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Dietary flavonol intake is associated with age of puberty in a longitudinal cohort of girls

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Abstract

Lignans and flavonols are dietary phytoestrogens found at high concentrations in the Western Diet. They have potential to influence the timing of puberty. We hypothesized that greater consumption of these two phytoestrogens would be related to later age at pubertal onset among girls. Pubertal assessment and 24-hour diet recall data were available for 1178 girls, ages 6-8 yr (mean 7.3 yr) in the Breast Cancer and Environment Research Project Puberty Study. Lignan and flavonol intakes were mainly derived from fruit and vegetable consumption. Average consumption was 6.5 mg/day for flavonols and 0.6 mg/day for lignans. Highest flavonol consumption (>5mg/day) was associated with later breast development (adjusted Hazards Ratio (HR): 0.74, 95% CI: [0.61-0.91]) compared to 2-5mg/day (adjusted HR: 0.84, 95% CI: [0.70-1.0]) and <2 mg/day (referent group; p-trend=0.006). Flavonol intake was not associated with pubic hair development. Lignan intake was not associated with either breast or pubic hair development. Dietary intake was only weakly correlated with urinary enterolactone, a biomarker for lignans ($R_s=0.13$). Consistent with biologic properties of phytoestrogens that indicate hormonal activity, their consumption may be associated with reproductive endpoints, even in childhood.

Keywords

longitudinal study; phytoestrogen; puberty; lignans; flavonols; Cox Proportional Hazard Ratio

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1. Introduction

Phytoestrogens (PEs) are naturally occurring plant-derived compounds found in many foods. They are hormonally active and have been postulated to have beneficial health effects. [1,2]. Numerous epidemiologic studies have suggested that dietary intake of phytoestrogens, particularly isoflavones, plays a role in preventing certain types of hormonally-dependent cancers, including breast and prostate [3-5]. Phytoestrogens may also protect against diabetes and cardiovascular disease [6,7]. Mechanisms of the protective effect are not fully understood, although they may be explained by the competition of PEs with endogenous estradiol for estrogen receptors [8] and an inhibitory effect on aromatase activity. In addition to their endocrine-related activities, other possible mechanisms include anti-proliferative and antioxidant properties [9,10].

Phytoestrogens are classified into several families including lignans, flavonoids and coumestans. Most of the research on health benefits of phytoestrogens has examined isoflavones, a flavonoid, which are found mainly in soy products. Lignans have lower hormonal activity compared to isoflavones; however they are the principal phytoestrogens in typical Western diets [11]. Lignans are found in many commonly consumed plant foods including flaxseeds, grains, nuts, fruits, berries, vegetables and tea. Flavonols such as quercetin, kaempferol and myricetin make up a large part of the flavonoid content in foods. They are present in large amounts in a variety of fruits (apples, berries), leafy green vegetables, onions and tea [12]. Lignans and flavonols are prevalent in the Western diet, but little is known about the magnitude and predictors of lignan and flavonol intake in children or adults.

Much attention has been given to estrogen-like exposures from the environment and their possible role in the declining age of puberty over the past 50 years [13]. Hormonal antagonists have the potential to delay pubertal onset. Limited epidemiologic evidence suggests that childhood intake of enterolactone [14] and isoflavones [15] may be associated with delayed pubertal timing in girls. In animal models, later puberty is observed for perinatal genistein administration [16]. However, some animal models have shown advanced female development after perinatal exposure to isoflavones and coumestrol [17,18] and peripubertal exposure to coumestrol [19]. Dose, timing and duration of intake may influence whether phytoestrogens have agonist or antagonist effects.

Evidence supports a protective effect of isoflavones on breast cancer in women who consume soy during adolescence or high levels as adults [2,20]. Additionally, studies suggest inverse relationships for both lignans and flavonols with breast cancer [3,21-24]. Because early puberty is an established breast cancer risk factor, prepubertal phytoestrogen intake may influence breast cancer risk through later pubertal development. Therefore, we hypothesized that increased intake of two phytoestrogen families, lignans and flavonols, would be associated with later pubertal development. We examined whether phytoestrogen intake was associated with timing of breast and pubic hair development in a prospective study of young girls.

