The potential health effects of dietary phytoestrogens

Phytoestrogens are plant-derived dietary compounds with structural similarity to 17-β-oestradiol (E2), the primary female sex hormone. This structural similarity to E2 enables phytoestrogens to cause (anti)oestrogenic effects by binding to the oestrogen receptors. The aim of the present review is to present a state-of-the-art overview of the potential health effects of dietary phytoestrogens. Various beneficial health effects have been ascribed to phytoestrogens, such as a lowered risk of menopausal symptoms like hot flushes and osteoporosis, lowered risks of cardiovascular disease, obesity, metabolic syndrome and type 2 diabetes, brain function disorders, breast cancer, prostate cancer, bowel cancer and other cancers. In contrast to these beneficial health claims, the (anti)oestrogenic properties of phytoestrogens have also raised concerns since they might act as endocrine disruptors, indicating a potential to cause adverse health effects. The literature overview presented in this paper illustrates that several potential health benefits of phytoestrogens have been reported but that, given the data on potential adverse health effects, the current evidence on these beneficial health effects is not so obvious that they clearly outweigh the possible health risks. Furthermore, the data currently available are not sufficient to support a more refined (semi) quantitative risk–benefit analysis. This implies that a definite conclusion on possible beneficial health effects of phytoestrogens cannot be made.

Abbreviations

ARE/EpRE, antioxidant/electrophile response element; E2, 17-β-oestradiol; EFSA, European Food Safety Authority; ERα, oestrogen receptor α; ERβ, oestrogen receptor β; ERs, oestrogen receptors (NRA3A); GPER, G protein-coupled oestrogen receptor; PPAR, peroxisome proliferator activated receptor (NR1C); TPO, thyroid peroxidase (EC number 1.11.1.8)
Introduction

Phytoestrogens are plant-derived dietary compounds, found in a wide variety of foods, especially in soy. They represent a diverse group of naturally occurring chemicals with structural similarity to 17-β-oestradiol (E2), the primary female sex hormone. Because the lack of phytoestrogens in the diet does not result in deficiency syndromes and because the phytoestrogens do not participate in any essential biological function, phytoestrogens are not considered nutrients. Their structural similarity to E2 enables them to cause (anti) oestrogenic effects by binding to the oestrogen receptors (ERs). This was already noticed in the previous century in Western Australia where sheep grazing on isoflavone-rich red clover fields showed fertility problems (Bennetts et al., 1946; Stafford, 1997; Scherr et al., 2009). It has been hypothesized that plants use phytoestrogens as part of their natural defence to control female fertility to prevent overpopulation and overgrazing by herbivore animals (Hughes, 1988). In line with this, Setchell (Setchell, 1998) suggested that the fertility problems of zoo animals could be related to the presence of isoflavone phytoestrogens in the standard animal diet. Besides these adverse effects, various beneficial health effects have been ascribed to phytoestrogens, such as a lowered risk of menopausal symptoms like hot flushes and osteoporosis. As a result, phytoestrogens are present in a large number of dietary supplements and widely marketed as natural alternatives to oestrogen replacement therapy. In addition, phytoestrogen exposure has been related to lowered risks of cardiovascular disease, obesity, metabolic syndrome and type 2 diabetes, brain function disorders, breast cancer and other forms of cancer including prostate cancer, bowel cancer and other cancers (Hughes, 1988; Adlercreutz, 2002; Bhathena and Velasquez, 2002; Karahalil, 2005; Cederroth and Nef, 2009; Patisaul and Jefferson, 2010; Zhao and Mu, 2011; Jungbauer and Medjakovic, 2014).

In the last decades, soy isoflavones have received attention because of the so called ‘Japanese Phenomenon’ connected to a lower incidence of specific chronic diseases in the Japanese compared with the Western population due to a higher intake of soy foods from early life onwards (Watanabe et al., 2002; Korde et al., 2004; Korde et al., 2009). The fact that the prevalence of breast cancer in daughters of migrated Japanese Americans became similar to that of Caucasian Americans after changing their food habits is in line with this observation.

In contrast to these beneficial health claims, the (anti) oestrogenic properties of phytoestrogens have also raised concerns since they might act as endocrine disruptors, indicating a potential to cause adverse health effects. Altogether, the health benefits or risks of isoflavones and other phytoestrogens are still controversial (Wuttke et al., 2007; Andres et al., 2011; Rietjens et al., 2013), and the question of whether phytoestrogens are beneficial or harmful to human health remains unresolved.

Keeping that in mind, the aim of the present review is to present a state-of-the-art overview of the potential health effects of dietary phytoestrogens. The paper presents an overview of the different phytoestrogens present in the diet and food supplements, their supposed mode(s) of action and recent evidence on their supposed beneficial effects.

To obtain an overview of the various health effects, we searched Web of Science, Scopus and PubMed for entries with the search terms ‘phytoestrogen(s)’, ‘review’, ‘meta-analysis’ and the respective health effects in titles, abstracts and keywords.

Phytoestrogens in the diet

Figure 1 presents an overview of the major types of phytoestrogens known to be present in the diet and food supplements, also including their chemical structure compared with that of E2. The major groups of phytoestrogens present in our diet are isoflavones, prenylflavanoids, coumestans and lignans. The main isoflavones are genistein, daidzein, glycitein, formononetin and biochanin A, which are mainly found in soy, soy-based food and legumes usually in their conjugated forms like genistin, daidzin, puerarin, glycitin, ononin and sissotrin. In countries in Asia where fermented soy products are part of the traditional diet, isoflavone intake levels may amount to about 15–50 mg isoflavones per day (Eisenbrand et al., 2007). In Western industrial countries, isoflavone intake has been reported to be less than 2 mg isoflavones per day (Eisenbrand et al., 2007), although it may be higher for menopausal women who take soy-based preparations as an alternative to hormone replacement therapy. The dosages recommended by the manufacturers may vary with the product, and have been reported to amount to values between 20 and 80 mg isoflavones per day (Eisenbrand et al., 2007).

