Published in final edited form as:
Sci Total Environ. 2011 November 15; 409(24): 5221–5227. doi:10.1016/j.scitotenv.2011.09.029.

URINARY MYCOESTROGENS, BODY SIZE AND BREAST DEVELOPMENT IN NEW JERSEY GIRLS

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Abstract

BACKGROUND—Despite extensive research and interest in endocrine disruptors, there are essentially no epidemiologic studies of estrogenic mycotoxins, such as zeranol and zearalenone (ZEA). ZEA mycoestrogens are present in grains and other plant foods through fungal contamination, and in animal products (e.g., meat, eggs, dairy products) through deliberate introduction of zeranol into livestock to enhance meat production, or by indirect contamination of animals through consumption of contaminated feedstuff. Zeranol is banned for use in animal husbandry in the European Union and other countries, but is still widely used in the US. Surprisingly, little is known about the health effects of these mycoestrogens, including their impact on puberty in girls, a period highly sensitive to estrogenic stimulation.

OBJECTIVES AND METHODS—We conducted a cross-sectional analysis among 163 girls, aged 9 and 10 years, participating in the Jersey Girl Study to measure urinary mycoestrogens and their possible relationship to body size and development.

RESULTS—We found that mycoestrogens were detectable in urine in 78.5% of the girls, and that urinary levels were predominantly associated with beef and popcorn intake. Furthermore, girls with detectable urinary ZEA mycoestrogen levels tended to be shorter and less likely to have reached the onset of breast development.

CONCLUSIONS—Our findings suggest that ZEA mycoestrogens may exert anti-estrogenic effects similar to those reported for isoflavones. To our knowledge, this was the first evaluation of urinary mycoestrogens and their potential health effects in healthy girls. However, our findings need replication in larger studies with more heterogeneous populations, using a longitudinal approach.

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Keywords

mycoestrogens; zearalenone; zeranol; thelarche; height; weight

1.1 INTRODUCTION

Epidemiologic studies have clearly identified puberty as a critical window in a woman's development during which environmental exposures can have a major impact on her future risk of developing breast cancer (Colditz et al., 1995). Estrogenic endocrine disrupting chemicals (e.g., DDT, phthalate esters, bisphenol A) have been the subject of considerable research and are now considered to be hazardous to human health (Roy et al., 2009) and capable of affecting onset and progression of puberty (Buck Louis et al., 2008). Of particular concern is the possibility that exposures during the female pubertal period, when sensitivity to hormonal signals is high and endogenous estrogen levels are still low, leads to the precocious onset of puberty, ultimately increasing the risk of breast cancer (Roy et al., 2009).

Zearalenone (ZEA) mycotoxins are present in grains and other plant foods through fungal contamination by *Fusarium* species, and in animal products (e.g., meat, eggs, dairy products) through deliberate introduction of zeranol into livestock to promote growth and improve beef/meat production, or by indirect contamination through consumption of contaminated feedstuff (Meucci et al., 2008). Zeranol (α-ZAL), a synthetic derivative of ZEA, is a United States FDA approved agent commonly used as a non-steroidal anabolic growth promoter in beef production, but banned in many countries, including those in the European Union (Massart et al., 2010). Because of their estrogenic activity, ZEA and its metabolites have been classified as phytoestrogens, mycoestrogens, and growth promoters (Bennett et al., 2003).

The metabolism of ZEA mycoestrogens in humans is poorly understood. Animal data suggest that these mycoestrogens are metabolized into all the other forms (Massart et al., 2010): ZEA is metabolized primarily to alpha-zearalenol (α-ZEL) and beta-zearalenol (β – ZEL), with further conversion to alpha-zearalanol (α-ZAL or zeranol) and beta-zearalanol (β-ZAL), respectively (Kleinova et al., 2002). Alpha-ZAL (zeranol) is primarily metabolized into β -ZAL and to a lesser extent into zearalanone (ZAN) (Kleinova et al., 2002).

Surprisingly, little is known about the health effects of these estrogenic mycotoxins, particularly their impact during puberty in girls, a period highly sensitive to estrogenic stimulation. There are several anecdotal reports of epidemics of precocious puberty, which were attributed to the use of anabolic estrogens in animal foods, such as zeranol, in Italy (Fara et al., 1979) and Puerto Rico (Comas, 1982; Schoental, 1983; Saenz de Rodriguez, 1984; Saenz de Rodriguez et al., 1985), although levels were not actually assessed. Serum mycoestrogens levels were assessed in small clinical studies of precocious puberty in Hungary (Szuets et al., 1997) and Italy (Massart et al., 2008). In the Italian study, mycotoxin-positive girls were taller and proportionally heavier than those who were mycotoxin-negative (Massart et al., 2008). Epidemiologic studies of the possible health effects of ZEA, zeranol and its metabolites in general, or on puberty are particularly lacking.

