Habitual Phytoestrogen Intake Is Associated with Breast Composition in Girls at 2 Years after Menarche Onset

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ABSTRACT

Background: High phytoestrogen intake during adolescence is associated with a reduced risk of breast cancer. Breast density (BD) is a strong predictor of breast cancer and can be considered an early marker. We aim to assess the association between the mean habitual intake of isoflavones, lignans, and total phytoestrogens intake during puberty until 2 years after menarche onset and absolute fibroglandular volume (AFGV) and percentage of fibroglandular volume (%FGV) in Hispanic girls at the end of puberty.

Methods: Longitudinal study set up in the Growth and Obesity Chilean Cohort Study (GOCS). We included 329 girls with dietary data (multiple 24-hours recalls) from puberty until 2 years after menarche onset (81% had 2–4 recalls). Two international datasets were used to estimate isoflavones, lignans, and total phytoestrogens in the diet. Breast composition was measured by dual energy X-ray absorptiometry at 2 years after menarche. Multiple linear regression models were used to assess the association between isoflavones, lignans, and total phytoestrogens intake and AFGV and %FGV.

Results: The average total phytoestrogen intake was 1 mg/day and %FGV was 50.7% (SD = 15.2) and AFGV 218.8 cm^3 (SD = 79.3). An inverse association was found between consumption of isoflavones and AFGV, as well as, with total phytoestrogens [Q4 vs. Q1 adjusted model ß = −49.2 cm^3; 95% CI (−85.5 to −13.0)].

Conclusions: Girls with a higher intake of total phytoestrogens and isoflavones during puberty until 2 years after menarche onset had significantly lower AFGV.

Impact: Although the intake of phytoestrogens is low in Western populations, higher consumption of them during a critical period of life like puberty could be beneficial to reduce breast cancer during adulthood.

Introduction

Estrogens play a fundamental role in the development, proliferation, and function of the mammary gland; however, prolonged exposure to estrogens is associated with an increased risk of breast cancer (1). Factors such as early menarche, late menopause (>55 years), nulliparity, late first pregnancy (>30 years), and hormone replacement therapy are associated with an increased breast cancer risk (2). On the other hand, the interval between menarche and first birth is an important window of susceptibility when undifferentiated breast tissue is particularly vulnerable to carcinogens (2), highlighting the importance of early life risk factors for this disease.

Phytoestrogens (isoflavones and lignans) are natural components present in plants that have a structure similar to 17β-estradiol (3). They can compete with and modulate the estrogenic response through their binding to the endogenous estrogen receptors ERα and Erβ. Binding through Erβ promotes apoptosis and inhibits the proliferative effect associated with ERα activation in breast tissue (4). Apparently, phytoestrogens have a greater affinity for Erβ, postulating that a higher intake of phytoestrogens could lower the risk of breast cancer (4).

Several meta-analyses have suggested an inverse relation between soy or isoflavone intake and breast cancer risk in Asian populations, who generally have high consumption of soy (5, 6). But in Western adult populations, the evidence is limited (7). In addition, in Asian population, they observed that the consumption of these products during adolescence may further decrease the breast cancer risk (8–10). These results point toward the concept of a "critical window" for exposure to phytoestrogens, specifically during the early stages of life, which could play a key role in the risk of developing breast cancer in adulthood (1). However, the time elapsed between this period and the diagnosis of breast cancer is long, so it is useful to evaluate intermediate variables that allow us to better quantify and understand these associations and their mechanisms.

Breast density (BD) is considered a strong surrogate of breast cancer risk; women with very dense breasts have a 5 times increased risk of breast cancer compared with women with nondense breasts (1). The correlation between phytoestrogens and BD is controversial and data are available only in adult women. Some studies have observed an inverse or null association with BD (11), while others have reported a positive association with BD (12, 13) and some have observed differences by ethnic group (13). Thus, our study aim to evaluate whether higher phytoestrogen intake during puberty is associated to breast density at 2 years after menarche onset as a marker of the end of pubertal development, a critical period for breast carcinogenesis. This study was performed in Latino adolescents, and BD was measured by dual X-ray absorptiometry (DXA), a novel approach to measure BD at young ages (14), where we estimated the % fibroglandular volume (%FGV) and the absolute fibroglandular volume (AFGV).

