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Effect of Dietary Flaxseed on Serum Levels of Estrogens and Androgens in Postmenopausal Women

Susan R. Sturgeon and Joanna L. Heersink
Division of Biostatistics and Epidemiology, School of Public Health and Health Sciences, University of Massachusetts Amherst, Amherst, Massachusetts, USA

Stella L. Volpe
Division of Biobehavioral and Health Sciences, School of Nursing, University of Pennsylvania, Philadelphia, Pennsylvania, USA

Elizabeth R. Bertone-Johnson and Elaine Puleo
Division of Biostatistics and Epidemiology, School of Public Health and Health Sciences, University of Massachusetts Amherst, Amherst, Massachusetts, USA

Frank Z. Stanczyk
Department of Obstetrics and Gynecology, Keck School of Medicine, University of Southern California, Los Angeles, California, USA

Sara Sabelawski
Department of Nutrition, School of Public Health and Health Sciences, University of Massachusetts Amherst, Amherst, Massachusetts, USA

Kristina Wähälä
Laboratory of Organic Chemistry, Department of Chemistry, University of Helsinki, Helsinki, Finland

Mindy S. Kurzer
Department of Food Science and Nutrition, University of Minnesota, Saint Paul, Minnesota, USA

Carol Bigelow
Division of Biostatistics and Epidemiology, School of Public Health and Health Sciences, University of Massachusetts Amherst, Amherst, Massachusetts, USA

Flaxseed is a rich source of dietary lignans. Experimental studies suggest lignans may exert breast cancer preventive effects through hormonal mechanisms. Our aim was to study the effects of flaxseed on serum sex hormones implicated in the development of breast cancer. Forty-eight postmenopausal women participated in a 12-wk preintervention–postintervention study. Participants consumed 7.5 g/day of ground flaxseed for the first 6 wk and 15.0 grams/day for an additional 6 wk. Nonsignificant declines were noted over the 12 wk (95% confidence intervals) for estradiol (pg/ml), estrone (pg/ml), and testosterone (pg/ml): –4.4 (–12.6 to 3.9), –3.3 (–7.7 to 1.2), –4.7 (–17.8 to 8.5), respectively. Changes tended to be more pronounced in overweight/obese women, particularly for estrone (–6.5, –11.9 to –1.2; $P = .02$). Our results suggest that dietary flaxseed may modestly lower serum levels of sex steroid hormones, especially in overweight/obese women.

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Address correspondence to Susan R. Sturgeon, Dr PH, Division of Biostatistics and Epidemiology, School of Public Health and Health Sciences, University of Massachusetts, 715 North Pleasant Street, Amherst, MA 01003-9304. E-mail: ssturgeon@schoolph.umass.edu

INTRODUCTION

Endogenous levels of estrogens and androgens are believed to play a central role in the etiology of breast cancer (1). After menopause, most estrogen is derived by the aromatase conversion of plasma androstenedione to estrone in adipose tissue (2). Some estrone is metabolized to estradiol. Free estradiol, unbound to sex hormone-binding globulin (SHBG) is hypothesized to be the most biologically active fraction in the breast. Estrone sulfate is an abundantly circulating estrogen that serves

as a reservoir for the more biologically active estrogens. Epidemiologic studies have indicated that elevated serum levels of total and free estradiol, estrone, estrone sulfate, and lower levels of SHBG after menopause each substantially increase the risk of breast cancer (1). Recent cohort studies (3,4), although not all (5,6), also have suggested that elevated serum levels of testosterone, free testosterone, and androstenedione may independently increase breast cancer risk.

Lignans, naturally occurring compounds structurally similar to sex steroid hormones, are found in low levels in a wide variety of grains, fruits, and vegetables (7). In humans, plant lignans are converted by colonic microflora to the mammalian lignans, enterolactone and enterodiol (8). Because breast cancer incidence rates are lower in Asian countries where women typically consume diets rich in fruits and vegetables, it has been hypothesized that lignans may have cancer preventative properties.

Lignans have been shown to inhibit cell proliferation and reduce mammary tumor incidence in rats (9,10). Experimental studies imply lignans exert breast cancer preventive effects through hormonal mechanisms. Specifically, lignans may contribute to decreased bioavailable estrogen in circulation through inhibition of aromatase activity in adipose tissue (11,12) and increased SHBG synthesis in the liver (9,13). Lignins, precursors to plant lignans, may be effective in binding to testosterone (14). Through this binding process, testosterone may be more readily excreted in the bile, thereby potentially lowering circulating levels.

