

## Dietary Flaxseed Alters Tumor Biological Markers in Postmenopausal Breast Cancer

Lilian U. Thompson,<sup>1</sup> Jian Min Chen,<sup>1</sup> Tong Li,<sup>2</sup> Kathrin Strasser-Weippl,<sup>2</sup> and Paul E. Goss<sup>3</sup>

**Abstract** **Purpose:** Flaxseed, the richest source of mammalian lignan precursors, has previously been shown to reduce the growth of tumors in rats. This study examined, in a randomized double-blind placebo-controlled clinical trial, the effects of dietary flaxseed on tumor biological markers and urinary lignan excretion in postmenopausal patients with newly diagnosed breast cancer. **Experimental Design:** Patients were randomized to daily intake of either a 25 g flaxseed-containing muffin ( $n = 19$ ) or a control (placebo) muffin ( $n = 13$ ). At the time of diagnosis and again at definitive surgery, tumor tissue was analyzed for the rate of tumor cell proliferation (Ki-67 labeling index, primary end point), apoptosis, c-erbB2 expression, and estrogen and progesterone receptor levels. Twenty-four-hour urine samples were analyzed for lignans, and 3-day diet records were evaluated for macronutrient and caloric intake. Mean treatment times were 39 and 32 days in the placebo and flaxseed groups, respectively. **Results:** Reductions in Ki-67 labeling index (34.2%;  $P = 0.001$ ) and in c-erbB2 expression (71.0%;  $P = 0.003$ ) and an increase in apoptosis (30.7%;  $P = 0.007$ ) were observed in the flaxseed, but not in the placebo group. No significant differences in caloric and macronutrient intake were seen between groups and between pre- and posttreatment periods. A significant increase in mean urinary lignan excretion was observed in the flaxseed group (1,300%;  $P < 0.01$ ) compared with placebo controls. The total intake of flaxseed was correlated with changes in c-erbB2 score ( $r = -0.373$ ;  $P = 0.036$ ) and apoptotic index ( $r = 0.495$ ;  $P < 0.004$ ). **Conclusion:** Dietary flaxseed has the potential to reduce tumor growth in patients with breast cancer.

A strong positive relationship has been established between high concentrations of plasma estrogen and an increased risk of developing breast cancer (1–3). Antagonizing the effects of estrogen with antiestrogenic therapies such as tamoxifen or aromatase (estrogen synthetase) inhibitors, decreases the incidence and the growth of invasive and noninvasive breast cancer (4, 5). However, adverse side effects from tamoxifen include thromboembolism and endometrial cancer, making its therapeutic index in chemoprevention of questionable value to women. Use of other antiestrogenic agents for breast cancer treatment and prevention is desirable. Mammalian lignans, mainly enterolactone and enterodiol, are produced from precursors such as secoisolariciresinol diglucoside in plant foods by the action of bacterial flora in the colon (ref. 6; Fig. 1). They undergo enterohepatic circulation and some are excreted

in the urine. The amount of dietary plant lignans ingested correlates directly with plasma levels and urinary excretion of mammalian lignans (6, 7). Lignans have chemical structures similar to estradiol and to the selective estrogen receptor modulator tamoxifen, suggesting that they may have hormonal (estrogenic or antiestrogenic) properties.

*In vitro* studies have shown that lignans induce: mRNA expression of the estrogen responsive protein pS2 in MCF-7 cancer cells (8); inhibit aromatase in placental microsomes, JEG-3 human choriocarcinoma cells, preadipocytes, and MCF-7 cells (9–11); bind to rat and human  $\alpha$ -fetoprotein, competing with estradiol and estrone for their binding site (12); stimulate growth of estrogen-dependent human breast cancer cells in the absence of estrogen and inhibit growth in the presence of estrogen (13–15); stimulate sex hormone-binding globulin synthesis (16); compete with estradiol for rat uterine nuclear estrogen type II binding site (16); and bind to the estrogen receptor (ER), particularly ER- $\beta$  (17). Lignans also have nonendocrine properties including antioxidant (18–21) and antiangiogenic effects (22). Epidemiologic studies have shown significant reductions in breast cancer risk in women with the highest versus the lowest quartile of urinary enterolactone excretion (23, 24), serum enterolactone levels (25), or lignan intake (26).

Flaxseed, more commonly known as linseed, is the richest source of mammalian lignan precursors, with levels 100 to 800 times higher than those in 66 other plant foods in the vegetarian diet (27). Flaxseed also has an exceptionally high concentration of  $\alpha$ -linolenic acid (57% of total fatty acids), which has been

**Authors' Affiliations:** <sup>1</sup>Department of Nutritional Sciences, <sup>2</sup>Princess Margaret Hospital, University of Toronto, Toronto, Ontario, Canada, and <sup>3</sup>Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, Massachusetts. Received 11/15/04; revised 2/4/05; accepted 2/23/05.

**Grant support:** American Institute for Cancer Research and Canadian Breast Cancer Research Foundation grants (L.U. Thompson and P.E. Goss).

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 U.S.C. Section 1734 solely to indicate this fact.

**Requests for reprints:** Paul E. Goss, Massachusetts General Hospital Cancer Center, Cancer Center Administration, 55 Fruit Street, Cox Building, Room 640, Boston, MA 02114. Phone: 617-724-3118; Fax: 617-724-3166; E-mail: pgoss@partners.org.

©2005 American Association for Cancer Research.

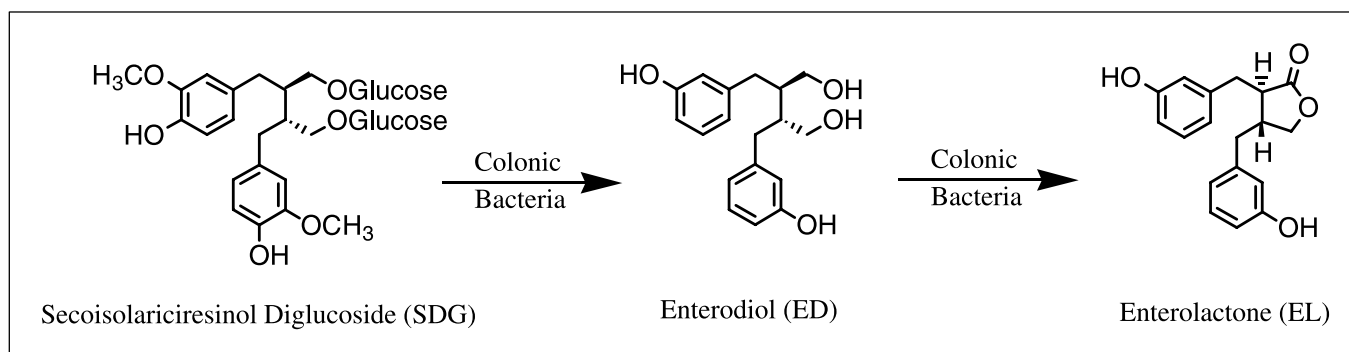


Fig. 1. Metabolism of plant lignans to mammalian lignans.