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Materials and Methods

Study design and population

The Growth and Obesity Chilean Cohort Study (GOCS) is a longitudinal study initiated in 2006 that recruited 1,195 children (≈50% female) between 2.6 and 4.0 years of age who attended 54 child-care centers in the southeastern area of Santiago de Chile. They are representative of low to middle socioeconomic levels. The inclusion criteria of the girls were as follows: single births born between 2002 and 2003, birth weights between 2,500 and 4,500 grams, and no physical or mental illness diagnosed at the time of recruitment (15). For this study, we included 329 nulliparous girls, for whom we had complete data on their breast composition at two years after menarche onset and 24-hour dietary recalls (24HR) during the period between the onset of menarche and 2 years later.

Data collection

Since 2006, GOCS participants attended an annual appointment for data collection at the Institute of Nutrition and Food Technology (INTA), University of Chile. During puberty, girls were evaluated every six months to determine their sexual maturation (breast and pubic hair development), and the date of their first menstrual bleeding (age at menarche) was asked at every visit.

Breast Composition

All girls who reached two years after menarche onset underwent a breast scan by DXA to assess their breast composition (%FGV and AFGV). We chose to measure breast composition 2 years after the menarche onset, because achieving menarche is an important factor for %FGV (16) and besides in unreported work from our group, %FGV increases until 2 years after menarche onset and remains stable thereafter (manuscript under preparation). This method was developed by Dr. Shepherd to be used in young women due to its low radiation (scan = 1.32 uSv) and lack of breast compression. It has been validated in Chilean women, with a precision of 2.8% and ICC greater than 0.9 (17). The procedure consists of the girl lying in a medial–lateral decubitus position (left side), the left arm rests below the head, and her right hand holds the right breast out of the X-ray field. The left breast is scanned first, and then the right breast is scanned, and finally the left breast is scanned again. All measurements were performed by a single trained DXA technician. For the analysis, breast composition was calculated using software according to the method developed by Shepherd and colleagues (14). The %FGV is estimated as the sum of the AFGV divided by the total breast volume. The GE Lunar Prodigy Bone Densitometer (GE Healthcare) is calibrated with a phantom (N17NS), which consists of seven densities to measure breast composition. More details have been published elsewhere (14).

Dietary data

We started collecting dietary information in 2013, when 86% of the sample was at Tanner stage II or higher and their mean age was 10.9 (SD 0.4), and this continued until 2 years after menarche onset (mean age = 13.9, SD = 1.0). We applied a 24-hour diet recall (24HR) at each visit to the participant to record their food consumption during the previous day. It was primarily answered by the participant (including food consumption at home, outside home and school) and the mother or caregiver could provide complementary data. We performed 24HR interviews using the United States Department of Agriculture (USDA) Automated Multiple-Pass Method (18, 19), which consists of asking about food and liquid intake in five steps to ensure adequate and complete data collection (18). We used the Photographic Atlas of the National Dietary Survey (19) to estimate the portion sizes of the consumed foods. The interviews were performed by trained dietitians to reduce any measurement error (20).

All data were recorded and analyzed with 24HR survey software (SER24H) developed in 2015 by our team. SER24H currently includes over 4,600 foods and 900 recipes commonly consumed by the Chilean population (21). Chile does not have an updated and comprehensive nutrient composition dataset; thus, each food recorded in the 24HR survey is assigned an energetic value and nutrient contents based on the USDA Food Composition Database, Release 28 (22). Assignments were based on information provided by the Guide on the Nutritional Composition of Natural Foods and Common Chilean Preparations (19), as well as data declared as part of the nutritional information on packaged products (collected during 2015–2016 for packaged products at supermarkets) (21). More details about the harmonization of the data have been published previously (20, 21).

We linked these data with databases containing isoflavones, lignans, and total phytoestrogens to Chilean foods. The total phytoestrogen and isoflavone data were extracted from the "USDA Database for the Isoflavone Content of Selected Foods, Release 2.1" (23) and the "Phytoestrogen Content of Foods Consumed in Canada, Including Isoflavones, Lignans, and Coumestan, 2006" (24). The latter dataset provided information on lignan content that is not included in the USDA database. Our 24HR datasets have been previously linked to the USDA dataset and ID (as detailed above); thus, we were able to extract the data on isoflavones and total phytoestrogens. However, we had to link the Canadian lignan content database with the USDA isoflavone content database. We used two criteria: (i) homologation of the foods by the content of isoflavones and (ii) homologation by the content of critical nutrients (macronutrients) when homologation by the first criteria was not possible. In both cases, it was considered that a food complies with the adequacy of nutrients when the food in the Canadian dataset had an adequacy for the content of isoflavones or critical macronutrients between 80% to 120% of that in the USDA dataset (±20%).