There is limited information on the effect of plant lignan intake on serum sex hormone levels in humans. Urinary levels of enterolactone were associated positively with serum levels of SHBG and inversely associated with serum levels of estradiol in one cross-sectional study (15). Another cross-sectional study reported an inverse association between serum testosterone levels and number of servings of foods rich in dietary lignans (i.e., dark brown bread) (16).

Dietary flaxseed is, by far, the richest dietary source of lignans (17). To date, three intervention studies have evaluated the impact of dietary flaxseed consumption on serum levels of sex hormones in postmenopausal women (18–20). One randomized cross-over study of 28 postmenopausal nuns reported serum levels of estradiol and estrone sulfate decreased significantly with flaxseed supplementation, although SHBG and testosterone concentrations were unchanged (18). Two other small intervention studies, each involving 20 or fewer women in the flaxseed treatment group, reported no changes in serum concentrations of estradiol, estrone, estrone sulfate, or SHBG associated with a flaxseed diet (19–20). By administering a well-tolerated flaxseed dose and employing a high-quality assay to measure hormone levels, this dietary flaxseed preintervention–postintervention study was designed to address potentially important methodologic limitations of prior studies, specifically noncompliance and hormone measurement error.

MATERIALS AND METHODS

Study Population and Experimental Design

The 48 study participants were recruited from the Amherst, Massachusetts area using newspaper advertisements and posted advertisements from December 2003 to June 2004. Eligible participants were defined as women who had undergone natural menopause (defined by cessation of menses for one or more years); were English speaking; did not have any bowel disease, cancer, or diabetes; had not taken antibiotics in the past 6 mo (because antibiotics may interfere with the microbial conversion of plant lignans to mammalian lignans); had not taken certain medications within the past 6 mo (i.e., hormone therapy, oral corticosteroids, anticoagulants); and were nonsmokers.

The study was explained at an in-person introductory visit, and written informed consent was obtained. Subjects were asked to follow certain dietary restrictions for at least 3 wk prior to a baseline visit and throughout the follow-up period. Specifically, participants were requested to avoid flaxseed and flaxseed-containing foods, all soy and soy products; dietary supplements, herbs, or teas known to have phytoestrogen activity (specifically isoflavones, natural estrogens, plant estrogens, genistein, dong quai, ginseng, black cohosh, *Vitex*, chastree, wild yam, motherwort, lemon balm, licorice, and red clover); and cruciferous vegetables (specifically broccoli, broccoli sprouts, brocoflower, brocolini, Brussels sprouts, cauliflower, cabbage, bok choy, Chinese cabbage, kale, Swiss chard, kohlrabi, rutabaga, turnips, collard greens, mustard greens, turnip greens, and watercress) because of their potential effects on endogenous hormone levels. Initially, participants were asked to refrain from alcohol consumption because it is known to alter endogenous hormone levels (21). However, this requirement made subject recruitment difficult. The alcohol requirements were therefore relaxed, requiring subjects to maintain their normal alcohol consumption but consume no more than two alcoholic beverages per day and no more than 7 alcoholic beverages per week. Study participants were asked to maintain their usual diet and exercise patterns throughout the study.

At the baseline visit, a 20 ml nonfasting blood specimen was drawn by a nurse phlebotomist (between the hours of 7:00 AM and 10:00 AM), and study participants submitted a 24-h urine specimen that had been collected over the previous day. In addition, study participants completed a 7-day dietary recall (7DDR; University of Massachusetts Medical School, Worcester), which queried dietary intake of 118 food categories or individual foods and 13 beverage items consumed over the previous week (22). The 7DDR also evaluates recreational and exercise activities, occupational activities, and household and childcare activities over the prior 28-day period. The 7DDR has been validated cross-sectionally against 24-h dietary recalls in three separate studies in a total of 261 subjects (23). Height and body weight was also measured at this visit, and a self-administered questionnaire was used to elicit information on sociodemographic factors and standard breast cancer risk factors.

At the baseline visit, study participants were instructed to consume one tablespoon of ground flaxseed (7.5 g) per day until the scheduled first follow-up visit, approximately 6 wk later. At the first follow-up visit, study participants were instructed to consume two tablespoons of ground flaxseed per day (15 g) until the second follow-up visit, 6 wk after the first follow-up visit. As with the baseline visit, at each of the follow-up visits, a 20 ml blood specimen was obtained, and participants provided a 24-h urine specimen that had been collected over the previous day. In addition, study participants completed a 7DDR and were weighed.