2. Methods and materials

This project was carried out as part of the NIEHS/NCI Breast Cancer and the Environment Research Project (BCERP). The observational longitudinal study of pubertal maturation is conducted at 3 sites: (1) Mount Sinai School of Medicine (NYC), which recruited through clinics, schools, and neighborhood centers in East Harlem, New York, with girls seen annually; (2) Cincinnati Children's Hospital/University of Cincinnati (Cincinnati), which recruited through schools in the Cincinnati metropolitan area and through the Breast Cancer

Registry of Greater Cincinnati, with girls seen semiannually; and (3) Kaiser Permanente Northern California (KPNC), which recruited KPNC Health Plan members in the San Francisco Bay Area, with girls seen annually. Enrollment of 1239 girls 6-8 years old occurred between the years 2004 and 2007. The study was approved by the institutional review board at each site and at the CDC. Participants were eligible if they were healthy and free of conditions affecting metabolism or growth. All sites obtained informed consent from parent or guardian and independently verified child assent. Parents or guardians of the participants identified girls' race as black, white, Asian, and ethnicity as Hispanic or non-Hispanic. At enrollment (baseline), and approximately annually for 5 years, parents or guardians were interviewed in person or were given a self-administered questionnaire, in either English or Spanish, about the children's environmental exposures, physical activity, medical history, and demographics. Anthropometry and pubertal staging were done at each visit. A complete description of the study design is available in Biro et. al 2010. Children provided a spot urine sample at baseline. Enterolactone, the major lignan metabolite was determined in urine; analytic methods and validation including quality control as well as comparison with NHANES data have been described [26]. Concentrations are reported corrected for urinary creatinine ($\mu\text{g/gC}$) to account for urine dilution; the median biomarker level was identical to that of children in NHANES 2003-2004 (411 vs. 414 $\mu\text{g/L}$) [27]

2a. Dietary Data

Dietary recall interviews for all sites were performed by trained dietitians at Cincinnati Center for Nutritional Research and Analysis using the Nutrition Data System for Research (NDSR). Multiple (2-4) 24-hour dietary recalls by telephone were conducted during the first year. Four calls are intended provide average intakes over a year's time, by including daily and seasonal variation in food consumed, which can capture type and quantity of all foods. Three recalls have been shown to reliably rank individuals on energy and most nutrients [28-30]. The majority of girls had a complete set of 4 calls ($N=984$); a small number had either 3 or 2 calls ($N=148$ and 46 , respectively). Calls with either extremely low or high total energy consumption values (<400 k/cal and >4000 k/cal per day) were excluded from analysis ($N=18$). We report on 1178 girls with 2-4 diet calls and at least one pubertal assessment exam.

2b. Construction of a Lignan and Flavonol Database for Assessing Intake

We first developed a dietary database to capture lignan and flavonol contents of several hundred foods that were unavailable in standard dietary databases including NDSR. We used the USDA 2011 database [31] for flavonol concentrations and the Phenol-Explorer for foods not included in the USDA database and to verify the USDA values [32]. When sources had very different values for the same food (more than 2SD) we took the mean of the 2 sources. Lignan values were gathered from earlier reports [33-35]. We assigned lignan and flavonol concentrations (mg/100g) for 2319 individual foods and components reported in 4472 dietary records. Lignan and flavonol intake was calculated for each reported food by multiplying quantity consumed (g/day) and phytoestrogen content (mg/g). Mean daily lignan and flavonol intake (mg/day) was calculated for each girl.

To identify the specific foods that had the highest contribution to our girls' overall lignan and flavonol intake, we calculated the total lignan (flavonol) intake associated with each food consumed by each participant and divided by the total lignan intake consumed from all foods. We present the percentage of specific food's contribution to the total lignan (flavonol) intake, separately. We also calculated the number (and %) of our study participants who consumed foods with the highest lignan and flavonol content, according to the USDA 2011.

2c. Pubertal Staging

Pubertal maturation for breast and pubic hair stages was assessed as previously reported [25] based on standard diagrams that depicted stages 1-5, as published by van Wieringen et al (1985). Pubertal onset is defined as breast stage 2: breast bud stage, as described as by Marshall et al. [36]. Clinical staff were trained by experts at each site following a standardized protocol as described [25] and interrater reliability was assessed. In summary, there was substantial agreement between examiners and 'master' trainers ($\kappa=0.67$) on 127 examinations during the first years of the study. Retraining and recertification were done throughout the study. Pubertal stages were assessed at baseline and thereafter at each visit. Staging data were available for as many as six years of visits.

