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Endocrine disruption by dietary phyto-oestrogens: impact on dimorphic sexual systems and behaviours

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A wide range of health benefits have been ascribed to soya intake including a lowered risk of osteoporosis, heart disease, breast cancer, and menopausal symptoms. Because it is a hormonally active diet, however, soya can also be endocrine disrupting, suggesting that intake has the potential to cause adverse health effects in certain circumstances, particularly when exposure occurs during development. Consequently, the question of whether or not soya phyto-oestrogens are beneficial or harmful to human health is neither straightforward nor universally applicable to all groups. Possible benefits and risks depend on age, health status, and even the presence or absence of specific gut microflora. As global consumption increases, greater awareness and consideration of the endocrine-disrupting properties of soya by nutrition specialists and other health practitioners is needed. Consumption by infants and small children is of particular concern because their hormone-sensitive organs, including the brain and reproductive system, are still undergoing sexual differentiation and maturation. Thus, their susceptibility to the endocrine-disrupting activities of soya phyto-oestrogens may be especially high. As oestrogen receptor partial agonists with molecular and cellular properties similar to anthropogenic endocrine disruptors such as bisphenol A, the soya phyto-oestrogens provide an interesting model for how attitudes about what is 'synthetic' *v.* what is 'natural,' shapes understanding and perception of what it means for a compound to be endocrine disrupting and/or potentially harmful. This review describes the endocrine-disrupting properties of soya phyto-oestrogens with a focus on neuroendocrine development and behaviour.

Soya: Isoflavones: Genistein: Equol: Endocrine disruption: Oestrogen: ERα: ERβ: Brain: Hypothalamus

A plant-based diet has many undeniable ecological and health benefits. As a food or food additive, soya is appealing because it is a complete protein that is cholesterol-free, lactose-free, high in fibre and rich in complex carbohydrates, antioxidants and unsaturated fats. Soya is also replete with phyto-oestrogens, which makes it a hormonally active food. For many, the consequences of this activity will be minimal, or even potentially beneficial, but for others the endocrine-disrupting properties of soya should not be discounted and health practitioners should be more broadly aware of this phenomenon and potential outcomes. The pros and cons of a phyto-oestrogen-rich diet on many aspects of human health, including breast and prostate cancer, reproductive maturation and function, cardiovascular health, bone health and menopausal symptoms have been reviewed previously by myself and others[1–6]. The present review specifically focuses on the endocrine-disrupting properties of soya isoflavones, particularly within the neuroendocrine system, and highlights our most recent findings along those lines.

Abbreviations: AVP, vasopressin; AVPV, anterior ventral periventricular nucleus; EDC, endocrine-disrupting compounds; ER, oestrogen receptor; HPG, hypothalamic–pituitary–gonadal; OT, oxytocin; PVN, paraventricular nucleus; SDN-POA, sexually dimorphic nucleus of the preoptic area.

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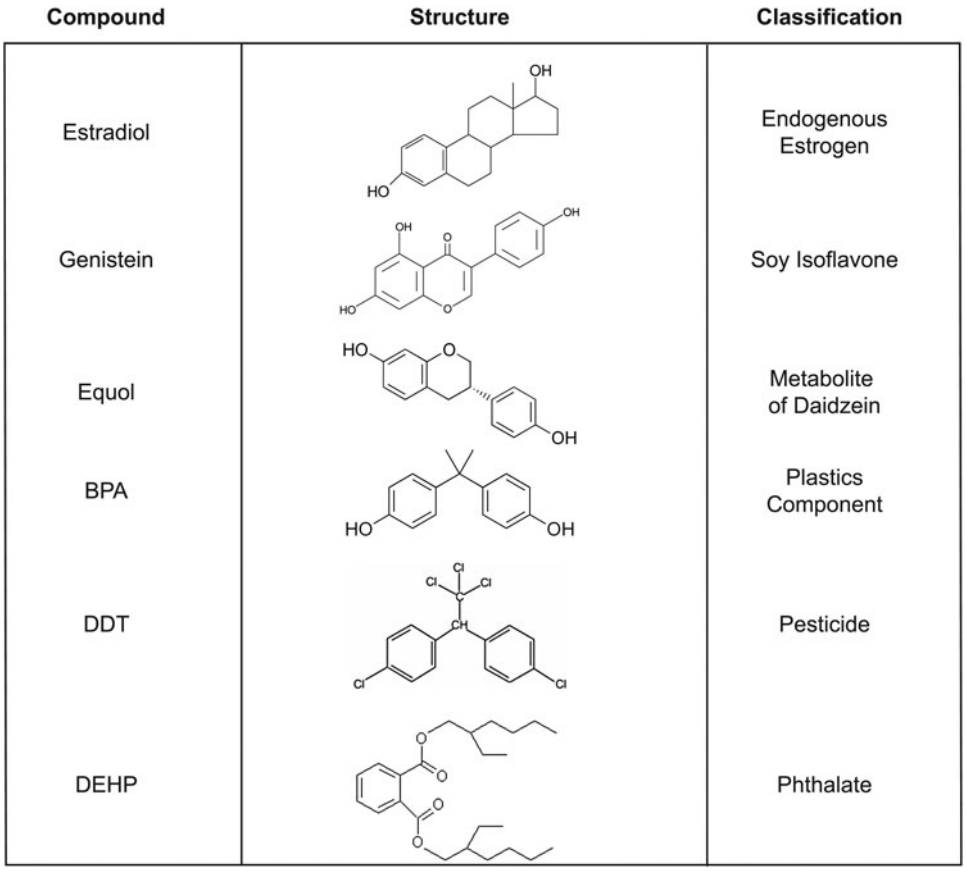

Fig. 1. Structures of some well-known anthropogenic and naturally occurring endocrine-disrupting compounds. BPA, bisphenol A; DDT, dichlorodiphenyltrichloroethane; DEPH, di(2-ethylhexyl)phthalate.

