistein conversion by strain HE8 was reported previously for strain HGH6 (Hur et al. 2000). Strain HE8 hardly converted daidzein and genistein when the isoflavones were added in the stationary growth phase. The conversion rates (standard deviations) were 0.16 μmol/h mg protein for daidzein and 0.20 μmol h/mg protein for genistein. When the cells were grown in the presence of daidzein, the conversion rate of daidzein, when added during the early stationary phase, increased tenfold. Similarly, the rate of genistein conversion in the stationary phase increased fivefold when the bacteria were grown with genistein. In fact, the conversion of genistein in the stationary phase was increased 12-fold when strain HE8 had been grown in the presence of daidzein. The findings suggest that the isoflavone conversion in other equol-forming intestinal bacteria is also inducible (Matthies et al. 2009).

S-Equol

Equol [7-hydroxy-3-(4′-hydroxyphenyl)-chroman], an isoflavan, belongs to the general class of compounds referred to as nonsteroidal estrogens. It has a molecular formula of $C_{15}H_{14}O_3$ and a molecular weight of 242.27 Daltons. The heterocyclic structure contains 2 reactive hydroxyls and 1 relatively inert and unreactive oxygen in the central pyran ring (Setchell and Clerici 2010). Physicochemically, equol is nonpolar and is relatively insoluble in water. As a result of the chiral carbon at position C-3 of the molecule, equol exists in 2 enantiomeric forms, *R*-equol and *S*-equol, and the latter is the natural diastereoisomer produced by intestinal bacteria in the intestine of humans, monkeys and rats (Schwen et al. 2012). The 2 enantiomers have been shown to differ in binding affinities and preferences for estrogen receptor α (ERα) and ERβ; *S*-equol has a high binding affinity for and preferentially binds to ERβ, whereas *R*-equol binds preferentially to ERα (Muthyala et al. 2004). This affinity is higher than that of daidzein (10–80 times) and it induces transcription more strongly than any other isoflavone. Therefore, because equol mediates many of its biological effects by binding to the estrogen receptors, in vitro studies suggest that equol is more biologically active than daidzein.

A concentration of >5–10 ng/mL has been associated with a positive outcome for vasomotor symptoms, osteoporosis (as measured by an increase in bone mineral density), prostate cancer, and the cardiovascular risk biomarkers low-density lipoprotein cholesterol and C-reactive protein.

Equol-producer phenotype

Those people who can metabolize daidzein to equol are designated ‘equol producers’. Individuals with a plasma equol concentration of >83 nmol/L (20 mg/L) are classified as ‘equol producers’ and those with concentrations <40 nmol/L (10 mg/L) can be classified as ‘equol non-producers’ (Setchell et al. 2002a). Cut-off levels can also be applied to urinary concentrations, with an equol producer being defined as excreting >1,000 nmol/L (Rowland et al. 2000).

Some studies have assessed demographic, anthropometric and other lifestyle factors in relation to equol producer phenotypes.

The percentage of a population that are equol producers varies according to geographic distribution. The prevalence of equol producers has been estimated to be 30–35 % in Western populations and up to 60 % in vegetarians or Asians (Gardana et al. 2009). This trend is mainly due to diet, because Asian populations intake higher amounts of soy products than Western populations. This fact has been confirmed in several studies, the results of which suggest that dairy soy product intake may be associated with the production of equol (Nagata et al. 2008). Microflora are stable and characteristic of each subject. Several studies suggest that the short-term administration of isoflavone supplements can convert equol non-producers to producers. For example, Tanaka et al. (2009) found that among 10 equol non-producers, 2 healthy volunteers became equol producers after supplementation for 3 months.

A person’s weight can have an influence on microflora. Recently, it has been reported that the ratio for equol producers in overweight or obese subject groups was lower (32.1 %) than the previously reported ratio of equol producers (approximately 50 %) in the Asian general population (Usui et al. 2013). This finding was confirmed in a study of 2,165

women who participated in the Shanghai Women's Health Study and it was found that urinary excretions of equol were inversely proportional to body mass index (Wu et al. 2012).

Although age does not seem to have an influence on equol production, Fujimoto et al. (2008) found significant differences in the daily intakes of genistein and daidzein between the teenager group and the other age groups ≥30 years ($P < 0.05$). In a study of Japanese populations, the proportion of equol producers in the teenager group was 10 % and this is significantly the lowest level among the age-stratified groups. The proportions of equol producers in the age-stratified groups from 10 to 49 years were also significantly lower than those in their fifties. The equol non-producers consumed significantly smaller amounts of isoflavones than the equol producers. In a Korean population study, the proportions of equol producers were 45 % in teenagers and 40 % for people in their twenties and thirties, with both levels significantly lower than in the groups in their forties (80 %) and fifties (65 %). The decreased intake of isoflavones, low serum level of equol and low incidence of equol production in the younger generation is probably due to changes in eating habits.