2d. Statistical Analyses

We examined bivariate relationships for lignan and flavonol intake in relation to selected demographic characteristics using adjusted general linear regression models and non-parametric statistics (Kruskal-Wallis). Correlations between urinary enterolactone and dietary lignan intake were examined using Spearman correlation coefficients adjusted for urinary creatinine. Lignan and flavonol intakes were modeled as residual energy-adjusted variables to remove variation caused by total energy intake [37]. In addition we examined models further adjusted for total energy intake by including total calories in the model; however we only report on results from models without further adjustment since there was little difference between these two approaches. To create lignan and flavonol intakes that are consistent with those reported in the literature as well as being representative of the wide intake range among our study population, we used the following groups, for lignans: <0.10mg, 0.10-0.50mg and >0.50mg/day and for flavonols <2mg, 2-5mg and >5mg/day.

Cox proportional-hazards models (PROC PHREG, SAS 9.2) were used to evaluate associations of phytoestrogens with age at pubertal onset. This model is used for longitudinal analyses, utilizing information from all girls who began the study. Analyses examining the association between lignan and flavonol intake and pubertal onset used longitudinal data on pubertal stages over 6 years of annual follow up visits. We defined age at breast or pubic hair stage 2 as the midpoint between the last visit at which stage 1 was observed with no prior visits at stage 2 and the first stage 2 visit with no subsequent visits at stage 1. Pubertal onset for girls who entered the study at breast stage 2 were assigned pubertal stage 2 as 6 months prior to their age at enrollment in the study. Girls who were still prepubertal at their last study visit or were lost to follow-up were right censored at that visit.

Models were adjusted for confounders that were selected based on the literature and on a conceptual model of diet and pubertal development which included: age, race, caregiver education, and BMI percentile (%) [38]. We did not include site in our final model because of possible overadjustment of the desired exposure differences that exist across sites and because of its collinearity with race and BMI%. However, we did examine the model with site because there are differences in dietary intake by site. We also examined interactions of site and BMI with dietary intake. Final models were adjusted for self-declared race/ethnicity and caregiver education (< high school). We examined models with and without BMI%, as it is related to breast development and to diet but may be in the causal pathway of pubertal onset.

We also examined accelerated failure time models with Weibull distributions using PROC LIFEREG. This interval-censored approach does not assign a specific time of onset but instead assumes that pubertal onset occurred in the interval between study visits. Results were almost identical to those from the Cox proportional hazards models.

3.Results

The study sample has been described elsewhere[25]. Briefly, girls were 6-8 years old at baseline (mean 7.3 yrs). There were similar proportions of Black, Hispanic and White girls, and a high percentage of caregivers had at least a high school education. At their baseline visit 168 (14%) of girls were breast stage 2 and 135 (12%) were pubic hair stage 2. Several baseline characteristics differed by site, with NYC having no White girls, a higher proportion of overweight girls and of caregivers with less than a high school degree [25]

The range for lignan intake was 0 –2.8 mg/day; for flavonols 0-28.1 mg/day. The distribution of both lignans and flavonols differed by demographic characteristics. Lignan intake at baseline was greater among older girls; flavonol intake at baseline was higher for White girls (Table 1). Energy intake was significantly higher in Cincinnati. Differences of phytoestrogen intake by site were due to the high proportion of White girls in California who ate more fruits, and vegetables and fiber (data not shown).

When we ranked foods by lignan or flavonol content (Tables 2 and 3), we found that at least some participants reported eating the 14 foods with the highest lignan and flavonol contents, with the exception of arugula and kale. However, fruits and vegetables high in lignan and flavonol content such as cranberries, blueberries, cherries, eggplant and cauliflower were not consumed by many of the girls. Orange juice, strawberries, potatoes, hamburgers/meat, carrots, broccoli and tomato products accounted for 75% of total lignan intake (Table 1), while onions, apples and tomato products accounted for more than 50% of flavonol intake (Table 2).

