



Review

Controversies concerning the use of phytoestrogens in menopause management: Bioavailability and metabolism

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ABSTRACT

It has been proposed that the use of phytoestrogens (PE) in menopausal therapy could be beneficial to woman health, particularly with respect to hot flushes. Indeed, PE may compensate the lack of endogenous 17 β -estradiol occurring during menopause. However, therapeutic benefits remain questionable, as highlighted by recent publications. Indeed, data are often subjected to controversy since a number of exogenous and endogenous factors influencing the responsiveness of patients are not sufficiently taken into account. In the present paper, we will discuss the role of bioavailability and metabolism in the instability of individual response to PE.

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1. Introduction

In menopausal women, osteoporosis and atherosclerosis result from deep physiological modifications that result from the lack

of production of 17 β -estradiol by ovaries. Hormone Replacement Therapy (HRT) that consists in taking estrogens to compensate this lack appears sometimes necessary. In this context, extracts from plants such as soybean, red clover or pueraria (kudzu), that are known to be rich in phytoestrogens (PEs) and particularly in flavones, flavanones, isoflavones and lignans (Fig. 1) are extensively used. However, potential benefits of these compounds are subject to controversies since their effect appear often poorly significant and, moreover, may dependent on individuals [1–9]. In fact, uncertainties concerning PE efficacy may be due to metabolism variations

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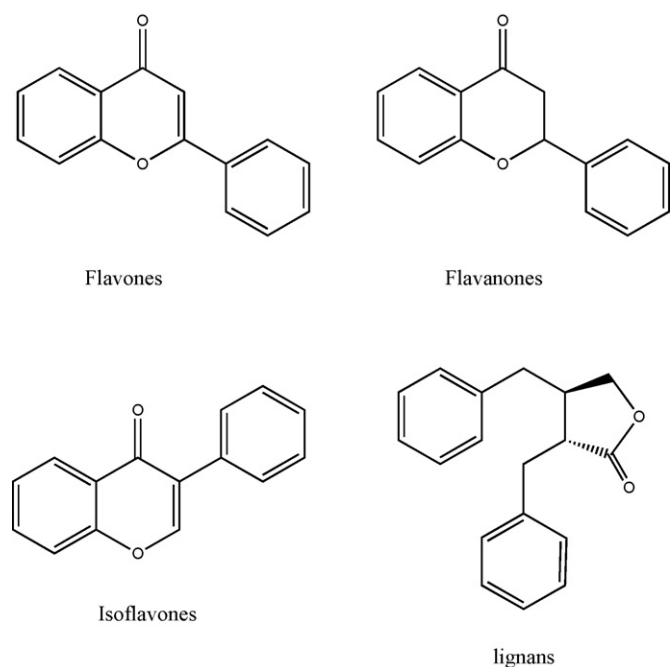


Fig. 1. General structure of flavonoids and lignans.

from one woman to another as well as to the lack of selectivity and specificity. In this first section, we focus on PE bioavailability and metabolism, the latter being influenced by enzyme polymorphism, diet and related gut bacteria. Based on recent publications, we argue in the present paper such obviousness.

2. Bioavailability

Except for flavanols, most of PE are absorbed under their glycoside form which is not easily assimilated by the intestine and the stomach [10,11]. After a hydrolysis step by endogenous and exogenous (gut microflora) enzymes, they are transformed into aglycones, allowing therefore a more efficient passing through the digestive barrier. Thus, the bioavailability of flavonoids varies in function of the chemical structure (aglycone/conjugated derivatives, chemical family) of the molecules that compose PE mixtures (exemplified in Ref. [12]) as well as of the presence/absence of required specific bacterial enzymes [13].

PE interact strongly with plasmatic proteins such as albumin but slightly with lipoproteins [14]. This suggests that they may interfere, in the context of poly-therapies, with drug delivery and clearance. Moreover, these features are, in part, relevant to the poor bioavailability and to the weak activity displayed by PE.

