Flaxseed and Its Lignan Precursor, Secoisolariciresinol Diglycoside, Affect Pregnancy Outcome and Reproductive Development in Rats[1,2,3]

Janet C. L. Tou, Jianmin Chen and Lilian U. Thompson[4]

Department of Nutritional Sciences, University of Toronto, Toronto, ON, Canada M5S 3E2

ABSTRACT Flaxseed is the richest source of the mammalian lignan precursor secoisolariciresinol diglycoside (SDG). Because lignans have estrogen agonist or antagonist properties, the objective of this study was to determine whether feeding flaxseed to rats during a hormone-sensitive period has reproductive effects. Rat dams were fed a basal diet or the basal diet supplemented with 10% flaxseed, 5% flaxseed or SDG at the level in 5% flaxseed during pregnancy and lactation. At weaning, the offspring were fed the basal diet. Flaxseed had no effect on pregnancy outcome except that the 10% flaxseed diet lowered birth weight ($P < 0.05$), compared with other treatments, and produced hormonal effects. The female offspring had shortened anogenital distance, greater uterine and ovarian relative weights, earlier age and lighter body weight at puberty, lengthened estrous cycle and persistent estrus ($P < 0.05$), whereas the males had reduced postnatal weight gain and, at postnatal d 132, greater sex gland and prostate relative weights ($P < 0.05$), suggesting estrogenic effects. In contrast, compared with the basal diet, 5% flaxseed reduced immature ovarian relative weight by 29% ($P < 0.05$), delayed puberty by ~5 d ($P < 0.05$) and tended to lengthen diestrus, indicating an antiestrogenic effect. The SDG produced results similar to those of 5% flaxseed, suggesting that lignans were responsible for the observed effects. Lignans were transferred to the offspring via rat dam's milk as indicated by the recovery of radioactivity in the offspring of lactating dams given ^{3}H-SDG. Because flaxseed affects the reproductive development of offspring, caution is suggested when consuming flaxseed during pregnancy and lactation. J. Nutr. 128: 1861–1868, 1998.

KEY WORDS: • *flaxseed* • *lignans* • *secoisolariciresinol diglycoside* • *rats* • *reproduction*

Phytoestrogens such as lignans and isoflavones, which act as either estrogen agonists or antagonists (Bandbury and White 1954, Farnsworth et al. 1975), have generated interest because of their potential use in hormone replacement therapy and cancer prevention. Mammalian lignans are produced by the action of gut microflora on precursors such as the plant lignan secoisolariciresinol diglycoside (SDG).[5] Flaxseed, the richest known source of SDG (Thompson et al. 1991 and 1997), has been observed to decrease the mammary tumor incidence, number and size in carcinogen-treated rats (Serraino and Thompson 1992, Thompson et al. 1996a and 1996b). This was attributed in part to the antiestrogenic properties of mammalian lignans produced from SDG in flaxseed (Orcheson et al. 1998). However, along with health benefits, flaxseed and its lignans may also have adverse effects because phytoestrogens have been reported to produce infertility and hyperestrogenization in a number of species (Price and Fenwick 1985, Rickard and Thompson 1997).

Dietary substances capable of altering hormone levels are of concern during pregnancy, a hormone-sensitive period for both the mother and offspring. During pregnancy, high estrogen levels are needed by the mother to establish and maintain pregnancy by stimulating uterine changes to allow implantation, enhancing uterine growth to accommodate a growing fetus and by acting as a trigger for parturition (Pasqualini et al. 1985). On the other hand, administration of estrogens to pregnant animals has been found to cause detrimental effects on pregnancy. These include decreased pregnancy weight gain and food intake, which result in increased resorption or abortion, prolonged gestation length and labor, leading to increased stillbirth incidence, decreased litter size and decreased birth weight, with the end result of decreased pup survival (Zimmerman et al. 1991).

Pregnancy is also a hormone-sensitive period for the reproductive development of the fetus. Sexual differentiation of the genitalia and central nervous system (CNS) into either male or female develops under the influence of hormones. In rats, critical sexual differentiation occurs from gestation d 18 and continues to postnatal day (PND) 10 (Manson and Kang 1989). Normally, the high maternal estrogen levels during pregnancy are prevented from exerting hormone toxicity on the fetus by both sex hormone-binding globulin (SHBG) and α-fetoprotein (AFP) in humans and by AFP in rats. By bind-

[1] Presented in part at Experimental Biology 97, April 6–9, 1997, New Orleans, LA [Tou, J.C.L. & Thompson, L.U. (1997) The effect of flaxseed on reproductive development and function. FASEB J. 11: A420 (abs.)].

[2] Support was provided by the Flax Council of Canada and the Natural Sciences and Engineering Research Council of Canada.

[3] The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

[4] To whom correspondence and reprint requests should be addressed.

[5] Abbreviations used: AA, arachidonic acid; AFP, α-fetoprotein; AGD, anogenital distance; ALA, α-linolenic acid; CNS, central nervous system; EPA, eicosapentanoic acid; LA, linoleic acid; PG, prostaglandin; PND, postnatal day, SDG, secoisolariciresinol diglycoside; SHBG, sex hormone binding globulin.

0022-3166/98 $3.00
Manuscript received 17 December 1997. Initial review completed 23 January 1998. Revision accepted 2 July 1998.

ing estrogen, these proteins prevent the free estrogen from interacting with receptors to produce hormonal effects. Those estrogens not extensively bound to AFP tend to be potent estrogenic toxins, capable of disrupting normal reproduction in the rat (Pasqualini et al. 1985). Whitten et al. (1993 and 1995) reported that the phytoestrogen coumestrol could be transferred to offspring postnatally via rat dam's milk and affect offspring reproduction at adolescence and later life. Reproductive abnormalities include altered puberty onset, disrupted estrous cycles and decreased fertility in female offspring, and decreased sexual behavior in male offspring. Environmental estrogens to which humans are most likely exposed include phytoestrogens in foods; there are trends toward their increased consumption as a result of their reported health benefits (Haumann 1997). The objectives of this study were to determine the following: *1*) the effect of feeding pregnant rats 5 or 10% flaxseed or its lignan precursor SDG at a level equivalent to that in 5% flaxseed on rat dam's pregnancy and pregnancy outcome; *2*) whether SDG can be transferred from mother to offspring via the milk to produce reproductive effects on the offspring; and *3*) the long-term reproductive effects and health implications of exposing offspring to flaxseed during a period when hormone-dependent development is occurring. The 5 and 10% flaxseed levels were chosen for this study because they have been shown previously to be colon and/or mammary cancer protective in rats (Jenab and Thompson 1996, Serraino and Thompson 1991 and 1992, Thompson et al. 1996a and 1996b).

MATERIALS AND METHODS

Chemicals. The SDG in flaxseed (Linott variety; Omega Products, Melfort, SK) was isolated and purified using a modification of the Klosterman method (Bakke and Klosterman 1956, Klosterman and Smith 1954) as described by Rickard et al. (1996). The benzyl methylenes of purified SDG were labeled with tritium by Amersham International (Little Chalfont, Buckinghamshire, UK) using a gas exchange method, resulting in a specific radioactivity of 999 GBq/mmol and radiochemical purity of >98.5%.