To explore alternative measures of lignan exposure, we assessed the correlation between urinary enterolactone concentrations and dietary lignan intake, which was poor ($R_s = 0.13$). The correlation was similar when we used the diet call closest to the urine sampling ($R_s = 0.12$). The mean (SD) urinary enterolactone concentrations were 5.8 $\mu\text{g/gC}$ (1.1), 6.2 $\mu\text{g/gC}$ (1.2), and 6.2 $\mu\text{g/gC}$ (1.2) for the diet lignan intake groupings of <0.1, 0.10-0.50 and >0.50 mg/day. Correlations were similar for urinary enterolactone with mean servings per day of fruits, vegetables, and fiber ($R_s = 0.13$ -0.19).

At the visit when they were last seen 998 (85%) of the girls had reached breast stage 2 or higher and 908 (78%) pubic hair stage 2. Compared to the lowest intake (<2mg/day), highest flavonol intake (>5mg/day) was associated with a later age at breast development (adjusted HR: 0.74, 95% CI: (0.61-0.91)) (p-trend=0.006)(Table 4). When we included site in the model the trend remained but the estimate and p-trend were attenuated (adjusted HR for high intake: 0.84, 95% CI: (0.69-01.04)) (p=0.107, p-trend=.133). Higher flavonol intake was not associated with the age at pubic hair development. Lignan intake was not associated with either breast or pubic hair development. Adjustment for BMI percentile did not change any of the results (results not shown). No significant interaction between BMI and either type of phytoestrogen intake on pubertal development was observed.

4.Discussion

In this prospective study of U.S. girls, findings confirmed our research hypothesis that phytoestrogens are associated with later pubertal onset. Breast development was almost 5 month later among girls with the highest flavonol intake compared to girls with lowest consumption. There were no associations between flavonols and pubic hair development, or lignans with either measure of pubertal development. An earlier report from this cohort found delayed breast development in relation to urinary enterolactone, a primary lignan metabolite [26].

Disparate diet intake may explain different results for these two phytoestrogens, but they may also have different potency and biologic mechanisms. The mean lignan intake in our population was in the lower range of studies that found inverse associations between lignan intake and breast cancer in women. The relatively low intake of lignans suggests that consumption in this population may not be sufficient to influence pubertal timing. Flavonol intake in our population was also lower than in many other studies [33,39]. Consistent with our present results, an earlier investigation using a cross sectional design identified later onset of breast development with higher urinary enterolactone but not with dietary lignan intake [14]. Another study also found later onset of breast development associated with higher dietary intake of isoflavones [15]. Studies suggesting that isoflavones are related to reduced breast cancer are consistent with our data [40,41].

Experimental comparisons of biologic potencies of these two phytoestrogen classes relative to estrogen are not definitive, although a range of phytoestrogens have been studied. Most studies find comparable, and very low estrogenic activity by flavonols and lignans. For example, comparison of two flavone-type chemicals and enterolactone demonstrated similar binding affinity for estrogen receptors (ER- α and - β) ($<1/1000$ that of estradiol). At the same time, genistein, a potent phytoestrogen, showed a stronger response to ER- β ($\sim 1/3$ that of estradiol) [42]. Other studies show similar responses of lignan and quercetin relative to estradiol [43,44].

Phytoestrogens, mainly isoflavones, have been associated with altered reproductive milestones in rodent models, including abnormal estrous cycles [19,45]. In premenopausal women given phytoestrogens (mainly isoflavones) in controlled experiments results have been mixed with regard to length of the menstrual cycle and hormone levels [46-50]. Timing of pubertal development in young girls has been associated with perinatal and pre-pubertal exposure to persistent halogenated aromatic compounds including polybrominated biphenyls (PBBs) [51], polychlorinated biphenyls (PCBs) [52] and dioxins [53], while other studies have reported no differences [54,55].

