100 vedeckých dôkazov o škodlivosti sóje

**Celé texty publikácií**


**Abstrakty odborných publikácií:**

Developmental effects of dietary phytoestrogens in Sprague-Dawley rats and interactions of genistein and daidzein with rat estrogen receptors alpha and beta in vitro.

Toxicol Sci 1999 Oct;51(2):236-44

Casanova M, You L, Gaido KW, Archibeque-Engle S, Janszen DB, Heck HA.

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Estrogenic isoflavones, such as genistein and daidzein, are present in virtually all natural-ingredient rodent diets that use soy as a source of protein. Since these compounds are endocrine-active, it is important to determine whether the amounts present in rodent diets are sufficient to affect sexual development. The present study consisted of in vitro and in vivo parts. In the in vitro portion, human hepatoma cells were transfected with either rat estrogen receptor (ER) alpha or beta plus an estrogen-responsive luciferase reporter gene. Genistein and daidzein were complete agonists at both ERs, genistein being more potent than daidzein, and both compounds were more potent at ER beta than ER alpha. In combined studies with estradiol, genistein exerted additive effects with estradiol in vitro. In the in vivo portion of the study, groups of six pregnant Sprague-Dawley females were fed one of the following four diets, and the pups were maintained on the same diets until puberty: (1) a natural-ingredient, open-formula rodent diet (NIH-07) containing 16 mg genistein and 14 mg daidzein per 100 g of feed;
Soy isoflavone supplements antagonize reproductive behavior and estrogen receptor alpha- and beta-dependent gene expression in the brain.

Endocrinology 2001 Jul;142(7):2946-52

Patisaul HB, Dindo M, Whitten PL, Young LJ.

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Epidemiological evidence suggests that isoflavone phytoestrogens may reduce the risk of cancer, osteoporosis, and heart disease, effects at least partially mediated by estrogen receptors alpha and beta (ERalpha and ERbeta). Because isoflavone dietary supplements are becoming increasingly popular and are frequently advertised as natural alternatives to estrogen replacement therapy, we have examined the effects of one of these supplements on estrogen-dependent behavior and ERalpha- and ERbeta-dependent gene expression in the brain. In the adult female rat brain, 17beta-estradiol treatment decreased ERbeta messenger RNA signal in the paraventricular nucleus by 41%, but supplement treatment resulted in a 27% increase. The regulation of ERbeta in the paraventricular nucleus is probably via an ERbeta-dependent mechanism. Similarly, in the ventromedial nucleus of the hypothalamus, supplement treatment diminished the estrogen-dependent up-regulation of oxytocin receptor by 10.5%. The regulation of oxytocin receptor expression in the ventromedial nucleus of the hypothalamus is via an ERalpha-dependent mechanism.

Effect of estradiol and soy phytoestrogens on choline acetyltransferase and nerve growth factor mRNAs in the frontal cortex and hippocampus of female rats.


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We report here the effects of oral micronized estradiol and soy phytoestrogens on uterine weight, choline acetyltransferase (ChAT) and nerve growth factor (NGF) mRNAs in the frontal cortex and hippocampus of ovariectomized young and retired breeder rats. Within each age category, 15 bilaterally ovariectomized rats were randomized equally into three groups: control (OVX), estradiol (E2), and soy phytoestrogens (SBE). The OVX rats were fed a casein/lactalbumin-based control diet; the E2 rats were fed with the control diet with added estradiol; and the SBE rats were fed with the control diet with added soy phytoestrogens. After 8 weeks of
treatment, blood, uteri, frontal cortex, and hippocampus were collected at necropsy. Results showed that the uterine weights and serum estradiol concentrations were significantly higher in the E2 group compared with those in the OVX and SBE groups. In the hippocampus of young rats, E2 treatment resulted in significantly higher NGF mRNA levels than no treatment (OVX), and NGF mRNA levels in the SBE group were intermediate between the E2 and OVX groups. ChAT mRNA levels were significantly higher in the frontal cortex of E2 and SBE-treated retired breeder rats compared to OVX retired breeder rats. There were no differences among treatment groups for ChAT mRNA levels in the frontal cortex of young rats and in the hippocampus of both young and retired breeder rats. Our data suggest that soy phytoestrogens may function as estrogen agonists in regulating ChAT and NGF mRNAs in the brain of female rats.


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Genistein, a phytoestrogen, was subcutaneously (s.c.) injected to pregnant Sprague-Dawley CD rats on gestational days 16-20 at either 25 mg (Group 1) or 5 mg/day (Group 2). Female offspring of mothers not exposed to genistein during pregnancy received 12.5 mg genistein s.c. at neonatal days 15 and 18 (Group 3), or received vehicle only (Group 4). At 35 days of age, 4-9 female offspring from each group were autopsied to observe the influence of genistein, and remainder of female offspring received 50 mg/kg N-methyl-N-nitrosourea (MNU) intraperitoneally and were sacrificed when mammary tumors were larger than 1 cm in size or when they reached 35 weeks of age. Genistein treatment during the perinatal period resulted in lower body weight and lower relative uterine-ovarian weight at 35 days, and a prolonged estrus cycle with a long estrus phase at 12-16 weeks. However, at 35 days (time at MNU administration), mammary gland development, cell proliferation rate (PCNA labeling index), and the number of estrogen receptor (ER)- and progesterone receptor (PgR)-positive cells were similar between genistein-treated and untreated rats. Twenty-five or 5 mg genistein/day in utero (between days 16 and 20 of gestation) or 12.5 mg genistein/day on neonatal days 15 and 18 did not affect the incidence of mammary tumors > 1 cm or the latency but did increase the number of mammary cancer lesions when MNU was administered at the time when the mammary gland growth in genistein-treated and untreated rats was similar. Thus, perinatal genistein is an endocrine disrupter and increases the multiplicity of MNU-induced mammary carcinoma in rats.

Neurobehavioral actions of coumestrol and related isoflavonoids in rodents.

Neurotoxicol Teratol 2002 Jan-Feb;24(1):47-54

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Isoflavonoids are plant estrogens that are increasingly advocated as a natural alternative to estrogen replacement therapy (ERT) and are available as dietary supplements. As weak estrogen agonists/antagonists with a range of other enzymatic activities, the isoflavonoids provide a useful model for the actions of endocrine disruptors. This paper reviews the responses of rodents to diets containing coumestrol or an isoflavone supplement in comparison to animals fed the phytoestrogen-free AIN76A diet. Neural mechanisms were investigated by examining isoflavonoid effects on ER(alpha)-dependent (regulation of oxytocin receptor [OTR] binding in the ventromedial nucleus of the hypothalamus [VMN]) and ERbeta-dependent (regulation of oxytocin receptor [OTR] binding in the ventromedial nucleus of the hypothalamus [VMN]) endpoints. Activational as well as organizational effects on sexual behavior and gonadotropin secretion were observed for coumestrol. Treatment of rat dams with a 100-ppm coumestrol diet from birth to postnatal day (PND) 21 induced premature anovulation in female offspring, and treatment from birth to PND 10 suppressed sexual behavior in male offspring. One-week treatment of ovariectomized (OVX) female rats with the same coumestrol diet increased ERbeta mRNA expression in the PVN, an effect opposite to that of estradiol. Ten days of treatment with a 200-ppm coumestrol diet increased LH secretion in OVX wild-type mice, an effect opposite to the normal negative feedback effects of estradiol. No effects were observed in ER(alpha) knockout (ER(alpha)KO)-OVX females, indicating that coumestrol's action on LH was mediated through ER(alpha). Similar activational effects were observed for the isoflavone diet. The
Lordotic response to estrogen was significantly reduced by 2 days of treatment of OVX adult females with an isoflavone diet providing 13 ppm genistein and 33 ppm daidzein. One week of treatment with the same isoflavone diet produced an effect opposite to that of estradiol in the PVN, increasing ERbeta mRNA expression above control levels. These investigations show that, in spite of their preferential affinity for ERbeta, isoflavonoids act through both ER(alpha) and ERbeta. Moreover, their neurobehavioral actions were antiestrogenic, either antagonizing or producing an action in opposition to that of estradiol. This work demonstrates that even small, physiologically relevant exposure levels can alter estrogen-dependent gene expression in the brain and complex behavior.

Cross-species and interassay comparisons of phytoestrogen action.
Whitten PL, Patisaul HB.
Environ Health Perspect 2001 Mar;109 Suppl 1:5-20
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This paper compiles animal and human data on the biologic effects and exposure levels of phytoestrogens in order to identify areas of research in which direct species comparisons can be made. In vitro and in vivo assays of phytoestrogen action and potency are reviewed and compared to actions, dose-response relationships, and estimates of exposure in human subjects. Binding studies show that the isoflavonoid phytoestrogens are high-affinity ligands for estrogen receptors (ERs), especially ER beta, but have lower potency in whole-cell assays, perhaps because of interactions with binding proteins. Many other enzymatic actions require concentrations higher than those normally seen in plasma. In vivo data show that phytoestrogens have a wide range of biologic effects at doses and plasma concentrations seen with normal human diets. Significant in vivoteresponses have been observed in animal and human tests for bone, breast, ovary, pituitary, vasculature, prostate, and serum lipids. The doses reported to be biologically active in humans (0.4–10 mg/kg body weight/day) are lower than the doses generally reported to be active in rodents (10–100 mg/kg body weight/day), although some studies have reported rodent responses at lower doses. However, available estimates of bioavailability and peak plasma levels in rodents and humans are more similar. Steroidogenesis and the hypothalamic–pituitary–gonadal axis appear to be important loci of phytoestrogen actions, but these inferences must be tentative because good dose-response data are not available for many end points. The similarity of reported proliferative and antiproliferative doses illustrates the need for fuller examination of dose-response relationships and multiple end points in assessing phytoestrogen actions.

Placental transfer of the soy isoflavone genistein following dietary and gavage administration to Sprague Dawley rats.
Reprod Toxicol 2001 Mar-Apr;15(2):105-10
Doerge DR, Churchwell MI, Chang HC, Newbold RR, Delclos KB.
Division of Biochemical Toxicology, National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, AR 72079, USA. ddoege@nctr.fda.gov
Genistein, the principal soy isoflavone, has estrogenic activity and is widely consumed by humans for putative beneficial health effects. The goal of the present study was to measure placental transfer of genistein in rats as a possible route of developmental exposure. Pregnant Sprague-Dawley rats were administered genistein orally, either by diet or by gavage. Concentrations of genistein aglycone and conjugates were measured in maternal and offspring serum and brain using HPLC with isotope dilution electrospray tandem mass spectrometry. Although fetal or neonatal serum concentrations of total genistein were approximately 20-fold lower than maternal serum concentrations, the biologically active genistein aglycone concentration was only 5-fold lower. Fetal brain contained predominately genistein aglycone at levels similar to those in the maternal brain. These studies show that genistein aglycone crosses the rat placenta and can reach fetal brain from maternal serum genistein levels that are relevant to those observed in humans.
Mass spectrometric determination of Genistein tissue distribution in diet-exposed Sprague-Dawley rats.

J Nutr 2000 Aug;130(8):1963-70

Chang HC, Churchwell MI, Delclos KB, Newbold RR, Doerge DR.

Division of Biochemical Toxicology, National Center for Toxicological Research, Jefferson, AR 72079, USA.

Genistein, the principal soy isoflavone, was administered in the diet to male and female Sprague-Dawley rats as part of a multigeneration study of potential endocrine modulation. The rats were exposed to genistein in utero, through maternal milk, and as adults through postnatal d 140 via essentially isoflavone-free feed (approximately 0.5 microg/g) fortified at 5, 100 and 500 microg/g with genistein aglycone. Analytical methods based on liquid chromatography, mass spectrometry and the use of deuterated genistein were developed and validated for use in measuring genistein in serum and tissues. Pharmacokinetic analysis of serum genistein showed a significant difference (P < 0.001) in the elimination half-life and area under the concentration-time curve between male [2.97 +/- 0.14 h and 22.3 +/- 1.2 micromol/(L. h), respectively] and female rats [4.26 +/- 0.29 h and 45.6 +/- 3.1 micromol/(L. h), respectively, +/- SEM]. Endocrine-responsive tissues including brain, liver, mammary, ovary, prostate, testis, thyroid and uterus showed significant dose-dependent increases in total genistein concentration. Female liver contained the highest amount of genistein (7.3 pmol/mg tissue) and male whole brain contained the least (0.04 pmol/mg). The physiologically active aglycone form was present in tissues at fractions up to 100%, and the concentration was always greater than that observed in serum in which conjugated forms predominated (95-99%). These results for measured amounts of genistein, present as aglycone and conjugates, in putative target tissues provide a link with other studies in which blood concentrations and physiologic effects of genistein are measured.

Genistein exerts estrogen-like effects in male mouse reproductive tract.

Mol Cell Endocrinol 1998 Sep 25;144(1-2):83-93

Strauss L, Makela S, Joshi S, Huhtaniemi I, Santti R.

University of Turku, Institute of Biomedicine, Department of Anatomy and Medicity Research Laboratory, Turku, Finland.

The aim of this study was to evaluate the estrogenicity of genistein in the neonatal and adult male mouse reproductive tract. In intact adults, genistein (2.5 mg s.c./kg of body weight/day for 9 days) reduced testicular and serum testosterone concentrations, pituitary LH-content and prostate weight. In castrated adults, genistein (0.025-2.5 mg s.c./kg of body weight) increased expression of c-fos gene in prostatic urethra. In adult, neonatally estrogenized mice showing an increased estrogen sensitivity, a 10-day treatment with genistein (2.5 mg s.c./kg of body weight) induced development of squamous epithelial metaplasia in prostatic collecting ducts. Neonatally, only a very high dose of genistein (1 mg/pup per day; i.e. approximately 500 mg/kg of body weight) induced persistent structural changes, similar to those seen in mice treated neonatally with diethylstilbestrol, in the urethroprostastic complex. These results suggest that in adult males, genistein induces the typical estrogenic effects in doses comparable to those present in soy-based diets, while in neonatal animals, considerably higher doses are required to show estrogen-like activity.

Maternal exposure to genistein during pregnancy increases carcinogen-induced mammary tumorigenesis in female rat offspring.

Oncol Rep 1999 Sep-Oct;6(5):1089-95

Hilakivi-Clarke L, Cho E, Onojafe I, Raygada M, Clarke R.

Research Bldg., Lombardi Cancer Center, Georgetown University, NW, Washington, DC 20007-2197, USA.

A high estrogenic environment in utero may increase subsequent breast cancer risk. It was therefore determined whether a maternal exposure during pregnancy to the phytoestrogen genistein or zearalenone, both of which exhibit estrogenic activities in vitro and in vivo, alters breast cancer risk among female offspring.
Pregnant rat dams were treated daily with subcutaneous injections of 20, 100 or 300 microgram genistein, 20 microgram zearalenone, or vehicle between days 15 and 20 of gestation. The offspring were given 7, 12-dimethylbenz(a)anthracene (DMBA) at the age of 2 months to induce mammary tumors. The results indicate that in utero exposure to genistein, but not to zearalenone, dose-dependently increased the incidence of DMBA-induced mammary tumors, when compared with the controls. Tumor growth characteristics were not altered. Prior to the carcinogen administration, the number of estrogen receptor (ER) binding sites, determined using a ligand binding assay, were significantly elevated in the mammary glands of genistein offspring. In contrast, the mammary protein kinase C (PKC) activity was significantly reduced in the genistein offspring. Our results suggest that a maternal exposure to subcutaneous administration of genistein can increase mammary tumorigenesis in the offspring, mimicking the effects of in utero estrogenic exposures. Furthermore, increased ER protein levels and reduced PKC activity in the mammary gland may be involved in increasing susceptibility to carcinogen-induced mammary tumorigenesis in rats exposed to genistein in utero.

Enhancement of experimental colon cancer by genistein.

Cancer Res 1997 Sep 1;57(17):3717-22
Division of Nutritional Carcinogenesis, American Health Foundation, Valhalla, New York 10595, USA.

Several phytochemicals and micronutrients that are present in fruits and vegetables are known to exert cancer chemopreventive effects in several organs, including the colon. Among them, the soybean isoflavonoid genistein received much attention due to its potential anticarcinogenic, antiproliferative effects and its potential role in several signal transduction pathways. The present study was designed to investigate the effect of genistein on azoxymethane (AOM)-induced colon carcinogenesis and to study its modulatory role on the levels of activity of 8-isoprostane, cyclooxygenase (COX), and 15-hydroxyprostaglandin F2alpha dehydrogenase (15-PGDH) in the colonic mucosa and colon tumors of male F344 rats. At 5 weeks of age, groups of male F344 rats were fed control (AIN-76A) diet or a diet containing 250 ppm genistein. Beginning 2 weeks later, all animals except those in the vehicle-treated groups were given weekly s.c. injections of AOM (15 mg/kg body weight) for 2 successive weeks. All rats were continued on their respective dietary regimen for 52 weeks after AOM treatment and were then sacrificed. Colon tumors were evaluated histopathologically. Colonic mucosae and tumors were analyzed for COX, 15-PGDH, and 8-isoprostane levels. Administration of genistein significantly increased noninvasive and total adenocarcinoma multiplicity (P < 0.01) in the colon, compared to the control diet, but it had no effect on the colon adenocarcinoma incidence nor on the multiplicity of invasive adenocarcinoma (P > 0.05). Also, genistein significantly inhibited the 15-PGDH activity (>35%) and levels of 8-isoprostane (50%) in colonic mucosa and in tumors. In contrast, genistein had no significant effect on the COX synthetic activity, as measured by the rate of formation of prostaglandins and thromboxane B2 from [14C]arachidonic acid. The results of this investigation emphasize that the biological effects of genistein may be organ specific, inhibiting cancer development in some sites yet showing no effect or an enhancing effect on the tumorigenesis at other sites, such as the colon. The inhibition of 8-isoprostane levels by genistein indicates its possible antioxidant potential, which is independent of the observed colon tumor enhancement, yet this agent may also possess several biological effects that overshadow its antioxidant potential. The exact mechanism(s) of colon tumor enhancement by genistein remain to be elucidated; it is likely that its colon tumor-enhancing effects may, at least in part, be related to inhibition of prostaglandin catabolic enzyme activities.

p53, mutations, and apoptosis in genistein-exposed human lymphoblastoid cells.

Mutat Res 1998 Aug 31;405(1):41-56
Morris SM, Chen JJ, Domon OE, McGarrity LJ, Bishop ME, Manjanatha MG, Casciano DA.
Division of Genetic and Reproductive Toxicology, National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR 72079, USA. smorris@nc.tr.fda.gov

The phytoestrogen, genistein, is a naturally occurring isoflavone found in soy products. On a biochemical basis, genistein is a competitive inhibitor of tyrosine kinases and the DNA synthesis-related enzyme, topoisomerase-II (topo-II). Exposure of mammalian cells to genistein results in DNA damage that is similar to that induced by the
to in order to determine the potential genotoxicity of genistein, human lymphoblastoid cells which differ in the functional status of the tumor suppressor gene, p53, were exposed to genistein and the induction of micronuclei quantified by microscopic analysis. In addition, the mutant fraction at the thymidine kinase (tk) locus (both the normal-growth and slow-growth phenotypes) was determined by resistance to trifluoroethymidine (TFT) and at the hypoxanthine phosphoribosyl transferase (hprt) locus by resistance to 6-thioguanine (6-TG). Flow cytometric analysis of the percentage of viable, apoptotic and degenerating cells was utilized to determine the rate and kinetics of cell death after genistein exposure. The detection of micronuclei in both cell lines indicated that genistein-induced damage had occurred in both AHH-1 tk+/- and L3. Linear regression analysis detected a significant increase in the number of 6-TG-resistant clones in both AHH-1 tk+/- (p53+/-) and L3 (p53+/+). A comparison of slopes revealed no difference between the lines. In contrast, a significant, concentration-dependent increase in the number of TFT-resistant clones with the slow-growth phenotype was detected in AHH-1 tk+/- (mutant p53), but not in L3 (wild-type p53). Cell death occurred primarily by apoptosis in both cell lines; however, a concentration-dependent decrease in the percentage of viable cells was detected immediately after exposure in L3, but not until 32 h after exposure in AHH-1 tk+/-.

Our results may be interpreted that genistein is a chromosomal mutagen and that p53 functional status affects the recovery of chromosomal mutants, possibly by signalling cells into the apoptosis pathways.

Placental transfer of the soy isoflavone genistein following dietary and gavage administration to Sprague Dawley rats.

Reprod Toxicol 2001 Mar-Apr;15(2):105-10

Doerge DR, Churchwell MI, Chang HC, Newbold RR, Delclos KB.

Division of Biochemical Toxicology, National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, AR 72079, USA. ddoege@nctr.fda.gov

Genistein, the principal soy isoflavone, has estrogenic activity and is widely consumed by humans for putative beneficial health effects. The goal of the present study was to measure placental transfer of genistein in rats as a possible route of developmental exposure. Pregnant Sprague-Dawley rats were administered genistein orally, either by diet or by gavage. Concentrations of genistein aglycone and conjugates were measured in maternal and offspring serum and brain using HPLC with isotope dilution electrospray tandem mass spectrometry. Although fetal or neonatal serum concentrations of total genistein were approximately 20-fold lower than maternal serum concentrations, the biologically active genistein aglycone concentration was only 5-fold lower. Fetal brain contained predominately genistein aglycone at levels similar to those in the maternal brain. These studies show that genistein aglycone crosses the rat placenta and can reach fetal brain from maternal serum genistein levels that are relevant to those observed in humans.

