Natural Estrogens

From the original article in 2008. Author: Ray Peat.

The fact that an extremely large number of naturally occurring compounds, and an unlimited number of synthetic compounds, have an estrogen-like activity has been exploited by the drug companies to produce patented proprietary drugs, especially the contraceptives.

The promotion of "natural estrogens" is a new marketing strategy that capitalizes on the immense promotional investment of the drug companies in the concept of estrogen replacement as "therapy."

> "**Whether weak or strong, the estrogenic response of a chemical, if not overcome, will add extra estrogenic burden to the system. At elevated doses, natural estrogens and environmental estrogen-like chemicals are known to produce adverse effects. The source of extra or elevated concentration of estrogen could be either endogenous or exogenous.** The potential of exposure for humans and animals to environmental estrogen-like chemicals is high."
> — D. Roy, et al., 1997

Estrogen marketing has entered a new phase, based on the idea of "specific estrogen-receptor modulators," the idea that a molecule can be designed which has estrogen's "good qualities without its bad qualities." This specific molecule will be "good for the bones, the heart, and the brain," without causing cancer of the breast and uterus, according to the estrogen industry. Meanwhile, soybeans are said to contain estrogens that meet that goal, and it is often said that "natural estrogens" are better than "synthetic estrogens" because they are "balanced."

Estrogen's effects on cells are immediate and profound, independent of the "estrogen receptors."

Japanese women's relative freedom from breast cancer is independent of soy products**:** traditional soy foods aren't the same as those so widely used in the US, for example, soy sauce doesn't contain the so-called soy estrogens, and tea is used much more commonly in Japan than in the US, and contains health protective ingredients. The "estrogenic" and "antioxidant" polyphenolic compounds of tea are not the protective agents (they raise the level of estrogen), but tea's *caffeine* is a very powerful and general anti-cancer protectant. The influential article in *Lancet* (D. Ingram, *Lancet* 1997;*350*:990-994. "Phytoestrogens and their role in breast cancer," *Breast NEWS: Newsletter of the NHMRC National Breast Cancer Centre, Vol. 3,* No. 2, Winter 1997) used a method known to produce false results, namely, comparing the phytoestrogens (found in large amounts in soybeans) in the urine of women with or without breast cancer. For over fifty years, it has been known that the liver excretes estrogens and other toxins from the body, and that when (because of liver inertia) estrogen isn't excreted by the liver and kidneys, it is retained in the body. This process was observed in both animals and humans decades ago, and it is *also well established that estrogen itself suppresses the detoxifying systems, causing fewer carcinogens to be excreted* in the urine. Ingram's evidence logically would suggest that the women who have cancer are failing to eliminate estrogens, including phytoestrogens, at a normal rate, and so are retaining a higher percentage of the chemicals consumed in their diets. Flavonoids and polyphenols, like our own estrogens, suppress the detoxifying systems of the body.

Our bodies produce estrogen in a great variety of tissues, not just in the ovaries. Fat cells are a major source of it. The tendency to gain weight after puberty is one of the reasons that women's estrogen levels rise with aging throughout the reproductive years, though this isn't the basic reason for estrogen's lifelong growing influence, even in men.

Our diets provide very significant, if not always dangerous, amounts of estrogen. "Weak estrogens" generally have the full range of harmful estrogenic effects, and often have additional toxic effects. American women who eat soy products undergo changes that appear to predispose them to cancer, making their tissues even more unlike those of the relatively breast-cancer resistant Japanese than they were before eating the soy foods.

People under stress, or who have a thyroid deficiency, or who don't eat enough protein, typically have elevated estrogen levels. The accumulation of the "essential fatty acids," the polyunsaturated oils, in the tissues promotes the action of estrogen in a variety of ways, and this effect of diet tends to be cumulative, and to be self-accelerating.

Science is a method that helps us to avoid believing things that are wrong, but there is a distinct herd instinct among people who "work in science," which makes it easy to believe whatever sounds plausible, if a lot of other people are saying it is true. This is just as evident in physics as it is in medicine. Sometimes powerful economic interests help people to change their beliefs, for example as the insurance industry helped to convince the public of the dangers of smoking. Two of the biggest industries in the world, the estrogen industry and the soy bean industry, spend vast amounts of money helping people to believe certain plausible-sounding things that help them sell their products. Sometimes they can achieve great things just by naming the substance.

Estrogenicity can be defined most simply as "acting the way estrogen does," (originally, the term "estrogen" meant "producing estrus," the female readiness to mate) and since our natural estrogen does many things, the definition is often, for practicality, based on the rapid changes produced in certain female organs by estradiol, specifically, the enlargement of the uterus by first taking up a large amount of water, and secondarily by the multiplication of cells and the production of specific proteins. A similar process occurring in the breast is also recognized as an important feature of the estrogen reaction, but as we try to define just what "estrogenicity" is, we see that there is something deeply wrong with this method of defining a hormone, because we are constantly learning more about the actions of estrogen, or of a specific form of the molecule. Calling it "the female hormone" distracted attention from its many functions in the male, and led to great confusion about its antifertility actions and its other toxicities. Many biologists called it "folliculin," because of the ovarian follicle's significant role in its production, but the pharmaceutical industry succeeded in naming it in relation to ***one*** of its functions, and then in

extending that idea of it as "the producer of female receptivity" to the even more misleading idea that it is "the female hormone." But when people speak about the "estrogenicity" of a substance, they mean that it has properties that parallel those of "folliculin," the particular group of ovarian hormones that includes estradiol, estrone, and estriol.

Over the last 100 years, thousands of publications about estrogen's toxicity have created a slight resistance to the consumption of the major estrogen products. One ploy to overcoming this resistance is to call certain products "natural estrogen," as distinguished from "synthetic estrogens." The **three main estrogens in our bodies are estradiol, estrone, and estriol, though there are many other minor variants on the basic molecule.** These three estrogens, singly or in combinations, are being sold as natural estrogens, with their virtues explained in various ways. Implicit in many of these explanations, is the idea that these are safer than synthetics. They are sometimes contrasted to the "horse estrogen" in Premarin, as if they are better because they are like the estrogens that people produce. But it was exactly the normal human estrogens, produced by the ovaries, that led to the basic discoveries about the toxicity of estrogen, its ability to produce cancer in any organ, to cause seizures, blood clots, birth defects, accelerated aging, etc.

Although I would suppose that a hormone from a horse might be "more natural" for a person's body than a hormone from a plant, the word "natural" as used in the phrases "natural food store," or "natural medicine," has come to be associated strongly with things derived from plants. The health food industry, now largely taken over by giant corporations to sell products that weren't producing as much revenue when sold in supermarkets and drugstores, has helped to create a culture in which botanical products are thought to be especially good and safe. Naturally grown free-range chickens used to be favored, because they could eat anything they wanted, but now eggs laid by factory chickens, eating an industrial corn-and-soy diet, are from "vegetarian chickens," because the marketers know the public will favor eggs that have the vegetarian mystique.

Biologically active molecules have both general and specific properties. Estrogenicity is a general property, but all molecules which have that property also have some other specific properties. Estriol is a little more water soluble than estrone, so it interacts with every body system in a slightly different way, entering oily environments with slightly less ease, etc.