Animals. Twenty-eight female and seven male Sprague-Dawley rats (77 d old; Charles River Canada, Montreal, Canada) were individually housed in polycarbonate cages in 22–24°C rooms with 50% humidity and a 12 h light:dark cycle. Animals were acclimated for 7 d during which they were given free access to water and fed a phytoestrogen-free basal diet. At the end of acclimation, males (353.71 ± 21.15 g) and females (249.82 ± 13.34 g) were mated. The female rat, determined to be in estrus from microscopic examination of vaginal smears, was placed with the male rat in a stainless steel cage with a wire-mesh floor. One male was mated with one female from each diet group. Successful mating was determined by the presence of copulation plugs in the cage's collection tray and confirmed by microscopic observation of sperm in vaginal smears. Pregnant female rats were individually housed in polycarbonate cages. Animal care and use conformed to the published guidelines (Canadian Council on Animal Care, 1984), and the experimental protocol was approved by the University of Toronto Animal Care Committee.

Diets. Pregnant rat dams were randomly assigned to the basal diet or basal diet supplemented with either 10% flaxseed, 5% flaxseed or a daily gavage of 1.5 mg SDG dissolved in 1 mL distilled water. The SDG level was estimated to be approximately equivalent to that given to rats in the 5% flaxseed diets on the basis of a SDG concentration of 2.93 μmol/g flaxseed according to HPLC analysis (Thompson et al. 1996a) and a diet intake of about 15 g/d. Rats not gavaged with SDG were gavaged daily with 1 mL saline. The diet composition was based on the AIN-93 G diet (Reeves et al. 1993). The basal diet supplemented with flaxseed was corrected for protein, fat and fiber contributed by flaxseed so that the energy value of the diets was the same (**Table 1**). All ingredients were from Dyets, (Bethlehem, PA). Diets were stored at 4°C and fresh diets were provided to the rats every 2 d.

TABLE 1

Composition of the diets

Ingredient	Basal[1]	5% flaxseed[2]	10% flaxseed[2]
		g/kg diet	
Cornstarch	397.48	394.40	391.20
Casein	200.00	188.80	177.60
Soybean oil (TBHQ)	70.02	51.70	33.50
Dextrose	132.00	132.00	132.00
Sucrose	100.00	100.00	100.00
Cellulose	50.00	32.60	15.20
Mineral mix 93G AIN	35.00	35.00	35.00
Vitamin mix 93G AIN	10.00	10.00	10.00
L-Cystine	3.00	3.00	3.00
Choline bitartrate	2.50	2.50	2.50
Flaxseed	—	50.00	100.00

[1] Semipurified AIN-93 G (Reeves et al. 1993).
[2] Modified semipurified AIN-93G.

Experimental design. Pregnant rat dams (7 per diet group) were given free access to their assigned diets, and pregnancy progression was monitored by measuring daily maternal weight gain and food intake throughout gestation. Parturition was classified as difficult if delivery exceeded 2 h, blood was present after delivery and rat dams failed to clean pups (Barrow 1990). Pregnancy outcomes measured were litter size, birth weight and stillbirth incidence, calculated as live birth index (a ratio of number of viable pups born/total number of pups born × 100) postnatal pup survival (the ratio of number of viable pups at PND 21/total number of pups born × 100), and percentage of females (the number of female pups/total number of pups born × 100). After birth, rat dams continued to consume their respective diets. Maternal weight, food intake and offspring weight were measured every other day during the lactation period. At the end of lactation, i.e., PND 21, all rat dams and two offspring (one male and one female) per litter were killed by CO_2 inhalation. The uterus and ovaries of female offspring, the testes and sex glands of the male offspring and all of the major organs of offspring were removed, blotted dry and weighed. Organ weights were expressed as relative weight, i.e., wet organ weight/body weight. All remaining offspring were weaned and fed the basal diet so that offspring were exposed to flaxseed or SDG only during the rat dam's pregnancy and lactation. At PND 50, and again at PND 132, a subgroup of male offspring (n = 6–10) and female offspring (n = 6–10) were killed by CO_2 inhalation to determine the effect of early flaxseed and SDG exposure on reproductive and major organs at puberty and adulthood. As on PND 21, each subgroup consisted of 2–3 offspring (1–2 of each sex) per litter from each of the dams.

Lignan transfer to offspring. To determine whether mammalian lignans from the rat dams could be transferred to the offspring through the milk, on PND 20, lactating rat dams (n = 3) from the SDG-treated group were gavaged with ^{3}H-SDG (3.7 kBq/g body weight) in 1 mL distilled water. A control group of lactating rat dams from the SDG-treated group (n = 3) were gavaged with 1.5 mg unlabeled SDG in 1 mL distilled water. Two offspring (one male and one female) per litter remained with their respective dam. After 24 h, the rat dams and offspring were killed by CO_2 inhalation. Trunk blood was collected and all tissues and gastrointestinal contents were removed, weighed and stored frozen at −20°C. Blood and tissue radioactivity were measured as described in detail by Rickard and Thompson (1998). Briefly, 0.5 mL of a 9:1 (v/v) solution of hyamine hydroxide (ICN Biomedicals, Aurora, Canada) in distilled water was added to the tissues and incubated in a 60°C shaking water bath. For blood samples, 300 μL hyamine hydroxide in ethanol (1:2, v/v) was added and samples incubated in a 60°C shaking water bath for 1 h. Corrections for chemical and color quenching of samples were done automatically by the LKB Wallac 1217 Rackbeta liquid scintillation counter (Fisher Scientific, Ottawa, Canada) by using the sample channels ratio technique. The ratio of counts for the sample in each

TABLE 2

The effect of exposure during pregnancy and lactation to flaxseed or secoisolariciresinol diglycoside (SDG) on maternal reproductive organ relative weights in rats[1]

Dietary treatment[2]	Uterus[3]	Ovaries[3]
	g/100 g body wt	
Basal diet	127.86 ± 8.93[a]	42.83 ± 3.18[a]
5% Flaxseed	152.64 ± 6.49[ab]	43.59 ± 4.71[ab]
SDG	155.80 ± 10.15[ab]	51.35 ± 5.27[ab]
10% Flaxseed	176.98 ± 15.40[b]	61.69 ± 5.13[b]

[1] Data are means ± SEM ($n = 7$). Different letters indicate significant differences ($P < 0.05$) by one-way ANOVA followed by Tukey's test.

[2] Pregnant rat dams were randomly assigned to basal diet, basal diet supplemented with 10% flaxseed, 5% flaxseed or a daily gavage of 1.5 mg SDG in distilled water. Assigned diets were fed throughout 22 d of gestation and 21 d of lactation.