Anthropometry

Information on birth weight was collected using medical records. Since 2006, weight and height have been measured during INTA visits by trained dietitians using standardized measurement protocols. Measurements were performed with a Tanita BC-418 with a precision of 0.1 kg and height using a Seca 222 with 0.1 cm accuracy. Body mass index (BMI) was obtained through the weight (kg)/height (m^2) formula, and we estimated the *Z*-score according to the growth curves of the World Health Organization (WHO) 2007 (25).

Maternal data

In 2010 to 2011, a maternal questionnaire was applied asking about the number of years of schooling of the mothers from which the socioeconomical level was derived.

Statistical analysis

Exposure, outcome, and confounders [girls' BMI *Z*-score at two years after menarche onset, the mean total energy intake (during the 2 years after menarche onset), birth weight, age at menarche and the mothers' years of formal education] were described as the means (SD), medians (IQR), or percentages as appropriate.

We estimated the phytoestrogens (total phytoestrogen, isoflavones, and lignans) and total energy as the average/day obtained from the R24H surveys carried out during puberty up to two years after menarche onset. We included all questionnaires provided by

the girl during this period, and we estimated the mean energy and phytoestrogen intake per girl. These were evaluated as continuous variables to assess the distribution of lignans, isoflavones, and total phytoestrogens. For the subsequent analyses, they were transformed into quartiles according to their distribution, in order to control for potential outliers.

A comparative analysis was performed between the quartiles of phytoestrogens (total, isoflavones, and lignans) and confounding and outcome variables. Categorical variables were evaluated by chi-square, and continuous variables were evaluated by Student *t* test. We estimate the *P* value for trend using a nonparametric test for trend across the quartiles of phytoestrogen intake.

We performed crude and covariate-adjusted linear regression models to evaluate the association between quartiles of intake of isoflavones, lignans, and total phytoestrogens (Q1 = reference group) and %FGV and AFGV at two years after menarche onset. Models were adjusted by previously established confounding variables, such as the girls' BMI *Z*-score at two years after menarche onset, mean energy intake during the 2 years after menarche onset, birth weight, age at menarche and mothers' years of formal education. All confounding variables were used as continuous variables in the models. We corroborated the assumptions of the residuals' normality in all of the linear regression models. Finally, we performed a sensitivity analysis restricting the final models to girls who had 2 or more 24HR during the period of study. The analyses were carried out using STATA 14.0 (StataCorp LP), and associations were considered significant at $P < 0.05$.

Ethics approval

The study protocol was conducted according to ethical guidelines of the Declaration of Helsinki, and it was approved by the Institutional Review Board of the Institute of Nutrition and Food Technology (INTA) of the University of Chile, which oversees research in human subjects. Written informed consent was obtained from all parents or guardians and assent from the girls.

Data availability

The data generated in this study are available upon request from the corresponding author.

Results

A total of 386 girls had dietary data during puberty until -2 years after menarche onset, but we evaluated breast composition in only 329 of them. Girls without breast composition data were not different from their counterparts in terms of total phytoestrogen consumption, total energy intake, age at menarche, BMI *Z*-score, and maternal education. A total of 1,075 24HR surveys were collected during puberty up to two years after menarche and only 13% of the girls answered one 24HR. As shown in **Table 1**, the average age of the girls at the time of DXA was 13.9 years (SD 1.0), and the age at menarche onset was 11.9 (SD = 0.9); both parameters increased across the quartiles of phytoestrogen intake. Approximately 50% were overweight or obese (BMI ≥ 1 SD) at 2 years after menarche onset; however, girls who reported the highest consumption of total phytoestrogens had significantly lower BMI *Z*-scores.