Major prenylflavanoids are 6-prenylnaringenin, 6-geranyl naringenin, 8-prenylnaringenin and isoxanthohumol,
which can all be found in hops and beer (Stevens and Page, 2004; Dhooghe et al., 2010). Of the prenyllavonoids, 8-prenylavonoids, 8-prenylavonoids (Figure 1) is the most potent phytoestrogen known. The main coumestans are coumestrol, 4′-methoxyconjugostrol, genistein and trifolol (Figure 1). Food sources high in coumestans include split peas, pinto beans, lima beans, and especially, alfalfa and clover sprouts. Lignans that are classified as phytoestrogens are enterodiol and enterolactone (Figure 1), which are formed from lignan precursors by intestinal bacteria (Lampe, 2003). Lignan precursors include pinoresinol, lariciresinol, secoisolariciresinol, matairesinol and others (Figure 2). Lignan precursors are found in a wide variety of foods, including flaxseeds, whole grains, fruits and vegetables, sesame seeds and legumes and present a principal source of dietary phytoestrogens in the Western diet (de Kleijn et al., 2002; Valsta et al., 2003).

Mode(s) of action: interaction with oestrogen receptors

The major mode of action by which phytoestrogens may exert their possible health effects, is based on their structural similarity to E2 enabling them to cause (anti)oestrogenic effects by binding to the ER. Two main ERs, that is oestrogen receptor α (ERα) (NR3A1) and oestrogen receptor β (ERβ) (NR3A2), have been identified in rats, mice, primates and humans (Kuiper et al., 1996; Ogawa et al., 1998). These ER subtypes have different roles in gene regulation, cancer biology and therapy (Nilsson et al., 2001; Williams et al., 2008; Thomas and Gustafsson, 2011). ERα activation in breast and uterus has been shown to enhance cell proliferation, necessary for growth and tissue maintenance (Pierce and Jordan, 2004; Harris, 2007; Thomas and Gustafsson, 2011) but may also play a role in the unlimited growth of, in particular, ERα-dependent breast tumours of which around 70% respond to anti-oestrogen therapy with, for example, the antagonist tamoxifen (Ali and Coombes, 2000). ERβ has been shown to counteract the ERα-mediated stimulation of cell proliferation (Bardin et al., 2004; Stossi et al., 2004; Strom et al., 2004; Sotoca et al., 2008b; Sotoca et al., 2008a; Thomas and Gustafsson, 2011). These opposite roles of ERα and ERβ in cellular responses to oestrogens have been illustrated by studies using the so-called T47D–ERβ cell line, a breast cancer cell line with a constant level of ERα, and a tetraclincine-dependent adjustable expression level of ERβ (Strom et al., 2004). When cells of this cell line express mainly ERα, genistein and E2 both appeared to induce a concentration-dependent increase in proliferation. When ERβ expression is induced, E2 and genistein no longer induce cell proliferation. These results support the conclusion that ERβ plays a role in counteracting ERα-mediated cell proliferation.

Because ERα and ERβ have different roles in gene regulation, cell proliferation and related health effects, their varying ratio and relative level within tissues may influence the cellular response towards different phytoestrogens. As a result, a certain phytoestrogen may have different effects in, for example, the uterus, in which ERα is the major isoform (Pearce and Jordan, 2004), than in the prostate, in which ERβ is dominant (Enmark et al., 1997; Pearce and Jordan, 2004). These tissue-specific effects may also result from differences in coactivators and corepressors activated upon activation of the two ERs in different tissues and/or the possible crosstalk of the ERs with other nuclear receptors (Wilson et al., 2004; Chang et al., 2008; Vanden Berghe and Haegeman, 2008; Evers et al., 2014a; Evers et al., 2014b). Furthermore, the actual mode of action of a phytoestrogen, either as an agonist or an antagonist, may also depend on the level of endogenous estrogens present (Barnes et al., 1995).

Using various in vitro models that have been developed for the detection of oestrogen activity, the relative oestrogenic potencies of a variety of phytoestrogens have been quantified. These in vitro assays include receptor binding studies (Kuiper et al., 1998; Gutendorf and Westendorf, 2001; Ikeda et al., 2002; De Angelis et al., 2005; Boue et al., 2011; Park et al., 2012; Djiogou et al., 2014; Liu et al., 2014), ERα and ERβ-dependent reporter gene assays (Kuiper et al., 1998; Casanova et al., 1999; Dornstauder et al., 2001; Gutendorf and Westendorf, 2001; Ikeda et al., 2002; Rickard et al., 2003; De Angelis et al., 2005; Harris et al., 2005; Escande et al., 2006; ter Veld et al., 2006; Chrzan and Bradford, 2007; Sotoca et al., 2008b; Chu et al., 2009; Kwack et al., 2009; Takeuchi et al., 2009; Boue et al., 2011; Park et al., 2012; Djiojou et al., 2014; Liu et al., 2014; Tiosano et al., 2014; Islam et al., 2015) and cell proliferation assays using oestrogen-sensitive human cell lines derived from three different female oestrogen-sensitive tissues, including breast (MCF-7/BOS and T47D), endometrial (ECC-1) and ovarian (BG-1) cells (Wang et al., 2012). Few studies also report on the oestrogenic effects of phytoestrogens in vivo in the so-called uterotrophic assay (Ding et al., 2010; Wang et al., 2012).

For the present review, we performed a literature research on the binding affinity of E2 and phytoestrogens to ERα and ERβ (expressed as IC50 values from competitive binding assays) and the ERα- and ERβ-mediated gene expression induced by E2 and phytoestrogens (expressed as EC50 values from reporter gene assays). To obtain this overview, we searched Web of Science, Scopus and PubMed for entries with the search terms ‘phytoestrogen(s)’, ‘ERα’, ‘ERβ’, ‘binding affinity’ and/or ‘reporter gene’ in titles, abstracts and keywords. In Figure 3, the reported IC50 and EC50 values for ERα are plotted against the reported IC50 and EC50 values for ERβ.
Tables S1 and S2, the IC$_{50}$ values and the EC$_{50}$ values, respectively, and the references to the literature are presented.

