

Reduction in Ki-67 in Benign Breast Tissue of High-Risk Women with the Lignan Secoisolariciresinol Diglycoside

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Abstract

Preclinical and correlative studies suggest reduced breast cancer with higher lignan intake or blood levels. We conducted a pilot study of modulation of risk biomarkers for breast cancer in premenopausal women after administration of the plant lignan secoisolariciresinol given as the diglycoside (SDG). Eligibility criteria included regular menstrual cycles, no oral contraceptives, a >3-fold increase in 5-year risk, and baseline Ki-67 of $\geq 2\%$ in areas of hyperplasia in breast tissue sampled by random periareolar fine-needle aspiration (RPFNA) during the follicular phase of the menstrual cycle. SDG (50 mg/d) was given for 12 months, followed by repeat RPFNA. The primary end point was change in Ki-67. Secondary end points included change in cytomorphology, mammographic breast density, serum bioavailable estradiol and testosterone insulin-like growth factor-I and IGF-binding protein-3, and plasma lignan levels. Forty-five of 49 eligible women completed the study with excellent compliance (median = 96%) and few serious side effects (4% grade 3). Median plasma enterolactone increased ~ 9 -fold, and total lignans increased 16-fold. Thirty-six (80%) of the 45 evaluable subjects showed a decrease in Ki-67, from a median of 4% (range, 2-16.8%) to 2% (range, 0-15.2%; $P < 0.001$, Wilcoxon signed rank test). A decrease from baseline in the proportion of women with atypical cytology ($P = 0.035$) was also observed. Based on favorable risk biomarker modulation and lack of adverse events, we are initiating a randomized trial of SDG versus placebo in premenopausal women. *Cancer Prev Res*; 3(10); 1342-50. ©2010 AACR.

Introduction

Secoisolariciresinol diglycoside (SDG) is a polyphenolic plant lignan, which, when administered orally, is hydrolyzed to secoisolariciresinol (SECO) and further metabolized by intestinal bacteria to the biologically active mammalian lignans enterodiol (END) and enterolactone (ENL) (1-4). In a high-estrogen environment, these lignans act as partial estrogen antagonists in a tissue-specific manner (5-9). SDG is found in highest concentration in flaxseed but is also present in other oil-rich seeds, nuts, whole grains, legumes, and certain fruits and vegetables (1, 10, 11). The typical Western diet provides <10 mg/d of lignans (11-13). Administration of flaxseed or SDG is

associated with reduced estrogen receptor-negative (ER⁻) and ER⁺ mammary cancers in preclinical studies (14-17).

Some human studies show an inverse correlation between lignan intake or blood levels and breast cancer incidence, but others do not (18-32). This inconsistency is not surprising given the inherent limitations of dietary recall, early use of intake questionnaires with incomplete validation for lignans, variation in lignan metabolism, single-point blood collections, and different populations studied (33-37). However, for premenopausal women, the preponderance of evidence suggests that there is a reduced cancer incidence with higher lignan intakes or plasma ENL levels (19-22, 24-26, 30). This is particularly in women with CYP17 A2 alleles that may result in higher endogenous estrogen levels (24, 38, 39). Correlative studies indicate reduction in risk of ER⁻ as well as ER⁺ breast cancer, including ER⁻ cancer in premenopausal women (23, 25, 27-29).

Given the likelihood that lignans act as partial estrogen antagonists, we undertook a pilot study of the plant lignan SDG in premenopausal women at increased risk for breast cancer. Our primary end point was change in the proliferation marker Ki-67 in hyperplastic benign breast tissue obtained by random periareolar fine-needle aspiration (RPFNA). Proliferation plays a fundamental role in carcinogenesis (40), and higher proliferation (Ki-67) in hyperplastic and atypical hyperplastic specimens is associated

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with cancer development (41, 42). Reduction in Ki-67 is also associated with response in early cancer treatment trials (43, 44). We had previously shown that cytomorphic evidence of atypia in tissue obtained by RPFNA from high-risk women is associated with a 5-fold increased risk of developing ductal carcinoma *in situ* (DCIS) or invasive cancer (45) and that Ki-67 in cytology specimens obtained by RPFNA is positively associated (46) with evidence of cytologic atypia (47, 48). Secondary end points measured included cytomorphology, percent mammographic density, serum bioavailable estradiol and testosterone, and insulin-like growth factor-I (IGF-I) and IGF-binding protein-3 (IGFBP-3; reviewed in ref. 49). We chose a commercial preparation (Brevail) to avoid the marked variability in SDG content and bioavailability observed with different batches of raw or ground flaxseed (50). Pharmacologic studies had shown that daily dosing with this formulation, which contains 50 mg of SDG, produced ENL levels (median, 63 nmol/L) similar to those found in the highest quintiles associated with reduction in cancer incidence in case-control studies (18, 51).