Phyto-oestrogens are naturally occurring plant compounds that are structurally and/or functionally similar to mammalian oestrogens and their active metabolites[7]. There are several phyto-oestrogen classes, but the most hormonally active are the phenolic compounds of which the isoflavones and coumestans are the most widely studied groups. Isoflavones are most abundant in soyabeans and other legumes but also found in berries, wine, grains, nuts and soya-fortified foods[8]. Although present as inactive glycoside conjugates (containing glucose or carbohydrate moieties) and unconjugated (aglycone) forms in food, only the latter are bioactive. Fermented soya, such as tempeh or miso, typically contains higher aglycone levels than other soya-based foods. Once consumed, isoflavones are rapidly metabolised and absorbed, entering systemic circulation predominantly as conjugates with limited bioavailability and bioactivity, leaving only a tiny fraction of the 'free' bioactive form in systemic circulation. Typically, metabolites are less bioactive than the parent compounds but equol, a metabolite of daidzein, is a notable exception[9]. At best, only 30–50 % of individuals are capable of bioconverting daidzein to its more oestrogenic metabolite equol with vegetarians and individuals of Asian origin being most likely[10,11]. Age and health status, particularly the use of antibiotics, can significantly impact the production and absorption of bioactive isoflavones, including equol.

Although they are structurally similar to anthropogenic endocrine-disrupting compounds (EDC) and behave similarly on numerous molecular and cellular targets, intake of soya phyto-oestrogens is broadly encouraged and regarded as healthy, while their synthetic counterparts are increasingly viewed with caution and met with repeated calls to ban or restrict their use (Fig. 1). This attitudinal discordance is almost entirely based on the source of the compounds (soya is 'natural', while synthetic chemicals are not) rather than the scientific evidence regarding their hormone-disrupting activities. While it is clear that for many people soya diets are a healthful option, particularly when meat and saturated fat intake is concomitantly reduced, a growing chorus of scepticism is cautioning that the health benefits popularly ascribed to soya may be overstated and minimally supported by robustly conducted, statistically sound scientific studies[12–14]. It has also been recognised for nearly a century that phyto-oestrogens have endocrine-disrupting properties in vertebrates, including human subjects, and that exposure to these compounds may pose a risk to some groups, particularly infants and the unborn[15–19].

Endocrine-disrupting activities of phyto-oestrogens in vertebrates and human subjects

An EDC is defined by the Endocrine Society as a compound that interferes with any aspect of hormone action[20]. The word 'interferes' is important because

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many things can have hormone action as part of maintaining homeostasis and interacting with the environment, such as the simple act of eating or standing in sunlight. An EDC is a compound that interferes with the way in which the pancreas responds to a meal, or disrupts the vitamin-D-producing capacity of sunbathed skin. In the case of isoflavones, the target of this 'interference' is primarily thyroid hormone and oestrogen. Although a formal definition has not yet been established, the term neuroendocrine disruption has been used to broadly describe chemical impacts on endocrine-related brain development and function[21,22]. In the case of phyto-oestrogens, the vast majority of research effort has concentrated on the reproductive neuroendocrine system, which includes the hypothalamic–pituitary–gonadal (HPG) axis. Importantly, neuroendocrine disruption is distinct from, and should not be conflated with, neurotoxicity, which characterises processes contributing to neuronal cell death and related downstream consequences (e.g. dopaminergic cell death and Parkinson's like symptoms) and peripheral neuropathies. Isoflavones are not neurotoxic.

That phyto-oestrogens are endocrine disrupting has been known since at least the 1940s when ewes grazing on clover rich pastures in Australia were observed to have abnormally high rates of infertility, abortion and reproductive abnormalities in their offspring[23–25]. Consequently, management of phyto-oestrogen levels has been the subject of grazing/feeding practices within the agricultural community for decades, including, most recently, in aquaculture[26,27]. Phyto-oestrogens have proven to be potently endocrine disrupting for a wide range of vertebrates, including rodents[1,28], birds[29], cheetahs[30], multiple species of fish[31,32], and grazing mammals such as cattle, sheep and even the southern white rhinoceros[23,25,33].

Evidence of endocrine disruption by soya in human subjects also dates back decades. Soya has been known to be goitrogenic for nearly a century[34,35] necessitating the addition of iodine to soya infant formula and other soya-rich foods. Both genistein and daidzein potently block thyroxine synthesis by serving as alternate substrates and blocking thyroid peroxidase catalysed tyrosine iodination. Soya also decreases absorption of synthetic thyroid hormone[36] potentially necessitating higher doses in hypothyroid patients. Thus, for these and other patients at risk for clinical or subclinical hypothyroid, compensatory iodine intake is advisable if soya is part of the regular diet. Additionally, although research regarding the relationship between soya intake and thyroid levels during pregnancy is extremely limited[37], because thyroid hormone is essential for normal brain development, pregnant women regularly consuming soya should be particularly mindful of this endocrine-disrupting property of soya.