Many such agents may influence reproductive function by acting as hormone agonists or antagonists, depending on the timing, dose and background level of hormones [56]. Both animal and epidemiology studies suggest that prenatal and perinatal exposures exert effects on later development [13,18]. Exposures occurring in specific “windows of susceptibility” may have influences that differ from those that occur in other windows. Therefore the timing of exposures may be critical in determining exposure-outcome association. For example, a protective effect of dietary phytoestrogen intake on breast cancer risk was observed for relatively high isoflavone consumption before puberty [2,3] and during adolescence [2,57], while soy intake during infancy altered the timing of menarche [58]. Thus phytoestrogen exposure may need to occur long before the outcome to have an influence. Birth may be a more critical window for environmental exposures related to pubertal development than later in childhood, as it is a time when the reproductive tract is susceptible to changes in the developmental process.

Research on the influence of dietary phytoestrogens on health outcomes in humans may be inconsistent due to the different methods of measuring phytoestrogens. Phytoestrogen exposure can be estimated from biomarkers in either urine or blood samples or through dietary databases where values are derived from measures in food content. Each method represents different windows of action as well as differences in absorbed dose. With a short half-life (hours) urinary enterolactone (excreted) generally represents recent exposure whereas a dietary measure is more reflective of annual average intake. Thus, the lignan exposure biomarkers for enterolactone have been shown to only weakly correlate with dietary lignan intake in populations, like ours, with very low intake levels (median <50 mg/

day); correlations between diet and urinary enterolactone range from 0.13-0.4 [22,59]. In populations with high dietary intake, correlations are somewhat better (0.33-0.65). In addition, higher correlations are reported for lignans with certain types of foods, including with vegetables (0.46-0.60) and fiber (0.45-0.6)[11,47,60-63]. Since the highest contributors to both lignans and flavonols are fruits and vegetables, it is not unexpected that servings of fruits and vegetables are correlated with both dietary lignan and urinary enterolactone in our data and others [47,60,62]. Low correlations of dietary intake with urinary metabolites are not surprising since there is substantial variability in urinary excretion patterns both within and between subjects due to genetic and metabolic factors involved in disposition of phytoestrogens in the body [64]. The low correlation offers additional support for the idea that lignan intake in our sample may have been too low to show a biologic effect.

Dietary methods and biomarkers have different strengths and limitations. Dietary recalls are more economical in large studies, but they have relatively large within-person random error because of high day-to-day variability in their intake. Multiple recalls such as those in our study cover different seasons and days of the week in order to obtain a better estimate of girls' usual dietary intake [65,66]. Further variability occurs because micronutrient content in food varies by analytic technique, geographic variations in food sources, and crop [34,67]. Furthermore, several other plant lignans (*e.g.*, pinoresinol, syringaresinol, lariciresinol), have been identified recently, but their concentrations in different foods are only now becoming available [35]. Although urinary biomarkers represent a relatively short window of exposure for phytoestrogens, they are considered a valid exposure measure because levels of several phytoestrogens are reasonably stable over a range of months to a year in several reports [68-70].

Research on sources of phytoestrogens in adults or children is limited. Besides fruits, vegetables and fiber intake, older age and being a participant in the California site were associated with higher lignan and flavonol intake in our study sample. In another report, non-Hispanic Black children generally had less healthy diets than Hispanic children when using servings of basic food groups [71]. Hispanic women 25-40 yrs old had higher phytoestrogen intake than non-Hispanics, and lignans accounted for 65% of the total phytoestrogen intake [72]. Among the lignans, legumes among Hispanics contributed to their high phytoestrogen intake. In our sample black girls have lower phytoestrogen levels and servings of fruits compared to other girls. Thus our observed association between higher flavonols with later breast development may simply reflect intake of beneficial plant-based foods and a generally healthy lifestyle.

There are several limitations of this study, including the low level of intake in our sample which may have weakened our ability to identify an association compared to other studies with higher intake levels that have reported associations [14,15]. There is also possible misclassification for both lignan and flavonol consumption, as a result of imprecisions in dietary intake methodologies and incomplete phytoestrogen databases. We used several available sources to construct an updated, more complete database; however, pre-prepared foods and dietary supplements are not fully captured. The timing of exposure with respect to puberty is important to consider regarding the effects of weak antagonists competing with endogenous hormones. The assessment of phytoestrogen exposure in relation to puberty may need to be done before the rise of sex hormones, which may have occurred before baseline (dietary assessment) in our study.