Materials and Methods

Eligibility for study

Premenopausal women with regular menstrual cycles, not on oral contraceptives for at least 6 months, were eligible for tissue screening by RPFNA provided they met risk criteria. Risk criteria included a 5-year Gail model risk of $\geq 1.7\%$ or 3-fold higher than the average Surveillance Epidemiology and End Results risk for age group, a prior breast biopsy of atypical hyperplasia or lobular carcinoma *in situ* (LCIS), or a prior contralateral treated breast cancer. A normal mammogram was required the day of or within 3 months before their RPFNA. Tissue eligibility required hyperplasia, with or without atypia, plus sufficient Ki-67 expression to enable detection of modulation. We selected a lower limit of Ki-67 as staining of 2% or more of epithelial cell nuclei assessed on a minimum of 500 hyperplastic epithelial cells. We had previously observed a median Ki-67 of 2% in high-risk premenopausal women for whom Ki-67 could be assessed (46). More recently, a Mayo Clinic study suggested that Ki-67 staining of 2% or higher in foci of atypia was associated with increased risk for development of breast cancer (42). Entry onto the intervention protocol was required within 3 months of the RPFNA along with normal renal, hepatic, and hematologic function. Subjects were also asked not to take antibiotics or flaxseed supplements for 6 weeks before baseline sampling for blood lignan levels and entry into the study protocol. Protocols for RPFNA and the flaxseed lignan intervention were approved by the University of Kansas Medical Center Human Subjects Committee.

Gail risk calculation

The 5-year projected probability of developing invasive cancer was calculated at the time of RPFNA according to the Gail risk Model at <http://bcra.nci.nih.gov/brc/> (52).

Biomarker assessments and assays

All biomarker assessments were done at baseline and 12 months. Additional plasma for lignan measurements was also obtained at 6 months. Sera and plasma were stored in aliquots at -80°C after processing.

Tissue acquisition and specimen processing

RPFNA was done between days 1 to 10 (follicular phase) of the menstrual cycle to reduce Ki-67 variability and minimize bleeding. Two sites per breast were aspirated under local anesthesia as previously described (49). The needle tip was preferentially guided to areas of increased resistance. Material from all aspiration sites was pooled in a single 15-cm³ tube with 9 mL of CytoLyt and 1 mL of 10% formalin. After 48 hours, cells were pelleted, washed in CytoLyt, and transferred to PreservCyt. Aliquots were then transferred to slides via ThinPrep methodology for pap staining for cytomorphology or Ki-67 (see below).

Cytomorphology

Cytomorphology was assessed by a single cytopathologist (C.M.Z.) and classified by both a categorical method (48) and a semiquantitative index score. Index scores of 11 to 14 generally correlate with hyperplasia without atypia, 15 to 18 with hyperplasia with atypia, and 19 to 24 as suspicious for malignancy (47). Cytomorphologic assessments were made without knowledge of the results of the Ki-67 assessment.

Ki-67

A categorical estimate of the number of ductal epithelial cells present was made as 500 to 1,000, 1,000 to 5,000, or $>5,000$, and only slides containing >500 cells were stained for Ki-67. Antigen retrieval was done with 10 mmol/L citrate buffer (pH 6) in a BioCare decloaking chamber for 2 minutes at 120°C . Slides were then stained with MIB-1 monoclonal antibody (M7240; DakoCytomation) at a 1:20 dilution in a Dako Autostainer. At baseline, only hyperplastic cell clusters were assessed, but at the 12-month follow-up, if no hyperplastic clusters were present, clusters containing the highest proportion of cells staining for Ki-67 were preferentially evaluated. The number of cells with unequivocal nuclear staining out of 500 cells assessed was recorded for each of two independent readers. In case of a difference between the two readers, the scores were averaged.