Soya can also impact reproductive function in women. Suppression of circulating steroid hormone levels and attenuation of the preovulatory gonadotropin surge have been repeatedly observed and a 2009 meta-analysis concluded that isoflavone intake moderately increases cycle length and suppresses luteinising hormone and follicle-stimulating hormone levels[38]. A 2008 clinical case report described three women (aged 35–56 years) experiencing a suite of symptoms related to excessive soya intake (estimated to exceed 40 g/d), including abnormal uterine bleeding, endometrial pathology and dysmenorrhea, all of which resolved when soya intake was discontinued or reduced[39]. Importantly, as for all EDC, timing of exposure is important when considering the potential for long-term effects. The youngest of the three patients had been on a soya-rich diet since age 14 years and was experiencing secondary infertility, a condition that resolved and resulted in a pregnancy once she cut back on her soya consumption. Of even greater concern is what might happen in infant girls who consume high levels of soya, while their reproductive systems are still developing. Exposure earlier in life may have more lasting effects because disruption of the organisational actions of hormones may produce permanent structural and/or functional changes[40].

The earliest evidence for developmental reproductive health effects came from two studies, conducted in the mid-1980s, which associated neonatal phyto-oestrogen exposure with thelarche before age 2 years in a population of Puerto Rican girls. A number of confounding factors, however, including the consumption of meat that had been fattened with potent oestrogens, including the notorious endocrine disruptor diethylstilbestrol, make the data problematic and difficult to interpret[41,42]. A highly cited retrospective cohort study of 952 women found that young women reared on soya-based infant formula (248 women) as part of a controlled, University of Iowa feeding study, reported longer menstrual bleeding and menstrual discomfort than those who were fed a non-soya based formula (563 women)[43]. At the time the study was conducted, the women were too young to comprehensively examine pregnancy or fertility outcomes, but, now that nearly a decade has past, this area is ripe for reevaluation. Soya formula consumption has also been linked to a greater risk of developing uterine fibroids[44]. A prospective study reported oestrogenised vaginal epithelium in female soya formula-fed infants, an important finding confirming soya infant formula is oestrogenic in human subjects[45]. Other studies, however, have found no link between soya infant formula and developmental reproductive parameters, including breast, ovarian or testes volume[45,46], and impacts of soya formula intake and on age at menarche are mixed[47,48]. That soya is hormonally active is irrefutable. Whether or not soya intake, particularly during infancy, can have long-term health effects remains the subject of debate, but parents should be made aware of possible oestrogenic effects if they choose to feed their infants a soya-based formula.

Mechanisms of endocrine disruption by isoflavones

EDC can act via a myriad of mechanisms but the most fundamental include: (1) mimicking the effects of natural hormones by acting as a ligand at their binding sites; (2) antagonising the effect of these hormones by blocking their interaction with their physiological binding sites;

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(3) reacting directly and indirectly with the hormone in question; (4) altering the natural pattern of synthesis/degredation of hormones; or (5) altering cellular hormone receptor levels[40,49,50]. Isoflavones have been shown to interfere with oestrogen action via all of these. They also have other biological activities, which is not atypical as one of the hallmarks of EDC is that they simultaneously affect multiple hormonal systems, and act by multiple mechanisms. Genistein is thought to slow tumourigenesis, for example, via inhibition of protein tyrosine kinases and inhibition of DNA topoisomerases I and II, along with other chemoprotective mechanisms[1,6,51]. Phyto-oestrogens are also good antioxidants and anti-inflammatory agents.

The primary mode of isoflavone endocrine disruption is interference with oestrogen. At almost the same instant that a second subform of the nuclear oestrogen receptor (ER) was discovered (termed ERβ) it was recognised that isoflavones bind and activate transcription via both forms (ERα and ERβ), but generally have a higher relative binding affinity for ERβ[52–55]. Potency estimates vary by assay, but most isoflavones bind nuclear ER far more readily than their synthetic endocrine-disrupting counterparts including bisphenol a[52]. Exposure is also consistently higher, often orders of magnitude higher, making them one of the most significant EDC in the human landscape[1,56]. Once bound, isoflavones act as partial agonists, with activity varying across tissue types and local levels of endogenous oestrogen. ER subtype distribution varies across tissues and cell types, particularly in the brain, changes over the lifespan, and is sexually dimorphic[57–59]. Because ERα and ERβ are differentially distributed throughout the body and the brain, including neuroendocrine pathways, which coordinate reproductive function, that isoflavones are more bioactive via ERβ is functionally significant[60–64]. ERα and ERβ regulate different aspects of reproduction, behaviour and neuroendocrine function across the lifespan, although their relative roles are more clearly elucidated in animal models than in human subjects, in some tissues than others and, in some cases, one sex than another[65–68]. For example, ERβ in the paraventricular nucleus of the hypothalamus (PVN), a region important for the coordination of reproductive, social and stress-related behaviours, suppresses anxiety-related behaviours and enhances production of the neuropeptide oxytocin (OT)[69–71]. ERβ is also expressed at higher levels than ERα in the basal forebrain, hippocampus, dorsal raphe and cerebral cortex in the adult[60,72,73], all brain regions critical to neuroendocrine function and mood-related behaviours. ERβ is particularly abundant in the prenatal brain and plays a key role in brain morphogenesis by affecting cortical layering and interneuron migration[73].

Once bound to ER, phyto-oestrogens can initiate transcription classically through interactions with the oestrogen response element or by binding early immediate genes, such as Jun and Fos[74]. Steroid hormones, particularly oestrogens, can also initiate rapid, non-genomic actions at the cell surface via a range of mechanisms, including the binding of specialised steroid membrane receptors or ion channel subunits[75–78]. The vast majority of rapid actions are thought to originate at oestrogen-binding sites at the extracellular surface of the cell membrane, meaning that a potential EDC does not have to enter the cell to be active. Binding then activates second messenger pathways leading to cellular responses such as increased intracellular calcium or cAMP levels, or promoting nitric oxide release resulting in the stimulation of signal transduction pathways important for neuronal signalling, differentiation and other cellular processes[79]. The best-known transmembrane ER, the G-protein-coupled oestrogen receptor, was cloned as the orphan receptor GPR30 two decades ago and is now known to be capable of binding a wide range of EDC, including genistein[80]. The functional significance of this pathway, or its disruption, has yet to be fully described but G-protein-coupled oestrogen receptor plays an important role in rapid vascular oestrogen signalling along with ERα and ERβ[81]. Emerging data reveals that phyto-oestrogens have epigenetic activity and can alter activities of DNA and histone methyltransferases, NAD-dependent histone deacetylases and other modifiers of chromatin structure[82–84].

