REVIEW

The effect of phytoestrogens on the female genital tract

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Environmental oestrogens have been implicated in the pathogenesis of hormonally treated cancers (such as breast and prostate cancer), male infertility, and abnormalities of the male and female reproductive tracts. They may be derived from plants (phytoestrogens), pharmaceuticals, or other synthetic compounds not originally intended to have oestrogenic activity (including soy based infant formulas). This review will discuss the evidence from both animal studies and humans for an effect of these ubiquitous compounds on the development of the human female genital tract, in addition to prolonging the menstrual cycle, alleviating symptoms of the menopause, and protecting against the development of endometrial carcinoma.

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Natural oestrogens are involved in the development and function of the male and female genital tract, neuroendocrine tissues, bone, and breast. At the cellular level, they promote cellular proliferation and hypertrophy of the female secondary sexual organs and induce the synthesis and secretion of cell type specific proteins.[1] It has been widely established that exposure to natural (endogenous) oestrogens is the principle risk factor for the development of endometrial cancer.[2] However, over the past 50 years an increasing body of evidence has accumulated indicating that several environmental chemicals exist that also have oestrogenic and/or potentially antioestrogenic effects both on aquatic and land based wildlife, and on humans. Although the effect of environmental oestrogens on the breast has been considered elsewhere,[3] there has been little attention paid to their effects on the human female reproductive tract. Therefore, we discuss the origin and nature of oestrogenic compounds within our environment, and review the evidence that such agents might affect the pathophysiology of the female genital tract.

THE NATURE OF ENVIRONMENTAL OESTROGENS
Environmental oestrogens derive from two main sources: phytoestrogens and synthetic (pharmaceutical and non-pharmaceutical) oestrogens.

Phytoestrogens
Phytoestrogens are naturally occurring phytochemicals found in plants and plant products, which are structurally and functionally similar to 17β-oestradiol (isoflavones) or synthetic oestrogens such as diethylstilboestrol (lignins). They form a diverse group with a variety of biochemical and biological properties.[4] [5] The principle phytoestrogens are the isoflavones (for example, coumestrol, genistein, daidzein, and equol[4–7]) and lignins (for example, enterolactone and enterodiol), derived from precursors in the diet by the gut microflora,[5] [8] and mycotoxins derived from fungal moulds or grain (for example, zearalenone).[9] The isoflavones have received the greatest attention with regard to female genital tract pathology and are low molecular weight diphenolic antimicrobial compounds (phytoalexins), which are synthesised de novo in plants in response to exposure to bacterial pathogens. Boué and colleagues[4]—for example, have shown that *Aspergillus sojae* induces the production of the coumestan phytoalexin, coumestrol, and the pterocarpan phytoalexins, glyceollins I–III in soybean cotyledons. Glyceollin prevents the accumulation of alfatoxin B_1 in cultures of *Aspergillus flavus*.

Coumestrol is the most potent of the phytoestrogens, but is some 100–200 times less potent than 17β-oestradiol, and almost 3000 times less potent than diethylstilboestrol.[10] [11] Using cytosolic preparations of rat endometrial adenocarcinoma cells, Hopert *et al* have shown that the affinity ranking to the oestrogen receptor (ER) is 17β-oestradiol >>> coumestrol > genistein > daidzein >>> mangostin, such that the affinity for the ER of coumestrol is approximately 2660 times that of mangostin.[11] Given that coumestrol is a fluorescent compound, it is possible to demonstrate directly a binding affinity with the ER that is approximately 20% of that seen for 17β-oestradiol.[12]

Phytoestrogens are present as glycosides in the diet in legumes, grains, nuts, and other fibre rich foods,[5] [7] [13] [14] and are present in the plasma and urine of both humans and animals eating a diet rich in such foods,[6] [7] [13–17] although there is a pronounced variation between individuals.[16] It has also been suggested that alcoholic beverages, especially bourbon, may contain phytoestrogens.[18] Soy is a particularly rich and prevalent source of phytoestrogens in the human diet because it is rich in the glycoside of daidzein, which is metabolised in the gut to equol.[19] [20] In the past decade, soymeal (rich in isoflavenoids) has been widely used in the preparation of processed foods, which feature highly in the Western diet. Setchell *et al* have also shown that commercially available soy based infant formulas contain considerable amounts of genistein and daidzein,

