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Review

Soy, phytoestrogens and their impact on reproductive health

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ABSTRACT

There is growing interest in the potential health threats posed by endocrine-disrupting chemicals (EDCs) to the reproductive system. Soybean is the most important dietary source of isoflavones, an important class of phytoestrogen. While consumption of soy food or phytoestrogen supplements has been frequently associated with beneficial health effects, the potentially adverse effects on development, fertility, and the reproductive and endocrine systems are likely underappreciated. Here we review the available epidemiological, clinical and animal data on the effects of soy and phytoestrogens on the development and function of the male and female reproductive system, and weigh the evidence as to their detrimental impact.

Contents

1. Soy and phytoestrogens as potential sources of endocrine-disrupting chemicals

The general human population is continuously exposed to various kinds of endocrine disrupting chemicals (EDCs). There is increasing concern about the long-term consequences of exposure to these chemicals due to the potential to perturb the hormonal system and thus cause adverse health effects (IPCS/WHO, 2002). EDCs are highly heterogeneous in structure and include synthetic organic compounds such as pesticides (e.g. organophosphates, methoxychlor, dichloro-diphenyl-trichloroethane or DDT), fungicides (vinclozolin) pharmaceutical agents (diethylstilbestrol, DES), dioxins, plasticizers (phthalates), plastics (bisphenol A or BPA), polychlorinated biphenyls (PCBs), flame-retardant polybrominated diphenyl ether (PBDE), and antifoulant paint additive (tributyltin), as well as natural plant-derived EDCs termed phytoestrogens.

Soy and soy products are the major source of isoflavones, a particular class of phytoestrogen that interacts with endogenous estrogen signaling pathways. Soybean food now forms part of our regular diet. Asian populations have long consumed soy, mostly in the form of unprocessed food such as tofu or tempeh, whereas Westerners more commonly eat it either as dietary supplement or as a source of edible oil and protein substitutes. Now-

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adays, proteins or oil of soy origin also appear in numerous food products – such as infant soy-based formula but more unexpectedly in bakery products (biscuits, doughnuts and cakes), fast foods (hamburger and hot dog buns, flour for frying nuggets, French fries and pizza doughs), cereal bars and salad dressings – to the point that it has become a struggle for people with soy allergies to find a product not containing soy.

The ability of phytoestrogens and isoflavones to bind and activate estrogen receptors (ERs) fits well within the definition of EDCs, which states that these substances are "exogenous agents that interfere with synthesis, secretion, transport, metabolism, binding action or elimination of natural hormones in the body that are responsible for homeostasis, reproduction and development and behavior". Exposure to these compounds may alter fertility by disrupting several aspects of reproduction such as sexual development, timing of puberty, sex-dependent behaviors, testicular and ovarian endocrine functions, gamete production, pregnancy and lactation. The initial recognition of the endocrine disrupting properties of phytoestrogens on reproduction was made in the 1940s, when it was found that ewes grazing in clover pasture developed infertility syndrome due to the exposure to high levels of formononetin, an isoflavone present in red clover (*Trifolium pratense* L.; Bennetts et al., 1946). This syndrome, referred as "clover disease" resulted in reduced ovulation rates, low lambing rates and structural defects in the reproductive tract (Adams, 1995). In the wild, one study found high levels of phytoestrogens in the leaves of stunted desert annuals in a dry year, leading ultimately to impaired reproduction when ingested by the California quail (*Lophortyx californicus*). In wet years, these quails bred normally and phytoestrogens were largely absent in these herbs (Leopold et al., 1976). Finally in captured cheetahs, cases of infertility and liver disease were attributed to the consumption of a soy-based diet and the exposure to high levels of isoflavones (~50 mg/day; Setchell et al., 1987).

The purpose of this review is to synthesize the available clinical, epidemiological and animal data concerning the potential association between exposure to soy-food and phytoestrogen complements and any subsequent effects on reproductive health. To provide a context and the requisite background, we begin with a brief overview about soy composition, the biosynthesis and metabolism of isoflavones, and a description of their potential mechanisms of action. We then present scientific evidence from both human and animal studies either supporting or refuting the potential detrimental effects of soy, soy-based formula and isoflavone exposure on the reproductive system. Finally, we put into perspective the discrepancies observed between human and animal data and raise the issue of the potential impact of simultaneous exposure of phytoestrogens and various EDCs that may cause additive or synergistic detrimental effects on both male and female reproductive systems.