Phyto-oestrogens have also been shown to interfere with the enzymes needed for steroid biosynthesis and/or degradation. Coumestrol, for example, attenuates the conversion of [3H]-estrone to [3H]-estradiol *in vitro* by inhibiting the enzyme 17β-hydroxysteroid oxidoreductase Type 1 in a dose-dependent fashion[85]. Genistein, though weaker, has a similar dose-dependent inhibitory effect. In rats, genistein can alter folliculogenesis, an outcome postulated to result, at least in part, from dysregulation of steroidogenic enzymes[86]. In porcine granulosa cells, genistein decreases the activity of cholesterol side-chain cleavage enzyme (P450scc) and 3β-hydroxysteroid dehydrogenase[87]. Genistein has also been characterised as a non-competitive inhibitor of 11β-hydroxysteroid dehydrogenase type 1, which produces bioactive glucocorticoids, such as cortisol, from inactive precursors[88]. Disruption of aromatase and 5α-reductase by a number of phyto-oestrogens has also been demonstrated *in vitro* but this potential activity in mammalian tissues remains controversial[2]. Disruption of biosynthetic/degradative enzymes could significantly alter local endogenous hormone levels but not manifest as a change in circulating hormone levels. This may be particularly important for brain and hormone-sensitive subregions such as the hypothalamus as growing evidence strongly suggests that neural cells have the capacity to synthesise steroid hormones *de novo*[89–91].

Another mechanism by which phyto-oestrogens can perturb steroid bioavailability and transport is by altering sex hormone-binding globulin synthesis and availability. Isoflavones have long been known to appreciably stimulate sex hormone-binding globulin production, particularly in individuals who have levels on the low range of normal[92]. Heightened sex hormone-binding globulin levels are thought to be one mechanism by which soya may lower breast cancer risk because bioavailable levels of circulating oestrogens are concomitantly reduced[93]. Similarly, suppression of circulating androgens, particularly dihydrotestosterone by equol, is hypothesised to

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be one way in which soya might protect against prostate cancer[51,94]. Notably, a subset of studies have found no impact of isoflavones on circulating sex hormone-binding globulin or steroid hormone levels in human subjects (e.g.[95]). One found suppressed luteal oestrogen levels following increased soya intake, but only in women of Asian descent[96], indicating ethnicity and/or the capacity to produce equol could be an underappreciated factor-mediating interindividual variability in responsiveness[97,98].

Endocrine-disrupting effects of soya isoflavones on the adult neuroendocrine system

Impacts on the mature reproductive axis in human subjects and other vertebrates have already been summarised and include altered serum hormone levels and suppression of ovulation. Elevated urine levels of genistein and daidzein have been associated with idopathic infertility and lower semen quality in Chinese men[99], and a slightly lower percentage of normal sperm in US men whose partners were attempting pregnancy[100], but supporting evidence in other populations or species for effects on spermatogenesis is limited. In contrast, the animal literature has explored a wider age range and a more diverse array of endocrine-disrupting effects.

Work in our laboratory focuses on neuroendocrine pathways underlying sexually dimorphic behaviours and, using a variety of animal models, we and others have shown that isoflavone intake interferes with oestrogen-mediated behaviours, including female sexual motivation. For example, consumption of a commercially prepared isoflavone supplement to adult female rats, at a dose that results in serum levels between those seen in Western and Asian (human) adults, attenuated lordosis (a reflexive posture indicating sexual receptivity) to the same degree as tamoxifen[101,102]. The supplement also suppressed proceptive behaviours even more profoundly than tamoxifen suggesting that soya isoflavones can suppress female sexual motivation and solicitation (Fig. 2). Administration of genistein alone did not recapitulate these effects[103]. Whether or not libido is altered in human subjects appears to be completely unknown. Remarkably, a Pubmed search with the keywords 'soya' and 'libido' produced only nine published papers, not all of which were relevant. One was a case report describing a case of bilateral gynecomastia, erectile dysfunction and loss of libido in a 60-year-old man, which resolved when he discontinued drinking three quarts of soya milk daily[104]. Another reported a beneficial effect of soya protein dietary supplements on libido in postmenopausal women but there was an equally beneficial placebo effect suggesting that the soya effect was spurious[105]. Further inquiry revealed no studies, which have tackled this question in younger populations, or with a large-enough sample size to achieve reasonably robust statistical power. Given that soya appears to have a consistently suppressive effect on circulating steroid hormone levels it is not implausible that libido may also be