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Abbreviations: DDT, dichlorodiphenyltrichloroethane; ER, oestrogen receptor; FSH, follicle stimulating hormone; LH, luteinising hormone; ppm, parts per million; SHBG, steroid hormone binding globulin

such that circulating concentrations of isoflavones in human infants fed these formulas may be 13 000–22 000 times higher than plasma oestradiol concentrations in early life.[21] Lactating females may pass dietary oestrogens to their offspring: animal studies have shown that milk from lactating females fed on a low dose coumestrol diet may also be a biologically important source of phytoestrogens.[22] Coumestrol is also present in certain food supplements, such as alfalfa tablets.[10]

The metabolism of phytoestrogens has been reviewed in detail elsewhere[13] and a detailed discussion is beyond the scope of this review. However, Sonnenschein and Soto[1] and Whitten and colleagues[7] have suggested that phytoestrogens may exert their biological activity by: (1) mimicking the action of endogenous oestrogens; (2) acting as oestrogen antagonists; (3) altering the pattern of synthesis and metabolism of endogenous hormones; and (4) modifying hormone receptor values. In addition, Aldercreutz et al have shown that there is a positive correlation between fibre intake, urinary excretion of enterolactone, and serum steroid hormone binding globulin (SHBG), and a negative correlation between urinary excretion of enterolactone and serum oestradiol.[23] Thus, the ingestion of phytoestrogens stimulates the hepatic synthesis of SHBG and indirectly reduces the amount of free (biologically active) oestradiol in the serum. Therefore, in the absence of oestrogen, isoflavones have a weakly oestrogenic effect, but may exhibit an antioestrogenic effect when oestrogen is present.[24]

However, it must be remembered that phytoestrogens may exert biological activity by other mechanisms. For example, the isoflavone genistein is a potent selective inhibitor of tyrosine kinase in both human[25] and rat myometrial cells.[26 27] A similar effect is also seen in human ovarian carcinoma cells[28] and in adenovirus infected cell lines.[29] It is of note that daidzein, which has a similar structure to genistein, has no effect on tyrosine kinase activity.[26] However, in human leiomyosarcoma cells, both genistein and daidzein inhibit fast Na^+ current by directly blocking fast Na^+ channels.[30] Kellis and Vickery[31] have shown that flavenoids inhibit the aromatisation of androstenedione and testosterone to oestrogens catalysed by human placental and ovarian microsomes.

Given that phytoestrogens are readily identified within the human diet, the lack of associated deleterious effects is at first glance surprising. However, this might be explained by adaptive phenomena during the coevolution of animals and plants. Thus, it is likely that sudden exposure to a phytoestrogen not normally present in an animal's ecosystem (for example, the recent widespread introduction of soymeal into the Western diet) is more likely to have deleterious effects on health than a phytoestrogen that is normally present and ingested over a long time course.[1 32] However, no studies have yet explored this in humans.

Xenoestrogens

A wide variety of synthetic chemicals present within the environment have oestrogenic activity. These may be subdivided into pharmaceutical oestrogens and other synthetic compounds that have been produced for other purposes, but have subsequently been found to have oestrogenic activity. Pharmaceutical oestrogens including diethylstilboestrol[33] and oral contraceptives can be found both in sewage and river water.