Stabilized flaxseed without any additives was purchased from the Essential Nutrient Research Corporation (ENRECO, Newton, WI). All of the flaxseed used in this study originated from a single production lot and was shipped to the University of Massachusetts Amherst in two separate shipments. Whole flaxseed was stored in cold storage and was ground immediately prior to shipping. A 1-lb bag of ground flaxseed and a tablespoon measure with instructions for standard measurement procedures was provided for each subject at the baseline visit, and any unused flaxseed was returned at the first follow-up visit. Two 1-lb bags of ground flaxseed were provided for each subject at the first follow-up visit, and any unused flaxseed was returned at the second follow-up visit. Study participants were instructed to sprinkle it on any cold food of their choice, for example, cottage cheese, yogurt, cereal, applesauce, pudding, or salad. They were instructed not to cook or bake with the flaxseed. Participants were provided with already ground flaxseed to maximize adherence, and were instructed to store the flaxseed in the refrigerator to prevent spoiling. The flaxseed that was returned at each follow-up visit was weighed to determine subject compliance.

Laboratory Analyses

Blood specimens were immediately processed and frozen at -80°C. Hormone assays were performed at the Reproductive Endocrine Research Laboratory at the University of Southern California, Los Angeles, directed by one of the authors (F. Z. Stanczyk). For each study participant, all three serum samples were included in the same analysis batch; the order in which samples were placed into a batch was randomized. Two replicate quality control blood specimens from two postmenopausal women were also inserted randomly into each of the 4 batches, for a total of 16 assessments of laboratory assay quality. Laboratory technicians were blinded to the identification of all samples from study and quality control subjects.

Concentrations of testosterone, estrone, and estradiol were measured by sensitive and specific radioimmunoassays (RIAs) after organic solvent extraction and Celite column partition chromatography as described previously (24,25). The sensitivities of the testosterone, estrone, and estradiol RIAs are 1.5 ng/dl, 4 pg/ml, and 3 pg/ml, respectively. SHBG was quantified by a solid-phase, 2-site, chemiluminescent immunoassay using the Immulite analyzer (Diagnostic Products Corp., Inglewood, CA). Bioavailable (non-SHBG bound) testosterone and estradiol concentrations were calculated using a validated algorithm on the basis of total testosterone estradiol, and SHBG concentrations, as well as an assumed albumin concentration (26–28). Based on the blinded quality control serum replicates inserted from two postmenopausal women, intra-assay coefficient of variations, respectively, were 9.4% and 8.2% for testosterone, 11.5% and 12.9% for estrone, 15.5% and 14.3% for estradiol, and 3.9% and 3.4% for SHBG.

Urine was collected in containers with ascorbic acid, and after recording the weight, 0.1% sodium azide was added as a preservative. Urine specimens were immediately processed and frozen at -20°C and shipped on dry ice to the Department of Nutrition and Food Science (University of Minnesota, St. Paul). Urinary concentrations of enterodiol, as a marker of flaxseed compliance, were measured using a modification of standard gas-chromatography/mass-spectrometry methods (29).

Statistical Analyses

Dietary and physical activity variables were created from the 7DDR administered at each of the three visits. Physical activity levels were calculated as total minutes per week and metabolic equivalent task (MET) hours per day following the method of Ainsworth and colleagues (30). Macronutrient and micronutrient intake in grams per day were adjusted for total energy intake using the residual method (31).

Repeated measures analysis of variance was performed to assess the significance of changes over time in hormone levels. Repeated measures analysis of covariance was used to determine changes in the hormone levels after adjustment for selected dietary factors, weight, and level of physical activity. Dietary factors evaluated included total energy intake and energy adjusted macronutrients and micronutrients (total fat, total saturated fat, total protein, vegetable protein, animal protein total carbohydrates, alcohol, total fiber, insoluble fiber, and linoleic acid). The decision to retain potential confounders in the repeated measures analysis of covariance was based on initial assessments of changes over time in each of the preceding covariates and utilized the F test ($P \leq 0.05$) in a repeated measures analysis.