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A high estrogenic environment in utero may increase subsequent breast cancer risk. It was therefore determined whether a maternal exposure during pregnancy to the phytoestrogen genistein or zearalenone, both of which exhibit estrogenic activities in vitro and in vivo, alters breast cancer risk among female offspring. Pregnant rat dams were treated daily with subcutaneous injections of 20, 100 or 300 microgram genistein, 20 microgram zearalenone, or vehicle between days 15 and 20 of gestation. The offspring were given 7, 12-dimethylbenz(a)anthracene (DMBA) at the age of 2 months to induce mammary tumors. The results indicate that in utero exposure to genistein, but not to zearalenone, dose-dependently increased the incidence of DMBA-induced mammary tumors, when compared with the controls. Tumor growth characteristics were not altered. Prior to the carcinogen administration, the number of estrogen receptor (ER) binding sites, determined using a ligand binding assay, were significantly elevated in the mammary glands of genistein offspring. In
contrast, the mammary protein kinase C (PKC) activity was significantly reduced in the genistein offspring. **Our results suggest that a maternal exposure to subcutaneous administration of genistein can increase mammary tumorigenesis in the offspring, mimicking the effects of in utero estrogenic exposures.** Further, increased ER protein levels and reduced PKC activity in the mammary gland may be involved in increasing susceptibility to carcinogen-induced mammary tumorigenesis in rats exposed to genistein in utero.

**Combined effects of dietary phytoestrogen and synthetic endocrine-active compound on reproductive development in Sprague-Dawley rats: genistein and methoxychlor.**

Toxicol Sci 2002 Mar;66(1):91-104

You L, Casanova M, Bartolucci EJ, Fryszymski MW, Dorman DC, Everitt JI, Gaido KW, Ross SM, Heck HD H.

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Humans and wildlife are frequently exposed to mixtures of endocrine active-compounds (EAC). The objective of the present study was to investigate the potential of the phytoestrogen genistein to influence the reproductive developmental toxicity of the endocrine-active pesticide methoxychlor. Three levels of genistein (0, 300, or 800 ppm) and two levels of methoxychlor (0 or 800 ppm) were used in this study. Sprague-Dawley rats were exposed to the two compounds, either alone or in combinations, through dietary administration to dams during pregnancy and lactation and to the offspring directly after weaning. Both compounds, methoxychlor in particular, were associated with reduced body growth at 800 ppm, but pregnancy outcome was not affected by either treatment. **An acceleration of vaginal opening (VO) in the exposed female offspring was the only observed effect of genistein at 300 ppm.** Exposure to 800 ppm genistein or 800 ppm methoxychlor caused accelerated VO **and also altered estrous cyclicity toward persistent estrus in the female offspring.** The estrogenic responses to genistein and methoxychlor administered together were apparently accumulative of the effects associated with each compound alone. **Methoxychlor, but not genistein, delayed preputial separation (PPS) in the male rats.** When administered with methoxychlor, genistein at 800 ppm enhanced the effect of methoxychlor on delaying PPS. Genistein and methoxychlor treatment did not change gender-specific motor activity patterns in either sex. To explore possible mechanisms for interaction between the two compounds on development, we performed estrogen receptor (ER)- and androgen receptor (AR)-based in vitro transcriptional activation assays using genistein and the primary methoxychlor metabolite 2,2-bis-(p-hydroxyphenyl)-1,1,1-trichloroethane (HPTPE). While the in vitro assays supported the estrogenic effects of genistein and methoxychlor and the antiandrogenic effects of methoxychlor, the reactivity of these compounds with ERs alpha and beta could not predict the greater in vivo estrogenic potency of methoxychlor over genistein; nor could the potentiation of the methoxychlor effect on PPS by genistein be predicted based on in vitro HPTPE and genistein reactions with the AR. **Data from this study indicate that phytoestrogens are capable of altering the toxicological behaviors of other EACs, and the interactions of these compounds may involve complexities that are difficult to predict based on their in vitro steroid receptor reactivities.**

**Effects of dietary genistein exposure during development on male and female CD (Sprague-Dawley) rats.**


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Genistein is a naturally occurring isoflavone that interacts with estrogen receptors and multiple other molecular targets. **Human exposure to genistein is predominantly through consumption of soy products, including soy-based infant formula and dietary supplements.** A dose range-finding study was conducted as a prelude to a multigeneration bioassay to assess potential toxicities associated with genistein consumption. Genistein was administered in a soy- and alfalfa-free diet at 0, 5, 25, 100, 250, 625, or 1250 ppm to pregnant dams starting on Gestation day 7 and continuing throughout pregnancy. Dietary exposure of the dams continued through lactation,
and pups were maintained on the same dosed feed as their mother after weaning until sacrifice at Postnatal day 50. Body weight and feed consumption of the treated dams prior to parturition showed a decreasing trend with a significant reduction at the highest dose. Litter birth weight was depressed in the 1250 ppm dose group, and pups of both sexes in that dose group had significantly decreased body weights relative to controls at the time of sacrifice. The most pronounced organ weight effects in the pups were decreased ventral prostate weight in males at the 1250 ppm dose and a trend toward higher pituitary gland to body weight ratios in both sexes. Histopathologic examination of female pups revealed ductal/alveolar hyperplasia of the mammary glands at 250 to 1250 ppm. Ductal/alveolar hyperplasia and hypertrophy also occurred in males, with significant effects seen at 25 ppm and above. Abnormal cellular maturation in the vagina was observed at 625 and 1250 ppm, and abnormal ovarian antral follicles were observed at 1250 ppm. In males, aberrant or delayed spermatogenesis in the seminiferous tubules relative to controls was observed at 1250 ppm. There was a deficit of sperm in the epididymis at 625 and 1250 ppm relative to controls, although testicular spermatid head counts and epididymal spermatozoa counts did not show significant differences from controls at these doses. Both sexes showed an increase in the incidence and/or severity of renal tubal mineralization at doses of 250 ppm and above. Dietary genistein thus produced effects in multiple estrogen-sensitive tissues in males and females that are generally consistent with its estrogenic activity. These effects occurred within exposure ranges achievable in humans.

Uterine Adenocarcinoma in Mice Treated Neonatally with Genistein

Cancer Research 61, 4325-4328, June 1, 2001

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The developing fetus is uniquely sensitive to perturbation with estrogenic chemicals. The carcinogenic effect of prenatal exposure to diethylstilbestrol (DES) is the classic example. Because phytoestrogen use in nutritional and pharmaceutical applications for infants and children is increasing, we investigated the carcinogenic potential of genistein, a naturally occurring plant estrogen in soy, in an experimental animal model previously reported to result in a high incidence of uterine adenocarcinoma after neonatal DES exposure. Outbred female CD-1 mice were treated on days 1–5 with equivalent estrogenic doses of DES (0.001 mg/kg/day) or genistein (50 mg/kg/day). At 18 months, the incidence of uterine adenocarcinoma was 35% for genistein and 31% for DES. These data suggest that genistein is carcinogenic if exposure occurs during critical periods of differentiation. Thus, the use of soy-based infant formulas in the absence of medical necessity and the marketing of soy products designed to appeal to children should be closely examined.

Incidence of squamous neoplasia of the cervix and vagina in women exposed prenatally to diethylstilbestrol (United States).


Abstract

Objectives: Women exposed prenatally to diethylstilbestrol (DES) have an excess risk of clear-cell adenocarcinoma of the vagina and cervix, but the effect on the incidence of squamous neoplasia is uncertain. The purpose of the current study was to evaluate the long-term risk of developing high-grade squamous neoplasia of the genital tract among women exposed prenatally to DES.

Methods: A cohort comprising 3899 DES-exposed and 1374 unexposed daughters was followed for 13 years (1982-1995) for pathology-confirmed diagnoses of high-grade squamous intraepithelial neoplasia (HSIL) of the genital tract. Poisson regression analysis was used to compute relative risks (RR) and 95% confidence intervals (95% CI), adjusting for age, calendar year, and other covariates.
Results: The RR (95% CI) among DES-exposed versus unexposed, based on 111 cases of high-grade disease, was 2.1 (1.2-3.8). Adjustment for screening history estimated by the number of years since the last Pap smear had little effect. Risk estimates were higher with earlier intrauterine exposure; the RR (95% CI) for exposure within 7 weeks of the last menstrual period was 2.8 (1.4-5.5). Only two cases of invasive squamous cervical cancer occurred in total, precluding separate analysis.

Conclusions: The findings support an association between in-utero DES exposure and high-grade squamous neoplasia, although a role for more intensive screening among DES-exposed women in the production of this excess could not be completely ruled out.

The effect of neonatal exposure to diethylstilbestrol, coumestrol, and beta-sitosterol on pituitary responsiveness and sexually dimorphic nucleus volume in the castrated adult rat.

Department of Obstetrics and Gynecology, Duke University Medical Center, Durham, North Carolina 27710.

The neonatal hormone environment influences the sexually differentiated patterns of development. Estrogens, derived from intracerebral aromatization, promote male pattern development of the central nervous system. The purpose of this study was to determine the effects of neonatal exposure to environmental estrogens on luteinizing hormone (LH) secretion and development of the sexually dimorphic nucleus of the medial preoptic area (SDN-POA) in castrated adult rats. Neonatal rats of both sexes received injections of either corn oil, 0.1 microgram diethylstilbestrol (DES), 3 micrograms beta-sitosterol (B1), 30 micrograms beta-sitosterol (B2), 0.1 microgram coumestrol (C1), 1 microgram coumestrol (C2), or 10 micrograms coumestrol (C3) on Day 1-10 of life and were castrated on Day 21. Right heart catheters were placed on Day 42, and GnRH (50 ng/kg) was administered. Blood was sampled for LH at 0-, 5-, 10-, 15-, and 30-min intervals. All doses of beta-sitosterol and coumestrol elicited increased basal levels of LH in females. In males, B1, B2, C2, and C3 increased basal levels of LH. The GnRH-induced LH increase was prevented in females treated with diethylstilbestrol and 10 micrograms of coumestrol. Males in all treatment groups exhibited GnRH-induced LH surges. The animals were sacrificed by decapitation on Day 49. Volumes of the SDN-POA of the groups were compared. Treatment with the agents did not result in significantly increased SDN volume in females; nor was there a difference in SDN size among the male groups. These data show that exposure to environmental estrogens early in development alters both postpubertal pituitary response to GnRH and basal LH secretion in females and alters only basal LH secretion in males. No significant enlargement (i.e., masculinization) of the SDN-POA was exhibited.

Quotes

B-sitosterol may compete with cholestrol and interfere with the synthesis of gonadal steroidal hormones.

B-sitosterol may create a neonatal environment with low endogenous levels of estrogens

The hormonal environment during the critical period exerts permanent organisational effects that may affect the behaviour in adult animals.

The phytoestrogen genistein induces thymic and immune changes: A human health concern?

Srikanth Yellayi*, Afia Naaz*, Melissa A. Szewczykowski*, Tomomi Sato*, Jeffrey A. Woods, Jongsoo Chang§, Mariangela Segre¶, Clint D. Allred§, William G. Helferich§, and Paul S. Cooke*

Departments of * Veterinary Biosciences, Kinesiology, ‡ Food Science and Human Nutrition, and § Veterinary Pathobiology, and Division of Nutritional Sciences, University of Illinois, Urbana, IL 61802
Use of soy-based infant formulas and soy/isoflavone supplements has aroused concern because of potential estrogenic effects of the soy isoflavones genistein and daidzein. Here we show that s.c. genistein injections in ovariectomized adult mice produced dose-responsive decreases in thymic weight of up to 80%. Genistein's thymic effects occurred through both estrogen receptor (ER) and non-ER-mediated mechanisms, as the genistein effects on thymus were only partially blocked by the ER antagonist ICI 182,780. Genistein decreased thymocyte numbers up to 86% and doubled apoptosis, indicating that the mechanism of the genistein effect on loss of thymocytes is caused in part by increased apoptosis. Genistein injection caused decreases in relative percentages of thymic CD4+CD8 and double-positive CD4+CD8+ thymocytes, providing evidence that genistein may affect early thymocyte maturation and the maturation of the CD4+CD8 helper T cell lineage. Decreases in the relative percentages of CD4+CD8 thymocytes were accompanied by decreases in relative percentages of splenic CD4+CD8 cells and a systemic lymphocytopenia. In addition, genistein produced suppression of humoral immunity. Genistein injected at 8 mg/kg per day produced serum genistein levels comparable to those reported in soy-fed human infants, and this dose caused significant thymic and immune changes in mice. Critically, dietary genistein at concentrations that produced serum genistein levels substantially less than those in soy-fed infants produced marked thymic atrophy. These results raise the possibility that serum genistein concentrations found in soy-fed infants may be capable of producing thymic and immune abnormalities, as suggested by previous reports of immune impairments in soy-fed human infants.

The phytoestrogen beta-sitosterol alters the reproductive endocrine status of goldfish.

MacLatchy DL, Van Der Kraak GJ.
Department of Zoology, College of Biological Science, University of Guelph, Ontario, Canada.

There is a growing awareness that chemicals in the environment may function as hormone mimics and affect endocrine function in wildlife. In this study, the effects of beta-sitosterol, a phytoestrogen present in high concentration in bleached kraft pulp mill effluent (BKME), on reproductive fitness of goldfish were investigated. Plasma reproductive hormone levels were measured in male and female goldfish on Day 4 following two intraperitoneal injections of beta-sitosterol or an oxidized sitosterol preparation. In some experiments, plasma hormone levels were also measured after fish were injected with Ovaprim, which contains a superactive analog of salmon GnRH and the dopamine receptor antagonist domperidone and leads to increased secretion of gonadotropin (GtH)-II (LH-type GtH). Plasma testosterone (T) and 11-ketotestosterone levels in males and T and 17 beta-estradiol levels in females were significantly decreased in beta-sitosterol-treated fish on Day 4 and 24 hr after an injection of Ovaprim. Plasma GtH-II levels were elevated in male fish treated with beta-sitosterol on Day 4 and further increased in response to Ovaprim, suggesting that reduced plasma steroid levels were not due to effects on pituitary function. In other studies, testes pieces from beta-sitosterol-treated goldfish produced reduced levels of T and pregnenolone in vitro both basally and in response to the GtH-II agonist human chorionic gonadotropin (hCG) when compared to the testes from control fish. Basal and hCG-stimulated pregnenolone and hCG-stimulated T were reduced in follicles from beta-sitosterol-treated fish; however, basal T production was not different from controls. These results suggest that beta-sitosterol reduces the gonadal steroid biosynthetic capacity through effects on cholesterol availability or the activity of the side chain cleavage enzyme P450SCC. These findings raise the possibility that beta-sitosterol could be a contributing factor to the reproductive dysfunction observed in fish exposed to BKME.

Potential value of plants as sources of new antifertility agents I.

Quotes
The world population explosion has pointed out the need for new and effective contraceptive agents...
...it can be seen that 565 species of plants are known to have a folkloric reputation for use as abortifacients, ecbolics, or emmenagogues. Of this 565 species, 225 have been shown to elicit a stimulant response when tested against uterine muscle either in vitro or in vivo.

Potential value of plants as sources of new antifertility agents II.


Quotes

A number of estrogenic sterols have been reported as being derived from higher plants

If one inspects the structures of the estrogenic sterols, isoflavones, and coumestans, one can see a striking similarity of the skeletal structures of these compounds with the structure of the synthetic estrogen diethylstilbestrol.

The order or degree of the biological activity of each of the three main groups of natural estrogens has been investigated. The sterol estrogens were found to be of the highest order of activity followed by the coumestrols and then the isoflavones.

Breast feeding and insulin dependent diabetes mellitus in children

Journal of the American College of Nutrition 1986; 5: 439-441


Department of Pediatrics, North Shore University Hospital, Manhasset and Department of Pediatrics, Cornell University Medical College, New York.

Abstract

We have evaluated the hypothesis of a protective effect of human milk on the development of insulin dependent diabetes mellitus (IDDM). We studied the feeding histories of 95 diabetic children and compared them with controls consisting of their non-diabetic siblings and a pair matched group of non-diabetic peers of the same age, sex, geographical location and social background. The incidence of breast feeding in diabetic children was 18%. This was similar to the control group. The duration of breast feedings was also similar among all three groups. There was no difference in the age of introduction of solid food between diabetic and non-diabetic children. Twice as many diabetic children, however, received soy containing formula in infancy as compared to control children. The mean age of onset of IDDM was not related to the type of feeding during infancy. The incidence of positive thyroid antibodies was two and one half times higher in formula-fed diabetic children that in breast-fed ones. In our studies we were unable to document any relationship between the history of breast feeding and subsequent development of IDDM in children.

Quotes

RECOMMENDATIONS

1. Breast-feeding is strongly endorsed as the primary source of nutrition during the first year of life for all infants.

2. In families with a strong history of IDDM, particularly if a sibling has diabetes, breast-feeding and avoidance of commercially available cow's milk and products containing intact cow's milk protein during the first year of life are strongly encouraged.
3. Since the antigenicity of infant formulas and cow’s milk may be different and there is no evidence against the use of formula for infants whose mothers do not breast-feed, commercial infant formulas utilising cow’s milk protein remain the approved alternate.

4. The substitution of soy-based formulas for milk-based formulas is not advised for either general or high-risk infant feeding practices because of animal studies linking the ingestion of soy protein intake to the development of diabetes.

Wood-derived estrogens: studies in vitro with breast cancer cell lines and in vivo in trout.

Toxicol Appl Pharmacol 1996 Feb;136(2):381-8
Institute of Biomedicine, University of Turku, Finland.

The wood-derived compound, beta-sitosterol (purity > 90%), was shown to be estrogenic in fish. It induced the expression of the vitellogenin gene in the liver of juvenile and methyltestosterone-treated rainbow trout. Structural similarities to beta-sitosterol notwithstanding, cholesterol, citrostadienol, beta-sitostanol, and 5-androstene-3 beta,17 beta-diol, an estrogenic member of the androstenic steroid group, were inactive. An abietic acid mixture (37% abietic acid, 6% dehydroabietic acid, and a remainder of unknown compounds) showed slight hormonal activity in feed, but it was completely inactive when given intraperitoneally in implants. The estrogenic component of the abietic acid preparation was not identified. In addition, to beta-sitosterol and abietic acid, several other wood-derived compounds including betulin, isorhapontigenin, isorhapontin, and pinosylvin were estrogenic in breast cancer cells (MCF-7 or T-47D). However, betulin and pinosylvin, available in sufficient amounts for in vivo testing, did not induce the expression of the vitellogenin gene. Differences in the primary sequences of human and fish estrogen receptors (hormone as well as DNA-binding regions) or uptake and metabolism of the compounds may explain the discrepancy between the two estrogen bioassays. Wood-derived compounds such as beta-sitosterol, present in pulp and paper mill effluents, may account for the weak estrogenicity of debarking effluent seen at the vitellogenin expression bioassay.

Quotes

Fish exposed to bleached pulp mill effluent (BKME) showed reduced plasma sex steroid levels, decreased egg and gonad size, decreased occurrence of secondary sexual characteristics, and an increased age to maturation.

Wood itself may also be a source of contaminants responsible for endocrine abnormalities.

The presence of high amounts of phytoestrogens in plants used for animal feed and the deleterious effect of excessive exposure of animals to phytoestrogens have been recognised for several decades.

The wood-derived compounds studied, beta-sitosterol and abietic acid mixture, were found to be estrogenic in juvenile and methyltestosterone-treated fish.

In addition to interaction with estrogen receptor, dietary estrogens or structurally related compounds may compete with endogenous hormones for active sites of metabolising enzymes, and thus reduce the concentrations of biologically active endogenous hormones.

Pulping processes


Rydholm, SA

Quotes
The unsaponifiable neutral substances of the wood extractives contain higher fatty alcohols such as lignoceryl alcohol, as well as plant hormones, the phytosterols, mainly β-sitosterol, CXXXIV, and β-sitostanol, CXXXV, in the approximate proportions of 30:63:7.

Their utilization in the production of sex hormones has been investigated on a fairly large scale, but was not found to be competitive with the alternative source, cholesterol from wool fat.

It further contains phytosterols, mainly β-sitosterol and β-sitostanol, which have been subject to interest for commercial production of sex hormones otherwise made from cholesterol.

**Hormonally Active Agents in the Environment**

National Research Council of the American Academy of Sciences,


**Quotes**

Potential exposure to plant estrogens found in wood has been assessed by various in vitro and in vivo bioassays. Wood-derived estrogens, such as beta-sitosterol, could represent environmental hormone exposures, particularly from pulp and paper mill effluents, downstream of wood-processing facilities. Mellanen et al. (1996) used two breast-cancer cell lines in vitro (MCF7 and T-47D) and expression of the vitellogenin gene in rainbow-trout livers to estimate estrogenic activity of wood-derived compounds. Some compounds, such as beta-sitosterol, were estogenic in human and fish bioassays, but some phytoestrogens, such as betulin and pinosylvin, were estogenic only in humans.

