Effect of Genistein on Steroid Hormone Production in the Pregnant Rhesus Monkey (44431)

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Abstract. **Genistein is a phytoestrogen found in soy beans. Phytoestrogens have been reported to cause reproductive problems in sheep and rats. This research was conducted to determine the effects of genistein fed to rhesus monkeys during pregnancy, with specific interest on fetal growth and steroidogenesis in the maternal-fetoplacental unit. Two groups of five monkeys each were selected in early stages of pregnancy. One group was administered genistein in a fruit treat each weekday until Cesarean section 10 days prior to term. The second, control group, received fruit treats without genistein. Maternal blood samples were collected on Tuesday and Friday of each week. At delivery, samples were collected from the maternal peripheral circulation, uterine veins, uterine-ovarian veins, and the fetal heart. Comparisons between control and genistein-treated monkeys revealed no differences in the maternal weight gained during pregnancy, or in fetal weights or placental weights at delivery. Serum was assayed by radioimmunoassay (RIA) for estradiol, progesterone, dehydroepiandrosterone sulfate (DHEA-S), and estrone. No significant differences ($P > 0.05$) were noted in progesterone or DHEA-S levels at delivery or during the pregnancy; however, estradiol levels were higher ($P < 0.05$) in the four areas studied at delivery and in the maternal blood with advancing gestation. Additionally, estrone levels tended to increase more rapidly ($P = 0.057$) in the maternal blood of monkeys receiving genistein than in untreated controls, suggesting that genistein may stimulate the deconjugation of estrone in the gut, thus allowing its reabsorption into the peripheral circulation and conversion to estradiol.** [P.S.E.B.M. 1999, Vol 222]

Phytoestrogens are estrogen-like chemicals that are diverse in their chemical structures as well as their plant origins (1). The discovery that some phytoestrogens compete with estradiol for binding to estrogen receptors and elicit estrogenic responses in estrogen-target tissues and cells suggests that they may share a common mechanism of action (2). Although some phytoestrogens have a low affinity for the estrogen receptor and a low estrogenic response (3), others are still of interest because their antiestrogenic action may provide anticarcinogenic activity (4).

Genistein (5,7,4′-trihydroxyisoflavone) is a type of phytoestrogen found as a glucoside in soy products (5). The glucoside is hydrolyzed by intestinal microflora to genistein (6, 7), a product that is similar in structure and molecular weight to estradiol. As with most hormones, the effects of genistein are cell-type specific and may be agonistic or antagonistic, depending on the tissue studied and dose administered. Genistein has been reported to have estrogenic properties, as indicated in uterine weight assays and estrogen receptor binding assays (8). Many of the studies on phytoestrogens have been conducted in rodents. A study by Levy *et al.* (9) found that the offspring of rats injected subcutaneously with genistein on gestational Days 16–20 exhibited lower birth weights, shorter anogenital distances, and delayed onset of vaginal openings. These effects were different from those found with neonatal treatment, and the difference between high (25 mg/day) and low (5 mg/day)

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doses suggests both agonist and antagonist roles. These results also differ from those caused by estradiol or diethylstilbestrol (DES) administration. Lamartiniere *et al.* (10) reported that genistein administered to neonatal rats at a dosage of 5 mg/20 μl on Days 2, 4, and 6 postnatally, caused increased latency and reduced incidence and multiplicity of chemically induced mammary tumors compared to vehicle-treated controls. This effect may be facilitated through a precocious maturation of the terminal end buds of the mammary glands, which are undifferentiated structures that are most susceptible to chemical carcinogens. Lamartiniere *et al.* (8) also reported that high concentrations of genistein administered to rats during the neonatal period adversely affected ovarian follicular development. A study (11) using ovariectomized rats treated with conjugated equine estrogens (CEE) and/or dietary soybean extract (SBE) found that the combination of CEE and SBE was not additive and that high doses of SBE decreased the uterine luminal epithelial height and the lactoferrin response induced by CEE alone. The SBE used in this study would contain a complex mixture of phytoestrogens, some of which might have antiestrogenic properties. Researchers concluded that dietary soybeans do not elicit a pattern of responses similar to that of the steroidal estrogens.