The overall trend emerging from this overview is that phytoestrogens are less potent oestrogens than E2 with higher IC$_{50}$ and EC$_{50}$ values for both receptors (Figure 3). Furthermore, the data show for most phytoestrogens that have been investigated, that the IC$_{50}$ values are higher for ER$\alpha$ than for ER$\beta$ (Figure 3A), indicating a higher binding preference for the ER$\beta$ than for the ER$\alpha$. Only for ferutinine, kievitone and for psoraldin the IC$_{50}$ values are higher for ER$\beta$ than for ER$\alpha$ (Table S1), indicating a higher binding preference for the ER$\alpha$ for these phytoestrogens. A higher preference of ferutinine for ER$\alpha$ was also found in reporter gene studies, whereas for the majority of phytoestrogens tested in reporter gene systems, a preference for ER$\beta$-mediated gene expression was observed (Figure 3B and Table S2). Most research on the binding of phytoestrogens to ER$\alpha$ and ER$\beta$, and the activation of ER$\alpha$- and ER$\beta$-mediated gene expression has been conducted with the major soy isoflavones genistein and daidzein (Kuiper et al., 1998; Casanova et al., 1999; Dornstauder et al., 2001; Gutendorf and Westendorf, 2001; Rickard et al., 2003; Bovee et al., 2004; De Angelis et al., 2005; Harris et al., 2005; Escande et al., 2006; Chrzan and Bradford, 2007; Sotoca et al., 2008b; Chu et al., 2009; Takeuchi et al., 2009; Tiosano et al., 2014; Beekmann et al., 2015; Islam et al., 2015). Figure 4 presents an example of such a study with genistein as measured in the human osteosarcoma (U2OS) ER$\alpha$ or ER$\beta$ reporter cell lines, comparing the activity of genistein with that of E2 (Sotoca et al., 2008b). The data presented in Figure 4 reveal that for E2, physiological concentrations may be such that only ER$\alpha$ is activated since the EC$_{50}$ value of E2 for activation of ER$\alpha$ is about 10-fold lower than that for activation of ER$\beta$. For the isoflavone genistein, the EC$_{50}$ for ER$\beta$ activation is lower than that for ER$\alpha$ activation, indicating that concentrations that activate ER$\alpha$ will at the same time activate ER$\beta$ that will counteract the ER$\alpha$-mediated effects on cell proliferation (Strom et al., 2004; Sotoca et al., 2008a; Rietjens et al., 2013). This may result in different physiological effects induced by E2 than those induced by phytoestrogens.

**Figure 3**

(A) Binding affinities to ER$\alpha$ and ER$\beta$ (expressed as IC$_{50}$ values) and (B) effect concentrations in ER$\alpha$ and ER$\beta$ reporter gene assays (expressed as EC$_{50}$ values) of E2 and phytoestrogens. In Tables S1 and S2, the IC$_{50}$ and EC$_{50}$ values, respectively, and the references to the literature are presented.

**Figure 4**

Induction of oestrogen responsive element mediated luciferase activity in the ER$\alpha$- and ER$\beta$-containing U2OS reporter cell lines by (A) E2 and (B) genistein. For further details, see Rietjens et al. (2013).
It is also of interest to note the possible role of G protein-coupled oestrogen receptors (GPERs), since many of the phytoestrogens including the soy isoflavone genistein and possibly equal activate GPERs (Prossnitz and Barton, 2011). In cell lines of thyroid, ovarian, endometrial and breast cancers, stimulation of GPERs with oestrogens including genistein, activates a signalling pathway that promotes proliferation, although inhibition of proliferation has also been reported. In particular, genistein has been reported to stimulate growth of MCF-7 cells through a GPER-dependent mechanism (Prossnitz and Barton, 2011).

**Mode(s) of action: epigenetic effects**

Another mode of action underlying the health effects of phytoestrogens may relate to epigenetic mechanisms. Various natural bioactive compounds have been shown to affect the epigenome, but for phytoestrogens, this has been mainly assessed for the soy isoflavone genistein, and to a lesser extent for the soy isoflavone daidzein and its microbial metabolite equol (Remely et al., 2015b). Especially for the reduction in cancer risk by the isoflavone genistein, a role for epigenetic changes resulting in alterations in the expression of genes that regulate cell proliferation and differentiation has been reported (Dolinoy et al., 2006; Hilakivi-Clarke et al., 2010). Genistein consumption is also among the factors that have been associated with epigenetic modifications in obesity (Remely et al., 2015a). Among these epigenetic changes were effects on DNA methylation, histone modification and microRNA regulation (Rietjens et al., 2013). Several studies have assessed the effects of genistein on DNA methylation.

Dolinoy et al. (2006) investigated, in the so-called agouti mouse model, the effect of genistein on DNA methylation in the offspring exposed during gestation. Genistein induced CpG hypermethylation of six CpG sites upstream of the agouti gene, resulting in a shift of the coat colour distribution towards pseudoagouti (brown), and a decrease in the incidence of the onset of obesity in the offspring at adult age (Dolinoy et al., 2006). The authors speculated that this ability of genistein to increase DNA methylation might provide an explanation for the lower incidence of certain cancers in Asians as compared with Westerners (Qin et al., 2009). This observation could provide another explanation for the different outcomes reported when studying effects of phytoestrogens on breast cancer incidence. It could be that beneficial effects result from epigenetic modifications that occur early in life perhaps even through soy intake by the mother, but that these beneficial effects may not be observed when intake of soy isoflavones starts later in life, for example when using hormone replacement therapy. Also, adolescence may reflect a sensitive period for phytoestrogens, and phytoestrogen intake during adolescence may reduce breast cancer risk later in life (Lee et al., 2009; Anderson et al., 2013; Molzberger et al., 2013).

In *in vitro* studies, genistein has been shown to decrease the methylation of several tumour suppressor genes (Pudenz et al., 2014), which may be mediated by the inhibition of DNA methyl transferase (DNMT) activity (Singh et al., 2013). This may allow silenced tumour suppressor genes to be re-expressed, indicating a potential beneficial effect of genistein. However, inhibition of DNMT may also lead to a reduced methylation of proto-oncogenes, as has been shown for coumestrol, daidzein and equol (Lyn-Cook et al., 1995; Koo et al., 2015), which may indicate a possible adverse effect. However, whether these findings from *in vitro* studies are relevant for the *in vivo* situation remains to be established, since genistein has been shown to cause an increase in DNA methylation upon exposure *in vivo*, such as in the agouti mouse model as mentioned above (Dolinoy et al., 2006) and in humans (Qin et al., 2009). This human study was a double-blind, randomized trial with 34 healthy premenopausal women, who received 40 or 140 mg isoflavones (including genistein, daidzein and glycitein) a day for the duration of one menstrual cycle, followed by characterization of the methylation status of five cancer related genes known to be methylated in breast cancer (p16, RASSF1A, RARβ2, ER and CCND2) assessed in isolated breast tissue samples (Qin et al., 2009). The results obtained revealed a treatment-related hypermethylation of the tumour suppressor genes RARβ2 and CCND2. The implications of these findings for human health are not clear yet, although this increase in methylation of tumour suppressor genes may indicate a possible adverse epigenetic change.