Antibacterial agents used for the treatment of infections can also alter the composition of bacterial populations in the colon and therefore can affect daidzein metabolism. Several antibiotics have been used to eliminate the colonic bacteria that were susceptible to these antibiotics. This allowed an evaluation of the effects of concentrations of antibiotics on daidzein metabolism by colonic bacteria in monkeys and enabled the subpopulation involved in daidzein metabolism to be identified. Tetracycline completely removed the equol-producing bacteria; the metabolism of daidzein was not altered in cultures of bacteria after ceftriaxone treatment; the bacteria metabolizing daidzein were enriched by ciprofloxacin. Therefore, some antibiotics can eliminate equol-producing bacteria in colonic microflora (Sutherland et al. 2012).

Antioxidant effects

Studies into the antioxidant activity of both producers and non-producers of equol have not been carried out to date. However, equol has shown antioxidant capacity in several in vivo and in vitro studies.

Antioxidant effects of equol were described in the 1990s and it showed more potent antioxidant activity than its parent compounds. For example, Arora et al. (1998) demonstrated that antioxidant activities for the isoflavone metabolites were comparable or superior to those of the isoflavone aglycones. Equol and its 4-hydroxy and 5-hydroxy derivatives were the most potent antioxidants in the study, suggesting that the absence of the 2,3-double bond and the 4-oxo group on the isoflavone nucleus enhances antioxidant activity. Additionally, the number and position of hydroxyl groups were determining factors for isoflavonoide antioxidant activity, with hydroxyl substitution being of high importance at the C-4′ position, of moderate importance at the C-5 position, and of little significance at the C-7 position.

The polyphenolic structures of isoflavonoids give these compounds the ability to scavenge free radicals and to chelate transition metals, a basis for their potent antioxidant activities. Is this context, equol significantly decreases O_2 and H_2O_2 production in a concentration-dependent manner (Pereboom et al. 1999). Thus, equol pretreatment caused a decrease in the H_2O_2-induced death of bovine aortic endothelial cells (bAECs) and led to a significant decrease in the number of cells with apoptotic morphology. This characteristic probably reduced the production of intracellular reactive oxygen species ($P < 0.05$). Incubation of bAECs with equol increased the expression of mitogen-activated protein kinases (MAPK) p38 and Bcl-2 (B cell lymphoma 2) after exposure to H_2O_2 compared with their expression without the equol pretreatment (Chung et al. 2008).

Equol can act to decrease or increase the availability of nitric oxide (NO). NO is a free radical generated by a family of enzymes called NO synthase (NOS). The overproduction of NO by inducible NOS (iNOS) is associated with the development of atherosclerosis. It has been reported that iNOS knock-out mice develop reduced levels of atherosclerotic lesion compared to wild-type mice (Buus et al. 2011).

Equol increases NO availability by inhibiting superoxide (O_2) production and this is manifested through enhanced levels of free NO, which prevents low-density lipoprotein (LDL) modification. Pretreatment of cells with 0.5 μM of equol inhibited production of O_2 by J774 monocyte/macrophages (Hwang et al. 2003). To clarify the effects of equol on oxidized low-density lipoprotein (OX-LDL), Kamiyama et al. (2009) studied OX-LDL-

stimulated apoptosis in human umbilical vein endothelial cells (HUVECs) and found that equol inhibited the induction of apoptosis in response to exposure of HUVECs to OX-LDL. Treatment of cells with equol led to a significant reduction in superoxide production by adenine dinucleotide phosphate (NAD(P)H) oxidase and also to a significant increase in NO production. Furthermore, inhibition of LDL oxidation depends on the isoflavone form. Thus, equol showed an inhibition of LDL oxidation that was 2.65-fold better than its parent compound daidzein. 8-Hydroxydaidzein was 12.5-fold better than daidzein while the parent aglycones also inhibited oxidation, albeit by only 5 %. However, monosulfated conjugates of genistein, daidzein and equol were much less effective and disulfates were completely ineffective. Since almost all isoflavones circulate as sulfates and glucuronides (conjugated), these data suggest that despite the increased potency produced by some metabolic changes, isoflavones may not be effective antioxidants in vivo unless until they are deconjugated again (Turner et al. 2004).