One concern is that exposure to phytoestrogens may pose a developmental hazard to infants (12). When given as directed by the manufacturers, soy formula provides the 4-month-old infant with approximately 6–9 mg/kg body weight of isoflavones, including genistein and 7,4′-dihydroxyisoflavone (daidzein). If an infant is fed soy-based formulas exclusively, the amount of isoflavones may exceed the amount shown in adults to significantly perturb the hormonal regulation of the menstrual cycle of western women (13); additionally, increased risk of goiter (14) and autoimmune thyroid disease (15) have been reported in infants fed soy-based formulas.

Alterations in mechanisms regulating steroidogenesis in the adult may also affect the endocrinology of the maternal-fetoplacental unit and may be critical as the normal maintenance of pregnancy is facilitated by placental progesterone biosynthesis. The progesterone production in the placental syncytiotrophoblast may be regulated by estrogen upregulation of the low-density lipoprotein (LDL) receptor (16, 17). Furthermore, placental estrogen production, *in vivo,* is dependent upon the availability of androgen precursors from the fetal adrenal. Therefore, the current study was conducted to determine if a supplemental phytoestrogen, genistein, in the diet of pregnant rhesus monkeys, affects steroidogenesis in the maternal-fetoplacental unit.

Materials and Methods

Animals. The monkeys used in this study were obtained from the research colony at the Tulane Regional Primate Research Center, an AAALAC accredited facility. All aspects of the study were approved by the IACUC of this facility. Ten adult female rhesus monkeys (*Macaca mulatta*) were timed bred using the standard progesterone recycling method developed at this Center (18, 19). Pregnancies were diagnosed by ultrasound examinations. The monkeys were housed in individual cages, fed a commercial diet (Teklad Monkey Diet, Harlan, Madison, WI) twice daily, with water available at all times. About 31% of the protein in this diet (minimum of 20% protein in the diet) came from soybeans. Since the phytoestrogen content in soybeans varies from source to source, potential effects of genistein from this diet were controlled by assuring that control and experimental monkeys received the same dietary intake, exclusive of the supplemental genistein. This diet is the same as provided to the monkeys prior to being placed on this study. The monkeys were anesthetized with ketamine hydrochloride anesthesia (10 mg/kg body weight) for all procedures that required direct handling of the animals.

The rhesus monkey was selected as the animal model because of the similarity of the anatomy and physiology of the female reproductive system and fetoplacental unit to that in the human. Only humans, apes, and Old World monkeys have menstrual cycles. In this research colony of rhesus monkeys the menstrual cycles are approximately 28 to 32 days in length and, as in the human, they generally produce a single fertile egg at each ovulatory period. The uterus is simplex, and the placenta is a hemochorial type, as in the human (20), a significant factor in studies involving transplacental passage of substances. Steroidogenic pathways in the maternal-fetoplacental unit are similar, as placental estrogen synthesis requires fetal androgen precursors, and placental progesterone production requires cholesterol from maternal lipoproteins in both women and rhesus monkeys. The monkeys were placed in two study groups, a control group and an experimental group. The experimental group monkeys received genistein as described below.

Genistein Administration. Genistein was purchased from Indofine Chemical Company, Inc. (Somerville, NJ; lot numbers CH114 and CH118) and was labeled as pure. No additional purification was attempted. The genistein was weighed out each day immediately before administration and was administered in a fruit treat, orange sections, Monday through Friday, between 0700 and 0900 hr, before the morning feeding. The orange sections were cut along the internal, core edge, a small amount of juice expressed, and the genistein powder poured into the section. The experimental monkeys were given the genistein section first and then a second untreated section. This assured the immediate consumption of the genistein-containing section. The dose administered was 8.0 mg/kg. Since genistein is reported to have only 1/1000th the estrogenic activity of estradiol (21), this represents an intermediate dose based on a per weight basis for a 55-kg woman taking 0.625 mg estradiol/day, the most common dose prescribed, and a dose of 0.3 mg/day, the lowest dose providing an estrogenic response in women. The 8 mg/kg of genistein is at the upper level of the amount of isoflavone consumed by a 4-month-old infant fed exclusively a soy-based formula (15) and

intermediate of the dose ranges used in rodent studies (9). Administration began on the day following pregnancy diagnosis and continued until the day of Cesarean section (C-section) delivery. Control monkeys also received two orange sections on the days when dosing took place.