Remarkably, an unpredictable release of PE from fatty tissues may occur since the latter is a major site of storage for PEs. Weight (and therefore lipid) excess which occurs in 40% of women in menopause may therefore correlate with an increase of PE in fatty tissues and, therefore, variable PE plasmatic concentrations due to unpredictable PE releases from fatty tissues to blood. Likewise, expression of aromatase whose activity (i.e. transformation of androgens into estrogens) is highly expressed in adipose tissues, increases as the body mass index (BMI) increases. Most PE, such as genistein, enterolactone or enterodiol and some other diphenols interfering with aromatase expression and activity as they are competitive inhibitors [15,16], they could inhibit the endogenous production of 17β -estradiol, inducing therefore opposite effects than those required! Indeed, this aromatase inhibitory effect, similar to that induced by the aromatase inhibitors used in the context

of breast cancer (i.e. exemestane, letrozole or anastrozole), may logically increase osteoporosis, a disease usually observed in post-menopausal women and against which PE are expected to be active. Thus, body mass index (BMI) should be considered to determine PE dosages in the context of HRT. Of note, the aromatase inhibitory effects displayed by PEs depending upon their chemical structure, the exact composition of PEs in preparations should be precisely indicated. Amazingly, these observations suggest that the anti-estrogenic component of PEs may depend on their aromatase inhibitory activity whereas their estrogenic activity would depend on their estrogen receptor agonist activity. In a physiological context, both effects may compensate each other (depending on the chemical structure of PEs), explaining therefore why PEs are weakly active *in vivo* when compared to their action *in vitro*.

3. Metabolism

3.1. Endogenous metabolism

3.1.1. Type I metabolism

Endogenous enzymes implied in type I (degradation) metabolism are crucial for PE assimilation. A number of these enzymes being subject to polymorphism and genetic instability, the production of active metabolites may logically depend upon the women's genotype. The main enzymatic mechanism related to type I metabolism are principally associated with deglycosylating and CYP enzymes.

After deglycosylation by lactate-phlorizin-hydrolases (which are present in the brush border of the small intestine [17]) or by small intestine and liver β -glucosidases [18], CYP enzymes (CYP17, CYP1A, CYP2A, CYP2B, CYP2C, CYP2D, CYP2E, CYP3A, CYP3B, CYP3C, CYP3D) degrade flavonoids into a variety of metabolites according to various reactions such as dehydrogenation, hydroxylation or O-demethylation (Fig. 2) [19–22]. Depending upon polymorphism, CYP affect differently the bioavailability of 17β -estradiol and flavonoids, making therefore bone metabolism and protection against breast cancer variable from one woman to one other, as observed in Japanese women [21]. In this same context, it should be stressed that CYP17, CYP2A and CYP2C17 polymorphisms may change metabolism of 17β -estradiol as well as of PE and may increase the susceptibility of patients to breast cancer, as shown in Thai women [23].

3.1.2. Type II metabolism

Type II (conjugation) metabolism contributes to the increase of the urinary excretion of PEs. It occurs principally in intestine and requires specific enzymes such as catechol-O-methyltransferase (COMT), sulfotransferases (SULTs) and β -glucuronidases [24].

- O-methylation has been observed on the catechol moiety of flavonoid derivatives such as 7-glucuronide quercetin and 3-O-glucuronide quercetin. This reaction is catalyzed by COMT, an enzyme that is subject to genetic polymorphism. Interestingly, this feature corroborates bone mineral density changes. Even if methyl transfer is, in this medical context, a minor route for PE metabolism, it constitutes an alternative to sulfation [24].
- Cytosolic sulfotransferases (principally SULT1A1, SULT1A2, SULT1A3 and SULT1C2 [25]) catalyze the transfer of sulfate ions from 3'-phosphoadenosine-5'-phosphosulfate to the hydroxyl in positions 7 or/and in position 4' of flavonoids (Fig. 3). As COMT, genetic polymorphism associated with SULTs modifies bone mineral density [26]. Remarkably, sulfation sites are decisive for estrogenicity: sulfation in position 4' leads to a modest estrogenicity whereas sulfation in position 7 induces a stronger estrogenicity [27]. This observation suggests that mono-sulfation/disulfation may have significant repercussions on transcription. In this context, it is noteworthy that acti-

