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Dietary Lignan Intake and Androgen Receptor Expression in Breast Tumors

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Abstract

Purpose—Lignans, a class of phytoestrogen commonly found in the Western diet, have been linked to decreased breast cancer risks in epidemiologic studies. Similar to estrogen receptors, the androgen receptor (AR), a prognostic factor in breast tumors, may be affected by lignans. However, few studies have investigated this link in the context of breast cancer etiology. We evaluated the relationship between dietary lignan intake and androgen receptor expression in incident breast tumors.

Methods—Tumor tissue, epidemiological, and clinical data were collected from 216 women with incident, primary, histologically-confirmed breast cancer enrolled in the Roswell Park Cancer Institute (RPCI) Data Bank and BioRepository (DBBR). On average, three tumor cores from each participant were assembled into a Tissue MicroArray (TMA). After immunohistochemical staining, a trained RPCI pathologist determined AR status of each core. Lignan intake was calculated from a food frequency questionnaire collected upon enrollment into the DBBR.

Results—We observed a weak positive association between dietary lignans and AR expression (β (SE) 27.6 (17.0), p 0.10) and there was no significant difference in lignan intake across categories of AR expression ($p=0.09$, $R^2=0.35$).

Conclusion—Our results do not support a clear relationship between dietary lignan intake and AR expression. This investigation is the first, to our knowledge, to examine dietary lignan intake and AR expression in breast tumors. Further research is needed within a larger, more representative sample to determine if lignan intake is truly associated with androgen receptor expression.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Keywords

Dietary lignans; androgen receptor; breast cancer; tissue microarray

Introduction

Breast cancer is the most common cancer and accounts for the second most cancer deaths in women [1]. Recently the androgen receptor (AR) has been considered alongside the estrogen and progesterone receptors as a possible prognostic factor in breast cancer [2]. AR expression may modify breast cancer risk, which is supported by epidemiological evidence that circulating androgens are higher in women with breast cancer compared to those without [3]. However, higher circulating androgens may reflect decreased AR expression, since fewer AR would result in more unbound, circulating, androgens. Conversely, increased AR expression may protect the mammary gland from stimulation by hormones, such as estrogen, thereby reducing proliferation [3]. In estrogen receptor (ER) positive cancers, AR expression has been associated with better overall survival and favorable tumor characteristics [4–6]; however, the evidence for the role of AR expression in ER negative tumors is less conclusive [2,6–8]. In triple negative tumors the presence of the AR is associated with increased mortality and less favorable tumor characteristics [9,6,10,11].

Lignans are a class of phytoestrogen commonly found in many foods such as fruits, vegetables, grains, and seeds. Dietary phytoestrogens, in particular lignans, have the ability to act as endogenous estrogens and possibly affect hormonal balance through competitive inhibition of hormone receptors, as well as other mechanisms [12,13]. The mechanistic relationship between dietary lignan intake and AR expression is relatively unexplored. If lignans affect AR similarly to ER, increased lignan intake may inhibit proliferative activity by lowering estrogen levels and up-regulating AR expression [13,14]. This would be beneficial in ER positive tumors where AR has been consistently shown to be a favorable prognostic factor. *In vitro* and animal models have demonstrated that lignans have the potential to decrease androgen receptor expression in ER positive prostate and ovarian cancer cells [15,16]. Only one study has examined the relationship between lignan concentration and AR expression in breast cancer cells *in vitro*. Using a line of ER positive MCF-7 breast cancer cells, Taxvig et al [17] reported no evidence of an agonistic or antagonistic effect of lignans on AR expression. To our knowledge no investigations have described the relationship between dietary lignans and the androgen receptor in breast tumors in women.

The mechanisms by which AR interacts with lignans to promote or inhibit cell growth in breast cancer are not completely understood. However, the AR is expressed in the majority of breast tumors and warrants further study. This cross-sectional study aims to evaluate the relationship between dietary lignan intake and androgen receptor expression in primary, incident, histologically confirmed breast cancer tumors.