Blood Collection. During gestation, blood was collected from the femoral vein of the anesthetized pregnant monkeys using a 21-gauge needle. The blood was immediately transferred into a blood collection tube without an anticoagulant. After the blood clotted, the tube was centrifuged and the serum removed, aliquoted, and frozen at –40°C until analyzed. A volume of 3.0 ml was collected twice weekly, between 0700 and 0900 hr from the day following pregnancy diagnosis, until the C-section. Frequent bleedings (twice weekly) were necessary to adequately record changes in maternal hormone levels, due to normal fluctuations in steroid profiles typical of advancing nonhuman primate pregnancy (20).

Radioimmunoassays for Hormones. The steroid hormones estradiol, progesterone, and dehydroepiandrosterone sulfate (DHEA-SO_4) were assayed in the sera using kits purchased from Diagnostic Products Corporation (San Diego, CA). Values obtained for estradiol standards to which genistein had been added did not differ from those of standards to which genistein had not been added, indicating that there was no cross-reaction of exogenous genistein with the estradiol-specific antibody. The estrone assays were conducted using kits purchased from Diagnostic Systems Laboratories, Inc. (Webster, TX). The kits from both suppliers have been validated in our lab by serum dilutions and spiking pooled sera with known amounts of hormones. The standard curves were inclusive of the range of hormones found in pregnant rhesus monkeys.

Cesarean Section and Collection of Blood and Tissues. Standard sterile techniques and surgical approach were used for C-sections. Pregnancies were terminated and tissues retrieved from rhesus monkeys on Day 155 of gestation, 10 days prior to the estimated time of normal parturition, 165 days. This period was chosen to acquire the placenta and fetuses near normal term. The surgeries were conducted using ketamine HCl/atropine for preanesthesia with isofluorane anesthesia and oxygen support for the procedure. At the time of the C-section the maternal weight was determined, and blood samples were collected from the maternal femoral veins, representative of maternal peripheral circulation, the uterine veins (UV), representative of the vasculature draining the uterofeto-placental unit, and the uterine-ovarian veins (UOV), which drain the ovaries. Following the delivery of the fetus, the fetal weight was determined, and fetal blood samples collected by cardiac puncture. Placental villous tissue was collected and snap-frozen in liquid nitrogen for later analysis of LDL receptor mRNA transcripts by the techniques described below. Also, villous tissue was enzymatically dispersed, and a fraction of purified syncytiotrophoblast cells were prepared by density gradient centrifugation. LDL receptor mRNA transcripts were also quantitated in this purified cell fraction, in the event that transcript abundance was divergently regulated in steroidogenesis (e.g., syncytiotrophoblasts versus nonsteroidogenic) (e.g., connective tissue, placental cell populations) (22). Blood was processed and stored as previously described.

Placental Histology. Morphological evaluation of placental villous tissue was conducted following hematoxylin-eosin staining (16), and appraisals were made by phase-contrast microscopy.

Quantitation of LDL Receptor mRNA. Total RNA was isolated according to Chomczynski and Sacchi (23) and Chirgwin *et al.* (24). Competitive reverse transcriptase-polymerase chain reaction (RT-PCR) was used to quantitatively assess mRNA transcripts in placental villous tissue and a purified fraction of syncytiotrophoblasts, as previously used in our laboratory (25). Thus, oligonucleotide primers were synthesized (Tulane Molecular Biology Consortium, Department of Biochemistry) according to the published sequence for LDL receptor (5′ primer: 5′-CAATGTCTCACCAAGCTCTG-3′; 3′ primer: 5′-TCTGTCTCGAGGGGTA GCTG-3′), (26). Quantitation of initial RT-PCR results was achieved, using the PCR MIMIC Construction Kit (Clontech, Palo Alto, CA) (27). Final concentrations were expressed as attomoles of LDL receptor mRNA/μg total RNA.