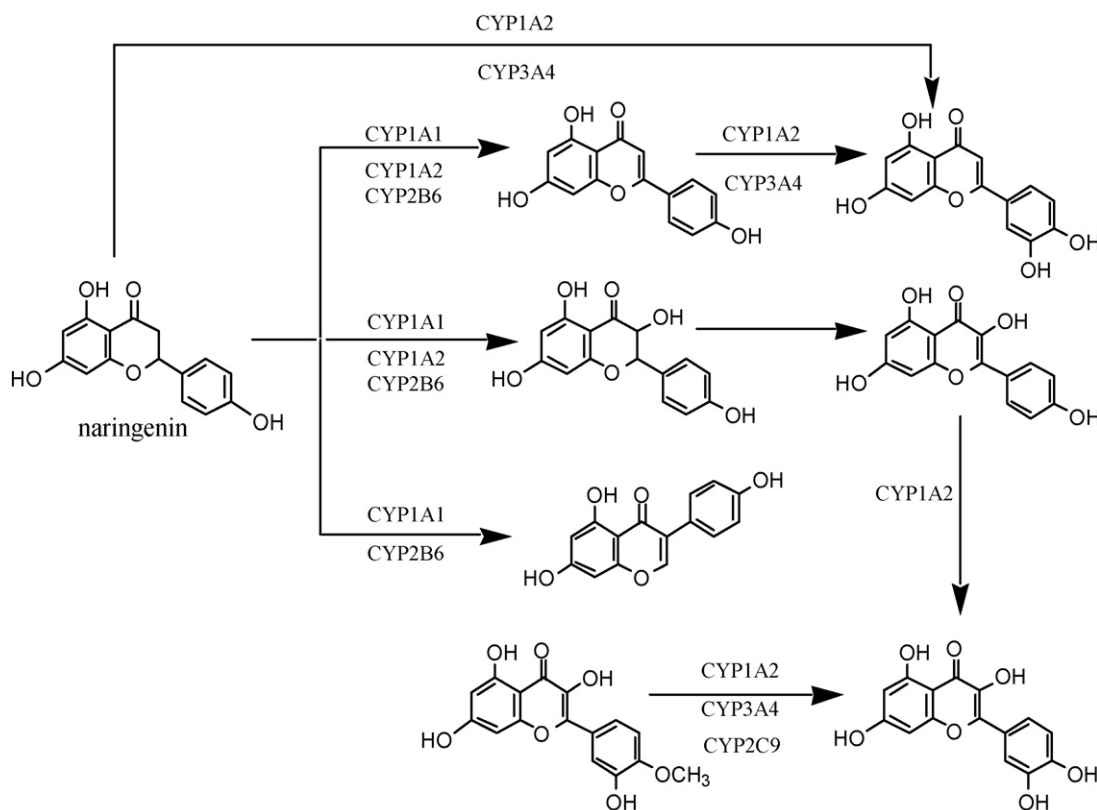


Fig. 2. Endogenous metabolism of flavanones and flavones (illustrated by naringenin) by CYP enzymes.

ated SULT subtypes depend upon PE species and plasmatic concentrations. For example, chrysin is sulfated by SULT1A1 at low concentration whereas it is sulfated by SULT1A3 at higher concentration [28]. Strikingly, SULTs are increased in hormone-dependent tumors and have been suspected in cell proliferation [25] by activating procarcinogen and promutagen xenobiotics. Such an observation suggests that SULT inhibitors could be *a priori* beneficial and may lead to novel anticancer approaches. In this regard, it is interesting to outline that flavon-5-ols, flavon-3-ols, quercetin and resveratrol inhibit the activity of SULT1A1. Unfortunately, the consequent increase of 17 β -estradiol compromises such perspectives [25,29]. Amazingly, these observations suggest that PE degradation is different between tumoral and normal tissues.

- Endogenous reticulum endoplasmic uridine diphosphoglucuronyltransferases (UGT1A1, UGT1A3, UGT1A6, UGT1A8 and UGT1A9 isoforms) that facilitate the urinary and biliary elimination of metabolites after glucurono-conjugation in position 7 and 4' of flavonoids are also subject to polymorphism, as highlighted with UGT1A3 [30]. In addition, free flavonoids can be regenerated by bacterial β -glucuronidases, leading therefore to enterohepatic recirculation and increase of PE half-life time [2].