Materials and Methods

Clinical, pathologic, and epidemiologic questionnaire data were obtained from the Roswell Park Cancer Institute (RPCI) Data Bank and Biorepository (DBBR) from women with breast cancer diagnosed between December 31, 2003 and December 31, 2008. The DBBR is a shared resource providing biospecimens and epidemiologic data, facilitating investigations of cancer etiology, prognosis, and prevention. The protocol for the DBBR is conducted according to the guidelines laid down in the Declaration of Helsinki, is approved by the RPCI Institutional Review Board, and all participants provide signed, informed consent. The methods for the DBBR have been described in detail elsewhere [18].

Upon enrollment into the DBBR, participants completed a food frequency questionnaire (FFQ) detailing the frequency of use in the year prior to diagnosis of 110 different foods and beverages. Additional questions were included to capture serving size, use of reduced fat or fortified foods, trimming of fat or skin from meat, frequency of meals, cooking techniques (such as grilling or frying), and vitamin or supplement use. Nutrient intake was calculated from the FFQ using USDA food composition data and standard nutrient calculation algorithms. For each nutrient, daily intakes were calculated as the product of the food-specific frequency of use, portion size in grams, and nutrient content summed across all contributing foods. Daily intakes of total lignan precursors (plant lignans) were calculated using published phytoestrogen data [19,20]. All lignan intakes were expressed as average daily intakes in micrograms.

Breast cancer tissue microarrays (TMAs) were prepared as follows: three 0.6-millimeter tissue cores from formalin-fixed paraffin embedded donor blocks were precisely arrayed into a new recipient paraffin block, including tumor specimens as well as controls. Controls within the TMA consisted of multiple cores of normal tissue from 10 different organs including heart, colon, kidney, adrenal, ovary, myometrium, brain, thyroid, lung, and prostate representing slightly more than 20% of all the cores in a TMA. In the present study, for the majority of cases three cores were taken from each participant to maximize the representation of the whole section and to minimize loss. After assembly, TMA cross sections were stained using standard IHC staining techniques for AR (Santa Cruz #SC-816 clone N-20 antibody for AR). A trained RPCI pathologist (HH) scored each core for intensity, on a scale of 0 to 3, and for the percent of cells stained in that core. Of the 608 women who enrolled in the DBBR during this time period and completed the food frequency questionnaire, 216 were also included on tissue microarrays assembled as part of the RPCI pathology shared resource and were used for this investigation.

Statistical Analysis

All statistical analyses were conducted with SAS 9.3. All statistical hypothesis testing was two sided and considered statistically significant at a $p < 0.05$. AR expression was evaluated both as a continuous variable and a dichotomous variable (positive or negative). Previous research employing TMAs to evaluate AR expression in breast tumors has relied on the average percent of cells stained [21,4,2,5,8,22,6]. AR positive was defined as an average percent of cells stained positive greater than 1%, similar to the cut points used for estrogen receptor to define positivity. Basic descriptive statistics were calculated for each of the study

sample characteristics and were compared according to AR status. We included both categorical and continuous parameterizations of AR expression in our analysis. We examined dietary lignan intake by category of AR expression (none, 30%, 31–59%, 60%) using analysis of variance. Dietary lignan intake was skewed and transformed using the natural log for descriptive analyses and linear regression. We used a linear regression model to evaluate the relationship between AR expression as a continuous variable (% cells stained) and dietary lignan intake. We considered the following variables as potential covariates: age at diagnosis, body mass index (BMI), energy intake, tumor size, menopausal status, smoking status, education, ER status, PR status, stage, and histological grade. None of these variables affected the estimates; therefore, the final models are minimally adjusted for age at diagnosis and total daily energy intake. The androgen receptor can interact with the ER and inhibit cancer growth [4], therefore, *a priori* we determined that estrogen receptor status may be an important effect modifier. Thus, analyses were stratified by ER status. Additionally, we examined whether women whose breast tumors were included on the TMAs differed from women who were not included on these covariates.