shown in animal and epidemiologic studies to be protective against breast cancer (28). Based on all these data, it is hypothesized that flaxseed might be effective in cancer treatment and prevention. When flaxseed was fed to carcinogen-treated rats at the preinitiation or promotion stages of carcinogenesis, significant reductions in mammary tumor incidence and size were observed (29–31). When it was fed at a time when mammary tumors were already established, the mammary tumors regressed in size (32). Tumor growth and/or the incidence of metastases were also reduced by dietary flaxseed in athymic mice injected with human ER-positive (MCF-7; ref. 33) or ER-negative (MDA MB 435; refs. 34, 35) breast cancer cells. The effect of secoisolariciresinol diglucoside, isolated and purified from flaxseed, was similar to that of flaxseed (32, 36), indicating that the effect of flaxseed was at least in part due to its lignans. Overall, these observations suggest that flaxseed may influence tumor development in breast cancer patients.

The ability to inhibit tumor growth kinetics in a neoadjuvant setting has previously been used as a measure of an agent's effectiveness in other clinical circumstances (37–39). The correlation between the cell proliferation marker Ki-67 and clinical outcome has been validated in several settings including in the comparison between the aromatase inhibitor letrozole and tamoxifen (40). Our study aimed to determine the effects of

dietary flaxseed on parameters reflecting tumor growth kinetics and urinary lignan excretion when given preoperatively to newly diagnosed postmenopausal breast cancer patients.

## Materials and Methods

**Patients and study design.** We conducted a randomized, placebo-controlled, double-blind, prospective study involving postmenopausal patients with primary breast cancer. The patients (from the University Health Network, Toronto) were women who presented with a newly diagnosed lump suspicious for cancer and needed a confirmatory breast core biopsy. Eligibility criteria included: menopause for at least 6 months; histologically diagnosed by core biopsy as having breast carcinoma; not having taken hormone therapy and soy foods or flaxseed within 90 days of the first biopsy; not having taken antibiotics within 3 days of the first biopsy; no known allergy to flaxseed, lactose, wheat or certain spices; had sufficient tissue specimen taken from core biopsy for assessing biomarkers. Sixty-five postmenopausal patients volunteered, but after the initial biopsy, 18 did not meet the criteria of inclusion because their initial tumor was evaluated as benign. Another 15 volunteers withdrew due to difficulty eating a bulky muffin, difficulty traveling to the hospital, depression due to a recent diagnosis of breast cancer, or a busy schedule. Thus, 32 eligible patients were entered into and completed this randomized trial of preoperative dietary intervention. The characteristics of the patients are summarized in Table 1. There were no significant differences in any of the baseline variables.

Table 1. Patient characteristics and treatment time

	Placebo	Flaxseed
<i>n</i>	13	19
Age, y (range)	64.5 ± 2.6 (53-79)	70.3 ± 2.1 (50-88)
Weight, kg (range)	64.9 ± 2.6 (52-84.9)	68.3 ± 2.5 (44.3-86.8)
Treatment time, days (range)	38.7 ± 4.4 (16-76)	32.1 ± 3.0 (13-55)
Tumor type (%)		
Ductal	11 (84.6)	17 (89.5)
Lobular	1 (7.7)	1 (5.3)
Mixed	1 (7.7)	1 (5.3)
Histology grade (%)		
3	6 (46.2)	8 (42.1)
2	5 (38.5)	11 (57.9)
1	2 (15.4)	0
ER+ and PR+ (%)	11 (84.6)	17 (89.5)
ER– or PR– (%)	2 (15.4)	2 (10.5)

NOTE: No significant differences between the two groups by  $\chi^2$  test.

Between the time of first biopsy and surgery, patients were randomized to either the treatment or the placebo group. The patients were not stratified but there was a balance in node-positive/negative patients on the two arms. The interval between the first core biopsy and the definitive surgical excision of the tumor was not prescribed by the research protocol but rather was determined by standard practice of care in our institution. Patients in the treatment group ( $n = 19$ ) ate one muffin daily containing 25 g of flaxseed included in their usual diet. Patients in the placebo (control) group ( $n = 13$ ) took the same type of muffin but without flaxseed. To maintain the double-blind status of the study, muffins were packaged in opaque wrappings so the different muffins could not be visually distinguished. The muffin packages were labeled with numerical code before delivery to the research assistant. The research assistant received a list indicating which coded muffin packages were to be dispensed to the subjects. Therefore, neither the patient nor the research assistant knew what muffins were being given. All patients received dietary counseling so that the muffin intake with or without flaxseed would not cause increased caloric intake or weight gain.

Core tumor biopsy tissues at the time of diagnosis and at the time of surgery were analyzed for cell proliferation (Ki-67 labeling index), apoptosis, c-erbB2 expression, and ER and progesterone receptor (PR) expression. Before and prior to the end of treatment, 24-hour urine samples and 3-day diet records were also collected for lignan and nutrient intake analysis, respectively.

The study was approved by the Human Subjects Ethics Committee of the University of Toronto and the Toronto Hospital Human Experimentation Committee. All patients provided written informed consent before participation.

**Study muffins.** Study muffins were prepared in the standard manner by Canada Bread Co. (Toronto, ON, Canada). They contained similar ingredients and were prepared to contain 20.7 g white wheat flour for flaxseed muffins or 20.7 g whole-wheat flour for placebo muffins. Flaxseed muffins contained 25 g ground flaxseed. Placebo muffins were prepared with whole-wheat flour instead of white wheat flour to raise the dietary fiber content closer to that of the flaxseed muffins. All muffins were formulated to be isocaloric and equivalent in fat, protein, and dietary fiber. Hence additional canola oil (10 g) was added to placebo muffins but not to the flaxseed muffins. Muffins were also flavored with nutmeg, cinnamon, and vanilla extract to help maintain subject blindness. All flaxseed came from the same source (Omega Products, Melfort, Saskatchewan, Canada) and batch, and contained 2 mg of secoisolaricresinol diglucoside per gram. The patients kept their weekly supply of muffins at  $-20^{\circ}\text{C}$  and defrosted them as needed. They ate one muffin per day at breakfast time. Any uneaten muffins or portions thereof were returned and weighed. The total intake of flaxseed was estimated as  $25\text{ g} \times \text{treatment days} - \text{uneaten amounts}$ .