Following the widespread use of hormonal contraception there has been increasing concern that contraceptive steroids may enter the water supply. Both natural and synthetic oestrogenic chemicals are present in raw domestic sewage.[34] Despite treatment in a modern sewage disposal plant, oestrogens can be detected, albeit at low concentrations, in the effluent from such plants.[35] Purdom et al have shown that both male and female trout exposed to the effluent from sewage treatment works have evidence of exposure to exogenous oestrogens, as determined by the measurement of plasma vitellogenin values, an oestrogen regulated egg yolk protein synthe-

sised in the liver.[36] The oral contraceptive steroid 17α-ethinyl oestradiol is a more potent inducer of vitellogenesis than 17β-oestradiol, primarily because it shows little binding to oestrogen binding plasma proteins.[37] In addition, they determined that the oral contraceptive steroid 17α-ethinyloestradiol is a more potent inducer of vitellogenesis than 17β-oestradiol. In The Netherlands, Belfroid et al have demonstrated the presence of oestrone and 17β-oestradiol (hormones that are naturally excreted in human and animal urine) in treated waste water at concentrations of 47 ng/litre (oestrone) and 1–12 ng/litre (17β-oestradiol).[38] The contraceptive steroid 17α-ethinyloestradiol has also been detected in treated waste water. The concentrations of such steroids are greater in domestic than in industrial effluent. In general, the concentrations of oestrogenic hormones within surface water in The Netherlands were low (1–4 ng/litre), and of these oestrone was the most prevalent.

Despite the evidence above that sewage plant effluent contains oestrogenic compounds that exert an effect on aquatic life, human health is only likely to be affected if such compounds re-enter the drinking water supply or the food chain from these sources. In Korea, where standards of water treatment are insufficient, upstream and downstream samples of river water have been shown to be contaminated with oestrogenic compounds.[39] In Israel, where water recycling is far more pronounced than in the UK, oestrogen has been detected in lake water used as a source of drinking water, possibly as a result of contamination by sewage water.[35] Studies in the UK have repeatedly failed to detect oestrogenic compounds in drinking water supplies.[40 41]

A detailed discussion of the nature and mechanism of action of the vast range of chemicals that have been found to have weak oestrogenic activity is beyond the scope of this review, and has been covered in detail elsewhere.[1 42] It is sufficient to note that such chemicals include pesticides, such as dichlorodiphenyltrichloroethane (DDT),[42 43] methoxychlor,[44] and kepone[1 42]; polycyclic aromatic hydrocarbons, such as 3,9-dihydroxybenz[a]anthracene; drugs, such as digoxin[45] and cimetidine; antioxidants in foods, such as butylated hydroxyanisole[46]; resin based composites and sealants used in dentistry[47]; and some polychlorinated industrial byproducts, such as polychlorinated biphenyls and chlorofluorocarbon.[42]

It is interesting to note that oestrogen-like substances may be released from plastic centrifuge tubes into plasma stored within them. Soto et al have shown that nonylphenol, an antioxidant used in the manufacture of plastics, is responsible for this.[48] In addition, Krishnan et al have shown that the oestrogenic compound bisphenol-A is released into cultures during the autoclaving of the polycarbonate plastic ware used to sterilise the culture medium.[49] Bisphenol-A is also released from the lacquer coatings of food cans[50] and certain sealants and resins used in dentistry.[47] Jobling et al have shown that phthalate esters (used in the production of various plastics) isolated from liquid sewage have oestrogenic activity and are able to combine with both piscine and mammalian ERs.[46] That such compounds may be released from plastics into specimens must be remembered when considering any study of environmental oestrogens where samples are collected into or stored in plastic containers, rather than into glass, and subsequently used in bioassays to determine their oestrogenicity. Nonylphenol is also known to leach from the PVC tubing used in milk processing, and from food packaging.[1]

THE EFFECT OF ENVIRONMENTAL OESTROGENS ON THE FEMALE GENITAL TRACTS OF ANIMALS

Most of what is known of the effects of environmental oestrogens on the female genital tract comes from iatrogenic human exposure to diethylstilboestrol and studies on laboratory animals and domestic livestock. In broad terms, these effects can

be divided into maturational and morphological abnormalities, and disruption of the oestrous cycle and infertility.