This study offered many advantages to examine our research question. The longitudinal design allowed for pubertal staging to be repeatedly assessed over the course of several years. We measured diet from detailed 24-hour calls and had a urinary biomarker, as well as a range of dietary and demographic factors.

In recent years, phytoestrogens have gained much attention due to their physiological properties, leading to an increase in research on their potential to improve many health conditions. Given the multitude of phytoestrogens reported in diverse dietary sources, a better appreciation of their innate biological activities along with timing and duration of intake may be able to identify specific health conditions associated with particular phytoestrogens.

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Abbreviations

PE	phytoestrogen
HR	Hazards Ratio
BCERP	Breast Cancer and the Environment Research Project

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Table 1

Lignan and Flavonol Intakes in Relation to Baseline Characteristics among BCERP participants, 2004-2010

		N	Flavonol mg/d		Lignan mg/d		Energy kcal/day	
			mean (SD)	p-value ¹	mean (SD)	p-value	mean (SD)	p-value
Age								
	6-6.9	377	5.9(3.3)	<.001	0.40(.29)	<.001	1548(357)	0.400
	7-7.9	628	6.9(3.7)		0.48(.34)		1577(342)	
	8-8.9	173	6.6(3.7)		0.53(.40)		1567(393)	
Site								
	New York City	369	6.1(3.2)	<.001	0.47(.37)	<.001	1473(405)	<.001
	Cincinnati	377	5.8(3.3)		0.40(.27)		1663(318)	
	Kaiser Permanente	432	7.5(3.9)					
	Northern California				0.50(.34)		1560(309)	
Child race/ethnicity								
	Black	361	6.3(3.5)	0.220	0.43(.28)	0.750	1650(334)	<.001
	Hispanic	345	6.3(3.5)		0.49(.37)		1423(403)	
	White	415	6.8(4.0)		0.46(.35)		1622(291)	
	Asian	57	6.4(2.8)		0.43(.25)		1492(283)	
Baseline BMI Percentile ²								
	<50th	392	6.5(3.7)	0.120	0.45(.32)	0.640	1570(354)	0.230
	50-85th	401	6.7(3.6)		0.47(.34)		1591(357)	
	85th	385	6.2(3.5)		0.46(.35)		1536(346)	
Parent or guardian education ³								
	High school	323	6.1(3.2)	0.020	0.46(.34)	0.680	1471(414)	<.001
	> High school	808	6.7(3.8)		0.46(.33)		1602(321)	

¹Test for difference was performed by the Kruskal-wallis test.²BMI (kg/mg²) % adjusted for age and gender according to CDC growth data.³missing on 43 girls There were no differences in models mutually adjusted except that flavonols were no longer different by education status.

There were no differences in models mutually adjusted except that flavonols were no longer different by education status.

Table 2

Consumption food items containing high lignan content by BCERP participants, 2004-2010

Food	Total Lignan (mg/100g) ¹	Serving Size ²	Total Lignan ³ (mg)/Serving	N (%) eating ⁴
Spinach	0.38	1 cup	0.76	129 (11)
Orange juice	0.3	3/4 cup	0.52	662 (56)
Grapefruit juice	0.3	3/4 cup	0.52	20 (2)
Eggplant	0.4	1/2 cup	0.4	5 (0.4)
Squash	0.38	1/2 cup	0.38	75 (6)
Carrots	0.35	1/2 cup	0.35	766 (65)
Yams/Sweet Potatoes	0.3	1/2 cup	0.3	45 (4)
Broccoli	0.23	1/2 cup	0.23	351 (30)
Hamburgers/Tacos	0.21	2-3 ounces	0.21	659 (56)
Cauliflower	0.1	1/2 cup	0.1	73 (5)
Strawberries	0.08	1/2 cup	0.08	279 (24)
French Fries/potatoes	0.08	1/2 cup	0.08	802 (69)
Banana	0.07	1 medium fruit	0.07	443 (38)
Tomato/Tomato	0.06	1/2 cup	0.06	1135 (96)
Products Catsup	0.35	1 tablespoon	0.05	663 (56)
Peanut butter	0.16	2 tablespoons	0.05	425 (36)
Apples/Applesauce	0.05	1 medium fruit	0.05	925 (79)
Pears, raw	0.05	1 medium fruit	0.05	118 (10)