Quanititation of Serum Lipoproteins. Serum concentrations of LDL- and high-density lipoprotein (HDL)-cholesterol were determined according to the method of Noma *et al.* (28) as adapted for use in our laboratory (17). Briefly, serum (0.2 ml) was first treated with 150 mg sodium heparin (Sigma), 7.35 g $CaCl_2$, and 475 mg $NiCl_2$ to isolate HDL, and then with 500 mg Amberlite anion exchange resin (Sigma) to isolate HDL plus LDL. Two milliliters of O-pthaldehyde solution (50 mg/100 ml glacial acetic acid (Sigma)) were added to both lipoprotein precipitates and cholesterol standards, and following addition of 1.0 ml concentrated H_2SO_4 (approximately 36 *N*), were incubated (room temperature, 10 min). Absorbance was determined at 550 nm. Serum LDL concentrations were calculated by substraction of cholesterol concentrations in HDL fractions from concentrations in HDL plus LDL fractions. The interassay coefficient of variation (n = 5 assays) for an LDL-cholesterol standard was 10.2% and for an HDL-cholesterol was 11.9%. The intraassay coefficient of variation (n = 3 replicates) was 1.7% for LDL-cholesterol and 2.3% for HDL-cholesterol.

Statistical Analysis

Comparisons of data from the control and genistein groups were made using independent sample, one-tail, Student's t test. Some data were also evaluated using the Mann-Whitney U test, the Kolmogorov-Smirnov test, the Wald-Wolfowitz Runs test, or the Kruskal Wallis ANOVA by

ranks test. P values presented are from the Student's t test unless otherwise noted. A significant difference was understood to exist when $P < 0.05$.

Results

The demand for pregnant monkeys by other investigators made it impossible for all the monkeys in this study to start on the same day or at exactly the same stage of gestation; however there was no significant difference ($P < 0.05$) in the mean gestational age between the control group and the experimental group. Because of this range in the gestational ages at the initial samplings, some of the following data will be presented for two study periods, midterm (gestational age of 82 days), and at the time of C-section, 155 days. At 82 days of gestation all the genistein-treated monkeys had received a minimum of 35 doses of genistein over a period of 7 weeks, and all the control monkeys had been on the study for a minimum of 3 weeks.

The differences between the control monkeys and the genistein-treated monkeys relative to the initial weights of the mothers, weights of the mothers at delivery, or in the fetal or placental weights at delivery were not significant. (Table I.) Histologic examination of placental sections stained with hematoxylin-eosin found no gross changes in placental villous morphology as a result of genistein treatment.

Progesterone levels in the control versus experimental maternal or fetal blood samples collected at delivery were not significantly different (Table II), although in each case the experimental group means were greater than the control group means.

Significant differences in the maternal estradiol levels at the time of the C-section were determined (see Table III). Thus control mean value was 212.9 pg/ml whereas the genistein-treated mean value was 335.8 pg/ml ($P = 0.04$). There were also significant differences regarding fetal mean values, 204.8 vs 358.3 pg/ml ($P = 0.04$) for untreated controls and genistein-treated monkeys, respectively. There were highly significant differences in the estradiol levels in the UV and in the UOV. UV values in the control group and the genistein-treated group were 529.4 and 1107.2 pg/ml respectively ($P = 0.02$), and the UOV values were 522.9 and 915.9 pg/ml ($P = 0.02$), respectively.

Table I. Maternal, Fetal, and Placental Weights in Control and Genistein-Treated Pregnant Rhesus Monkeys

Variable	Control	Genistein[a]
MWT 1[b]	6.83 ± 0.83[f]	6.52 ± 0.37
MWT 2[c]	8.33 ± 0.91	8.03 ± 0.47
FWT[d]	500.58 ± 17.34	501.34 ± 9.47
PWT[e]	155.46 ± 8.39	157 ± 8.51

[a] Received genistein orally 5 days each week, 8 mg/kg.
[b] MWT 1 = Initial maternal weight.
[c] MWT 2 = Maternal weight at delivery.
[d] FWT = Fetal weight at delivery.
[e] PWT = Placental weight at delivery.
[f] kg, Mean ± SEM.