The purpose of this study was to evaluate urinary levels of ZEA, zeranol, and related metabolites, as well as their relationship with body size and breast development among participants in the Jersey Girl Study. To our knowledge, this is the first study evaluating urinary levels of mycoestrogens, their food sources, and their possible impact on growth and development in healthy peripubertal girls.

2.1 MATERIALS AND METHODS

The Jersey Girl Study was designed as a feasibility study to establish a cohort with the overall aim of evaluating the association between environmental factors and the onset of breast development and menarche in New Jersey girls. Girls are recruited through pediatric practices, media, and community recruitment efforts. Healthy girls, aged 9–10 years, residing in New Jersey, regardless of ethnic/racial background, and living with their biological mothers are eligible to participate in the study. As the study requires a relatively high level of involvement, only mothers and children with no cognitive impairments and who can speak and read English are eligible. Initially, we focused recruitment to pediatric practices in the Princeton area, but because of large public interest in the study prompted by media coverage, we decided to expand to all counties in New Jersey. The study is approved by the Institutional Review Board of the University of Medicine and Dentistry of New Jersey. Parental consent and girls' assent are obtained before data collection begins. In these analyses we included baseline data and urine samples from the first 163 girls recruited into the study.

2.1.1. Data Collection

Data collection was carried out by phone and during a clinical visit and included:

Questionnaires—completed by mothers, including a brief eligibility questionnaire (by phone) and the main questionnaire (self-administered), which was based on some of the baseline instruments used in the Cohort Study of Young Girls' Nutrition, Environment, and Transitions (CYGNET) Study, one of three studies under the NIEHS/NCI-funded Breast Cancer and the Environment Research Program (BCERP)(Hiatt et al., 2009). The phone and self-administered questionnaires included questions on major factors suspected or known to affect the onset of puberty, including demographic characteristics (e.g., race), environmental exposures, medical history, family structure, physical activity, prenatal and early childhood factors, as well as maternal and paternal factors.

Anthropometric measurements—Trained research personnel conducted body measurements using standardized procedures and the same instruments. Methods were based on the National Health and Nutrition Examination Survey III Study Procedures (National Health and Nutrition Examination Survey III, 1988), modified for the Jersey Girl Study. Height and waist and hip circumferences were measured to the nearest 0.1 cm. Weight and body composition (% body fat, fat mass, and fat free mass) were measured by bioelectrical impedance analysis using a Tanita® TBF-300A scale. Body Mass Index (BMI) –for age and gender percentiles were computed using the Centers for Disease Control and Prevention online calculator (BMI Percentile Calculator for Child and Teen) (Centers for Disease Control and Prevention).

Puberty staging—The Tanner scale for breast and pubic hair development, which ranges from Tanner stage 1 (pre-pubertal) to 5 (post-pubertal)(Coleman et al., 2002) was administered using standardized forms with pictures representing each stage. Girls were given the choice of having their pediatricians complete the form during their physical exam or having one of our female physicians affiliated with the study conduct Tanner staging. The pediatricians who recruited girls into the study were provided with the same Tanner forms and reminded to assess breast development by palpation. Our study physicians (pediatric residents or breast fellows) obtained Tanner staging for breast development by palpation and were trained in procedures by the same pediatric endocrinologist (IM). We also asked mothers to report current Tanner stage for breast and pubic hair development using the same forms.

Preliminary analyses to evaluate the agreement between physician and mom assessment among 118 girls (Bandera et al., 2010) indicated substantial agreement for the onset of breast development, defined as Tanner stage B2 or higher, (kappa; 0.7; 95% CI: 0.6, 0.8) and pubic hair (kappa: 0.8; 95% CI: 0.7–0.9). Based on these results, we adjusted procedures to make participation more comfortable for girls by relying on visual inspection to assess Tanner stage. For the few girls for whom breast development assessment by physician could not be obtained (n=16), we used mother's assessment.

Dietary intake assessment and analyses—Three 24-hour dietary recalls on different days of the week (one of them during the weekend) directly with the girls and their mothers were conducted by the Cincinnati Center for Nutritional Research and Analysis. Nutrition Data System (NDS-R) was used for nutrient analyses (Schakel et al., 1988; Harnack et al., 2008). We computed the average of the three 24-hour recalls to represent "usual intake" on the 162 girls with complete assessments. Urine collection was not initially coordinated with dietary assessment so that at least one of the 24-hour recalls could capture dietary intake the day before the morning urine was collected. Therefore, this was available only in a subset of the girls (n=58).