¹Total Lignan Values from USDA 2011²Serving Sizes based on USDA Guidelines³% contribution of foods to total lignan intake: orange juice 37%, strawberries 13%, carrots 6%, potatoes 6%, hamburgers/meat 5% , broccoli 5%, tomato products 3%⁴Ever reported eating

Table 3

Consumption food items containing high flavonol content by BCERP participants 2004-2010

Food	Total Flavonol ¹ (mg/100g)	Serving Size ²	Total Flavonol ³ (mg)/Serving	N (%) eating ⁴
Arugula	79.4	1 cup	158.8	0
Kale	34.45	1 cup	68.9	0
Cranberries, raw	21.96	1/2 cup	21.96	21 (2)
Onions	19.51	1/2 cup	19.51	1029 (87)
Spinach	9.01	1 cup	18.02	129 (11)
Blueberries	10.59	1/2 cup	10.59	94 (8)
Pears	4.81	1 medium fruit	4.81	118 (10)
Plums	4.01	1 medium fruit	4.01	65 (6)
Apple w/ skin	3.38	1 medium fruit	3.38	925 (78)
Cherries	2.92	1/2 cup	2.92	38 (3)
Tomato/tomato products ¹	2.88	1/2 cup	2.88	1135 (96)
Cocoa dry powder, unsweetened	20.13	1 tablespoon	2.88	229 (19)
Grapes	2.1	1/2 cup	2.1	327 (28)
Strawberries	1.69	1/2 cup	1.69	279 (24)

¹Total Flavonol Values from USDA 2011²Serving Sizes based on USDA Guidelines³% contribution of foods to total flavonol intake: apples 24%, onions 20%, tomato/products 11%, strawberries 5%, grapes 4%, lettuce 3%, blueberries 2%.⁴Ever reported eating

Table 4

Cox Proportional Hazards Ratios (HRs) and 95% Confidence Intervals (CI) for age in months at breast (B2) and pubic hair (PH2) development in relation to two dietary phytoestrogens, BCERP 2004-2010

		HR for Age at Breast Stage 2			p-trend¹
		<0.1	0.1-0.5	>0.5	
Lignans	mg/day				
	N per group	151	849	178	
	Median mg/day	0.07	0.24	0.7	
	Median age B2 ²	9.04	9.17	9.13	
	HR (CI)	1.00 (Ref)	0.90 (0.75-1.09)	0.85 (0.67-1.07)	0.222
	aHR ³ (CI)	1.00 (Ref)	0.89 (0.74-1.08)	0.88 (0.69-1.11)	0.422
Flavonols	mg/day	<2.0	2-5	>5.0	p-trend¹
	N per group	174	646	358	
	Median mg/day	1.5	3.5	6.5	
	Median Age B2 ²	8.92	9.13	9.38	
	HR (CI)	1.00 (Ref)	0.86 (0.72-1.01)	0.73 (0.60-0.89)	0.001
	aHR ³ (CI)	1.00 (Ref)	0.84 (0.70-1.04)	0.74 (0.61-0.91)	0.006
		HR for Age at Pubic Hair Stage 2			p-trend¹
		<0.1	0.1-0.5	>0.5	
Lignans	mg/day				
	N per group	151	849	178	
	Median Age PH2 ²	9.75	9.58	9.75	
	HR (CI)	1.00 (Ref)	1.09 (0.89-1.32)	1.02 (0.80-1.30)	0.694
	aHR ³ (CI)	1.00 (Ref)	1.07 (0.88-1.31)	1.08 (0.84-1.38)	0.759
Flavonols	mg/day	<2.0	2-5	>5.0	p-trend¹
	N per group	174	646	358	
	Median Age PH2 ²	9.4	9.7	9.7	
	HR (CI)	1.00 (Ref)	0.94 (0.77-1.13)	0.88 (0.71-1.08)	0.196
	aHR ³ (CI)	1.00 (Ref)	0.95 (0.78-1.15)	0.98 (0.79-1.21)	0.891

¹ p-trend calculated using the median of each category as continuous variable

² Median B2 age among non-censored girls within each nutrient category

³ aHR estimated from model adjusted for race, education