Hormone and growth factors

Assays were done using commercially available kits. Each subject's pretreatment and posttreatment samples were run together in duplicate on the same 96-well plate, along with a pooled sera control, plus the standards and controls of the kit. Estradiol [and sex hormone binding globulin (SHBG) done with estradiol] assays were to be done on blood samples collected at the time of the initial screening and final poststudy RPFNAs during the follicular phase of the menstrual cycle, as this phase is associated with the least fluctuations in estradiol levels. However,

due to oversight, blood for baseline estradiol was collected on only half of the subjects. IGF-I, IGFBP-3, progesterone, testosterone, and SHBG assays were done on sera collected from all eligible subjects immediately before starting study agent and then after 12 months of study agent, between days 21 and 24 of the menstrual cycle. Levels of IGF-I and IGFBP-3 are highest during the luteal phase (53), and progesterone and testosterone are most reproducible in the midluteal phase (54). Specimens were thawed once for estradiol, IGF-I, and IGFBP-3 and twice for progesterone and testosterone. Bioavailable estradiol and testosterone results were computed using values for estradiol, testosterone, and SHBG according to standard formulas (55, 56).

Estradiol, progesterone, testosterone, and SHBG assay kits were purchased from Diagnostics Biochem Canada, Inc. All were competitive enzyme immunoassays done except for SHBG, which was a direct capture ELISA. Limits of detection for each assay were as follows: estradiol, 10 pg/mL; progesterone, 0.1 ng/mL; testosterone, 0.022 ng/mL; and SHBG, 0.1 nmol/L. IGF-I and IGFBP-3 assay kits were purchased from Diagnostic Systems Laboratories, Inc. Limits of detection were 0.01 ng/mL (for IGF-I) and 0.04 ng/mL (for IGFBP-3).

Mammographic breast density

Baseline and month 12 mammograms were done between days 1 and 10 of the menstrual cycle, similar to that for RPFNA, and generally on the same day as RPFNA. Images were digitally scanned or downloaded from a PACS system and cropped to remove any identifying information. Digital images were assembled in batched sets for an assessment of percent density using the Cumulus software program (57). The single reader (C.J.F.) knew which two files were from the same subject but did not know the sequence. Because there was a hospital conversion midway through the study from analog to digital mammography, a large number of subjects had prestudy and poststudy mammograms acquired using different technology and a secondary analysis was also done for the 25 subjects who had the same type of mammogram at baseline and 12 months.

Lignans

Baseline, 6-month, and 12-month plasma samples from the same subject were assessed together for analysis of lignans (SECO, END, and ENL). Samples were run in two batches ~2 months apart. Samples were thawed once and underwent solid-phase extraction, hydrolysis (58), and high-performance liquid chromatography analysis with Waters Quattro Micro UPLC system coupled to electrospray tandem mass spectrometry (59). The lower limit of quantification of lignans was 1 ng/mL (1 ng/mL is ~2.5 nmol/L). For analysis, samples classified as non-detectable for lignans were coded as 0, whereas samples with detectable signal but below the lower limit of quantification were coded as 0.5 ng/mL. The within-batch reproducibility for the assay of ENL, END, and SECO based on repeat measures of a quality assurance plasma

sample over six batches was 11.9%, 5.8%, and 10.1%, respectively, expressed as coefficient of variation.

Data capture and statistical methods

The study design called for accrual of 50 subjects, with 40 evaluable for the primary end point biomarker. Based on assumptions of a mean baseline Ki-67 of 4% and a SD of the change of 0.9%, the study had 86% power with a two-sided α of 0.05 to detect a 50% effect size for change in Ki-67 using a paired *t* test. Because change in Ki-67 was not normally distributed, the nonparametric Wilcoxon signed rank test was used. Changes in plasma SECO, END, and ENL were correlated with changes in Ki-67, percent mammographic breast density, progesterone and plasma IGF-I/IGFBP-3, SHBG, bioavailable estradiol, and bioavailable testosterone. Because the data were highly skewed, a nonparametric Spearman's correlation was used. For secondary end point markers for which the study was not specifically powered, no corrections for multiple comparisons were formally used; however, this was taken into consideration in the interpretation of the results.