Urine sample collection, processing, and analyses—Plastic urine containers were provided to collect a first morning void and samples were brought to in-person appointments. Samples were aliquoted and stored in a −70°C freezer. One aliquot from each of the girls was transferred to the Chemical Analysis Facility of the Occupational and Environmental Health Sciences Institute (EOHSI) for mycoestrogen analyses.

2.1.2 Analytical Method

Samples were transferred to EOHSI de-identified and, therefore, analyses were blinded. Levels of zeranol and ZEA and their main metabolites, α-ZEL, β-ZEL, β-ZAL, and zearalanone (ZAN) were measured using an HPLC/MS/MS method, optimized by hybridizing two literature methods (van Bennekom et al., 2002; Schmidt et al., 2008) and replacing the quadrapole based mass spectrometric platform with an ion trap. The values measured were for free zeranol and metabolites. No glucoronidase was used. Briefly, ZAL and ZEL analytes were extracted from approximately two ml of urine using a Chem Elut™ solid phase extraction (SPE) cartridge and eluted with MTBE. The eluent was evaporated under N_2 and the residue reconstituted with one ml methanol and applied to an amino cartridge. The analytes were eluted with one ml of methanol. A five uL sub-sample was separated on a Thermo Surveyor high performance liquid chromatograph using a Hypersil C18 column. Each individual ZAL and ZEL analyte was quantified against a calibration curve generated from commercial standards with an MS^2 experiment on a Thermo LTQ linear ion trap mass spectrometer. All quality standard control parameters were followed throughout the analysis, including blanks and recoveries for each sample run. Analyte spikes were used for quality control and run with each sample batch. Recoveries of ±20% were used to validate the quality of the run. The method detection limit was 0.05 ng/ml. Concentrations below the limit of detection (<LOD) were given zero values in data analysis. The inter-day assay variability (% RSD) was 4.5 for zearalenone, 3.2 for α-zearalenol, 2.6 for β-zearalenol, 2.8 for zeranol, 3.0 for zearalanone, and 2.7 for β-zearalanol. The intra-day assay variability (% RSD) was 4.0 for zearalenone, 3.1 for α-zearalenol, 2.5 for β-zearalenol, 2.5 for zeranol, 2.8 for zearalanone, and 2.7 for β-zearalanol. Urinary mycoestrogen values were corrected for urine dilution by specific gravity (SG) using the formula (Pearson et al., 2009):

$$\text{Mycoestrogen-SG corrected} = \text{Mycoestrogen value} / [(\text{SG} - 1) \times 100]$$

This method has been found to be more appropriate than using creatinine values in normalizing children's urine samples when assessing environmental exposures (Pearson et al., 2009).

2.1.3 Statistical Analyses

Descriptive statistics for urinary levels of zearalenone, zeranol, and their main metabolites, as well as for other study variables were derived. The value for total mycoestrogens was computed as the sum of ZEA, zeranol, α-ZEL, β-ZEL, β-ZAL, and ZAN. We conducted log transformation of the data for girls with detectable levels to approximate normality and computed geometric means and 95% confidence intervals. Mycoestrogens levels were evaluated according to consumption of the most important food sources (e.g., grains, animal products) and means of the log transformed variables were compared using ANOVA and two sample t-tests. Association between food consumption categories and mycoestrogen status was evaluated using Chi-Square test, Fisher's Exact test, and Mantel-Haenszel Chi Square test, as appropriate. Mycoestrogen-positive girls were further categorized into two levels, based on the median value. We then examined means for anthropometric variables (weight, height, BMI, waist-to-hip ratio, lean mass, fat mass, % body fat) after adjusting for age, according to mycoestrogen status (negative, positive low, positive high) using ANCOVA, as these variables seemed to approximate a normal distribution as confirmed by histograms. We also evaluated intake of relevant food groups according to mycoestrogen status using non-parametric tests (Kruskal-Wallis) as appropriate.

Prevalence ratios (PR) and 95% CI for having reached the onset of breast development (Tanner stage B2+) for mycoestrogen-positive vs. mycoestrogen-negative girls were computed. Crude and adjusted PRs were computed using modified Poisson regression using Proc Genmod in SAS, which has been found to be more appropriate than logistic regression for outcomes that are not rare (Zou, 2004). Covariates considered included age, BMI, isoflavone intake, beef intake, and recruitment year. We also computed odds ratios (OR) and 95% CI using logistic regression for comparison purposes. Analyses were repeated excluding overweight and obese girls, as they may have been more likely to have been misclassified regarding the onset of breast development. All analyses were computed using SAS version 9.2 (SAS Institute, Cary NC).