RESULTS

As shown in Table 1, the study population was mainly non-Hispanic White (95%) and college educated (74%), with a mean age of 57 $\pm$ 4.8 yr. Based on an analysis of the weights of distributed and returned flaxseed, mean daily flaxseed intake ($\pm$ SD) was 7.8 $\pm$ 1.6 g/day and 15.4 $\pm$ 2.8 g/day during the first and second follow-up periods, respectively. Mean urinary enterodiol levels at baseline, 6 wk, and 12 wk were 0.6 mg/day, 2.39 mg/day, and 5.75 mg/day, respectively, showing a rise as expected when higher doses of flaxseed were ingested. There were no changes in study subject weight or level of physical activity over the intervention period (results not shown). Mean

TABLE 1
Baseline Characteristics of Study Participants[a]

Characteristic	Mean (SD)	No.[b]	%
Age (yr)	57 (4.8)		
MET (h/day)	2.8 (0.5)		
Minutes of activity per week	260 (47)		
Total energy intake (kcal/day)	1,732 (139.1)		
Race			
White		46	94.8
Other		1	2.1
Education			
High school or some college		13	27.1
College graduate		35	72.9
Body mass index (kg/m^2)			
< 25		25	53.2
≥ 25		22	46.8
Menopausal hormone use (yr)			
Never		27	56.3
< 5		13	27.1
≥ 5		7	16.4
Ever smoked cigarettes			
No		31	64.6
Yes		17	35.4
Family history of breast cancer			
No		39	81.3
Yes		9	18.8
History of benign breast disease			
No		37	77.1
Yes		11	22.9

[a]n = 48. Abbreviation is as follows: MET = metabolic task equivalent.
[b]May not add up to 48 (100%) due to missing data.

body weights (kg) were 67.5, 67.5, and 67.2 at the baseline visit, first follow-up visit, and second follow-up visit, respectively.

There were small declines in serum levels of testosterone, estrone, and estradiol after 6 and 12 wk of flaxseed ingestion among all women, although these declines were not statistically significant (Table 2). Body mass index (BMI) is a recognized breast cancer risk factor; the elevated risk in overweight women likely reflects higher circulating estrogens due to increased aromatase activity in adipose tissue (32). At baseline, circulating levels of estradiol across 3 weight groups (<25, 25–30, >30 kg/m^2) were 15.1, 11.1, and 15.6 pg/ml, respectively ($P = 0.86$), whereas comparable estrone levels were 24.6, 25.9, and 58.6 pg/ml ($P < 0.001$), respectively. Because dietary lignans have been shown to inhibit aromatase activity (11,12), we examined the effects of flaxseed on serum sex hormone levels separately in normal weight (BMI < 25 kg/m^2) and overweight/obese (BMI ≥ 25 kg/m^2) women. Among overweight/obese women, we observed a pattern of more substantial declines in serum levels of testosterone, estrone, and estradiol associated with consuming flaxseed. The decline among overweight/obese women was statistically significant for testosterone at 6 wk and for estrone at 6 and 12 wk. Among normal weight women, we also observed small suggestive declines in serum sex hormone levels, but these were not statistically significant.

A total of 6 study participants had estradiol concentrations $\geq$ 30 pg/ml at any of the 3 study visits. All study subjects had follicle-stimulating hormone levels in the postmenopausal range, and none reported use of hormone therapy during the study period (results not shown). All analyses were rerun excluding these participants to determine their impact on the study findings. Results were generally similar to those reported above. Among overweight/obese women, for example, there continued to be statistically significant declines in estrone from baseline to Follow-Up 1, and from baseline to Follow-Up 2 (–5.9 pg/ml and –3.9 pg/ml, respectively). There were also statistically significant declines from baseline to Follow-Up 1 for testosterone (–15.9 pg/ml) and estradiol (–1.5 pg/ml), but changes in these hormones were modest and not statistically significant from baseline to Follow-Up 2 (–6.6 pg/ml and –0.6 pg/ml, respectively).

When we further restricted the data set to 6 obese women ($\geq$30 kg/m^2; results not shown), we observed statistically significant declines between baseline and Follow-Up 1 for testosterone (–34.2 pg/ml) and estrone (–18.4 pg/ ml). Comparable values between baseline and Follow-Up 2 were –15.6 pg/ml and –10.9 pg/ml, respectively, although these were not statistically significant.

Overall, there was little evidence that consuming a higher dose of flaxseed for 12 wk resulted in a greater decline in serum sex hormone levels than consumption of flaxseed for 6 wk in either subgroup. Serum levels of SHBG were essentially unchanged between the first and second follow-up visits. Although mean intake of vegetable protein, carbohydrate, and linoleic acid changed significantly over the intervention periods, the results in Table 2 were essentially unchanged after adjustment for these factors.