Another possible contributory mechanism toward the antioxidant activities of these compounds is their ability to stabilize membranes by decreasing membrane fluidity. The effects of equol on membrane fluidity and their preferential localization in the membrane were investigated using large unilamellar vesicles as the membrane models. The results of the study suggest that equol is partitioned into the hydrophobic core of the membrane and causes a dramatic decrease in lipid fluidity in this region of the membrane. Localization of equol within the membrane interior and the resulting restrictions on fluidity of membrane components could sterically hinder diffusion of free radicals and thereby decrease the kinetics of free radical reactions (Arora et al. 2000).

Antioxidant species may act in vivo to decrease oxidative damage to DNA, protein and lipids, thus reducing the risk of coronary heart disease and cancer. It has been demonstrated in human lymphocytes that pre-treatment of the cells with equol offered protection against this damage at concentrations within the physiological range (Sierens et al. 2001). In another study it was found that pre-treatment with equol at doses of 0.01–100 micromol/L significantly protected sperm DNA against oxidative damage and that equol is a more potent antioxidant than ascorbic acid and alpha-tocopherol. It was concluded that this compound could protect against male infertility (Sierens et al. 2002).

Currently, the most relevant antioxidant mechanism for equol is protection from photocarcinogenesis, inflammation and immune suppression induced by ultraviolet (UV) radiation, so this compound might be effective in preventing skin cancer or as a sun-protective cosmetic ingredient. Several models in animals have been developed to demonstrate the protective effects of this compound. In hairless mouse, the daily topical applications of equol lotions significantly protected against skin carcinogenesis induced by chronic exposure to solar-simulated UV radiation (SSUV), topical treatment with the chemical carcinogen DMBA or by the combined cocarcinogenic treatment of DMBA followed by chronic SSUV (Widyarini et al. 2005). Additionally, equol was found to have strong antioxidant action against acute UVA-induced lipid peroxidation of mouse skin (Reeve et al. 2005). The photoimmune protective property was shown to depend on equol's activation of estrogen receptor (ERβ) signaling in the skin, which in turn enhances the expression of antioxidant enzymes and inhibits the expression of snail, a transcription factor that regulates keratinocyte cell proliferation and migration (Jackson et al. 2011). UVB irradiation has also been used to induce inflammation in mouse skin. It was found that the effect of topical applications of equol dose-dependently inhibited the UVB induction of cutaneous tumor necrosis factor (TNF)-α mRNA and protein, a cytokine critical for the initiation of psoriatic inflammation. Expression of IL-6 mRNA and protein was also decreased and the number of infiltrating mast cells into the dermis was reduced. Furthermore, the upregulated mRNA and protein levels of P-cadherin, a marker characteristic of cutaneous hyperproliferation, were also normalized. These results suggested that equol has the potential for useful, innocuous anti-inflammatory therapy from topical application in human cutaneous diseases (Bandara et al. 2010).

Equol may act as an antioxidant through the inhibition of oxidative stress and stimulation of catalase and superoxide dismutase (SOD), but it can also cause prooxidant effects such as reduction of the glutathione/glutathione disulfide (GSH/GSSG) ratio depending on the treatment period. This was demonstrated in a mouse model in which equol was orally

administered at either 5 or 25 mg/kg body weight/day for 1, 3 or 7 weeks. Equol administration significantly inhibited biomarkers of oxidative stress (i.e., thiobarbituric acid-reactive substances value, carbonyl content, and serum 8-hydroxydeoxyguanosine) at all doses and for all durations of administration. This phenomenon was most pronounced at 3 weeks. Moreover, catalase and total SOD activities and their mRNA expression were significantly increased by equol. Although equol increased the glutathione peroxidase (GSH-px) activity in mice treated with equol for 1 week, long-term administration of equol (7 weeks) caused a decrease in the ratio of GSH/GSSG and the activities of GSH-px and glutathione reductase (Choi 2009b). These findings are consistent with the results of another study performed in mouse brain, in which the long-term or higher dose of equol caused apoptosis (Choi 2009a).

Menopause

Postmenopausal women suffer several symptoms, such as hot flushes and osteoporosis. Soy food has proven to be beneficial in postmenopausal subjects, mainly in Asian countries.