3.1 RESULTS

Uncorrected and SG-corrected mycoestrogen values are shown in Table 1. In subsequent analyses we used SG-corrected values. Detectable mycoestrogen levels were found in 78% of the samples, with the highest levels found for ZEA, which was detected in 55% of the samples (Table 1). Zeranol was detected in over 20% of the samples, but levels were low. Levels of mycoestrogens according to selected study characteristics (Table 2) tended to suggest some individual differences by geographical area and race (e.g., they tended to be higher in African American girls). However, these analyses were based on small numbers and need to be replicated. Mycoestrogen levels by recruitment season were similar (data not shown).

We evaluated food sources by examining mycoestrogen levels according to consumption of the major food sources of ZEA and zeranol, including corn and grains, as well as animal products (i.e., beef, pork, poultry, veal, milk, eggs, and dairy) in the subset of girls for which we had a dietary recall the day before urine was collected. When we compared levels and food consumption on the previous day, only beef and popcorn consumption seemed to be related to urinary ZEA and total mycoestrogens (Table 3). While analyses were only based on six girls who reported popcorn consumption the day before urine collection, all were mycoestrogen positive and both ZEA and total mycoestrogen levels were significantly

higher among them. We also evaluated levels of mycoestrogens according to the combined exposure to beef and popcorn (data not shown). The geometric means (95% CI) of total mycoestrogens were 7.11 ng/ml (1.51–33.41) for girls consuming beef and popcorn the day before urine sample collection, and 0.07 ng/ml (0.02–0.20) for girls not consuming these two foods. When we used the three-day average intake, representing "usual intake", the association between total mycoestrogens and beef intake persisted (data not shown). However, there was no association with any other foods, including popcorn.

We also evaluated anthropometric characteristics according to urinary mycoestrogen status (Table 4). Mycoestrogen-negative girls tended to be significantly taller and to have higher adiposity, compared to mycoestrogen-positive girls. They were also more likely to have reached the onset of breast development. These findings were not explained by differences in food consumption or age. These analyses again indicated a relationship with beef, as consumption was significantly lower in mycoestrogen negative girls, compared to girls with the highest level of urinary mycoestrogens. Stratified analyses by puberty status (having reached the onset of puberty yes/no) yielded similar results (data not shown).

Girls with mycoestrogen-positive urine were less likely to have reached the onset of breast development (PR: 0.79; 95% CI: 0.60–1.04) after adjusting for age, BMI, isoflavone intake, and recruitment year (Table 5). Adding beef consumption as a covariate did not change PR estimates. We also computed ORs and 95% CI (data not shown), and as expected, point estimates were of stronger magnitude, as logistic regression tends to overestimate the association for outcomes that are not rare. Overall, the results were similar. Compared to mycoestrogen-negative, mycoestrogen-positive girls had an OR of 0.53 (95% CI: 0.21–1.34) for onset of breast development, after adjusting for age, BMI, recruitment year, and isoflavone intake. Analyses were repeated excluding overweight and obese girls, who may be more likely to be misclassified according to breast development status, with similar results (adjusted PR: 0.80; 95% CI: 0.55–1.18) (data not shown). Notably, the association for the group of overweight and obese girls was stronger and statistically significant (adjusted PR: 0.72; 95% CI: 0.53–0.99). We also repeated analyses excluding the 16 girls for which breast development status was based on mother's Tanner assessment, rather than physician assessment, with similar results (adjusted PR: 0.81; 95% CI: 0.62–1.07).

4.1 DISCUSSION

We found that mycoestrogens were detectable in a large proportion of participants, with girls having mycoestrogen-positive urine being significantly of shorter stature and less likely to have reached the onset of breast development.

There is substantial evidence pointing to the peripubertal period as a critical window of susceptibility for breast cancer risk (Colditz et al., 1995), and both linear growth velocity from childhood through adolescence and an earlier age at menarche have been consistently found to increase risk (Forman et al., 2005). Experimental studies have shown that ZEA administration during different windows of susceptibility is capable of affecting breast development (Hilakivi-Clarke et al., 1998; Belli et al., 2010) and carcinogenesis (Schoental, 1985; Hilakivi-Clarke et al., 1999). ZEA has been shown to bind to both ERα and ERβ, acting as a full agonist for ERα and a mixed agonist-antagonist for ERβ, exhibiting much higher binding affinity than that found for other well-known endocrine disruptors, such as bisphenol A, or DDT, in both receptor subtypes (Kuiper et al., 1998). The estrogenic potency of ZEA in ERα is less than 17-β-estradiol, but similar to coumestrol and higher than genistein and other isoflavones (Kuiper et al., 1998). Findings from our study seem to contradict the experimental evidence, which tend to suggest estrogenic effects. However,

mycoestrogen levels from dietary consumption are probably considerably lower than the doses used in experimental studies.