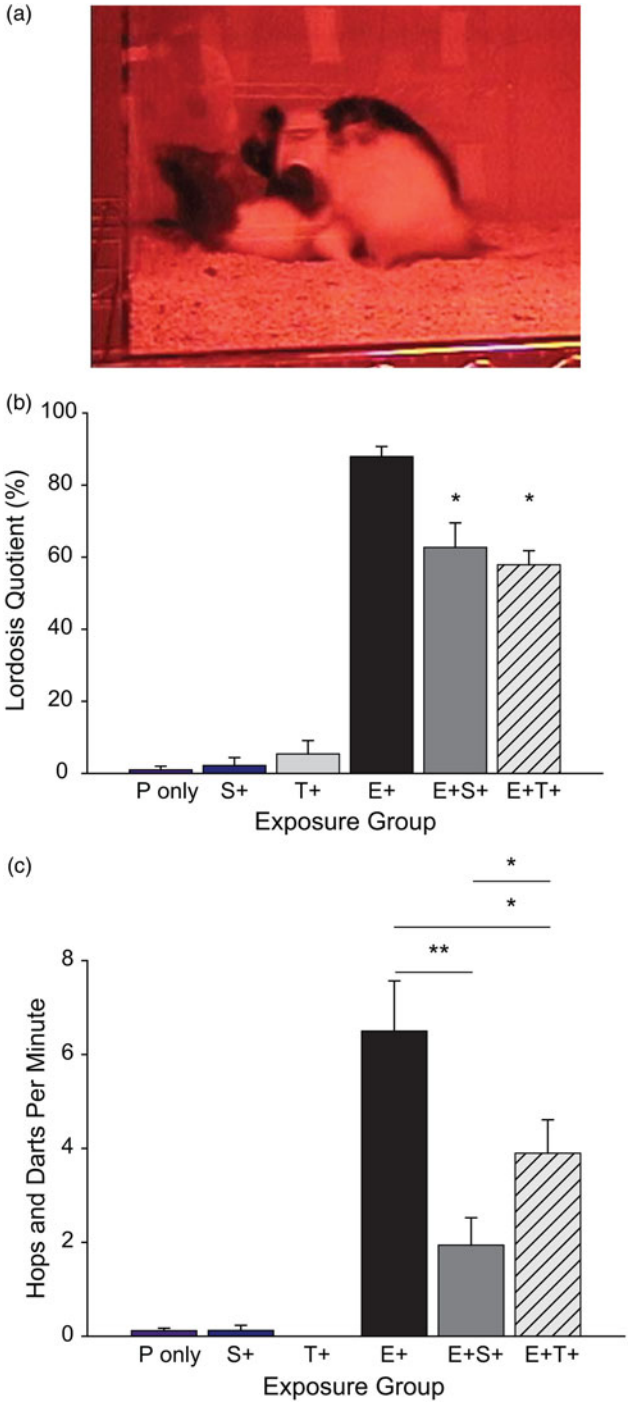

Fig. 2. (Colour online) In ovariectomised, hormone replaced female rats, sexual behaviour is suppressed by a soya isoflavone supplement. (a) Lordosis is a hallmark receptive posture in the rat and the frequency of lordosis in response to male mounting, which can be induced in ovariectomised females with progesterone (P) and estradiol benzoate (E), but not P alone. (b) In the presence of E and P tamoxifen (E + T+) or a soya supplement (E + S+) significantly decrease lordosis in female rats. (c) Similarly, proceptive behaviour, including hopping and darting, is also suppressed in hormonally replaced female rats on tamoxifen (E + T+) and, to an even greater degree, the soya isoflavone supplement (E + S+). $^{*}P \leq 0{\cdot}05$; $^{**}P \leq 0{\cdot}01$; means ± SEM. Figure adapted from Patisaul *et al.*[101]

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suppressed but this appears to be, by and large, an unanswered question.

Mood and anxiety-related behaviours may also be impacted by adult soya intake[106]. In human subjects, nearly all studies along these lines have focused on postmenopausal women and evidence for improvement of mood is minimal and sporadic[107]. Results across animal studies are mixed and sex dependent with females generally showing decreased anxiety and males showing heightened[108,109]. This pattern tends to abrogate or reverse expected sex differences in assessments of anxiety-related behaviours[110] and appears to involve the neuropeptides OT and vasopressin (AVP). For example, male cynomolgus monkeys fed soya protein isolate containing 1·88 mg isoflavones/g protein over 18 months demonstrated higher frequencies of intense aggressive (67 % higher) and submissive (203 % higher) behaviours as well as a decreased proportion of time (68 % reduction) spent in physical contact with other monkeys[111]. Male rats maintained on a diet containing 150 μg/g genistein and daidzein displayed increased anxiety and elevated stress-induced plasma AVP and corticosterone levels[112]. Increased hypothalamic AVP has also been reported in Sprague–Dawley rats fed a diet containing 1250 ppm genistein across the lifespan in a study run at a US Food and Drug Association research center[113]. We found that the same isoflavone supplement found to suppress reproductive behavior and motivation in female rats (described earlier) abrogated the oestrogen-dependent up-regulation of OT receptors in the ventromedial nucleus of the hypothalamus and heightened ERβ expression levels in the PVN, an effect opposite to that of estradiol[102].

Involvement of the PVN is consistent with both the oestrogen and thyroid-disrupting properties of soya, and the high potency of isoflavones at ERβ. The PVN, which contains little to no ERα but high levels of ERβ[114], is a primary site of OT and AVP production, peptide hormones important for social behaviour and the facilitation of sexual behaviour[115], as well as thyroid hormone releasing hormone and corticotropin-releasing hormone. Oestrogen-dependent stimulation of PVN OT and AVP production requires ERβ[116,117]. OT then binds to its receptor throughout the brain, including the ventromedial nucleus, a nucleus critical for mediating the lordosis response in females[118]. Isoflavone-related effects on these and other oestrogen dependent systems in the adult rodent brain have previously been reviewed[109,119–121] but a concerted focus on OT/AVP systems in human subjects remains lacking.