**Tumor analysis by immunohistochemistry.** For Ki-67, c-erbB2, ER, and PR analysis, 5  $\mu\text{m}$  sections of formalin-fixed, paraffin-embedded tissues were stained immunohistochemically (33, 34). Briefly, sections were treated with 3% aqueous  $\text{H}_2\text{O}_2$  and microwaved in 10 mmol/L citrate buffer (pH 6.0). The primary antibodies (Dako, Mississauga, Ontario, Canada) were diluted with the Dako Diluent buffer as follows: Ki 67 (MIB-1) 1:100, c-erbB2 (rabbit anti-human) 1:500, ER (ID5) 1:100, and PR (PgR 636) 1:100. The sections with antibody were incubated at  $4^{\circ}\text{C}$  overnight, and then treated with Dako's labeled streptavidin-biotin system kit (Universal LSAB 2 Kit) and AEC+ substrate-chromogen (Dako) with a light counterstain of hematoxylin.

For the apoptosis assay, *in situ* terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling was used with ApopTag Detection Kit (Intergen, Purchase, NY) according to the manufacturer's protocol. Briefly, sections were pretreated with proteinase K (20  $\mu\text{g}/\text{mL}$ ), and then incubated in a reaction mixture containing terminal transferase and digoxigenin dUTP at  $37^{\circ}\text{C}$  for 1 hour, followed by antidigoxigenin antibody coupled to horseradish

peroxidase for 30 minutes. Diaminobenzidine was used to detect the immunoreactivity, with counterstain of methyl green.

Positive and negative (diluent buffer) controls were used for each staining batch. The pre- and posttreatment sections of the same subject were stained at the same time. Slides were read under a light microscope at  $400\times$ , and a minimum of 1,000 cells from 5 to 10 randomly selected fields were counted. The specimens were blinded from the reader in terms of treatment groups and being pre- or posttreatment samples. The Ki-67 labeling index and apoptotic index were calculated as the percentage of positive cells over total cells counted. The H-score was used to assess the expression of c-erbB2, ER, and PR. The staining intensity was evaluated as negative (0), weak (1), medium (2), and strong (3) and multiplied by the number of cells with such intensity. The score was then calculated as:  $[(1 \times \text{cell number} + 2 \times \text{cell number} + 3 \times \text{cell number}) / \text{total cell number}]$  (34, 41). The scoring system, which combined the fraction of positive cells and staining intensity, provides more meaningful changes elicited by the treatment than staining intensity alone.

**Diet record and urinary lignan analysis.** Flaxseed intake was recorded daily. At baseline and before surgery, three food records (2 weekdays and 1 weekend day) were recorded by subjects and analyzed for intake of calories and macronutrients based on the condensed Canadian Nutrient File (42). Urinary lignan analysis was conducted by gas chromatography-mass spectrometry, as previously described by our group (7, 43).

**Statistical analysis.** Study subjects were randomly assigned to receive either flaxseed or placebo muffins using a technique of random permutation. The study was designed to detect a minimum change of 20% within the specified pre- and posttreatment arms, and a 30% difference between the two treatment groups in percent change in biological markers, specifically Ki-67 labeling index as the primary end point. Thus, the aim was to accrue a minimum of 10 to 15 patients in each group to reach a power of 80% for a two-sided test with  $\alpha$ -value of 0.05.

Baseline (pretreatment) differences between the two treatment arms were assessed by the Mann-Whitney test. The changes in each of the biological variables during the pre- and posttreatment within the specified treatment arms were analyzed by the Wilcoxon signed rank test, which takes into account the magnitude of the changes seen as well as the direction (increase or decrease). Percentage change in the values of each of the biological markers was calculated for each patient in the placebo and flaxseed groups, and was compared by using the Mann-Whitney test. Spearman's correlation coefficient test was used to determine the relationships among paired variables such as biomarkers versus demographic characteristics or length of treatment. All statistical tests of significance ( $P < 0.05$ ) were two-tailed. Statistical analyses were done using SigmaStat (Jandel Scientific, San Rafael, CA).

## Results

Muffin intake compliance was good (95.4% in the placebo and 92.5% in the flaxseed group) and did not differ significantly between the groups. This translated into a significant increase (1,300%;  $P < 0.01$ ) in mean urinary lignans in the flaxseed group but not in the placebo group (Table 2). The only side effects reported were abdominal fullness and increased bowel movements. No significant differences in caloric and macronutrient intake were observed between the treatment groups and between the pre- and posttreatment periods (data not shown). The mean treatment times were 32 and 39 days (median 30 and 37 days) for the flaxseed and placebo groups, respectively (Table 1).

Table 2 and Fig. 2 summarize the results of biological markers measured in tumor specimens pre- and posttreatment. No significant differences between the treatment groups were