Results

Baseline characteristics

Between December 2005 and April 2008, 78 women were screened by RPFNA for consideration of participation on the study. A total of 49 subjects were enrolled between February 2006 and June 2008. The last subject completed study in June 2009. Characteristics of both the 49 enrolled and the 45 evaluable subjects are given in Table 1 and were not different. Median age was 43, 73% had one or more first-degree relatives with breast cancer, and 22% had a prior biopsy with atypical ductal hyperplasia (ADH) or LCIS. Baseline RPFNA indicated hyperplasia with atypia in 59% of subjects (62% of evaluable) and a median Ki-67 of 4%. Baseline median mammographic density was 40.9%.

Change in biomarkers over the course of the study

Ki-67. Our primary end point was change in Ki-67 over the 12-month study. Median Ki-67 was 4% at baseline (range, 2-16.8%) and 2% (range, 0-15.2%) at 12 months in the 45 women who completed the trial (median decrease, 2.4%; range, -13.0% to +8.2%). Thirty-six (80%) of the 45 women showed a decrease in Ki-67 with a mean relative reduction of 0.67 ($P < 0.001$, Wilcoxon signed rank test; see Fig. 1).

Cytomorphology. The proportion of evaluable women with atypical cytomorphology was greater at baseline (62%) than at the conclusion (42%) of the study ($P = 0.035$, two-sided McNemar's test), although there was not a significant change in median semiquantitative Masood score. Consistent with the reduction in Ki-67, a significant shift in cell number category was also observed (see Table 2).

Mammographic breast density. Mammographic density declined over the 12-month period by a nonsignificant 6.3% (median). During the trial, our hospital switched

Table 1. Baseline key variables of all 49 subjects enrolled and of the 45 subjects evaluable for the primary end point

Variable	n = 49	n = 45
Race (non-White)	2%	2%
Ethnicity (Hispanic/Latino)	2%	2%
Age (y)		
Median	43	43
Mean ± SD	41.8 ± 6.5	42.3 ± 6.3
Range	27-51	29-51
Education		
High school/vocational	8 (16%)	7 (15%)
College graduate	27 (55%)	25 (56%)
Post-graduate	14 (29%)	13 (29%)
Height (in)		
Median	66	66
Mean ± SD	65.2 ± 2.8	65.3 ± 2.9
Range	56-70	56-70
Weight (lb)		
Median	138	137
Mean ± SD	148 ± 33	148 ± 34
Range	101-234	101-234
Body mass index (kg/m ²)		
Median	23.2	22.8
Mean ± SD	24.6 ± 5.2	24.5 ± 5.3
Range	17.4-37.2	17.4-37.2
5-y Gail risk (%)		
Median	1.6	1.6
Mean ± SD	2.0 ± 1.5	2.0 ± 1.3
Range	0.1-6.5	0.1-5.7
Age at menarche (y)		
Median	13	13
Mean ± SD	12.8 ± 1.5	12.8 ± 1.5
Range	10-16	10-16
Age first live birth, y (42 parous = 86%)		
Median	29	29
Mean ± SD	28.6 ± 4.3	28.7 ± 4.2
Range	19-43	19-43
Prior biopsy with ADH or LCIS	11 (22%)	8 (18%)
	(20 with any biopsy, 41%)	(17 with any biopsy, 38%)
No. first-degree relatives with breast cancer		
0	13 (27%)	11 (24%)
1	30 (61%)	29 (64%)
≥2	6 (12%)	5 (11%)
Family history consistent with hereditary breast cancer	16 (33%)	16 (36%)

from analog to digital mammography, and digital mammography is generally associated with less density than analog. Restricting the analysis to the 25 individuals who had the same type of mammogram on and off study, there was no change either in median density or in the proportion of individuals having increases or decreases in density (Fig. 2).

Hormone levels and growth factors. With the exception of a borderline decrease in IGFBP-3 and increase in bioavailable testosterone, there were no changes in hormone or

growth factor levels during the study (see Table 3). Although there was a marginal decrease in SHBG when collected early in the menstrual cycle, no such effect was observed when collected at days 20 to 24 of the cycle.