[3] At the end of lactation (postnatal d 21), rat dams were killed and uterine and ovarian weights measured.

channel, i.e., section of the energy spectrum for tritium, varies linearly with efficiency for quenching solutions (Evans 1974). Extensive color quenching of samples was avoided by bleaching colored tissue samples with 100 μL of 9.7 mol/L hydrogen peroxide and blood samples with 0.5 μL of 9.7 mol/L hydrogen peroxide and then incubating the samples for another 30 min. After cooling, 15 mL of the cocktail scintillator Cytoscint ES (ICN Biomedicals) and 0.5 mL of 0.5 mol/L acetic acid were added. Tissue samples were placed in the dark for 2 h; blood samples were placed in the dark for 4 d to equilibrate before counting in the liquid scintillation counter. To determine radioactivity in gastrointestinal contents, samples were homogenized in 30 mL distilled water using a Polytron homogenizer (Brinkman Instruments Canada, Mississauga, Canada). Aliquots (100 μL) were added to 1 mL Cytoscint ES and incubated in a 60°C shaking water bath for 2 h. After cooling, 15 mL of Cytoscint ES and 0.5 mL 0.5 mol/L acetic acid were added. Gastrointestinal samples were counted after being in the dark for 4 d to eliminate chemiluminescence from fecal porphyrins. Radioactivity values were adjusted for background radioactivity and counting efficiency. Total body radioactivity was calculated as the sum of tissue radioactivity × sample tissue weight/aliquot weight.

Growth and anogenital distance (AGD). Offspring weight gain was measured every other day. Immature male rats were distinguished from females by their longer AGD, the distance from the papilla to the anus. Hormone imbalance in male or female offspring due to in utero diet treatment was determined from the difference in the AGD at PND 3 when compared with rats receiving the basal diet (controls). Dietary effect on pup genitalia during lactation was determined from the difference in AGD at PND 21 and at PND 3. AGD measurements were also adjusted for body size by calculating relative AGD, i.e., (AGD at PND 21 - AGD at PND 3)/body weight gain from PND 3 to 21.

Puberty onset and estrous cycle. In female rats, the age and weight at puberty onset as indicated by visible opening of the vaginal aperture was determined. Vaginal smears were taken on PND 40–50 and PND 100–132 and examined microscopically to determine estrous cycling. The estrous phase of animals was classified as follows: *1*) proestrus (mainly epithelial cells present); *2*) estrus (mainly cornified cells present); *3*) metestrus (cornified and leukocytes present in large numbers); or *4*) diestrus (mainly leukocytes present). Rat estrous cycle length was determined as the number of days required to complete the four phases of proestrus, estrus, metestrus and diestrus. Rats were considered acyclic if they remained in one phase for >10 d.

Histological examination of the prostate. The prostates of male rats killed at PND 132 were fixed in 10% buffered formalin, processed routinely and embedded in paraffin. Paraffin sections of 5 μm thickness were serially cut at 200-μm intervals. After deparaffinization in toluene and rehydration in a graded series of ethyl alcohol, the sections were stained with hematoxylin and eosin and then examined under a light microscope.

Statistical analysis. All statistical analyses were done using SigmaStat Version 2.0 by Jandel Scientific (San Rafael, CA). Differences among diet treatment groups within the same age and gender group were analyzed by one-way ANOVA. Two-way ANOVA was used to determine the differences in weight due to diet treatment or gender, the differences in AGD or relative AGD due to diet treatment or gender, the difference in female relative sex organ weights (i.e., uterine and ovarian weights) due to diet treatment or age and the differences in male relative sex organ weights (i.e., testes and accessory sex glands) due to diet treatment or age. Data not normally distributed were analyzed nonparametrically using Kruskal-Wallis one-way ANOVA on ranks. Post-hoc multiple comparison tests included Tukey's test (parametric) or Dunn's test (nonparametric). Differences were considered significant at $P < 0.05$. Results are expressed as means ± SEM.

RESULTS

Pregnancy outcomes. Compared with the basal diet (control), the 5% flaxseed, SDG at the level found in 5% flaxseed or 10% flaxseed diet fed to pregnant rat dams throughout gestation produced no significant differences in maternal food intake, weight gain, gestation length or parturition and pregnancy outcomes such as litter size, live birth index, postnatal pup survival and number of female vs. male offspring. An exception was the 10% flaxseed diet, which caused significantly lowered ($P < 0.05$) mean birth weight (4.73 ± 0.07 g) compared with the basal diet (5.14 ± 0.10 g), 5% flaxseed (5.06 ± 0.08 g) and 1.5 mg SDG (5.24 ± 0.07 g). All rat dams killed at the end of lactation (PND 21) showed no organ weight abnormalities, except for the significantly ($P < 0.05$) larger relative uterine and ovarian weights in those fed 10% flaxseed compared with the basal diet (**Table 2**).

Lignan transfer via milk. The mean total body radioactivity (995.98 ± 201.61 Bq) in nursing male and female offspring

TABLE 3

The effect of exposure during pregnancy to flaxseed or secoisolariciresinol diglycoside (SDG) on body weight, anogenital distance (AGD) and relative AGD in rats at postnatal day 3[1]

Treatment[2]	*n*	Body weight	AGD	Relative AGD[3]
		g	*mm*	*mm/g*
Females				
Basal diet	54	6.61 ± 0.17	3.0 ± 0.1[b]	0.46 ± 0.01[b]
5% Flaxseed	54	6.55 ± 0.17	3.1 ± 0.1[b]	0.48 ± 0.02[b]
SDG	44	6.75 ± 0.20	3.2 ± 0.1[b]	0.49 ± 0.02[b]
10% Flaxseed	58	6.23 ± 0.14	2.3 ± 0.1[a]	0.39 ± 0.02[a]
Males				
Basal diet	42	6.50 ± 0.10[b]	5.0 ± 0.1[b]	0.78 ± 0.02
5% Flaxseed	43	6.80 ± 0.15[b]	5.0 ± 0.1[b]	0.75 ± 0.02
SDG	45	6.95 ± 0.15[b]	5.1 ± 0.1[b]	0.74 ± 0.01
10% Flaxseed	47	5.84 ± 0.12[a]	4.2 ± 0.1[a]	0.73 ± 0.02

[1] Data are means ± SEM. Different letters indicate significant differences ($P < 0.05$) within the same gender by one-way ANOVA followed by Dunn's test (nonparametric). Two-way ANOVA showed diet treatment ($P < 0.001$) and gender effects ($P < 0.001$) on AGD and relative AGD but insignificant treatment and gender interaction.

[2] Pregnant rat dams were randomly assigned to basal diet, basal diet supplemented with 10% flaxseed, 5% flaxseed or a daily gavage of 1.5 mg SDG in distilled water.

[3] Relative AGD was calculated as anogenital distance/body weight.

TABLE 4

The effect of exposure during pregnancy and lactation to flaxseed or secoisolariciresinol diglycoside (SDG) on weight gain, change in anogenital distance (ΔAGD) and relative ΔAGD in rats[1]

Treatment	*n*	Weight gain[2]	ΔAGD[3]	Relative ΔAGD[4]
		g	*mm*	*mm/g*
Females				
Basal diet	54	35.55 ± 0.94	6.8 ± 0.1[b]	0.20 ± 0.01[b]
5% Flaxseed	54	33.96 ± 0.70	7.5 ± 0.2[b]	0.23 ± 0.01[c]
SDG	44	34.16 ± 0.69	7.5 ± 0.2[b]	0.23 ± 0.01[c]
10% Flaxseed	58	33.07 ± 0.86	5.4 ± 0.2[a]	0.17 ± 0.01[a]
Males				
Basal diet	42	37.85 ± 0.96[b]	10.6 ± 0.3[b]	0.28 ± 0.01
5% Flaxseed	43	35.90 ± 1.06[b]	10.8 ± 0.4[b]	0.31 ± 0.02
SDG	45	37.22 ± 1.43[b]	11.2 ± 0.4[b]	0.34 ± 0.02
10% Flaxseed	47	31.31 ± 1.02[a]	9.0 ± 0.2[a]	0.30 ± 0.01

[1] Data are means ± SEM. Different letters indicate significant differences ($P < 0.05$) within the same gender by one-way ANOVA followed by Dunn's test (nonparametric). Two-way ANOVA showed diet treatment ($P < 0.001$) and gender effects ($P < 0.001$) of AGD and relative AGD but insignificant treatment and gender interaction.