**Table 2.** Effect of flaxseed on urinary lignan excretion and breast tumor biomarkers

	Placebo		Flaxseed	
	Pre	Post	Pre	Post
Urinary lignans ( $\mu\text{mol/L/d}$ )				
Median (25-75%)	2.8 (2.3-3.3)	0.9 (0.2-2.3)	1.0 (0.9-10.0)	58.7 (9.0-128.5)*
(Mean $\pm$ SE)	(2.8 $\pm$ 0.3)	(1.2 $\pm$ 0.8)	(5.1 $\pm$ 2.5)	(71.5 $\pm$ 28.4)
Ki-67 index (%)				
Median (25-75%)	15.1 (9.1-21.0)	12.0 (9.5-16.2)	15.2 (12.1-23.8)	10.0 (6.9-17.4)*
(Mean $\pm$ SE)	(15.8 $\pm$ 2.5)	(13.7 $\pm$ 2.6)	(18.1 $\pm$ 2.0)	(12.6 $\pm$ 1.6)
Apoptosis index (%)				
Median (25-75%)	0.87 (0.75-1.1)	0.94 (0.77-1.26)	0.88 (0.6-1.07)	1.15 (0.89-1.72)*
(Mean $\pm$ SE)	(1.04 $\pm$ 0.15)	(1.08 $\pm$ 0.15)	(0.89 $\pm$ 0.09)	(1.44 $\pm$ 0.24)
C-erbB2 score				
Median (25-75%)	0.2 (0.09-1.17)	0.31 (0.06-0.69)	0.31 (0.12-0.84)	0.09 (0.04-0.6)*
(Mean $\pm$ SE)	(0.58 $\pm$ 0.20)	(0.53 $\pm$ 0.18)	(0.47 $\pm$ 0.09)	(0.34 $\pm$ 0.10)
ER score				
Median (25-75%)	1.01 (0.13-1.27)	0.70 (0.17-1.06)	0.78 (0.48-1.0)	0.81 (0.53-0.98)
(Mean $\pm$ SE)	(0.72 $\pm$ 0.17)	(0.65 $\pm$ 0.13)	(0.75 $\pm$ 0.10)	(0.79 $\pm$ 0.12)
PR score				
Median (25-75%)	0.1 (0.02-0.26)	0.07 (0.01-0.40)	0.11 (0.01-0.28)	0.14 (0.09-0.33)
(Mean $\pm$ SE)	(0.19 $\pm$ 0.07)	(0.17 $\pm$ 0.06)	(0.2 $\pm$ 0.05)	(0.21 $\pm$ 0.04)

Abbreviations: Pre, pretreatment; Post, posttreatment; ER, estrogen receptor; PR, progesterone receptor.  
\*  $P < 0.01$  posttreatment versus pretreatment by Wilcoxon signed rank test (details in Fig. 2).

observed in baseline values of all tumor variables. After completion of treatment, decreases in tumor cell proliferation and c-erbB2 expression, and an increase in cell apoptosis were observed in both groups but a greater number of patients showed these changes in the flaxseed (74-84%) than in the placebo group (54-61%). Tumor cell proliferation (Ki-67 labeling index; Fig. 2A) significantly decreased by 34.2% (median), the apoptotic index (Fig. 2B) significantly increased up to 30.7% (median) and the expression of c-erbB2 (Fig. 2C) significantly decreased by 71.0% (median) in the flaxseed, but not in the placebo group. No significant differences in ER and PR levels were seen between the pre- and posttreatment periods in any group.

A comparison between the two groups for the percentage of changes in pre- and posttreatment in individual subjects is illustrated in Fig. 3. The percentage of changes in urinary lignan excretion (Fig. 3A), apoptotic index (Fig. 3C), and c-erbB2 score (Fig. 3D) were significantly higher ( $P < 0.05$ ) in the flaxseed group than in the placebo group. The percent change in Ki-67 labeling index (Fig. 3B) was also higher in the flaxseed than in the placebo group, but did not reach statistical significance. No significant differences in the percentage of changes in ER and PR levels were found between the two groups (Fig. 3E and F).

The total intake of flaxseed was significantly correlated with changes in c-erbB2 score ( $r = -0.373$ ;  $P = 0.036$ ) and apoptotic index ( $r = 0.495$ ;  $P < 0.004$ ), but not with changes in Ki-67 labeling index, ER, or PR. No significant relationship was observed between patient age and weight, baseline tumor characteristics such as tumor grade and ER or PR status, and the changes observed during the treatment period (data not shown).

## Discussion

Our study shows that daily intake of 25 g flaxseed can significantly reduce cell proliferation, increase apoptosis, and affect cell signaling by reducing c-erbB2 expression of human breast cancer cells. The percentage of reduction in tumor c-erbB2 expression and percentage of increase in cell apoptosis did not relate to baseline tumor characteristics, i.e., patient age, weight, tumor grade, ER, and PR status, but they significantly correlated with the total amount of flaxseed eaten. Although reductions in cell proliferation and c-erbB2 expression and an increase in apoptosis were also observed in some patients in the placebo group; they were small and insignificant. They may be attributed to other phytochemicals such as antioxidants, phytic acid, phytoesters, minerals, and vitamins in the whole wheat flour used in the placebo muffin, which have been related to reduced cancer risk (44).

Expression of c-erbB2 (HER2) has been associated with more aggressive phenotypes of breast cancer and an increased potential for forming metastases (45-47). In addition, it plays a role in cell differentiation, adhesion, and motility (48, 49). In recent studies, a negative correlation between HER1/2 expression and response to antiestrogenic, but not aromatase inhibitor therapy, has been shown (39). Our results therefore suggest that the intake of flaxseed has the potential to delay disease progression of preinvasive or invasive breast cancer by changing the phenotype of the cancer cells to a less aggressive form. The changes caused by flaxseed have also been shown by other endocrine agents such as tamoxifen (37, 40, 50), faslodex (51), raloxifene (41), and the aromatase inhibitors vorozole (52), letrozole (37, 38, 40), and anastrozole (38). A recent study concluded that the magnitude of

Ki-67 reduction can be taken as a surrogate end point biomarker for the efficacy of endocrine agents in breast cancer treatment, and that the efficacy of breast cancer endocrine therapy is dependent on the successful induction of the arrest of cell proliferation (40). This is the basis for numerous ongoing trials of novel endocrine agents and cell signaling inhibitors in the preoperative setting.