2. Soybean composition and isoflavone biosynthesis

Understanding the precise composition of soybean is essential since soy may exert its health benefits or detrimental effects through the concerted action of several of its components (Barrett, 2006). Soybean (*Glycine max*) is composed of macronutriments such as proteins, carbohydrates and lipids. Protein content varies between 36% and 46% depending on the variety (Garcia et al., 1997; Grieshop and Fahey, 2001; Grieshop et al., 2003). The major portion of the protein component is formed by storage proteins such as 7S globulin (β-conglycinin) and 11S globulin (glycinin), which represent about 80% of the total protein content (Garcia et al., 1997). Other proteins or peptides present in lower amounts include enzymes such as lipoxygenase, chalcone synthase and catalase. Peptides such as lunasin (a 43 amino acid protease inhibitor) and the Bowman-Birk inhibitor (a 71 amino acid protease inhibitor) have been reported to have an anticancer capacity or an *in vitro* chemopreventative effect (Armstrong et al., 2003; Galvez et al., 2001; Lam et al., 2003). Some of the health benefits attributed to soy may result from the release of biologically active peptides by enzymatic proteolysis during gastrointestinal digestion or during the fermentation of soy proteins. For example, bioactive peptides encrypted in the amino acid sequence of soy proteins, in particular β-conglycinin, have been found to exert anticancer, antihypertensive, hypocholestorolemic, antiobesity and antioxidant activities (Martinez-Villaluenga et al., 2008, 2009; Martinez-Villaluenga et al., 2010; Wang et al., 2008). Soybean also contains a wide range of micronutrients and phytochemicals including phytic acid (1.0–2.2%), sterols (0.23–0.46%), and saponins (0.17–6.16%), the effects of which have recently been reviewed in detail by Kang et al. (2010). One important group of compounds present in soybean that has received considerable attention is a class of phytoestrogen called the isoflavones. Phytoestrogens are non-steroidal compounds that bind to and activate estrogen receptors (ERs) α and β, due to the fact that they mimic the conformational structure of estradiol (Kuiper et al., 1997, 1998). Phytoestrogens are naturally occurring plant compounds found in numerous fruits and vegetables, and are categorized into three classes: the isoflavones, lignans and coumestans. Genistin and daidzin are the predominant isoflavones found in soybeans and make up the most important dietary source of phytoestrogens for humans, cattle and rodents.

In plants, the synthesis of isoflavones coincides with environmental stresses such as pest infection, drought or lack of nutriments (Howitz and Sinclair, 2008). Isoflavones play important roles as signal molecules for inhibiting pathogen attacks (Lozovaya et al., 2007; Zabala et al., 2006) and for plant–microbe symbiotic interactions (Subramanian et al., 2006; Sugiyama et al., 2007). Genistin and daidzin is initiated by the conversion of the essential amino acid L-phenylalanine into p-Coumaroyl-CoA. This then enters one of two branches of the phenylpropanoid (PP) pathway, which require several enzymes as described in Fig. 1 (Du et al., 2010). Ultimately both genistin and daidzin are then glycosylated, malonylated or acetylated to their corresponding glycoconjugates, which are stored in vacuoles.

3. Soy consumption, metabolism and absorption of isoflavones

Soy and soy-based food represent the most important dietary source of phytoestrogens in humans. As one might expect, people consuming more soy-derived products are exposed to higher levels of isoflavones. However, the quantity of ingested isoflavone also depends on agronomic factors such as soy varieties, culture conditions and soybean processing methods. Since isoflavones are tightly associated with proteins, alcohol extraction and soy processing tend to significantly decrease phytoestrogen content (Bhathena and Velasquez, 2002). For example, the total isoflavone content is high in soy flour (150–170 mg/100 g), soy protein isolate (SPI, 91 mg/100 g), natto (fermented soybean, 82 mg/100 g) and edadame (raw green soybean, 49 mg/100 g) but significantly lower in alcohol extracted soy protein concentrate (SPC, 11 mg/100 g), tofu (25–30 mg/100 g) or soymilk (1–3 mg/100 g; see Table 1; (US Department of Agriculture, 2008). Soy is abundant in traditional Asian diets: the daily intake is from 7–8 g/day in Hong-Kong and China, up to 20–30 g/day in Korea and Japan. Most Europeans and North Americans, however, consume less than 1 g/day (Ho et al., 2000; Nagata, 2000). The corresponding plasma isoflavone levels in Japanese men are around 493 and 283 nM for genistein and daidzein respectively, whereas in British men, or in individuals consuming soy-free diets, these fall around 33 and 18 nM (Morton et al., 1994, 2002; van Erp-Baart et al., 2003). Acute ingestion of dietary soy may lead to increases up to the micromolar range in plasma isoflavone levels (Adlercreutz et al., 1993; King and Bursill, 1998; Watanabe et al.,