[2] Lactation period weight gain was determined as weight at postnatal d 21 − postnatal d 3.

[3] Anogenital distance was calculated as anogenital distance at postnatal d 21 − postnatal d 3.

[4] Relative AGD was calculated as anogenital distance (postnatal d 21 − postnatal d 3)/body weight gain (postnatal d 21 − postnatal d 3).

of rat dams gavaged with ^{3}H-SDG in distilled water was significantly higher ($P < 0.05$) than that in the nursing offspring of control rat dams gavaged with unlabeled SDG (34.56 ± 14.65 Bq), which in turn was not significantly different than the value obtained for the distilled water controls (32.56 ± 8.23 Bq).

Body weight and anogenital distance. The body weight, AGD and relative AGD measured at PND 3 were affected by in utero exposure to flaxseed (**Table 3**). Female offspring had shorter AGD compared with male offspring, and in utero, 10% flaxseed exposure caused shortening ($P < 0.05$) of the AGD in both sexes. Only the male offspring treated with 10% flaxseed in utero had significantly ($P < 0.05$) lower body weights than those in the basal diet, 5% flaxseed and 1.5 mg SDG diet groups. Body weight was correlated to AGD ($r = 0.85$, $P < 0.01$); thus, when calculated as relative AGD (AGD/body weight), the effect of 10% flaxseed on AGD persisted only in the female offspring. A two-way ANOVA showed both diet and gender effects ($P < 0.001$) on AGD and relative AGD. Weight gain, change in AGD and change in relative AGD from PND 3 to 21 were also affected by lactation exposure to flaxseed (**Table 4**). Lactation exposure to 10% flaxseed significantly ($P < 0.05$) lowered body weight gain in male offspring and shortened AGD in both sexes compared with other diet groups. When adjusted for body weight by calculation of relative change in AGD, diet effects on AGD persisted only in females. Compared with the other diet treatments, female offspring exposed to 10% flaxseed had lower relative AGD. In contrast, rats consuming 5% flaxseed or SDG at levels equivalent to that in the 5% flaxseed diet had higher relative AGD compared with the basal diet and the 10% flaxseed treatment groups. A two-way ANOVA indicated both gender and diet effects ($P < 0.001$) on AGD and relative AGD.

Puberty onset. Flaxseed and SDG altered the timing of puberty (**Table 5**). Exposure of female offspring to 10% flaxseed during pregnancy and lactation resulted in puberty onset at a significantly ($P < 0.05$) earlier age and lighter body weight, whereas 5% flaxseed resulted in puberty onset at an older age but at the same body weight as the basal diet group. Effects similar to those produced by 5% flaxseed occurred when SDG was given at the level found in 5% flaxseed.

TABLE 5

The effect of exposure during pregnancy and lactation to flaxseed or secoisolariciresinol diglycoside (SDG) on puberty onset in female rat offspring[1]

Dietary treatment	*n*	Age	Weight
		d	*g*
Basal diet	13	30.1 ± 0.8[b]	93.69 ± 1.85[b]
5% Flaxseed	13	34.9 ± 0.6[c]	98.23 ± 2.17[b]
SDG	15	34.7 ± 0.7[c]	99.60 ± 1.96[b]
10% Flaxseed	17	25.5 ± 0.3[a]	77.77 ± 1.84[a]

[1] Data are means ± SEM. Different letters indicate significant differences ($P < 0.05$) by one-way ANOVA followed by Tukey's test (parametric).

Estrous cycles. All female offspring were cycling at PND 50 (**Table 6**). However, compared with other diet treatments, those exposed to 10% flaxseed during pregnancy and lactation had significantly ($P < 0.05$) lengthened estrous cycles because of prolonged time in the estrus phase. By PND 132, 20% of the 10% flaxseed-treated rats were acyclic due to persistent estrus, whereas 14.3% of the SDG-treated rats and 16.7% of the 5% flaxseed-treated rats were acyclic as a result of persistent diestrus. Of the animals still cycling at PND 132, only the 10% flaxseed-treated animals had significantly lengthened estrous cycles due to prolonged time in estrus phase compared with the basal diet group.

Reproductive organ and major organ weights. Except for the reproductive organs, there were no significant effects on the major organ weights in the offspring killed at PND 21, 50

TABLE 6

The effect of exposure during pregnancy and lactation to flaxseed or secoisolariciresinol diglycoside (SDG) on estrous cycling and length in female rat offspring[1]

	Postnatal day 40–50		Postnatal day 100–132	
Treatment	Rats cycling[2]	Cycle length[3]	Rats cycling[2]	Cycle length[3]
	n	*d*	*n*	*d*
Basal diet	13/13	4.9 ± 0.2[a]	7/7	5.4 ± 0.2[a]
5% Flaxseed	13/13	6.3 ± 0.3[a]	5/6	6.8 ± 0.9[ab]
SDG	15/15	6.5 ± 0.3[a]	6/7	7.3 ± 0.2[ab]
10% Flaxseed	17/17	7.5 ± 0.3[b]	8/10	7.7 ± 0.5[b]

[1] Data are means ± SEM. Different letters indicate significant differences ($P < 0.05$) by one-way ANOVA followed by Dunn's test (nonparametric).

[2] Estrous cycles were determined by vaginal smears. More than 10 d in one phase indicated acyclicity.

[3] Estrous cycles were determined by vaginal smears. Time required to complete the proestrus, estrus, metestrus and diestrus phases indicated estrous cycle length. Vaginal smears were taken at postnatal d 40–50 and in older rats at postnatal d 100–132.

TABLE 7

The effect of exposure during pregnancy and lactation to flaxseed or secoisolariciresinol diglycoside (SDG) on female rat offspring reproductive organ relative weights at the different developmental stages[1]

Treatment[2]	*n*	Body weight	Uterus[2]	Ovaries[2]
		g	*mg/100 g body weight*	
21 d				
Basal diet	7	48.05 ± 3.35	80.16 ± 6.15[a]	64.16 ± 5.66[b]
5% Flaxseed	7	40.70 ± 2.88	125.53 ± 14.34[b]	43.96 ± 1.95[a]
SDG	7	46.07 ± 1.90	138.03 ± 8.82[b]	44.01 ± 4.54[a]
10% Flaxseed	7	39.98 ± 2.06	147.95 ± 8.14[b]	59.10 ± 5.05[ab]
50 d				
Basal diet	6	179.92 ± 4.51	195.81 ± 21.89	58.31 ± 3.40[a]
5% Flaxseed	7	191.03 ± 8.09	178.51 ± 10.33	60.08 ± 4.18[a]
SDG	8	187.73 ± 7.41	171.19 ± 14.78	58.89 ± 2.46[a]
10% Flaxseed	7	191.83 ± 9.39	170.34 ± 14.31	87.23 ± 5.82[b]
132 d				
Basal diet	7	283.17 ± 15.86	175.79 ± 18.10	37.15 ± 2.79[a]
5% Flaxseed	6	335.02 ± 17.36	156.87 ± 12.00	40.77 ± 2.13[ab]
SDG	7	326.66 ± 12.79	159.43 ± 16.71	41.11 ± 2.34[ab]
10% Flaxseed	10	304.94 ± 8.24	168.96 ± 6.34	47.51 ± 2.50[b]