Maturational and morphological effects

It has now been well established that a low dose phytoestrogen diet can induce developmental and maturational abnormalities in both laboratory animals and domestic livestock. In rats, Whitten *et al* have shown that a low dose (0.01%) coumestrol diet (a concentration of coumestrol found naturally in human food) fed to lactating dams induces vaginal opening within female pups at a lower body weight than in pups from dams fed with a control diet, although the age at vaginal opening did not differ across treatment groups.[22 51] Although coumestrol treated animals were significantly lighter than the controls, coumestrol was shown to exert a significant effect on weight at vaginal opening, independent of its effects on growth rate. Murthy *et al* found that a benzene extract from the flowers *Hibiscus rosa sinensis* (a plant purported to act as an antifertility agent in rural folk practice in India) induces precocious vaginal opening in mice.[52] Similarly, Burroughs *et al* found that the administration of coumestrol subcutaneously to newborn mice in the first 5 days of life induced eye opening and vaginal opening at an earlier age than was seen in untreated mice, and that these effects were similar to those seen in mice given diethylstilboestrol, although vaginal opening was more advanced in the group treated with diethylstilboestrol.[53 54] In contrast, they observed no difference in body weights between the treated and untreated groups. However, Awoniyi *et al* were unable to demonstrate precocious vaginal opening in rats born to dams fed genistein on day 17 of gestation,[55] although these animals did show prominent ovarian follicular atresia.

Human concentration doses of coumestrol are uterotrophic in rats.[56] Uterine wet and dry weights are increased above controls in neonatal rats receiving either 10 µg or 100 µg coumestrol for the first 5 days of postnatal life. This effect is confined to the first 10 days of postnatal life. After 10 days, the uterine weight is significantly lower than in untreated animals.[57 58] Genistein has a similar uterotrophic effect.[59] In addition, after 5 days of 10 µg/day coumestrol treatment ER values are lowered to 60% of controls by 5 days, and to 15–20% of controls by 10 days, the level eventually stabilising to 40–70% below controls.[57] Equol may induce a transient reduction in ER values.[57] Baker *et al* have also shown coumestrol to be uterotrophic, with a clear dose response in rats at doses of 20, 40, and 80 mg/kg/day.[60] Ashby and colleagues[61] and Tinwell and colleagues[62] have shown that the uterotrophic effect of coumestrol results not only from an increase in uterine fluid content, but also hyperplasia of the endometrium. Animals treated with coumestrol show a rise in uterine DNA content that mimics that seen in animals treated with oestradiol. In contrast, the work of Markaverich *et al* showed that immature ovariectomised rats dosed subcutaneously on a single occasion with coumestrol had rapidly increased wet and dry uterine weights but no increase in uterine DNA content.[63] Furthermore, they found that coumestrol did not cause cytosolic ER depletion, nuclear accumulation, or the stimulation of nuclear type II sites, which characteristically precede oestrogenic stimulation of DNA synthesis. However, coumestrol did increase ER induction.[63]

Burroughs *et al* have shown that neonatal exposure to coumestrol or diethylstilboestrol in mice can induce significant long term reproductive tract abnormalities, including vaginal cysts (which may represent Wolffian duct remnants), persistent vaginal cornification, endometrial squamous metaplasia, absence of corpora lutea, increased ceroid deposition in the ovaries, and the presence of haemorrhagic follicles in coumestrol treated animals.[64] Mice treated neonatally with coumestrol are also less likely to develop endometrial cystic glandular hyperplasia.[54]

Morphological effects as a result of environmental oestrogens are also seen in larger mammals. Nwannenna *et al* observed a uterotrophic effect of formononetin rich red clover silage on ovariectomised ewes, which was associated with changes in teat length and colour of the vulva similar to those seen in sheep exposed to oestradiol implants.[65] Cantero *et al* have shown that non-lactating adult sheep grazing lucerne (*Medicago sativa*), a crop containing coumestrol at 17–30 parts per million (ppm), for prolonged periods have morphologically abnormal reproductive organs.[66] Macroscopically, such ewes display cysts in the endometrium with petechiae and ecchymoses in the uterine mucosa. Two of the ewes studied also had paraovarian cysts. Histologically, the ovaries were unremarkable. The cervix uteri had complex hypertrophied endometrial folds with cystic glandular hyperplasia and the endometrium displayed more glandular activity than was seen in controls. Adams[67] found that sheep grazing a pasture of subterranean clover (*Trifolium subterraneum*, isoflavone content 1.9% formononetin, 0.89% genistein, 1.04% biochanin A on a dry weight basis) developed excessive numbers of small and medium sized ovarian follicles, in many of which antrum formation was deficient. This was associated with early atresia of the follicles. In addition, the uterine submucosa and muscle layer were oedematous and subacute endometritis was present. Cervical crypt complexity and squamous metaplasia were also present.