DISCUSSION

Consuming 7.5 g of flaxseed per day for 6 wk and 15 g of flaxseed for an additional 6 wk resulted in modest, nonstatistically significant declines in serum levels of testosterone, estrone, and estradiol but not SHBG in this group of postmenopausal women. In the subset of overweight women, the mean reduction of 6.5 pg/ml for estrone was statistically significant. In the randomized cross-over trial of 28 postmenopausal women by Haggans and colleagues (18), 5 and 10 g/day flaxseed diets for 7 wk reduced serum concentrations of estradiol and estrone sulfate but not estrone. By contrast, dietary flaxseed at much higher prescribed doses (25 g/day for 16 weeks, and 40 g/day for 3 mo, respectively) have not been shown to alter serum levels of estrogens or SHBG in 2 small intervention studies (19,20). The difference between our findings and those from prior studies may reflect noncompliance to high-dose flaxseed regimens,

TABLE 2
Serum Sex Hormone Levels at Baseline and Change Associated With Dietary Flaxseed Intervention Among Postmenopausal Women and by Category of Body Mass Index[a]

	Baseline Level	Change from Baseline to Follow-Up 1			Change from Baseline to Follow-Up 2[b]		
	Mean	Mean (95% CI)	*P* Value	%	Mean (95% CI)	*P* Value	%
All women ($n = 48$)[c]							
Testosterone (pg/ml)	181	−9.9 (−23.8 to 4.0)	0.16	−5.5	−4.7 (−17.8 to 8.5)	0.48	−2.6
Estrone (pg/ml)	30.0	−2.6 (−6.7 to 1.5)	0.21	− 8.7	−3.3 (−7.7 to 1.2)	0.15	−11.0
Estradiol (pg/ml)	14.1	−2.7 (−6.6 to 1.2)	0.17	−19.1	−4.4 (−12.6 to 3.9)	0.29	−31.2
SHBG (nmol/l)	49.1	0.6 (−2.5 to 3.7)	0.71	+1.2	.04 (−2.7 to 2.0)	0.98	0.0
Bioavailable estradiol (pg/ml)	8.9	−0.2 (−4.1 to 3.7)	0.92	−2.2	−2.8 (−7.5 to 2.0)	0.25	−31.4
Bioavailable testosterone (pg/ml)	9.1	−0.7 (−1.5 to 0.14)	0.10	−7.7	−0.4 (−1.1 to 0.4)	0.33	−4.4
Overweight/obese ($n = 22$)[d]							
Testosterone (pg/ml)	180	−14.6 (−28.1 to 1.1)	0.03	−8.1	−7.0 (−18.2 to 4.2)	0.20	−3.9
Estrone (pg/ml)	36.3	−7.6 (−14.2 to 0.9)	0.03	-19.4	−6.5 (−11.9 to 1.2)	0.02	−18.0
Estradiol (pg/ml)	12.5	−3.6 (−9.4 to 2.2)	0.21	-20.9	−3.4 (−9.3 to 2.4)	0.24	−27.2
SHBG (nmol/l)	38.0	−0.1 (−2.1 to 1.9)	0.93	-0.0	−1.1 (−3.0 to 0.8)	0.25	−2.9
Bioavailable estradiol (pg/ml)	8.7	1.1 (−7.0 to 9.3)	0.77	+12.6	−2.2 (−5.6 to 1.3)	0.21	−25.3
Bioavailable testosterone (pg/ml)	10.3	−1.0 (−1.7 to −0.2)	0.02	-10.9	−0.3 (−1.1 to 0.5)	0.38	−2.5
Normal Weight ($n = 25$)[e]							
Testosterone (pg/ml)	182	−6.7 (−31.6 to 18.2)	0.58	− 3.7	−2.1 (−25.9 to 21.7)	0.86	−1.2
Estrone (pg/ml)	24.6	1.4 (−3.8 to 6.6)	0.58	+5.7	−0.5 (−7.8 to 6.8)	0.88	−2.0
Estradiol (pg/ml)	15.6	−2.1 (−7.9 to 3.7)	0.47	−13.5	−5.3 (−20.6 to 10.0)	0.48	−34.0
SHBG (nmol/l)	59.9	1.3 (−4.5 to 7.2)	0.64	+2.2	1.1 (−5.7 to 7.9)	0.74	+1.8
Bioavailable estradiol (pg/ml)	9.2	−1.4 (−4.7 to 1.8)	0.38	−15.2	−3.3 (−12.0 to 5.4)	0.44	−35.9
Bioavailable testosterone (pg/ml)	8.0	−0.5 (−2.0 to 0.9)	0.47	−6.3	−0.4 (−1.7 to 0.9)	0.56	−5.0

[a] Abbreviations are as follows: SHBG, sex hormone-binding globulin; BMI, body mass index.
[b] Excludes 1 woman without a follow-up 2 visit.
[c] Includes 1 woman with missing height.
[d] BMI $\geq$ 25kg/m^2.
[e] BMI $<$ 25 kg/m^2.

hormone measurement variability, or differences in body weight across studies.