**Other possible modes of action**

In addition to the action of phytoestrogens via the oestrogen receptors or epigenetic modes of action, some studies suggested other possible modes of action of phytoestrogens. This includes, for example, the effects of genistein resulting in activation of AMP-activated kinase (EC number 2.7.11.31) in cells exposed to genistein *in vitro* (Hwang et al., 2005; Park et al., 2010). The exact mechanism underlying this activation has not been fully elucidated (Hsu et al., 2011) but may be related to genistein-mediated stimulation of intracellular production of ROS (Hwang et al., 2005).

Another mode of action may relate to the action of phytoestrogens as kinase inhibitors (Dubey et al., 1999; Kang et al., 2007; Van et al., 2010). Given that the human kinome is composed of hundreds of protein kinases of which many have been reported to be disease associated (Manning et al., 2002; Lahiry et al., 2010), one could suggest that this is a possible mode of action underlying the effects of phytoestrogens on health. It is important to note, however, that EC50 values generally reported for this inhibition of protein kinases by isoflavones seem to be around the two-digit and lower three-digit μM range (Dubey et al., 1999; Kang et al., 2007; Kim et al., 2009), which is substantially higher than generally reported physiological plasma concentrations of the respective phytoestrogens, which tend to be in the two- to three-digit nM range, rarely reaching low μM concentrations (King and Burns, 1998; Verkasalo et al., 2001; Setchell et al., 2003).

Phytoestrogens may also activate the PPAR (NR1C1) family including, particularly PPARα (NR1C1), and PPARγ (peroxisome proliferator activated receptor γ) (NR1C3) (Jungbauer and Medjakovic, 2014). It has been suggested that this mode of action mainly contributes to the possible effects of phytoestrogens on obesity, metabolic syndrome and type 2 diabetes, as PPARγ agonists are effective drugs for patients with type 2 diabetes. Specifically, isoflavones and their metabolites appear to be PPAR agonists with EC50 values in the two-digit μM range (Jungbauer and Medjakovic, 2014). It remains to be established whether physiological
concentrations are actually high enough to induce these PPAR-mediated effects.

Another mode of action, suggested mainly for the beneficial health effects of phytoestrogens, is the induction of antioxidant/electrophile response element (ARE/EpRE)-mediated gene expression by activation of nuclear factor erythroid 2-related factor 2-Keap 1 signalling (Jungbauer and Medjakovc, 2014). EpRE-mediated induction of gene expression leads to increased cellular defence against the toxicity of electrophiles and ROS and related adverse health effects. Activation of this pathway may be related to protection against tumour induction and oxidative stress in cardiovascular disease.

Finally, it is of interest to consider the possible role of polymorphisms in the differential effects of phytoestrogens. So far, differences in health effects of phytoestrogens have not been related to specific gene polymorphisms. However, since ERs seem to play an important role in several of the health effects of phytoestrogens, it can be expected that phytoestrogen-related health effects may differ between persons with differences in ER function, for example resulting from ER gene polymorphisms. Nott et al. (2008) concluded that an increasing body of evidence implicates ERα polymorphisms as one of the contributing factors for differential responses to oestrogen competitors and that polymorphisms in the ER genes may influence the individual response to hormone replacement therapy (Gennari et al., 2005). Polymorphisms of genes that play a role in the metabolism of (phyto)oestrogens may also play a role in differences in health effects of phytoestrogens in the human population (Wang et al., 2011). Also, polymorphisms related to interindividual differences in the gut microbiota and the consequences of these differences for formation of (in)active metabolites may play a role. An adequate example is the formation of equol as a metabolite of daidzein only in a part of the population. Equol has been reported to be more active as an oestrogen-active compound than daidzein itself (Magee, 2011). Another example is the formation of enterodiol and enterolactone from ingested lignans through the gut microbiota in only a part of the population (Kuijsten et al., 2005; Hullar et al., 2015). Also, these differences can be an underlying reason for different responses to phytoestrogen exposure in the population.

Effects of phytoestrogens on menopausal symptoms
Several meta-analyses investigated the effects of phytoestrogens or soy isoflavone extracts or supplements on menopausal symptoms. These studies reported not only a reduction in the frequency and severity of hot flashes (Howes et al., 2006; Taku et al., 2012; Chen et al., 2015) but also no conclusive evidence or only some indications for a reduction in hot flush frequency or severity (Nedrow et al., 2006; Lethaby et al., 2007; Jacobs et al., 2009; Bolanos et al., 2010; Eden, 2012; Lethaby et al., 2013), varying effects on spine bone mineral density and no effects on femoral neck, hip total and trochanter bone mineral density (Taku et al., 2010), and no protection against bone fracture (Kreijkamp-Kaspers et al., 2004; Tempfer et al., 2007). The European Food Safety Authority (EFSA) evaluated the health claims related to the reduction of vasomotor symptoms and the maintenance of bone mineral density by soy isoflavones during menopause (EFSA NDA Panel, 2012). It was concluded that the available evidence was not sufficient to establish a relationship between the maintenance of bone mineral density and the consumption of soy isoflavones. Also, a relationship between the reduction of vasomotor symptoms associated with menopause and the consumption of soy isoflavones could not be established. EFSA evaluated 14 long-term (>12 months) intervention studies on the effects of soy isoflavones on bone mineral density in post-menopausal women. Of these studies, only two, reported an effect of soy isoflavones on bone mineral density and on markers of bone formation or resorption at doses of 54 mg per day (Morabito et al., 2002; Marini et al., 2007). The remaining 12 studies, testing doses of isoflavones up to 200 mg per day, showed no effects of soy isoflavones on bone mineral density or markers of bone formation or resorption [EFSA NDA Panel, 2012] and references therein], although EFSA indicated that for some studies this negative result may have been due to the limited number of subjects included, resulting in an underpowered study.