It has been observed that Asian women have fewer hot flushes than women of other ethnic groups and this may be explained, at least partially, by both the higher overall consumption of soy products and the higher percentage of women able to harbor equol-producing bacteria in Asia (Gold et al. 2000). In a study of 96 menopausal women from Taiwan, 66 were included in the isoflavone group and 30 in the placebo group (52 % equol producers). Compared with the women in the placebo group, those with the ability to produce equol experienced a greater and more rapid improvement of their menopausal symptoms after they began taking a daily dose of 135 mg of isoflavones. Thus, in the equol producer group only, the scores for hot flushes and excessive sweating were significantly reduced after 3 months, as were the scores for weakness, palpitations, limb paresthesia and total symptoms after 6 months (Jou et al. 2008). In another randomized study on 130 perimenopausal (no menses in the past 3 months) and postmenopausal ($\geq$12 months of amenorrhea) North American women with a mean of five or more moderate/severe hot flushes per day, the subjects were treated with varying total daily isoflavone doses and dosing frequency, with the study carried out separately for equol producers and non-producers. Hot flush intensity scores were lower in women with the highest total daily dose (100–200 mg) and the highest dosing frequency (2–3 times daily), with greater benefits on nighttime scores than on daytime scores. Dose and frequency related differences were somewhat larger in equol producers than in non-producers (Crawford et al. 2013).

To demonstrate effects of equol on hot flushes, two studies with doses of 10 mg/day of *S*-equol in postmenopausal North American and Japanese women have been carried out. This dose reduced hot flush frequency and it was even more effective for relieving muscle and joint pain. Thus, *S*-equol ($\geq$20 mg/day) alleviates hot flushes to a greater extent than soy isoflavones in those women who experience >8 hot flushes/day (Aso et al. 2012; Jenks et al. 2012). This finding is consistent with the results of a previous study in an ovariectomized rat model (Yoneda et al. 2011).

It is critical for women to maintain a high bone mineral density (BMD) prior to menopause to prevent osteoporosis. In an effort to examine and clarify the mechanism of the bone-protective effects of isoflavones in humans, several studies were carried out on both premenopausal and postmenopausal women. A study of 200 premenopausal women in the United States without isoflavone supplement intake did not show differences in hip, spine, femoral neck or head BMD, or in body composition between equol producers (27.5 %) and non-producers. This result may be due to low soy consumption and the low number of daidzein-metabolizing bacteria in Western premenopausal women (Atkinson et al. 2012). This trend is consistent with the results of another study of 128 healthy Japanese postmenopausal women aged 45–60 years who were randomly assigned to 4 groups: placebo; placebo combined with walking (3 times per week); isoflavone intake (75 mg of isoflavones per day); and isoflavone combined with walking. The subjects were classified as producers (55.7 %) or non-producers of equol. The percentage changes in BMD in equol producers were -0.53 and $+0.13$ % in the sub-whole body and total hip, respectively. These results are significantly different to the values of -1.35 and -1.77 % for the sub-whole body and total hip, respectively, in non-producers in the isoflavone group ($P = 0.049$ and 0.040, respectively). The findings of this study suggest that the preventive

effects of isoflavones on bone loss depend on the individual's intestinal flora for equol production (Wu et al. 2006). This finding is similar to that obtained in a study by Ishimi, in which 54 early postmenopausal Japanese women were classified by their equol-producer phenotype. It was found that the annualized changes in BMD in the total hip and intertrochanteric regions in the isoflavone-treated equol producers (−0.46 and −0.04 %, respectively) were lower than in the non-producers (−2.28 and −2.61 %, respectively) and the annualized change in fat mass was also lower in the equol producers compared with the non-producers in the isoflavone group. Equol also inhibited bone loss and fat accumulation in estrogen-deficient osteoporotic mice (Ishimi 2010). Furthermore, *S*-equol formed from daidzein may play a critical role in preventing bone loss. In this respect, Tousen et al. (2011) performed a 1-year double-blind, randomized, placebo-controlled trial with natural *S*-equol supplements on 93 non-equol-producing menopausal Japanese women. These women showed a significant decrease in urinary deoxypyridinoline (a specific marker of bone resorption and osteoclastic activity) with a −23.94 % change in the group that received 10 mg of equol supplement per day, compared with a −2.87 % change in the group that received a placebo, after 12 months of intervention ($P = 0.020$). Thus, 10 mg/day of equol supplement markedly inhibited bone resorption and prevented a decrease in bone mineral density in the entire body in postmenopausal women after 12 months.