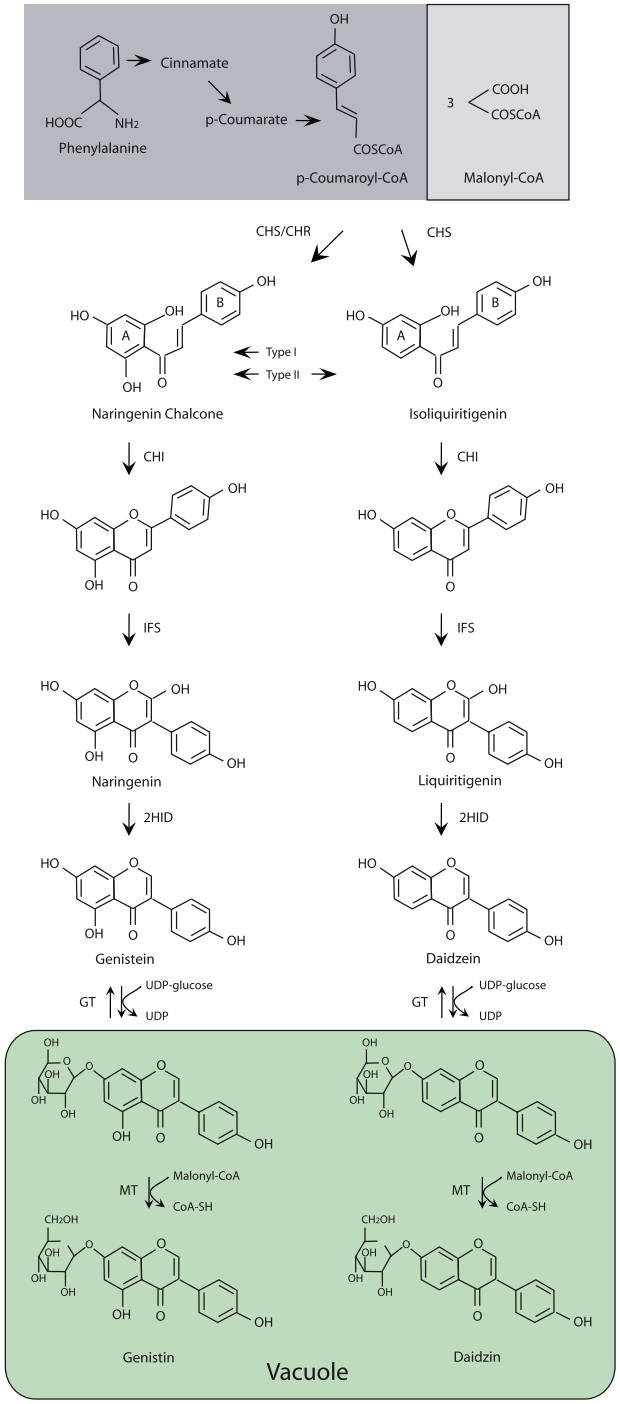

Fig. 1. Schematic representation of the isoflavone biosynthesis pathway in soybean. The first steps (in the dark gray box) consist of eliminating ammonia from phenylalanine to generate cinnamic acid (cinnamate). The phenylpropanoid pathway is further divided into diverse branches, of which only the synthesis of genistin and daidzin are shown here. CHS (chalcone synthase) activity supplies the downstream enzymes with chalcone, the critical metabolite of the phenylpropanoid pathway. In soybean, two types of CHS are present. Type I CHS catalyses naringenin chalcone, whereas type II produces both isoliquiritigenin and naringenin chalcone. CHR (chalcone reductase) contributes to naringenin chalcone synthesis. CHI (chalcone isomerase) catalyses the intramolecular cyclization of bicyclic chalcones to their corresponding tricyclic (2S)-isoflavones. IFS (isoflavone synthase) hydroxylates the isoflavanone at the C2 position, which subsequently leads to the migration of the aryl moiety from C2 to C3. The final step in the formation of the isoflavanoid skeleton is mediated by HID (2-hydroxyisoflavone dehydratase). Daidzein is then a major precursor for various phytoalexins such as medicarpin, biochanin A, glyceollin and equol. Isoflavone conjugates are subsequently synthesized within the vacuole from isoflavone aglycones by the combined action of MT (methyltransferases) and GT (glycosyltransferases).

1998; Xu et al., 1994). Interestingly, in infants fed with soy formula, levels can rise up to 1,640 and 1,160 nM for genistein and daidzein, respectively (Setchell et al., 1997).