Results

Compared to DBBR participants not included on the TMAs, women included on the TMAs were more likely to be younger at the time of enrollment (55.81 years vs. 59.36 years respectively, $p<0.001$) as well as at the time of diagnosis (55.60 years vs. 59.25 years respectively, $p<0.001$) and to be premenopausal (43.4% vs. 30.7% respectively, $p=0.001$). Women included on the TMAs were also more likely to have a larger tumor (1.80 mm vs. 1.20 mm respectively, $p<0.001$), higher stage (14.4% stage 3 or 4 vs. 6.7 % respectively, $p<0.001$), and higher grade (68.7% grade 3 vs. 55.8% respectively, $p=0.003$). Women included on the TMAs were also more likely to be ER negative (35.7% vs. 10.8% $p<0.001$) and PR negative (44.3% vs. 23.7%, $p<0.001$) but HER2 Positive (20.8% vs. 11.3% $p=0.001$). Generally women with tumor samples did not differ from the women without on BMI ($p=0.24$), energy ($p=0.71$), or lignan intake ($p=0.07$) (data not shown).

Approximately 87% of tumors had any AR staining with 82% falling into the AR positive category when defined as greater than 1% of cells stained. Age, age at diagnosis, BMI, energy, menopausal status, education, marital status, and smoking status did not differ significantly by AR status (Table 1). Women with AR positive tumors were more likely to also have ER positive ($p<0.001$) and PR positive ($p<0.001$) tumors, with a lower histological grade ($p=0.004$). Tumor size, HER 2 status, and tumor stage did not differ significantly by AR status. Overall dietary lignan intake did not differ by AR status ($p=0.273$).

We present descriptive statistics for dietary lignan intake across category of AR expression in Table 2. When average percent of cells stained positive was expressed as categories (none, 30%, 31–59%, 60%) no association between AR expression and dietary lignan intake was observed after adjustment for energy intake and age ($p=0.093$, $R^2 = 0.35$). Similarly, when restricted to ER positive tumors ($p=0.287$, $R^2=0.47$) or ER negative tumors ($p=0.366$, $R^2=0.31$) no association was observed. Among women with any positive staining, when both lignan intakes and AR expression were expressed as continuous variables, we observed a weakly positive relationship between lignan intake and average percent of cells

stained, after adjustment for age and energy intake (β (SE) 27.6 (17.0), p 0.10, data not shown). However, this association further attenuated when stratified by ER status (ER positive β (SE) 13.5 (19.7), p 0.49, ER negative β (SE) 44.0 (33.5), p 0.20 data not shown).

Discussion

Dietary phytoestrogens have been shown to affect estrogen metabolism and receptor binding in previous studies; however, relatively little has been reported for the androgen receptor. In this cross-sectional study of dietary lignan intake and AR expression, we observed little evidence for an association between lignan intakes and AR positivity or AR status in breast tumors, nor did we observe associations between dietary lignans and AR expression by ER status. Over 80% of our sample was AR positive regardless of definition. While this is consistent with current literature [23,24] the small AR negative group may have limited our ability to detect a significant difference in dietary lignan intake between AR positive and AR negative participants. We hypothesized that increased dietary lignan intake would be associated with increased AR expression, and that association would vary by ER status. In our sample of AR positive women we did observe the expected increase in AR expression with increase in dietary lignan intake, although the association was borderline significant. However, contrary to expectation, there was no effect of ER status on the association. Sample size may have limited our ability to detect an interaction with ER status.

Consistent with our findings of higher dietary lignan intake in women with AR negative tumors, *in vitro* evidence from prostate cell lines [15] and Chinese hamster ovary cells [16] support AR down regulation upon the addition of varying concentrations of lignans. Mammalian lignans inhibit aromatase, an enzyme involved in steroid synthesis. An inhibition of aromatase would putatively decrease the amount of androgens converted into estrogens thereby resulting in higher androgen levels [13]. *In vitro* evidence using MDA453 AR positive breast cancer cells demonstrates AR down-regulation in the presence of increased androgen levels [25]. However, among AR positive women, we observed increasing expression of AR with increasing dietary lignan intake, suggesting that alternate mechanisms may be responsible. Mammalian lignans competitively bind to the ER and AR in breast tissue; preferential binding of lignans to ER may be reflected in up-regulation of AR.