Change in lignan blood levels over the course of the study

Forty-two subjects had plasma obtained for lignans at 0, 6, and 12 months. Plasma lignan levels were below the

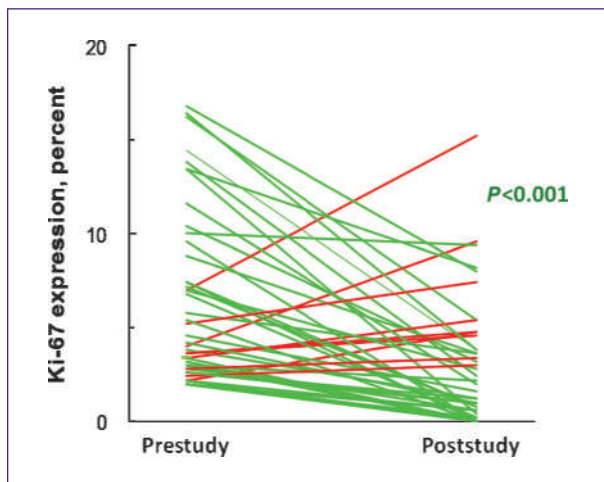


Fig. 1. Change in expression of Ki-67 assessed by immunocytochemistry over the course of the 12-mo study. The difference in expression is statistically significant by Wilcoxon signed rank test.

limit of quantification or undetectable at baseline for SECO in 94%, END in 84%, and ENL in 16% of specimens. All three lignans showed a statistically significant ($P < 0.001$, Wilcoxon signed rank test) increase in levels between baseline and 6 or 12 months (see Table 3), but no difference between 6 and 12 months. There was a 9-fold increase in median levels of ENL, the most biologically relevant lignan, and a 16-fold increase in median total lignan levels from baseline to 12 months. There

Table 2. Assessment of change in cell number and cytomorphology

Variable and time/change	Frequency	P
Change in cell number per slide		
Increase	6 (13%)	0.002
No change	19 (42%)	
Decrease	20 (44%)	
Change in categorical descriptor		
Worsen	9 (20%)	0.16
No change	19 (42%)	
Improve	17 (38%)	
Change in presence of cytologic atypia		
Worsen (no atypia→atypia)	3 (7%)	0.013
No change (same at both RPFNAs)	30 (67%)	
Improve (atypia→no atypia)	12 (27%)	
Masood score, median (range)		
Baseline	15 (11-17)	0.13
12 mo	14 (10-20)	
Change from baseline	0 (-5 to +5)	
Change in Masood score by ≥ 2 points (frequency)		
Worsen	5 (11%)	0.090
No change	28 (62%)	
Improve	12 (27%)	

was no significant relationship between reported compliance and change in ENL levels or ENL levels and change in Ki-67.

Compliance

Our preset criterion for compliance was ingestion of 70% of prescribed capsules and was met by 44 of 45 biomarker evaluable subjects. Median compliance as assessed by capsule count was 96% in biomarker evaluable subjects.

Adverse events

Reported adverse events were mild and, for the most part, probably unrelated to drug. There were no grade 4, grade 3 in only 4%, grade 2 in 47%, and grade 1 in 35%. Four subjects discontinued study prematurely: 1 with a grade 3 adverse event from pelvic pain at 3 months, 1 with pregnancy at 9 months, 1 with DCIS detected on her regularly scheduled 12-month mammogram before RPFNA, and another failed to return for any follow-up visit. The majority of the grade 2 adverse events were considered to be probably unrelated to the study agent, including minor teeth, sinus, and respiratory infections. Gastrointestinal (GI) symptoms such as nausea, flatulence, or diarrhea provided only 11% grade 2 events. Grade 1 adverse events were predominately related to transient GI complaints and alteration of the menses. Table 4 gives frequency of reported adverse events that might be expected from SDG. Half of the subjects reported some GI symptoms during the 12 months and 26% reported irregular menses. Only one subject reported becoming amenorrheic, halfway through study, but then had a period 1 week before the poststudy RPFNA. There was no correlation between side effects and ENL levels.