Evidence for developmental neuroendocrine disruption in animals and human subjects

Neuroendocrine disruption by soya isoflavones in mature neuroendocrine systems is by and large reversible with dietary modification and thus, with the exception of some hypersensitive groups such as hypothyroid and oncology patients, soya likely poses no long term health risk and may even confer modest benefits. Of greater concern is that phyto-oestrogens may interfere with the organisational role of oestrogen in the developing brain and reproductive system. Data from a diversity of animal models have repeatedly shown that manipulation of oestrogen during specific critical windows of development throughout gestation and early infancy leads to a myriad of adverse outcomes in the HPG axis including malformations in the ovary, uterus, mammary gland and prostate, early puberty, reduced fertility, disrupted brain organisation, and reproductive tract cancers[66,122–126]. The disruptor diethylstilbestrol story also starkly illustrates the broad spectrum of sex-specific consequences on neuroendocrine systems following fetal oestrogen exposure[127,128]. Although isoflavones and other EDC are far less potent than disruptor diethylstilbestrol, human exposure is ubiquitous and there is growing acceptance that EDC are contributing to adverse reproductive health trends in Western nations including median age at menarche, first breast development, and sexual precocity[40,129–131]. Advanced pubertal onset in girls adopted from developing countries by Western parents supports a role for environmental factors[129]. Emerging but controversial data suggest that EDC may also be shifting age at puberal onset in boys[132]. Among men, sperm counts in the USA and Europe appear to have declined by approximately half over the past 50 years[133,134] with upwards of 30 % in the subfertile range in places like Denmark where exposure to persistent environmental pollutants is particularly high[135]. A provocative but limited study associated increased incidence of hypospadias (malformation of the male external genitalia) with maternal vegetarianism[136] but this effect has not been replicated. Synthetic EDC which interfere with androgen biosynthesis or activity are also associated with disorders of male genital development[137,138] thus it is not implausible that equol may be endocrine disrupting in this regard. Increased prevalence of reproductive health disorders is likely not attributable to a single factor, not even a single environmental factor, but EDC are causal to some degree and isoflavones are hypothesised to play a contributing role[5,40,139–141].

Disruption of reproductive tract development

The vast majority of studies exploring the impact of early life isoflavone exposure on HPG differentiation and function have used rodent models, with the compounds administered either prenatally to the pregnant dam or postnatally to the pups. This aspect of the literature has been extensively reviewed and will thus not be recapitulated in detail here but adverse outcomes in female rodents include disrupted timing of vaginal opening (pubertal onset), altered ovarian development, impaired oestrous cyclicity and ovulation, and disrupted HPG steroid feedback[1,2,5,142]. We have recently shown, for example, that female rats reared on a soya-rich diet across the lifespan (gestation through adulthood) have earlier pubertal onset (defined as the day of vaginal opening in the rat), and a greater number of corpera lutea post-puberty but took longer to establish regular oestrus cycles than their conspecifics on soya-free diet. Cycle regularity

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then degraded with time and soya-reared animals had a greater number of cystic follicles in early adulthood[143]. Notably, not all pathology is readily obvious. Emerging evidence suggests that the oviductal and uterine environments in mice developmentally exposed to human-relevant gensitein levels are not suitable to maintain pregnancy, which manifests as the incapacity of the uterus to support implantation and embryonic development[144]. Moreover, embryo transfer experiments have shown that the uterus of genistein-treated mice is not capable of sustaining pregnancy even if the blastocysts arise from control mice[145]. These data are consistent with effects seen in sheep and other species suggesting that developmental isoflavone exposure induces permanent changes in the function of the female reproductive tract that may be subtle but can result in complete infertility, particularly as the animal ages.

There is a surprising paucity of data on the impact of developmental isoflavone exposure on male neuroendocrine physiology (reviewed in[146]). There is some sporadic evidence in animal models that developmental isoflavone exposure affects testicular function, but many studies find no effects, which makes it challenging to draw definitive conclusions[4]. A transformative pair of high-impact studies, which greatly contributed to health advisories in Europe, was conducted in marmosets. Twins were fed either soya or milk formula. Males on the soya diet had lower serum testosterone concentrations and higher numbers of Leydig cells then their milk-fed twins. As adults, the soya fed marmosets had larger testes and lower serum testosterone levels, demonstrating that the impacts were persistent[147,148], but fertility was not compromised. Two rat studies conducted using classical toxicological testing parameters and long-term multigenerational oral exposure protocols spanning gestation through adulthood linked genistein with abnormalities in spermatogenesis[149,150]. One also found genistein-related alterations in sperm motility and a reduction in litter size accompanied by evidence of post-implantation embryo loss when the adult rats underwent fertility testing[149]. Chronically exposed males have also been shown to develop mammary gland hypertrophy at doses at or above 11 mg/kg, and mammary gland hyperplasia at doses at or above 29 mg/kg (ductal/alveolar hyperplasia was observed in females as well)[151]. This effect was confirmed in a subsequent study by a different research group even though exposure was restricted to the perinatal period, suggesting that the sensitive period of exposure is pre-pubertal[152]. The male mammary gland may be one of the most sensitive targets for endocrine disruption but is rarely examined in EDC studies, leading some to advocate for its inclusion in chemical test guideline studies and risk assessment[153,154].