Our study may have been affected by low inter-person variability in dietary lignan intake. The mean dietary lignan intake in this sample was 140 micrograms per day whereas other studies using similar methodologies, report values as high as 1700 micrograms per day [26–29]. Additionally, a study of Canadian women that used the same food composition database used in our study reported a mean dietary lignan intake of 857 micrograms per day (interquartile range, 305–4005) [30]. The FFQ used for this study was not designed to specifically capture lignan intake and as such may be missing key lignan rich foods (flaxseed, sesame seeds, whole grain bread, tea). On the other hand, previous research conducted in Western New York identified broccoli, apricots, berries, coffee, and red wine as primary sources of lignan intake, items that were all included on the FFQ which increases our confidence that we are capturing the exposure adequately in this population [31]. In addition, in the past 2–3 years, additional data on flaxseed use has been collected in the

DBBR; these data suggest that approximately 15% of women would have reported flaxseed use but that there were no obvious differences in reported intake between participants with a cancer diagnosis compared to those without a cancer diagnosis (personal communication).

Several limitations need to be considered. First, this study was based on a small, convenience sample from a larger pool of women recruited into RPCI's DBBR. Our limited sample size may have affected our ability to detect associations in our categorical analysis. Tumor characteristics of women who were included on TMAs for AR analysis were different from women who were not included. Women included had significantly greater tumor size, higher stage, and grade. Construction of TMAs requires an ample amount of biospecimen, the source could not be depleted thus requiring a larger tumor size to be included, and larger tumors tend to be of higher stage and grade. Women included on TMAs were also more likely to be ER-, PR-, or HER2+, which may be due to the fact that some TMAs were constructed for research purposes, with special interest in rare or aggressive tumors that were likely ER and PR negative. In the larger sample, from the entire DBBR with complete FFQ data, higher dietary lignan intake was significantly associated with tumors with more favorable characteristics [31]. AR has consistently been demonstrated to be associated with favorable tumor characteristics as well [8,21,5,10] which were not well-represented in our TMAs.

Heterogeneity of breast tumors may result in cores from each tumor that may not accurately represent the overall tumor. However, previous research has validated that two TMA cores are predictive of the whole tumor cross section 95% of the time [32]. Three cores, on average, were taken from each participant to maximize the representativeness of the cores with the whole tumor, and there was low intraperson variability in AR staining across an individual's cores (ICC= 0.89 95%CI 0.87, 0.90) confirming that heterogeneity of the breast tissue likely did not influence our findings. Additionally, positive controls were included on every TMA to ensure accurate staining and minimize outcome misclassification.

To our knowledge this is the first investigation to examine the association between androgen receptor (AR) expression and dietary lignan intake in women with primary histologically-confirmed invasive breast cancers. Previous research has relied on cellular models and therapeutic doses of mammalian lignans and does not inform on typical dietary intakes of lignans in women with breast cancer. By examining dietary intake of lignans, we represent a more realistic exposure dose as no therapeutic lignan-based agent exists. A strength of this investigation is that we have extensive dietary and clinical data allowing us to extensively examine potential confounders. Also, dietary lignan intake was calculated using a database that directly measured lignans from typically prepared foods assuring a more accurate representation of lignan intake in our sample. These women completed the FFQ at the time of diagnosis; therefore, we can be reasonably sure that we are capturing their lignan intake as accurately as possible close to diagnosis and as close as possible to disease onset. Lastly, this investigation is utilizing primary, incident cases of breast cancer so there are no concerns of previous treatment confounding the results of our assay. In summary, we examined the association between lignan intake and the androgen receptor a number of different ways in an attempt to further the knowledge surrounding this topic in breast cancer. We did not demonstrate a significant relationship between dietary lignan intake and AR

expression. Future research is needed to concretely define the relationship between dietary lignan intake and AR at the cellular and population level. Further exploration of the relationship of AR positive breast cancers and dietary lignan intake should be done in a larger, more representative sample. Specific attention should be placed on stratifying the sample by tumor subtype, where possible, which we were not able to do in this study. Prospective studies are also needed to explore the effect of dietary lignan intake on risk of having AR positive breast cancer. As the understanding of the importance of the AR in breast cancer risk and prognosis increases, understanding its relationship with lignans may be important in identify new treatment options, such as anti-androgens, and a potential modifiable prognostic factor.