The mean total energy consumption of the girls during this period was 1,792 kcal/day. The mean intake of isoflavones and lignans was 0.75 mg and 0.21 mg, respectively, obtaining a total of 0.96 mg for both phytoestrogens combined (**Table 1**). Overall, 94 (28.6%) girls reported no consumption of phytoestrogens, and 51 (15.5%) reported consumption greater than 1 mg/day.

In the girls, the mean AFGV was 218.8 cm^3 (SD = 79.3), and %FGV was 50.7% (SD = 15.2) and girls who reported a higher intake of total phytoestrogens had a lower AFGV and we observed a trend within the quartiles ($P = 0.037$; **Table 1**).

Table 1. Characteristics of the 329 GOCS girls included in this study by quartiles of phytoestrogen intake.

		Total phytoestrogens			
	Total	**Q1 (0)**	**Q2 (>0–0.28)**	**Q3 (0.29–0.76)**	**Q4 (0.77–19.48)**
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Age at DXA (years)	13.9 (1.0)	12.9 (0.5)	14.1 (0.8)	14.3 (0.8)	14.5 (0.8)
Age at menarche (years)	11.9 (0.9)	11.0 (0.6)	12.1 (0.7)	12.3 (0.7)	12.4 (0.8)
Anthropometry					
Birth weight (kg)	3.4 (0.4)	3.4 (0.4)	3.3 (0.4)	3.3 (0.4)	3.4 (0.4)
BMI *Z*-score at 2 years after menarche onset	1.0 (1.0)	1.3 (0.9)	1.0 (0.9)	0.9 (1.2)	0.7 (1.0)
Nutritional status at 2 years after menarche onset [*n*, (%)]					
Underweight (≤−1 SD)	8 (2.1)	0 (0)	1 (1.1)	5 (5.2)	2 (2.1)
Normal (>−1 y <1 SD)	184 (47.7)	37 (37)	46 (49.5)	48 (49.5)	54 (56.3)
Underweight (≥1 y <2 SD)	130 (33.7)	41 (41)	33 (35.5)	27 (27.8)	28 (29.2)
Obese (≥2 SD)	64 (16.6)	22 (22)	13 (14.0)	17 (17.5)	12 (12.5)
Diet data					
Total energy (Kcal)	1,792 (561.2)	1,898 (730)	1,690 (456)	1,753 (509)	1,822 (482)
Total phytoestrogens (mg)	1.0 (2.6)	0 (0)	0.1 (0.1)	0.5 (0.1)	3.2 (4.5)
Isoflavones (mg)	0.7 (2.5)	0 (0)	0 (0.1)	0.3 (0.2)	2.6 (4.6)
Lignans (mg)	0.2 (0.4)	0 (0)	0.01 (0.02)	0.2 (0.2)	0.6 (0.6)
DXA breast density					
%FGV	50.7 (15.2)	50.2 (15.3)	51.2 (14.3)	49.5 (14.8)	52.1 (16.4)
AFGV	218.8 (79.3)	229.9 (82.9)	218.6 (74.5)	221.2 (83.2)	202.0 (73.0)
Maternal data					
Year of formal education [n, (%)]					
<12 years	251 (76.7)	75 (75.0)	65 (70.7)	82 (84.5)	71 (75.5)
12 or more years	76 (23.2)	25 (25.0)	27 (29.4)	15 (15.5)	23 (24.5)

As shown in the linear regression models (**Table 2**), girls within the highest quartile of isoflavone consumption had significantly lower levels of AFGV at two years after menarche onset, but the results were significant only in the fully adjusted model [Q4 vs. Q1: ß: −30.5 cm^3, (95% CI: −60.7, −0.4)]. We did not observe any association between lignan intake and AFGV; however, assessing the total intake of both phytoestrogens, we observed a strong inverse association in the group of highest quartiles, either in the crude or adjusted models (ß: −36.2 cm^3; 95% CI: −66.2, −6.1). We did not observe any association with %FGV.

Table 2. Crude and adjusted linear regression models of isoflavones, lignans, and total phytoestrogens intake during puberty up to 2 years after menarche onset and %FGV and AFGV at 2 years after menarche onset.