EFSA also evaluated five human intervention studies in which soy isoflavones were administered for 6–9 months, and effects on bone mineral density were measured in peri-/post-menopausal women. One of these intervention studies revealed a beneficial dose–response effect on bone mineral density at the lumbar spine and femoral neck. Two of the studies reported a beneficial effect of soy isoflavones on bone mineral density at the lumbar spine, and two studies showed no effect of soy isoflavones on bone mineral density. EFSA concluded that ‘these studies provide some evidence for an effect of soy isoflavones on the attenuation of bone mineral density loss at the lumbar spine in post-menopausal women when consumed for 6-9 months’. Given the effects reported on parameters for bone formation and bone resorption, EFSA also concluded that these effects were possibly mediated by a decrease in bone resorption. However, taking all the evidence together, EFSA considered ‘the evidence insufficient to establish a cause and effect relationship between the consumption of soy isoflavones and maintenance of bone mineral density in post-menopausal women’.

A later study reported by Greendale et al. (Greendale et al., 2015) investigated cross-sectional and longitudinal relations between dietary intake of isoflavones and bone mineral density at the lumbar spine and femoral neck in Black, White, Chinese and Japanese women during the menopausal transition. In Japanese women, higher isoflavone intake appeared to be associated not only with higher peak femoral neck bone mineral density but also with greater rates of lumbar spine and femoral neck bone mineral density loss during the menopausal transition. Results for the other racial/ethnic groups did not support a relationship between dietary intake of isoflavones and either peak bone mineral density, or bone mineral density loss during the menopausal transition.

Altogether, it seems that the evidence for a beneficial effect of isoflavones and other phytoestrogens on bone mineral density in post- and peri-menopausal women is limited and not convincing, and that they may even cause adverse effects, as indicated by the study of Greendale et al. (2015).
Potential health effects of dietary phytoestrogens

Effects of phytoestrogens on cardiovascular disease
In particular, the increase in cardiovascular risk and cardiovascular mortality upon sudden loss of ovarian function has provided a basis for the proposed role of oestrogens and phytoestrogens in the reduction in cardiovascular disease (Colditz et al., 1987; Parker et al., 2009). Reduced oestrogen levels during menopause may influence the development of obesity, fat distribution, the lipid profile in plasma, and rheological properties of plasma and platelet function (Gorodeski, 1994). These observations suggest that oestrogen deficiency may promote cardiovascular disease in women and trigger the idea that phytoestrogens may reduce the risk. The hypothesis is also supported by the low rates of cardiovascular diseases in Asian populations where the diet is particularly rich in soy, followed by the loss of this protection among the groups that have moved to Western societies (Nagata, 2000; Zhang et al., 2003). A meta-analysis reported by Toked et al. (2015) concluded that isoflavone supplementation had no effect on serum lipid profiles.

Oestrogens have been shown to influence atherosclerosis and the related clinical events in a differential way (Rossouw et al., 2007; Cano et al., 2010). They may act not only as protectors against atherosclerosis but also as potential disruptors of established atherosclerotic plaques, the latter being important hallmarks in the pathogenesis of the arterial forms of cardiovascular disease. The concept that phytoestrogens may act in a similar way comes from the observation that in Asian populations with high levels of their consumption, the prevalence of cardiovascular disease is lower than in populations in Western countries (Gonzalez Canete and Duran Aguero, 2014).

Cano et al. (2010) and also Gonzalez Canete and Duran Aguero (2014) reviewed the main evidence on the effects of isoflavones on the cardiovascular system at both the experimental and the clinical level. The review indicated that two clinical studies (van der Schouw et al., 2005; Kokubo et al., 2007) have examined the association of isoflavone intake with clinical cardiovascular events in a population without cardiovascular disease at baseline. One study concluded that high isoflavone intake was associated with reduced risk of cerebral and myocardial infarctions in Japanese women, with the risk reduction being especially pronounced in postmenopausal women (Kokubo et al., 2007). The second study was performed in a Western population and investigated whether a low intake of phytoestrogens would be associated with increased risks of cardiovascular disease (van der Schouw et al., 2005). The study concluded that an intake of isoflavones or lignans was not associated with decreased cardiovascular disease risk. However, when stratifying for ever versus never smokers, the risk of cardiovascular disease appeared to decrease with increased intake of lignans for ever smokers. The authors concluded that their data do not support the presence of a protective effect on cardiovascular disease risk of higher habitual intake of phytoestrogens, although for smokers a small risk reduction with higher lignan intake could not be excluded. In clinical practice, none of the phytoestrogens investigated have been proven to protect against cardiovascular disease (Tempfer et al., 2007).

Altogether, it can be concluded that it is not really clear if the administration of phytoestrogens in the early postmenopausal period may be protective for cardiovascular disease. For phytoestrogens, the current evidence appears poor compared to that available for oestrogens, and it is possible that the potential effect of oestrogens on the risk for stroke is not reproduced by isoflavones (Cano et al., 2010).

Effects of phytoestrogens on obesity, metabolic syndrome and type 2 diabetes
Dietary phytoestrogens have also been suggested to play a beneficial role in obesity, metabolic syndrome and type 2 diabetes (Bhathena and Velasquez, 2002; Cederoth and Nef, 2009; Crespillo et al., 2011; Jungbauer and Medjakovic, 2014; Struja et al., 2014). The meta-analysis by Fang et al. (2016) reported an improvement in glucose metabolism and a significant reduction in insulin levels and insulin resistance in menopausal women, by especially genistein. Also, the meta-analysis reported by Zhang et al. (2013) concluded that soy isoflavone supplementation could improve glucose metabolism and insulin control in non-Asian postmenopausal women. Nutritional intervention studies in both animals and humans suggested that an intake of soy protein with isoflavones and flaxseed results in improved glucose control and insulin resistance (Bhathena and Velasquez, 2002; Cederoth and Nef, 2009; Jungbauer and Medjakovic, 2014). It is important to note that at present, it often remains unclear whether the beneficial effects are really due to the phytoestrogens or to some other dietary component in the soy protein and flax seed diet. The activation of PPAR-mediated effects already mentioned above may provide a possible mode of action underlying these effects. However, as already concluded by Bhathena and Velasquez (2002), further investigations are needed to evaluate the long-term effects of phytoestrogens on obesity, metabolic syndrome and type 2 diabetes.