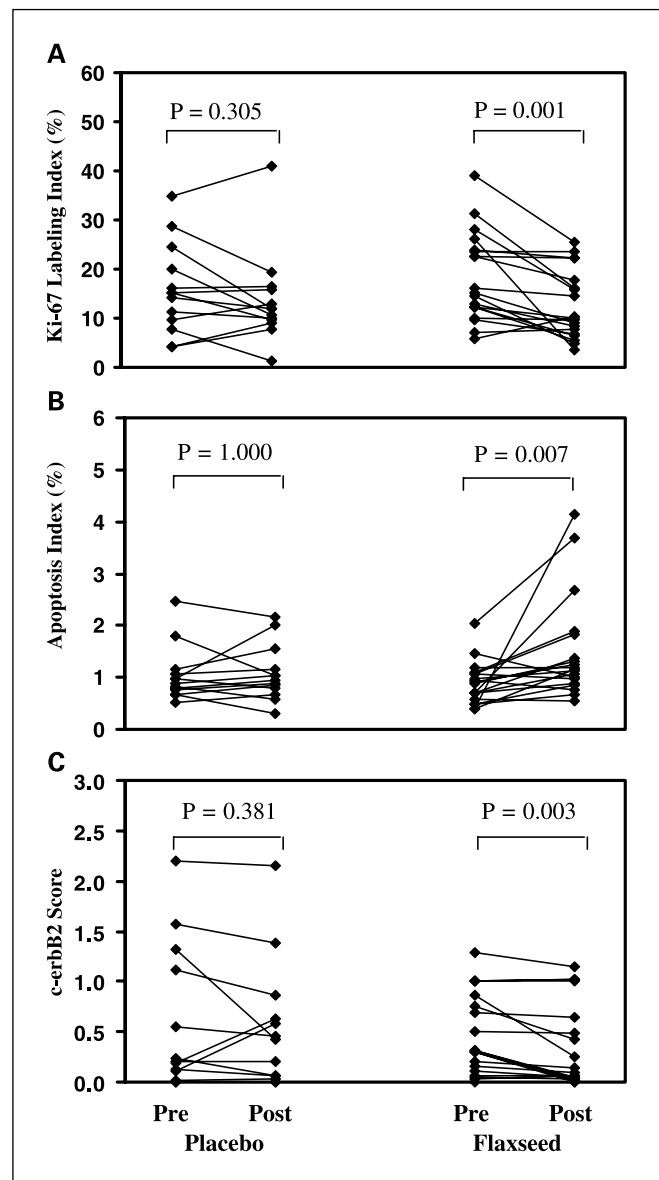
The results of most of the previous studies are not comparable with the present study because the treatment duration in neoadjuvant trials is usually at least 12 weeks. In the studies with shorter treatment duration (i.e., preoperative studies), the reduction in Ki-67 labeling index induced by tamoxifen was higher than that achieved by flaxseed in our study (50). For example, using a similar experimental design, daily treatment with 20 mg tamoxifen for a median of 21 days (range 6-65 days), resulted in a 46.4% reduction in Ki-67

labeling index (50). This is higher than the 34.2% decrease observed with 25 g flaxseed treatment in our study. In another study, treatment with raloxifene for 14 days resulted in a reduction in Ki-67 labeling index of 21% (41). However, flaxseed is a well-tolerated dietary intervention rather than a medication with concomitant side effects. The only side effects reported by subjects were increased abdominal fullness and bowel movements due to the high fiber content of flaxseed. As an increase in bowel movement may be desirable for patients with low fiber intake and chronic constipation, this effect may not be considered adverse compared to other side effects seen with breast cancer drugs such as tamoxifen or aromatase inhibitors (4). In ovariectomized athymic mice, no adverse effect on the major organs including the uterus (33) and bones<sup>4</sup> were observed after feeding 10% dietary flaxseed for up to 21 weeks, indicating that moderate intake of flaxseed is relatively safe.

The results of this clinical trial are in agreement with previous clinical and preclinical studies showing antitumor effects of flaxseed in prostate cancer patients (53), carcinogen-treated rats (32), athymic mice with established ER-positive (33), or ER-negative breast cancers (34, 35), and tumor-bearing transgenic mice (54). The 5% or 10% flaxseed diet used in the animal studies is approximately equivalent to 25 to 30 g of flaxseed given to patients with breast or prostate cancer, depending on the amount of other foods consumed. Our results are also in line with epidemiologic studies showing that high levels of lignan intake (26), plasma or urinary excretion of mammalian lignans (23–25) or high levels of  $\alpha$ -linolenic acid in adipose tissues (28) are associated with a reduced risk of breast cancer.

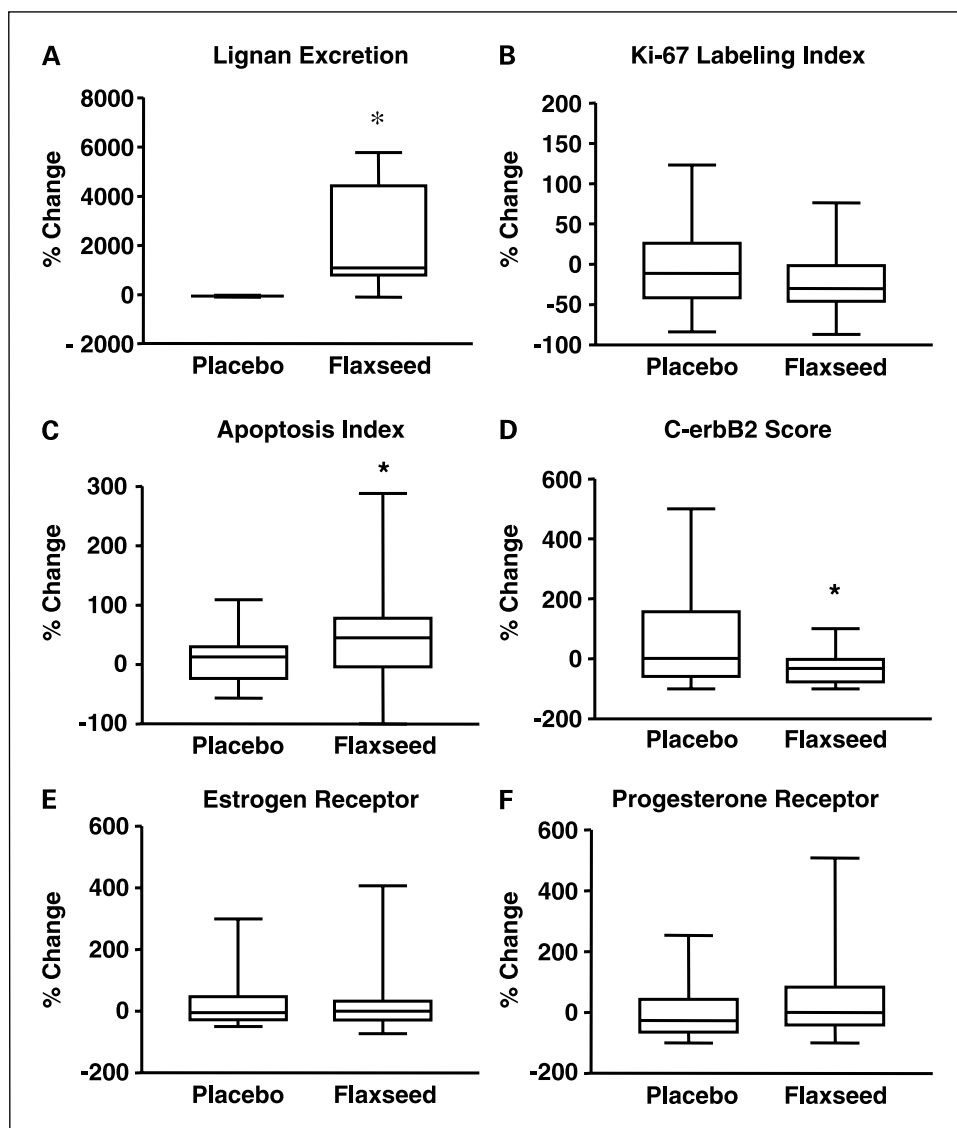
The mechanisms by which lignans exert their protective effects against hormone-related cancers have not yet been definitively clarified. In previous studies, investigators have focused on the ability of lignans to antagonize estrogen metabolism and action (9–11, 55, 56). The daily intake of 25 g flaxseed by postmenopausal women has been shown in our previous studies to increase the urinary levels of 2/16 $\alpha$ OHE1, indicating a reduction in estrogenic and potentially carcinogenic effects (56). It has been hypothesized that the lignans act as anti-estrogens by competing with estradiol for binding to the ER. The mammalian lignan enterolactone has also been shown to inhibit the activity of aromatase (9–11), leading to a reduction of endogenous estrogen synthesis.