The estrogen which occurs in yeasts, estradiol, is identical to the major human estrogen, and it is thought to have a reproductive function in yeasts, though this isn't really understood. A feature of this molecule, and of all other molecules that "act like estrogen," is the phenolic function, an oxygen and hydrogen group attached to a resonant benzene ring. Phenol itself is estrogenic, and the phenolic group is so extremely common in nature that the number of existing estrogenic substances is great, and the number of potential molecules with estrogen-function is practically infinite.

The phenolic group has many biological functions. For example, it commonly functions as an "antioxidant," though something which functions as an antioxidant in one situation is often a pro-oxidant in another situation. The molecule can have catalytic, germicidal, aromatic, neurotropic, and other functions. But it also always has, to some degree, the "estrogenic" function. This overlap of functions probably accounts for why so many plants have significant estrogenic activity. (Natural estrogens, like other phenolics, including the flavonoids, are also mutagenic.)

The estrogenic properties of legumes were studied when sheep farmers found that their sheep miscarried when they ate clover. (I think it's interesting how this terribly toxic effect has been neglected in recent decades.) All legumes have this property, and all parts of the plant seem to contain some of the active chemicals. In beans, several substances have been found to contribute to the effect. The estrogenic effects of the seed oils and the isoflavones have been studied the most, but the well-known antithyroid actions (again, involving the oils, the isoflavones, and other molecules found in legumes) have an indirect estrogen-promoting action, since hypothyroidism leads to hyperestrogenism. (Estrogens are known to be thyroid suppressors, so the problem tends to be self-accelerating.)

The various specific actions of the many estrogenic substances in beans and other legumes haven't been throughly studied, but there is evidence that they are also--like estrogen itself--both mutagenic and carcinogenic.

The estrogen-promoting actions of soy oil apply to **all of the commonly used polyunsaturated fatty acids. The same fatty acids that suppress thyroid function, have estrogenic effects.**

The isoflavones (many of which are now being promoted as "antioxidants" and "cancer preventives") are toxic to many organs, but they have clear estrogenic effects, and are active not only immediately in the mature individual, but when they are present prenatally, they cause feminization of the male genitalia and behavior, and early maturation of the female offspring, with the tissue changes that are known to be associated with increased incidence of cancer.

There are interesting associations between vegetable "fiber" and estrogens. Because of my own experience in finding that eating a raw carrot daily prevented my migraines, I began to suspect that the carrot fiber was having both a bowel-protective and an antiestrogen effect. Several women who suffered from premenstrual symptoms, including migraine, had their serum estrogen measured before and after the "carrot diet," and they found that the carrot lowered their estrogen within a few days, as it relieved their symptoms.

Undigestible fiber, if it isn't broken down by bowel bacteria, increases fecal bulk, and tends to speed the transit of material through the intestine, just as laxatives do. But some of these "fiber" materials, e.g., lignin, are themselves estrogenic, and other fibers, by promoting bacterial growth, can promote the conversion of harmless substances into toxins and carcinogens. When there is a clear "antiestrogen" effect from dietary fiber, it seems to be the result of accelerated transit through the intestine, speeding elimination and preventing reabsorption of the estrogen which has been excreted in the bile. Laxatives have this same effect on the excretion of estradiol.

Some of the isoflavones, lignins, and other phytoestrogens are said to prevent bowel cancer, but some of them, e.g., lignin, appear to sometimes increase its likelihood.

The phytoestrogens appear to pose a risk to organs besides the breast and uterus, for example the liver, colon, and pancreas, which isn't surprising, since estrogen is known to be carcinogenic for every tissue. And carcinogenesis, like precancerous changes, mutations, and reduced repair of DNA, is probably just an incidental process in the more general toxic effect of acceleration of aging.

References

"Stimulatory influence of soy protein isolate on breast secretion in pre- and postmenopausal women," Petrakis NL; Barnes S; King EB; Lowenstein J; Wiencke J; Lee MM; Miike R; Kirk M; Coward L Department of Epidemiology and Biostatistics, University of California, San Francisco 94143-0560, USA. Cancer Epidemiol Biomarkers Prev, 1996 Oct, 5:10, 785-94 "Soy foods have been reported to have protective effects against premenopausal breast cancer in Asian women. No studies have been reported on potential physiological effects of dietary soy consumption on breast gland function. We evaluated the influence of the long-term ingestion of a commercial soy protein isolate on breast secretory activity. We hypothesized that the features of nipple aspirate fluid (NAF) of non-Asian women would be altered so as to resemble those previously found in Asian women. At monthly intervals for 1 year, 24 normal pre- and postmenopausal white women, ages 30 to 58, underwent nipple aspiration of breast fluid and gave blood and 24-h urine samples for biochemical studies. No soy was administered in months 1-3 and 10-12. Between months 4-9 the women ingested daily 38 g of soy protein isolate containing 38 mg of genistein. NAF volume, **gross cystic disease fluid protein (GCDFP-15) concentration**, and NAF cytology were used as biomarkers of possible effects of soy protein isolate on the breast. In addition, plasma concentrations of estradiol, progesterone, sex hormone binding globulin, prolactin, cholesterol, high density lipoprotein-cholesterol, and triglycerides were measured. Compliance was assessed by measurements of genistein and daidzein and their metabolites in 24-h urine samples. Excellent compliance with the study protocol was obtained. Compared with NAF volumes obtained in months 1-3, **a 2-6-fold increase in NAF volume ensued during months 4-9 in all premenopausal women.** A minimal increase or no response was found in postmenopausal women. No changes were found in plasma prolactin, sex hormone binding globulin, cholesterol, high density lipoprotein cholesterol, and triglyceride concentrations. Compared with concentrations found in months 1-3 (no soy), **plasma estradiol concentrations were elevated erratically throughout** a "composite" menstrual cycle during the months of soy consumption. No significant changes were seen in plasma progesterone concentrations. No significant changes were found in plasma estrogen levels in postmenopausal women. A moderate decrease occurred in the mean concentration of GCDFP-15 in NAF in premenopausal women during the months of soy ingestion. **Of potential concern was the cytological detection of epithelial hyperplasia in 7 of 24 women (29.2%) during the months they were consuming soy protein isolate. The findings did not support our a priori hypothesis. Instead, this pilot study indicates that prolonged consumption of soy protein isolate has a stimulatory effect on the premenopausal female breast, characterized by increased secretion of breast fluid, the appearance of hyperplastic epithelial cells, and elevated levels of plasma estradiol.** These findings are suggestive of an estrogenic stimulus from the isoflavones genistein and daidzein contained in soy protein isolate.