**Estrogen-specific 17 beta-hydroxysteroid oxidoreductase type 1 (E.C. 1.1.1.62) as a possible target for the action of phytoestrogens.**


Makela S, Poutanen M, Lehtimaki J, Kostian ML, Santti R, Vihko R.

Institute of Biomedicine, University of Turku, Finland.

Several plant estrogens, especially coumestrol and genistein, were found to reduce the conversion of [3H]estrone to [3H] 17-beta-estradiol catalyzed by estrogen-specific 17 beta-hydroxysteroid oxidoreductase Type 1 (E.C. 1.1.1.62) in vitro. Coumestrol, the most potent inhibitor in our experiments, is the best inhibitor of the enzyme known to date. All compounds with inhibitory effects were also estrogenic. However, structural demands for 17 beta-HSOR Type 1 inhibition and estrogenicity of tested compounds in breast cancer cells (judged by increased cell proliferation) were not identical. Zearalenone and diethylstilbestrol, both potent estrogens, did not inhibit 17 beta-HSOR Type 1. Thus, changes in the estrogen molecule may discriminate between active sites of 17 beta-HSOR Type 1 and estrogen binding sites of the ER. The effects of these compounds in vivo cannot be predicted on the basis of these results. Inhibition of 17 beta-HSOR Type 1 enzyme could lead to a decrease in the availability of the highly active endogenous estrogen. However, these compounds are estrogenic per se, and they may thus replace endogenous estrogens. Additional studies are needed to further understand the role of these plant estrogens in the etiology of hormone-dependent cancers. It is not easily conceivable how the chemopreventive action of Asian diets, possibly mediated by phytoestrogens in soya products, can be based on the inhibition of estrone reduction at the target cells by phytoestrogens or related compounds, unless they are "incomplete estrogens" (i.e., unable to induce all effects typical of endogenous estrogens).

**Quotes**

Compounds with high binding affinities for ER (a specific intracellular endocrine receptor) are generally the most active biologically and enhance proliferation of estrogen-responsive tumor cells.
None of the phytoestrogens we studied earlier (coumestrol, genistein, biochanin A, and zearalenone reduced the proliferation rate of 17β-estradiol-stimulated breast cancer cells...

In addition to their interaction with ER (a specific intracellular endocrine receptor), dietary estrogens or structurally related compounds might compete with endogenous estrogens for the active site of the estrogen biosynthesizing and metabolizing enzymes and thus reduce the concentration of biologically active endogenous estrogens.

**Tumor Sterols**

Metabolism 969; 18 (8): 646-651.

Day EA, Gray T, Beeler M, Beeler MF.

Tumor tissue, including breast cancer, thyroid carcinoma, uterine carcinoma and granulosa thecal cell ovarian tumor, from twelve different patients, was analysed for sterol content by thin-layer and gas-liquid chromatography. Cholesterol was present in large amounts in all. A sterol with the retention time of desmosterol was present in 11; one with the retention time of stigmasterol in 3; of which, 2 also contained a sterol with the retention time of campesterol, and 1 of B-sitosterol. Thus, the finding of osteolytic sterols in breast cancer by Gordan and associates is partially confirmed and extended.

**Quotes**

The present study tends to confirm, in part, reports by Gordan and associates of the presence of osteolytic phytosterols in breast cancers, postulated by them to account for the frequent serious degree of hypercalcemia seen in these patients. We have demonstrated evidence for their presence in one of eight breast cancers as well as in a follicular carcinoma of the thyroid metastatic to the lung, and in a malignant mixed Mullerian tumor of the uterus.

We found no evidence of the presence of these sterols in normal tissue.

**Identification of osteolytic sterols in human breast cancer**


Gordan GS, Fitzpatrick ME, Lubich WP.

**Quotes**

Hypercalcemia is a serious, life-threatening emergency. The most common cause of hypercalcemia is malignancy, and the one tumor accounting for two-thirds of all cases of the hypercalcemia of malignancy is breast cancer. In most cases, the hypercalcemia of breast cancer is associated with osteolytic metastases. As with other malignancies, however, hypercalcemia occasionally occurs in the absence of osseous lesions.

Hypercalcemia is a frequent cause of death and disability in breast cancer, occasionally even in the absence of osseous metastases.

It is not caused by parathyroid hormone or vitamin D

A series of plant sterols has been identified in breast cancer extracts and in the plasma of patients with breast cancer. Some of these, campesterol, stigmasterol and sitosterol, are also found in normal plasma. Breast cancer is characterised by an abnormality of sterol metabolism leading to the accumulation of sterol esters, especially...
the osteolytic, short chained fatty acid esters of stigmasterol and 7-dehydrositosterol. Either or both was found in the plasma of all 25 cases - 19 disseminated and 6 local. They were not found in normal non-lactating women.

It is suggested that the esters of stigmasterol and of 7-dehydrositosterol may play a role in the hypercalcemia of breast cancer.

**Significance of dietary plant sterols in man and experimental animals**


**M. T Ravi Subbiah.**

**Quotes**

Plant sterols and their esters have been isolated from plasma and breast extracts of a number of patients with breast cancer in concentrations much higher than is found in normal persons. This has been confirmed in patients with thyroid or renal carcinoma. Indeed, the esters of certain plant sterols have been shown to possess high osteolytic activity.

**Taber's Cyclopedic Medical Dictionary**

18th edition, 1999 pp 1367

**Hypercalcemia**

An excessive amount of calcium in the blood. The causes of this condition include primary hyperthyroidism, lithium therapy, malignancies including solid tumors and hematological malignancies, vitamin D intoxication, hyperthyroidism, vitamin A intoxication, aluminium intoxication; and milk-alkali syndrome.

**The Merck Manual of Diagnosis and Therapy**

17th edition, 1999 pp 145-151

**M. H Beers, R. Berkow, Editors**

**Hypercalcemia Quotes**

Hypercalcemia usually results from excessive bone resorption of which the principle causes are:

- Parathyroid hormone excess
- Humoral hypercalcemia of malignancy
- Malignancy with bone metastases
- Hyperthyroidism
• Vitamin D toxicity
• Vitamin A toxicity
• Immobilisation

The clinical manifestations of hypercalcemia include constipation, anorexia, nausea and vomiting, abdominal pain and ileus.

Elevation of plasma Ca is associated with emotional lability, confusion, delirium, psychosis, stupor and coma. Neuromuscular involvement may cause prominent skeletal muscle weakness.

**Phytosterols in Aortic Tissue in Adults and Infants.**


**M. J. Mellies, T. T. Ishikawa, C. J. Glueck, K. Bove, J. Morrison**

Quotes

Plant sterols are structurally similar to cholesterol, all having a cyclopentenophenanthrene ring with 3-beta hydroxy substitution and a 5-6 double bond. Beta-sitosterol is proportionately the predominant plant sterol in edible oils and grains, with smaller amounts of campesterol and stigmasterol. In adult diets, plant sterols are estimated to account for about one tenth of 1 per cent of total calories and 20 per cent of total sterols. Studies in adults using labelled beta-sitosterol suggest that less than 5 per cent of orally administered sitosterols are absorbed, representing about one tenth of the amount of cholesterol absorbed under similar conditions.

There is however an apparent increase in absorption of dietary phytosterols in infancy and childhood. In infants and children fed diets rich in phytosterols, plasma phytosterols rose from less than 2 to 9 mg. per 100 mL. The implications of long-term three- to fivefold elevations of the plasma phytosterols in infancy and childhood are unknown.

In adults with mature atheromatous lesions, plant sterols are present in appreciable amounts. The implications of these findings are unknown and their relationship to deposition of cholesterol in atheromatous and in normal aortic tissues remains to be elucidated.

Although balance studies of phytosterol absorption have not been done in human infants, it appears that phytosterol-rich diets induce elevations of plasma phytosterol five- to 15-fold above that observed in adults. Once absorbed, phytosterols are catabolised, esterified, and handled in a fashion similar to, but not exactly the same as cholesterol. ...there is no endogenous synthesis of beta-sitosterol.

...the correlation between cholesterol and phytosterols (excluding campesterol) suggests parallel accumulation of these sterols.

...it is speculated that phytostereols may accumulate in infants faster than cholesterol.

In contrast, the ratio of plasma cholesterol to plant sterol in vegetable-oil formula-fed infants was considerably lower than relative tissue ratios. The early increments of aortic tissue phytosterol in infants on phytosterol-rich diets might be speculatively related to relatively low cholesterol and relatively high plasma phytosterol.

In the mature adult atheromatous lesion, where large amounts of cholesterol were present, there were notable increases in plant sterols.
Adverse effects of phytoestrogens-7. Effect of beta-sitosterol treatment on follicular development, ovarian structure and uterus in the immature female sheep.


El Samannoudy FA, Shareha AM, Ghannudi SA, Gillaly GA, El Mougy SA.

Abstract

The daily subcutaneous injection of β-sitosterol for 20 days in the immature female sheep resulted in a preliminary increase in the ovarian and uterine weights, which was then followed at a dose of 20 mg by a marked reduction in their weights. In the ovary, with increase in the dose of β-sitosterol, follicular growth was inhibited and large Graaffian follicle exhibited signs of atresia or induced ovulation. Petechial haemorrhages were evident in the ovarian structure and on the outer layer of the uterine endothelium. Disturbances in the alkaline phosphatase distribution were seen in the zona pellucida and interstitial gland of the ovary. In the uterus, the alkaline phosphatase distribution increased in the uterine glands and endothelium at doses of 0.5 and 1 mg. At a dose of 20 mg there was a sudden significant decrease in the uterine alkaline phosphatase distribution. The activity of the acid phosphatase decreased continuously with dose and time of β-sitosterol injection. Moreover, there was a slight modification in the reactivity of the oxidative enzymes (SD.NADH2-TR and NADPH2-TR) as well as the cholinesterases after β-sitosterol injection.

Quotes

From the developing egg to the delivery of a foetus, the female reproductive cycle is a complex and delicately balanced interplay of events, any one of which, if distorted, may interrupt the succeeding processes. Therefore the addition of steroids to the body imposes alterations on the reproductive system.

...observations that increasing the dose of oestradiol beyond the optimal levels result in a decreased uterine growth response and that continued treatment with high doses of oestrogen first leads to a disproportionate overgrowth of the myometrium and eventually to a refractory state during which both endometrium and myometrium undergo atrophy. This was an obvious observation in our case with the use of β-sitosterol.

The similarity between β-sitosterol and oestrone on the uterus does not hold with the effect of β-sitosterol on the ovary. The present result of the general significant decrease of sheep ovarian weight under the influence of β-sitosterol is a repetition for a previous picture on the effect of β-sitosterol on the rabbit ovarian weight...

Yet in our experiment, β-sitosterol inactivated the process of follicular development.

The antifertility effectiveness of phytoestrogens has been thought to reside in their ability to suppress ovulation by interference with the normal interactions of the hypothalamus and pituitary, which results in alterations in the secretion of gonadotrophins. These findings have been substantiated. Thus the β-sitosterol action, as demonstrated by failure of growth of the follicles, increased percentage of atresia and the formulation of cystic ovaries (which could resemble the multiple cysts of Stein-Loeventhal ovary in humans) could be explained on the basis that β-sitosterol could have blocked the synthesis or/and production of gonadotrophins from the pituitary or their releasing factors from the hypothalamus.

Therefore, β-sitosterol will disturb the cell permeability and the absorption of nutrient material by the uterine cells, through the disturbance occurring on the alkaline phosphatase which is responsible for such an action.

In addition, the influence of β-sitosterol on the milieu medic of the uterus presumably affects the transmission of nerve impulses in the uterus.

Masculinization of female mosquitofish by exposure to plant sterols and Mycobacterium smegmatis.

Bull Environ Contam Toxicol 1985 Nov;35(5):627-32

Denton TE, Howell WM, Allison JJ, McCollum J, Marks B
A study by Howell et al. (1980) revealed a population of the sexually dimorphic poeciliid mosquitofish, *Gambusia affinis Holbrooki*, in a stream receiving papermill effluents. In this population, the females were strongly masculinised showing both physical secondary sex characteristics and reproductive behaviour of males.

It is well documented that plant sterols are widespread within the plant kingdom, especially among pine trees used in the pulping industry, and that sitosterols are the most abundant of these sterols.

At one time, the biocconversion of soybean sterols to β-sitosterol, campesterol, and stigmasterol was considered an alternative source of intermediates for steroid manufacture.

Howell et al. (1980) found masculinised *Gambusia affinis Holbrooki* as far downstream as 4 miles from the location where pulp chemicals were being discharged. Masculinised forms were not found above the point where papermill effluents were entering the stream.

It is well known that the quantity of plant sterols are greatly elevated in papermill effluents. The majority of the sitosterols are found in the resin fractions of conifers.

All *Gambusia* exposed to plant sterols and Mycobacterium developed male-like gonopodia.

These characteristics did not regress when transformed fish were removed to their natural environment which was free of plant sterol products.

It is our considered opinion that both B-sitosterol and stigmastanol are capable of being degraded by Mycobacterium into substances capable of modifying anal fin rays of Gambusia. Both of these substances are present in different amounts in the two soybean extracts used in this study.

It is probable that all three compounds are degraded into androstane-like compounds.

The removal of sitosterols during the pulping process presents great difficulty as they are only slightly hydrophyllic at all pH levels. Papermill effluents released into settling ponds can possibly contain enough sitsterols and micro-organisms to produce androgens which are released into streams.

**Gonopodial Morphogenesis in Female Mosquitofish, *Gambusia affinis affinis*, Masculinized By Exposure to Degradation Products From Plant Sterols.**

Environmental Biology of Fishes 1989 24(1):43-51

**Howell WM, Denton TE**

Female mosquitofish, *Gambusia affinis affinis*, were masculinised by exposure to degradation products (presumably steroids) of the plant sterol, stigmasterol.

In 1980, a puzzling situation was presented regarding a population of *Gambusia affinis holbrooki* inhabiting a stream polluted by pulp wastes from a paper-mill (Howell et al. 1980). All females within this population were abnormal in that they possessed a well-developed, male-like gonopodium and displayed typical male reproductive behaviour.

For example tall oil, an abundant substance in papermill effluent, contains 3% plant sterols, consisting of 17 different compounds, of which sitosterol and campesterol comprise 85%. Laboratory experiments have duplicated the same masculinising effects that occur in female *Gambusia* living in streams receiving wastes from pulping of pinewood chips.

We know of no hormone treatment system that will produce the 3-4-5 termination complex in female Gambusia as rapidly as exposure to biodegraded stigmasterol. The degraded stigmastanol apparently acts as a stimulus to turn on specific inactive gene complexes which otherwise would never be active during the life of a female *Gambusia*. This would never occur in a normal female *Gambusia*. Animal behaviourists could use this model to investigate changes in behaviour and social interactions in masculinised female *Gambusia*.  


Effect of beta-sitosterol on uterine biochemistry: a comparative study with estradiol and progesterone.


Malini T, Vanithakumari G.

Department of Endocrinology, Dr. ALM Post Graduate Institute of Basic Medical Sciences, Taramani, Madras, India.

Administration of estradiol/progesterone to ovariectomized animals significantly increased the uterine weight, RNA, DNA and protein concentrations. Similarly, administration of beta-sitosterol alone or in combination with estradiol caused a marked increase in the above parameters and the maximum influence was evident only after median and high dose treatments. However, administration of median/high dose of beta-sitosterol along with progesterone accentuated only the RNA and protein concentrations but exerted an inhibitory effect on sitosterol-induced increment in uterine weight and DNA concentrations.

It is well-established that mammalian uterus is both an estrogen and progesterone responsive organ. Endogenous steroids as well as synthetis estrogens, progestins and androgens are effective in stimulating the growth of uterus. ...it is also well known that depending on dose and time of administration, one steroid may augment or antagonise the activity of another sex steroid.

In the present study also, estradiol administration induced a significant increase in the uterine weight, associated with hyperemia and hyperplasia of the uterine tissue. B-sitosterol provoked a similar increase in uterine wet weight, suggesting the possible estrogenicity of the compound. The observation confirms and extends the reports from earlier workers. Further the uterine growth response to B-sitosterol was found to be dose-dependent and only a high dose produced a greater increase in uterine weight with massive oedema comparable to that of estradiol stimulation.

The observed increase in tissue wet weights of rats treated with B-sitosterol either individual or concurrently with estradiol may primarily be attributed to the hypertrophy and hyperplasia of uterine tissue as evidenced histologically.

Goitrogenic and estrogenic activity of soy isoflavones.


Division of Biochemical Toxicology, National Center for Toxicological Research, Jefferson, Arkansas, USA. Soy is known to produce estrogenic isoflavones. Here, we briefly review the evidence for binding of isoflavones to the estrogen receptor, in vivo estrogenicity and developmental toxicity, and estrogen developmental carcinogenesis in rats. Genistein, the major soy isoflavone, also has a frank estrogenic effect in women. We then focus on evidence from animal and human studies suggesting a link between soy consumption and goiter, an activity independent of estrogenicity. Iodine deficiency greatly increases soy antithyroid effects, whereas iodine supplementation is protective. Thus, soy effects on the thyroid involve the critical relationship between iodine status and thyroid function. In rats consuming genistein-fortified diets, genistein was measured in the thyroid at levels that produced dose-dependent and significant inactivation of rat and human thyroid peroxidase (TPO) in vitro. Furthermore, rat TPO activity was dose-dependently reduced by up to 80%. Although these effects are clear and reproducible, other measures of thyroid function in vivo (serum levels of triiodothyronine, thyroxine, and thyroid-stimulating hormone; thyroid weight; and thyroid histopathology) were all normal. Additional factors appear necessary for soy to cause overt thyroid toxicity. These clearly include iodine deficiency but may also include additional soy components, other defects of hormone synthesis, or additional goitrogenic dietary factors. Although safety testing of natural products, including soy products, is not required, the possibility that widely consumed soy products may cause harm in the human population via either or both estrogenic and goitrogenic activities is of concern. Rigorous, high-quality experimental and human research into soy toxicity is the best way to address these concerns. Similar studies in wildlife populations are also appropriate.
The effect of neonatal exposure to diethylstilbestrol, coumestrol, and beta-sitosterol on pituitary responsiveness and sexually dimorphic nucleus volume in the castrated adult rat.


The neonatal hormone environment influences the sexually differentiated patterns of development. Estrogens, derived from intracerebral aromatization, promote male pattern development of the central nervous system. The purpose of this study was to determine the effects of neonatal exposure to environmental estrogens on luteinizing hormone (LH) secretion and development of the sexually dimorphic nucleus of the medial preoptic area (SDN-POA) in castrated adult rats. Neonatal rats of both sexes received injections of either corn oil, 0.1 microgram diethylstilbestrol (DES), 3 micrograms beta-sitosterol (B1), 30 micrograms beta-sitosterol (B2), 0.1 microgram coumestrol (C1), 1 microgram coumestrol (C2), or 10 micrograms coumestrol (C3) on Day 1-10 of life and were castrated on Day 21. Right heart catheters were placed on Day 42, and GnRH (50 ng/kg) was administered. Blood was sampled for LH at 0-, 5-, 10-, 15-, and 30-min intervals. All doses of beta-sitosterol and coumestrol elicited increased basal levels of LH in females. In males, B1, B2, C2, and C3 increased basal levels of LH. The GnRH-induced LH increase was prevented in females treated with diethylstilbestrol and 10 micrograms of coumestrol. Males in all treatment groups exhibited GnRH-induced LH surges. The animals were sacrificed by decapitation on Day 49. Volumes of the SDN-POA of the groups were compared. Treatment with the agents did not result in significantly increased SDN volume in females; nor was there a difference in SDN size among the male groups. These data show that exposure to environmental estrogens early in development alters both postpubertal pituitary response to GnRH and basal LH secretion in females and alters only basal LH secretion in males. No significant enlargement (i.e., masculinization) of the SDN-POA was exhibited.

Safety Issues of Soy Phytoestrogens in Breast Cancer Patients


de Lemos, M.

To the Editor:

The results of Van Patten et al confirmed previous findings that soy phytoestrogens have minimal efficacy for menopausal symptoms in breast cancer patients. However, I am concerned that patients in neither study were apparently informed of the potential stimulatory effects of phytoestrogens on breast tumor. Similar omission would have raised ethical concerns if pharmaceutical drugs were involved.

At concentrations below 10 µmol/L, phytoestrogens can stimulate breast tumor growth and antagonize the antitumor effects of tamoxifen, particularly in an environment of low endogenous estrogen. In contrast, phytoestrogens inhibit breast tumor growth and enhance the antitumor effects of tamoxifen at concentrations above 10 µmol/L. In humans, serum phytoestrogen concentrations attained after acute or chronic intake of phytoestrogens were much lower than 10 µmol/L.

Without long-term human data, cancer risk assessments need to be cautious and assume that substances that promote tumor growth in preclinical studies may pose similar risks clinically. Hence, to weigh the potential risks versus benefits before using phytoestrogens for unproven indications, breast cancer patients should be informed that phytoestrogens have the potential to stimulate tumor growth.

Mário de Lemos

Effects of the dietary phytoestrogens daidzein and genistein on the incidence of vulvar carcinomas in 129/J mice.