3.2. Exogenous (gut microflora) metabolism

PE are easily metabolized by intestine bacteria through O-deglycosylation, O-demethylation, dehydroxylation, hydrogenation, lactonisation or ring cleavage reactions. Composed of more

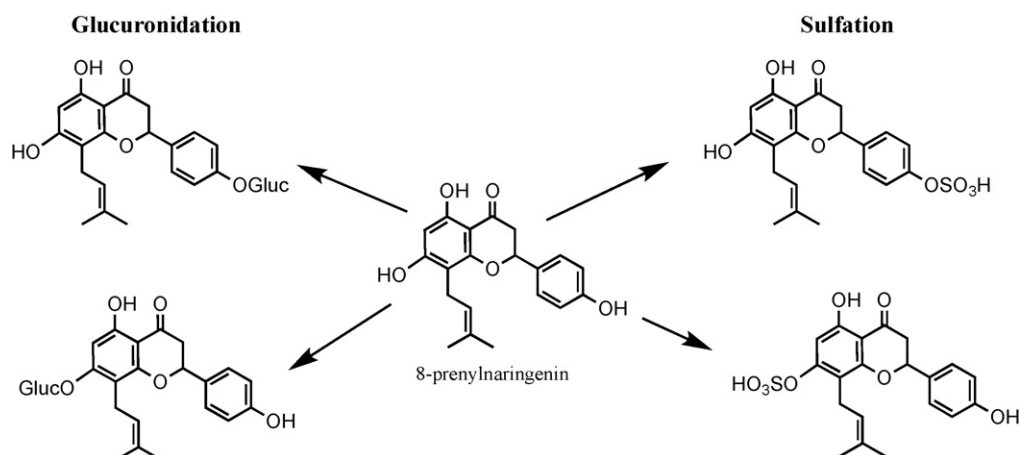


Fig. 3. Type II (conjugation) metabolism of flavanones (exemplified with 8-prenylnaringenin).