The metabolism and absorption of isoflavones is a complex process. As described above, genistin and daidzin, the two major isoflavones present in soy, are biologically inactive β-D-glycosides. Once ingested, isoflavone glycosides are hydrolyzed to their corresponding bioactive aglycone forms (genistein and daidzein) by bacterial β-glucosidases in the intestinal wall. Once hydrolyzed, aglycone forms can then be absorbed in the upper small intestine by passive diffusion (King et al., 1996). In contrast, isoflavones glycosides are poorly absorbed in the small intestine, due to their higher molecular weight and hydrophilicity (Izumi et al., 2000; Piskula et al., 1999). Nonetheless, pharmacokinetic studies confirm that healthy adults absorb isoflavones rapidly and efficiently (Setchell et al., 2001). The average time taken after ingesting the aglycones to reach peak plasma concentrations is 4–7 h, which is delayed to 8–11 h for the corresponding β-glycosides. The half-lives of daidzein and genistein were reported to be 9.3 and 7.1 h respectively, indicating that isoflavones or their metabolites are rapidly excreted. However, despite differences in peak levels and pharmacokinetics, whether circulating isoflavone levels differ between aglycones and isoflavone glycosides after their ingestion is still controversial (Cassidy, 2006; Kwon et al., 2007; Nielsen and Williamson, 2007). A recent report even shows a stronger effect of genistin than genistein when orally administered (Jefferson et al., 2009a), suggesting that isoflavone glycosides might be biologically more active than aglycones, despite delayed absorption. Daidzein can be further converted by the gut microflora into equol or O-desmethylangolensin, a process that is blocked upon antibiotic treatment *in vitro* (Atkinson et al., 2004). When analyzing the plasma and urine of humans or animals after soy ingestion, genistein, daidzein, equol and O-desmethylangolensin are the major isoflavones usually detected (Setchell et al., 1998). The detection of these metabolites in plasma and urine is dependent on the time following ingestion, presumably reflecting the time required for conversion, intestinal absorption and secretion (Rowland et al., 2003). While all rodents are equol producers, only 30–50% of humans are able to metabolize daidzein into equol (Atkinson et al., 2005). Factors affecting equol production include the host's genetic background, gut microflora and diet. Interestingly, 50–60% of Asians are capable of producing equol after soy ingestion, compared to 30% of the adult Western population (Bolca et al., 2007). It remains unclear whether the ability of individuals to metabolize daidzein into equol is required to observe either the health benefits (Lampe, 2009) or detrimental effects associated with consuming soy-based food. However, it may represent a significant source of variability and may contribute to the inter-individual differences observed in numerous clinical trials dealing with soy consumption and phytoestrogen exposure.

4. Mechanisms of action

Phytoestrogens may exert their effects through a variety of mechanisms. The most characterized mode of action involves the capacity of isoflavones to bind both estrogen receptors (ER) α and β, and to mimic estrogenic actions (Kuiper et al., 1997, 1998). Isoflavones bind to and activate ER-dependent gene transcription through both ER isoforms, generally with a higher binding affinity for ERβ than ERα. For example, the relative estrogenic potency of genistein for ERβ is 30-fold higher than for ERα. Due to their relative estrogenic potencies, genistein, daidzein and other non-soy phytoestrogens have been described as Selective Estrogen Receptor Modulators (SERMs). At this point, it is relevant to note that the relative binding affinities of these isoflavones for both ERs are far greater than for many of the classical synthetic EDCs of concern, such as bisphenol A, nonylphenol, DDT and methoxychlor (Kuiper

Table 1
Isoflavones content in selected soy-based products.

Food description	Daidzein mg/100 g	Genistein mg/100 g	Glycitein mg/100 g	Total mg/100 g
Western soy-products				
Soy meal, defatted	80.8	114.7	16.1	209.6
Soy flour, full fat	72.9	98.8	16.1	178.1
Soy protein isolate	30.8	57.3	8.5	91.1
Soy protein conc., aqueous extract	38.3	52.8	4.9	94.7
Soy protein conc., alcohol extract	5.8	5.3	1.6	11.5
Soy yogurt	13.8	16.6	2.8	33.2
Infant soy-based formula	7.2	14.8	3.0	25.0
Soy milk	2.8	5.1	nd	7.9
Traditional Asian food				
Soybeans, raw	20.4	22.6	7.6	49.0
Soybeans, sprouted cooked, steamed	5.0	6.7	0.8	12.5
Natto	33.2	37.7	10.6	82.3
Tempeh	22.7	36.2	3.8	60.6
Miso	16.4	23.2	3.0	41.5
Tofu, cooked	12.8	16.2	2.4	31.4
Miso soup	0.8	0.7	0.0	1.5
Soy sauce	0.8	0.4	0.1	1.2

Example of isoflavone content in Western and Asiatic food products. Values were obtained from the USDA Database for the Isoflavone Content of Selected Foods, Release 2.0, September 2008. The table represents the mean values of isoflavone content obtained from multiple experiments. Values are reported as mg/100 g of food weight. Source: USDA Database for the Isoflavones Content of Selected Foods, Release 2.0, 2008.

et al., 1998). The fact that ERα and β display overlapping but distinct expression patterns in the male and female reproductive systems and throughout development (Matthews and Gustafsson, 2003; Meltser et al., 2008; Merchenthaler et al., 2004) may explain why phytoestrogens do not act as classical estrogen agonists. The estrogenic activity of isoflavones may in fact depend of the presence and/or recruitment of co-activator or co-repressor proteins present in particular cell types or tissues at specific times of development. Phytoestrogens act either by initiating transcription via a classical mode of action involving the interaction of nuclear ERs with estrogen response elements (EREs), or by non-genomic effects mediated by membrane-associated or cytoplasmic ERs. The non-genomic effects mediated by ERs usually involve rapid cellular responses leading to nitric oxide release, calcium flux, and/or activation of different signaling pathways, such as the AMP-activated protein kinase (AMPK), mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) pathways, as observed in various cell lines (D'Eon et al., 2005; Ropero et al., 2006). In addition to their estrogenic actions, isoflavones have been reported to possess antioxidant (Rufer and Kulling, 2006) and tyrosine kinase inhibitor (Akiyama et al., 1987) properties. They may also act as peroxisome proliferator-activated receptor gamma (PPAR)γ ligands (Rosen et al., 1999).