[1] Data are means ± SEM. Means with different letters are significantly different ($P < 0.05$) within the same age groups by one-way ANOVA followed by Tukey's test (parametric).
[2] Relative organ weights were calculated as wet weight/body weight.

or 132. In female offspring (PND 21), the immature uterine relative weight was higher in 10% flaxseed, 5% flaxseed and SDG diet groups compared with the basal diet group, but this difference disappeared by PND 50 and 132 (**Table 7**). At PND 21, offspring exposed to 5% flaxseed and SDG had lower relative ovarian weights than the controls, but the difference disappeared at PND 50. On the other hand, the relative ovarian weight was greater in the 10% flaxseed diet group at PND 50 compared with the other diet treatments, and this persisted into adulthood (PND 132) relative to the basal diet group. A two-way ANOVA indicated significant ($P < 0.001$) diet and age effects on female reproductive organ weights.

Pregnancy and lactation exposure to flaxseed or SDG had no effect on male accessory sex gland or testes weight at PND 21 or 50 but at a later age (PND 132), male offspring that had been treated with 10% flaxseed had greater accessory sex gland and prostate relative weights than those of the basal, 5% flaxseed and 1.5 mg SDG diet groups (**Table 8**). A two-way ANOVA indicated significant ($P < 0.001$) age and diet effects on sex gland weight.

Prostate histology. The rat prostate consists of ventral lobe, dorsal-lateral lobes and coagulating glands, but morphologic alterations were detected only in the ventral lobe. Microscopic examination of the ventral prostate of male offspring exposed to the basal diet from pregnancy and lactation until killed at PND 132 showed that prostate acini was composed mainly of columnar epithelial cells surrounded by small stroma that formed infoldings that projected into the lumen (**Fig. 1***A*). At PND 132, the ventral lobe of male offspring exposed to 10% flaxseed during the pregnancy and lactation period exhibited extensive cell proliferation (Fig. 1*B*). There were increased amounts of secretory epithelial cells forming more papillary infoldings and secondary projections. The epithelial cells of the acini were taller columnar-shaped cells with some nuclei located in apical regions. This proliferation was not restricted to the focal region but occurred in most areas of the ventral lobe. In contrast to the histologic appearance of the 10% flaxseed group, the male offspring exposed to 5% flaxseed during the pregnancy and lactation periods exhibited mild inhibition of prostate growth at PND 132 (Fig. 1C). The epithelial cells of the acini were cuboidal or flattened cuboidal with fewer infoldings. In some areas, the acini had more stroma. At PND 132, the histologic appearance of the ventral

TABLE 8

The effect of exposure during pregnancy and lactation to flaxseed or secoisolariciresinol diglycoside (SDG) on male rat offspring reproductive organ relative weights at postnatal day 132[1]

Treatment	*n*	Body weight	Sex glands[2]	Testes	Seminal vesicle	Prostate
		g	*mg/100 g body weight*			
Basal diet	6	570.12 ± 14.56	701.89 ± 13.84[a]	565.02 ± 32.31	243.21 ± 13.96	279.31 ± 15.99[a]
5% Flaxseed	6	584.03 ± 23.19	708.37 ± 32.84[a]	571.47 ± 28.48	258.66 ± 14.81	270.12 ± 16.97[a]
SDG	10	578.91 ± 14.12	706.30 ± 26.18[a]	543.46 ± 20.40	244.50 ± 11.88	290.31 ± 13.95[a]
10% Flaxseed	6	522.85 ± 12.22	827.62 ± 29.49[b]	627.06 ± 39.10	259.79 ± 14.18	359.79 ± 23.43[b]

[1] Data are means ± SEM. Means with different letters are significantly different ($P < 0.05$) by one-way ANOVA followed by Tukey's test (parametric).
[2] Relative organ weights were calculated as wet weight/body weight.

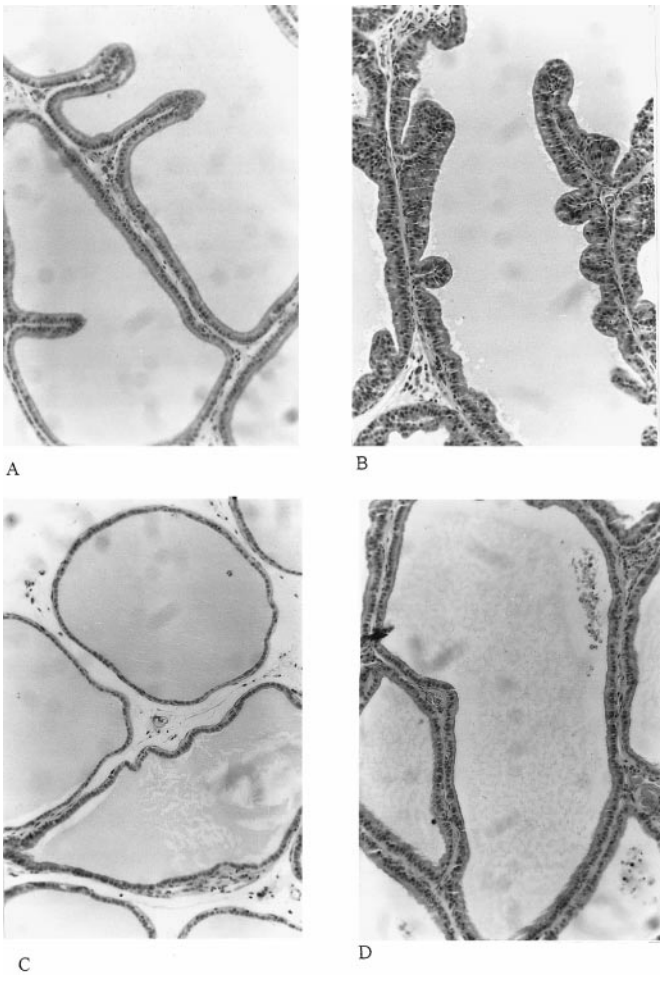

FIGURE 1 Typical histologic sections of the ventral prostate at postnatal d 132 in male rats exposed to either basal diet, basal diet supplemented with 10% flaxseed, 5% flaxseed or secoisolariciresinol diglycoside (SDG) at the level found in 5% flaxseed during pregnancy and lactation. (*A*) Basal diet group: acini are composed of columnar epithelial cells surrounded by small stoma; (*B*) 10% flaxseed group: acini are lined by tall columnar cells with more infoldings and projecting into the lumen. Some of the nuclei are located in the apical region of the cell; (C) the 5% flaxseed group: acini are lined mainly with cuboidal or flattened cuboidal epithelial cells with fewer infoldings and surrounded by more stoma; (*D*) the SDG group: features are similar to those of basal diet group (magnification X125).

lobe of male offspring exposed to SDG was similar to that of the basal diet group (Fig. 1*D*).