The effects of flaxseed in patients with ER-positive tumors may in part be attributed to the high concentration of lignans and  $\alpha$ -linolenic acid in flaxseed (32) and their effects on estrogen metabolism. However, ER-negative tumors also showed a reduction in cell proliferation and increased apoptosis, indicating that flaxseed may have influenced the tumor through both endocrine and nonendocrine mechanisms. The hormone-independent effects of flaxseed may be due to the antioxidant and antiangiogenic properties of its lignans or due to other mechanisms. Enterodiol and enterolactone as well as their precursors secoisolariciresinol and hydroxymatairesinol have hydroxyl-radical scavenging properties (18–20). For example, short-term feeding of rats with flaxseed or secoisolariciresinol diglucoside has a sparing effect on hepatic endogenous



**Fig. 2.** Individual change in (A) Ki-67 labeling index; (B) apoptosis index; (C) c-erbB2 score in patients following treatment with placebo or flaxseed (Wilcoxon signed rank test).

<sup>4</sup>K. Power, N. Saarinen, J.M. Chen, W.E. Ward, L.U. Thompson, unpublished data.



**Fig. 3.** Median percentage change of urinary lignan excretion and biomarkers in subjects during the period of pre- and posttreatment in placebo and flaxseed. *A*, urinary lignan; *B*, Ki-67 labeling index; *C*, apoptosis index; *D*, c-erbB2 score; *E*, estrogen receptor index; *F*, progesterone receptor index. \*,  $P < 0.05$  versus placebo by Mann-Whitney test.

antioxidant status in young rats (21). The antiangiogenic effects of flaxseed are supported by numerous preclinical studies. For example, we have observed a reduction in human tumor growth, metastases, and vascular endothelial growth factor in athymic mice fed flaxseed (33–35). The mammalian lignan enterolactone also decreases the proliferation of endothelial cells *in vitro* (22) and inhibits aromatase (9–11).

The nonendocrine effect of flaxseed on tumor cells could also include inhibition of growth factor pathways. In addition to reducing tumor c-erbB2 expression in our study and in the mammary gland of rats (57), we have also found previously that flaxseed can significantly decrease the plasma levels of insulin-like growth factor 1 in carcinogen-treated rats (58) and tumor insulin-like growth factor 1 and epidermal growth factor receptor levels when athymic mice were injected with ER-negative breast cancer cells (34).

Another possible antitumorigenic effect of flaxseed is related to  $\alpha$ -linolenic acid, an *n*-3 fatty acid. An equivalent amount of  $\alpha$ -linolenic acid-rich oil in 5% flaxseed has been shown to reduce the growth of established tumors in carcinogen-treated

rats (32), possibly by contributing to the antiangiogenic effects of flaxseed (59–61) or by other antitumor mechanisms such as effects on humoral and cell-mediated immunity, alteration of growth factor-binding sites, and formation of cytotoxic lipid peroxidation products (62).

It was previously hypothesized that flaxseed might interfere with the antiestrogenic effects of tamoxifen due to its endocrine properties (33). However, our studies in athymic mice with established ER-positive human breast tumors (MCF-7) have shown that, in the short-term, flaxseed enhances the effectiveness of tamoxifen in reducing tumor growth, both in the presence of high and low levels of estrogen (33). *In vitro* studies have also shown that the steps involved in cancer metastases, i.e., invasiveness and adhesiveness of human tumor cells, are reduced to a greater extent by the lignans in combination with tamoxifen than by either one alone (63).

Our study is small and the results need to be confirmed in a larger number of patients for a longer treatment period before it can be definitively concluded that flaxseed has the potential to reduce the growth and invasiveness of breast cancer. Its

excellent tolerability, however, may make flaxseed particularly attractive for studies in breast cancer prevention, where healthy women should be offered well-tolerated interventions for long-term use. The interaction of flaxseed and its lignan and oil components with other hormonally active agents also needs to be addressed in the future. If the therapeutic index seen in this short-term study can be sustained over a long-term period, flaxseed, which is inexpensive and readily

available, may be a potential dietary alternative or adjunct to currently used breast cancer drugs.

## Acknowledgments

We thank L. Orcheson, F. Cheung, and M. Chen for technical assistance; Omega Products, Ltd., for providing the flaxseed; Canada Bread, Co., for the muffin preparation; and all the physicians, research staff, and patients who participated in the study.