Phytoestrogens have also been reported to affect steroid biosynthesis by regulating aromatase and 5α reductase activity *in vitro* (Almstrup et al., 2002; Evans et al., 1995). In addition, isoflavones were shown to regulate plasma sex hormone binding globulin (SHBG) levels (Adlercreutz et al., 1992) and displace *in vitro* testosterone and 17β estradiol from hSHBG binding sites. This potentially affects the availability of free bioactive steroids and the androgen-to-estrogen balance (Dechaud et al., 1999). These findings suggest that this potential capacity of phytoestrogens to interfere with steroid biosynthesis and transport should be taken into consideration when analyzing the impact of such compounds on the development and function of the reproductive and endocrine system.

5. Soy, phytoestrogens and the reproductive system

There are inherent challenges and confounding factors in understanding the risks or benefits that soy and phytoestrogens may pose to human health. First, clinical studies investigating the impact of soy on reproductive development and function are scarce, and those available are hampered by limited sample size, disparities in experimental design and absence of adequate reproductive measurements that may only manifest years after the initial exposure. In addition, comparisons between different animal and clinical studies are hampered by the lack of standardization in experimental design: soy nomenclature, formulations (soy proteins, pure isoflavones, etc), doses, routes (dietary, injection and gavage), durations and times of exposure all vary considerably between studies, making them difficult to compare. Crucial factors such as the differences in isoflavone metabolism between animal models and humans may also represent confounding factors. Finally, comparisons are further hampered by major disparities in the subsequent analyzes performed to evaluate the effects of phytoestrogens and soy and elucidate the mechanisms by which they potentially affect reproductive and endocrine functions. All of these parameters should be kept in mind when reviewing scientific reports assessing the impact of soy on the reproductive system.

5.1. Soy-based infant formula: A source for concern?

Over the past 40 years, millions of infants have been fed with soy-based formula without any apparent detrimental effects (Badger et al., 2009). Soy protein-based formulas are routinely used to provide an adequate source of nutrition for a term infant. These formulas contain a soy protein isolate, supplemented with additional amino acids, minerals, vitamins and fat necessary to support growth and development. In the US, it is estimated that 20–25% of infants are fed on soy-based formula sometime during the first year of life (Bhatia and Greer, 2008; NTP, 2010). Guidelines issued by societies such as the American Academy of Pediatrics (AAP) (Bhatia and Greer, 2008) or the European Society for Pediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) Committee on Nutrition (Agostoni et al., 2006) do not recommend the exclusive use of soy formula over non-soy and/or breast milk. In fact, the use of these soy-based formulas is only recommended for infants that develop cow milk allergies or lactose intolerance, although either a parental conviction that soy consumption provides health benefits, or a vegetarian lifestyle might well represent the major reasons. As might be expected, infancy is a very sensitive period

for endocrine disruption, and exposure to significant levels of phytoestrogens during this critical period of development can have potentially detrimental long-term effects on reproduction fertility and behavior. The mean daily consumption of total isoflavones in babies fed on soy formula ranges from 6 to 9 mg/kg, resulting in a much higher plasma isoflavone concentration (980 μg/L) than their breast-fed or cow-based formula counterparts (9.4 and 4.7 μg/L, respectively; Badger et al., 2002; Setchell et al., 1997, 1998). By comparison, the circulating concentration of phytoestrogens in infants fed with soy formula is 13,000–22,000 times higher than the endogenous estrogen levels, or 50–100 times higher than the estradiol levels, present in pregnant women (Badger et al., 2002; Setchell et al., 1998).

Unfortunately, very few epidemiological or clinical studies have investigated the potential long-term effects of soy-based formula and/or isoflavone exposure on reproduction, fertility and behavior. Available studies are hampered not only by limited sample size, variation in soy formula composition and subsequent analyzes but also by the fact that many of the reproductive effects may only manifest themselves years or even decades after the initial exposure, and may be subtle enough to escape detection even then. A recent cross-sectional study reported a higher prevalence of breast buds during the second year of life in female infants fed soy-based formula, when compared to their breast or cow-based formula fed counterparts (Zung et al., 2008). Similarly, a prospective study showed that human infants at times exhibited an estrogenized vaginal epithelium when fed soy-based formula as opposed to cow-based formula or breast-milk (Bernbaum et al., 2008). These data suggest that soy formula may prolong the physiologic estrogenization of the newborn. Among the rare reports evaluating the long term reproductive health impact of soy formula, a retrospective cohort study found that women who participated in a soy formula study as infants reported no significant differences in the onset of puberty or in reproductive functions, but did report slightly longer menstrual bleedings and more discomfort than those who were fed with milk formulas (Strom et al., 2001). However, the true relevance of this study has been the subject of controversy, due to the lack of direct measurements of hormone levels and reproductive functions (Goldman et al., 2001; Tan et al., 2006). Recently, a large study involving 19,972 women found that an increased risk of developing benign smooth-muscle tumors of the uterus (uterine leiomyomata or fibroids) is associated with being fed soy formula during infancy (D'Aloisio et al., 2010). Concerning male reproductive function, a study led by Richard Sharpe compared the long term male reproductive functions of marmosets fed either with a standard cow's milk-based formula or a soy-based formula during the first 6 weeks of life (Sharpe et al., 2002; Tan et al., 2006). Although soy-fed marmosets had a lower neonatal testosterone rise, and an increased Sertoli and Leydig cell number and adult testicular weight, no adverse reproductive consequences were observed in adulthood, including the timing of puberty and overall fertility.