DISCUSSION

The 10 or 5% flaxseed or SDG at levels found in 5% flaxseed had no apparent effect on rat dam's pregnancy but exerted reproductive changes in the offspring. Inadequate or excessive estrogen disrupts pregnancy by altering the proper estrogen balance required for the establishment and maintenance of pregnancy. Estrogenization occurred in pregnant rat dams fed 10% flaxseed as indicated by greater maternal uterine and ovarian relative weights, but it was not enough to impair pregnancy. In contrast, feeding the phytoestrogen coumestrol at 150–900 μg/kg diet estrogenized the reproductive tract of rat dams and impaired pregnancy by increasing embryo degeneracy (Fredericks et al. 1981). The absence of pregnancy effects by flaxseed or SDG suggests a weaker estrogenic property of the mammalian lignans compared with coumestrol. Coumestrol has the strongest estrogenic potency of the several phytoestrogens that have been investigated (Mayr et al. 1992).

Pregnancy and lactation are more hormone-sensitive periods for offspring than for the pregnant rat dams because sexual differentiation of the offspring's reproductive tract and CNS is occurring during those periods under the influence of hormones (Manson and Kang 1989). Pregnant rat dams fed 10% flaxseed had offspring with lower birth weights than those fed the other diets. This suggests estrogenization because high dose estrogen has been reported to inhibit pituitary growth hormones (Wiedemann 1981). Continued growth suppression in nursing offspring indicated that estrogen exposure occurred via lignan transfer through rat dams' milk. Lower body weight in the male but not the female offspring suggests that decreased growth was not the result of decreased milk production.

Other early evidence of estrogenization of female offspring in response to flaxseed or SDG includes shortening of AGD, which usually occurs in the presence of estrogen, and the higher immature uterine growth, which is a classic measure of estrogenic potency. Significant changes in timing of puberty onset, ovarian relative weight, premature cycle irregularity and acyclicity occurred after flaxseed or SDG exposure ended, indicating that changes exerted during sexual differentiation resulted in permanent effects. Whether reproductive effects exerted were estrogenic or antiestrogenic depended on the dosage of flaxseed given.

Compared with the other diet groups, the female offspring exposed to 10% flaxseed had earlier age and lighter weight at puberty onset, greater ovarian relative weight at PND 50, which persisted into adulthood, lengthened estrous cycles, and by PND 132, persistent estrus in 20% of the offspring. These reproductive effects were determined to be estrogenic on the basis of reports of similar results in neonatal female rats treated with synthetic and endogenous estrogens (Sheehan et al. 1980). This was also in agreement with results of feeding 0.01% coumestrol to lactating rats dams whose offspring showed persistent estrus at PND 132 (Whitten et al. 1993). It is possible that 10% flaxseed may exert effects on the ovarian weight and ovarian cycles indirectly through estrogenization of the developing CNS. Normally, the CNS produces cyclical release of hormones regulating the ovarian cycle but early estrogenization of the CNS produces acyclic hormone release, resulting in ovarian follicle growth but failure to ovulate and lack of estrous cyclicity due to persistent estrous (MacLusky and Naftolin 1981).

The 5% flaxseed or 1.5 mg SDG diet group had significantly higher relative AGD, greater immature uterine relative weight but lower ovarian relative weight compared with the basal diet group. At a later stage, puberty onset was delayed and estrous cycles were lengthened due to prolonged diestrus. By PND 132, ~15% of adult animals were acyclic due to persistent diestrus. Diestrus occurs when estrogen stimulation of the vaginal epithelium is blocked or stopped (Nalbandov 1976). These reproductive effects were determined to be antiestrogenic based on the Gellert and Wilson (1979) study, which reported similar effects of delayed puberty onset, decreased regularity of ovarian cycles and cessation of cyclicity at an earlier age in neonatal androgenized female rats. Compared with the basal diet group, the lower immature ovarian relative weight and premature acyclicity in female offspring exposed to 5% flaxseed suggests that the mechanism whereby 5% flaxseed exerted its antiestrogenic action was by direct effects on the reproductive tract. According to Manson and Kang (1989),

delayed puberty results from ovary damage and the delay in puberty onset is proportional to the time required for the follicle to repopulate. At a later age, this partial destruction of the follicle pool can result in premature cessation of ovarian cycles. Flaxseed had effects on the sex organs but no gross effect on other major organs based on the absence of weight changes.

Male offspring exposed to 10% flaxseed during the pregnancy and lactation periods were estrogenized as indicated by shortened AGD. AGD was not significantly longer when expressed relative to body weight. Immature testes and accessory sex gland relative weights were also unaffected. By PND 132, there were greater accessory sex gland and prostate relative weights and cell proliferation in the prostate of male offspring exposed to 10% flaxseed during pregnancy and lactation compared with the other diet treatment groups. Evidently, reproductive effects are not always immediately observed but may manifest when the offspring develop reproductive capacity upon reaching puberty or adulthood (Whitten et al. 1993 and 1995).

In the rodent model, early exposure to estrogen has been reported to alter prostatic growth and response to androgens during adulthood, and to promote preneoplastic lesions and tumor formation in the aging animals (Rajfer and Coffey 1978, Santti et al. 1994). Similar observations of higher prostate relative weight and hyperplasia at PND 132 in the 10% flaxseed diet group suggest that early exposure to high dose flaxseed may potentially increase the risk of prostate cancer. It is noted, however, that soybean, a rich source of isoflavone phytoestrogens, increased prostate weight when fed to untreated mice but decreased prostate dysplasia when fed to neonatal mice that had been estrogenized by 3 d treatment with diethylstilbestrol (Makela et al. 1995). If the lignans act in the same way as the isoflavones, then 10% flaxseed in the presence of a more potent estrogen may provide cancer protective effects by acting as an estrogen antagonist.

In contrast to 10% flaxseed, 5% flaxseed resulted in a mild inhibition of prostate growth. A similar inhibition of epithelial cell proliferation was observed in the mammary gland of rats fed 5% flaxseed (Serraino and Thompson 1991). In addition, lower nuclear aberration and decreased tumor number and size were observed in the SDG- or 5% flaxseed–treated rats (Serraino and Thompson 1992, Thompson et al. 1996a and 1996b). Female offspring exposed to 5% flaxseed during pregnancy and lactation had delayed puberty onset followed by increased incidence of premature acyclicity due to diestrus. Epidemiologic evidence suggests that late menarche, early menopause and hormone deprivation are beneficial in reducing breast cancer risk (Kelsey et al. 1993). According to Whitten et al. (1995) phytoestrogen exposure during sexual differentiation can alter sex-specific patterns of development, and these changes may be cancer protective. Therefore, depending on the dose, flaxseed exposure during pregnancy and lactation results in reproductive changes that may either reduce or increase cancer incidence in the offspring.