Table II. Terminal Progesterone Levels in Control and Genistein-Treated Pregnant Rhesus Monkeys and their Fetuses

Serum Source[a]	Control	Genistein[b]
Maternal	5.25 ± 0.81[c]	6.57 ± 1.14
Fetal	8.41 ± 1.58	18.59 ± 8.74
UV[d]	17.04 ± 3.22	30.45 ± 8.37
UOV[e]	23.10 ± 4.54	26.64 ± 7.29

[a] Maternal and fetal samples collected at time of C-section, 155 days gestation.
[b] Genistein administered 5 days each week, 8 mg/kg.
[c] ng/ml, mean ± SEM.
[d] UV = uterine veins.
[e] UOV = uterine-ovarian veins.

Table III. Terminal Estradiol Levels in Control and Genistein-Treated Pregnant Rhesus Monkeys and their Fetuses

Serum Sources[a]	Control	Genistein[b]	Probability
Maternal	212.9 ± 33.01[c]	335.8 ± 32.57	0.04
Fetal	204.8 ± 35.19	358.3 ± 80.12	0.04
UV[d]	529.4 ± 21.08	1107.2 ± 54.73	0.02
UOV[e]	522.9 ± 19.00	915.9 ± 42.03	0.02

[a] Maternal and fetal samples collected at time of C-section, 155 days gestation.
[b] Genistein administered 5 days each week, 8 mg/kg.
[c] pg/ml, mean ± SE.
[d] *UV* = uterine veins.
[e] UOV = uterine-ovarian veins.

Estradiol levels in the maternal peripheral blood were higher in the genistein-treated group compared to the control by 82 days of gestation (see Table IV). At the time of the C-sections, the estradiol levels were significantly higher ($P = 0.039$) in the genistein-treated group. Estrone levels were not significantly different between the control and genistein-treated mothers (Table IV); however there was a trend for the estrone levels in the genistein group to increase more between Day 82 of pregnancy and the time of C-section (390.2%) compared to the increase in the control group of 258.3% ($P = 0.0574$).

DHEA-SO_4 levels were not significantly different between the two groups although the maternal levels and the rate of increase in the genistein group did tend to be higher than the control group (see Table V).

Quantitative RT-PCR indicated no differences in LDL receptor mRNA in either raw villous tissue or in purified syncytiotrophoblast cells between the two groups (control, 0.990 ± 0.084; genistein, 1.055 ± 0.169, mean ± SEM, attomoles/μg total RNA). The mean concentrations of LDL and HDL in maternal sera and in the fetal sera (Table VI) were not significantly different between the control group and the genistein-treated group; however, the mean values

Table IV. Estradiol and Estrone Levels in Control and Genistein-Treated Pregnant Rhesus Monkeys at Gestational Age 82 and 155 Days

Group	Estradiol			Estrone		
	GA 82[a]	GA 155[b]	% Increase[c]	GA 82[a]	GA 155[b]	% Increase[c]
Control	245.03[d] ± 75.53	212.90 ± 66.02	99.40 ± 60.7	169.99 ± 22.3	430.74 ± 22.33	258.3[e] ± 84.6
Genistein	318.22 ± 104.86	335.80[f] ± 65.13	112.2 ± 29.8	132.14 ± 47.32	513.18 ± 243.59	390.2[e,g] ± 114.0

[a] Gestational age, 82 days.
[b] Gestational age, 155 days.
[c] Hormone values at 155 days/values at 82 days × 100.
[d] pg/ml, SD.
[e] Compared to estradiol, same group, $P < 0.006$.
[f] Compared to control, $P < 0.04$.
[g] Compared to control, $P = 0.0573$.

Table V. DHEA-SO_4 Levels in Control and Genistein-Treated Pregnant Rhesus Monkeys at Ages 82 and 155 Days of Gestation

Group	82 Days Gestation	155 Days Gestation	% Increase
Control	16.54 ± 3.47[a]	21.28 ± 3.90	132.0 ± 29.3[b]
Genistein	21.86 ± 4.47	34.92 ± 7.45	167.4 ± 3.7
Probability[c]	0.195	0.059	0.054

[a] Mean ± SEM, μg/dl.
[b] Values at 155 days/values at 82 days × 100.
[c] *P* values from Student's *t* test.

from the genistein-treated monkeys tended to be greater in both the maternal and fetal sera.