In immature sheep, El Samannoudy *et al* showed that the phytoestrogen β-sitosterol leads to reduced ovarian weight with haemorrhage into cystic atretic follicles (comparable with the Stein-Loeventhal ovary in humans) when administered subcutaneously. β-Sitosterol is also uterotrophic in immature sheep.[68]

Effects on the oestrous cycle and fertility

In addition to producing the maturational and morphological abnormalities described above, environmental oestrogens can affect the oestrous cycle and cause infertility. Neonatal rats exposed to a low dose phytoestrogen in the first 5 days of postnatal life exhibit persistent cornification of vaginal epithelial cells on cytological examination by 4 months of age; evidence of a persistent oestrous state.[22] Such animals fail to respond to oestrogen priming followed by progesterone with a luteinising hormone (LH) surge at the expected time, suggesting a role for hypothalamic or pituitary responses in the acyclic condition.[22] A similar pattern of acyclicity with vaginal cornification is seen in mice treated with coumestrol in the neonatal period. Burroughs *et al* have repeatedly shown that this state persists even after bilateral oophorectomy, further evidence that exposure to phytoestrogens in the neonatal period has lasting effects on the hypothalamo–hypophysial–ovarian axis.[53 54 64] Similar effects are seen when neonatal mice receive natural or synthetic oestrogens.[64] Hughes *et al* found that orally administered genistein had no effect on tonic LH values in ovariectomised rats, but that low dose genistein (0.1 mg/kg) administered intravenously suppresses LH concentrations.[69] Genistein blocked the post gonadotrophin releasing hormone LH surge in such animals. In a follow up study, Hughes *et al* showed that neither genistein nor the mycoestrogens zearalenone or zearalenol provided oestrogenic priming for progesterone induced LH secretion, even though both genistein and zearalenol blocked gonadotrophin releasing hormone induced LH secretion.[70]

More recently, Murthy *et al* showed that a benzene extract from flowers of *H rosa sinensis* administered intraperitoneally to adult albino mice induced an irregular oestrous cycle, with a dose dependent prolonged oestrous and metestrus.[52] The ovaries of affected mice show a decrease in the number of Graafian follicles, an absence of corpora lutea, and an increased number of atretic follicles. In addition, the ovaries show a significant increase in cholesterol and ascorbic acid,

indicating the non-availability of pituitary gonadotrophins for steroidogenesis.

The effect of phytoestrogens on fertility in sheep (so called clover disease) and cattle is well described, with low birth rates, uterine prolapse, hydrops uteri, and pyometron. Phytoestrogen mediated infertility in sheep may be temporary or permanent. Temporary infertility occurs when adult ewes are grazed on oestrogenic pasture at the time of mating, with reduced ovulation and conception rates. Coumestrol concentrations as low as 25 ppm may be sufficient, but fertility returns to normal within four to six weeks of removal of the ewes to non-oestrogenic pasture. Prolonged exposure to oestrogenic pasture may cause permanent infertility as a result of the permanent redifferentiation of the cervix to resemble uterus, both microscopically and functionally. This is associated with loss of sexual characteristics, which is possible in sheep because the genes that control sexual differentiation are not fully deactivated at birth.[71]

The effect of phytoestrogens on the female genital tract is dependent upon the age at exposure and the duration of exposure. From the discussion above it is evident that neonatal exposure results in acyclicity and a persistent oestrous state, as demonstrated by vaginal cornification. In contrast, Cline and colleagues[72] have shown that although an oestrogenic response can be produced in postmenopausal macaques by conjugated equine oestrogens and by tamoxifen, the vaginal cytological pattern in animals treated with soybean phytoestrogens (genistein) at doses comparable to a human diet showed no difference to control animals. Similarly, Anthony et al have shown that a soybean isoflavone rich diet fed to peripubertal rhesus monkeys lowers their cardiovascular risk factors but does not affect the reproductive tract.[73] Interestingly, the serum SHBG concentration was also unaffected.