The long-term hormonal effects exerted by flaxseed may also alter fertility. The 10% flaxseed–treated female offspring had an earlier age at puberty onset, suggesting that the animal's reproductive lifespan may increase. However, cycle irregularity, premature cessation of cyclicity and increased incidence of persistent estrus with 10% flaxseed treatment suggests that, ultimately, the reproductive lifespan may be shortened. The 5% flaxseed delayed puberty and produced early acyclicity, which may compromise reproduction because this can decrease the potential mating that can occur over an animal's lifespan. The similarity of the SDG results to 5% flaxseed on reproductive markers indicates that the lignans produced from SDG were the components responsible for the partial antiestrogenic properties of 5% flaxseed. Unfortunately, SDG at the level found in 10% flaxseed was not tested to determine whether estrogenic effects observed were due to lignans.

The high (n-3) fatty acid, α-linolenic acid [18:3(n-3), ALA] content of flaxseed (Cunnane 1995) has been suspected to be responsible for the reproductive effects observed, particularly at the 10% flaxseed level. The (n-3) competes with the (n-6) fatty acids for the same desaturase/elongase enzyme, with the (n-3) fatty acids having greater affinity for these enzymes (Drevon 1992). Therefore, raising the (n-3)/(n-6) ratio can inhibit the elongation/desaturation of the (n-6) fatty acid linoleic acid [18:2(n-6), LA] to arachidonic acid [20:4(n-6), AA], the precursor for series-2 prostaglandins (PG) (Horrobin 1983, Willis 1981), which are important for normal reproductive function in males and females (Bygedeman et al.1987). However, we believe that the (n-3) fatty acid was not primarily responsible for the effects seen. First, the fatty acid composition of the liver of offspring in this study (unpublished data) showed higher ALA (4.65 $\pm$ 0.50 g/100 g) in the 10% flaxseed group compared with ALA (1.36 $\pm$ 0.18 g/100 g) in the basal diet group. However, the greater (n-3)/(n-6) fatty acid ratio produced by 10% flaxseed was not high enough to compromise the elongation/desaturation of LA to AA, as indicated by the absence of significant differences in liver LA (19.01 $\pm$ 0.85 g/100 g) and AA (12.18 $\pm$ 0.90 g/100 g) in the 10% flaxseed group compared with the LA (21.20 $\pm$ 0.82 g/100 g) and AA (14.73 $\pm$ 0.78 g/100 g) in the control group. Second, some other studies have shown that raising dietary (n-3) fatty acids to levels that suppressed production of series-2 PG from AA improved rather than compromised reproduction. Truijillo and Broughton (1995) reported that increased dietary (n-3) fatty acids increased the number of ova released during ovulation, whereas raising dietary (n-6) fatty acids reduced ovulation in rats. Pregnant women given a diet supplemented with fish oil, a rich source of long-chain (n-3) fatty acids, had decreased production of the series-2 PG from AA but improved reproduction as indicated by a lower incidence of toxemia, fewer premature deliveries and prolonged gestation, resulting in higher birth weights (Olsen et al 1986, Olsen and Secher 1990). In contrast, pregnant rats treated with 10% flaxseed in this study had offspring with lower birth weights and no changes in pregnancy outcomes (i.e., offspring survival, gestation length and littersize). Third, Pandalai et al. (1996) found that high concentrations of eicosapentanoic acid [20:5(n-3), EPA] inhibited, whereas low concentrations promoted proliferation of both prostate cancer and normal cells. In contrast, 10% flaxseed, which would have produced higher EPA from ALA than the 5% flaxseed or basal diet, resulted in higher prostate cell proliferation, whereas 5% flaxseed inhibited prostate cell proliferation compared with the basal diet group. This suggests that the effects in the prostate were not due to the flaxseed oil but to some other component(s) present in flaxseed such as the lignans.

In conclusion, feeding rat dams 5 or 10% flaxseed or SDG at levels present in 5% flaxseed produced no significant effect on pregnancy except for the lower birth weights of offspring in those fed 10% flaxseed. However, feeding flaxseed or SDG during pregnancy and lactation had hormone-related dose-dependent effects on the offspring with potential implications for reproduction and cancer risk in the long term. The effects of flaxseed at the levels used are more likely due to the mammalian lignans produced from SDG than from its ALA-rich oil. Lignans likely were transferrred from the rat dams through the milk. Thus, caution is suggested when consuming

flaxseed at high doses during the hormone-sensitive periods of pregnancy and lactation. It is also suggested that the dose and timing of flaxseed exposure be considered when adopting flaxseed for chronic disease prevention and therapeutic application. Similar cautions have been suggested to nursing mothers consuming soy products because soy can increase the isoflavone phytoestrogen concentration in their milk up to 10-fold (Setchell et al. 1997) and daily exposure of infants to phytoestrogen-rich milk or soy-based infant formula containing phytoestrogen may be sufficient to exert biological effects (Haumann 1997, Sheehan 1998).

LITERATURE CITED

Bakke, J. E. & Klosterman, H. J. (1956) A new diglycoside from flaxseed. Proc. Natl. Acad. Sci. U.S.A. 10: 18–22.

Barrow, P. (1990) Technical procedures in reproductive toxicology. Lab. Anim. Handb. 11: 9–25.

Bradbury, R. B. & White, D. C. (1954) Oestrogens and related substances in plants. Vitam. Horm. 12: 207–233.

Bygedeman, M., Gottlieb, C., Swanborg, K. & Swahn, M. L. (1987) Role of prostaglandins in human reproduction recent advances. In: Advances in Prostaglandin, Thomboxane and Leukotriene Research, Vol. 17 (Samuelsson, B., Paoletti, R. & Ramwell, P. W., eds.), pp. 1112–1116. Raven Press, New York, NY.

Canadian Council on Animal Care (1984) Guide to the Care and Use of Experimental Animals. Ottawa, ON, Canada.

Cunnane, S. C. (1995) Metabolism and function of alpha-linolenic acid in humans. In: Flaxseed in Human Nutrition (Cunnane, S. C. & Thompson, L. U., eds.), pp. 99–127. AOCS Press, Champaign, IL.

Drevon, C .A. (1992) Marine oils and their effects. Nutr. Rev. 50: 38–45.

Evans, E .A. (1974) Tritium and Its Compounds (Evans, E. A., ed.), 2nd ed., pp. 489–490. Wiley, New York, NY.

Farnsworth, N. R., Bingel, A. S., Cordell, G. A., Crane, F. A. & Wong, H. H. (1975) Potential value of plants as sources of new antifertility agents II. J. Pharm. Sci. 64: 717–754.

Fredricks, G. R., Kincaid, R. L., Bondioli, K. R. & Wright, R. W., Jr. (1981) Ovulation rates and embryo degeneracy in female mice fed the phytoestrogen coumestrol. Proc. Soc. Exp. Biol. Med. 167: 237–241.

Gellert, R. J. & Wilson, C. (1979) Reproductive function in rats exposed prenatally to pesticides and polychlorinated biphenyls (PCB). Environ. Res. 18: 437–443.

Haumann, B. F. (1997) Soy protein foods gain storage space. INFORM 6: 588–596.

Horrobin, D. F. (1983) The regulation of prostaglandin biosynthesis by the manipulation of essential fatty acid metabolism. Rev. Pure Appl. Sci. 4: 339–383.

Jenab, M. & Thompson, L. U. (1996) The influence of flaxseed and lignans on colon carcinogenesis and beta-glucuronidase radioactivity. Carcinogenesis 17: 1343–1348.