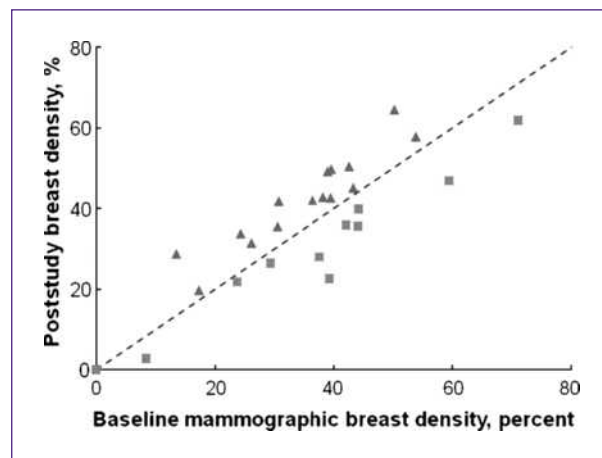


Fig. 2. Change in mammographic breast density (expressed as percent of breast area that is considered to be at increased density) over the course of the 12-mo study. The triangles indicate subjects where density increased; the squares indicate subjects where density decreased. The dashed line indicates equivalence (i.e., no change over the course of the study). The difference in density is not statistically significant by Wilcoxon signed rank test.

Table 3. Change in serum hormones and growth factors (median, mean, and SD)

Variable or biomarker	n	Prestudy	Poststudy	Difference	P (Wilcoxon)
Collected at time of RPFNA (days 1-10 of menstrual cycle)					
Estradiol (pg/mL)	22	97.5 (154 ± 117)	102 (144 ± 90)	1.29 (-10.8 ± 53.4)	0.55
Estradiol (nmol/L)	22	0.36 (0.57 ± 0.43)	0.38 (0.53 ± 0.33)	0.00 (-0.04 ± 0.20)	
Bioavailable (free) estradiol (pmol/L)	22	4.6 (6.3 ± 4.4)	4.9 (6.4 ± 4.1)	0.32 (0.12 ± 1.84)	0.90
SHBG, with estradiol (nmol/L)	22	80.2 (81.2 ± 28.6)	67.9 (72.9 ± 26.3)	-11.7 (-8.3 ± 30.1)	0.031
Collected at days 20 to 24 of menstrual cycle					
IGF-I (ng/mL)	44	194 (201 ± 78)	180 (196 ± 67)	0.28 (-5.1 ± 47.8)	0.48
IGF-I (nmol/L)	44	25.3 (26.2 ± 10.1)	23.4 (25.5 ± 8.7)	0.04 (-0.66 ± 6.21)	
IGFBP-3 (ng/mL)	44	5,854 (5,978 ± 1,009)	5,672 (5,729 ± 1,022)	-220 (-249 ± 790)	0.029
IGFBP-3 (nmol/L)	44	205 (209 ± 35)	199 (201 ± 36)	-7.7 (-8.7 ± 27.6)	
IGF-I/IGFBP-3 molar ratio	44	0.12 (0.12 ± 0.04)	0.12 (0.13 ± 0.03)	0.001 (0.003 ± 0.029)	0.65
Progesterone (ng/mL)	44	6.1 (8.0 ± 8.2)	6.4 (8.8 ± 9.8)	0.4 (0.8 ± 8.5)	0.58
Progesterone (nmol/L)	44	19.3 (25.5 ± 26.2)	20.3 (28.0 ± 31.2)	1.3 (2.5 ± 27.2)	
Testosterone (ng/mL)	44	1.1 (1.6 ± 2.4)	1.1 (1.6 ± 2.0)	0.07 (0.00 ± 1.32)	0.11
Testosterone (nmol/L)	44	3.7 (5.7 ± 8.3)	3.8 (5.7 ± 7.0)	0.25 (0.00 ± 4.57)	
Bioavailable (free) testosterone (pmol/L)	44	39.3 (59.8 ± 63.3)	43.3 (73.9 ± 101.3)	3.9 (17.2 ± 74.8)	0.061
SHBG (nmol/L)	44	76.9 (70.1 ± 25.5)	70.9 (72.2 ± 30.6)	0.82 (2.03 ± 23.5)	0.99
SECO (nmol/L)	42	0.0 (0.62 ± 1.15)	5.7 (33.7 ± 66.5)	5.5 (32.8 ± 66.5)	<0.001
END (nmol/L)	42	0.0 (0.69 ± 1.29)	23.3 (84.3 ± 133.0)	23.3 (83.7 ± 133.0)	<0.001
ENL (nmol/L)	42	11.1 (15.8 ± 17.4)	99.2 (132.7 ± 120.3)	74.8 (117.0 ± 117.7)	<0.001
Total lignans (nmol/L)	42	11.1 (16.8 ± 17.9)	183.3 (248.8 ± 216.7)	165.9 (233.5 ± 213.9)	<0.001