Effects of phytoestrogens on breast cancer
The use of soy-based preparations has been proposed for the prevention and treatment of certain types of cancer, such as for the prevention and treatment of breast cancer in women and prostate cancer in men (Eisenbrand et al., 2007). In contrast, clinical studies have reported data that suggest that isoflavones may via their oestrogenic and proliferative effects possibly raise breast cancer incidence in sensitive individuals (Petrakis et al., 1996; Hargreaves et al., 1999).

Meta-analyses of epidemiological studies conducted in women consuming high-soy diets concluded that there is a significant trend of decreased risk for breast cancer upon increasing intake of soy food (Trock et al., 2006; Wu et al., 2008; Dong and Qin, 2011; Chen et al., 2014). High lignan exposure has also been associated with a reduced breast cancer risk in postmenopausal women (Velentzis et al., 2009; Buck et al., 2010). Fritz et al. (2013) reported a systematic review and meta-analysis on the potential effects of soy, red clover and isoflavone intake on breast cancer incidence and recurrence. The analysis included 40 randomized controlled trials, 11 uncontrolled trials and 80 observational studies. The authors concluded that soy consumption may be associated with reduced risk of breast cancer incidence, recurrence and mortality. Soy intake consistent with a traditional Japanese diet (2–3 servings a day containing 25–50 mg isoflavones)
may also be protective against breast cancer and recurrence. Nevertheless, the authors also indicate that better evidence confirming the safety of isoflavones would be required before the use of high doses (≥100 mg) of isoflavones can be recommended for breast cancer patients. In addition, a nested case-control study and meta-analysis of epidemiological studies revealed an inverse correlation between genistein intake and breast cancer (Taylor et al., 2009). It is important to note that this protective effect may originate from soy intake early in life (Warri et al., 2008; Hilakivi-Clarke et al., 2010). Also of interest is the possible involvement of equol, a metabolite of daidzein produced by the human intestinal microflora, which may have similar beneficial effects on the incidence of breast cancer (Ingram et al., 1997; Goodman et al., 2009), although other studies have reported no or even adverse effects of equol (Magee, 2011). Given that only a limited percentage (30–40%) of the population has the ability to convert daidzein to equol, and taking into account that in vitro studies suggest equol to be more biologically active than its parent compound daidzein (Magee, 2011), it can be suggested that inter-individual variation in response to daidzein may in part be related to variability in gut microflora composition resulting in inter-individual differences in conversion of daidzein to equol. This existence of equol producers and non-producers could provide an alternative explanation for interindividual differences in the response to phytoestrogens, since phytoestrogen metabolite formation by the gut microbiota can vary significantly between individuals with metabolites sometimes being more active than the parent phytoestrogen.

Also, the lignans enterodiol and enterolactone have been suggested to exert protective effects on breast cancer, possibly not only by oestrogen receptor-dependent but also oestrogen receptor-independent mechanisms (Mueller et al., 2004; Adlercreutz, 2007; Penttinen et al., 2007; Mense et al., 2008; Saarinen et al., 2010; Buck et al., 2011). There are also significant inter-individual differences for enterodiol and enterolactone production due to different compositions of the gut microflora, which can cause a variation in the response of individuals to lignan precursors (Yoder et al., 2015). While coumestans are reported to be potent phytoestrogens in vitro, their effect on breast cancer is not known (Scarlata and Miksicek, 1995; Kuiper et al., 1998).

Recently, EFSA published a risk assessment for peri- and post-menopausal women consuming food supplements containing isolated isoflavones (EFSA ANS Panel, 2015). EFSA evaluated especially the possible association between the isoflavone intake from food supplements and harmful effects observed in peri- and post-menopausal women for the mammary gland, uterus and thyroid (EFSA ANS Panel, 2015). For the evaluation, EFSA selected 43 human studies and 62 animal studies. Among these studies were four epidemiological studies investigating breast cancer incidence (Rebechkin et al., 2007; Obi et al., 2009; Brasky et al., 2010; Boucher et al., 2013), eight interventional controlled studies, measuring mammographic density (Morabito et al., 2002; Atkinson et al., 2004; Marini et al., 2008; Powles et al., 2008; Verheus et al., 2008; Maskarinec et al., 2009; Colacurci et al., 2013; Delmanto et al., 2013), and two interventional controlled studies, investigating histopathological changes (Cheng et al., 2007; Khan et al., 2012). These studies did not reveal an association between isoflavone exposure and adverse effects in the mammary gland (EFSA ANS Panel, 2015). Of the 11 animal studies that investigated histopathological changes in the mammary gland upon treatment with isoflavones and the 10 studies in ovariectomized animals that investigated breast cell proliferation, the majority did not reveal an effect (EFSA ANS Panel, 2015). Only in two of the studies in ovariectomized rats, a stimulating effect on the mammary gland was observed after 90 days of dosing with genistein at 5.4 and 54 (Rimoldi et al., 2007) and 221 mg.kg bwt⁻¹.day⁻¹ (Wuttke et al., 2006). EFSA concluded that these findings were ‘consistent with the results from the US National Toxicology Program study conducted in non-ovariectomized animals administered genistein at doses ranging 0.3–44 mg.kg bwt⁻¹.day⁻¹ (NTP, 2008), in which there was some evidence of carcinogenic activity of genistein in female rats based on an increased incidence of mammary gland adenoma or adenocarcinoma’. EFSA also indicated that the human data from observational studies did not point at an increased risk of breast cancer in menopausal women but specifically also noted that this conclusion cannot be extended to the risk of oestrogenic isoflavones-based food supplements for postmenopausal women who have a diagnosis or history of oestrogen-dependent cancer. Also, other endpoints considered did not reveal any effect except for some non-malignant histopathological changes in mammary tissue after 60 months supplementation at 150 mg.day⁻¹ of soy isoflavones which is high compared with the current estimated daily intakes.