	AFGV (cm^3) β (95% CI)	%FGV β (95% CI)
Isoflavones		
Model 1		
1st quartile	Ref	Ref
2nd quartile	−9.11 (−33.24, 15.02)	−2.05 (−6.67, 2.57)
3rd quartile	−15.58 (−39.05, 7.89)	2.02 (−2.47, 6.51)
4th quartile	−21.88 (−45.83, 2.08)	1.80 (−2.79, 6.38)
Model 2		
1st quartile	Ref	Ref
2nd quartile	−17.32 (−43.98, 9.34)	−1.16 (−5.08, 2.76)
3rd quartile	−22.48 (−51.12, 6.16)	−0.20 (−4.41, 4.00)
4th quartile	−29.13 (−59.37, 1.11)	−1.24 (−5.68, 3.20)
Model 3		
1st quartile	Ref	Ref
2nd quartile	−17.85 (−44.50, 8.80)	−1.42 (−5.31, 2.47)
3rd quartile	−21.66 (−50.30, 6.98)	−0.62 (-4.80, 3.56)
4th quartile	−30.54 (−60.66, −0.42)[a]	−1.53 (−5.92, 2.87)
Lignans		
Model 1		
1st quartile	Ref	Ref
2nd quartile	−11.31 (−40.41, 17.80)	0.39 (−5.19, 5.96)
3rd quartile	−17.63 (−39.43, 4.17)	1.86 (−2.32, 6.03)
4th quartile	−4.87 (−27.26, 17.51)	2.80 (−1.49, 7.08)
Model 2		
1st quartile	Ref	Ref
2nd quartile	−8.01 (−38.50, 22.49)	−0.57 (−5.04, 3.89)
3rd quartile	−16.71 (−41.20, 7.78)	−0.78 (−4.36, 2.81)
4th quartile	−4.52 (−29.79, 20.75)	0.80 (−2.90, 4.50)
Model 3		
1st quartile	Ref	Ref
2nd quartile	−8.58 (39.18, 22.01)	−0.44 (−4.88, 4.00)
3rd quartile	−16.86 (−41.49, 7.77)	−1.19 (−4.77, 2.38)
4th quartile	−3.08 (−28.49, 22.33)	0.69 (3.00, 4.37)
Total Phytoestrogens		
Model 1		
1st quartile	Ref	Ref
2nd quartile	−11.36 (−35.60, 12.89)	0.97 (−3.70, 5.64)
3rd quartile	−8.69 (−31.75, 14.37)	−0.68 (−5.12, 3.75)
4th quartile	−27.93 (−52.09, −3.78)[a]	1.92 (−2.73, 6.57)
Model 2		
1st quartile	Ref	Ref
2nd quartile	−17.39 (−44.64, 9.87)	0.11 (−3.90, 4.13)
3rd quartile	−16.98 (−44.50, 10.53)	−1.93 (−5.98, 2.12)
4th quartile	−37.31 (−67.20, −7.42)[a]	−1.03 (−5.43, 3.37)
Model 3		
1st quartile	Ref	Ref
2nd quartile	−17.30 (−44.72, 10.13)	−0.31 (−4.31, 3.69)
3rd quartile	−17.39 (−45.02, 10.23)	−1.88 (−5.92, 2.15)
4th quartile	−36.19 (−66.24, −6.14)[a]	−1.14 (−5.53, 3.25)

Note: Model 1: Crude ($n = 329$).
Model 2: Model 1 + mean kcal (total energy between puberty until 2 years after menarche onset), BMI *Z*-score at 2 years after menarche onset, age at menarche and age at DXA and birthweight ($n = 329$).
Model 3: Model 2 + maternal education ($n = 325$).
[a]$P < 0.05$.

Our sensitivity analysis, restricting the final models to girls with 2 or more 24HR, showed similar results. In the fully adjusted models, we observed an inverse association of AFGV with isoflavone intake (Q4 vs. Q1: ß: −28.4 cm^3; 95% CI: −59.0, −3.1) and total phytoestrogen intake (Q4 vs. Q1: ß: −33.1; 95% CI: −64.8, −1.3).

Discussion

Our results suggest that girls with the highest quartile of total isoflavone and total phytoestrogen intake have significantly lower levels of AFGV than girls with a lower intake. The estimated total phytoestrogen intake of the girls during the two years postmenarche was on average 1 mg/day (80% provided by isoflavone), similar to other Western countries where the average isoflavone intake is less than 2 mg/day (26). These values are much lower than those reported in Asian countries, where intake ranges between 20 and 50 mg/day, mainly due to the high consumption of soy and derived products (27).