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The objective of this study was to determine the effect of dietary phytoestrogens on the incidence of spontaneous vulvar carcinomas in 129/J mice using three natural ingredient diets and two purified diets containing predetermined levels of daidzein and genistein. Eighty weanling female mice without clinical evidence of vulvar carcinomas were randomly assigned 16 per diet to each of 5 test diets. Mice were clinically examined for vulvar masses weekly for 3 months and at monthly intervals thereafter. Vulvar carcinomas in representative groups of mice were confirmed using routine histological procedures. The incidence of vulvar carcinomas increased sharply in mice on all test diets during the first 2 months with minor changes during the remainder of the study. Within one month, the incidence of vulvar carcinomas in mice fed the AIN-76A modified soy protein diet was significantly (P < .05) increased over those of mice fed the AIN-76A modified casein diet, the #5K96, or the # 5058 diet. At three months, the incidence of vulvar carcinomas in mice fed the soy protein diet was significantly (P < .05) increased over those of mice fed the NIH-31 diet or the PMI #5K96 diet. There was a marginally significant (P < .10) correlation between the total daidzein and genistein levels in the five test diets and the incidence of vulvar carcinomas in mice as determined by clinical examination. We concluded that dietary levels of daidzein and genistein were associated with an increase in the incidence of vulvar carcinomas in mice and that the 129/J mouse may provide an animal model for studying the development of vulvar carcinomas.

Effects of soy phytoestrogens genistein and daidzein on breast cancer growth.


de Lemos ML

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OBJECTIVE: To determine whether genistein and daidzein, the major phytoestrogens in soy, can stimulate breast cancer growth. DATA SYNTHESIS: Systematic search through primary English-language literature on MEDLINE (1966-January 2001), EMBASE (1982-January 2001) and Current Contents (1998-January 2001). DATA SOURCES: Genistein and daidzein at low concentrations were found to stimulate breast tumor growth in vitro and in vivo animal studies, and antagonize the antitumor effect of tamoxifen in vitro. At high concentrations, genistein inhibited tumor growth and enhanced the effect of tamoxifen in vitro. CONCLUSIONS: Genistein and daidzein may stimulate existing breast tumor growth and antagonize the effects of tamoxifen. Women with current or past breast cancer should be aware of the risks of potential tumor growth when taking soy products.

Infant feeding with soy formula milk: effects on the testis and on blood testosterone levels in marmoset monkeys during the period of neonatal testicular activity.

Hum Reprod 2002 Jul;17(7):1692-703

Sharpe RM, Martin B, Morris K, Greig I, McKinnell C, McNeilly AS, Walker M.

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BACKGROUND: This study has addressed concerns about possible effects of feeding human infants soy formula milk (SFM). METHODS: This is a feeding study in marmosets, using a mainly co-twin design. From 4-5 until 35-45 days of age, co-twin males were fed by hand with either standard (cow) formula milk (SMA = controls) or with SFM for approximately 8 h each day (2 h at weekends) and intake related to bodyweight. Blood samples were collected at 18-20 and at 35-45 days of age in 13 sets of co-twins plus two non-twin males per group and, at the later age, seven sets of co-twins were killed and the testes and pituitary gland fixed for cell counts. RESULTS:
Weight gain and formula intake were similar in both feeding groups. SMA-fed males had mean testosterone levels of 2.8-3.1 ng/ml, typical of the 'neonatal testosterone rise', whereas SFM-fed males exhibited consistently lower mean levels (1.2-2.6 ng/ml); paired comparison in SMA-and SFM-fed co-twins at day 35-45 revealed 53-70% lower levels in 11 of 13 co-twins fed with SFM (P = 0.004). Further evidence for suppression of testosterone levels in SFM-fed males came from comparison of the frequency of low testosterone levels (<0.5 ng/ml). In historical controls aged 35-45 days, two out of 22 values were <0.5 ng/ml, a similar frequency as found in control SMA-fed males (one out of 15 values <0.5 ng/ml). In contrast, 12 out of 15 values for SFM-fed males were <0.5 ng/ml (P < 0.001). There was no consistent relationship between SFM intake/g and testosterone levels. Paradoxically, the mean number of Leydig cells per testis was increased by 74% (P < 0.001) in co-twins fed SFM, when compared with their SMA-fed brothers, whereas no significant changes were found in numbers of Sertoli and germ cells. Because of the lack of gonadotrophin assays, the number of immunopositive LHbeta and FSHbeta cells in the pituitary gland, and their ratio, were determined but no consistent difference was found between SMA- and SFM-fed twins.

CONCLUSIONS: Based on the average isoflavone content of the SFM brand used, intake of isoflavones was estimated at 1.6-3.5 mg/kg/day in the SFM-fed marmosets which is 40-87% of that reported in 4 month human infants fed on a 100% SFM diet. It is therefore considered likely that similar, or larger, effects to those shown here in marmosets may occur in human male infants fed with SFM. Whether the changes described result in longer-term effects is under investigation.

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.

Some 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.
Dietary genistein negates the inhibitory effect of tamoxifen on growth of estrogen-dependent human breast cancer (MCF-7) cells implanted in athymic mice.

Cancer Res 2002 May 1;62(9):2474-7

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The use of dietary isoflavone supplements by postmenopausal women with breast cancer is increasing. We investigated interactions between the soy isoflavone, genistein, and an antiestrogen, tamoxifen (TAM), on the growth of estrogen (E)-dependent breast cancer (MCF-7) cells implanted in ovariectomized athymic mice. We hypothesized that weakly estrogenic genistein negate/overwhelm the inhibitory effect of TAM on the growth of E-dependent breast tumors. Six treatment groups were used: control (C); 0.25 mg estradiol (E2) implant (E); E2 implant + 2.5 mg TAM implant (2.5 TE); E2 implant + 2.5 mg TAM implant + 1000 ppm genistein (2.5 TEG); E2 implant + 5 mg TAM implant (5 TE), and E2 implant +5 mg TAM implant +1000 ppm genistein (5 TEG). Treatment with TAM (2.5 TE and 5 TE) suppressed E2-stimulated MCF-7 tumor growth in ovariectomized athymic mice. Dietary genistein negated/overwhelmed the inhibitory effect of TAM on MCF-7 tumor growth, lowered E2 level in plasma, and increased expression of E-responsive genes (e.g., pS2, PR, and cyclin D1). Therefore, caution is warranted for postmenopausal women consuming dietary genistein while on TAM therapy for E-responsive breast cancer.

Physiological concentrations of dietary genistein dose-dependently stimulate growth of estrogen-dependent human breast cancer (MCF-7) tumors implanted in athymic nude mice.

J Nutr 2001 Nov;131(11):2957-62

Ju YH, Allred CD, Allred KF, Karko KL, Doerge DR, Helferich WG.

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Previously our laboratory has shown that the soy isoflavone, genistein, stimulates growth of human breast cancer (MCF-7) cells in vivo and in vitro. In this study, the dose-response analysis of genistein at the physiologically achievable concentration range between 125 and 1,000 microg/g in the diet was conducted in ovariectomized athymic nude mice implanted with MCF-7 cells. We hypothesized that genistein at this concentration range can stimulate dose-dependently the breast tumor growth, cell proliferation and an estrogen-responsive pS2 gene induction. Tumor size and body weight were monitored weekly. At completion of the study, we analyzed cellular proliferation of tumors using incorporation of BrdU, pS2 expression of tumors using a Northern blot analysis and total genistein level in plasma using liquid chromatography-isotope dilution mass spectrometry (LC-ES/MS). Dietary genistein (> or = 250 microg/g) increased tumor size in a dose-dependent manner [8.4x the negative control (NC) group in the 250 microg/g group, 12.0x in the 500 microg/g group, 20.2x in the 1,000 microg/g group and 23.2x in the positive control (PC) group]. The percentage of proliferating cells was significantly increased by genistein at and above 250 microg/g (5.3x the NC group in the 250 microg/g, 5.6x in the 500 microg/g, 5.0x in the 1,000 microg/g and 4.8x in the PC group). Expression of pS2 mRNA was also significantly increased with increasing dietary genistein levels (11.25x the NC group in the 500 microg/g group and 15.84x in the 1,000 microg/g group). Total plasma genistein concentrations were between 0.39 and 3.36 micromol/L in mice fed between 125 and 1,000 microg/g genistein. In conclusion, dietary treatment with genistein at physiological concentrations produces blood levels of genistein sufficient to stimulate estrogenic effects, such as breast tumor growth, cellular proliferation and pS2 expression in athymic mice in a dose-responsive manner similar to that seen in vitro.
Dietary genistin stimulates growth of estrogen-dependent breast cancer tumors similar to that observed with genistein.

Carcinogenesis 2001 Oct;22(10):1667-73

Allred CD, Ju YH, Allred KF, Chang J, Helferich WG.

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The estrogenic soy isoflavone, genistein, stimulates growth of estrogen-dependent human breast cancer (MCF-7) cells in vivo. Genistin is the glycoside form of genistein and the predominant form found in plants. It is generally believed that genistin is metabolized to the aglycone genistein in the lower gut. However, it is unclear if the rate of metabolism of genistin to genistein is sufficient to produce a level of genistein capable of stimulating estrogen-dependent breast cancer cell growth. Our hypothesis was that dietary genistin would stimulate tumor growth similar to that observed with genistein in athymic mice. To test this hypothesis, genistin or genistein was fed to athymic mice containing xenografted estrogen-dependent breast tumors (MCF-7). Mice were fed either genistein at 750 p.p.m. (parts per million) or genistin at 1200 p.p.m., which provides equal molar concentrations of aglycone equivalents in both diets. Tumor size was measured weekly for 11 weeks. At completion of the study, half of the animals per treatment group were killed and tumors collected for evaluation of cellular proliferation and estrogen-responsive pS2 gene expression. Incorporation of bromo-deoxyuridine into cellular DNA was utilized as an indicator of cellular proliferation. Dietary genistin resulted in increased tumor growth, pS2 expression and cellular proliferation similar to that observed with genistein. The remaining mice were switched to diets free of genistin and genistein. When mice were placed on isoflavone free diets, tumors regressed over a span of 9 weeks. Next, we examined how effectively and where metabolism of genistin to genistein occurred in the digestive tract. We present evidence that demonstrates conversion of genistin to its aglycone form genistein begins in the mouth and then continues in the small intestine. Both human saliva and the intestinal cell-free extract from mice converted genistin to genistein. In summary, the glycoside genistin, like the aglycone genistein, can stimulate estrogen-dependent breast cancer cell growth in vivo. Removal of genistin or genistein from the diet caused tumors to regress.

Soy diets containing varying amounts of genistein stimulate growth of estrogen-dependent (MCF-7) tumors in a dose-dependent manner.

Cancer Res 2001 Jul 1;61(13):5045-50

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We have demonstrated that the isoflavone, genistein, stimulates growth of estrogen-dependent human breast cancer (MCF-7) cells in vivo (C. Y. Hsieh et al., Cancer Res., 58: 3833-3838, 1998). The isoflavones are a group of phytoestrogens that are present in high concentrations in soy. Whether consumption of genistein from soy protein will have similar effects on estrogen-dependent tumor growth as pure genistein has not been investigated in the athymic mouse tumor implant model. Depending on processing, soy protein isolates vary widely in concentrations of genistein. We hypothesize that soy isolates containing different concentrations of genistein will stimulate the growth of estrogen-dependent cells in vivo in a dose-dependent manner. To test this hypothesis we conducted experiments in which these soy protein isolates were fed to athymic mice implanted s.c. with estrogen-dependent tumors. Genistein content (aglycone equivalent) of the soy isolate diets were 15, 150, or 300 ppm. Positive (with 17beta-estradiol pellet implant) and negative (no 17beta-estradiol) control groups received casein-based (isoflavone-free) diets. Tumor size was measured weekly. At completion of the study animals were killed and tumors collected for evaluation of cellular proliferation and estrogen-dependent gene expression. Incorporation of bromodeoxyuridine into cellular DNA was used as an indicator of cell proliferation, and pS2 mRNA was used as an estrogen-responsive gene. Soy protein diets containing varying amounts of genistein increased estrogen-dependent tumor growth in a dose-dependent manner. Cell proliferation was greatest in tumors of animals given estrogen or dietary genistein (150 and 300 ppm). Expression of pS2 was increased in tumors from animals consuming dietary genistein (150 and 300 ppm). Here we present new information that soy protein isolates containing increasing concentrations of genistein stimulate the growth of estrogen-dependent breast cancer cells in vivo in a dose-dependent manner.
Detection of phytoestrogens in samples of second trimester human amniotic fluid.

Toxicol Lett 2002 Mar 28;129(3):199-205

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There is widespread concern that fetal exposure to hormonally active chemicals may adversely affect development of the reproductive tract. Therefore, the present study was performed to develop the necessary analytical methods and test the hypothesis that dietary phytoestrogens can be quantified in second trimester human amniotic fluid. Amniotic fluid samples (n=59) from women (n=53) undergoing routine amniocentesis between 15 and 23 weeks of gestation were analyzed by gas chromatography/mass spectrometric (GC/MS). Analytes included the phytoestrogens daidzein, genistein, formononetin, biochanin A, and coumestrol. Dietary phytoestrogens were quantified in 96.2% of second trimester amniotic fluid samples tested. The mean (+/- standard deviation (S.D.)) concentration of daidzein and genistein in amniotic fluid was 1.44 +/- 1.34 and 1.69 +/- 1.48 ng/ml with maximum levels of 5.52 and 6.54 ng/ml, respectively. Second trimester amniotic fluid contains quantifiable levels of dietary phytoestrogens and thus is a marker of mid pregnancy fetal exposure.

Acute and chronic effects of genistein, tyrphostin and lavendustin A on steroid synthesis in luteinized human granulosa cells.

Hum Reprod 2002 Mar;17(3):589-94

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BACKGROUND: Phytoestrogens, including genistein and other inhibitors of tyrosine kinases (TKs), inhibit specific steroidogenic enzymes. This study was designed to compare the effects of genistein, with two other TK inhibitors, on steroid synthesis in human granulosa luteal (GL) cells and to identify which steroidogenic enzymes they may affect. METHODS: GL cells, obtained from women undergoing IVF procedures, were cultured for various periods of time with and without substrates for progesterone and estradiol synthesis, in the presence or absence of the TK inhibitors. RESULTS: The TK inhibitors significantly suppressed progesterone and estradiol synthesis in a dose-dependent manner over a 48 h culture period. Progesterone production in the presence of 10(-7) mol/l pregnenolone during a 4 h period was inhibited by both acute (4 h) and chronic (24 h) exposure of GL cells to 50 micromol/l genistein (P < 0.05) whilst no significant effects of 50 micromol/l tyrphostin A23 were observed. Genistein (4 and 24 h exposure) inhibited the production of estradiol using 10(-7) mol/l estrone as a substrate, but inhibition of estradiol synthesis using androstenedione or testosterone as substrates was only observed after a 24 h exposure. In contrast, tyrphostin acutely stimulated estradiol synthesis when androstenedione and testosterone were used as substrates (P < 0.05) but not estrone. CONCLUSIONS: Genistein directly inhibits 3 and 17beta-hydroxysteroid dehydrogenase activity, whilst tyrphostin has an acute stimulatory effect on aromatase activity. Over a longer time (24 and/or 48 h period), both TK inhibitors suppress steroid synthesis.

Neonatal exposure to genistein reduces expression of estrogen receptor alpha and androgen receptor in testes of adult mice.

Endocr J 2001 Dec;48(6):655-63

Shibayama T, Fukata H, Sakurai K, Adachi T, Komiyama M, Iguchi T, Mori C.
We investigated the long-term estrogenic influence of genistein on the male reproductive system in mice. Newborn ICR male mice were treated with genistein (10, 100, or 1,000 microg/mouse) for neonatal 5 days. As positive control, administration of diethylstilbestrol (0.5-50 microg/mouse) was carried out. In mice exposed to genistein, we examined weight of testes, sperm counts, sperm motility, and mRNA expression levels of estrogen receptor a (ERalpha) and androgen receptor (AR) at 4, 8 or 12 weeks after birth. Moreover, at 12 weeks of age, we evaluated protein level of ERalpha. In our conventional reproductive-toxicological study (weight of testes, sperm counts and sperm motility), neonatal transient exposure to genistein did not show adverse effects on the male reproductive system in 4, 8 or 12 week old mice. However, in mice treated with genistein mRNA expression levels of ERa and AR were reduced at 8 weeks. This reduction was recovered at 12 weeks in mice treated with a lower dose (10 microg) of genistein but not in those with higher doses (100 microg and 1,000 microg). In addition, ERa protein levels tended to decrease in 12 weeks of adulthood. Our results exhibited that the disruption of gene expression continued for long term such as 3 months after administration of genistein, even if no effect was found at conventional reproductive-toxicological level. We have shown that neonatal administration of weak estrogenic compound (genistein) affects male reproductive organs at molecular levels in adulthood.

**Dietary soy phytoestrogen effects on brain structure and aromatase in Long-Evans rats.**


Lephart ED, Adlercreutz H, Lund TD.

Phytoestrogens are estrogen-like (plant-derived) molecules that protect against age-related diseases (cardiovascular disease and osteoporosis), hormone-dependent (breast and prostate) cancers and selectively bind estrogen receptors. However, little is known about the influence of phytoestrogens on brain. Using diets containing either high phytoestrogen levels, derived from soy, or very low phytoestrogens we quantified phytoestrogen concentrations of daidzein, genistein and equol in brain. We found that dietary phytoestrogens: significantly decrease body and prostate weights, do not alter brain aromatase levels and significantly change during adulthood the structure of the sexually dimorphic brain region (i.e. anteroventral periventricular nucleus; AVPV) in male, but not in female rats. Since most commercial animal diets contain significant concentrations of phytoestrogens their influence on brain structure should be considered.

**The effect of isoflavone extract ingestion, as Trinovin, on plasma steroids in normal men.**

Steroids 2002 Jan;67(1):25-9

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Plasma testosterone, dihydrotestosterone, androstenedione, dehydroepiandrosterone sulfate, androsterone and epiandrosterone sulfates, cortisol and sex hormone binding globulin were measured in six adult men before and during daily isoflavone extract ingestion (40 mg) in the form of Trinovin tablets. Although modest plasma genistein levels were achieved following three weeks of Trinovin ingestion (106-356 nmol/l) there were no significant changes in most of the analytes tested. However plasma levels of dihydrotestosterone showed an increase that reached significance when combined basal levels were compared to levels following Trinovin treatment. The results suggest that the daily ingestion of isoflavones in the form of Trinovin (1 tablet/day), over a short term, does not alter most plasma steroid levels. We therefore question the value of Trinovin, at the recommended dosage, as offering protective effects against prostate disease by mechanisms involving either significant modulation of plasma steroid or SHBG levels. In contrast the increase in dihydrotestosterone plasma levels could be seen as possibly detrimental.
Oxidative metabolism and genotoxic potential of major isoflavone phytoestrogens.


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The soy isoflavones daidzein, genistein and glycitein are extensively metabolized by rat liver microsomes to a variety of catechol metabolites. Hydroxylated metabolites of daidzein and genistein have also been demonstrated in incubations with human hepatic microsomes and in the urine of humans after ingestion of soy food. Although the microsomal metabolism of formononetin and biochanin A is dominated by demethylation to daidzein and genistein, respectively, catechols of the parent isoflavones and of the demethylation products are also formed. Thus, oxidative metabolism appears to be common among isoflavones and may have implications for their biological activities. As genistein but not daidzein exhibits clastogenic activity in cultured mammalian cells, the role of oxidative metabolism for the genotoxicity of isoflavones is of particular interest.

Reproductive effects in male and female rats of neonatal exposure to genistein.


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Sprague-Dawley rats were administered genistein orally at doses of 12.5, 25, 50, or 100 mg/kg on postnatal days 1 through 5 to examine its effects on reproductive function after puberty. In addition, preputial separation and vaginal opening as endpoints of sexual maturation, estrous cycling, sperm count, serum testosterone concentration, and histopathologic changes of reproductive organs of male and female rats were examined. Body weights of male and female rats exposed to genistein at any dose level examined were lower than those of controls. Timing of preputial separation in males and timing of vaginal opening were not affected by genistein treatment. The number of females showing estrous cycle irregularities was increased by genistein treatment. The fertility of female rats exposed neonatally to genistein at 100 mg/kg was disrupted, while neonatal exposure to genistein did not affect male fertility. Neither sperm counts nor serum testosterone concentration were changed by neonatal exposure to genistein. Female rats exposed neonatally to genistein at 100 mg/kg showed histopathologic changes in the ovaries and uterus, while male rats showed no histopathologic alterations in the gonads. The results of this study indicate that early neonatal exposure to genistein caused dysfunction of postpubertal reproductive performance as well as abnormal development of gonads in female but not in male rats.

Developmental estrogenization and prostatic neoplasia.