As we have seen, the scarcity of human data available and the apparent lack of significant long-term detrimental effects may explain why consumers, clinicians and health organizations usually consider soy formula as a safe alternative to breastfeeding (Bhatia and Greer, 2008; COT, 2003; NTP, 2006, 2010). It should be noted however that a recent safety assessment of soy infant formula by the review panel from the Center for the Evaluation of Risks to Human Reproduction (CERHR, established by the National Toxicology Program, NTP and the National Institute of Environmental Health Sciences, NIEHS) failed to issue a conclusive recommendation on the reproductive and developmental toxicity because of the limited utility of available humans studies (NTP, 2010). Since absence of evidence is not an evidence of absence, it simply emphasizes the need for additional human studies to clarify whether or not concerns over soy-based formula are justified.

5.2. Soy, phytoestrogens and female fertility

If we set aside the particular case of soy-based formula, numerous epidemiological and clinical studies have evaluated the relationship between soy and phytoestrogen consumption and its impact on female reproductive health. There have been reports in humans which reveal a modest suppressant effect of soy and isoflavone exposure on the hypothalamic–pituitary–gonadal axis. Although available data are highly heterogeneous, a recent meta-analysis of 47 studies concluded that consumption of soy and isoflavones in premenopausal women reduces circulating LH and FSH, and increases menstrual cycle length (Hooper et al., 2009). In contrast no statistical effects were observed on hormonal status in postmenopausal women. Because soy food is increasingly part of the female diet, the clinical relevance of these modest hormonal changes must be examined in further robust studies.

As compared with human studies, a large body of literature clearly shows that developmental exposure to purified genistein can induce adverse reproductive effects in female rodents. These include alterations in ovarian development (increased percentage of multi-oocyte follicles (MOFs)), the timing of vaginal opening, estrous cyclicity, ovarian function, HPG axis, subfertility and an increased incidence of uterine adenocarcinoma (Chen et al., 2007; Delclos et al., 2001, 2009; Jefferson et al., 2006, 2002, 2005; Kouki et al., 2003; Lewis et al., 2003; Nagao et al., 2001; Newbold et al., 2001; Nikaido et al., 2004; NTP, 2008). For example, in mice neonatal injection of genistein at doses of 0.5, 5 and 50 mg/kg/day on postnatal days 1–5 leads to prolonged estrous cycles with a dose-dependent and age-related increase in severity (Jefferson et al., 2002). These doses span the range of human exposure levels: a treatment of 50 mg/kg/day resulted in serum genistein levels of 6.8 μM, comparable to the circulating levels of 1.4–4.5 μM found in the serum of infants consuming soy-based formulas (Setchell et al., 1997). Interestingly, similar alterations of the estrous cycle have been observed in other rodents studies involving exposure to EDCs such as genistein, zearalenone, and bisphenol A (Kouki et al., 2003; Nikaido et al., 2004). In addition to the adverse effects on the reproductive tract and ovaries, pregnancy in mice exposed to genistein as neonates was also affected in a dose and time-dependent manner (Jefferson et al., 2005). While females exposed to low doses of genistein (0.5 and 5 mg/kg) showed signs of reduced fertility with age, mice treated with the highest dose of genistein (50 mg/kg) became pregnant at 2 months of age but were unable to support pregnancy and did not deliver any pups. Neonatal genistein treatment also results in a dose-dependent increase in the number of multi-oocyte follicles (MOFs) present in immature ovaries (Jefferson et al., 2006, 2002). These effects appear to be mediated by ERβ as MOFs are not observed in genistein-treated mice lacking ERβ (Jefferson et al., 2002). Interestingly, several other xenoestrogens including 17β-estradiol, DES and Bisphenol A have also been reported to affect ovarian development and cause MOFs in rodents (Iguchi et al., 1990; Suzuki et al., 2002). However, a recent study found that most of the oocytes ovulated from females treated orally with high doses (50 mg/kg/day) of genistein had normal morphology and were developmentally competent (Jefferson et al., 2009b). Infertility is in fact a consequence of the incapacity of the uterus to support preimplantation embryo development and implantation, rather than poor oocyte quality (Jefferson et al., 2009b).

