

Developmental Effects of Dietary Soy Phytoestrogens

Project Scope

Evidence suggests that naturally occurring compounds in diet may disrupt or mimic normal hormone action. Developmental exposure to such endocrine active chemicals (EACs) may adversely affect reproductive, neurobehavioral, and immunological development or predispose exposed individuals to certain cancers later in life. Increased soy consumption in the North American diet has resulted in higher dietary phytoestrogen exposure in reproductive-aged women, and has raised concerns about possible adverse effects on the fetus and neonate. Studies have shown developmental effects can be produced by EAC exposures in rodent models.

The main objectives of this research were to determine:

- Whether intake of dietary soy phytoestrogens by rodents at environmentally relevant concentrations from mid-gestation throughout lactation to weaning affects sexual development in the progeny.
- If the human fetus receives EAC doses at critical developmental stages that are comparable to those that cause developmental effects in animal models.

To address these objectives, studies were conducted in rats to evaluate the effects of *in utero* and lactational exposure to EACs, including dietary soy phytoestrogens, at levels comparable to the upper range of potential dietary intake in North America, on reproductive development. These data were used to estimate the possible effects of equivalent human exposures. Another study was performed to determine whether man-made chemicals with potential endocrine-disrupting activity and natural phytoestrogens could be quantified in human amniotic fluid during the second trimester of pregnancy.

Grant Title and Principal Investigator

Developmental Effects of Dietary Soy Phytoestrogens

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Key Findings and Implications

- Developmental exposure of rats to the potent reference estrogen diethylstilbestrol (DES) at low doses and/or the common phytoestrogen genistein at levels comparable to the upper range of human exposure affected some markers of sexual development in both male and female pup.
- Similar exposure of mice to DES or the phytoestrogens genistein or daidzein was found to alter many reproductive outcomes in both males (e.g., testicular weight) and females (e.g., estrogen cycling). Timing of exposure also was an important modifier of the observed effects.
- Neither *In utero* nor lactational exposure to genistein nor daidzein affected mammary tumor latency or the number of tumors in mice predisposed to development of such tumors.
- In the Los Angeles areas, approximately one in three fetuses are exposed to xenobiotic EACs (e.g., PCBs, the pesticide hexachlorobenzene) and about half of all fetuses are exposed to the phytoestrogens daidzein, genistein, formononetin, biochanin-A, and coumestrol *in utero*. Amniotic fluid sampling may serve as a useful biomarker of exposure to these agents.
- The results in rodents in conjunction with the detection of EACs in amniotic fluid sustain concerns about potential adverse health effects of developmental exposures to EACs for the human fetus/neonate.

Project Period: November 1996 to October 1999

Relevance to ORD's Multi-Year Research Plan

This project contributes to two important long-term goals of the ORD's MYP for endocrine disruptors: (1) provide a better understanding of the science underlying the effects, exposure, assessment, and management of endocrine disruptors, and (2) determine the extent of the impact of endocrine disruptors on humans, wildlife, and the environment.

The results of this project provide a better understanding of the developmental/reproductive effects of several common dietary soy phytoestrogens in rodents exposed from mid-gestation throughout lactation to levels comparable to the upper range of potential dietary intake in humans. The research demonstrated the successful use of an animal model of the effects of EACs at properly scaled levels of exposure during sensitive developmental stages, Shedding light on the potential for similar effects in humans.