J Clin Endocrinol Metab 1995 May;80(5):1685-1690 **Dietary intervention study to assess estrogenicity of dietary soy among postmenopausal women.** Baird DD, Umbach DM, Lansdell L, Hughes CL, Setchell KD, Weinberg CR, Haney AF, Wilcox AJ, Mclachlan JA. National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709, USA. We tested the hypothesis that postmenopausal women on a soy-supplemented diet show estrogenic responses. Ninety-seven postmenopausal women were randomized to either a group **that was provided with soy foods for 4 weeks or a control group that was instructed to eat as usual.** Changes in urinary isoflavone concentrations served as a measure of compliance and phytoestrogen dose. Changes in serum FSH, LH, sex hormone binding globulin, and vaginal cytology were measured to assess estrogenic response. **The percentage of vaginal superficial cells (indicative of estrogenicity) increased for 19% of those eating the diet compared with 8% of controls** (P = 0.06 when tested by ordinal logistic regression). FSH and LH did not decrease significantly with dietary supplementation as hypothesized, nor did sex hormone binding globulin increase. Little change occurred in endogenous estradiol concentration or body weight during the diet. Women with large increases in urinary isoflavone concentrations were not more likely to show estrogenic responses than were women with more modest increases. On the basis of published estimates of phytoestrogen potency, a 4-week, soy-supplemented diet was expected to have estrogenic effects on the liver and pituitary in postmenopausal women, but estrogenic effects were not seen. At most, there was a small estrogenic effect on vaginal cytology.

Oncol Rep 1998 May-Jun;5(3):609-16 **"Maternal genistein exposure mimics the effects of estrogen on mammary gland development in female mouse offspring."** Hilakivi-Clarke L, Cho E, Clarke R Lombardi Cancer Center, Research Bldg., Room W405, Georgetown University, 3970 Reservoir Road, NW, Washington, DC, 20007-2197, USA. **Human and animal data indicate that a high maternal estrogen exposure during pregnancy increases breast cancer risk among daughters. This may reflect an increase in the epithelial structures** that are the sites for malignant transformation, i.e., terminal end buds (TEBs), and a reduction in epithelial differentiation in the mammary gland. Some **phytoestrogens, such as genistein which is a major component in soy-based foods, and zearalenone, a mycotoxin found in agricultural products, have estrogenic effects on the reproductive system, breast and brain.** The present study examined whether in utero exposure to genistein or zearalenone influences mammary gland development. Pregnant mice were injected daily with i) 20 ng estradiol (E2); ii) 20 microg genistein; iii) 2 microg zearalenone; iv) 2 microg tamoxifen (TAM), a partial estrogen receptor agonist; or v) oil-vehicle between days 15 and 20 of gestation. E2, **genistein, zearalenone, and tamoxifen all increased the density of TEBs in the mammary glands. Genistein reduced, and zearalenone increased, epithelial differentiation.** Zearalenone also increased epithelial density, when compared with the vehicle-controls. None of the treatments had permanent effects on circulating E2 levels. **Maternal exposure to E2 accelerated body weight gain, physical maturation (eyelid opening), and puberty onset (vaginal opening) in the female offspring. Genistein and tamoxifen had similar effects on puberty onset than E2.** Zearalenone caused persistent cornification of the estrus smears. These findings indicate that **maternal exposure to physiological doses of genistein mimics the effects of E2 on the mammary gland and reproductive systems in the offspring. Thus, our results suggest that genistein acts as an estrogen in utero, and may increase the incidence of mammary tumors if given through a pregnant mother.** The estrogenic effects of zearalenone on the mammary gland, in contrast, are probably counteracted by the permanent changes in estrus cycling.

[The effects on the thyroid gland of soybeans administered experimentally in healthy subjects] Ishizuki Y; Hirooka Y; Murata Y; Togashi K Nippon Naibunpi Gakkai Zasshi, 1991 May 20, 67:5, 622-9 To elucidate whether soybeans would suppress the thyroid function in healthy adults, we selected 37 subjects who had never had goiters or serum antithyroid antibodies. They were given 30g of soybeans everyday and were divided into 3 groups subject to age and duration of soybean administration. In group 1, 20 subjects were given soybeans for 1 month. Groups 2 and 3 were composed of 7 younger subjects (mean 29 y.o.) and 10 elder subjects (mean 61 y.o.) respectively, and the subjects belonging to these groups received soybeans for 3 months. The Wilcoxon-test and t-test were used in the statistical analyses. In all groups, the various parameters of serum thyroid hormones remained unchanged by taking soybeans, however TSH levels rose significantly although they stayed within normal ranges. The TSH response after TRH stimulation in group 3 revealed a more significant increase than that in group 2, although inorganic iodide levels were lowered during the administration of the soybeans. We have not obtained any significant correlation between serum inorganic iodide and TSH. Hypometabolic symptoms (malaise, constipation, sleepiness) and goiters appeared in half the subjects in groups 2 and 3 after taking soybeans for 3 months, but they disappeared 1 month after the cessation of soybean ingestion. These findings suggested that excessive soybean ingestion for a certain duration might suppress thyroid function and cause goiters in healthy people, especially elderly subjects.

Exp Clin Endocrinol Diabetes 1996;104 Suppl 4:41-5 **Iodolactones and iodoaldehydes--mediators of iodine in thyroid autoregulation.** Dugrillon A Central Clinical Laboratory, University of Heidelberg, Germany. "Within the last decades multiple iodolipid-classes have been identified in thyroid tissue. For a long time they have been supposed to be involved in thyroid autoregulation, but for the time being no specific compounds could be isolated. A new approach was stimulated by the finding that **thyroid cells were able to iodinate polyunsaturated fatty acids** to form iodolactones and by the identification of alpha-iodohexadecanal (alpha-IHDA) as the major compound of an iodolipid fraction."

Plasma free fatty acids, inhibitor of extrathyroidal conversion of T4 to T3 and thyroid hormone binding inhibitor in patients with various nonthyroidal illnesses. Suzuki Y; Nanno M; Gemma R; Yoshimi T Endocrinol Jpn, 1992 Oct, 39:5, 445-53.

[Endemic goiter in Austria. Is iodine deficiency the primary cause of goiter?] Grubeck-Loebenstein B; Kletter K; Kiss A; Vierhapper H; Waldhäusl W Schweiz Med Wochenschr, 1982 Oct 30, 112:44, 1526-30 **In spite of government-regulated iodide admixture to table salt, the incidence of goiter is still high in Austria.** Iodine excretion and thyroid function were therefore investigated in 80 patients suffering from ordinary goiter in whom thyroid size and resulting symptoms had increased lately. 25 euthyroid non-goitrous subjects served as controls. 48% of the goitrous patients investigated presented with iodine excretion of less than 70 micrograms/24 h, suggesting an insufficient iodine supply. Thyroid I131 uptake, basal and TRH-stimulated plasma TSH concentrations, and serum T3 levels were higher, whereas serum T4 levels were lower in these patients than in goitrous patients with higher iodine excretion and non-goitrous controls. Iodine deficiency thus appears to be of pathogenetic relevance in about half of the goitrous Austrian population. **Other factors enhancing goiter development seem to assume particular importance in goitrous patients with a sufficient iodine supply.**