We observed a decline in testosterone after flaxseed consumption, consistent with a report that plant lignins can bind and promote excretion of testosterone in the bile (14). In contrast to our findings, the randomized cross-over study in postmenopausal women by Haggans and colleagues (18) observed no change in testosterone levels. However, in one cross-sectional study of approximately 250 postmenopausal women in Wisconsin (16), serum testosterone was inversely associated with number of servings per week of whole grain products from the dark bread group ($r = –.20$, $P = 0.01$). This association was attributed to lignan content because no overall association between dietary fiber and testosterone concentration was observed.

One cross-sectional study of approximately 40 women reported a positive association between urinary excretion of enterolactone and serum SHBG, an inverse association between enterolactone and serum testosterone levels, and an inverse association between enterolactone and serum estradiol levels (31). Similarly to the other intervention studies (18–20), we did not observe an association between SHBG and flaxseed ingestion in the present study.

Ziegler (34) highlighted the methodological challenges in interpreting findings from observational studies on the relationship between lignan exposure and risk of breast cancer. Three case-control studies (35–37) and 6 cohort studies (38–43) have examined the relationship between enterolactone in serum or urine, as markers of lignan exposure, and risk of breast cancer. The three case-control studies reported substantial reductions in breast cancer risk after menopause associated with urinary excretion of enterolactone (35–37). Interestingly, our data are consistent with observational evidence that an inverse association between urinary excretion of lignans and risk of breast

cancer may be more pronounced among women with a higher BMI (35). Among overweight women, Dai and colleagues (37) found that breast cancer risk decreased steadily with increasing tertiles of urinary lignan levels (relative risk = 1.0, 0.72, and 0.27). By contrast, the comparable relative risks among overweight women were 1.0, 1.53, and 0.70. It is possible that changes in metabolism or in dietary intake after a breast cancer diagnosis may account for the observed associations in case-control studies. Of the 5 cohort studies, only one observed an inverse association between enterolactone exposure and risk of breast cancer (38). As noted by Ziegler (34), however, single measures of enterolactone in biological specimens such as those used in cohort studies, although capturing potentially important individual variation in colonic metabolism of dietary lignans, may result in missed associations, as they do not reflect integrated long-term exposure.

Three other case-control studies (44–46) and two cohort studies (47,48) have examined the relation between dietary lignan intake and risk of breast cancer. Women with higher dietary lignan intake tended to have reduced breast cancer risk in three studies (44,45,48). Although dietary measures of lignans may be better at integrating exposure over a long time period, databases for lignan content of food may not be comprehensive. In addition, estimates of breast cancer risk vary depending on which measures of lignan intake is employed: absolute quantity of plant lignans (matairesinol and secoisolaciresinol) or estimated bioavailable lignans (enterolactone and enterodiol) using a conversion factor derived from an in vitro model for colonic fermentation (34). Other more general limitations of research in this field have also been noted, including that the level and variation in lignan exposure is low in most populations studied to date, and many dietary questionnaires do not include foods that are especially high in lignan content, including dietary flaxseed. These limitations would tend to bias the association between lignans and risk of breast cancer toward the null.

This study has several limitations. First, it was relatively small and thus small changes in serum sex hormones due to flaxseed are difficult to detect. In addition, the study lacks a placebo control group. Nevertheless the findings suggest that consumption of one to two tablespoons of flaxseed per day for 12 wk may favorably influence estrogen and androgen concentrations, particularly in overweight/obese women. It is also important to emphasize flaxseed is a whole food that is notably rich in lignans, the plant-based omega-3 fatty acid (i.e., alpha-linolenic acid) and fiber, and it cannot be assumed that a single dietary component is responsible for observed biological effects. In summary, additional research focused on clarifying the effects of flaxseed on breast biology is warranted. Such research is important because of the promotion of flaxseed as having breast cancer preventative effects and its increasing widespread availability.

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