Most of these studies were performed with subcutaneous injection of the aglycone form genistein, which different than consuming soy-based diets containing biologically inactive conjugated isoflavones such as genistin and daidzin. The method of administration raises questions about the potential relevance of such experiments, as it represents an alternative route that bypasses gut metabolism. Furthermore, the limited metabolic capacity of the neonate mice

could potentially affect the peak levels and pharmacokinetics of isoflavone glycosides (Rozman et al., 2006). Several studies have examined the effects of oral exposure to glycosylated or aglycone forms of isoflavones or dietary soy on female reproductive function and fertility. Chronic dietary coumestrol treatment (0.01% of the diet) was associated with precocious vaginal opening in rats (Whitten and Naftolin, 1992). Similarly, female rats receiving soy extract supplement at weaning also showed accelerated vaginal opening and an increased length of the oestrus cycle (Gallo et al., 1999). Recently a study demonstrated that oral exposure to the glycosylated form genistin during neonatal life leads to detrimental effects on the female reproductive system similar to those caused by either the subcutaneous or oral administration of the aglycone form genistein (Jefferson et al., 2009a). Mice exposed to oral genistin on neonatal days 1–5, at doses of 25 or 37.5 mg/kg/day, had increased estrogenic activity as reflected by the increased uterine wet weight at day 5 and the induction of the estrogen-responsive gene *LF*. These estrogenic effects were similar to those found in mice treated with subcutaneous injections of genistein (25 or 37.5 mg/kg/day). Additional detrimental effects included altered ovarian development (increased percentage of MOFs), delayed vaginal opening, altered length of the estrous cycle, decreased fertility and delayed parturition.

These findings indicate that the dose of the biologically active compound, rather than the route of exposure, is the important parameter. However infants consuming soy formulas ingest large amounts of soy protein isolates rather than pure compounds such as genistein or genistin. It is important to investigate whether neonatal exposure to isoflavones from dietary soy (instead of purified compounds) causes similar adverse consequences in the female reproductive system of rodents.

5.3. Soy, phytoestrogens and male reproductive function

As is the case for women, there is a surprising paucity of studies evaluating the effects of soy and phytoestrogens on fertility and reproductive parameters in men. To our knowledge, only three studies have investigated the impact of soy food or isoflavone intake on semen quality with contradictory results. In a cross-sectional study involving 99 men, Chavarro et al. (2008) found that dietary intake of soy food and isoflavones was inversely related to sperm concentration. There were no appreciable changes in semen quality parameters such as sperm motility, sperm morphology or ejaculate volume. However, the lack of a precise evaluation of soy-consumption and serum isoflavone levels, and the fact that the participants enrolled in this study were male partners in subfertile couples, raises caution and renders the interpretation of the findings difficult. In a similar study performed with 48 men with abnormal semen parameters and 10 control patients, Song et al. (2006) found instead that isoflavone intake was positively correlated to sperm count and motility. A report from Mitchell et al. limited to only 14 individuals, showed no influence on semen quality or sexual hormones in young males orally consuming 40 mg/day of soy-isoflavones (i.e. genistein, daidzein, glycitein), for 2 months (Mitchell et al., 2001). To add more confusion, an *in vitro* study showed that low concentrations of genistein (1, 10 and 100 nM) caused an accelerated capacitation and acrosome loss in human spermatozoa (Fraser et al., 2006).

Studies focusing on hormonal levels also found no clear effects (or borderline significance) when consuming tofu (70 mg/day isoflavones), soymilk (48 mg/day isoflavones), or soy products (22 mg/day isoflavones; Habito et al., 2000; Nagata et al., 2000, 2001). A recent meta-analysis, which included studies that evaluated testosterone levels as a bio-indicator of risks for prostate cancer, suggested out of 32 reports that soy foods or isoflavone intake do not alter free testosterone levels (Hamilton-Reeves et al., 2010). Overall, there is an apparent lack of notable effects, suggesting that regular adult soy-product or isoflavone intake in men causes little detrimental effect on reproduction and fertility. However, this near absence of documented impact emphasizes once again the need for long term, large scale comprehensive human studies.

In contrast, the role of soy consumption and isoflavone exposure has been extensively evaluated in animal models. In rodents, exposure to isoflavone during gestation and lactation (dietary exposure) or perinatally (oral gavage) yielded, once again, inconsistent results. While some reports found no reproductive defects (Fielden et al., 2003; Kang et al., 2002; Nagao et al., 2001), others reported variable persistent phenotypic and behavioral reproductive abnormalities such as decreased testicular weight or size (Atanassova et al., 2000; West et al., 2005; Wisniewski et al., 2003), decreased spermatogenesis (Atanassova et al., 2000; West et al., 2005), lower FSH (Atanassova et al., 2000) and testosterone levels (Wisniewski et al., 2003), smaller ano-genital distance (Wisniewski et al., 2003, 2005) and alterations of reproductive and aggressive behavior (Wisniewski et al., 2003, 2005).