Biochemical and molecular changes at the cellular level in response to exposure to environmental estrogen-like chemicals. Roy D; Palangat M; Chen CW; Thomas RD; Colerangle J; Atkinson A; Yan ZJ Environmental Toxicology Program, University of Alabama, Birmingham 35294, USA. J Toxicol Environ Health, 1997 Jan, 50:1, 1-29. Estrogen-like chemicals are unique compared to nonestrogenic xenobiotics, because in addition to their chemical properties, the estrogenic property of these compounds allows them to act like sex hormones. **Whether weak or strong, the estrogenic response of a chemical, if not overcome, will add extra estrogenic burden to the system. At elevated doses, natural estrogens and environmental estrogen-like chemicals are known to produce adverse effects. The source of extra or elevated concentration of estrogen could be either endogenous or exogenous.** The potential of exposure for humans and animals to environmental estrogen-like chemicals is high. Only a limited number of estrogen-like compounds, such as diethylstilbestrol (DES), bisphenol A, nonylphenol, polychlorinated biphenyls (PCBs), and dichlorodiphenyltrichloroethane (DDT), have been used to assess the biochemical and molecular changes at the cellular level. Among them, DES is the most extensively studied estrogen-like chemical, and therefore this article is focused mainly on DES-related observations. In addition to estrogenic effects, environmental estrogen-like chemicals **produce multiple and multitype genetic and/or nongenetic hits.** Exposure of Syrian hamsters to stilbene estrogen (DES) produces several changes in the nuclei of target organ for carcinogenesis (kidney): (1) Products of nuclear redox reactions of DES modify transcription regulating proteins and DNA; (2) transcription is inhibited; (3) tyrosine phosphorylation of nuclear proteins, including RNA polymerase II, p53, and nuclear insulin-like growth factor-1 receptor, is altered; and (4**) DNA repair gene DNA polymerase beta transcripts are decreased and mutated.** Exposure of Noble rats to DES also produces several changes in the mammary gland: proliferative activity is drastically altered; the cell cycle of mammary epithelial cells is perturbed; telomeric length is attenuated; etc. It appears that some other estrogenic compounds, such as bisphenol A and nonylphenol, may also follow a similar pattern of effects to DES, because we have recently shown that these compounds **alter cell cycle kinetics, produce telomeric associations, and produce chromosomal aberrations.** Like DES, bisphenol A after metabolic activation is capable of binding to DNA. However, it should be noted that a particular or multitype hit(s) will depend upon the nature of the environmental estrogen-like chemical. The role of individual attack leading to a particular change is not clear at this stage. Consequences of these multitypes of attack on the nuclei of cells could be (1) nuclear toxicity/cell death; (2) repair of all the hits and then acting as normal cells; or (3) sustaining most of the hits and acting as unstable cells. Proliferation of the last type of cell is expected to result in transformed cells.

Potential adverse effects of phytoestrogens. Whitten PL; Lewis C; Russell E; Naftolin F Department of Anthropology, Emory University, Atlanta, GA 30322. J Nutr, 1995 Mar, 125:3 Suppl, 771S-776S Evaluation of the potential benefits and risks offered by naturally occurring plant estrogens requires investigation of their potency and sites of action when consumed at natural dietary concentrations. Our investigations have examined the effects of a range of natural dietary concentrations of the most potent plant isoflavonoid, coumestrol, using a rat model and a variety of estrogen-dependent tissues and endpoints. Treatments of immature **females demonstrated agonistic action in the reproductive tract, brain, and pituitary at natural dietary concentrations. Experiments designed to test for estrogen antagonism demonstrated that coumestrol did not conform to the picture of a classic antiestrogen.** However, coumestrol did suppress estrous cycles in adult females. Developmental actions were examined by neonatal exposure of pups through milk of rat dams fed a coumestrol, control, or commercial soy-based diet during the critical period of the first 10 postnatal days or throughout the 21 days of lactation. The 10-day treatment did not significantly alter adult estrous cyclicity, but the 21-day treatment produced in a **persistent estrus state in coumestrol-treated females by 132 days of age.** In contrast, the 10-day coumestrol treatments produced **significant deficits in the sexual behavior of male offspring.** These findings illustrate the broad range of actions of these natural estrogens and the variability in potency across endpoints. This variability argues for the importance of fully characterizing each phytoestrogen in terms of its sites of action, balance of agonistic and antagonistic properties, natural potency, and short-term and long-term effects.

Am J Obstet Gynecol 1987 Aug;157(2):312-317 **Age-related changes in the female hormonal environment during reproductive life.** Musey VC, Collins DC, Musey PI, Martino-Saltzman D, Preedy JR. Previous studies have indicated that serum levels of follicle-stimulating hormone rise with age during the female reproductive life, but the effect on other hormones is not clear. We studied the effects of age, independent of pregnancy, by comparing serum hormone levels in two groups of nulliparous, **premenopausal women aged 18 to 23 and 29 to 40 years. We found that increased age during reproductive life is accompanied by a significant rise in both basal and stimulated serum follicle-stimulating hormone levels. This was accompanied by an increase in the serum level of estradiol-17 beta and the urine levels of estradiol-17 beta and 17 beta-estradiol-17-glucosiduronate.** The serum level of estrone sulfate decreased with age. Serum and urine levels of other estrogens were unchanged. The basal and stimulated levels of luteinizing hormone were also unchanged. There was a significant decrease in basal and stimulated serum prolactin levels. Serum levels of dehydroepiandrosterone and dehydroepiandrosterone sulfate decreased with age, but serum testosterone was unchanged. It is concluded that significant age-related changes in the female hormonal environment occur during the reproductive years.

Rodriguez, P; Fernandez-Galaz, C; Tejero, A. **Controlled neonatal exposure to estrogens: A suitable tool for reproductive aging studies in the female rat.** Biology of Reproduction, v.49, n.2, (1993): 387-392. The present study was designed to determine whether the modification of exposure time to large doses of estrogens provided a reliable model for early changes in reproductive aging. Silastic implants containing estradiol benzoate (EB) in solution were placed into 5-day-old female Wistar rats and removed 1 day (Ei1 group) or 5 days (Ei5) later. In addition, 100 mu-g EB dissolved in 100 mu-l corn oil was administered s.c. to another group (EI). Control rats received either vehicle implants or 100 mu-l corn oil. Premature occurrence of vaginal opening was observed in all three estrogenized groups independently of EB exposure. However, females bearing implants for 24 h had first estrus at the same age as their controls and cycled regularly, and neither histological nor gonadal alterations could be observed at 75 days.. Interestingly, they failed to cycle regularly at 5 mo whereas controls continued to cycle. On the other hand, the increase of EB exposure (Ei5, EI) resulted in a gradual and significant delay in the onset of first

estrus and in a high number of estrous phases, as frequently observed during reproductive decline. At 75 days, the ovaries of these last two groups showed a reduced number of corpora lutea and an increased number of large follicles. According to this histological pattern, ovarian weight and **progesterone (P) content gradually decreased whereas both groups showed higher estradiol (E-2) content** than controls. This resulted in **a higher E-2:P ratio, comparable to that observed in normal aging rats.** The results allow us to conclude that the exposure time to large doses of estrogens is critical to the gradual enhancement of reproductive decline. Furthermore, exposures as brief as 24 h led to a potential early model for aging studies that will be useful to verify whether neuroendocrine changes precede gonadal impairment.