Prostate 1994;24(2):67-78

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The association of estrogens with benign prostatic hyperplasia and prostatic cancer has been widely studied, but no conclusive evidence exists for a role of estrogens in prostatic disease. This paper reviews the literature and describes studies which have sought to show a correlation of estrogens and alterations in the prostates of humans and experimental animal models. Using the developmentally estrogenized mouse model, we propose an alternative role for estrogens as a predisposing factor for prostatic diseases: estrogen exposure during development may initiate cellular changes in the prostate which would require estrogens and/or androgens later in life for promotion to hyperplasia or neoplasia. Thus, the critical time for estrogen action would be during the development of the prostatic tissue. We further suggest that estrogen-sensitive cells may remain in the prostate and be more responsive to estrogens later in life or less responsive to the normal controlling mechanisms of prostatic growth.

**Neurobehavioral effects of dietary soy phytoestrogens.**

Neurotoxicol Teratol 2002 Jan-Feb;24(1):5-16


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Phytoestrogens, plant-derived nonsteroidal estrogens found in high abundance in most soy food products, have been studied for their potential beneficial effects against hormone-dependent cancers and age-related diseases. However, little is known about the influence of phytoestrogens on the brain or behavior. This brief review describes mainly our own studies in rodents that have examined the influence of dietary soy isoflavones on certain aspects of brain structure, learning, memory and anxiety along with the brain androgen-metabolizing enzyme, aromatase. These studies used a commercially available diet rich in phytoestrogens (Phyto-rich) vs. a custom diet relatively free of phytoestrogens (Phyto-free). The phytoestrogen content of each diet was determined by high-performance liquid chromatography analysis, circulating plasma phytoestrogen levels were quantified by gas chromatography mass spectroscopy and concentrations of phytoestrogens in specific brain regions were measured by time-resolved fluoroimmunoassay (TR-FIA). Our studies showed that brain aromatase levels were not significantly altered by phytoestrogen diet treatments in perinatal, maternal or adult rats. However, volumes of the sexually dimorphic nucleus of the preoptic area (SDN-POA) were significantly affected by the Phyto-free diet treatment in male rats during adulthood, where SDN-POA volumes were smaller compared to Phyto-rich male values. Additionally, the Phyto-rich diet fed to adult male and female rats produced anxiolytic effects as assessed in the elevated plus maze vs. Phyto-free fed animals. Finally, when learning and memory parameters were examined in a radial arm maze testing visual-spatial memory (VSM), the diet treatments significantly changed the typical sexually dimorphic pattern of VSM. Specifically, adult Phyto-rich fed females outperformed Phyto-free fed females, while in males on the same diets, the opposite pattern of maze performance was observed. When female vs. male performance was compared, Phyto-rich females executed the VSM task in a manner similar to that of Phyto-free fed males, while Phyto-free fed female's VSM was comparable to Phyto-rich males. **These results indicate that consumption of dietary phytoestrogens resulting in very high plasma isoflavone levels (in many cases over a relatively short interval of consumption in adulthood) can significantly alter sexually dimorphic brain regions, anxiety, learning and memory. The findings of these studies identify the biological actions of phytoestrogens, specifically isoflavones and their metabolites, found in animal soy-containing diets on brain and behavior and implicate the importance of phytoestrogens given the recognized significance of estrogens in brain and neural disorders, such as Alzheimer's disease, especially in women.**

**Inactivation of thyroid peroxidase by soy isoflavones, in vitro and in vivo.**


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Soy-containing foods and dietary supplements are widely consumed for putative health benefits (e.g. cancer chemoprevention, beneficial effects on serum lipids associated with cardiovascular health, reduction of
osteoporosis, relief of menopausal symptoms). However, studies of soy isoflavones in experimental animals suggest possible adverse effects as well (e.g. enhancement of reproductive organ cancer, modulation of endocrine function, anti-thyroid effects). This paper reviews the evidence in humans and animals for anti-thyroid effects of soy and its principal isoflavones, genistein and daidzein.

**Estrogen and spermatogenesis.**


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Although it has been known for many years that estrogen administration has deleterious effects on male fertility, data from transgenic mice deficient in estrogen receptors or aromatase point to an essential physiological role for estrogen in male fertility. This review summarizes the current knowledge on the localization of estrogen receptors and aromatase in the testis in an effort to understand the likely sites of estrogen action. The review also discusses the many studies that have used models employing the administration of estrogenic substances to show that male fertility is responsive to estrogen, thus providing a mechanism by which inappropriate exposure to estrogenic substances may cause adverse effects on spermatogenesis and male fertility. The reproductive phenotypes of mice deficient in estrogen receptors alpha and/or beta and aromatase are also compared to evaluate the physiological role of estrogen in male fertility. The review focuses on the effects of estrogen administration or deprivation, primarily in rodents, on the hypothalmo-pituitary-testis axis, testicular function (including Leydig cell, Sertoli cell, and germ cell development and function), and in the development and function of the efferent ductules and epididymis. The requirement for estrogen in normal male sexual behavior is also reviewed, along with the somewhat limited data on the fertility of men who lack either the capacity to produce or respond to estrogen. This review highlights the ability of exogenous estrogen exposure to perturb spermatogenesis and male fertility, as well as the emerging physiological role of estrogens in male fertility, suggesting that, in this local context, estrogenic substances should also be considered "male hormones."

**The phenotype of the aromatase knockout mouse reveals dietary phytoestrogens impact significantly on testis function.**

Endocrinology 2002 Aug;143(8):2913-21

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Estrogen is synthesized in the testis, both in Leydig cells and seminiferous epithelium, and its importance in spermatogenesis is highlighted by the phenotype of the aromatase knockout (ArKO) mouse. These mice are unable to synthesize endogenous estrogens. The males develop postmeiotic defects by 18 wk of age. We hypothesized that maintenance of spermatogenesis in younger animals may be mediated by exogenous estrogenic substances. Dietary soy meal, contained in almost all commercial rodent diets, provides a source of estrogenic isoflavones. We thus investigated spermatogenesis in wild-type and ArKO mice raised on a diet containing soy, compared with a soy-free diet, to elucidate the biological action of phytoestrogens on the testis. In ArKO mice, dietary phytoestrogens could partially prevent disruptions to spermatogenesis, in that they prevented the decline in germ cell numbers. They also seemed to maintain Sertoli cell function, and they blocked elevations in FSH. The impairment of spermatogenesis seen in soy-free ArKOs occurred in the absence of a decreased gonadotropic stimulus, suggesting that the effects of dietary phytoestrogens are independent of changes to the pituitary-gonadal axis. Our study highlights the importance of estrogen in spermatogenesis and shows that relatively low levels of dietary phytoestrogens have a biological effect in the testis.

Climacteric 1999 Mar;2(1):6-12

Nagata C, Shimizu H, Takami R, Hayashi M, Takeda N, Yasuda K.

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OBJECTIVE: To examine the relationships between dietary intake of soy products and hot flushes and other menopausal symptoms. METHODS: Subjects were 284 women aged 40-59 years who attended a health check-up program provided by a general hospital in Gifu, Japan. They completed a health questionnaire including the Kupperman test of menopausal distress. Diet was assessed by a semiquantitative food frequency questionnaire. RESULTS: Fermented soy product intake but not total soy product intake was significantly negatively correlated with hot flush severity ($r = -0.16$, $p = 0.01$) after controlling for age and menopausal status. Neither total soy product intake nor fermented soy product intake was significantly correlated with menopausal index score. Estimated isoflavone intake from total and fermented soy products was significantly lower by 15% ($p = 0.02$) and 19% ($p = 0.01$), respectively, in women with hot flushes, compared to those without hot flushes after controlling for covariates. CONCLUSION: The data support a hypothesis that intake of fermented soy products alleviates the severity of hot flushes.

Early exposure to genistein exerts long-lasting effects on the endocrine and immune systems in rats.


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Background: Although the immunologic effects of endogenous and synthetic estrogens are well studied, few studies have examined the hormonal effects of phytoestrogens (i.e., plant-derived estrogens) on the immune system. The primary goal of this study was to compare the effects of perinatal exposure with life-long exposure to genistein, an estrogenic compound in soy, on the endocrine and immune system in adulthood. Materials and Methods: Pregnant female rats were exposed to no, low (5 mg/kg diet), or high (300 mg/kg diet) genistein diets throughout gestation and lactation. At weaning, male offspring exposed to genistein perinatally were either switched to the genistein-free diet or remained on the genistein-dosed diets. At 70 days of age, immune organ masses, lymphocyte subpopulations, cytokine concentrations, and testosterone concentrations were assessed in male offspring. Results: Data were analyzed based on the diets that males were exposed to during gestation and lactation because life-long exposure to genistein had no additional effect on any of the dependent measures. Relative thymus masses were greater among males exposed to the high genistein diet than among males exposed to no genistein. Although the proportions of splenic and thymic CD4+ T cells were not altered by genistein, the percentages of CD4+CD8+ thymocytes, CD8+ splenocytes, and total T cells in the spleen were higher and the percentages of CD4-CD8- thymocytes were lower among males exposed to genistein than among males not exposed to genistein. Synthesis of interferon-gamma (IFN-gamma) was marginally higher and testosterone concentrations were lower among genistein-exposed than genistein-free males. Discussion: These data illustrate that exposure to genistein during pregnancy and lactation exerts long-lasting effects on the endocrine and immune systems in adulthood. Whether exposure to phytoestrogens during early development affects responses to infectious or autoimmune diseases, as well as cancers, later in life requires investigation.

Exposure to Genistein During Gestation and Lactation Demasculinizes the Reproductive System in Rats.

J Urol 2003 Apr;169(4):1582-1586
Wisniewski AB, Klein SL, Lakshmanan Y, Gearhart JP.

PURPOSE: Exposure to the phytoestrogen genistein (Indofine Chemical Co., Somerville, New Jersey) can disrupt normal male sexual differentiation. To determine if perinatal (that is gestation and lactation) genistein exposure at doses common in human diets alters masculinization we examined the development of the external genitalia, testes, wolffian ducts and sexual behavior in male rats exposed to genistein supplemented diets during early development. MATERIALS AND METHODS: Female rats were fed a phytoestrogen-free diet supplemented with no genistein (free), a low genistein dose (low) or a high genistein dose (high) throughout gestation and lactation. Anogenital distance of male offspring was measured weekly from postnatal days 2 to 21. At puberty (postnatal day 40 to 45) preputial separation, and tests length and width of male offspring were measured. At age 70 days reproductive organ masses, plasma testosterone concentration, sperm counts and sexual behavior were assessed in male offspring. RESULTS: Exposure to genistein resulted in temporary, prepubertal urogenital abnormalities at postnatal days 21 and 40. Males exposed to genistein had smaller anogenital distance and testis size, and delayed preputial separation. Perinatal exposure to genistein also caused long-term dysfunction in reproductive behavior, in which adult males exposed to genistein were less likely to mount, intromit and ejaculate during mating tests. Males exposed to genistein also had lower testosterone concentrations in adulthood. CONCLUSIONS: Perinatal genistein exposure results in transient and lasting alterations in masculinization of the reproductive system. These results extend our knowledge of the effects of early genistein exposure on male development and may have implications for human health in terms of potential relationships of endocrine disrupters and urogenital abnormalities thought to be increasing in incidence in boys and men.

The soya isoflavone content of rat diet can increase anxiety and stress hormone release in the male rat.

Psychopharmacology (Berl) 2003 Mar 5;


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RATIONALE. Most commercial rodent diets are formulated with soya protein and therefore contain soya isoflavones. Isoflavones form one of the main classes of phytoestrogens and have been found to exert both oestrogenic and anti-oestrogenic effects on the central nervous system. The effects have not been limited to reproductive behaviour, but include effects on learning and anxiety and actions on the hypothalamo-pituitary axis. It is therefore possible that the soya content of diet could have significant effects on brain and behaviour and be an important source of between-laboratory variability.

OBJECTIVES. To determine whether behaviour in two animal tests of anxiety, and stress hormone production, would differ between rats that were fed a diet which was free of soya isoflavones and other phytoestrogens (iso-free) and those that were fed a diet which contained 150 &mgr;g/g of the isoflavones genistein and daidzein (iso-150). This controlled diet has an isoflavone concentration similar to that in the maintenance diet routinely used in our institution. METHODS. Male rats were randomly allocated to the iso-free and iso-150 diets and their body weights and food and water consumption were recorded for 14 days. They were then maintained on the same diets, but housed singly for 4 days, before testing in the social interaction and elevated plus-maze tests of anxiety. Corticosterone concentrations in both dietary groups were determined under basal conditions and after the stress of the two tests of anxiety. Vasopressin and oxytocin concentrations were determined after brief handling stress. RESULTS. The groups did not differ in food or water intake, body weight or oxytocin concentrations. Compared with the rats fed the iso-free diet, the rats fed the iso-150 diet spent significantly less time in active social interaction and made a significantly lower percentage of entries onto the open arms of the plus-maze, indicating anxiogenic effects in both animal tests. The groups did not differ in their basal corticosterone concentrations, but the iso-150 group had significantly elevated stress-induced corticosterone concentrations. Stress-induced plasma vasopressin concentrations were also significantly elevated in the iso-150 diet group compared with the iso-free rats. CONCLUSIONS. Major changes in behavioural measures of anxiety and in stress hormones can result from the soya isoflavone content of rat diet. These changes are as striking as those seen following drug administration and could form an important source of variation between laboratories.
Effects of Genistein Isoflavone (4',5',7-Trihydroxyisoflavone) and Dexamethasone on Functional Characteristics of Spermatozoa.

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Caudal epididymal spermatozoa were used to study the influence of genistein isoflavone and dexamethasone (dxm) on the functional characteristics of spermatozoa. The effects of genistein alone and in combination with dxm on sperm motility, sperm morphology, spontaneous acrosome reaction (AcR), and ionophore A23187-induced AcR were investigated. The FITC-PSA/Hoechst 33258 staining procedure was used to assess sperm cell viability and AcR status and thus to differentiate between true AcR and acrosome degeneration. The overall results indicated that (1) lower doses of genistein alone, or in combination with dxm, did not significantly influence sperm motility or sperm morphology; (2) ionophore A23187 induced AcR in rat spermatozoa; (3) there appeared to be no direct correlation between sperm motility and AcR, (4) higher doses of genistein, alone or in combination with dxm, significantly interfered with percentage sperm motility and caused significant detachment of sperm heads but did not cause morphological defects; and (5) higher doses of genistein caused significant decrease in sperm acrosome reactivity with long duration of exposure. In view of the fact that sperm capacitation and AcR are physiological prerequisites for successful fertilization of oocytes, the findings suggest that chronic exposure of spermatozoa to high doses of genistein could be associated with infertility problems through suppression/inhibition of AcR and sperm motility. Dexamethasone did not appear to influence the effect of genistein on the functionality of post spermatogenic spermatozoa.

Cytotoxic potential of the phytochemical genistein isoflavone (4',5',7-trihydroxyisoflavone) and certain environmental chemical compounds on testicular cells.

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The effects of genistein (Gn), sodium azide (naz), and dexamethasone (dxm) on testicular cells TM3, TM4 and GC-1 spg were studied in vitro. First, a series of experiments were performed to assess the response of the cells to the exposure of Gn, naz, dxm, a combination of Gn with naz and Gn with dxm. Trypan blue exclusion assay was used to determine the percentage of viability, and LDH-cytotoxicity test was used to assess the degree of treatment-induced cytotoxicity on each cell type. A second series of experiments were performed to study cytomorphology and determine the type and percentage of treatment-induced cell death (apoptosis and necrosis) on each cell line, using fluorescent dye technique to detect apoptotic and necrotic cells, and tunnel assay to confirm apoptosis. The results from the data obtained demonstrated: i) that incubation of testis cells with each of the agents (Gn, dxm, naz) alone and in two combinations (Gn-dxm, and Gn-naz) induced significant testicular cell death; ii) that both genistein and dexamethasone mostly and significantly induced apoptotic cell death while sodium azide induced necrotic cell death; iii) that addition of dexamethasone to genistein demonstrated synergism in apoptosis on testis cells; and iv) that combination of naz with Gn demonstrated synergism in necrosis on testis cells even though Gn alone did not induce significant necrosis. It is concluded that the synergistic actions of genistein and dxm, and of genistein + sodium azide in induction of apoptosis and/or necrosis may be of clinical and pathophysiological research interest considering the chemopreventive and chemotherapeutic potential of genistein; and the clinico-pharmacological application of dexamethasone and sodium azide.

Influence of genistein (4',5,7-trihydroxyisoflavone) on the growth and proliferation of testicular cell lines.
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The effects of genistein on testicular cells, TM3, TM4, and GC-1 spg, were studied in vitro. First, each cell line was cultured with pre-determined concentrations of genistein for a maximum of 72 h to assess the effects of genistein on in vitro growth of the test cells. A second series of experiments were performed to determine the degree of genistein-induced apoptosis in these cells, using Apop-Tag kit reagents, to detect apoptotic cells in situ by specific end labeling, and detection of DNA fragments produced by the apoptotic process. The results obtained indicate that: i) genistein inhibits the growth and proliferation of testicular cells; ii) growth inhibition and proliferation is dose- and exposure-time dependent; iii) there is significant difference in sensitivity of the different testicular cells to genistein; iv) genistein induces apoptosis in testicular cells in a concentration-dependent manner. Genistein-induced apoptosis identifies genistein as a potential diagnostic and therapeutic tool in testicular pathophysiological research.

Impact of exposure to endocrine disrupters in utero and in childhood on adult reproduction.

Best Pract Res Clin Endocrinol Metab 2002 Jun;16(2):289-309

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Recent reports have demonstrated a decline in human male reproductive health: high and probably increasing prevalence of cryptorchidism and hypospadias, low and probably decreasing semen quality, a rising incidence of testicular cancer and a growing demand for assisted reproduction. These changes seem to be interrelated and may be symptoms of a common underlying entity, the testicular dysgenesis syndrome, with foundations in fetal life due to adverse environmental influences. Wildlife experience and animal studies have provided evidence that fetal or perinatal exposure to endocrine disrupters results in disturbed sexual differentiation and urogenital malformations followed by decreased reproductive health in adult life. This chapter reviews existing evidence for a connection between (i) exposure to endocrine disrupters in fetal life and childhood and (ii) adult reproductive health in humans. This topic is not only relevant to basic scientists but also to clinical endocrinologists, who should also be encouraged to participate in research concerning this problem.

Regulation of male sex hormone levels by soy isoflavones in rats.

Nutr Cancer 2002;42(2):206-10

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Several studies have suggested that soybean intake is associated with a lower risk of prostate cancer. However, the mechanism of prostate cancer prevention by soybeans remains unclear. Because prostate cancer is reported to have an association with an increased level of dihydrotestosterone (DHT) and soybean isoflavones are known to inhibit 5 alpha-reductase, which is involved in the conversion of testosterone to DHT, the effects of soybean extract and isoflavones on the plasma levels of male sex hormones were investigated using male rats. In Experiment I, Sprague-Dawley rats were fed diets with and without soy flour; in Experiment II, rats were fed diets containing 2% soy methanol extract or 0.2% semipurified isoflavones or a control diet. The study showed a reduction of plasma DHT along with an increase in total plasma androgen in rats fed soy flour or semipurified isoflavones for 1 wk. These results suggest that soy isoflavone intake may reduce plasma DHT level.
Dietary soy and increased risk of bladder cancer: the Singapore Chinese Health Study.


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The association between soyfood consumption and subsequent bladder cancer risk was investigated in a population-based cohort study, the Singapore Chinese Health Study. As of December 31, 2000, 329,848 person-years of follow-up were accrued. Sixty-one histologically confirmed incident bladder cancer cases were identified. Information on soyfood consumption at baseline was obtained through in-person interviews using a validated dietary questionnaire. Relative risks and 95% confidence intervals were calculated using the Cox proportional hazard regression method. High intake of soyfood was statistically significantly related to an elevated risk of bladder cancer. Relative to the lowest quartile of energy-adjusted total soy intake (<36.9 g/1000 Kcal), the highest quartile of total soy intake (≥92.5 g/1000 Kcal) was associated with a 2.3-fold increase in bladder cancer risk (95% confidence interval = 1.1-5.1) after adjustment for cigarette smoking and level of education. Similar results were obtained for intakes of soy protein and soy isoflavones. The soyfood-bladder cancer risk association did not differ significantly between men and women and was not explained by other dietary factors. The soy-cancer relationship became stronger when the analysis was restricted to subjects with longer (≥3 years) duration of follow-up. To our knowledge, this is the first epidemiological report on the effect of dietary soy on bladder cancer risk.

Phytoestrogen supplements for the treatment of hot flashes: the Isoflavone Clover Extract (ICE) study: a randomized controlled trial.