Disruption of brain sexual differentiation and neuroendocrine organisation

Work in our laboratory focuses on sexually dimorphic, oestrogen-sensitive hypothalamic systems and we have repeatedly shown that the sex-specific ontogeny of these systems is vulnerable to synthetic and naturally occurring EDC including soya isoflavones[1,121,155]. In rodents, hormone mediated morphological and functional organisation within the neuroendocrine system occur during a series of well-defined critical periods spanning gestation through puberty[66,124,156]. Although most sex differences are established during prenatal and neonatal development, in the rat new cells (neurons and glia) are added to sexually dimorphic nuclei during adolescence in response to steroid hormone treatments[157,158], demonstrating the long-term sensitivity of sexually dimorphic brain regions to steroid hormone-mediated signalling. Interference with the hormone-sensitive organisation of neuroendocrine pathways could result in irreversible developmental defects and disruption of sex-typical behaviours, emphasising that development is likely the most susceptible periods for EDC exposure over the lifespan. Although it does not readily transfer lactationally, genistein efficiently crosses the rat placenta and the bioactive aglycone form of genistein is present in the fetal brain at levels comparable to circulating levels in the dam[159,160]. Moreover, the transfer of genistein to the brain from systemic circulation appears to be more efficient in prenatal animals than adults[161] demonstrating that it and other isoflavone phyto-oestrogens are capable of directly interfering with the organisation of neuroendocrine signalling pathways in the developing brain.

The sexually dimorphic brain region most frequently used as a biomarker of endocrine disruption in rats is the sexually dimorphic nucleus of the preoptic area (SDN-POA). The volume of the SDN-POA is enhanced by estradiol aromatised from perinatal, testicular androgen[124], is five to six times larger in males than females[162], and is thought to play a role in male reproductive behaviours and mate choice. Although both ERα and ERβ are expressed in the SDN-POA across the lifespan, ERα appears to play a dominant role in masculinising SDN-POA morphometrics[61,163,164], a process which has now been elucidated in detail and is largely complete by the second week of life[67,165]. In rats, numerous studies have queried the extent to which soya isoflavones alter SDN-POA volume in both sexes and, while not always in complete accordance, the data are generally consistent with oestrogenic effects. For example, when administered prenatally through adulthood, genistein increases SDN-POA volume in males but not females[166]. No enhancement, however, was observed in males exposed from birth through weaning[167] or in males exposed on only the first few days of life[168] suggesting that exposure must be ongoing to maintain the enlargement. Masculinising effects on female SDN-POA volume have only been observed following high-dose exposure[169] and some studies have not found genistein to be endocrine disrupting in the female rat SDN-POA, even at doses high enough to be uterotrophic[167,170].

An additional area of focus for our studies is the anterior ventral periventricular nucleus (AVPV), which, like the SDN-POA, is sexually differentiated by endogenous gonadal hormones during a series of pre- and perinatal critical periods but is larger in females than males[171,172]. The presence and density of the two ER

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subtypes varies across species but both are present in the rat[58]. The AVPV is essential for coordinating the preovulatory gonadatropin surge and plays a central role in female sexual behaviour[173–175]. In human subjects, the neural machinery controlling gonadotropin pulsatility is functional by the end of the first trimester[176], while in rodents this system does not fully sexually differentiate until the first few days of the neonatal period[171]. In male rodents, testicular androgen is aromatised to oestrogen in the brain, and it is this locally derived oestrogen, working primarily through ERα-dependent pathways, that is primarily responsible for defeminising/masculinising the AVPV[67,177,178]. At birth exogenous oestrogen administration can defeminise the female AVPV and surrounding structures thereby eliminating lordosis and the capacity for steroid-positive feedback. By extension, if endogenous oestrogen is blocked in males, either by castration, by aromatase inhibition, or antagonism of hypothalamic ER, the AVPV and surrounding structures fails to defeminise and the capacity to elicit lordosis and a gonadal surge remains. Therefore, interference with oestrogen at birth, in either sex, can result in the improper differentiation and function of the HPG axis across the lifespan.

We have shown that subcutaneous administration of 10 mg/kg genistein, a dose that is approximately equivalent to the total amount of isoflavones ingested by infants fed soya formula, over the first 4 d of life, advances vaginal opening and compromises the ability to maintain a regular oestrous cycle in female rats[179]. This outcome was accompanied by an impaired ability to stimulate gonadotropin releasing hormone activity (as measured by the co-immunoreactivity of gonadotropin releasing hormone and Fos) following ovariectomy and hormone priming. We have further shown that neonatal exposure to 10 mg/kg genistein significantly decreases the density of kisspeptin immunoreactive fibres in the AVPV of female rats[180,181]. Exciting work over the past decade has identified kisspeptin neurons as the primary gatekeepers of gonadotropin releasing hormone release in many species, including human subjects[182,183]. Therefore, our findings suggest that disrupted organisation of kisspeptin signalling pathways may be a novel mechanism by which isoflavones and other EDC may induce a suite of HPG-related abnormalities, including advanced pubertal onset, irregular oestrous cycles and premature anovulation[184].

How much is too much: human isoflavone intake, metabolism and excretion

Ultimately risk of harm comes down to two primary factors: dose and timing of exposure. Undoubtedly, development is the most sensitive period for the endocrine-disrupting consequences of soya isoflavone exposure, thus it is not surprising that concerns have been expressed regarding the safety of soya-based infant formula. Initially developed as an alternative to bovine milk formulas for babies with milk allergy, use of soya infant formula in the USA is a popular choice and constitutes an estimated 25 % of the formula market[185–187]. The safety of soya formula has been rigorously discussed from several perspectives, and a litany of review articles and position papers have been published on the subject[188–195]. Societies including the American Academy of Pediatrics and the European Society for Pediatric Gastroenterology Hepatology and Nutrition Committee on Nutrition have issued guidelines recommending against the exclusive use of soya formula except in the rare cases of true milk allergy or lactose intolerance. The US National Toxicology Programme completed its most recent safety assessment of soya infant formula in 2010 (monograph available at http://ntp.niehs.nih.gov/pubhealth/hat/noms/formula/index.html) and concluded there is 'minimal concern for adverse developmental effects.' For comparison, this is the same level of concern initially expressed for bisphenol a until the Food and Drug Association elevated that advisory to 'some concern' in January, 2010 based on new data (and then subsequently lowered it again). Notably, the National Toxicology Programme could not issue a conclusive recommendation regarding potential long-term reproductive effects of soya infant formula largely because of limited and poor-quality human data. An apparent lack of adverse effects is one reason why so many consumers, clinicians and public health agencies consider regular use of soya formula to be safe, even beneficial. However, the absence of evidence is not evidence of safety so this problematic data gap regarding the long-term impacts of soya formula use remains in serious need of attention.