Project Results and Implications

This first study focused on the effects of the common dietary phytoestrogen genistein at levels comparable to the upper range of potential dietary intake and a potent reference estrogen, diethylstilbestrol (DES), at marginally effective doses on some markers of sexual development in rats. Pregnant dams were randomly assigned to each of five treatment (daily oral doses) groups: control (corn oil), low DES, high DES, genistein, and genistein + DES. None of the treatments affected the duration of gestation, litter size, or anogenital distance (AGD; an indicator of maleness or femaleness), controlled for birth weight. However, the ratio of AGD to body weight at weaning differed significantly between the control group and each of the estrogenic treatments in both sexes, with larger AGD/body ratios associated with all of the estrogenic treatments. Males exposed to high DES and genistein alone exhibited earlier onset of puberty by about three days compared to controls. In females, the onset of puberty was unaffected by estrogen treatment in all groups of females except low DES, in which onset was about one day earlier than in controls. Post-pubertal ratios of male reproductive organ weights/body weight (testes, epididymis, seminal vesicle, and prostate) were unaffected by estrogen treatment in all groups except high DES, where increased testicular weight and decreased epididymis, seminal vesicle, and prostate weights were observed. Initial vaginal cycles were four days long for all groups of females except those exposed to high DES, who had mean cycle length of six days. This study showed that low doses of estrogen, and the genistein doses comparable to those in upper range of human exposure, affect some markers of sexual development in rats.

Researchers also studied the developmental effects of *in utero* or lactational exposure to phytoestrogens in mice. Dams were treated with DES, daidzein, or genistein (low and high doses) during pregnancy and/or lactation (birth-weaning). As expected, the reference estrogen DES was found to alter many reproductive outcomes in both male and female mice. For some of these endpoints, daidzein and genistein, mimicked DES; however, unique or opposite responses were also evident for each of the phytoestrogens, including low doses of genistein. Effects were seen in both male and female pups receiving *in utero* and/or lactational exposures to all of the agents tested. The timing of exposure also affected the type of response elicited by the treatments; *in utero* and lactational exposure often resulted in different effects. Affects on estrogen cycling and uterine and testicular weight persisted after sexual maturity. These results indicate that *in utero* and lactational exposure to phytoestrogens can influence reproductive development of mice.

Experiments were also performed that examined the effects of the phytoestrogens on the development of mammary tumors in mice. Wild-type dams were mated with MMTV-*neu* transgenic males, yielding offspring (*c-neu*) in which the frequency of spontaneous mammary tumors is increased. The dams were treated with daidzein or genistein during pregnancy or lactation, and their female offspring monitored for tumor development for 13 months. Neither genistein nor daidzein affected average mammary tumor latency or the average number of tumors per female offspring after *in utero* or lactational exposure. Thus,

developmental exposure to these phytoestrogens did not appear to have a protective effect, as had been previously reported for carcinogen-induced tumors in other animal models.

Research under this grant also investigated whether man-made chemicals and phytoestrogens can be quantified in human amniotic fluid during the second trimester of pregnancy. Two groups of amniotic fluid samples obtained at routine amniocentesis of pregnant women in the Los Angeles, California region were assayed by gas chromatographic/mass spectrometric (GC/MS) analysis. The first group of amniotic fluid samples (n = 53) were analyzed for common polychlorinated biphenyl (PCB) congeners, two metabolites of the banned pesticide dichlorodiphenyltrichloroethane (DDT), as well as for the pesticides hexachlorobenzene (HCB) and three isomers of hexachlorocyclohexane (HCH). The contaminants HCH and one DDT metabolite were detected in all amniotic fluid samples, while specific PCB congeners were detected much less frequently. Approximately one-third of the amniotic fluid samples tested positive for at least one environmental contaminant assayed. A second group of amniotic fluid samples (n=62) was analyzed for the common phytoestrogens daidzein, genistein, formononetin, biochanin-A, and coumestrol. Overall, approximately 41 and 61 percent of the samples were found to contain quantifiable levels of daidzein and genistein, respectively. There were no significant differences in the ethnic background, age, or gestational age of women who had detectable levels of phytoestrogens compared to those who did not. These results suggest that amniotic fluid analyses may be a useful biomarker of fetal exposures to manmade estrogenic substances, as well as to phytoestrogens. Although the consequences of these exposures for the human fetus remain unknown, this study supports concerns about potential adverse health effects of developmental exposures to EACs for the human fetus and neonate.

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For More Information

<http://www.ag.uiuc.edu/~stratsoy/soyhealth/welcome.html>

NCER Project Abstract and Reports:

http://cfpub2.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/140