Discussion

Although not significant, the increase in estrone in the genistein-treated mothers compared to the control mothers may suggest a mechanism responsible for the increased estradiol levels found in this study. Genistein may stimulate the deconjugation of estrone in the gut that would allow for reabsorption of the estrone into the peripheral circulation and the conversion of estrone to estradiol (7). More importantly, since estradiol produced in the placenta is preferentially secreted toward the maternal compartment as a fetal protective mechanism (20), the elevated estradiol levels in the blood of fetuses from the genistein-treated monkeys suggest that the genistein has crossed the placental barrier. This finding may be of great physiological relevance to fetuses exposed to high levels of phytoestrogens in early gestation, as there may be a potential for induced feminization during early embryonic development due to the genistein-induced increase in estradiol levels. The present study does not investigate this potential directly. If genistein does cross the placental barrier and does bind to estrogen receptors, then the elevated fetal estradiol levels may be necessary for the endogenous estradiol to competitively bind to these receptors. If exogenous estrogens, either synthetic or from plant sources, are present in the circulation they may bind to the intracellular estrogen receptors more rapidly than endogenous estrogens because they are not bound as strongly to plasma proteins (29, 30). Nagel *et al.* (29) reported that because of this difference in binding affinity to plasma proteins, genistein biological activity relative to estradiol would be 10 times greater in serum than would be estimated in serum-free assays. They suggested that if genistein reached the fetus, the biological bioactivity might be greater than in the *in vitro* assays. Wang *et al.* (31) showed that 1) genistein competed with estradiol binding to the estrogen receptor; 2) genistein, although estrogenic, can interfere with the effects of estradiol; and 3) prolonged exposure to genistein resulted in a decrease in estrogen receptor mRNA, as well as a decreased response to stimulation by estradiol. Increased production of estradiol by the fetus may be necessary to maintain the required estrogenic responses.

In the present study, LDL- and HDL-cholesterol levels did not appear to change significantly as a result of genistein treatment. Divergently, in a previous study, female rhesus monkeys fed a high-isoflavone diet exhibited lower total plasma cholesterol and lower LDL-cholesterol than those fed a low-isoflavone diet (32). In surgically induced postmenopausal cynomolgus monkeys fed diets containing either CEE or an SBE with normal levels of genistein and daidzein (33), the same beneficial effects on plasma lipids and lipoproteins were manifested. Further, the SBE diet did not result in hypertriglyceridema and did cause an increase in apolipoprotein A-1 as compared to the CEE diet. A similar study (34) reported that the SBE effects on coronary artery dilation were equal to that of CEE and superior to CEE plus a progestin, the combination commonly used in postmenopausal hormone replacement. Indeed, the isoflavones in soy protein improved cardiovascular risk factors without apparent deleterious effects on the reproductive system of peripubertal rhesus monkeys (35). It may be significant that these studies used soybean extracts or soy-based diets to administer the phytoestrogens to nonhuman primates. The mixture of phytoestrogens in such a diet may cause either synergistic or attenuated responses. Until the present study there were no previous reports on the effects of a purified single dietary phytoestrogen on the primate maternal-fetoplacental unit. In this regard, estrogen has been reported to upregulate placental progesterone biosynthesis in the nonhuman primate *via* enhancement of LDL receptors in syncytiotrophoblast cells (16, 17); however,

Table VI. LDL and HDL Levels in Maternal and Fetal Sera from Pregnant Rhesus Monkeys Receiving Dietary Supplemental Genistein

Group	Maternal		Fetal	
	LDL[a]	HDL[b]	LDL	HDL
Control	29.80 ± 10.10[c]	40.02 ± 3.69	34.92 ± 7.37	34.62 ± 3.36
Genistein[d]	35.34 ± 3.39	46.80 ± 4.96	38.32 ± 6.78	36.34 ± 3.11

[a] LDL = low density lipoprotein.
[b] HDL = high density lipoprotein.
[c] mg/dl, mean ± SEM.
[d] Received genistein, 8 mg/kg, 5 days each week.

genistein administered in the present study did not elicit any effect on production of progesterone or placental LDL receptor mRNA. This lack of response may have resulted from either an inadequate dosage or specificity of the purified phytoestrogen. A permissive, rather than a dose-dependent regulation of the LDL receptor by endogenous estrogen may also have masked any potential effects.