## References

- Rose DP. Diet, hormones and cancer. *Annu Rev Public Health* 1993;14:1–17.
- Clemons M, Goss PE. Mechanisms of disease: estrogen and the risk of breast cancer. *N Engl J Med* 2001;344:276–85.
- Adlercreutz H. Western diet and Western disease: some hormonal and biochemical mechanisms and associations. *Scand J Clin Lab Invest* 1990;50:3–23.
- Clemons M, Danson S, Howell A. Tamoxifen (Nolvadex): a review. *Cancer Treat Rev* 2002;28:165–80.
- Smith IE, Dowsett M. Aromatase inhibitors in breast cancer. *N Engl J Med* 2003;348:2431–42.
- Thompson LU. Analysis and bioavailability of lignans. In Thompson LU, Cunnane SC, editors. *Flaxseed in human nutrition*, 2nd ed. Champaign: AOCS Press; 2003, p. 92–116.
- Nesbitt PD, Lam Y, Thompson LU. Human metabolism of mammalian lignan precursors in raw and processed flaxseed. *Am J Clin Nutr* 1999;69:549–55.
- Sathyamoorthy N, Wang TTY, Phang TM. Stimulation of pS2 expression by diet-derived compounds. *Cancer Res* 1994;54:957–61.
- Adlercreutz H, Bannwart K, Wahala T, et al. Inhibition of human aromatase by mammalian lignans and isoflavonoid phytoestrogens. *J Steroid Biochem Mol Biol* 1993;44:147–53.
- Wang C, Makela T, Hase T, Adlercreutz H, Kurzer MS. Lignans and flavonoids inhibit aromatase enzyme in human preadipocytes. *J Steroid Biochem Mol Biol* 1994;50:205–12.
- Brooks JD, Thompson LU. Mammalian lignans and genistein decrease the activities of aromatase and 17 $\beta$ -hydroxysteroid dehydrogenase in MCF-7 cells. *J Steroid Biochem Mol Biol*. In press 2005.
- Garreau B, Vallette G, Adlercreutz H, et al. Phytoestrogens: new ligands for rat and human  $\alpha$ -fetoprotein. *Biochem Biophys Acta* 1991;1094:339–45.
- Mousavi Y, Adlercreutz H. Enterolactone and estradiol inhibit each other's proliferative effect on MCF-7 breast cancer cells in culture. *J Steroid Biochem Mol Biol* 1992;41:615–9.
- Welshons WV, Murphy CS, Koch R, Calaf G, Jordan VC. Stimulation of breast cancer cells *in vitro* by the environmental estrogen enterolactone and the phytoestrogen equol. *Breast Cancer Res Treat* 1987;10:169–75.
- Wang C, Kurzer MS. Effect of phytoestrogens on DNA synthesis in MCF-7 cells in the presence of estradiol or growth factors. *Nutr Cancer* 1998;31:90–100.
- Adlercreutz H, Mousavi Y, Clark J, et al. Dietary phytoestrogens and cancer: *in vitro* and *in vivo* studies. *J Steroid Biochem Mol Biol* 1992;41:331–7.
- Saarinen NM, Warri A, Makela SI, et al. Hydroxymatairesinol, a novel enterolactone precursor with antitumor properties from coniferous tree (*Picea abies*). *Nutr Cancer* 2000;36:207–16.
- Kitts DD, Yuan YV, Wijewickreme, Thompson LU. Antioxidant activity of the flaxseed lignan secoisolariciresinol diglycoside and its mammalian lignan metabolites enterodiol and enterolactone. *Mol Cell Biochem* 1999;202:91–100.
- Prasad K. Hydroxyl radical-scavenging property of secoisolariciresinol diglycoside (SDG) isolated from flaxseed. *Mol Cell Biochem* 1997;168:117–23.
- Kangas L, Saarinen N, Mutanen M, et al. Antioxidant and antitumor effects of hydroxymatairesinol (HM-3000, HMR), a lignan isolated from the knots of spruce. *Eur J Cancer Prev* 2002;11:548–57.
- Yuan YV, Rickard SE, Thompson LU. Short term feeding of flaxseed or its lignan has minor influence on *in vivo* hepatic antioxidant status in young rats. *Nutr Res* 1999;19:1233–43.
- Fotsis T, Pepper M, Adlercreutz H, et al. Genistein, a dietary-derived inhibitor of *in vitro* angiogenesis. *Proc Natl Acad Sci U S A* 1993;90:2690–4.
- Ingram D, Sanders K, Kolybaba M, Lopez D. Case-control study of phyto-oestrogens and breast cancer. *Lancet* 1997;350:990–4.
- Dai Q, Franke AA, Jin F, et al. Urinary excretion of phytoestrogens and risk of breast cancer among Chinese women in Shanghai. *Cancer Epidemiol Biomarkers Prev* 2002;11:815–21.
- Pietinen P, Stumpf K, Mannisto S, Kataja V, Uusitupa M, Adlercreutz H. Serum enterolactone and risk of breast cancer: a case-control study in eastern Finland. *Cancer Epidemiol Biomarkers Prev* 2001;10:339–44.
- McCann SE, Moysich KB, Freudenheim JL, Ambrosone CB, Shields PG. The risk of breast cancer associated with dietary lignans differs by CYP17 genotype in women. *J Nutr* 2002;132:3036–41.
- Thompson LU, Robb P, Serraino M, Cheung F. Mammalian lignan production from various foods. *Nutr Cancer* 1991;16:43–52.
- Bougnoux P, Chajes V.  $\alpha$ -linolenic acid and cancer. In Thompson LU, Cunnane SC, editors. *Flaxseed in human nutrition*, 2nd ed. Champaign: AOCS Press; 2003, p. 232–44.
- Serraino M, Thompson LU. The effect of flaxseed supplementation on early risk markers for mammary carcinogenesis. *Cancer Lett* 1991;60:135–42.
- Serraino M, Thompson LU. The effect of flaxseed supplementation on the initiation and promotional stages of mammary tumorigenesis. *Nutr Cancer* 1992;17:153–9.
- Rickard SE, Yuan YV, Chen J, Thompson LU. Dose effects of flaxseed and its lignan on methyl-*N*-nitrosourea-induced mammary tumorigenesis in rats. *Nutr Cancer* 1999;35:50–7.
- Thompson LU, Rickard S, Orcheson L, Seidl MM. Flaxseed and its lignan and oil components reduce mammary tumor growth at a late stage of carcinogenesis. *Carcinogenesis* 1996;17:1373–6.
- Chen J, Hui E, Yip T, Thompson LU. Dietary flaxseed enhances the inhibitory effect of tamoxifen on the growth of estrogen-dependent human breast cancer (MCF-7) in nude mice. *Clin Cancer Res* 2004;10:7703–11.
- Chen J, Stavro M, Thompson LU. Dietary flaxseed inhibits breast cancer growth and metastasis and downregulates expression of epidermal growth factor receptor and insulin-like growth factor. *Nutr Cancer* 2002;43:187–92.
- Dabrosin C, Chen J, Wang L, Thompson LU. Flaxseed inhibits metastasis and decreases extracellular vascular endothelial growth factor in human breast cancer xenografts. *Cancer Lett* 2002;186:31–7.
- Thompson LU, Seidl M, Rickard S, Orcheson L, Fong HH. Antitumor effect of mammalian lignan precursor from flaxseed. *Nutr Cancer* 1996;26:159–65.
- Miller WR, Dixon JM, Macfarlane L, Cameron D, Anderson TJ. Pathological features of breast cancer response following neoadjuvant treatment with either letrozole or tamoxifen. *Eur J Cancer* 2003;39:462–8.
- Miller WR, Dixon JM, Cameron DA, Anderson TJ. Biological and clinical effects of aromatase inhibitors in neoadjuvant therapy. *J Steroid Biochem Mol Biol* 2001;79:103–7.
- Ellis MJ, Rosen E, Dressman J, Marks J. Neoadjuvant comparisons of aromatase inhibitors and tamoxifen: pre-treatment determinants of response and on-treatment effect. *J Steroid Biochem Mol Biol* 2003;86:301–7.
- Ellis MJ, Coop A, Singh B, et al. Letrozole inhibits tumor proliferation more effectively than tamoxifen independent of HER1/2 expression status. *Cancer Res* 2003;63:6523–31.
- Dowsett M, Bundred NJ, Decensi A, et al. Effect of raloxifene on breast cancer cell Ki67 and apoptosis: a double-blind, placebo-controlled, randomized clinical trial in postmenopausal patients. *Cancer Epidemiol Biomarkers Prev* 2001;10:961–6.
- Health and Welfare Canada. *Nutrient value of some common foods*. Ottawa, Canada: Supply and Services; 1991.
- Rickard SR, Orcheson LJ, Seidl MM, Luyengi L, Fong HH, Thompson LU. Dose-dependent production of mammalian lignans in rats and *in vitro* from the purified precursor secoisolariciresinol diglycoside from flaxseed. *J Nutr* 1996;126:2012–9.
- Slavin J. Why whole grains are protective: biological mechanisms. *Proc Nutr Soc* 2003;62:129–34.
- Gullick WJ, Love SB, Wright C, et al. c-erb B-2 protein overexpression in breast cancer is a risk factor in patients with involved and uninvolved lymph nodes. *Br J Cancer* 1991;63:434–8.
- Tan M, Yao J, Yu D. Overexpression of the c-erbB2 gene enhanced intrinsic metastasis potential in human breast cancer cells without increasing their transformation abilities. *Cancer Res* 1997;57:1199–205.
- DiGiovanna MP, Carter D, Fly SD, Stern DF. Functional assay for HER-2/neu demonstrates active signaling in a minority of HER-2/overexpressing invasive human breast tumors. *Br J Cancer* 1996;74:802–6.
- De Potter CR, Schellhout AM. The neu-protein and breast cancer. *Virchows Arch* 1995;426:107–15.
- Hanna W, Kahn HJ, Trudeau M. Evaluation of HER-2/neu (erbB-2) status in breast cancer: from bench to bedside. *Mod Pathol* 1999;12:827–34.
- Clarke RB, Laidlaw IJ, Jones LJ, Howell A, Anderson E. Effect of tamoxifen on Ki67 labeling index in human breast tumors and its relationship to oestrogen and progesterone receptor status. *Br J Cancer* 1993;67:606–11.
- Robertson JF, Nicholson RI, Bundred NJ, et al. Comparison of the short-term biological effects of 7 $\alpha$ -[9-(4,4,5,5,5-pentafluoropentylsulfanyl)-nonyl]estra-1,3,5,(10)-triene-3,17 $\beta$ -diol (Faslodex) versus tamoxifen in postmenopausal women with primary breast cancer. *Cancer Res* 2001;61:6739–46.
- Harper-Wynne CL, Sacks NP, Shenton K, et al. Comparison of the systemic and intratumoral effects of tamoxifen and the aromatase inhibitor vorozole in postmenopausal patients with primary breast cancer. *J Clin Oncol* 2002;20:1026–35.
- Demark-Wahnefried W, Price DT, Polascik TJ, et al. Pilot study of dietary fat restriction and flaxseed supplementation in men with prostate cancer before surgery: exploring the effects on hormonal levels,