Modulation of cardiovascular risk

Cardiovascular disease (CVD) mortality rates are lower in Asian countries where dietary patterns are very different from those in Western countries. Previous studies have suggested that the daidzein metabolite equol rather than daidzein itself contributes to the beneficial effect of soya foods in the prevention of CVD. Early studies showed that the intake of isoflavones reduced some lipid markers in hypercholesterolemic and/or hypertensive volunteers or postmenopausal women. A study of Asian populations (202 Chinese adults, 63 % equol producers and 37 % non-producers equol) showed that equol producers have lower serum uric acid (−10.2 %, $P = 0.001$), TAG (−29.5 %, $P = 0.007$) and waist:hip ratio (−2.6 %, $P = 0.032$), and that they also tended to have higher high-density lipoprotein (HDL) cholesterol (6.3 %, $P = 0.069$) compared with equol non-producers (Guo et al. 2010). Other similar work demonstrated that the benefits of soya isoflavones in preventing CVD may be apparent among equol producers only. Thus, in 572 Chinese adults, compared with non-producers, equol producers showed significantly lower serum TAG (−38.2, $P = 0.012$) and common carotid artery intima-media thickness (CCA-IMT) (−4.9 %, $P = 0.033$) (Cai et al. 2012). However, in a recent study of 85 hypercholesterolemic American men and postmenopausal women, the soy food intake reduced serum LDL cholesterol equally in both equol producers and non-producers. However, in equol producers, i.e. ∼35 % of this study population, soy consumption had the added cardiovascular benefit of maintaining higher HDL-cholesterol concentrations than those seen in equol non-producers (Wong et al. 2012). Studies in Western hypercholesterolemic and/or hypertensive volunteers revealed significant reductions in total cholesterol, LDL cholesterol, LDL:HDL ratio, plasma triglycerides and lipoprotein(a) with the soy diet. Systolic and diastolic blood pressure of postmenopausal women also decreased and endothelial function improved in the equol producers, whereas systolic and diastolic blood pressure increased and endothelial function deteriorated in the equol non-producers (Meyer et al. 2004; Kreijkamp-Kaspers et al. 2005). The results of another study showed that the differences between the groups in concentrations of plasma lipids, glucose or insulin, or in blood pressure according to equol-producing status were not significant. The postmenopausal women in this study were younger (aged 46–70) than those in the study carried out by Krijkamp-Kaspers et al. (aged 60–75). The subjects in this study, therefore, were more likely to have a low risk of CVD at the outset and, consequently, differences with respect to equol status may not be detected (Hall et al. 2006). The use of isoflavones to supplement diet has led to excellent results between equol producers and non-producers. Thus, a randomized, controlled, parallel study design was carried out on 62 adults with hypercholesterolemia who consumed a novel soy germ pasta, naturally enriched in isoflavone aglycones. The pasta delivered 33 mg of isoflavones and negligible soy protein and led to a serum isoflavone concentration of 222 ± 21 nmol/L. Soy germ pasta reduced serum total and LDL cholesterol by 0.47 ± 0.13 mmol/L

($P = 0.001$) and 0.36 ± 0.10 mmol/L ($P = 0.002$) more than conventional pasta, which represent reductions from baseline of 7.3 % ($P = 0.001$) and 8.6 % ($P = 0.002$), respectively. Arterial stiffness ($P = 0.003$) and high-sensitivity C-reactive protein (hsCRP) ($P = 0.03$) decreased and improvements in all of the above risk markers were greatest in equol producers. It is likely that pasta naturally enriched with isoflavone aglycones and lacking soy protein had a significant hypocholesterolemic effect. Thus, the number of equol producers was very high (69 %), greater than in early studies, because daidzein-metabolizing bacteria could help to improve risk markers (Clerici et al. 2007).

The most recent study aimed at confirming the importance of being an equol producer was carried out on 54 overweight or obese Japanese outpatients, who orally ingested placebo or natural *S*-equol tablets containing 10 mg *S*-equol for 12 weeks. This treatment led to a significant decrease in glycosylated hemoglobin (HbA1c), serum LDL cholesterol levels and cardio-ankle vascular index (CAVI) score, indicating that equol might have a role in glycemic control and in the prevention of cardiovascular disease (Usui et al. 2013). In this study, differences between equol producers and non-producers were not found, because equol intake did not allow an estimation of the presence of daidzein-metabolizing bacteria, but it did confirm the importance of being an equol producer due to the beneficial effect of *S*-equol.