Several epidemiologic studies have suggested an inverse association between soy/isoflavone intake and breast cancer; however, few have evaluated the effect when exposure occurs early in life. "The Shanghai Breast Cancer Study" found that women who reported a higher soy intake during adolescence (13–15 years) had a significantly lower risk of developing breast cancer [Q5 vs. Q1 OR: 0.51; 95% CI (0.40–0.65)], even after adjusting for reported soy intake during adulthood (8). Korde and colleagues (2009) found in Asian American women that high soy consumption during childhood (5–11 years) decreased the risk of developing breast cancer by approximately 60% [RR: 0.40; 95% CI (0.18–0.86)], and the effect was not attenuated after adjusting for dietary soy intake during adolescence or adulthood (28). Similar results were observed in Chinese, Japanese, and Filipino women, where the greater the consumption of tofu during adolescence decreased the risk of developing breast cancer [>4 times/week: OR 0.51, 95% CI (0.31–0.84)]. Furthermore, this effect was higher if consumption persisted into adulthood (9). In addition, a study performed in Canada (a Western population, with low intake of phytoestrogens) observed an inverse association with isoflavones, lignans, and total phytoestrogen intake during adolescence (10–15 years) and breast cancer. Particularly, they found that high consumption of lignans during adolescence reduced the risk of breast cancer [OR (Q4 vs. Q1): 0.74, 95% CI (0.64–0.85); ref. 29]. These studies support the hypothesis that intake of phytoestrogen during adolescence might be beneficial for breast cancer prevention, a period in which undifferentiated breast tissue is particularly vulnerable to carcinogenesis (1). However, most of these observations are obtained in the Asian population, where the intake of phytoestrogens is high (20–50 mg/day; ref. 27).

Observational studies assessing the intake of phytoestrogens and BD are limited and restricted to adult populations. Data from 406 adult women belonging to two cohorts in Singapore observed that higher intake of soy protein and isoflavones (highest quartile of intake vs. lowest quartile) was inversely associated with women with a high-risk parenchymal pattern (pattern IV and V according to Tabar's classification system; ref. 30) compared withj women with a low-risk pattern [pattern I; OR 0.41, 95% CI (0.18–0.94); ref. 31). In contrast, a study carried out in Hawaii (2001) observed a positive association between soy intake (mean age 53.9 and SD 10.1) and % FGV (>34.8 g: 33.4% vs. >6.7 g: 30.0%, $P = 0.04$) and a nonsignificant negative trend with AFGV after adjusting for BMI, energy, age, and parity (a difference of 18 cm^3 between Q4 vs. Q1; ref. 32). Randomized clinical trials to evaluate the consumption of phytoestrogens and BD are not conclusive. A meta-analysis of randomized clinical trials (5 trials, $n = 519$) evaluating the effect of isoflavones and BD in pre- and postmenopausal Western women showed no association in postmenopausal women and in premenopausal women, only a modest, nonsignificant increase in %FGV (12). The main limitation of this meta-analysis is that despite considering intakes of isoflavones equivalent to or higher than those reported in Asian populations (40–120 mg/day), the duration of exposure to the isoflavones measured was short (6 months–3 years). In other study, a double-blind clinical trial was conducted in adult premenopausal women [multiethnic cohort (38% Caucasian)] who were supplemented with 100 mg of isoflavones over 12 months to match the intakes of Asian populations and analyze its association with mammographic BD. No significant changes were found in the mammographic densities (%FGV and AFGV). However, the small sample size ($n = 34$) limits the power to detect significant changes, and the short duration of the intervention does not permit evaluation of the effect of consuming isoflavones sustained over time (33).

To our knowledge, no other studies have assessed phytoestrogen intake during the pubertal period until first birth and BD, a critical period for breast development (34). We used a validated (35) novel approach to measure breast density at young ages with low-dose exposure (14). The % of FGV is a relative measure that assesses the amount of fibroglandular tissue in relation to the total breast volume or area, and it was the first parameter to be identified as a strong risk factor for breast cancer. Afterward, new studies have tested whether the direct measure of the absolute fibroglandular volume is also associated with increased breast cancer (36). Interestingly, we observed an inverse association between phytoestrogen consumption and breast composition only with AFGV, but we observed a nonsignificant inverse association with %FGV. Our results suggest that the intake of phytoestrogens during the pubertal period may modulate the breast composition at young ages, decreasing the total amount of fibroglandular volume. Thus, if the results of this study are confirmed in larger and prospective studies; a reduction in breast cancer in adulthood associated with the intake of phytoestrogens in adolescence could occur through modulation of the breast composition at young ages.