THE EFFECT OF ENVIRONMENTAL OESTROGENS ON THE HUMAN FEMALE GENITAL TRACT

As we have seen, there is considerable evidence to indicate that several naturally occurring and synthetic chemicals have weak oestrogenic activity that can affect the female reproductive tract of fish, rodents, and livestock. Despite the large body of literature describing the occurrence of such compounds within the human diet, there have been surprisingly few studies exploring the effects of phytoestrogens and xenoestrogens on the human female genital tract. However, there are some indications that xenoestrogens may play a role in the pathogenesis of breast cancer[74–76] and male genital tract abnormalities.[75 77] It is tempting to draw inferences from the animal work above, but it must remembered that there are pronounced interspecies variations in the metabolism of dietary oestrogens, notably the effects of ruminant digestion on phytoestrogens, which leads to their pronounced effects on sheep.[78] Similarly, the effects of coumestrol on the development of the neonatal rodent hypothalamic–pituitary axis[22] described above cannot be directly transposed to humans because the development of this axis in humans occurs in utero and rats have no SHBG.[56]

That such compounds are not currently regarded as an important cause of human female reproductive tract pathology may indicate that co-adaptive evolution has gradually rendered such agents effectively harmless, or that insufficient research has been undertaken to date. It is interesting to note that in evolutionary terms the chemical content of our diet has changed dramatically in recent times, the introduction of soy based infant formula and our exposure to DDT being notable examples. However, the metabolism of dietary isoflavones in humans is poorly understood and shows pronounced individual variability.[20]

It cannot be doubted that humans are exposed to phytoestrogens and synthetic oestrogens, and bioassays have been developed to determine the oestrogenicity of such agents isolated from human serum.[79 80] In one such analysis of human serum, Soto et al have recently shown that cumulative exposure to DDT metabolites has a synergistic oestrogenic effect.[81] Furthermore, Markiewicz et al have shown that isoflavenoids enhance alkaline phosphatase activity in a human endometrial adenocarcinoma cell line (Ishikawa-Var 1) and that the complexes formed between the human ER, oestradiol, and the isoflavenoids are functionally equivalent.[79]

Those studies that have been undertaken to explore the effect of exposure to environmental oestrogens in humans can be divided into two categories. The first body of evidence deals with the association between environmental oestrogen exposure and the risk of neoplasia in the genital tract. Of these, the most prominent is the now well established role of exposure to diethylstilboestrol in utero in the subsequent development of adenocarcinoma of the vagina and cervix. One in 1000 women exposed to diethylstilboestrol in utero will develop the disease.[82] Such exposure is iatrogenic, has been discussed elsewhere,[83 84] and given that it is not our aim in this review to discuss the effects of iatrogenically administered oestrogens, falls outside the scope of this review. The remainder of the literature explores the effects of environmental oestrogens on fertility and the oestrous cycle. The existing literature is confined to the effect of phytoestrogens on the female genital tract. To the best of our knowledge, the role of xenoestrogens in human female infertility and gynaecological neoplasia has not been studied.