There are few reports of dietary intake of ZEA mycotoxins in humans and substantial uncertainty and large variability in reported levels in different food groups. While there are no US regulations for the ZEA levels in foods, the European Union has established limits ranging from 20 μg/kg for processed maize-based foods for infants and young children, to 200 μg/kg for maize products(Richard, 2007). The Joint FAO/WHO 2000 Codex Committee on Food Additives and Contaminants established a provisional maximum tolerable daily intake for ZEA of 0.5 μg/kg (Massart et al., 2010). Our preliminary analyses suggested that the strongest predictor of ZEA was beef intake and to some extent, popcorn. Both corn cereal and popcorn were found in a Canadian survey to have high levels of ZEA (33 ng/g and 18.6 ng/g, respectively)(Kuiper-Goodman et al., 1987). It is uncertain whether ZEA levels from beef consumption come from the metabolism of zeranol or from animal's consumption of contaminated grains. The findings for popcorn need replication as they were based on only six girls consuming popcorn the day before urine collection.

To our knowledge, no epidemiologic studies have evaluated the impact of mycoestrogens on healthy girls. In a series of 36 cases of early thelarche in Hungary, ZEA was detected in 5 of the patients, with serum levels ranging from 18.9 to 103 μg/L (Szuets et al., 1997). Massart et al. (Massart et al., 2008) also assessed serum levels of mycotoxins in 32 girls with idiopathic central precocious puberty under treatment with triptorelin (GnRH agonist) and 31 controls in Italy. Out of the 63 subjects, ZEA and α-ZEL were detected in 6 cases and in none of the controls, with mean serum values of 933.7 pg/mL and 106.5 pg/mL, respectively. They also found that mycotoxin-positive girls (n=6) had higher growth rate after the 12-month treatment than those who were mycotoxin-negative. In contrast, in our study mycoestrogen-negative girls were significantly taller and heavier than mycoestrogen-positive girls. However, the study by Massart et al. was conducted in Italy where use of zeranol and ZEA levels are regulated, was based on serum levels in a small sample (results were based on only 6 mycotoxin-positive girls), and included girls under treatment for precocious puberty.

Given the cross-sectional nature of our data, the results are difficult to interpret as 9–10 year-old girls have not reached their final height. Nevertheless, they are in agreement with findings for phytoestrogens from other studies (Wolff et al., 2008; Cheng et al., 2010). A longitudinal study suggested delayed onset of puberty and age at take off (ATO) for those with the highest dietary intake and urinary levels of isoflavones (Cheng et al., 2010). A cross-sectional study also suggested that phytoestrogens may delay breast development (Wolff et al., 2008). It is possible that mycoestrogens act in a similar fashion as phytoestrogens, which have been shown to exhibit antiestrogenic effect by competing with endogenous estrogens at the estrogen receptor level and by inhibiting aromatase and 17 beta-hydroxysteroid dehydrogenase (17-beta-HSD) (Cheng et al., 2010). In our study, isoflavone intake did not seem to explain our findings. Estrogens have been shown to accelerate linear growth at low concentrations and to close the epiphyseal plates and stop linear growth at higher concentrations(Aksglaede et al., 2006). Taken together, our data suggest that mycoestrogens may be acting as anti-estrogenic agents, by delaying the height spurt takeoff, which occurs around age 9 yrs in US girls (Abbassi, 1998) as well as the onset of breast development.

5.1 CONCLUSIONS

The experimental and epidemiologic data, while limited, clearly indicate that mycoestrogens act as endocrine disruptors. Estrogenic and anti-estrogenic effects have been reported, and

their action seems to depend on dose, hormonal environment, and critical window of administration. Despite these effects, data in humans are surprisingly lacking, given the fact that mycoestrogens are present in our food supply as much or more than isoflavones are, which have been studied extensively. Given the fact that ZEA is not regulated in the U.S. and the use of zeranol has been banned in other countries but is widely used in the U.S., the potential health effects of these mycoestrogens need to be urgently elucidated. This study is the first one to evaluate urinary mycoestrogens and breast development in healthy girls and suggest potentially important health effects. However, our findings need to be replicated in larger studies, with a more heterogeneous population, using a longitudinal design, so that the role of mycoestrogens on pubertal markers can be fully understood.

Highlights

- Mycoestrogens were detected in urine of girls in NJ, particularly zearalenone.
- Girls with detectable urinary mycoestrogens tended to be of shorter stature.
- They were also less likely to have reached the onset of breast development.
- Zearalenone mycoestrogens may exert anti-estrogenic effects in peripubertal girls.

Acknowledgments

Funding: This work was funded by the Cancer Institute of New Jersey, Komen Foundation Central and South Jersey Affiliate, The New Jersey Commission on Cancer Research, NIEHS P30ES005022, and NIH-K22CA138563.