When considering the potential safety of soya formula, it is frequently argued that Asian populations have been consuming soya for centuries, with no obvious consequences. This argument fails to recognise, however, that exposure patterns differ in key ways between Asians consuming a traditional soya-rich diet and Caucasians eating a typical Western diet[185]. This timing of exposure is critical. In a traditional Asian diet, soya consumption is moderate across the entire lifespan, but because isoflavones do not effectively transfer via lactation, exposure in breastfeeding infants is extremely low. By contrast, Western babies on soya infant formula have their highest exposures in the first year of life then exposure rapidly plummets. In that regard the two populations are not comparable because their exposure patterns during a critical window of development are so dramatically different. Other diet and lifestyle differences may also be confounding when evaluating the potential health benefits and risks of soya. For example, Asian populations on traditional diets eat less processed foods, considerably higher levels of seafood and lower levels of animal fat than Western populations.

So how much is too much? There is no 'typical' level of isoflavone intake as consumption patterns vary widely across populations, and geographic regions. For Asians, vegetarians and other groups in which soya is foundational to the diet, isoflavone consumption can be as high as 100 mg/d (intake range is about 0·3–1·5 mg/kg body weight)[6,192,196–198]. Western diet intake estimates range from 1 to 3 mg/d[198–201]. For their weight, infants exclusively fed soya-based formula have the

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highest mean daily consumption of total isoflavones, ranging from 6 to 9 mg/kg body weight per d in 4-month-old infants, an amount that is up to seven times higher than Asians consuming a traditional soya-based diet.

The isoflavone content of an array of foods and food products is now available via online databases (reviewed in:[202]) including one maintained by the USDA[203]. Food isoflavone content varies widely, even in the same foods, because of local and/or seasonal differences in growing conditions so its difficulty to accurately estimate intake[198]. Additionally, soya is found in upwards of 60 % of processed foods and ground meats[204]. Textured soya protein (50–70 % soya protein) is used as a meat substitute or filler for hotdogs, hamburgers, sausages and other meat products[205,206], while soya protein isolate (90 % soya protein) is frequently used to enrich energy bars and sports drinks (particularly those advertising high protein levels), cereals, granola bars, infant formula, imitation dairy products, ice cream and cheese. Soya isoflavones and other phyto-oestrogens are also widely available as dietary supplements[207,208], typically containing concentrations far higher than those found in food[209].

Not surprisingly, blood isoflavone levels also vary widely, and can be orders of magnitude different between individuals based on dietary preferences and individual differences in phyto-oestrogen absorption and metabolism[210–212]. Blood genistein levels are generally in the range of 25 ng/ml for Asian women, slightly less for vegetarian women, and under 2 μg/ml for US women[213]. Isoflavones can pass from mother to fetus through the placenta, and have been found in human umbilical cord blood and amniotic fluid at levels comparable with concentrations seen in maternal plasma, demonstrating that fetal exposure can be significant[214]. Infants on soya formula can have plasma levels exceeding 1000 ng/ml[209] which is 13 000–22 000 times higher than their own endogenous oestrogen levels, 50–100 times higher than oestradiol levels in pregnant women, and 3000 times higher than oestradiol levels at ovulation[185,215,216]. In contrast, infants fed cow's milk formula or human breast milk have plasma isoflavone levels of 9·4 and 4·7 ng/ml, respectively[192,196,216]. Notably, levels in infants and vegetarians easily far surpass, sometimes by several orders of magnitude, internal levels other endocrine disruptors of concern, including bisphenol a and phthalates[126].

Conclusions and recommendations

Soya isoflavones are clearly endocrine disrupting, but although they are similar to their synthetic brethren in terms of their cellular and molecular mechanisms of action on neuroendocrine structure and function, and the scope of adverse outcomes they can inflict, society embraces these compounds at the same time it rejects, often with vigour, exposure to their synthetic brethren. Thus, phyto-oestrogens both challenge our attitudes regarding EDC and highlight how profoundly the direction and interpretation of research and available data can be influenced by source. While some beneficial effects might be conferred by including moderate levels of dietary soya, particularly in adults eating a diet high in saturated fat and animal protein, the potentially adverse effects of these compounds for some groups are likely underappreciated. An abundance of animal data unequivocally demonstrates that soya isoflavone exposure, at doses and plasma concentrations attainable in human subjects, including soya-reared infants, can permanently alter the structure and function of neuroendocrine pathways in both sexes. Infants fed soya formula have the highest exposure to any non-pharmacological source of oestrogen-like compounds, and yet greater anxiety surrounds compounds like bisphenol a and the phthalates which have far lower potency on neuroendocrine targets and to which exposure is far lower. Although relatively few adverse effects have been reported, that is somewhat a consequence of lack of data rather than lack of measurable effects. Although unsatisfying, a parsimonious approach to soya intake is to follow the classic adage and consume in moderation. Development of dietary guidelines should consider the endocrine-disrupting properties of soya and other hormonally active foods, particularly for vulnerable groups such as pregnant women and hypothyroid individuals.

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Conflicts of Interest

None.

Authorship

The author had sole responsibility for all aspects of preparation of this paper.

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