There are several potential biological mechanisms explaining the inverse association between the consumption of phytoestrogens and breast cancer. Isoflavones have a higher affinity for ERβ than for ERα, decreasing cell proliferation in the breast and reducing breast cancer risk and potentially AFGV (37). In addition, animal studies showed that prepubertal exposure to isoflavones and lignans promotes the differentiation of the mammary epithelial tree, which decreases the sensitivity of the mammary gland to carcinogens and therefore breast cancer risk in adulthood (38). Childhood and adolescence are key periods for breast development, and during this period, there is a greater susceptibility to carcinogens compared to breast tissue in adulthood (8, 9, 28, 29). Furthermore, approximately 30% to 50% of individuals can metabolize the isoflavone daidzein to equol, which has been shown to have a higher estrogenic activity than its precursor. Tseng and colleagues (2013) observed an inverse association between isoflavone intake and %FGV only in equol-producing women [mean difference: 29.3% (23.4–35.2); ref. 39].

Our study is not free of limitations. First, the phytoestrogen consumption was self-reported, which could be affected by recall bias; however, we used 24-hour recall data obtained by trained dietitians and a standardized technique to diminish this error. In order to diminish potential measurement error, we perform a sensitivity analysis including girls only with two or more recall and results were similar, and we model phytoestrogen intake in quartiles to avoid the influential effect of outliers. However, we further excluded girls who reported an intake of phytoestrogens higher than five times the

standard deviation, and the results did not differ compared to the whole sample. In addition, dietary questionnaires do not consider differences in metabolism, so we could not differentiate them according to equol production, nor were we able to estimate the concentrations of biologically active forms through plasma or urinary measurements. However, a significant correlation has been reported among the different measurement methods (plasma concentration, urinary concentration, and dietary phytoestrogen surveys; ref. 40). Moreover, 13% of the girls had only one 24-hour recall data, but the results were similar after restricting the analysis to girls with two or more questionnaires. Other dietary factors could be considered as confounders, such as fat intake, which has been related to breast density (41) in other populations. However, in our study, fat intake was not associated with phytoestrogen intake and breast density in the girls, and for this reason, we did not consider it as a potential confounder. In addition, we do not have Chilean data in relation to the concentration of phytoestrogens in common foods; however, we chose two international dataset that have information on phytoestrogens and which to our knowledge are the most complete. Besides these limitations, our study also highlights several strengths. It has a longitudinal design allowing us to measure the intake of phytoestrogens prospectively during puberty and adolescence, which are key periods for breast development. In addition, we used breast density at the end of pubertal development, which is recognized as a strong risk factor for breast cancer and, in this case, an early marker of disease.

In conclusion, we found that girls who reported a higher intake of total phytoestrogens during puberty had a significantly lower AFGV. This study is one of the first to evaluate this association in a Western adolescent population. These findings support the hypothesis that small amounts of phytoestrogens consumed early in life may provide protection from breast carcinogenesis, and the timing of these intakes matters for the early prevention of breast cancer. This is a potentially modifiable environmental factor, and it could have a significant impact on public health; however, additional studies are needed to confirm these findings (including studies that evaluate the change in phytoestrogen intake and change in breast composition data during the life course) and elucidate their implications.

Authors' Disclosures

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Authors' Contributions

C. Lesser: Conceptualization, data curation, formal analysis, writing–original draft. **V. Mericq:** Supervision, methodology, writing–review and editing. **M. Reyes:** Supervision, methodology, writing–review and editing. **M.L. Garmendia:** Data curation, methodology, writing–review and editing. **J.A. Shepherd:** Software, writing–review and editing. **K.B. Michels:** Funding acquisition, writing–review and editing. **C. Corvalán:** Funding acquisition, writing–review and editing. **A. Pereira:** Formal analysis, supervision, funding acquisition, methodology, project administration, writing–review and editing.

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