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CONTEXT: Clinical trials demonstrating increased risk of cardiovascular disease and breast cancer among women randomized to hormone replacement therapy have increased interest in other therapies for menopausal symptoms. Dietary supplements containing isoflavones are widely used as alternatives to hormonal therapies for hot flashes, but there is a paucity of data supporting their efficacy. OBJECTIVE: To compare the efficacy and safety of 2 dietary supplements derived from red clover with placebo in symptomatic menopausal women. DESIGN, SETTING, AND PARTICIPANTS: Randomized, double-blind, placebo-controlled trial of menopausal women, aged 45 to 60 years, who were experiencing at least 35 hot flashes per week. The study was conducted between November 1999 and March 2001 at 3 US medical centers and included women who were recently postmenopausal (mean [SD], 3.3 [4.5] years since menopause) experiencing 8.1 hot flashes per day. Women were excluded if they were vegetarians, consumed soy products more than once per week, or took medications affecting isoflavone absorption. INTERVENTION: After a 2-week placebo run-in, 252 participants were randomly assigned to Promensil (82 mg of total isoflavones per day), Rimostil (57 mg of total isoflavones per day), or an identical placebo, and followed-up for 12 weeks. MAIN OUTCOME MEASURE: The primary outcome measure was the change in frequency of hot flashes measured by participant daily diaries. Secondary outcome measures included changes in quality of life and adverse events. RESULTS: Of 252 participants, 246 (98%) completed the 12-week protocol. The reductions in mean daily hot flash count at 12 weeks were similar for the Promensil (5.1), Rimostil (5.4), and placebo (5.0) groups. In comparison with the placebo group, participants in the Promensil group (41%; 95% confidence interval [CI], 29%-51%; P = .03), but not in the Rimostil group (34%; 95% CI, 22%-46%; P = .74) reduced hot flashes more rapidly. Quality-of-life improvements and adverse events were comparable in the 3 groups. CONCLUSION: Although the study provides some evidence for a biological effect of Promensil, neither supplement had a clinically important effect on hot flashes or other symptoms of menopause.
Dietary topoisomerase II-poisons: contribution of soy products to infant leukemia?

EXCLI Journal 2002;1:8-14

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DNA topoisomerases are nuclear enzymes inducing transient breaks in the DNA allowing DNA strands or double helices to pass through each other. The clinically used DNA topoisomerase II-poison etoposide is known to induce DNA double strand breaks leading to chromosomal aberrations and leukemias. Recently, some alarming studies have been published, suggesting that maternal exposure to low doses of dietary topoisomerase II poisons, including bioflavonoids such as genistein or quercetin, may contribute to the development of infant leukemia: approximately 80% of infants with acute myelogenous leukemia (AML) and acute lymphoblastic leukemia (ALL) have chromosome translocations involving the MLL (mixed lineage leukemia) gene. It has been shown that antineoplastic chemotherapy with the leukemogenic topoisomerase II-poison etoposide induced identical chromosomal aberrations involving the MLL gene compared to children with infant leukemia. Interestingly, the MLL cleavage sites induced by etoposide colocalized with the cleavage sites observed in infant leukemia. In addition, an almost 10-fold higher risk of infant AML has been reported for mothers consuming relatively high levels of topoisomerase II-poison containing foods. These observations are relevant, since many foods contain topoisomerase II poisons, predominantly soy and soy products, but also coffee, wine, tea, cocoa, as well as some fruits and vegetables. Further studies on the role of dietary topoisomerase II-poisons are urgently required. If the causal relationship between dietary exposure to topoisomerase II poisons and infant leukemia will be confirmed, care should be taken to reduce exposure to critical foods during pregnancy. Full Paper Available Here

Effect of soy-derived isoflavones on hot flushes, endometrial thickness, and the pulsatility index of the uterine and cerebral arteries.

Fertil Steril 2003 May;79(5):1112-1117

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To determine the effect of soy-derived isoflavones on hot flushes, endometrial thickness, and the vascular reactivity of uterine and cerebral arteries. Double-blind, randomized, placebo-controlled trial. Healthy volunteers in an academic research environment. Sixty-two postmenopausal women aged 45-60 years attending the Outpatient Menopause Clinic of our gynecological departments. The patients were administered 72 mg of soy-derived isoflavones or placebo under double-blind conditions. The daily number of hot flushes was recorded in a diary. Endometrial thickness was measured by means of transvaginal ultrasound; the uterine, internal carotid, and middle cerebral arteries were evaluated using Doppler ultrasound. The daily number of hot flushes, endometrial thickness, and arterial pulsatility index (PI). Both treatments led to a 40% reduction in the number of hot flushes. Soy-derived isoflavones had no effect on endometrial thickness or the PI of the uterine and cerebral arteries. The daily administration of 72 mg of soy-derived isoflavones is no more effective than placebo in reducing hot flushes in postmenopausal women. It also has no effect on endometrial thickness or the PI of the uterine and cerebral arteries.
Dietary supplements of soya flour lower serum testosterone concentrations and improve markers of oxidative stress in men.

Eur J Clin Nutr 2003 Jan;57(1):100-6

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OBJECTIVE:: We examined the effects on serum sex steroids, lipids and markers of oxidative stress of supplementing the diets of healthy male volunteers with scones made with soya flour. DESIGN:: A randomized placebo controlled cross-over trial. SETTING:: University Hospital of Wales. SUBJECTS:: Twenty volunteers recruited by advertisement. INTERVENTIONS:: Male volunteers ate three scones a day in addition to their normal diet for a period of 6 weeks. The scones were made with either wheat or soya flour (containing 120 mg/day of isoflavones). Blood was analysed for sex steroids (testosterone, dihydro-testosterone, oestradiol, oestrone, sex hormone binding globulin, albumin and the concentration of non-protein bound sex steroids were calculated), lipid profile (total cholesterol, high density lipoprotein cholesterol and triglycerides) and measures of oxidative stress (hydroperoxides, susceptibility of LDL to oxidation with copper and myeloperoxidase). RESULTS:: The volunteers' mean age was 35.6 (s.d. 11.2) y. Total serum testosterone fell in volunteers taking the soya scones (19.3-18.2 nmol/l; 95% CI 1.01, 1.12; P=0.03). No significant changes were seen in the concentrations of the other serum sex steroids, albumin or sex hormone binding globulin throughout the study. Significant improvements in two of the three markers of oxidative stress were seen in volunteers taking soya scones. Lag time for myeloperoxidase rose from 55.0 to 68.0 min (95% CI -16.0, -3.5; P=0.009) and the presence of hydroperoxides decreased from 2.69 to 2.34 micro mol/l (95% CI 0.12, 0.71; P=0.009). There were no changes seen in serum triglycerides or cholesterol. CONCLUSIONS:: We have shown that soya supplements reduce serum testosterone and improve markers of oxidative stress. These findings provide a putative mechanism by which soya supplements could protect against prostatic disease and atherosclerosis. Further dietary studies with clinical end points are warranted. SPONSORSHIP:: The Mason medical research foundation.

Maternal exposure to potential inhibitors of DNA topoisomerase II and infant leukemia (United States): a report from the Children's Cancer Group.


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Nearly 80 percent of infant leukemias present with an abnormality involving the MLL gene at 11q23. Moreover, secondary acute myeloid leukemias (AML) that occur as the result of chemotherapy agents, which are known to inhibit DNA topoisomerase II, often manifest the same MLL abnormalities. It has been hypothesized that de novo infant leukemias may occur as a result of maternal exposure to agents in diet and medications that inhibit DNA topoisomerase II. Three epidemiologic studies of childhood leukemia with similar methodologies were conducted in the United States and Canada over the past 10 years by the Children's Cancer Group (CCG). Of the total 771 mothers of infants diagnosed at one year of age or less (< 12.5 months) who originally were interviewed (303 infant cases and 468 matched controls) across the three studies, follow-up questionnaire data on maternal exposure to potential DNA topoisomerase II inhibitors during pregnancy were available on 84 cases and 97 matched controls in the US. For maternal diet, a composite variable was created that consisted of 10 foods identified alpha priori as containing DNA topoisomerase II inhibitors. There were no significant trends with increasing maternal consumption for either the overall group, or the acute lymphoblastic leukemia (ALL) stratum. However, within the AML stratum, there was a statistically significant positive association (P trend = 0.04) with increasing consumption of DNA topoisomerase II-inhibitor containing foods (odds ratio [OR] = 9.8, 95 percent confidence interval [CI] = 1.1-84.8; OR = 10.2, CI = 1.1-96.4; for medium and high consumption, respectively). Other potential topoisomerase II inhibitors were explored; no significant findings were found. Results of this preliminary study, in combination with molecular data, should be used in future investigations of childhood leukemia (particularly, infant) to justify the incorporation of a detailed dietary history.
The phytoestrogens coumoestrol and genistein induce structural chromosomal aberrations in cultured human peripheral blood lymphocytes.


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The clastogenic potential of the phytoestrogens coumoestrol (COUM), genistein (GEN) and daidzein (DAI) has been studied in human peripheral blood lymphocytes in vitro. After exposure of the cultured lymphocytes to 50 to 75 microM COUM or 25 microM GEN for 6 h, a clear induction of structural chromosomal aberrations was observed by cytogenetic analysis. The major alterations were chromatid breaks, gaps and interchanges. In contrast, DAI did not induce chromosome aberrations even at 100 microM. These results, together with previously published reports on the induction of micronuclei and DNA strand breaks in cultured Chinese hamster V79 cells by COUM and GEN, but not DAI, suggest that some but not all phytoestrogens have the potential for genetic toxicity.

Cell-transforming activity and mutagenicity of 5 phytoestrogens in cultured mammalian cells.

Int J Cancer 2003 Jun 20;105(3):312-20

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For the simultaneous assessment of in vitro carcinogenicity and mutagenicity of phytoestrogens, the abilities of 5 phytoestrogens, daidzein, genistein, biochanin A, prunetin, and coumestrol, to induce cell transformation and genetic effects were examined using the Syrian hamster embryo (SHE) cell model. Cellular growth was inhibited by all phytoestrogens in a concentration-related manner. The growth inhibitory effect of the compounds was ranked: genistein, prunetin > coumestrol > biochanin A > daidzein, which did not correspond to their apoptosis-inducing abilities. Morphological transformation in SHE cells was elicited by all phytoestrogens, except, prunetin. The transforming activities were ranked as follows: genistein > coumestrol > daidzein > biochanin A. Somatic mutations in SHE cells at the Na(+)/K(+) ATPase and hprt loci were induced only by genistein, coumestrol, or daidzein. Chromosome aberrations were induced by genistein or coumestrol, and aneuploidy in the near diploid range was occurred by genistein or biochanin A. Genistein, biochanin A or daidzein induced DNA adduct formation in SHE cells with the abilities: genistein > biochanin A > daidzein. Prunetin was negative for any of these genetic endpoints. Our results provide evidence that genistein, coumestrol, daidzein and biochanin A induce cell transformation in SHE cells and that the transforming activities of these phytoestrogens correspond to at least 2 of the mutagenic effects by each phytoestrogen, i.e., gene mutations, chromosome aberrations, aneuploidy or DNA adduct formation, suggesting the possible involvement of mutagenicity in the initiation of phytoestrogen-induced carcinogenesis.

A pilot study of the effects of phytoestrogen supplementation on postmenopausal endometrium.


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OBJECTIVE: This study was designed to assess endometrial histology in postmenopausal women not taking hormone replacement therapy, to evaluate side effects and efficacy of phytoestrogens in treating menopause-associated symptoms, and to determine whether 6 months of phytoestrogen supplementation altered endometrial histology. METHODS: We performed a prospective, double-blinded, randomized, placebo-controlled trial comparing the effects of 6 months of dietary phytoestrogen supplementation versus placebo in postmenopausal women. Baseline endometrial biopsies were performed and, if adequate, nonhyperplastic, noncancerous, and nonovulatory, subjects were randomly assigned to receive daily placebo or soy cereal supplementation for 6 months. Study subjects completed baseline and weekly dietary, symptom, and side effect logs. Repeat endometrial biopsies were obtained at 6 months. RESULTS: Subjects were recruited from January 1998 through June 2000. Twenty-seven subjects were randomized, and 19 completed the study. One (3.7%) baseline endometrial sample was weakly proliferative. All other baseline and final biopsies were consistent with atrophic, inactive endometrium. The maximum risk of endometrial stimulation with phytoestrogens is 35%. Hot flushes, night sweats, and vaginal dryness were significantly less severe at the final week of the study compared with baseline in the placebo group. Insomnia was more common in the treated group. There were no other statistically significant differences in symptoms or side effects. CONCLUSION: Phytoestrogens did not cause stimulation of the endometrium. Insomnia was more frequent over the 6-month study in the soy group, whereas hot flushes, night sweats, and vaginal dryness improved from baseline in the placebo group but not in the soy group.

Soya phytoestrogens change cortical and hippocampal expression of BDNF mRNA in male rats.


File SE, Hartley DE, Alom N, Rattray M.

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Adult male hooded Lister rats were either fed a diet containing 150 microg/g soya phytoestrogens or a soya-free diet for 18 days. This concentration of phytoestrogens should have been sufficient to occupy the oestrogen-beta, but not the oestrogen-alpha, receptors. Using in situ hybridisation, significant reductions were found in brain-derived neurotrophic factor (BDNF) mRNA expression in the CA3 and CA4 region of the hippocampus and in the cerebral cortex in the rats fed the diet containing phytoestrogens, compared with those on the soya-free diet. No changes in glutamic acid decarboxylase-67 or glial fibrillary acidic protein mRNA were found. This suggests a role for oestrogen-beta receptors in regulating BDNF mRNA expression.

This abstract should be read in conjunction with that of Connor et al. 1997 below.

Brain-derived neurotrophic factor is reduced in Alzheimer's disease.


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Alzheimer's disease may be due to a deficiency in neurotrophin protein or receptor expression. Consistent with this hypothesis, a reduction in BDNF mRNA expression has been observed in human post-mortem Alzheimer's disease hippocampi. To further investigate this observation, we examined whether the alteration in BDNF expression also occurred at the protein level in human post-mortem Alzheimer's disease hippocampi and temporal cortices using immunohistochemical techniques. We observed a reduction in the intensity and number of BDNF-immunoreactive cell bodies within both the Alzheimer's disease hippocampus and temporal cortex when compared to normal tissue. These results support and extend previous findings that BDNF mRNA is reduced in the human Alzheimer's disease hippocampus and temporal cortex, and
suggest that a loss of BDNF may contribute to the progressive atrophy of neurons in Alzheimer's disease.

**Brain Aging and Midlife Tofu Consumption**


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Objective: To examine associations of midlife tofu consumption with brain function and structural changes in late life.

Methods: The design utilized surviving participants of a longitudinal study established in 1965 for research on heart disease, stroke, and cancer. Information on consumption of selected foods was available from standardized interviews conducted 1965–1967 and 1971–1974. A 4-level composite intake index defined "low-low" consumption as fewer than two servings of tofu per week in 1965 and no tofu in the prior week in 1971. Men who reported two or more servings per week at both interviews were defined as "high-high" consumers. Intermediate or less consistent "low" and "high" consumption levels were also defined. Cognitive functioning was tested at the 1991–1993 examination, when participants were aged 71 to 93 years (n=3734). Brain atrophy was assessed using neuroimage (n=574) and autopsy (n=290) information. Cognitive function data were also analyzed for wives of a sample of study participants (n=502) who had been living with the participants at the time of their dietary interviews.

Results: Poor cognitive test performance, enlargement of ventricles and low brain weight were each significantly and independently associated with higher midlife tofu consumption. A similar association of midlife tofu intake with poor late life cognitive test scores was also observed among wives of cohort members, using the husband’s answers to food frequency questions as proxy for the wife’s consumption. Statistically significant associations were consistently demonstrated in linear and logistic multivariate regression models. Odds ratios comparing endpoints among "high-high" with "low-low" consumers were mostly in the range of 1.6 to 2.0.

Conclusions: In this population, higher midlife tofu consumption was independently associated with indicators of cognitive impairment and brain atrophy in late life.

Full Paper Here

**Aluminum and bone disorders: with specific reference to aluminum contamination of infant nutrients.**


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Aluminum (Al) impairment of bone matrix formation and mineralization may be mediated by its direct effect on bone cells or indirectly by its effect on parathyroid hormone and calcium metabolism. Its toxic effects are proportional to tissue Al load. Al contamination of nutrients depends on the amount of Al present naturally in chemicals or from the manufacturing process. Intravenous calcium, phosphorus, and albumin solutions have high Al (greater than 500 micrograms/L), whereas crystalline amino acid, sterile water, and dextrose water have low Al (less than 50 micrograms/L) content. Enteral nutrients including human and whole cow milk have low Al, whereas highly processed infant formulas with multiple additives, such as soy formula, preterm infant formula, and formulas for specific disorders are heavily contaminated with Al. Healthy adults are in zero
Severe nutritional deficiencies in toddlers resulting from health food milk alternatives.


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It is widely appreciated that health food beverages are not appropriate for infants. Because of continued growth, children beyond infancy remain susceptible to nutritional disorders. We report on 2 cases of severe nutritional deficiency caused by consumption of health food beverages. In both cases, the parents were well-educated, appeared conscientious, and their children received regular medical care. Diagnoses were delayed by a low index of suspicion. In addition, nutritional deficiencies are uncommon in the United States and as a result, US physicians may be unfamiliar with their clinical features. Case 1, a 22-month-old male child, was admitted with severe kwashiorkor. He was breastfed until 13 months of age. Because of a history of chronic eczema and perceived milk intolerance, he was started on a rice beverage after weaning. On average, he consumed 1.5 L of this drink daily. Intake of solid foods was very poor. As this rice beverage, which was fallaciously referred to as rice milk, is extremely low in protein content, the resulting daily protein intake of 0.3 g/kg/day was only 25% of the recommended dietary allowance. In contrast, caloric intake was 72% of the recommended energy intake, so the dietary protein to energy ratio was very low. A photograph of the patient after admission illustrates the typical features of kwashiorkor: generalized edema, hyperpigmented and hypopigmented skin lesions, abdominal distention, irritability, and thin, sparse hair. Because of fluid retention, the weight was on the 10th percentile and he had a rotund sugar baby appearance. Laboratory evaluation was remarkable for a serum albumin of 1.0 g/dL (10 g/L), urea nitrogen <0.5 mg/dL (<0.2 mmol/L), and a normocytic anemia with marked anisocytosis. Evaluation for other causes of hypoalbuminemia was negative. Therapy for kwashiorkor was instituted, including gradual refeeding, initially via a nasogastric tube because of severe anorexia. Supplements of potassium, phosphorus, multivitamins, zinc, and folic acid were provided. The patient responded dramatically to refeeding with a rising serum albumin and total resolution of the edema within 3 weeks. At follow-up 1 year later he continued to do well on a regular diet supplemented with a milk-based pediatric nutritional supplement. The mortality of kwashiorkor remains high, because of complications such as infection (kwashiorkor impairs cellular immune defenses) and electrolyte imbalances with ongoing diarrhea. Children in industrialized countries have developed kwashiorkor resulting from the use of a nondairy creamer as a milk alternative, but we were unable to find previous reports of kwashiorkor caused by a health food milk alternative. We suspect that cases have been overlooked. Case 2, a 17-month-old black male, was diagnosed with rickets. He was full-term at birth and was breastfed until 10 months of age, when he was weaned to a soy health food beverage, which was not fortified with vitamin D or calcium. Intake of solid foods was good, but included no animal products. Total daily caloric intake was 144% of the recommended dietary allowance. Dietary vitamin D intake was essentially absent because of the lack of vitamin D-fortified milk. The patient lived in a sunny, warm climate, but because of parental career demands, he had limited sun exposure. His dark complexion further reduced ultraviolet light-induced endogenous skin synthesis of vitamin D. The patient grew and developed normally until after his 9-month check-up, when he had an almost complete growth arrest of both height and weight. The parents reported regression in gross motor milestones. On admission the patient was unable to crawl or roll over. He could maintain a sitting position precariously when so placed. Conversely, his language, fine motor-adaptive, and personal-social skills were well-preserved. Generalized hypotonia, weakness, and decreased muscle bulk were present. Clinical features of rickets present on examination included: frontal bossing, an obvious rachitic rosary (photographed), genu varus, flaring of the wrists, and lumbar kyphoscoliosis. The serum alkaline phosphatase was markedly elevated (1879 U/L). phosphorus was low (1.7 mg/dL), and calcium was low normal (8.9 mg/dL). The 25-hydroxy-vitamin D level was low (7.7 pg/mL) and the parathyroid hormone level was markedly elevated (114 pg/mL). The published radiographs are diagnostic of advanced rickets, showing diffuse osteopenia, frayed metaphyses, widened epiphysial plates, and a pathologic fracture of the ulna. The patient was treated with ergocalciferol and calcium supplements. The published growth chart demonstrates the dramatic response to therapy. Gross motor
milestones were fully regained within 6 months. The prominent neuromuscular manifestations shown by this patient serve as a reminder that rickets should be considered in the differential diagnosis of motor delay.

**Hypocalcemic tetany in 'alternative' soy milk nutrition in the first months of life**


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A 14 weeks old infant was admitted to the intensive care unit with life-threatening hypocalcemic-hyperphosphatemic spasms. Hypocalcemia-hyperphosphatemia was found to have been caused by feeding a high phosphate/low calcium soy milk. The daily uptake of calcium was calculated to have been 3.3-6 mmol that of phosphate 30 mmol. The parents strongly believed that soy milk formulas were equivalent to breast milk and cow's milk formulas and lived on a strictly vegetarian diet. Therapy with calcium (at an initial dose of 2.25 mmol/kg/day) and 1.25 OH vitamin D3 (Rocaltrol, 0.25 microgram/day) normalized Ca, PO4, vitamin D and parathyroid hormone levels rapidly. Vegetarian feeding had led to life-threatening hypocalcemic hyperphosphatemic spasms in the infant. We conclude that malnutrition and false nutritional beliefs have to be included as a potential cause of early hypocalcemia in infants.