We would like to thank Drs. Bozena Winnik, Stephen Marcella, Kathy Black, Jeanne Ferrante, Paul Lioy, Clifford Weisel, Yi Chu, Kim Hirshfield, Abigail Donaldson, Marlene Mancuso, Winnie Polen, and Luanne Labian, and Vicky Bandera for their contribution to the Jersey Girl Study; and Drs. William Hait and Arnold Rabson for supporting the study. We are also indebted to the many individuals and organizations that helped us with recruitment, including Princeton Nassau Pediatrics (Princeton and West Windsor), Hunterdon Family Practice and Obstetrics, (Flemington NJ), and RWJ/UMG Somerset Pediatrics (Somerset NJ); Michele Fisher and Candace Botnick from the CINJ Office of Communications; Kiameesha Evans and CINJ Office of Community Outreach, Terry Falco and the NJ Family Medicine Research Network; Debra Raines and the YMWCA Princeton Breast Cancer Resource Center; Michele Tropper and the American Cancer Society; Rutgers Cooperative Extension; Morris Center YMCA, CINJ-LIFE Center; and Montgomery High School. We are particularly grateful to all the girls and their moms for their time and their initiative to contribute to research.

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Sci Total Environ. Author manuscript; available in PMC 2012 November 15.

Table 1

Urinary levels of zearalenone, zeranol, and related mycoestrogens among 163 participants with detectable levels in the Jersey Girl Study.

		Uncorrected values						Specific gravity corrected values					
Mycoestrogen (ng/ml)	**n >LOD (%)**	**Mean**	**SD**	**Median**	**75th**	**Min**	**Max**	**Mean**	**SD**	**Median**	**75th**	**Min**	**Max**
Zearalenone (ZEA)	90 (55.2)	1.82	4.80	0.38	0.90	0.05	33.12	1.28	3.14	0.32	0.77	0.04	22.34
Alpha-zearalanol (zeranol)	35 (21.5)	0.25	0.13	0.25	0.32	0.02	0.57	0.20	0.21	0.17	0.20	0.01	1.23
Alpha-zearalenol (α-ZEL)	60 (36.8)	0.63	1.87	0.11	0.18	0.003	10.69	0.41	1.18	0.06	0.13	0.003	7.16
Beta-zearalenol (β-ZEL)	39 (23.9)	0.35	0.23	0.30	0.46	0.05	1.10	0.21	0.18	0.16	0.29	0.02	1.02
Beta-zearalanol (β-ZAL)	17 (10.4)	0.29	0.15	0.27	0.34	0.04	0.60	0.40	0.64	0.21	0.31	0.02	2.76
Zearalanone (ZAN)	29 (17.8)	0.33	0.59	0.25	0.30	0.07	3.31	0.22	0.29	0.17	0.23	0.04	1.57
Total mycoestrogens	128 (78.5)	1.86	5.73	0.52	1.03	0.03	48.22	1.32	3.66	0.31	0.91	0.03	29.88

LOD: Limit of Detection

Table 2

Specific gravity corrected values for zearalenone (ZEA), zeranol, and total ZEA mycoestrogens (ng/ml) according to selected characteristics among the Jersey Girl Study participants included in these analyses (n=163).

	n (%)	ZEA Median (Min-Max)	Zeranol Median (Min-Max)	Total ZEA mycoestrogens Median (Min-Max)
Age at recruitment				
9 yrs.	95 (58.3)	0.08 (0–8.58)	0 (0–1.23)	0.21 (0–11.90)
10 yrs	68 (41.7)	0.12 (0–22.34)	0 (0–0.50)	0.23 (0–29.88)
p value		*0.68*	*0.65*	*0.90*
Girls' race				
White	146 (92.4)	0.08 (0–22.34)	0 (0–1.23)	0.21 (0–29.88)
African American	7 (4.4)	0.32 (0–0.62)	0 (0–0.32)	0.37 (0–1.08)
Asian	5 (3.2)	0.12 (0–0.80)	0 (0–0.30)	0.31 (0.12–1.84)
p value		*0.63*	*0.15*	*0.38*
Mothers' education				
High school/some college	34 (20.9)	0.05 (0–15.72)	0 (0–0.21)	0.23(0–22.89)
Bachelor's degree	63 (38.7)	0.12 (0–8.58)	0 (0–0.50)	0.22 (0–11.25)
Graduate education	66 (40.5)	0.08 (0–22.34)	0 (0–1.23)	0.21 (0–29.88)
p value		*0.70*	*0.07*	*0.31*
Family's income				
<100,000	40 (26.5)	0.21 (0–15.72)	0 (0–0.32)	0.32 (0–22.89)
>100,000	111 (73.5)	0.09 (0–22.34)	0 (0–1.23)	0.20 (0–29.88)
p value		*0.11*	*0.49*	*0.28*
County of residence				
Mercer	63 (38.7)	0.10 (0–22.34)	0 (0–1.23)	0.22 (0–29.88)
Middlesex	29 (17.8)	0.12 (0–3.72)	0 (0–0.32)	0.33 (0–4.86)
Other	71 (43.6)	0.08 (0–15.72)	0 (0–0.30)	0.18 (0–22.89)
p value		*0.63*	*0.36*	*0.09*
BMI –for-age and gender percentile(CDC definition)				
Underweight (< 5th percentile)	9 (5.5)	0 (0–0.76)	0 (0–0.15)	0.30 (0–1.25)
Healthy weight (5th – <85th percentile)	115 (70.6)	0.11 (0–22.34)	0 (0–1.23)	0.22 (0–29.88)
Overweight (85th – < 95th percentile)	20 (12.3)	0.11 (0–4.90)	0 (0–0.20)	0.24 (0–6.13)
Obese (≥ 95th percentile)	19 (11.7)	0 (0–4.53)	0 (0–0.07)	0.13 (0–5.29)
p value		*0.39*	*0.52*	*0.77*
Puberty stage at recruitment				
Breast: Tanner B2+				
Age 9 years (*n*=95)				
Yes	52 (54.7)	0.04 (0–4.53)	0 (0–1.23)	0.21 (0–5.85)