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Table 1

Descriptive characteristics of Caucasian women enrolled in Roswell Park's Databank and BioRepository stratified by Androgen Receptor (AR) status.

	AR Positive ^a	AR Negative	P-value ^b
N	177	39	
	Mean ± SD		
Age, y	56.19 ± 12.33	53.21 ± 13.37	0.179
Age at Diagnosis, y	55.96 ± 12.39	53.05 ± 13.58	0.193
BMI, kg/m ²	28.14 ± 5.71	27.07 ± 6.37	0.297
Energy, kcal/d	1724.94 ± 711.16	1815.90 ± 696.93	0.468
Lignans, mcg/d ^c	122.76 (95.57)	115.51(109.96)	0.273 ^d
< 83.8350	65.40(28.04)	59.89 (40.40)	0.326
83.8350 to 122.255	106.15 (20.08)	100.11 (26.18)	0.392
122.256 to 182.311	149.71(22.16)	136.32 (13.37)	0.192
>182.311	218.71 (70.25)	206.83(70.22)	0.767
	Frequency (%)		
Menopausal Status			
Pre	78 (44.07)	16 (41.03)	0.728
Post	99 (55.93)	23 (58.97)	
Education			
Less than High School	10 (5.65)	3 (7.69)	0.842
High School	51 (28.81)	12 (30.77)	
Greater than High School	116 (65.54)	24 (61.54)	
Marital Status ^e			
Married/Cohabiting	120 (68.34)	26 (66.67)	0.723 ^f
Widowed	20 (11.30)	4 (10.26)	
Separated Divorced	21 (11.86)	7 (17.95)	
Single	15 (8.47)	2 (5.13)	
Smoking Status			
Never	97 (54.80)	21 (53.85)	0.254
Former	56 (31.64)	16 (41.03)	
Current	24 (13.56)	2 (5.13)	
ER status			
Positive	136 (76.84)	3 (7.69)	<0.0001
Negative	41 (23.16)	36 (92.31)	
PR status			
Positive	120 (67.80)	2 (5.13)	<0.0001
Negative	57 (32.20)	37 (94.87)	

^a AR positive defined as average percent of cells stained greater than one,

^b unless otherwise stated p-values are from a two sample independent t-test and chi-square tests.,

^c median and interquartile range reported,

^d Wilcoxon Rank Sum test,

^e one women missing marital status was excluded from this analysis,

^f Fisher's exact test

Table 2

Mean (Standard Deviations) of dietary lignan intake across categories of AR expression. Means are adjusted for daily energy intake and age.

	Mean Lignan Intake	p-value ^a	R ^{2a}
Categories of AR expression			
0%	136.39 (115.13, 161.56)	0.093	0.353
>0 to 33%	104.35 (90.94, 119.74)		
>33 to 66%	121.73 (99.76, 148.55)		
>66%	122.40 (112.70, 132.93)		
ER Negative Disease Only			
0%	143.27 (119.29, 172.08)	0.287	0.47
>0 to 33%	110.77 (90.07, 136.24)		
>33 to 66%	113.39 (76.45, 168.50)		
>66%	130.39 (105.34, 161.41)		
ER Positive Disease Only			
0%	158.65 (65.42, 384.73)	0.366	0.31
>0 to 33%	101.91 (84.69, 122.63)		
>33 to 66%	127.05 (101.52, 159.01)		
>66%	117.79 (108.07, 128.39)		

^aP-value and R² for Analysis of Variance adjusted for age and daily energy intake.