Soy-based diets have also been reported to protect against the development of atherosclerosis, another cardiovascular risk factor. To examine the potential benefit of these compounds on atherosclerosis progression, Curtis et al. (2013) designed a double-blind, parallel-design, placebo-controlled trial that was conducted on 93 postmenopausal women with type 2 diabetes mellitus. The subjects were randomly assigned to a split dose of 27 g flavonoid-enriched chocolate/d [850 mg flavan-3-ols (90 mg epicatechin) + 100 mg isoflavones (aglycone equivalents)/d] or matched placebo. It was found that equol producers (n = 17) experienced larger reductions in diastolic blood pressure, mean arterial pressure and pulse wave velocity (-2.24 ± 1.31, -1.24 ± 1.30 mm Hg, and -0.68 ± 0.40 m/s, respectively; $P < 0.01$) compared with non-equol producers (n = 30).

Studies in animals have demonstrated that soy-based diets containing phytoestrogens afford protection against CVD. Several models have been developed to study different risk factors. In some studies it was established that feeding a soy isoflavone-rich diet during pregnancy, weaning and postweaning affords cardiovascular protection in aged male rats. Notably, rats exposed to a soy isoflavone-deficient diet throughout pregnancy and adult life exhibited increased oxidative stress, diminished antioxidant enzyme and endothelial nitric oxide synthase (eNOS) levels, endothelial dysfunction, and elevated blood pressure in vivo. The beneficial effects of refeeding isoflavones to isoflavone-deficient rats include an increased production of nitric oxide and endothelium-derived hyperpolarizing factor (EDHF), an upregulation of antioxidant defense enzymes and lowering of blood pressure in vivo (Bonacasa et al. 2011). In another study performed on human platelets, it was found that equol has biological effects attributable to thromboxane A(2) receptor antagonism and this compound could therefore have beneficial effects in the prevention of thrombotic events (Munoz et al. 2009).

Estrogen or estrogen-related molecules as such equol seem to protect women from left ventricular hypertrophy, and recent evidence suggests that this effect is mediated by estrogen receptor (ER) (Joy et al. 2006).

The mechanisms that contribute to the atheroprotective effects of a soy-based diet were addressed using apolipoprotein E knock-out (apoE−/−) mice fed with soy protein isolate associated with or without phytochemicals or casein. The results showed that atherosclerotic lesions were reduced in aortic sinus. Plasma lipid profiles did not differ among the 3 groups, suggesting that alternative mechanism(s) could have contributed to the atheroprotective effect of soy-based diets. Analysis of proximal aorta showed reduced expression of monocyte chemoattractant protein-1 (MCP-1), a monocyte chemokine, in mice fed with both soy-based diets compared with the casein fed mice. In an in vitro lipopolysaccharide (LPS) induced inflammation model, equol dose-dependently inhibited LPS-induced MCP-1 secretion by macrophages, suggesting a role for soy isoflavones in the protective in vivo effects. Collectively, these findings suggest that the reduction in atherosclerotic lesions observed in mice fed with the soy-based diet is mediated in part by inhibition of MCP-1, which could result in reduced monocyte migration, an early event during atherogenesis (Nagarajan et al. 2008).

Prostate cancer chemoprevention

The incidence rate of prostate cancer has been reported to be lower in Asian than Western populations. Traditional Asian food, which is high in soybean products, may be associated with this decreased risk of prostate cancer (Akaza 2012; Sugiyama et al. 2013). The results of some studies have attributed to equol a lower risk of prostate cancer (Jackson et al. 2010; Miyanaga et al. 2012), whereas other studies have implicated other isoflavones (Park et al. 2009; Travis et al. 2009). Only two assays have been carried out on the influence that isoflavones have on the risk of prostate cancer in equol producers. Jackson et al. (2010) evaluated the relationship between spot urinary concentrations of phytoestrogens and total prostate cancer and tumor grade in a hospital-based case–control study in Jamaica and they found that producers of equol have a lower risk of total- and high-grade prostate cancer, although these patients did not intake supplemented isoflavones. Another case–control study concerned the prostate cancer risk based on isoflavone intake (60 mg/day) and equol production for 12 months in 158 Japanese men aged between 50 and 75 years. The results showed that, for the 53 patients aged 65 or more, the incidence of cancer in the isoflavone group was significantly lower than that in the placebo group (28.0 vs. 57.1 %, $P = 0.031$). Isoflavone intake proved to have an effect on prostate cancer, even though Japanese ordinarily ingest considerable amounts of isoflavone in daily life. One would expect that this effect would be even more marked in Europe and America, where the isoflavone intake is much lower (Miyanaga et al. 2012). However, studies performed in these regions did not find any relation between equol and the risk of prostate cancer, probably due to the low isoflavone levels present in the diet (Venkitaraman et al. 2008; Ward et al. 2008a; Park et al. 2009). The preventive effect of isoflavones could be due to the fact that prostate tissue may have the ability to concentrate dietary soy isoflavones to potentially anti-carcinogenic levels (Gardner et al. 2009).