Thus, concerns still exist that the oestrogenic activity of phytoestrogens may present a risk to patients with oestrogen-sensitive breast cancer and to women that are at a high risk of developing breast tumours (Messina, 2008; Hilakivi-Clarke et al., 2010), and it remains to be established whether exposure to isoflavones reduces or increases breast cancer risks.

**Effects of phytoestrogens on other forms of cancer including prostate cancer, bowel cancer, uterine cancer and other cancers**

Epidemiological studies and a nested case-control study carried out in Japan reported that isoflavone intake might be associated with a decreased risk of lung cancer (Shimazu et al., 2011). This observation also follows from a meta-analysis indicating that the consumption of soy food is associated with lower lung cancer risk (Yang et al., 2011). Another nested case-control study performed with the Korean Multicenter cancer cohort revealed that high serum concentrations of isoflavones were associated with a decreased risk for gastric cancer (Ko et al., 2010). Furthermore, several studies report a negative correlation between prostate cancer incidence and a phytoestrogen rich diet containing lignans and/or isoflavones (Severson et al., 1989; Adlercreutz, 1995; Kurahashi et al., 2007), and a meta-analysis revealed that the consumption of soy foods or genistein and daidzein is associated with a reduction in prostate cancer risk in men (Hwang et al., 2009; Yan and Spitznagel, 2009; He et al., 2015). Another meta-analysis reported by Van Die et al. (2014) concluded that there is a potential role for soy and soy isoflavones in reducing risks for prostate cancer, but that
a clear understanding could not be derived from the data because of limitations in sample size and study duration in the individual trials. Also equal, the gut microbiome metabolite of the soy isoflavone daidzein was suggested to have beneficial effects on the incidence of prostate cancer (Akaza et al., 2004; Ozasa et al., 2004). Epidemiological studies suggest that high dietary intake of phytoestrogens in pre- and post-menopausal women is correlated with reduced thyroid cancer risk (Horn-Ross et al., 2002; Haselkorn et al., 2003). For oestrogens in general, increased exposure has been linked to an increased risk of developing uterus cancer in women (Persson, 2000), and of prostate and testicular cancer in men (Bonkhoff et al., 1999; Maffini et al., 2006). Women who consume a diet rich in isoflavones or soy may have a lower risk of endometrial and ovarian cancer (Eden, 2012; Qu et al., 2014; Zhang et al., 2015).

The risk assessment for peri- and post-menopausal women taking isoflavone-containing food supplements performed by EFSA (EFSA ANS Panel, 2015) also evaluated the possible association between the intake of isoflavones from food supplements and harmful effects on the uterus. EFSA reported that there was no study on the association between isoflavone intake and risk of uterine cancer in the target population. As surrogate markers, data on endometrial thickness reported in 25 interventional controlled studies and on histopathology of the endometrium presented in nine interventional controlled studies were evaluated (EFSA ANS Panel, 2015). No significant adverse effects were observed in any of the human intervention studies, at dose levels up to 150 mg isoflavone·day⁻¹ for 2.5 years. Thirteen studies in animals investigated cell proliferation in the uterus, and 22 animal studies studied uterus histopathological changes. No adverse effects were seen in any of the studies. EFSA concluded that the human and animal studies support that in post-menopausal women adverse effects on the uterus were not noted for soy isoflavones, but that in the absence of data on the effect on uterine cancer, it was not possible to draw conclusions on effects of isoflavones on the uterus in peri-menopausal women (Horn-Ross et al., 2002; Haselkorn et al., 2003).

Effects of phytoestrogens on the thyroid and thyroid function
The soy isoflavones daidzein and genistein are reported to inhibit thyroid peroxidase (TPO) in vitro (EC number 1.11.1.8), an enzyme involved in the synthesis of T3 and T4 (Divi et al., 1997). Also in rats in vivo, daidzein and genistein inhibit TPO activity; however, no other adverse effects on thyroid functions could be observed (Chang and Doerge, 2000; Doerge and Sheehan, 2002). In addition, oestrogens are implied to have indirect effects on thyroid function, which has led to the concern that phytoestrogens may adversely affect thyroid function. However, human studies on the effect of soy isoflavones on thyroid function reviewed by EFSA (EFSA ANS Panel, 2015) are not conclusive. Some studies suggest that risk factors, such as iodine deficiency and subclinical hypothyroidism, might increase susceptibility of individuals to potential adverse effects of soy isoflavones on thyroid function (Doerge and Sheehan, 2002; Sathyapalan et al., 2011).

Effects of phytoestrogens on brain function
Most studies on the effect of phytoestrogens on neurological endpoints are conducted with soy isoflavones, while few studies also addressed the effects of dietary lignans. The rationale behind such studies is that oestrogens play an important role in brain health. The brain controls oestrogen release through the hypothalamus–pituitary–gonadal axis and also responds to oestrogens. In addition, oestradiol plays a key role in the neurobiology of ageing, because endocrine and neural senescence overlap in time and are mechanistically intertwined in complex feedback loops (Morrison et al., 2006). For oestrogen therapy, there are conflicting results on whether protective or detrimental effects on brain health are exerted. The reasons for the different observations appear to lie in the time at which oestrogen therapy is initiated, the neurological status of the brain at the time of oestrogen therapy initiation and the type of therapy used (Brinton, 2004). Interestingly, the loss of especially ERα seems to be associated with reduced neuroprotection by E2, and there appears to be a beneficial role for ERβ in the aging brain (Schreihof and Ma, 2013). A literature review on the neuroprotective effects of phytoestrogens found in soy reports that, while it has been demonstrated in animal research and cell culture studies that phytoestrogens from soy can exert neuroprotective effects, clinical trials and observational studies in humans have produced inconclusive findings (Soni et al., 2014). In this review, seven observational studies were reviewed; of which, three found positive effects of isoflavones on cognition (Hogervorst et al., 2008; Hogervorst et al., 2011; Greendale et al., 2012). Out of the 12 randomized control trials reviewed, six reported beneficial effects of isoflavone treatment on cognitive function (File et al., 2001; Duffy et al., 2003; Kritz-Silverstein et al., 2003; Casini et al., 2006; Gleason et al., 2009; Thorp et al., 2009). Despite positive findings of studies investigating phytoestrogens and cognition, approximately half of the reviewed studies demonstrate negative or null effects (Soni et al., 2014). Just like for oestrogen therapy, also for phytoestrogens, there are many factors that have an effect on the outcomes, like age, gender, ethnicity, and menopausal status, as well as duration of consumption, and the cognitive test used. One important characteristic of the study population is the metabolic capacity to produce equol, which differs between Asian and non-Asian study populations, and generally declines with age (Soni et al., 2014).