Breast biopsies and events

Six of the 49 high-risk subjects underwent breast biopsy as a result of breast imaging (5, mostly new microcalcifications) or the RPFNA (1, suspicious cytomorphology) at

the 12-month visit. Biopsies showed fibrocystic change or proliferative breast disease in four and LCIS in two. Four of the six women had baseline cytologic atypia, and one of the two with LCIS had a prior history of LCIS. Subsequent to these biopsies shortly after going off study, two women were diagnosed with DCIS and another with invasive cancer.

Table 4. Frequency of anticipated adverse events

Adverse event	Frequency, n (%)	
	Single events	Per subject
Diarrhea		
Grade 1	4 (8%)	2 (4%)
Grade 2	2 (4%)	1 (2%)
Flatulence		
Grade 1	10 (20%)	10 (20%)
Grade 2	2 (4%)	2 (4%)
GI symptom		
Grade 1	22 (44%)	18 (36%)
Grade 2	3 (6%)	3 (6%)
Irregular menses		
Grade 1	15 (30%)	13 (26%)
Rash		
Grade 1	1 (2%)	1 (2%)
Grade 2	10 (20%)	6 (12%)
Hot flashes		
Grade 1	2 (4%)	2 (4%)
Grade 2	1 (2%)	1 (2%)

Discussion

To our knowledge, this is the first report of a significant reduction in Ki-67 in benign breast tissue, with sufficient SDG to raise plasma lignan levels ~10-fold. We also observed a reduction in the proportion of women with cytologic atypia. Our median ENL of 99 nmol/L following supplementation with 50 mg/d SDG was higher than the mean ENL in the highest quintile of the Finnish case-control study associated with a 62% breast cancer risk reduction when compared with the lowest quintile (18). The mean level for the lowest quintile in the Finnish study (3 nmol/L) was similar to the baseline ENL (11 nmol/L) in our study. Our findings in premenopausal women at high risk for development of breast cancer parallel those of Thompson et al. (60), who observed a reduction in Ki-67 in tumor tissue after ~30 days of muffins supplemented with 25 g of flaxseed versus muffins alone in a cohort of 32 premenopausal and postmenopausal women with newly diagnosed breast cancer.

Although the mechanism of action of SDG is not clear, several possibilities have emerged from preclinical studies

and include (a) antioxidant effects (61), (b) increase in BRCA1 protein and differentiation (62, 63), (c) reduced breast aromatase with reduction in tissue estrogen production and altered ER-related signaling (64–66), (d) activation of peroxisome proliferator-activated receptor- γ with an increase in adiponectin and resulting suppression of AKT/mammalian target of rapamycin activity (67, 68), (e) downregulation of epidermal growth factor receptor with resultant decrease in mitogen-activated protein kinase and reduction in IGF-I with downregulation of phosphatidylinositol 3-kinase signaling (17, 69, 70), and (f) reduced vascular endothelial growth factor secretion and angiogenesis (3).

Lack of modulation of mammographic density (71) despite an increase in plasma lignans and a reduction in tissue Ki-67 is in line with findings of Stuedal et al. (72), who found no correlation of plasma ENL and mammographic density, and findings by ourselves and others indicating a lack of correlation between Ki-67 obtained from cytologic or histologic specimens and mammographic density (73–75). Because modulation of mammographic breast density has been observed with tamoxifen and other selective ER modulators but not aromatase inhibitors (76–79), demonstration of modulation of mammo-

graphic density after a short-term intervention may be drug class specific.

The primary limitation of our pilot study is the lack of a placebo control group. However, cytomorphology and Ki-67 are reasonably stable over time when a stable hormonal milieu is maintained (80, 81).

Given the favorable safety profile, prior studies indicating lignan-associated reduction in breast pain and breast tumor cell proliferation (60, 82, 83), and the current study suggesting reduction in proliferation and atypia, SDG warrants further testing in premenopausal women in a phase II placebo controlled trial.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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