Initial studies in animals, such as that carried out by Landström et al. (1998) showed that soy flour inhibits implanted prostate cancer growth in prostate tumors transplanted in 125 rats. This effect is due to the significant increase in daily urinary excretion of the isoflavonoids, daidzein, O-desmethylangolensin, equol and genistein in the rats fed with soy flour. Other authors showed that equol administration in rats reduces prostate-specific antigen levels from human prostate cancer (LNCap) cells under 5alpha-dihydrotestosterone (5alpha-DHT) stimulation, decreases rat prostate size, decreases serum 5alpha-DHT levels and androgen hormone action, while not altering other circulating sex steroids or luteinizing hormone levels (Lund et al. 2011).

A model of benign and malignant prostatic epithelial cells in vitro suggests that equol inhibited growth of benign human prostatic epithelial cells by 37 % at 10^{-6} M and 80 % at 10^{-5} M (Hedlund et al. 2003). This effect could be due to the presence of equol-modulated genes in multiple cellular pathways, including the cell-cycle pathway genes. Equol effect androgen-responsive genes, IGF-1 pathway gene and MAP kinase-related pathway gene are known to be active mediators of prostate cancer cell proliferative activity and the inhibition of these pathways would be consistent with an inhibitory effect on LNCaP cell growth (Magee et al. 2006; Takahashi et al. 2006). Equol also enhanced the growth inhibitory effect of estrogen-related receptor (ERR)-γ on prostate cancer (PC)-3 cells, indicating that it inhibits invasion in prostate cancer DU145 cells possibly via down-regulation of matrix metalloproteinase-9, matrix metalloproteinase-2 and urokinase-type plasminogen activator by antioxidant activity (Hirvonen et al. 2011; Zheng et al. 2012).

Breast cancer chemoprevention

It appears that equol producer patients have a lower risk of developing breast cancer than those subjects who do not have this capability. This behavior has been demonstrated in several Meta-analyses, where the protective effect appears to be more evident in Asian women than in those living in Western populations (Wu et al. 2008; Dong and Qin 2011; Zhong and Zhang 2012). While these studies showed that the intake of isoflavones reduced the risk of developing breast cancer, another study of 237 European women with estrogen receptor-positive tumors indicated that the risk of breast cancer was increased with higher levels of urinary equol [odds ratio 1.07 (95 % confidence interval 1.01–1.12), $P = 0.013$] (Ward et al. 2008b). This finding is consistent with the findings of another study, where isoflavone supplements were found to increase the progression of estrogen-dependent tumors (Tonetti et al. 2007).

One of the hypothesized protective mechanisms of soy against breast cancer involves changes in estrogen metabolism to 2-hydroxy (OH) and 16α-OH estrogens. In this respect, Morimoto et al. (2012) examined the effect of soy foods on the 2:16α-OH E(1) ratio among premenopausal women during a randomized, crossover intervention study in which women were stratified by equol producer status. Similar non-significant increases in the 2:16α-OH E(1) ratio were observed in equol producers ($P = 0.13$) and non-producers ($P = 0.23$). These findings suggest a beneficial influence of soy foods on estrogen metabolism regardless of equol producer status.

In an effort to confirm the results of an earlier study showing premenopausal equol producers to have hormone profiles associated with reduced breast cancer risk, and to investigate whether equol excretion status and plasma hormone concentrations can be influenced by consumption of probiotics, Bonorden et al. (2004) performed a randomized, single-blind, placebo-controlled, parallel-arm trial in 34 subjects. It was found that consumption of *Lactobacillus acidophilus* and *Bifidobacterium longum* did not alter urinary equol excretion and plasma reproductive hormones in premenopausal women, probably because these bacteria did not have the capacity to metabolize daidzein. Other study in postmenopausal women with an intake of a particular probiotic supplement did not generally show an effect on plasma isoflavones, although the large differences between plasma and urinary equol in some subjects suggest that equol producer status may be modifiable in some individuals (Nettleton et al. 2004).