Cancer Lett 1992 Oct 30;67(1):55-59 **Evidence of hypothalamic involvement in the mechanism of transplacental carcinogenesis by diethylstilbestrol.** Smith DA, Walker BE Anatomy Department, Michigan State University, East Lansing 48824-1316. Disruption of hypothalamic sex differentiation in the fetus is one hypothesis to explain female reproductive system anomalies and cancer arising from prenatal exposure to diethylstilbestrol (DES). To further test this hypothesis, breeding performance and behavior were monitored in a colony of mice exposed prenatally to DES, using a schedule previously shown to produce anomalies and cancer of the female reproductive system. **Fertility decreased with age more rapidly in DES-exposed females than in control females.** DES-exposed females were less accepting of the male than control females. These observations support the hypothesis of abnormal hypothalamic sex differentiation as a basic mechanism in DES transplacental carcinogenesis.

Int J Cancer 1980 Aug;26(2):241-6 **The influence of the lipid composition of the feed given to mice on the immunocompetence and tumour resistance of the progeny.** Boeryd B, Hallgren B. In inbred CBA mice, the immunocompetence of adult progeny from breeding pairs fed three different diets was compared. **Substitution of soy oil for animal fat in the feed of the mice during gestation or lactation significantly decreased the PFC response to SRBC in the adult offspring.** Addition of 2-methoxy-substituted glycerol ethers to the feed of mothers deprived of animal fat during lactation partly restored the PFC response of the male offspring. In the adult mice fed differently pre- and perinatally the resistance to a transplanted syngeneic sarcoma was similar. The growth of offspring from mice fed the three diets was similar. In mice deprived of animal fat at weaning and for the following 21 days the immune reactivity to SRBC, tested about 3 months after stopping the diet, was not influenced. However, the resistance to a transplanted tumour in similarly fed mice was increased and this resistance was brought approximately to the control level by methoxy-substituted glycerol ethers.

Cancer Res 1987 Mar 1;47(5):1333-8. **Effects of dietary fats and soybean protein on azaserine-induced pancreatic carcinogenesis and plasma cholecystokinin in the rat.** Roebuck BD, Kaplita PV, Edwards BR, Praissman M **Both dietary unsaturated fat and raw soybean products are known to enhance pancreatic carcinogenesis when fed during the postinitiation phase. A comparison of these two dietary components was made to** evaluate the relative potency of each ingredient for enhancing pancreatic carcinogenesis and to determine if this enhancement was correlated with an increase in plasma cholecystokinin (CCK) levels. Male Wistar rats were initiated with a single dose of azaserine (30 mg/kg body weight) at 14 days of age. The rats were weaned to test diets formulated from purified ingredients. Dietary protein at 20% by weight was either casein or soy protein isolate (heat treated or raw).. Corn oil was the unsaturated fat of major interest and it was fed at either 5 or 20% by weight. Pancreases were quantitatively evaluated for carcinogen-induced lesions at 2- and 4-month postinitiation. In a second experiment designed to closely mimic the above experiment, rats were implanted with cannulae which allowed plasma to be repetitively sampled over a 2.5-week period during which the test diets were fed. Plasma was collected both prior to introduction of the test diets and afterwards. Plasma CCK was measured by a specific radioimmunoassay. Both the 20% corn oil diet and the raw soy protein isolate diet enhanced pancreatic carcinogenesis. The effects of the raw soy protein isolate on the growth of the carcinogen-induced lesions were significantly greater than the effects of the 20% corn oil diet. Plasma CCK values were not elevated in the rats fed the 20% corn oil diet, but they were significantly elevated in the rats fed the raw soy protein isolate. Heat-treated soy protein isolate neither enhanced carcinogenesis nor elevated the plasma CCK level. This **study demonstrates that certain plant proteins enhance the growth of carcinogen-induced pancreatic foci and that this effect is considerably greater than the enhancement by high levels of dietary unsaturated fat. Furthermore, the enhancement by the raw soy protein isolate may be mediated by CCK; but this does not appear to be the mechanism by which the unsaturated fat, corn oil, enhances pancreatic carcinogenesis.**

J Biol Chem 1988 Mar 15;263(8):3639-3645 **Dynamic pattern of estradiol binding to uterine receptors of the rat. Inhibition and stimulation by unsaturated fatty acids.** Vallette G, Christeff N, Bogard C, Benassayag C, Nunez E

J Biol Chem 1986 Feb 25;261(6):2954-2959 **Modifications of the properties of human sex steroid-binding protein by nonesterified fatty acids.** Martin ME, Vranckx R, Benassayag C, Nunez EA The effect of unsaturated and saturated nonesterified fatty acids (NEFAs) on the electrophoretic, immunological, and steroid-binding properties of human sex hormone-binding protein (SBP) were investigated. Tests were carried out on whole serum from pregnant women and on purified SBP using polyacrylamide gel electrophoresis, crossed immunoelectrophoresis with autoradiography, and equilibrium dialysis. All three methods showed that NEFAs influence the binding of sex steroids to SBP both in whole serum and with the purified protein. Saturated NEFAs caused a 1.5-2-fold increase in binding of **dehydrotestosterone, testosterone, and estradiol to SBP, while unsaturated NEFAs, such as oleic (18:1) and docosahexaenoic (22:6) acids inhibited the binding of these steroids to SBP. Thus, unsaturated NEFAs** in the concentration range 1-100 microM are more inhibitory for estradiol binding than for testosterone or dehydrotestosterone binding. In addition to these binding changes, polyacrylamide gel electrophoresis and immunoelectrophoretic studies revealed a shift in SBP from the slow-moving active native form to a fast-moving inactive one. There was also a reduction in the apparent SBP concentration by Laurell immunoelectrophoresis in the presence of unsaturated NEFA (5.5 nmol of NEFA/pmol of protein). These studies indicate that unsaturated NEFAs induce conformational changes in human SBP which are reflected in its electrophoretic, immunological, and steroid-binding properties. They suggest that the fatty acid content of the SBP environment may result in lower steroid hormone binding and thus increased free hormone levels.

J Biol Chem 1986 Feb 25;261(6):2954-2959 **Modifications of the properties of human sex steroid-binding protein by nonesterified fatty acids.** Martin ME, Vranckx R, Benassayag C, Nunez EA The effect of unsaturated and saturated nonesterified fatty acids (NEFAs) on the electrophoretic, immunological, and steroid-binding properties of human sex hormone-binding protein (SBP) were investigated. Tests were carried out on whole serum from pregnant women and on purified SBP using polyacrylamide gel electrophoresis, crossed immunoelectrophoresis with autoradiography, and equilibrium dialysis. All three methods showed that NEFAs influence the binding of sex steroids to SBP both in whole serum and with the purified protein. Saturated NEFAs caused a 1.5-2-fold increase in binding of **dehydrotestosterone, testosterone, and estradiol to SBP, while unsaturated NEFAs, such as oleic (18:1) and docosahexaenoic (22:6) acids inhibited the binding of these steroids to SBP.** Thus, unsaturated NEFAs in the concentration range 1-100 microM are more inhibitory for estradiol binding than for testosterone or dehydrotestosterone binding. In addition to these binding changes, polyacrylamide gel electrophoresis and immunoelectrophoretic studies revealed a shift in SBP from the slow-moving active native form to a fast-moving inactive one. There was also a reduction in the apparent SBP concentration by Laurell immunoelectrophoresis in the presence of unsaturated NEFA (5.5 nmol of NEFA/pmol of protein). These studies indicate that unsaturated NEFAs induce conformational changes in human SBP which are reflected in its electrophoretic, immunological, and steroid-binding properties. They suggest that the fatty acid content of the SBP environment may result in lower steroid hormone binding and **thus increased free hormone levels.**

J Steroid Biochem 1986 Feb;24(2):657-659 **Free fatty acids: a possible regulator of the available oestradiol fractions in plasma.** Reed MJ, Beranek PA, Cheng RW, James VH Consumption of dietary fats has been linked to the high incidence of breast cancer found in Western women. In vitro studies we have carried out show that **unsaturated free fatty acids can increase the biologically available**

oestradiol fractions in plasma. It is possible therefore that the increased risk for breast cancer associated with a diet high in fats may be related to an elevation in the biologically available oestradiol fractions in plasma.