	n (%)	ZEA Median (Min-Max)	Zeranol Median (Min-Max)	Total ZEA mycoestrogens Median (Min-Max)
No	43 (45.3)	0.10 (0–8.58)	0 (0–0.32)	0.22 (0–11.90)
p value		*0.54*	*0.20*	*0.29*
Age 10 years. (n=68)				
Yes	49 (72.1)	0.11 (0–15.72)	0 (0–0.32)	0.24 (0–22.89)
No	19 (27.9)	0.13 (0–22.34)	0 (0–0.50)	0.23 (0–29.88)
p value		*0.58*	*0.16*	*0.92*
Pubic Hair: Tanner PH2+				
Age 9 years (n=95)				
Yes	32 (33.7)	0 (0–4.53)	0 (0–0.20)	0.12 (0–5.85)
No	63 (66.3)	0.11 (0–8.58)	0 (0–1.23)	0.23 (0–11.90)
p value		*0.28*	*0.01*	*0.25*
Age 10 years (n=68)				
Yes	36 (52.9)	0 (0–4.51)	0 (0–0.20)	0.16 (0–4.91)
No	32 (47.1)	0.18 (0–22.34)	0 (0–0.50)	0.33 (0–29.88)
p value		*0.02*	*0.36*	*0.03*
Menarche at recruitment				
Started menarche	3 (1.8)	0.11 (0–0.58)	0.07 (0–0.32)	0.11 (0.07–1.06)
Not started menarche	160 (98.2)	0.10 (0–22.34)	0 (0–1.23)	0.22 (0–29.88)
p value		*0.90*	*0.05*	*0.98*

p values based on Kruskal Wallis test

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Table 3

Urinary mycoestrogens (95% confidence interval) according to intake of main food sources (day before sample, n=58)

	Zearalenone (ng/ml)			Zeranol (ng/ml)			Total Mycoestrogens (ng/ml)		
	Negative	Positive		Negative	Positive		Negative	Positive	
	n (%)	n (%)	Geometric Mean (95% CI)	n (%)	n (%)	Geometric Mean (95% CI)	n (%)	n (%)	Geometric Mean (95% CI)
Beef									
No	17 (43.6)	22 (56.4)	0.33 (0.22–0.49)	27 (69.2)	12 (30.8)	0.13 (0.07–0.22)	7 (18)	32 (82)	0.34 (0.23–0.49)
Yes	2 (10.5)	17 (89.5)	0.76 (0.38–1.53)	14 (73.7)	5 (26.3)	0.21 (0.17–0.25)	1 (5.3)	18 (94.7)	1.11 (0.59–2.11)
p value		*0.02*[1]	*0.04*[2]		*0.73*[1]	*0.14*[2]		*0.25*[1]	*0.002*[2]
Popcorn									
No	18 (34.6)	34 (65.4)	0.38 (0.26–0.55)	37 (71.2)	15 (28.9)	0.17 (0.11–0.25)	8 (15.4)	44 (84.6)	0.42 (0.30–0.59)
Yes	1 (16.7)	5 (83.3)	1.93 (0.44–8.39)	4 (66.7)	2 (33.3)	0.05 (0.03–0.07)	0	6 (100)	2.36 (0.67–8.35)
p value		0.65[1]	0.01[2]		>0.99[1]	0.05[2]		0.58[1]	0.002[2]
Total grains									
Low	11 (37.9)	18 (62.1)	0.32 (0.21–0.50)	21 (72.4)	8 (27.6)	0.22 (0.12–0.41)	4 (13.8)	25 (86.2)	0.38 (0.25–0.58)
High	8 (27.6)	21 (72.4)	0.65 (0.35–1.21)	20 (69)	9 (31)	0.10 (0.06–0.16)	4 (13.8)	25 (86.2)	0.71 (0.40–1.27)
p value		*0.40*[1]	*0.08*[2]		*0.77*[1]	*0.05*[2]		>0.99[1]	*0.09*[2]

[1] p value based on Chi-Square test or Fisher's Exact Test, as appropriate.