Future investigations, employing more experimental animals than the present study ($n = 5$) may reveal that the trends noted in some of the parameters studied are significant and may define the mechanism by which the purified phytoestrogen, genistein, elicits the increase in the maternal and fetal estradiol levels. These future studies will also investigate the concentration of phytoestrogen in the maternal circulation and in the neonate.

1. Price KR, Fenwick GR. Naturallay occurring oestrogens in foods: A review. Food Addit Contam **2:**73–106, 1985.
2. Shemesh M, Lindner HR, Ayalon N. Affinity of rabbit uterine oestradiol receptor for phyto-oestrogens and its use in a competitive protein-binding radioassay for plant coumestrol. J Reprod Fertil **29:**1–9, 1972.
3. Sheehan DM. The case for expanded phytoestrogen research. Proc Soc Exp Biol Med **208:**3–5, 1995.
4. Setchell KD, Adlercreutz H. Mammalian lignans and phyto-oestrogens: Recent studies on their formation, metabolism, and biological role in health and disease. In: Rowland IR, Ed. Role of the Gut Flora in Toxicity and Cancer. New York: Academic Press, pp315–345, 1988.
5. Miksicek RJ. Estrogenic flavonoids: Structural requirements for biological activity. Proc Soc Exp Biol Med **208:**44–50, 1995.
6. Adlercreutz H, Honjo H, Higashi A, Fotsis T, Hämäläinen E, Hasegawa T, Okada H. Urinary excretion of ligans and isoflavoid phytoestrogens in Japanese men and women consuming a traditional Japanese diet. Am J Clin Nutr **54:**1093–1100, 1991.
7. Adlercreutz H. Evolution, nutrition, intestinal microflora, and prevention of cancer: A hypothesis. Proc Soc Exp Biol Med **217:**241–246, 1998.
8. Lamartiniere CA, Murrill WB, Manzolillo PA, Zhang J-Z, Barnes S, Zhang X, Wei H, Brown NM. Genistein alters the ontogeny of mammary gland development and protects against chemically induced mammary cancer in rats. Proc Soc Exp Biol Med **217:**358–364, 1998.
9. Levy JR, Faber KA, Ayyash L, Hughes CL Jr. The effect of prenatal exposure to the phytoestrogen genistein on sexual differentiation in rats. Proc Soc Exp Biol Med **208:**60–66, 1995.
10. Lamartiniere CA, Moore J, Holland M, Barnes S. Neonatal genistein chemoprevents mammary cancer. Proc Soc Exp Biol Med **208:**120–123, 1995.
11. Tansey G, Hughes CL Jr, Cline JM, Krümmer A, Walmer DK, Scholtzer S. Effects of dietary soybean estrogens on the reproductive tract in female rats. Proc Soc Exp Biol Med **217:**340–344, 1998.
12. Irvine CH, Fitzpatrick MG, Alenander SL. Phytoestrogens in soy-base infant foods: Concentrations, daily intake, and possible biological effects. Proc Soc Exp Biol Med **217:**247–253, 1998.
13. Cassidy A, Bingham S, Setchell KD. Biological effects of soy proteins rich in isoflavones on the menstrual cycle of premenopausal women. Am J Clin Nutr **60:**333–340, 1994.
14. Shepard TH, Pyne GE, Kirchvink JF, McLean M. Soybean goiter: Report of three cases. New Engl J Med **262:**1099–1103, 1960.
15. Fort P, Moses M, Goldberg T, Lifschitz F. Breast and soy-formula feeding in early infancy and the prevalence of autoimmune thyroid disease in children. J Am Coll Nutr **9:**164–167, 1990.
16. Albrecht ED, Henson MC, Pepe GJ. Regulation of placental low-density lipoprotein uptake in baboons by estrogen. Endocrinology **128:**450–458, 1991.
17. Henson MC, Pepe GJ, Albrecht ED. Developmental increase in placental low-density lipoprotein uptake during baboon pregnancy. Endocrinology **130:**1698–1706, 1992.
18. Davison-Fairburn B, Blanchard J, Hu F-S, Martin L, Harrison RM, Ratterree M, Murphey-Corb M. Experimental infection of timed-pregnant rhesus monkeys with simian immunodeficiency virus (SIV) during early, middle, and late gestation. J Med Primatol **19:**381–393, 1990.
19. Harrison RM. Exogenous progesterone recycling and early detection of pregnancy in rhesus monkeys. J Med Primatol **20:**323–324, 1991.
20. Walsh SW. Regulation of progesterone and estrogen production during rhesus monkey pregnancy. In: Albrecht, ED, Pepe GJ, Eds. Perinatal Endocrinology. Ithaca, NY: Perinatology Press, pp219–241, 1985.
21. Adlercreutz H, Goldin BR, Gorbach SL, Höckertedt KAV, Watanane S, Hämäläinen EK, Markkanen MH, Mäkelä TH, Wähälä KT, Adlercreutz CH. Soybean phytoestrogen intake and cancer risk. J Nutr **125:**757S–770S, 1995.
22. Henson MC, Greene SJ, Reggio BC, Shi W, Swan KF. Effects of reduced maternal lipoprotein-cholesterol availability on placental progesterone biosynthesis in the baboon. Endocrinology **138:**1385–1391, 1997.
23. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocynate-phenol-chloroform extraction. Anal Biochem **162:**156–159, 1997.
24. Chirgwin JM, Przybla AE, MacDonald RJ, Rutter WJ. Isolation of biologically active ribonucleic acid from sources rich in ribonuclease. Biochemistry **18:**5294–5299, 1979.
25. Henson MC, Swan KF, O'Neil JS. Expression of placental leptin and leptin receptor transcripts in early pregnancy and term. Obstet Gynecol **92:**1020–1028, 1998.
26. Yammamoto T, Davis CG, Brown MS, Schneider WJ, Casey ML, Goldstein JL, Russell DW. The human LDL receptor: A cysteine-rich protein with multiple Alu sequences in its mRNA. Cell **39:**27–38, 1979.
27. Siebert PD, Larrick JW. PCR MIMICS: Competitive PCR fragments