Mammographic density is often used as a biomarker for breast cancer risk. This characteristic represents the amount of stromal and glandular tissue in the female breast. High mammographic density has been associated with a three- to six-fold increase in breast cancer risk. Only a few intervention studies concerning isoflavones from soy or other sources and mammographic density have been published. Equol excretion status could be related with mammographic density. In a trial that involved 55 sedentary, American postmenopausal women, aged 50–75 years, mammographic density was 39 % lower in equol producers compared with non-producers ($P = 0.04$). This result suggests that particular intestinal bacterial profiles are associated with postmenopausal mammographic density (Frankenfeld et al. 2004). This study does have some limitations, such as the small sample of women, and the authors were not able to estimate precisely the magnitude of the association between daidzein-metabolizing phenotypes and mammographic density. Another study was carried out on 175 Dutch postmenopausal women, aged 60–75 years, to compare the effects of soy protein intake, with 99 mg isoflavones administered daily with an intake of milk protein (placebo) for 1 year. The results showed that mammographic density decreased in both study arms, but the decrease did not differ significantly between intervention and placebo groups, where equol producer status did not modify the results (Verheus et al. 2008). Differences in the microflora between overweight subjects and those with normal weights could be responsible for these contradictions. Atkinson et al. (2009) did not find any associations between daidzein-metabolizing phenotypes and breast density in 200 premenopausal women in the United States, probably because of low-soy consumption.

New insights into the benefits of equol-producing bacteria

Equol producer status has been linked with the prevention of other pathologies, such as gastrointestinal disorders, and a healthy diet based on soy food intake appears to maintain health in subjects over many years.

Considering the gastrointestinal tract, it is known that hydrogen gas produced during colonic fermentation is excreted in breath and flatus, or removed by hydrogen-consuming bacteria such as methanogens and sulfate-reducing bacteria. A recent study showed that the equol-producing activity of the microbial consortium EPC4 attenuated the methanogenesis and sulphidogenesis of active hydrogen-consuming bacteria under in vitro simulated gastro-intestinal conditions. These findings are relevant in terms of abdominal discomfort such as bloating and flatulence and highlight the potential of these bacteria as a probiotic for the stimulation of equol production and the concomitant decrease of undesirable methane and hydrogen sulfide production (Bolca and Verstraete 2010). This factor could be important in other diseases of the gastrointestinal tract, such as inflammatory bowel disease, irritable bowel disease and obesity, in which methane and hydrogen sulfide producer bacteria could be involved.

A recent study (Hozawa et al. 2013) has related isoflavone intake with disability and death. The researchers used a nested case–control study to compare serum isoflavone (daidzein, genistein, glycitein and equol) levels in 165 participants that died or were certified as disabled and 177 controls. It was found that higher serum equol levels, but not any other isoflavones, were inversely associated with the composite endpoint of disability and death. The proportion of cases was lower in the group with the highest levels of equol (34/91, 37 %) compared with equol nonproducers (84/161, 52 %). The risk of disability or death among equol producers remained reduced after adjusting for age and sex. However, the correlation between equol levels and the bone mineral density of the calcaneus was not significant. Furthermore, adjusting for bone mineral density did not alter the risk of the composite endpoint of death and disability. Therefore, other mechanisms may play a role and should also be considered. Unfortunately, the researchers' did not have any information regarding the causes of disability or mortality and, consequently, they were not able to clarify the factors associated with reducing the risk of the composite endpoint in the higher equol group than the other groups. According to the Health Report published in 2004 by the World Health Organization, both healthy life expectancy at age 0 and 60 years were the highest in Japan compared with all other countries in the world and, although it cannot be concluded that equol producer status is involved, higher equol levels appear to be associated with better health. Further studies, including randomized controlled trials, aimed at clarifying the role of equol in overall health are warranted.

Conclusions

The importance of equol has increased in recent years due to the beneficial effects on human health, since it has antioxidants, improve the symptoms associated with the menopause (both hot flushes and osteoporosis), modulate cardiovascular risk, and are chemopreventive in prostate cancer. The chemoprevention in breast cancer is more controversial, whereas the intake of isoflavones over time can prevent breast cancer or, one established, it may accelerate the progression of estrogen-dependent tumor. It is clear that the long-term intake of soy food is necessary to obtain higher yields of these compounds, so their effects are more evident in Asian populations than in Western populations. Microflora play an important role because they metabolize these parent compounds to more active metabolites. The different compositions of the microflora according to sex, ethnic race, age, obesity and the geographic distribution affect the metabolism of isoflavones. The *Coriobacteriaceae* and *Clostridiaceae* families have been linked with the metabolism of these compounds, but it is necessary to carry out further studies to investigate these bacteria. The recently developed pyrosequencing techniques could help to discover equol producer bacteria that cannot be cultured and also the genes responsible for the metabolism of these compounds. The isolation of equol-producing probiotic bacteria could also be used to convert equol nonproducers to equol producers, since the effects on human health of these compounds are stronger in the equol producer population.

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