**Manipulation of prenatal hormones and dietary phytoestrogens during adulthood alter the sexually dimorphic expression of visual spatial memory.**


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BACKGROUND:. In learning and memory tasks, requiring visual spatial memory (VSM), males exhibit higher performance levels compared to females (a difference attributed to sex steroid hormonal influences). Based upon the results from our companion investigation, this study examined the influence of prenatal sex steroid hormone manipulations on VSM in adulthood, as assessed in the radial arm maze. Additionally, the influence of dietary soy phytoestrogens (i.e., the presence of high or low estrogen-like compounds present in the animal's diet) on VSM was examined in combination with the prenatal hormonal manipulations. RESULTS:. Radial arm maze performance on a phytoestrogen-rich diet: 1) females treated prenatally with testosterone were masculinized and acquired/performed in a manner similar to control or oil-treated males and 2) males treated prenatally with an androgen receptor blocker (flutamide) were feminized and acquired/performed in a fashion typical of control or flutamide-treated females. When a diet change was initiated in adulthood, control phytoestrogen-rich fed females outperformed control females switched to a phytoestrogen-free diet. Whereas, in control males the opposite diet effect was identified. Furthermore, flutamide-treated males fed a phytoestrogen-rich diet outperformed flutamide-treated males switched to a phytoestrogen-free diet. CONCLUSIONS:. These results suggest that prenatal hormonal manipulations significantly sex-reverse the normal sexually dimorphic expression of VSM. Specifically, VSM was enhanced in females treated with testosterone and inhibited in males treated with flutamide. Finally, dietary soy phytoestrogens set a bias on learning and memory in these hormonally manipulated animals in a predictable manner and these data confirm and extend the findings in our companion paper.

**Serum plant sterols as a potential risk factor for coronary heart disease.**


Department of Clinical Pharmacology, University of Bonn, Bonn, Germany. In patients with the inherited disease of phytosteroolemia, elevated concentrations of plant sterols (eg, campesterol and sitosterol)
have been implicated as a risk factor for premature atherosclerosis. Whether plasma concentrations of campesterol and sitosterol are risk factors for coronary heart disease (CHD) in non-phytosterolemia subjects has not been established. Therefore, the present study examined the role of plant sterols in patients admitted for elective artery coronary bypass graft (ACBG). Serum concentrations of campesterol and sitosterol, as well as lathosterol, desmosterol, cholestanol, and lipoproteins were analyzed in 42 men and 11 women without lipid-lowering treatment during the past. Twenty-six patients reported a positive family history in their first-degree relatives for CHD. Lipid profile and other risk factors were comparable in both groups. Patients with a positive family history for CHD had significant higher plasma levels of campesterol (0.50 +/- 0.17 vs. 0.38 +/- 0.16 mg/dL; P = 0.011), sitosterol (0.40 +/- 0.11 v. 0.31 +/- 0.11 mg/dL; P = 0.004) and their ratios to cholesterol. Lathosterol, desmosterol, cholestanol, and their ratios to cholesterol were not significantly different. Analysis of covariance (ANCOVA) analysis showed no influence of sex, age, triglycerides, total-, low-density lipoprotein (LDL)-, and high-density lipoprotein (HDL)-cholesterol on the results, but confirmed a strong influence of plant sterols. These findings support the hypothesis that plant sterols might be an additional risk factor for CHD.

Review Article: Soy infant formula and phytoestrogens


Soy infant formula contains high levels of the isoflavones, genistein and daidzein, which are commonly referred to as phytoestrogens. These are non-steroidal chemicals with structural similarities to estrogen. Infants consuming soy formula have high levels of circulating isoflavones. These are an order of magnitude greater than the levels of isoflavones which have been shown to produce physiological effects in adult women consuming a high soy diet. There is conflicting evidence about the risks and benefits of soy phytoestrogens, with research presenting a contradictory picture. Some reviewers suggest that early exposure to soy may prevent cancer and heart disease. However, there is very little research on the effects of consumption of soy phytoestrogens by human neonates. Against this generally positive view there is an increasing number of recent reports that suggest that in experimental animals, phytoestrogens have adverse effects with respect to carcinogenesis, reproductive function, immune function, and thyroid disease. Despite the absence of adequate scientific research that quantifies the level of risk to infants, most would argue for a precautionary approach to be taken in situations where there are potential developmental effects from the consumption of pharmacologically active compounds in infancy and childhood.

Dietary genistein inactivates rat thyroid peroxidase in vivo without an apparent hypothyroid effect.


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Biological effects of genistein are currently under investigation by the National Toxicology Program because of widespread and increasing soy consumption by humans and evidence for modulation of endocrine function. Rats were exposed to genistein aglycone in soy-free feed fortified at 0, 5, 100, and 500 ppm starting in utero through 20 weeks. Thyroid glands and serum were analyzed for total genistein (aglycone + conjugates) using HPLC with electrospray mass spectrometric detection. Microsomal thyroid peroxidase (TPO) activity was measured spectrophotometrically. The total genistein content in rat serum was as high as 8 microM, and significant dose-dependent increases of genistein in thyroid tissue up to 1 pmol/mg were found in male and female rats. The activity of TPO in male and female rats was found to be reduced by up to 80% in a dose-dependent manner. Male and female rats consuming a standard soy-based rodent diet (NIH 31) had TPO activity approximately 50% lower than rats consuming a soy-free diet and this loss was commensurate with measured serum levels of isoflavones. Suicide inactivation of rat, porcine, and human TPO was observed in vitro at concentrations of genistein aglycone comparable to those measured in rat thyroids. Thyroid hormone levels (T3, T4, TSH) in serum, thyroid weights, and histopathology showed no differences between treated and untreated groups. These findings suggest that, even though substantial amounts of TPO activity are lost concomitant to soy
Isoflavone consumption by normal rats, the remaining enzymatic activity is sufficient to maintain thyroid homeostasis in the absence of additional perturbations.

Neonatal exposure to genistein induces estrogen receptor (ER) alpha expression and multioocyte follicles in the maturing mouse ovary: evidence for ERbeta-mediated and nonestrogenic actions.


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Outbred CD-1 mice were treated neonatally on Days 1-5 with the phytoestrogen, genistein (1, 10, or 100 micro g per pup per day), and ovaries were collected on Days 5, 12, and 19. Ribonuclease protection assay analysis of ovarian mRNA showed that estrogen receptor beta (ERbeta) predominated over ERalpha in controls and increased with age. Genistein treatment did not alter ERbeta expression, however, ERalpha expression was higher on Days 5 and 12. ERbeta was immunolocalized in granulosa cells, whereas ERalpha was immunolocalized in interstitial and thecal cells. Genistein treatment caused a dramatic increase in ERalpha in granulosa cells. Genistein-treated ERbeta knockout mice showed a similar induction of ERalpha, which is seen in CD-1 mice, suggesting that ERbeta does not mediate this effect. Similar ERalpha induction in granulosa cells was seen in CD-1 mice treated with lavendustin A, a tyrosine kinase inhibitor that has no known estrogenic actions, which suggests that this property of genistein may be responsible. As a functional analysis, genistein-treated mice were superovulated and the number of oocytes was counted. A statistically significant increase in the number of ovulated oocytes was observed with the lowest dose, whereas a decrease was observed with the two higher doses. This increase in ovulatory capacity with the low dose coincided with higher ERalpha expression. Histological evaluations on Day 19 revealed a dose-related increase in multioocyte follicles (MOFs) in genistein-treated mice. Tyrosine kinase inhibition was apparently not responsible for MOFs because they were not present in mice that had been treated with lavendustin; however, ERbeta must play a role, because mice lacking ERbeta showed no MOFs. These data taken together demonstrate alterations in the ovary following neonatal exposure to genistein. Given that human infants are exposed to high levels of genistein in soy-based foods, this study indicates that the effects of such exposure on the developing reproductive tract warrant further investigation.

Soy formula complicates management of congenital hypothyroidism

Archives of Disease in Childhood 2004;89:37-40

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Aims: To test the hypothesis that feeding soy formula to infants with congenital hypothyroidism (CH) leads to prolonged increase of thyroid stimulating hormone (TSH).

Methods: The study was a review of 78 patients seen during their first year of life between 1990 and 1998. Data regarding clinical diagnosis, date of treatment initiation, TSH, levothyroxine dose, weight, length, and diet information from each visit were collected from the charts.

Results: There were eight patients in the soy diet group and 70 in the non-soy diet group. There was no significant difference between the two groups in the starting dose of levothyroxine or the change in this dose over one year. There was a significant difference between the two groups in the following areas: time to TSH normalisation, first TSH on treatment, percentage with increased TSH at 4 months of age, percentage with increased TSH throughout the first year of life, and in the overall trend of TSH at each visit.
Conclusions: Infants fed soy formula had prolonged increase of TSH when compared to infants fed non-soy formula. These infants need close monitoring of free thyroxine and TSH measurements, and they may need increased levothyroxine doses to achieve normal thyroid function tests.

Physiological concentrations of dietary genistein dose-dependently stimulate growth of estrogen-dependent human breast cancer (MCF-7) tumors implanted in athymic nude mice.


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Previously our laboratory has shown that the soy isoflavone, genistein, stimulates growth of human breast cancer (MCF-7) cells in vivo and in vitro. In this study, the dose-response analysis of genistein at the physiologically achievable concentration range between 125 and 1,000 microg/g in the diet was conducted in ovariectomized athymic nude mice implanted with MCF-7 cells. We hypothesized that genistein at this concentration range can stimulate dose-dependently the breast tumor growth, cell proliferation and an estrogen-responsive pS2 gene induction. Tumor size and body weight were monitored weekly. At completion of the study, we analyzed cellular proliferation of tumors using incorporation of BrdU, pS2 expression of tumors using a Northern blot analysis and total genistein level in plasma using liquid chromatography-isotope dilution mass spectrometry (LC-ES/MS). Dietary genistein (> or = 250 microg/g) increased tumor size in a dose-dependent manner [8.4x the negative control (NC) group in the 250 microg/g group, 12.0x in the 500 microg/g group, 20.2x in the 1,000 microg/g group and 23.2x in the positive control (PC) group]. The percentage of proliferating cells was significantly increased by genistein at and above 250 microg/g (5.3x the NC group in the 250 microg/g, 5.6x in the 500 microg/g, 5.0x in the 1,000 microg/g and 4.8x in the PC group). Expression of pS2 mRNA was also significantly increased with increasing dietary genistein levels (11.25x the NC group in the 500 microg/g group and 15.84x in the 1,000 microg/g group). Total plasma genistein concentrations were between 0.39 and 3.36 micromol/L in mice fed between 125 and 1,000 microg/g genistein. In conclusion, dietary treatment with genistein at physiological concentrations produces blood levels of genistein sufficient to stimulate estrogenic effects, such as breast tumor growth, cellular proliferation and pS2 expression in athymic mice in a dose-responsive manner similar to that seen in vitro.

Increased aggressive behavior and decreased affiliative behavior in adult male monkeys after long-term consumption of diets rich in soy protein and isoflavones.

Horm Behav. 2004 Apr;45(4):278-84.

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Estrogen produced by aromatization of gonadal androgen has an important facilitative role in male-typical aggressive behavior that is mediated through its interaction with estrogen receptors (ER) in the brain. Isoflavones found in soybeans and soy-based dietary supplements bind ER and have dose- and tissue-dependent effects on estrogen-mediated responses. Yet, effects of isoflavone-rich diets on social and aggressive behavior have not been studied. We studied the effects of long-term (15 months) consumption of diets rich in soy isoflavones on spontaneous social behavior among adult male cynomolgus macaques (Macaca fascicularis) (n = 44) living in nine stable social groups. There were three experimental conditions which differed only by the source of dietary protein: casein and lactalbumin (no isoflavones), soy protein isolate containing 0.94 mg isoflavones/g protein, and soy protein isolate containing 1.88 mg isoflavones/g protein. In the monkeys fed the higher amount of isoflavones, frequencies of intense aggressive (67% higher) and submissive (203% higher) behavior were elevated relative to monkeys fed the control diet (P’s < 0.05). In addition, the proportion of time spent by these monkeys in physical contact with other monkeys was reduced by 68%, time spent in proximity to
other monkeys was reduced 50%, and time spent alone was increased 30% (P’s < 0.02). There were no effects of treatment on serum testosterone or estradiol concentrations or the response of plasma testosterone to exogenous gonadotropin-releasing hormone (GnRH). The results indicate that long-term consumption of a diet rich in soy isoflavones can have marked influences on patterns of aggressive and social behavior.

**beta-Sitosterol, beta-Sitosterol Glucoside, and a Mixture of beta-Sitosterol and beta-Sitosterol Glucoside Modulate the Growth of Estrogen-Responsive Breast Cancer Cells In Vitro and in Ovariectomized Athymic Mice.**


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We hypothesized that the phytosterols beta-sitosterol (BSS), beta-sitosterol glucoside (BSSG), and Moducare (MC; BSS:BSSG = 99:1) could modulate the growth of estrogen-dependent human breast cancer cells in vitro and in vivo. The present study evaluated the estrogenic and antiestrogenic effects of BSS, BSSG, and MC (0.001 to 150 micro mol/L) on the proliferation of Michigan Cancer Foundation 7 (MCF-7) cells in vitro. Both BSS (>1 micro mol/L) and MC (>50 micro mol/L) increased MCF-7 cell proliferation. Treatment with 150 micro mol/L of BSS and MC increased cell growth by 2.4 and 1.5 times, respectively, compared to the negative control (NC) group. However, BSSG had no effect at the concentrations tested. The effects of dietary BSS, BSSG, and MC on the growth of MCF-7 cells implanted in ovariectomized athymic mice were also evaluated. Estrogenic effects of the phytosterols were evaluated in the NC, BSS, BSSG, and MC treatment groups, and antiestrogenic effects were evaluated in the 17beta-estradiol (E(2)), E(2) + BSS, E(2) + BSSG, and E(2) + MC treatment groups. Mice were treated with dietary BSS (9.8 g/kg AIN93G diet), BSSG (0.2 g/kg diet), or MC (10.0 g/kg diet) for 11 wk. Dietary BSS, BSSG, and MC did not stimulate MCF-7 tumor growth. However, dietary BSS, BSSG, and MC reduced E(2)-induced MCF-7 tumor growth by 38.9% (P < 0.05), 31.6% (P = 0.08), and 42.1% (P < 0.05), respectively. The dietary phytosterols lowered serum E(2) levels by 35.1, 30.2, and 36.5% in the E(2) + BSS, E(2) + BSSG, and E(2) + MC groups, respectively (P < 0.05), compared to that of the E(2) treatment group. Estrogen-responsive pS2 mRNA expression in tumors did not differ among groups, but expression of the antiapoptotic marker B-cell lymphoma/leukemia-2 (bcl-2) in tumors from the E(2) + MC group was downregulated, compared to that of the E(2) treatment group. In summary, BSS and MC stimulated MCF-7 cell growth in vitro. Although BSSG comprises only 1% of MC, BSSG made MC less estrogenic than BSS alone in vitro. However, dietary BSS and MC protected against E(2)-stimulated MCF-7 tumor growth and lowered circulating E(2) levels.

**Paediatric group position statement on the use of soya protein for infants.**

J Fam Health Care. 2003;13(4):93

The British Dietetic Association.

Breast feeding should be strongly encouraged as providing the safest, most nutritionally adequate form of feeding for most infants. Dietitians should discourage the use of soya protein in children with atopy or cow’s milk allergy in the first six months of life to avoid sensitisation to soya protein and exposure to phytoestrogens while organ systems remain at their most vulnerable. This would include soy infant formula and soya products such as desserts etc. When a soy-based infant formula is used, parents should be informed of current findings relating to phytoestrogens and health and on the clinical need for soy formula. Any parent choosing to refuse soya for their infant should be supported in their decision. More research into the long-term effects of phytoestrogen exposure in infants is needed and into whether any adverse effects are dose related. This position statement will be updated as further evidence becomes available.
New guidelines on infant feeding in the first 12 months of life.


More J.

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Paediatric dietitian Judy More discusses several recent directives and guidelines on infant feeding in the first year of life. These include recommendations that babies should be fed exclusively on breast milk for their first six months and that soy formulae should normally be avoided for babies under six months old. There is also specific new advice on restricting salt intake for infants.

Adrenocortical effects of oral estrogens and soy isoflavones in female monkeys.

J Clin Endocrinol Metab. 2004 May;89(5):2319-25.

Wood CE, Cline JM, Anthony MS, Register TC, Kaplan JR.

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The goal of this study was to evaluate the long-term adrenocortical effects of premenopausal oral contraceptives (OC) and postmenopausal conjugated equine estrogens (CEE) and soy isoflavones in a female cynomolgus monkey model. Half of the animals received a triphasic OC for a period of 26 months, after which all monkeys were ovariectomized and randomized to one of three diet groups for 36 months: 1). isoflavone-depleted soy protein (control) (n = 54); 2). soy protein with isoflavones (129 mg/d equivalent) (SPI+) (n = 56); or 3). isoflavone-depleted soy protein with CEE (0.625 mg/d equivalent) (n = 59). In the premenopausal phase, OC treatment resulted in significantly higher cortisol (F) and lower dehydroepiandrosterone sulfate, androstenedione, and testosterone relative to intact controls. In the postmenopausal phase, CEE treatment resulted in significantly higher basal F and lower dehydroepiandrosterone sulfate, androstenedione, and testosterone when compared with control and SPI+ diets. Serum F and androgens in the SPI+ group did not differ significantly from the control group. The SPI+ group had significantly lower adrenal weight than either control or CEE groups, and this effect was localized primarily to the zona fasciculata region of the adrenal cortex. These findings suggest that long-term estrogen treatment may contribute to an androgen-deficient and hypercortisolemic state.

Endometrial effects of long-term treatment with phytoestrogens: a randomized, double-blind, placebo-controlled study.


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OBJECTIVE: To determine the effects of 5 years of treatment with soy phytoestrogens on histological characteristics of endometrium in postmenopausal women. DESIGN: Randomized, double-blind, placebo-controlled study. SETTING: Centre of Perinatal and Reproductive Medicine, Department of Gynecological, Obstetrical, and Pediatric Sciences, University of Perugia, Italy. PATIENT(S): Three hundred seventy-six postmenopausal healthy women, all with intact uterus. INTERVENTION(S): Women were distributed in two different groups using randomized criteria: group A (n = 179) patients received soy tablets (150 mg of isoflavones per day) for 5 years; group B (n = 197) patients received identical appearing placebo tablets for 5 years. MAIN OUTCOME MEASURE(S): Results of endometrial histology from biopsies obtained at baseline, 30 months, and 5 years after the beginning of the treatment. RESULT(S): Two hundred ninety-eight women completed the 5-year treatment. No cases of malignancy were detected during biopsy. Seventy percent of women undergoing
treatment with soy phytoestrogens had an endometrium classified as atrophic or nonassessable versus 81% receiving placebo. The occurrence of endometrial hyperplasia was significantly higher in group A (3.37% vs. 0%).

CONCLUSION(S): Long-term treatment (up to 5 years) with soy phytoestrogens was associated with an increased occurrence of endometrial hyperplasia. These findings call into question the long-term safety of phytoestrogens with regard to the endometrium.

Effect of soy protein containing isoflavones on cognitive function, bone mineral density, and plasma lipids in postmenopausal women: a randomized controlled trial.


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The Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, The Netherlands.

CONTEXT: Postmenopausal estrogen therapy has been posited to have some beneficial effects on aging processes, but its use has risks. Isoflavones, estrogenlike compounds naturally occurring in plant foods, might confer positive effects with fewer adverse effects. OBJECTIVE: To investigate whether soy protein with isoflavones improves cognitive function, bone mineral density, and plasma lipids in postmenopausal women.

DESIGN, SETTING, AND PARTICIPANTS: Double-blind, randomized, placebo-controlled trial of 202 healthy postmenopausal women aged 60 to 75 years, recruited from a population-based sample in the Netherlands, conducted between April 2000 and September 2001. INTERVENTION: Participants were randomly assigned to receive 25.6 g of soy protein containing 99 mg of isoflavones (52 mg genistein, 41 mg daidzein, and 6 mg glycitein or total milk protein as a powder on a daily basis for 12 months. MAIN OUTCOME MEASURES: Cognitive function was assessed using the following instruments: dementia, Mini-Mental State Examination; memory, Rey Auditory Verbal Learning Test, immediate recall, delayed recall, and recognition, the Digit Span forward and reversed, and the Doors test; complex attention tasks, Digit Symbol Substitution and Trailmaking, A1, A2, and B; and verbal skills, Verbal Fluency A and N, animals and occupations, and the Boston Naming Task. Bone mineral density of the hip and lumbar spine was assessed using dual-energy x-ray absorptiometry scanning. Lipid assessment included lipoprotein(a), total cholesterol, low-density lipoprotein, high-density lipoprotein, and triglycerides. RESULTS: A total of 175 women completed the baseline and at least 1 postintervention analysis and were included in the modified intent-to-treat analysis. Adherence was good (median plasma genistein levels, 17.2 and 615.1 nmol/L for placebo and soy group, respectively). Cognitive function, bone mineral density, or plasma lipids did not differ significantly between the groups after a year. CONCLUSION: This double-blind randomized trial does not support the hypothesis that the use of soy protein supplement containing isoflavones improves cognitive function, bone mineral density, or plasma lipids in healthy postmenopausal women when started at the age of 60 years or later.