Similarly, animal data regarding adult exposure to soy and phytoestrogens also yielded conflicting results, with only a few studies revealing detectable detrimental effects, although these did not result in any major long term consequence in terms of male fertility. In a recent review of the literature (Cederroth et al., 2010a), we found that out of 32 studies in mice, rats, rabbits, mink, and monkeys concerning the effects of soy or isoflavone exposure (including, gestational, post-natal, and adult chronic exposure), 11 out of 18 of them reported changes (either positive or negative) in the level of reproductive hormones such as testosterone, LH or FSH, but without significant effects on sperm production or litter size. Among the 12 studies that investigated the consequences on sperm production and fertility, only two reported negative effects (Cederroth et al., 2010b; Eustache et al., 2009). It is interesting to note that these two studies were the only ones to assess the effects of life-long exposure (from conception to adulthood). In addition, both studies reported a decrease in litter size (21–40%), whereas the other three studies evaluating litter size effects at precise windows of exposure found no effects (Atanassova et al., 1999; Nagao et al., 2001; Tan et al., 2006). These findings suggest that negative effects on sperm production and male fertility in rodents are only observed during a life-long exposure to soy and phytoestrogens.

6. Importance of assessing the combined effects of phytoestrogens and other EDCs on the reproductive system

Although the detrimental impacts of EDCs are typically evaluated on a compound-by-compound basis, human exposure is never limited to one chemical, but includes a mixture of various EDCs and other environmental pollutants of diverse origins, which may have additive or synergistic effects (Gray et al., 2006; Kortenkamp, 2008). The available experimental evidence shows that this is indeed the case. For example, Christiansen et al. demonstrated that a combined administration of three anti-androgenic chemicals (vinclozolin, flutamide and procymidone) at doses where each of the individual chemicals caused no observable effects, resulted in major impairment of masculinization, such as increased frequency of hypospadias in the male offspring (Christiansen et al., 2008). Phytoestrogens appear to act in a similar manner. *In vitro* experiments have shown that low concentrations of genistein, nonylphenol and 8-pre-nylnaringenin were more effective when used in combinations, rather than alone, in causing adverse effects on the key processes of capacitation and acrosome reaction both in human and mouse spermatozoa (Fraser et al., 2006). Recently, it has been reported in rat studies that alterations in male reproductive functions were more pronounced when co-exposed to a low

dose of genistein (1 mg/kg/day) and the fungicide vinclozolin (1 mg/kg/day) from conception to adulthood (Eustache et al., 2009). The significance of these findings for humans must be highlighted, because it suggests that simultaneous exposure to phytoestrogens and various EDCs at sensitive periods of development or adulthood are likely to cause more pronounced detrimental effects on the reproductive system. Such results also emphasize the need for further studies to assess the additive or synergistic effects of isoflavones and other EDC cocktails on both male and female reproductive systems.

7. Importance of identifying the bioactive constituents of soy

Another important issue when trying to understand the mechanisms of action and consequences of soy consumption concerns the controversy surrounding the potential bioactive constituents of soy. It is usually assumed that if soy-based food is bioactive, this is a consequence of isoflavone exposure. In most studies using whole soybean or soy proteins, beneficial effects could not be specifically attributed with certainty to soy isoflavones. Although most of the attention has been focused on isoflavones, soybeans contain numerous potentially bioactive constituents including saponins, protease inhibitors, phytic acids, and cryptic peptids (see Section 2, dealing with soybean composition). One should not consider soybean, soy protein isolate or isoflavone as equivalent. It is possible that whole soybean or soy protein as an intact product might have a higher bioactive potential, in particular for metabolic parameters (see review by Cederroth and Nef, 2009), than isolated compounds taken individually. For instance, the consumption of isoflavone supplements alone does not provide all of the beneficial effects observed with soy proteins, as exemplified with metabolic and cardiovascular parameters (Demonty et al., 2003).

8. General conclusions and research need

We have attempted to summarize the current state of the scientific literature regarding the potential detrimental effects of soy consumption on the reproductive health of both humans and animals. Overall, there is a paucity of large-scale comprehensive clinical studies examining the potentially adverse effects of soy consumption in human reproductive health. Available studies have found either no impact or revealed only minor detrimental effects. This is particularly the case for the few studies which have assessed the effects of soy-based formula on sexual development and adult reproductive health. Compared with humans, the evidence that animals are affected by soy consumption or isoflavone exposure is extensive. It is generally well accepted that female fertility is disrupted by *in utero* or neonatal exposure to isoflavones. Multiple animal studies have documented alterations in the timing of vaginal opening, estrous cyclicity, ovarian function, HPG axis, an increased number of multi-oocyte follicles (MOFs) and subfertility. Concerning male reproductive health, studies have found that consumption of soy or phytoestrogens resulted in alteration of androgens, LH and FSH levels. Negative effects on litter size and sperm production are only observed during life-long exposure, suggesting that shorter, specific periods of exposure may not cause detrimental effects on male reproduction in rodents.

The lack of consistent results across all species is puzzling, but may well result from a combination of factors including differences in the type of soy foods/isoflavone supplements ingested, route of administration, window of exposure and isoflavone metabolism. Nevertheless, it highlights the importance of conducting large-scale comprehensive prospective studies in humans to examine pubertal development and reproductive function in infants fed soy-based formula. Animal studies are also required to assess the additive or synergistic effects of isoflavones and other EDC cocktails on both male and female reproductive systems.

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