Endocrinology 1986 Jan;118(1):1-7 **Potentiation of estradiol binding to human tissue proteins by unsaturated nonesterified fatty acids.** Benassayag C, Vallette G, Hassid J, Raymond JP, Nunez EA Nonesterified fatty acids (NEFAs) have been recently shown in the rat to be involved in steroid hormone expression, having effects on plasma transport and **intracellular activity.** This study examines the influence of saturated and unsaturated NEFAs on estradiol (E2) binding to cytosol from human uterus, breast, and melanoma. Binding was analyzed after separation with dextran-coated charcoal or hydroxylapatite and by sucrose density gradient centrifugation. **Unsaturated NEFAs induced a 2- to 10-fold increase (P less than 0.001) in E2 binding to cytosol** from normal, fibromatous, and neoplastic uteri, while saturated NEFAs **had a slight inhibitory effect** (P less than 0.05). Similar effects were seen with cytosol from metastatic melanoma lymph nodes and neoplastic breast tissues. By contrast, unsaturated NEFAs did not increase E2 binding to serum from these patients. Density gradient centrifugation indicated that the increased binding was associated with the proteins present in the 2- to 4 S region. Analysis of E2 metabolites in the presence of unsaturated NEFAs showed the formation of water-soluble derivatives. Seventy percent of these E2 derivatives were trichloracetic acid precipitable, suggesting a covalent link between the steroid and a protein. The existence of such water-soluble metabolites could be erroneously interpreted as a true binding to soluble cytoplasmic receptors.

Ann N Y Acad Sci 1988;538:257-264 **Possible relevance of steroid availability and breast cancer.** Bruning PF, Bonfrer JM Netherlands Cancer Institute (Antoni van Leeuwenhoekhuis), Amsterdam. "The as yet circumstantial evidence for a central role of estrogens in the promotion of human breast cancer is supported by many data. However, it has not been possible to identify breast cancer patients or women at risk by abnormally elevated estrogen levels in plasma. **The concept of available, i.e., non-SHBG bound sex steroid seems to offer a better understanding than total serum steroid levels do. We demonstrated that sex steroid protein binding is decreased by free fatty acids."**

J Surg Oncol 1993 Feb;52(2):77-82. **The effect of the fiber components cellulose and lignin on experimental colon neoplasia.** Sloan DA, Fleiszer DM, Richards GK, Murray D, Brown RA Department of Surgery, University of Kentucky College of Medicine, Lexington. Sixty Sprague-Dawley rats were pair-fed one of three nutritionally identical diets. One diet contained "low-fiber" (3.8% crude fiber); the others contained "high fiber" (28.7% crude fiber) composed of either cellulose or lignin. Although both "high fiber" diets had similar stool bulking effects, **only the cellulose diet** was associated with a reduction in 1,2-dimethylhydrazine (DMH)-induced colon neoplasms. The cellulose diet was also associated with distinct changes in the gut bacterial profile and with a lowered serum cholesterol.

Nutr Cancer 1984;6(2):77-85 **Enhancement of 1,2-dimethylhydrazine-induced large bowel tumorigenesis in Balb/c mice by corn, soybean, and wheat brans.** Clapp NK, Henke MA, London JF, Shock TL This study was designed to determine the effects of four well-characterized dietary brans on large bowel tumorigenesis induced in mice with 1,2-dimethylhydrazine (DMH). Eight-week-old barrier-derived male Balb/c mice were fed a semisynthetic diet with 20% bran added (either corn, soybean, soft winter wheat, or hard spring wheat) or a no-fiber-added control diet. Half of each group was given DMH (20 mg/kg body weight/week, subcutaneously for 10 weeks) beginning at 11 weeks of age. Surviving mice were killed 40 weeks after the first DMH injection. Tumors were not found in mice not subjected to DMH. In DMH-treated mice, tumors were found almost exclusively in the distal colon. Tumor incidences were as follows: **controls, 11%; soybean group, 44%; soft winter wheat group, 48%; hard spring wheat group, 58%; and corn group, 72%.** Tumors per tumor-bearing mouse ranged from 1.4 to 1.6, except in the corn group, which had 2.1. **A positive correlation was found between percentage of neutral detergent fiber in the brans and tumor incidences** but not between the individual components of cellulose, hemicellulose, or lignin. The **enhancement of DMH-induced large bowel tumorigenesis by all four bran types may reflect a species and/or mouse strain effect that is bran-source related. These data emphasize the importance of using well-defined bran in all "fiber" studies.**

Prev Med 1987 Jul;16(4):540-4 **Fiber, stool bulk, and bile acid output: implications for colon cancer risk.** McPherson-Kay R Dietary fiber has direct effects on stool bulk and bile acid output that may be of relevance in the etiology of colon cancer. Most types of fiber increase the total volume of stool and reduce the concentration of specific substances, including bile acids, that are in contact with the bowel wall. However, fibers differ in their effect on stool bulk, with wheat fiber being a more effective stool bulking agent than fruit and vegetable fibers. In addition, the extent to which a specific fiber reduces bile acid concentration will be modified by its concomitant effects on total fecal sterol excretion. Whereas wheat bran reduces fecal bile acid concentration, **pectin, lignin, and oat bran do not. These three fibers significantly increase total bile acid output. Bile acids act as promoters of colonic tumors in mutagenesis assay systems and in various animal models.** Human epidemiological studies show a relationship between various dietary variables, including fat and fiber intake, fecal concentration of bile acids, and colon cancer risk.

Eur J Gastroenterol Hepatol 1998 Jan;10(1):33-9 **Intestinal absorption of oestrogen: the effect of altering transit-time.** Lewis SJ, Oakey RE, Heaton KW University Department of Medicine, Bristol Royal Infirmary, UK. OBJECTIVE: The mechanism by which a high fibre diet may reduce serum oestrogens is unknown. We hypothesized that time is a rate-limiting factor in oestrogen absorption from the colon so that changes in colonic transit-rate affect the proportion of oestrogen that is deconjugated and/or absorbed. AIM: To determine if alteration of intestinal transit rate would influence the absorption of an oral dose of oestradiol glucuronide. PARTICIPANTS: Twenty healthy postmenopausal women recruited by advertisement. SETTING: Department of Medicine, Bristol Royal Infirmary. METHODS: Volunteers consumed, in turn, wheat bran, senna, loperamide and bran shaped plastic flakes, each for 10 days with a minimum 2 week washout period between study periods, dietary intake being unchanged. Before and in the last 4 days of each intervention whole-gut transit-time, defecation frequency, stool form, stool beta-glucuronidase activity, stool pH and the absorption of a 1.5 mg dose of oestradiol glucuronide were measured. RESULTS: Wheat bran, senna and plastic flakes led to the intended reduction in whole-gut transit-time, increase in defecatory frequency and increase in stool form score. Loperamide caused the opposite effect. **The length of time the absorbed oestrogen was detectable in the serum fell with wheat bran and senna, although this was only significant for oestradiol.** Oestrone, but not oestradiol, was detectable for a longer time with loperamide. Plastic flakes had no effect on either oestrogen. Areas under the curve did not change significantly but tended to fall with the three transit-accelerating agents and to rise with loperamide. CONCLUSION: Our data indicate there is likely to be an effect of intestinal transit on the absorption of oestrogens but more refined techniques are needed to characterize this properly.