Effects of environmental oestrogens on the menstrual cycle

Evidence is emerging that phytoestrogens may possibly exert an effect on the human menstrual cycle, although the findings are inconsistent.[75] In a study of six premenopausal women evaluated in a metabolic suite, Cassidy and colleagues[85] showed that a diet containing 60 mg soy protein/day (equivalent to approx 25 mg daidzein and 20 mg genistein/day) delays menstruation by one to five days and prolongs the follicular phase of the menstrual cycle by 2.5 ± 1.6 days. Such a prolongation of the menstrual cycle was significant when compared with the cycle length in the same women on a diet containing no soy protein. There was no change in the duration of the luteal phase. In addition, soy protein ingestion results in a significant suppression of the midcycle LH and follicle stimulating hormone (FSH) peak plasma concentrations, whereas the plasma concentration of oestradiol is increased and the concentration of cholesterol is reduced in the follicular phase. Thus, in premenopausal women, phytoestrogens apparently exert an antioestrogenic effect. However, the phytoestrogen diet did not affect the plasma SHBG concentration.[85] The addition of flax seeds (which contain the lignins enterodiol and enterolactone) to an omnivorous diet similarly lengthens the luteal phase of the menstrual cycle, although the overall cycle length remained unchanged.[8] This matter is far from settled, however. In a study of 14 women, Duncan et al found that a diet rich in soy isoflavones (128 mg $\pm$ 16mg/day) resulted in a modest reduction in plasma oestrone concentrations, but had no impact on the length of the menstrual cycle, follicular phase, or luteal phase.[86] Other groups have suggested that soy isoflavone ingestion in premenopausal women leads to unchanged follicular phase length, midcycle LH, and FSH[87]; increased,[88] unchanged,[87] or decreased[89] oestradiol; decreased[89] dehydroepiandrosterone sulfate; and decreased[89] or unchanged[85 88] luteal phase progesterone (reviewed in Cassidy and colleagues[85]).

It has been suggested that phytoestrogens may also modify the symptoms of the menopause. Lock[90] reported that in comparison with Canadian women, Japanese women are less likely to suffer from hot flushes during the menopause. Aldercreutz et al have hypothesised that the high isoflavenoid phytoestrogen content of the Japanese diet might be responsible

for this, noting that the urinary excretion of genistein, daidzein, and equol is much greater in Japanese than in North American women.[14 15] Albertazzi *et al* found that menopausal women given a daily dietary supplement of 60 g soy protein had a significant reduction (p = 0.01) in the mean number of hot flushes compared with a placebo group.[91] Dietary supplementation with wheat flour (which contains zearalenone) similarly reduces the frequency of hot flushes.[9] However, the findings are mixed and a series of studies has failed to demonstrate any effect of phytoestrogens on menopausal symptoms.[75]

In postmenopausal hysterectomised women receiving transdermal oestrogen, Nicholls *et al* have shown that dietary soy phytoestrogens mildly suppress the LH surge following a gonadotrophin releasing hormone challenge, and thus have a mild antioestrogenic effect.[92] The results failed to achieve significance however (possibly a function of the small sample size), and must be interpreted with caution. Baird and colleagues[93] noted that a diet rich in soy was associated with minor oestrogenic changes in vaginal cytology, but observed no alteration in LH or FSH. Duncan *et al* have shown that postmenopausal women receiving a high isoflavone diet (2.00 mg/kg/day) had modestly reduced serum oestradiol and oestrone values, although there was no significant effect on vaginal cytology or endometrial biopsy.[94] In contrast, Wilcox *et al* found that dietary supplementation with phytoestrogens led to increased vaginal cytological maturation.[95]

Environmental oestrogens and the risk of gynaecological malignancy

It is generally agreed that the high fat, low fibre diet of the industrialised world is associated with an increased risk of cancer, whereas a diet rich in fruit, vegetables, whole grains, and legumes is protective.[96 97] In a study comparing women with endometrial cancer with women admitted with non-gynaecological disorders, Levi *et al* have shown that a diet rich in vegetables, fresh fruit, whole grain bread, and pasta, independent of total energy intake, reduces the risk of endometrial carcinoma by 40–60%.[98]