- prostate-specific antigen, and histopathologic features. *Urology* 2001;58:47–52.
54. Lin X, Gingrich JR, Li J, Haroon ZA, Demark-Wahnefried W. Effect of flaxseed supplementation on the development of prostatic neoplasia and metastasis in transgenic (TRAMP) mice. *J Nutr* 2001;131:3136–7S.
55. Bradlow H. 2-hydroxyestrone: the “good” estrogen. *J Endocrinol* 1996;150:S259–65.
56. Brooks JD, Ward WE, Lewis JE, et al. Diet supplemented with flaxseed alters estrogen metabolism in postmenopausal women to a greater extent than with an equal amount of soy. *Am J Clin Nutr* 2004;79:318–25.
57. Tan KP, Chen J, Ward WE, Thompson LU. Mammary gland morphogenesis is enhanced by exposure to flaxseed or its major lignan during suckling in rats. *Exp Biol Med (Maywood)* 2004;229:147–57.
58. Rickard SR, Yuan YV, Thompson LU. Plasma insulin-like growth factor I levels in rats are reduced by dietary supplementation of flaxseed or its lignan secoisolariciresinol diglycoside. *Cancer Lett* 2000;161:47–55.
59. Rose DP, Connolly JM. Regulation of tumor angiogenesis by dietary fatty acids and eicosanoids. *Nutr Cancer* 2000;37:119–27.
60. Rose DP, Connolly JM, Yayburn J, Coleman M. Influence of diets containing eicosapentanoic or docosahexanoic acid on growth and metastasis of breast cancer in nude mice. *J Natl Cancer Inst* 1995;87:587–92.
61. Natarajan R, Nadler J. Roles of lipoygenase in breast cancer. *Front Biosci* 1998;8:E81–8.
62. Rose DP. Dietary fatty acids and prevention of hormone responsive cancer. *Proc Soc Exp Biol Med* 1997;216:224–33.
63. Chen J, Thompson LU. Lignans and tamoxifen, alone or in combination, reduce human breast cancer cell adhesion, invasion and migration *in vitro*. *Breast Cancer Res Treat* 2003;80:163–70.