for use as internal standards in quantitative PCR. Biotechniques **14:**244–249, 1993.
28. Noma A, Okabe H, Netsu-Nakayama K, Ueno Y, Shinohara H. Improved method for simultaneous determination of cholesterol in high- and low-density lipoproteins. Clin Chem **25:**1480–1481, 1979.
29. Nagel SC, vom Saal FS, Welshons WV. The effective free fraction of estradiol and xenoestrogens in human serum measured by whole cell uptake assays: Physiology of delivery modifies estrogenic activity. Proc Soc Exp Biol Med **217:**300–309, 1998.
30. McLachlan JA, Arnold SF. Environmental estrogens. Amer Sci **84:**452–461, 1996.
31. Wang TT, Sathyamoorthy N, Phang JM. Molecular effects of genistein on estrogen receptor–mediated pathways. Carcinogenesis **17:**271–275, 1996.
32. Honoré EK, Williams JK, Anthony MS, Clarkson TB. Soy isoflavone enhances coronary vascular reactivity in atherosclerotic female macaques. Fertil Steril **67:**148–154, 1997.
33. Clarkson TB, Anthony MS, Williams JK, Honoré EK, Cline JM. The potential of soybean phytoestrogens for postmenopausal hormone replacement therapy. Proc Soc Exp Biol Med **217:**365–368, 1998.
34. Williams JK, Honoré EK, Washburn SA, Clarkson TB. Effects of hormone replacement therapy on reactivity of atherosclerotic coronary arteries in cynomolgus monkeys. J Am Coll Cardiol **24:**1757–1761, 1994.
35. Anthony MS, Clarkson TB, Hughes CL Jr, Morgan TM, Burke GL. Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys. J Nutr **126:**43–50, 1996.