Br J Cancer 1997;76(3):395-400. **Lower serum oestrogen concentrations associated with faster intestinal transit.** Lewis SJ, Heaton KW, Oakey RE, McGarrigle HH University Department of Medicine, Bristol Royal Infirmary, UK. Increased fibre intake has been shown to reduce serum oestrogen concentrations. We hypothesized that fibre exerts this effect by decreasing the time available for reabsorption of oestrogens in the colon. We tested this in volunteers by measuring changes in serum oestrogen levels in response to manipulation of intestinal transit times with senna and loperamide, then comparing the results with changes caused by wheat bran. Forty healthy premenopausal volunteers were placed at random into one of three groups. The first group took senna for two menstrual cycles then, after a washout period, took wheat bran, again for two menstrual cycles. The second group did the reverse. The third group took loperamide for two menstrual cycles. At the beginning and end of each intervention a 4-day dietary record was kept and whole-gut transit time was measured; stools were taken for measurement of pH and beta-glucuronidase activity and blood for measurement of oestrone and oestradiol and their non-protein-bound fractions and of oestrone sulphate. **Senna and loperamide caused the intended alterations in intestinal transit, whereas on wheat bran supplements there was a trend towards faster transit. Serum oestrone sulphate fell with wheat bran (mean intake 19.8 g day(-1)) and with senna; total- and non-protein-bound oestrone fell with senna.** No significant changes in serum

oestrogens were seen with loperamide. No significant changes were seen in faecal beta-glucuronidase activity. Stool pH changed only with senna, in which case it fell. In conclusion, speeding up intestinal transit can lower serum oestrogen concentrations.

J Steroid Biochem Mol Biol 1991 Aug;39(2):193-202 **Influence of wheat bran on NMU-induced mammary tumor development, plasma estrogen levels and estrogen excretion in female rats.** Arts CJ, de Bie AT, van den Berg H, van 't Veer P, Bunnik GS, Thijssen JH TNO Toxicology and Nutrition Institute, The Netherlands. In our animal experiments the hypothesis was tested that a high-fiber (HF) diet reduces tumor promotion **by interruption of the enterohepatic circulation resulting in lowered estrogen exposure of the estrogen-sensitive tissue.** In the first experiment the development of N-nitrosomethylurea (NMU) induced mammary tumors was investigated. One group of rats (HF) was fed a HF diet (11% fiber, based on wheat bran), the other group (LF) fed a low-fiber diet (0.5% fiber, based on white wheat flour). Tumor incidence (90 and 80%, respectively) and latency (121 and 128 days, respectively) were similar in the HF and LF groups. Compared to the LF group, HF rats had lower tumor weights (0.16 vs 0.55 g; P less than 0.01) and a slightly lower tumor multiplicity (1.8 vs 2.8 tumors per tumor-bearing rat). These differences were reduced after adjustment for body weight. In a second experiment rats, not treated with the carcinogen, were kept on the same HF and LF diets. From these rats 24-h urine and feces and orbital blood samples were **collected for analysis of (un)conjugated estrogens. The excretion of both free and conjugated estrogens in fecal samples was about 3-fold higher in HF rats than in LF rats. During the basal period of the cycle urinary excretion of estrone was lower in HF rats (mean 9.7 ng/day) than in LF rats (mean 13.0 ng/day; P less than 0.05). It is concluded that wheat bran interrupts the enterohepatic circulation of estrogens, but plasma levels are not affected. Whether the development of mammary tumors is reduced by the introduction of specific components of wheat bran, or by a reduced body weight due to a lower (effective) energy intake remains to be determined.**

Nutr Cancer 1998;31(1):24-30 **Dietary lignin, and insoluble fiber, enhance uterine cancer but did not influence mammary cancer induced by N-methyl-N-nitrosourea in rats.** Birt DF, Markin RS, Blackwood D, Harvell DM, Shull JD, Pennington KL Eppley Institute for Research in Cancer and Allied Disease, University of Nebraska Medical Center, Omaha 69198, USA. Previous investigations suggested potential breast cancer-preventive properties of dietary fiber from cabbage. The purpose of the present investigation was to determine whether lignin, a component of cabbage fiber, would protect against mammary carcinogenesis by N-methyl-N-nitrosourea (MNU) in Sprague-Dawley rats. A six-week study was conducted using diets containing 0.5-5% dietary wood lignin (a readily available, purified source). These diets were well tolerated by the rats, and a carcinogenesis study using 5 mg MNU/100 g body wt i.v. at 50 days of age was conducted, with the 2.5% lignin diet fed from 6 through 8 weeks of age followed by 5% lignin diet until 20 weeks after MNU. Dietary lignin and MNU treatment increased food consumption ($p < 0.05$), and body weight was slightly reduced at 10 and 20 weeks after MNU in the MNU-5% lignin diet group ($p < 0.05$). Serum estradiol was not altered by dietary lignin or MNU treatment, but uterine weights were highest in the MNU-control diet group 4 and 12 weeks after MNU. Expression of creatine kinase B, an estrogen-responsive gene, was lower in the uteri of the MNU-lignin diet group than in other groups at 20 weeks. Mammary carcinogenesis was not altered by dietary lignin. **However, uterine endometrial adenocarcinoma was observed only in the MNU-lignin diet group (4 carcinomas/40 effective rats) ($p < 0.05$).**

Ginecol Obstet Mex 1998 Mar;66:111-8 **[Estrogens of vegetable origin].** [Article in Spanish] Rubio Lotvin B Reproduccion y de Ginecologia y Obstetricia Facultad de Medicina, UNAM Depto. de Ginecologia y Obstetricia Hospital Americano, Britanico Cowdray. Mexico, D.F. In recent years, estrogens of vegetable origin have acquired some importance that justify the presentation of the available data. The compounds that have estrogenic effect when ingested as food through **vegetables include isoflavones, lignines and lactones. The review comprises their chemical structure,** metabolism and excretion as well as their effect on plasmatic levels of estrogens FSH, LH and SHBG as well as their activity over lipoproteins and, naturally, their action on menopause symptoms and breast cancer.