Evidence for genistein mediated cytotoxicity and apoptosis in rat brain.


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The effects of chronic treatment with high doses of genistein, a major isoflavone of soybeans and soy-based products, have yet to be determined and what is known remains controversial. The present study was undertaken to investigate the cytotoxic effects of chronic ingestion of genistein on rat brain in vivo and the observations were compared with results from in vitro studies with primary cultures of cortical neurons. Sprague-Dawley rats were given 2 or 20 mg/day genistein (p.o.) for four weeks. The high dose of genistein (20 mg/day) significantly increased lactate dehydrogenase (LDH) in rat brain tissue homogenates, whereas the low dose of genistein (2 mg/day) decreased LDH. In addition, DNA fragmentation was detected in homogenates of
brain tissue from rats receiving either dose of genistein. These results are consistent with those of in vitro studies indicating that high concentrations of genistein caused cytotoxicity and DNA ladder formation in primary cultures of cortical neurons. Genistein decreased the expression of the 32 kDa caspase-3 precursor and increased the levels of cleaved caspase-3 (18 kDa) in both rat brain tissue homogenates and in primary cultures of cortical neurons. Furthermore, expression of poly (ADP-ribose) polymerase (PARP) was also decreased in both experimental systems. These results suggest that chronic administration of genistein at high doses may induce cytotoxicity and apoptosis in the rat brain.

The effect of soy protein isolate on bone metabolism.


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OBJECTIVE: This double-blind, 15-month pilot study was designed to investigate the effect of soy protein isolate with varying concentrations of isoflavones on early postmenopausal bone loss and lipids. DESIGN: A total of 65 women, with a mean age of 55 years and 7.5 years since menopause, were randomized to one of three groups; soy protein with 96 mg isoflavones/day, soy with 52 mg isoflavones/day, or soy without isoflavones (< 4 mg isoflavones/day). Soy was given for 9 months and then discontinued; participants were followed for an additional 6 months. Bone mineral density (BMD) and blood lipids were measured during this time. RESULTS: Measurement of serum isoflavones at 3 months showed dose-related increases in the three groups. There was no significant effect of the soy supplements on BMD of the spine or femoral neck in any of the three groups. BMD increased significantly in the trochanter at 9 months (P = 0.0219) and at 15 months (P < 0.05) in the group given isoflavone-free soy compared with the other two groups. There was no significant effect of soy on lipid metabolism at the end of the intervention. CONCLUSION: The present study did not find a significant positive effect of soy protein isolate supplemented with isoflavones on BMD and the serum lipid profile in early postmenopausal women.

Genistein at a concentration present in soy infant formula inhibits Caco-2BBe cell proliferation by causing G2/M cell cycle arrest.


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Fifteen percent of all U.S. infants are fed soy formulas containing up to 47 mg/L of isoflavones (>65% as genistin + genistein); thus, these infants' intestines are exposed to a high dose of genistein, a phytoestrogen and tyrosine kinase inhibitor. Little attention has been focused on genistein's impact on the developing intestine. We hypothesized that a high dose of genistein would inhibit intestinal cell growth. Caco-2BBe human intestinal cells were exposed to 0, 3.7, and 111 micro mol/L (0, 1, and 30 mg/L) genistein in DMEM + 0.5% fetal bovine serum for 24-48 h. Cell number, thymidine incorporation, apoptosis, and cell cycle analyses were performed. The low genistein concentration increased intestinal cell proliferation by 28% (P = 0.001), but did not affect cell number or caspase-3 activity compared to the control. Furthermore, the addition of ICI, an estrogen receptor antagonist, negated the proliferative effect of the low genistein. In contrast, the high genistein concentration reduced cell number by 40%, proliferation by 94%, and caspase-3 activity by 50% compared to the control (P < 0.05). Cell cycle analysis after 48 h exposure to high genistein revealed 37% of cells in G0/G1 and 35% in G2/M vs. 71% in G0/G1 and 17% in G2/M for the control and low genistein groups. Thus, a biphasic effect of genistein was seen with a low dose stimulating intestinal cell proliferation through the estrogen receptor, whereas a high dose of genistein inhibited intestinal cell proliferation and altered cell cycle dynamics. A high dose of genistein may potentially compromise intestinal growth.
Bioavailability of zinc in milk and soy protein-based infant formulas.


Momcilovic B, Belonje B, Giroux A, Shah BG.

Total femur zinc of young rats was used to evaluate the biological availability of zinc in milk and soy protein-based infant formulas. A zinc deficient diet (0.8 mug Zn/g) containing egg white protein was supplemented with graded levels of zinc from zinc sulfate, milk and soy protein-based infant formulas. A plot of total femur zinc (log) after feeding the diet for 3 weeks versus the zinc added to the diet gave a linear relationship over the range of 0, 3, 6, 9 and 12 mug/g added zinc. By using a slope-ratio bioassay model, the relative biological availability of endogenous and added zinc in milk-based formula was estimated to be 0.86 and that of soy-based formula 0.67 (zinc sulphate = 1.00) with corresponding 95% fiducial limits being 0.82 to 0.91 and 0.62 to 0.71. Thus, to provide equivalent amounts of available zinc, the total zinc content of the soy protein-based formula would need to be at least 20% higher than that of the formula containing milk protein.

Genotoxicity of the isoflavones genistein, daidzein and equol in V79 cells.


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Hormonally active chemicals in the human diet, such as man-made estrogenic chemicals or plant-derived compounds (phytoestrogens), have become a matter of public concern. A significant part of human exposure to phytoestrogens is attributable to soy isoflavones. Besides their estrogenic properties, soy isoflavones also exert genotoxic actions. In this paper, the micronucleus (MN) assay in V79 cells was used to study chromosomal genotoxicity. Genistein caused a clear dose-related induction of MN within the range of 5-25 microM; MN rates were declining at higher genistein concentrations. This was probably due to cytotoxicity of genistein since reduced neutral red uptake and MTT formation with an IC(50) of about 75 microM occurred. Daidzein induced a comparatively shallow increase in the number of MN between 25 and 100 microM. In contrast, the daidzein metabolite equol caused an increase in the number of MN up to 25 microM with no further increase at higher concentrations. Additional staining with anti-kinetochore (CREST) antibodies served to determine if the micronuclei contain whole chromosomes or acentric fragments. Genistein induced mostly CREST(+) micronuclei, i.e. MN with chromosomal fragments, thus indicative of a clastogenic mode of action. MN induced by high concentrations of daidzein were partly CREST(+) and CREST(-), whilst equol induced mostly CREST(+) micronuclei indicative of an aneugenic action. These results point to a differential genotoxicity of phytoestrogens.

Dietary soy and increased risk of bladder cancer: A prospective cohort study of men in Shanghai, China.

Int J Cancer. 2004 Nov 1;112(2):319-23.

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To verify our previous finding of a positive association between dietary soy and bladder cancer risk, we examined the association in a second, geographically distinct prospective cohort of Chinese subjects, the Shanghai Cohort Study. Briefly, 18,244 men aged 45-64 years were recruited between January 1986 and September 1989. As of December 31, 2002, 61 incident bladder cancer cases were identified. Information on soy consumption was obtained through in-person interviews at baseline using a food frequency questionnaire. Cox proportional hazard regression methods were used to estimate relative risks (RR) and their corresponding 95% confidence intervals (CI), with adjustment for age (years) at baseline interview, level of education and other potential confounders.
Compared to men consuming soy less than once a week, the RR (95% CI) for those who consumed soy 1-<3 times per week, 3-<7 times a week and daily were 2.05 (0.80-5.29), 2.45 (0.89-6.76) and 4.61 (1.57-13.51), respectively (p for trend = 0.004), after adjustment for age, cigarette smoking and level of education. The soy-bladder cancer risk associations in smokers and non-smokers were comparable. The soy-bladder cancer relationship became stronger when the analysis was restricted to subjects with 2 or more years of follow-up.

Effects of Soy-Derived Isoflavones and a High-Fat Diet on Spontaneous Mammary Tumor Development in Tg.NK (MMTV/c-neu) Mice.


Phytoestrogens such as isoflavonoids and lignans have been postulated as breast cancer protective constituents in soy and whole-grain cereals. We investigated the ability of isoflavones (IFs) and flaxseed to modulate spontaneous mammary tumor development in female heterozygous Tg.NK (MMTV/c-neu) mice. Two different exposure protocols were applied, either from 4 wk of age onward (postweaning) or during gestation and lactation (perinatal). In the postweaning exposure study, mice were fed IFs or flaxseed in a high-fat diet. In addition, flaxseed in a low-fat diet was tested. Postweaning exposure to IFs and flaxseed tended to accelerate the onset of mammary adenocarcinoma development, although tumor burden at necropsy was not changed significantly. Perinatal IF exposure resulted in enhanced mammary gland differentiation, but palpable mammary tumor onset was not affected. However, tumor burden at necropsy in the perinatal exposure study was significantly increased in the medium- and high-IF dose groups. Comparison of both exposure scenarios revealed a strongly accelerated onset of tumor growth after perinatal high-fat diet exposure compared with the low-fat diet. This study shows that breast cancer-modulating effects of phytoestrogens are dependent both on the background diet and on the timing of exposure in the life cycle.

Mammary gland morphology in Sprague-Dawley rats following treatment with an organochlorine mixture in utero and neonatal genistein.

Foster WG, Younglai EV, Boutross-Tadross O, Hughes CL, Wade MG.

In a related reproductive toxicology study designed to investigate the effects of in utero exposure to environmental toxicants and potential interaction with postnatal genistein, gross enlargement of thoracic mammary glands was observed in female offspring at 200 days of age. Therefore, the objective of this study was to analyze the effect of in utero exposure to a mixture of toxicants on mammary gland morphology. Time-mated Sprague-Dawley rats were treated on days 9-16 of gestation with vehicle or a mixture of environmental toxicants at 1x the acceptable daily intake. Furthermore, it is unclear whether postnatal exposure to phytoestrogens in soy formulas poses breast cancer benefit or risk, and potential interactions with environmental toxicants are unknown. Therefore, half the female pups from each treatment group received either subcutaneous vehicle or genistein (10 microg/g body weight [bw]/day) on postnatal days 2-8. Following necropsy at 200 days of age, a pathologist, blinded to treatment groups, examined mammary gland histopathology. Only mild histological changes were found in mammary glands of rats exposed to the mixture in utero while pronounced ductal hyperplasia, lactational changes, and fibrosis were observed in mammary glands from the genistein group and were more prominent in the mixture + genistein group. Mammary glands of the control group were histologically normal. Collectively, our results reveal that postnatal exposure to pharmacological levels of genistein induces profound morphological changes in the mammary glands of adult female rats, and that high levels of phytoestrogens possess the potential to modulate the toxicological effects of toxicant mixtures.
Dietary soy protein and isoflavones have no significant effect on bone and a potentially negative effect on the uterus of sexually mature intact Sprague-Dawley female rats.


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OBJECTIVE: To evaluate the effect of dietary soy protein and isoflavones on bone and the reproductive tract in premenopausal rats. DESIGN: Three-month-old intact Sprague-Dawley female rats (N = 50) were fed diets containing casein, soy protein, or casein with isoflavone extract for 12 weeks. The amount of casein, soy protein, and extract (per kilogram diet) in each group was: (1) 200 g casein (control); (2) 100 g casein plus 100 g soy protein (low soy); (3) 200 g soy protein (high soy); 4) 200 g casein plus 17.2g extract (low extract); and (5) 200 g casein plus 34.4 g extract (high extract). Diet consumption, body weight, uterine wet weight, urinary deoxypyridinoline concentration, and bone mineral density of the femur and lumbar vertebrae were measured. Femur rigidity was evaluated by histomorphometry. The uterus and vagina were studied histologically.

RESULTS: Rats in all treatment groups had lower body weights and lower deoxypyridinoline concentrations compared with controls, but none of the differences was statistically significant. There was no significant difference in femur and lumbar bone mineral density, uterine wet weights, or histomorphometry between the control and treatment groups. Histologically, uteri and vaginae were normal in all groups except that 1 of 10 rats in the high-soy group and 2 of 10 rats in the high-extract group showed extensive squamous metaplasia in the uterine gland. CONCLUSION: These results suggest that dietary isolated soy protein and isoflavones have no effect on bone and the vagina during premenopausal period, but may have an adverse effect on the uterus.

Pet Abstracts

Effect of dietary soy on serum thyroid hormone concentrations in healthy adult cats.


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OBJECTIVE: To compare effects of short-term administration of a soy diet with those of a soy-free diet on serum thyroid hormone concentrations in healthy adult cats. ANIMALS: 18 healthy adult cats. PROCEDURE: Cats were randomly assigned to receive either a soy or soy-free diet for 3 months each in a crossover design. Assays included CBC, serum biochemical profile, thyroid hormone analysis, and measurement of urinary isoflavone concentrations. RESULTS: Genistein, a major soy isoflavone, was identified in the urine of 10 of 18 cats prior to dietary intervention. Compared with the soy-free diet, cats that received the soy diet had significantly higher total thyroxine (T4) and free T4 (fT4) concentrations, but unchanged total triiodothyronine (T3) concentrations. The T3/fT4 ratio was also significantly lower in cats that received the soy diet. Although the magnitudes of the increases were small (8% for T4 and 14% for fT4), these changes resulted in an increased proportion of cats (from 1/18 to 4/18) that had fT4 values greater than the upper limit of the laboratory reference range. There was no significant effect of diet on any other measured parameter. CONCLUSIONS AND CLINICAL RELEVANCE: Short-term administration of dietary soy has a measurable although modest effect on thyroid hormone homeostasis in cats. Increase in T4 concentration relative to T3 concentration may result from inhibition
of 5'-iodothyronine deiodinase or enhanced T3 clearance. Soy is a common dietary component that increases serum T4 concentration in cats.

**Induction of micronuclei, DNA strand breaks and HPRT mutations in cultured Chinese hamster V79 cells by the phytoestrogen coumoestrol.**


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Coumoestrol (COUM), genistein (GEN) and daidzein (DAI) are major phytoestrogens present in numerous plants eaten by humans and food-producing animals. Little is known about the genotoxicity of these natural compounds. The effects of COUM, GEN and DAI were studied in cultured Chinese hamster V79 cells at various endpoints. None of the substances affected the cytoplasmic microtubule complex or the mitotic spindle. However, COUM and GEN but not DAI proved to be strong inducers of DNA strand breaks and micronuclei containing acentric fragments, as shown with antikinetochore antibodies. The clastogenicity of GEN may be due to its non-intercalative inhibitory effect on topoisomerase II, whereas COUM may act through topoisomerase II inhibition and/or DNA intercalation. COUM was also a clear inducer of hypoxanthine guanine phosphoribosyltransferase (HPRT) mutations in V79 cells; GEN was only marginally active and DAI inactive at this endpoint. This is the first report on the clastogenicity and mutagenicity of COUM in mammalian cells.

**Phytoestrogens: adverse effects on reproduction in California quail.**


Leopold AS, Erwin M, Oh J, Browning B.

Phytoestrogens, largely formononetin and genistein, are produced in the leaves of stunted desert annuals in a dry year. When ingested by California quail, these compounds apparently inhibit reproduction and prevent the production of young that will not have adequate food. In a wet year, forbs grow vigorously and phytoestrogenic substances are largely absent. Quail then breed prolifically and the abundant seed crop carries the enlarged population through the winter.

**Evaluation of environmental, nutritional, and host factors in cats with hyperthyroidism.**


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The pathologic changes associated with hyperthyroidism (adenomatous hyperplasia, adenoma of the thyroid gland) have been well characterized in cats, but the pathogenesis of these changes remains unclear. In this research, we undertook a case-control study to search for potential risk factors for this disease. Owners of 379 hyperthyroid and 351 control cats were questioned about their cats' exposure to potential risk factors including breed, demographic factors, medical history, indoor environment, chemicals applied to the cat and environment, and diet. The association between these hypothesized risk factors and outcome of disease was evaluated by
conditional logistic regression. Two genetically related cat breeds (ie, Siamese and Himalayan) were found to have diminished risk of developing hyperthyroidism. Cats that used litter had higher risk of developing hyperthyroidism than those that did not. Use of topical ectoparasite preparations was associated with increased risk of developing hyperthyroidism. **Compared with cats that did not eat canned food, those that ate commercially prepared canned food had an approximate 2-fold increase in risk of disease.** When these 4 variables (breed, use of cat litter, consumption of canned cat food, and use of topical ectoparasite preparations) from the univariate analysis were selected for further study as candidate risk factors and analyzed by multivariate conditional logistic regression, a persistent protective effect of breed (ie, Siamese or Himalayan) was found. In addition, results suggested a 2- to 3-fold increase in risk of developing hyperthyroidism among cats eating a diet composed mostly of canned cat food and a 3-fold increase in risk among those using cat litter. In contrast, the use of commercial flea products did not retain a strong association. The results of this study indicate that further research into dietary and other potentially important environmental factors (eg, cat litter) is warranted.

**Identification of phytoestrogens in the urine of male dogs.**


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It is becoming increasingly apparent that dietary factors may play a role in the etiology of hormone dependent neoplasias. It has been hypothesized that estrogens play some role in the etiology of benign prostatic hyperplasia (BPH) in the canine. The presence of estrogen receptor binding activity in a fraction of canine urine purified by high performance liquid chromatography (HPLC) that did not correspond to estriol, estradiol, estrone or any of their primary metabolites was observed in the present study. **We used thermospray-mass spectrometry and GC-MS to identify the phytoestrogens daidzein, equol, formononetin and genistein in HPLC purified fractions of urine obtained from male beagles.** Using the same techniques we also confirmed the presence of daidzein and genistein in the commercial diet fed to these same dogs. Using the immature rat uterine cytosol estrogen receptor assay, relative binding affinities of 0.08, 1.1, less than 0.01 and 3.9% were obtained for daidzein, equol, formononetin and genistein, respectively when compared to estradiol (100%). **In conclusion, phytoestrogens are present in urine of male beagles. Moreover, the commercial diet fed to these dogs contain isoflavones which can be converted to equol by intestinal microflora.** These results suggest the need for investigations of phytoestrogens (e.g. equol) excreted into the urine daily and its relationship to the incidence and severity of BPH in the dog.

**Identification and concentration of soy isoflavones in commercial cat foods.**


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**OBJECTIVE:** To determine the absolute and relative soy isoflavone content in commercial cat foods. **SAMPLE POPULATION:** 14 dry, 6 semimoist, and 22 moist commercial cat foods. **PROCEDURE:** Soy isoflavone content of each food was determined by use of acid-methanol hydrolysis and high-pressure liquid chromatography with ultraviolet absorbance detection. Isoflavones were identified and quantified by reference to authentic standards. **RESULTS:** Genistein and daidzein were the major soy isoflavones identified in 24 of 42 foods, with concentrations ranging from 1 to 163 microg/g of food. Foods labeled as containing soybean solids (16/42) had isoflavone concentrations > 11 microg/g. More dry (13/14) and semimoist (6/6) foods contained isoflavones than moist foods (5/22). Isoflavone content and food cost were negatively correlated for dry and semimoist foods but not for moist foods. Total amount of isoflavone consumed by cats fed these soy-containing foods as a sole maintenance diet was estimated to be between 0.6 and 4.5 mg/kg of body weight/d, which is comparable to concentrations in humans that result in a measurable although modest effect on serum concentrations of steroid and thyroid hormones. **CONCLUSIONS AND CLINICAL RELEVANCE:** Genistein and daidzein are common constituents of commercial cat foods. Predictors of isoflavone content included ingredient labeling, food
type, and food cost. Soy isoflavones in some commercial cat foods were detected in amounts predicted to have a biological effect.

**Effects of the protein phosphorylation inhibitor genistein on maturation of pig oocytes in vitro.**


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In vitro maturation of cumulus enclosed and denuded pig oocytes was reversibly inhibited by the protein kinase inhibitor genistein. The half-maximal effect on maturation was observed at 40 micrograms ml⁻¹. Genistein inhibited total protein phosphorylation and synthesis with the same dose-response relationship (ED₅₀: 40 micrograms ml⁻¹). Protein phosphorylation and synthesis patterns were changed by effective concentrations of genistein. Pig oocytes were sensitive to genistein during the first 12 h of in vitro maturation. This genistein sensitive period corresponds closely with the period of sensitivity to the protein synthesis inhibitor cycloheximide. Whereas the inhibition of protein synthesis affects only nuclear membrane breakdown and not chromatin condensation, genistein inhibits both events. The results of these experiments suggest that protein phosphorylation and synthesis play major roles during pig oocyte maturation in vitro. **It is concluded that genistein inhibited protein phosphorylation is a regulator of chromatin condensation, whereas both new protein synthesis and phosphorylation appear to be required for nuclear membrane disassembly.** Caution about this second conclusion is, however, necessary because of the dual action of genistein on both protein phosphorylation and indirectly on protein synthesis.