Kelsey, J. L., Gammon, M. D. & John, E. M. (1993) Reproductive factors and breast cancer. Epidemiol. Rev. 15: 7–16.

Klosterman, H. J. & Smith, F. (1954) The isolation of β-hydroxy-β-methylglutaric acid from the seed of flax. J. Am. Chem. Soc. 76: 1229–1230.

MacLusky, N. J. & Naftolin, F. (1981) Sexual differentiation of the central nervous system. Science (Washington, DC) 211: 1298–1306.

Makela, S. I., Pylkkanen, L. H., Santii, R.S.S. & Adlercruetz, H. (1995) Dietary soybean may be antiestrogenic in male mice. J. Nutr. 124: 437–445.

Manson, J. M. & Kang, Y. J. (1989) Test methods for assessing female reproductive and developmental toxicology. In: Principles and Methods of Toxicology (Hayes, A. W., ed.), 2nd ed., pp. 311–359. Raven Press Ltd., New York, NY.

Mayr, U., Butsch, A. & Schneider, S. (1992) Validation of two in vitro test systems for estrogenic activities with zearalenone, phytoestrogens and cereal extracts. Toxicology 74: 135–49.

Nalbandov, A. V. (1976) The estrous cycle. In: Reproductive Physiology of Mammals and Birds: The Comparative Physiology of Domestic and Laboratory Animals and Man (Nalbanov, A. V., ed.), pp. 98–124. W. H. Freeman & Co., San Francisco, CA.

Olsen, S .F., Hansen, H. S., Sorensen, T. I., Jensen, B., Secher, N. J., Sommer, S. & Knudsen, L. B. (1986) Intake of marine fat, rich in (n-3) polyunsaturated fatty acids may increase birthweight by prolonged gestation. Lancet 2: 367–369.

Olsen, S. F. & Secher, N. J. (1990) A possible preventative effect of low dose fish oil on early delivery and pre-eclampsia indications from a 50 year old controlled trial. Br. J. Nutr. 64: 599–609.

Orcheson, L. J., Rickard, S. E., Seidl, M. M. & Thompson, L. U. (1998) Flaxseed and its mammalian lignan precursor cause lengthening or cessation of estrous cycling in rats. Cancer Lett. 125: 69–76.

Pandalai, P. K., Pilat, M. J., Yamazaki, K., Naik, H. & Pienta, K. J. (1996) The effects of omega-3 and omega-6 fatty acids on in vitro prostate cancer growth. Anticancer Res. 16: 815–820.

Pasqualini, J. R., Kind, F. A. & Sumida, C. (1985) The binding of homones in maternal and fetal biological fluids. In: Hormones and the Fetus (Pasqualini, J. R., ed.), pp. 1–50. Pergamon Press, New York, NY.

Price, K .R. & Fenwick, G. R. (1985) Naturally occurring oestrogens in foods—a review. Food Addit. Contam. 2: 73–106.

Rajfer, J. & Coffey, D. S. (1978) Sex steroid imprinting of the immature prostate. Investig. Urol. 16: 186–192.

Reeves, P. G., Nielson, F. H. & Fahey, G. C., Jr. (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-96A rodent diet. J. Nutr. 123: 1939–1951.

Rickard, S. E., Orcheson, L. J., Seidl, M. M., Luyengi, L., Fong, H.H.S. & Thompson, L. U. (1996) Dose-dependent production of mammalian lignans in rats and in vitro from the purified precursor secoisolariciresinol diglycoside in flaxseed. J. Nutr. 126: 2012–2019.

Rickard, S. E. & Thompson, L. U. (1997) Phytoestrogens and lignans: effects on reproduction and chronic disease. In: Antinutrients and Phytochemicals in Food (Shahidi, F., ed.), pp. 273–293. American Chemical Society, Washington, DC.

Rickard, S. E. & Thompson, L. U. (1998) Chronic exposure to secoisolariciresinol diglycoside alters lignan desposition in rats. J. Nutr. 128: 615–623.

Santti, R., Newbold, R. R., Makela, S., Pylkkanen, L. & Lachlan, J. A. (1994) Developmental estrogenization and prostatic neoplasia. Prostate 24: 67–78.

Serraino, M. R. & Thompson, L. U. (1991) The effect of flaxseed supplementation on early risk markers for mammary carcinogenesis. Cancer Lett. 60: 135–142.

Serraino, M. R. & Thompson, L. U. (1992) The effect of flaxseed supplementation on the initiation and promotional stages of mammary tumorigenesis. Nutr. Cancer 17: 153–159.

Setchell, K. D., Zimmer-Nechemias, L., Cai, J. & Heubi, J .E. (1997) Exposure of infants to phyto-oestrogens from soy-based infant formula. Lancet 350: 23–27.

Sheehan, D. M. (1998) Herbal medicines, phytoestrogens and toxicity: risk: benefit considerations. Proc. Soc. Exp. Biol. Med. 217: 379–385.

Sheehan, D. M., Branham, W. S., Medlock, K. L., Olson, M. E. & Zehr, D. (1980) Estrogen plasma binding and regulation of development in the neonatal rat. Teratology 21(2): 68A (abs).

Thompson, L. U., Rickard, S. E., Cheung, F., Kenaschuk, E. O. & Obermeyer, W. R. (1997) Variability in anticancer lignan levels in flaxseed. Nutr. Cancer 27: 26–30.

Thompson, L. U., Rickard, S. E., Orcheson, L. J. & Seidl, M. M. (1996a) Flaxseed and its lignan and oil components reduce mammary tumor growth at a late stage of carcinogenesis. Carcinogenesis 17: 1373–1776.

Thompson, L. U., Robb, P., Serraino, M. & Cheung, F. (1991) Mammalian lignan production from various foods. Nutr. Cancer 16: 43–52.

Thompson, L. U., Seidl, M. M., Rickard S. E., Orcheson, L. J. & Fong, H.H.S. (1996b) Antitumorigenic effect of mammalian lignan precursor from flaxseed. Nutr. Cancer 26: 159–165.

Trujillo, E. P. & Broughton, K. S. (1995) Ingestion of n-3 polyunsaturated fatty acids and ovulation in rats. Journal of Reproduction and Fertility 105: 197–203.

Whitten, P .L., Lewis, C. & Naftolin, F. (1993) A phytoestrogen diet induces the premature anovulatory syndrome in lactationally exposed female rats. Biol. Reprod. 49: 1117–1121.

Whitten, P. L., Lewis, C., Russel, E. & Naftolin, F. (1995) Potential adverse effects of phytoestrogens. J. Nutr. 125: 771S–776S.

Wiedemann E. (1981) Adrenal and gonadal steriods. In: Endocrine Control of Growth (Daughaday, W. H., ed.), pp. 67–119. Elsevier, New York, NY.

Willis, A. L. (1981) Nutritional and pharmacological factors in eicosanoid biology. Nutr. Rev. 39: 289–301.

Zimmermann, S. A., Clavenger, W. R., Brimhall, B. B. & Bradshaw, W.S. (1991) Diethylstilbestrol induced perinatal lethality in the rat. II. Perturbation of parturition. Biol. Reprod. 44: 583–589.