We cannot immediately assume that the antioestrogenic effect of a diet rich in fruit, vegetables, and legumes (and hence fibre) is a result of the presence of phytoestrogens. Goldin and colleagues[99] and Rose and colleagues[100] have shown that a high fibre diet itself reduces the serum oestrogen concentration in premenopausal women. Supplementation of the diet with wheat bran significantly reduces the serum oestrone and oestradiol concentrations without altering the SHBG concentration.[100] The presence of fibre within the gut alters the enterohepatic circulation of oestrogens. Oestrogens conjugated in the liver to form biologically inactive glucuronides and sulfoglucuronides are excreted in the bile. These are metabolised by bacterial glucuronidase and sulfatase to active metabolites, which are reabsorbed. In vegetarian women, who consume less fat and more fibre than omnivorous women, there is a positive correlation between faecal weight and faecal excretion of oestrogens. Vegetarian women have significantly lower faecal β-glucuronidase activity, and a higher faecal weight. Thus, a greater proportion of the oestrogen excreted in the bile is lost in the faeces, and such women have a lower plasma oestrone and oestradiol concentration.[98] Postmenopausal women have a lower urinary excretion of oestradiol and a higher plasma SHBG concentration.[101]

Nonetheless, there is evidence to suggest that phytoestrogens themselves are cancer preventative agents.[102] Circumstantial evidence is available in the lower prevalence of endometrial cancer in Japanese women (who eat a diet rich in phytoestrogens) compared with American women.[103] Furthermore, irrespective of the amount of fibre consumed, women with endometrial cancer eat less vegetables, fruit, and dairy products than controls.[104] Goodman *et al* found that although

there was a negative correlation between dietary fibre intake and the risk of endometrial cancer, there was also an inverse correlation between soy protein consumption and the risk of endometrial cancer.[105] This also held true for other sources of phytoestrogens such as whole grains, vegetables, fruits, and seaweeds.

CONCLUSION

There is considerable evidence that environmental oestrogens impact upon the development of the female genital tract and subsequent fertility in fish, laboratory rodents, and livestock. Such oestrogenic compounds may be (1) naturally occurring compounds synthesised by plants to resist pathogens, (2) natural steroidal oestrogens or synthetic oestrogens excreted into the environment, or (3) chemicals synthesised for another purpose and found subsequently to have oestrogenic activity. In humans, there is growing evidence that such compounds may have an impact on male fertility and on the risk of developing breast cancer. The literature on the effect of environmental oestrogens on the human genital tract is surprising by its absence. It is evident that such compounds are in our environment, and that we ingest them. Furthermore, it is clear that they may affect the length of the human menstrual cycle. The true effect of such compounds on the pathophysiology of the female genital tract remains unknown. The animal data suggest that the timing of exposure to such compounds is crucial, with neonatal exposure having the most pronounced effects. Given the exposure of neonates to phytoestrogens, this should be a cause for concern. The impact of exposure to phytoestrogens in adulthood is also worthy of further study. Because there is clear evidence that natural and synthetic steroid oestrogens excreted into the environment may affect wildlife and also enter the human water supply, the impact of such agents deserves attention. Clearly, however, large scale longitudinal studies will be needed to separate the effects of environmental oestrogens from those of the abundant confounding factors.

Take home messages

- Environmental oestrogens have been implicated in the pathogenesis of hormonally treated cancers (such as breast and prostate cancer), male infertility, and abnormalities of the male and female reproductive tracts
- Environmental oestrogens may be derived from plants (phytoestrogens), pharmaceuticals, or other synthetic compounds not originally intended to have oestrogenic activity
- Exposure to these compounds results in structural and functional abnormalities in the female genital tract of fish, rodents, and livestock
- The age at first exposure and the duration of exposure are important, neonatal exposure having the potential to produce lasting morphological abnormalities and a persistent (gonad independent) oestrous state
- The human diet is rich in phytoestrogens, and such compounds are also present in soy based infant formulas, which may be a cause for concern
- To date, there is little evidence that such compounds affect human female genital tract development or fertility, probably because of the ubiquitous nature of such compounds in the environment and a lack of investigation, rather than the absence of a correlation
- Evidence is emerging, however, that phytoestrogens prolong the menstrual cycle, alleviate symptoms of the menopause, and protect against the development of endometrial carcinoma

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