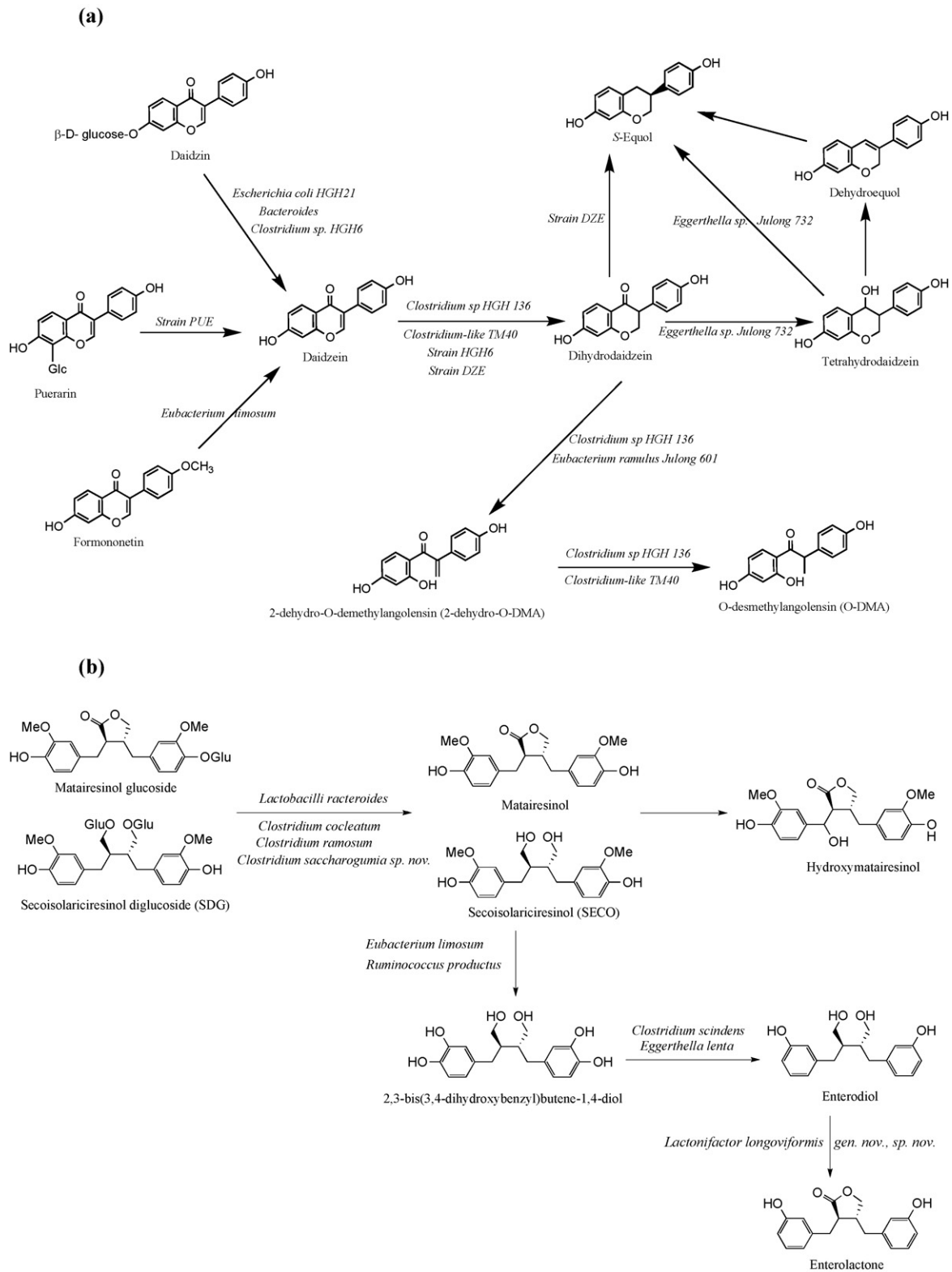


Fig. 4. (a) Gut microflora metabolism of isoflavonoids (exemplified with daidzein). (b) Gut microflora metabolism of lignans (exemplified by matairesinol glucoside and secoisolariciresinol diglucoside).

than 400 different species, gut microflora depends upon age, ethnic origin, diet, intestine transit, pH, acetate production, endogenous metabolism, as well as a number of pathologies. Amazingly, these observations support the concept of “*in vivo* gut microflora instability” and, therefore, PE metabolism instability.

- As previously highlighted, O-deglycosylation is required for efficient absorption of flavonoids. Catalyzed by a number of Gram+/Gram– bacteria, this reaction occurs in the jejunum and is partially achieved by bacterial β -glucosidases (Fig. 4a) [31–35]. To a lesser extent, C-deglycosylation occurs during the biocon-

version of puerarin into daidzein by the strain PUE (Fig. 4a) [36].

- As observed with isoflavonoids and enterolignans, O-demethylation reactions imply intestinal bacteria such as *bifidobacteria*, *Eubacterium limosum* (*Eubacterium* sp. ARC-2) or *Ruminococcus productus* (Fig. 4b) [37–39].
- Dehydroxylation is relevant to isoflavonoids (after the reduction of the carbonyl in position 4 into the hydroxyl analog by *Eggerthella* sp. Julong 732 or directly by the strain DZE, Fig. 4a) and to enterolignans, as exemplified by the transformation of 2,3-bis(3,4-dihydroxybenzyl)butene-1,4-diol into enterodiol by *Clostridium* species and *Eggerthella lenta* (Fig. 4b). Remarkably, *Eggerthella* sp. SDG-2 and the strain ARC-1 participate in enantioselective dehydroxylation. *Eggerthella* sp. SDG-2 dehydroxylates (–) dihydroxyenterolactone whereas the strain ARC-1 dehydroxylates (+) dihydroxyenterolactone [40].
- The reduction of the 2–3 double-bond of a number of flavonoids is mediated by bacteria that utilize hydrogen such as methane producing and sulfate reducing bacteria. According to this statement, *Clostridia* species [41,42] (Fig. 4a) or *Eubacterium rectale* [42] afford the active metabolites equol, O-DMA, enterodiol, enterolactone and 8-prenylaringenin [39,40,43].
- Lactonisation and ring cleavage reactions are two additional metabolism pathways that are specific of lignans and flavonoids, respectively. Whereas the first is observed in the presence of *Lactonifactor longoviformis* gen. nov., sp. nov., the second is mediated by *clostridia* species or *Eubacterium ramulus* Julong 601 [44] with, however, a preference for flavonoids with a hydroxyl in position 5 (Fig. 4a and b) [34].