Consumption of soy or soy isoflavones has been shown to affect various endpoints related to neuronal health in rats (Zeng et al., 2004; Azcoitia et al., 2006; Huang and Zhang, 2010; Neese et al., 2010; Pan et al., 2010; Bagheri et al., 2012), and in mice (Zhao and Brinton, 2009; Bansal and Parle, 2010; Zhao et al., 2011; Yao et al., 2013), while consumption of large doses are also reported to have negative effects on the brain of rats (Choi and Lee, 2004). It is of interest to note that the protective effects of genistein on the cerebrovascular cortex of ageing rats were suggested to have a different mode of action than that of oestradiol (Moran et al., 2013).

Different lignans have shown positive effects on cognitive performance and markers of Alzheimer’s disease in mice (Um et al., 2009; Giridharan et al., 2011; Hu et al., 2012; Jeong et al., 2013; Li et al., 2014; Mao et al., 2015). In observational studies in humans, higher dietary intake of lignans is associated with better cognitive function (Franco et al., 2005; Kreijkamp-
Kaspers et al., 2007; Greendale et al., 2012; Nooyens et al., 2015), while coumestrol ingestion was unrelated to cognitive performance (Greendale et al., 2012).

Altogether, the data presented on possible beneficial effects of phytoestrogens on neurological health are inconclusive and seem to be affected by various factors. Furthermore, it is not clear, whether the observed beneficial effects are due to an oestrogen-like mode of action, or whether other mechanisms underlie these effects.

**Discussion**

Many health effects including both benefits and risks have been related to exposure to phytoestrogens. Reported benefits include a lowered risk of menopausal symptoms, cardiovascular disease, breast cancer, other forms of cancer including prostate cancer, bowel cancer, uterine cancer and other cancers, and brain function disorders. On the other hand, phytoestrogens are also considered endocrine disruptors, indicating that they have the potential to cause adverse health effects such as infertility and increased risks of cancer in oestrogen-sensitive organs. These adverse effects have been mainly suggested based on data from in vitro, animal or epidemiological studies. Clinical studies often report the absence of adverse effects. Tempfer et al. (2009) reported a meta-analysis on possible side effects of phytoestrogens. Based on 174 randomized controlled trials, it was concluded that phytoestrogen supplements have a safe side effect profile with moderately elevated rates of gastrointestinal side effects. It is important to note, however, that these studies were generally not designed to study the safety of the phytoestrogens.

Consequently, the question of whether phytoestrogens are beneficial or harmful to human health remains of importance. The present overview reveals that the answer is rather complex and may depend on age, health status, and even the presence or absence of specific gut microflora in the population of concern. To further illustrate this complexity, Figure 5 presents a cartoon summarizing the possible health effects of phytoestrogens and the potential underlying modes of action as presented in the present paper.

Given the rapid increase in global consumption of phytoestrogens and the fact that phytoestrogens are present in a wide range of dietary food supplements and widely marketed as a natural alternative to oestrogen replacement therapy, further insight into the risks and benefits of these phytoestrogens seems essential. Additional issues to be taken into account in the near future include (i) effects on children since phytoestrogens may be present in for example soy based infant foods; (ii) the role of the gut microbiota in phytoestrogen metabolism and differences in this microbial metabolism and the consequenses for the health effects; (iii) the role of possible other modes of action than the oestrogenic activity in the health effects of phytoestrogens; (iv) the physiological levels of the phytoestrogens and their metabolites and how these relate to concentrations required in in vitro cellular models to actually induce the different effects; (v) the role of polymorphisms in the differential biological effects of phytoestrogens and the possible contribution of -omics technologies to elucidate these effects; and (vi) the fact that most phytoestrogens are weak oestrogens with anti-oestrogenic effects when they compete with endogenous oestrogens for binding to the ERs, but pro-oestrogenic effects in the absence of endogenous oestrogens and uncertain effects in the presence of low concentrations of endogenous oestrogens as occurs in the menopause.

The current review presents an overview of the potential health benefits of dietary phytoestrogens. It is of interest to

**Figure 5**
Schematic presentation summarizing the possible health effects of phytoestrogens and the potential underlying modes of action as presented in the present paper.
put the observations within the guidance developed by EFSA for performing risk–benefit assessments of food. Given that in Europe food supplements are considered food this seems appropriate. EFSA recommended a stepwise approach for the risk–benefit assessment including first an assessment of whether the health risks clearly outweigh the health benefits or vice versa. The literature overview presented in this paper illustrates that at the current state-of-the-art the beneficial health effects are not so obvious that they clearly outweigh the possible health risks. This implies that a further refinement of the assessment would be required, aiming at providing semi-quantitative or quantitative estimates of risks and benefits at relevant exposures by using common metrics, and a comparison of risks and benefits using a composite metric such as disability-adjusted life years or quality-adjusted life years to express the outcome of the risk–benefit assessment as a single net health impact value. The currently available data set is not sufficient to support such a refined (semi) quantitative analysis. Taking all together, it can be concluded that several potential health benefits of phytoestrogens have been reported but that, given the data on potential adverse health effects, the current evidence on these beneficial health effects is not so obvious that they clearly outweigh the possible health risks. This implies that a definite conclusion on possible beneficial health effects of phytoestrogens cannot be made.

Author contributions
All authors contributed equally to writing the review.

Conflict of interest
The authors declare no conflicts of interest.

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Potential health effects of dietary phytoestrogens


Potential health effects of dietary phytoestrogens


**Supporting Information**

Additional Supporting Information may be found in the online version of this article at the publisher's web-site: http://dx.doi.org/10.1111/bph.13622

**Table S1** binding affinity of E2 and phytoestrogens to ERα and ERβ (expressed as IC50 values from competitive binding assays).

**Table S2** ERα- and ERβ- mediated gene expression induced by E2 and phytoestrogens (expressed as EC50 values from reporter gene assays).