Proc Soc Exp Biol Med 1995 Jan;208(1):6-12 **Chemical studies of phytoestrogens and related compounds in dietary supplements: flax and chaparral.** Obermeyer WR, Musser SM, Betz JM, Casey RE, Pohland AE, Page SW Division of Natural Products, Food and Drug Administration, Washington, District of Columbia 20204. High-performance liquid chromatographic (HPLC) and mass spectrometric (MS) procedures were developed to determine lignans in flaxseed (Linum usitatissimum) and chaparral (Larrea tridentata). **Flaxseed contains high levels of phytoestrogens. Chaparral has been associated with acute nonviral toxic hepatitis and contains lignans that are structurally similar to known estrogenic compounds.** Both flaxseed and chaparral products have been marketed as dietary supplements. A mild enzyme hydrolysis procedure to prevent the formation of artifacts in the isolation step was used in the determination of secoisolariciresinol in flaxseed products. HPLC with ultraviolet spectral (UV) or MS detection was used as the determinative steps. HPLC procedures with UV detection and mass spectrometry were developed to **characterize the phenolic components, including lignans and flavonoids,** of chaparral and to direct fractionation studies for the bioassays.

Brain Res 1994 Jul 25;652(1):161-3 **The 21-aminosteroid antioxidant, U74389F, prevents estradiol-induced depletion of hypothalamic beta-endorphin in adult female rats.** Schipper HM, Desjardins GC, Beaudet A, Brawer JR Department of Anatomy and Cell Biology, Bloomfield Centre for Research in Aging, Jewish General Hospital, McGill University, Montreal, Que., Canada. **A single intramuscular injection of 2 mg estradiol valerate (EV) results in neuronal degeneration** and beta-endorphin depletion in the hypothalamic arcuate nucleus of adult female rats. We have hypothesized that peroxidase-positive astrocytes in this brain region oxidize estrogens and catecholestrogens to semiquinone radicals which mediate oxidative neuronal injury. In the present study, dietary administration of the potent antioxidant 21-aminosteroid, U-74389F, completely blocked EV-induced beta-endorphin depletion in the hypothalami of adult female rats. Neither EV nor 21-aminosteroid treatment had any effect on hypothalamic concentrations of neuropeptide Y and Met-enkephalin, **confirming that the estradiol lesion is fairly selective for the beta-endorphin cell population.** The present findings support the hypothesis that the toxic effect of estradiol on hypothalamic beta-endorphin neurons is mediated by free radicals.

J Steroid Biochem Mol Biol 1998 Feb;64(3-4):207-15, **"Effects of tea polyphenols and flavonoids on liver microsomal glucuronidation of estradiol and estrone."** Zhu BT, Taneja N, Loder DP, Balentine DA, Conney AH "Administration of 0.5 or 1% lyophilized green tea (5 or 10 mg tea solids per ml, respectively) as the sole source of drinking fluid to female Long-Evans rats for 18 days stimulated liver microsomal glucuronidation of estrone, estradiol and 4-nitrophenol by 30-37%, 15-27% and 26-60%, respectively. Oral administration of 0.5% lyophilized green tea to female CD-1 mice for 18 days stimulated liver microsomal glucuronidation of estrone, estradiol and 4-nitrophenol by 33-37%, 12-22% and 172-191%, respectively. The in vitro addition of a green tea polyphenol mixture, a black tea polyphenol mixture or (-)-epigallocatechin gallate inhibited rat liver microsomal glucuronidation of estrone and estradiol in a concentration-dependent manner and their IC50 values for inhibition of estrogen metabolism were approximately 12.5, 50 and 10 microg/ml, respectively. Enzyme kinetic analysis indicates that the inhibition of estrone glucuronidation by 10 microM (-)-epigallocatechin gallate was competitive while inhibition by 50 microM (-)-epigallocatechin gallate was noncompetitive. Similarly, several flavonoids (naringenin, hesperetin, kaempferol, quercetin, rutin, flavone, alpha-naphthoflavone and beta-naphthoflavone) also inhibited rat liver microsomal glucuronidation of estrone and estradiol to varying degrees. Naringenin and hesperetin displayed the strongest inhibitory effects (IC50 value of approximately 25 microM). These two hydroxylated flavonoids had a competitive mechanism of enzyme inhibition for estrone glucuronidation at a 10 microM inhibitor concentration and a predominantly noncompetitive mechanism of inhibition at a 50 microM inhibitor concentration."

Toxicology 1997 Sep 26;122(1-2):61-72, **"Effects of co-administration of butylated hydroxytoluene, butylated hydroxyanisole and flavonoids on the activation of mutagens and drug-metabolizing enzymes in mice."** Sun B, Fukuhara M Effects of co-administration of food additives and naturally occurring food components were studied on the activation of mutagens. Male mice (ddY) were given diets containing butylated hydroxytoluene (BHT) or butylated hydroxyanisole (BHA) and flavone or flavanone (2,3-dihydroflavone) for two weeks and the ability of hepatic microsomes to activate aflatoxin B1, benzo[a]pyrene and N-nitrosodimethylamine was determined by the

mutagenicity test. Co-administration of an antioxidant (0.1% BHT or 0.2% BHA in diet) and a flavonoid (0.1% flavone or 0.1% flavanone) **resulted in additive effects on the activation of aflatoxin B1 and benzo[a]pyrene,** while the activation of N-nitrosodimethylamine was not elevated significantly by the co-administration. To understand the mechanism for the additive effects, induction of specific isozymes of cytochrome P450 involved in the activation of the mutagens was studied. Co-administration of BHT (0.1%) and flavone (0.1%) increased markedly the levels of proteins and the activities of the enzymes related to the isozymes of CYP2A and CYP2B, while co-administration of BHA (0.2%) and flavanone (0.1%) elevated those related to CYP1A. Further, the activation of aflatoxin B1 and benzo[a]pyrene in hepatic microsomes was inhibited by the antibodies against these isozymes, which suggested that the enhanced activation of the mutagens by the co-administration might be mediated by the induction of these isozymes.

Biochem Soc Trans 1977;5(5):1489-92. **Frameshift mutagenicity of certain naturally occurring phenolic compounds in the 'Salmonella/microsome' test: activation of anthraquinone and flavonol glycosides by gut bacterial enzymes.** Brown JP, Dietrich PS, Brown RJ

Mutagenesis 1997 Sep;12(5):383-90 **"Involvement of rat cytochrome 1A1 in the biotransformation of kaempferol to quercetin: relevance to the genotoxicity of kaempferol.**" Silva ID, Rodrigues AS, Gaspar J, Maia R, Laires A, Rueff J. "Kaempferol is a flavonoid widely distributed in edible plants and has been shown to be genotoxic to V79 cells in the absence of external metabolizing systems. The presence of an external metabolizing system, such as rat liver homogenates (S9 mix), leads to an increase in its genotoxicity, which is attributed to its biotransformation to **the more genotoxic flavonoid quercetin**, via the cytochrome P450 (CYP) mono-oxygenase system."

Environ Health Perspect 1997 Apr;105 Suppl 3:633-6 **Dietary estrogens stimulate human breast cells to enter the cell cycle.** Dees C, Foster JS, Ahamed S, Wimalasena J. **"Our findings are consistent with a conclusion that dietary estrogens at low concentrations do not act as antiestrogens, but act like DDT and estradiol to stimulate human breast cancer cells to enter the cell cycle."**