Amazingly, these observations suggest that not only enzyme polymorphism but also gut microflora population may have crucial repercussions on the release of active PE. Gut microflora depending upon diet habits, the latter may have crucial repercussions on PE-responsiveness [42,45,46].

Most of PE metabolites produced by intestinal bacteria being active, their stereochemistry must be also taken into account. For example, the bacterial metabolism of isoflavones [36] leads to the very active only metabolite *S*-equol [34]. It is of note that *S*-equol (Fig. 4a) binds to ER β with a 13 times higher affinity than *R*-equol and a two times higher than (\pm) equol [41,47]. ER α distribution in tissues depending upon the age and the pathophysiological context of patients, these factors should be also taken into account.

Hence, the activity of intestinal bacteria with regard to possible tumor promoting effects in women with breast cancer predispositions seems likely, the latter being implied in the biosynthesis of active metabolites.

O-Dma is also a very active metabolite. O-Dma is produced by 80–90% of the population, whereas equol is produced by 30–40% of the population (20–30% of adults from western populations and 50% of Japanese women), suggesting strong variations towards therapeutic responsiveness and toxic effects [48]. Thus, isoflavone consumers should be systematically classified as “equol producers” or “equol non-producer” as well as “O-Dma producers” or “O-Dma non-producers” [1,35]. At least, it is of note that O-DMA producers have a 6% higher total (leg and head) bone mineral density when compared to non-O-DMA producers [49].

4. Conclusion

In the present review, we have explored the metabolic factors that make *in vivo* pharmacology of PE extremely variable and benefits resulting from their use quite ambiguous and most of time inconclusive. In respect to PE metabolism discussed in this

section, it would be necessary to consider the physiopathological context, diet habits, ethnic origins as well as the phenotype and gut microflora population (based on questionnaire) for each women. Such requests being arduous and difficult to implement, innocuousness and efficacy of PE will be subject to debates, since moreover chemical nature of PE in commercial botanical extracts are not well defined. The presence of the products as aglycones or conjugates should be precisely stated. In this aim, dosage methods should be perfected. Indeed, Boniglia et al. [50] have recently shown by high-performance liquid chromatography coupled with an UV detector that most preparations do not contain the content of isoflavones declared by suppliers. Thus, the exact qualitative/quantitative composition of PE in such preparations would be precisely provided by the supplier, as for drugs. Finally, we recommend to patients to use PE under a strict medical survey.

Contributors

Dr. Patricia de Cremoux, Associate Professor, University René Diderot - Paris 7, PhD, physician, Head of Pharmacology Unit (Tumor Biology Department), Institut Curie, Paris (France) has contributed to this work by participating to the discussion concerning the effects of diet on gut microflora. Also, she has participated to the discussion concerning enzymatic polymorphism and the associated effects on phytoestrogen bioavailability and metabolism.

Dr. Pascale This, physician in endocrinology and gynecology in Institut Curie (Paris, France) and in the Centre de la Femme (Service of Gynecology – Obstetrics) in the Hospital of Versailles has participated to this work by developing the clinical consequences of phytoestrogen bioavailability and metabolism changes.

Pr. Guy Leclercq, Professor – holder of the agrégation, Director of the Laboratoire J.-C. Heuson de Cancérologie Mammaire, Université Libre de Bruxelles (U.L.B.) - Institut Jules Bordet, Brussels (Belgium) is a biochemist and is a world-famous specialist in endocrinology. Its critical view on estrogens and related mechanisms was indispensable. He has participated to all aspects of this work.

Dr. Yves Jacquot, Pharmacist, Professor Assistant in the University Pierre et Marie Curie (Paris 6), is researcher in bioorganic chemistry in the University Pierre et Marie Curie (CNRS - UMR 7203) and in the Ecole Normale Supérieure (ENS) in Paris (France). As a pharmacologist specialised in estrogens and the biomolecular aspects of the estrogen receptor, he works among others on the synthesis of estrogen receptor modulators that share peptidic structures or benzopyranic and coumarinic motifs. Yves Jacquot has initiated the project and is the head of project. In this work, he has developed the bioavailability of phytoestrogens as well as the molecular and enzymatic aspects of the metabolism of phytoestrogens.

Conflict of interest

No competing interest has to be declared.

Provenance and peer review

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