[2] p value based on t-test.

Table 4

Participant characteristics and food consumption according to mycoestrogen status in the Jersey Girl Study (n=163).

	Mycoestrogen Negative (n=35)	Mycoestrogen Positive (Low[a]) (n=64)	Mycoestrogen Positive (High[a]) (n=64)	p value
Age (yrs.)	9.72	9.81	9.84	0.51
Tanner Stage B2+[b] (%)	68.6 %	62.5 %	57.8%	0.57
Anthropometrics (age-adjusted means ± SE)				
BMI	18.61 (0.53)	18.40 (0.39)	17.82 (0.39)	0.42
Weight (kg)	38.36 (1.44)	36.65 (1.06)	34 (1.06)	0.001
Height (cm)	143.14 (1.22)	140.28 (0.90)	137.38 (0.90)	<0.0001
Fat mass (kg)	8.94 (0.90)	8.40 (0.67)	7.00 (0.67)	0.07
Fat free mass (kg)	29.42 (0.66)	28.45 (0.49)	26.98 (0.49)	<0.0001
Percent body fat (%)	21.57 (1.52)	21 (1.13)	18.75 (1.12)	0.24
Waist circumference (cm)	68.10 (1.50)	66.90 (1.10)	64.63 (1.11)	0.07
Hip circumference (cm)	78.71 (1.31)	78.38 (0.96)	76.23 (0.97)	0.01
Waist-to-hip ratio	0.86 (0.01)	0.85 (0.01)	0.85 (0.01)	0.45
Food intake (3-day average) in daily servings/ 1000 kcal[c] (means ± SD)	**(n=35)**	**(n=64)**	**(n=63)**	
Beef	0.39 (0.57)	0.30 (0.43)	0.54 (0.57)	0.02
Total grains	3.95 (0.96)	3.84 (0.97)	4.01 (1.20)	0.88
Total corn products	0.14 (0.18)	0.18 (0.33)	0.18 (0.23)	0.40
Popcorn	0.05 (0.13)	0.07 (0.19)	0.06 (0.18)	0.78
Total isoflavones (mg/1000 kcal)	0.85 (2.1)	0.89 (2.32)	0.89 (2.44)	0.25
%calories from fat	32.39 (10.78)	32.12 (9.91)	31.20 (11.24)	0.48
Total calories	1636 (317.44)	1754.54 (395.69)	1724.51 (396.89)	0.36
Food intake (day before) in daily servings/ 1000 kcal[d] (means ± SD)	**(n=8)**	**(n=25)[e]**	**(n=25)[e]**	
Beef	0.12 (0.34)	0.29 (0.64)	0.97 (1.24)	0.03
Total grains	4.47 (1.22)	3.74 (0.84)	3.78 (1.61)	0.28
Total corn products	0	0.07 (0.24)	0.10 (0.20)	0.17
Popcorn	0	0.01 (0.05)	0.08 (0.18)	0.10
Total isoflavones (mg/1000 kcal)	2.54 (6.34)	1.12 (2.76)	1.83 (5.35)	0.99
%calories from fat	38.70 (33.33)	31.57 (6.54)	30.79 (6.26)	0.60
Total calories	1531.26 (302.90)	1593.75 (483.66)	1846.93 (489.18)	0.14

p values based on ANCOVA analyses for anthropometric variables and non-parametric tests (Kruskal-Wallis) for the food variables.

[a] Based on median value for total mycoestrogens (0.31 ng/ml).

[b] Physician assessment of Tanner stage was missing in 16 girls and mother's assessment was used.

[c] Conducted with n=162 girls (one girl not included because she did not complete the three recalls)

[d] Conducted with n=58 girls who had a dietary recall before the urine collection.

[e] Based on median value for total mycoestrogens (0.46 ng/ml).

Table 5

Prevalence ratios (PR) and 95% confidence intervals (CI) for onset of breast development (B2+) according to mycoestrogen status at baseline.

	B2+	B1	Crude PR (95% CI)	PR1 (95% CI)	PR2 (95% CI)
Total mycoestrogen					
Negative	24 (23.8)	11 (17.7)	Ref	Ref	Ref
Positive	77 (76.2)	51 (82.3)	0.88 (0.67–1.14)	0.80 (0.61–1.05)	0.79 (0.60–1.04)

B1: Tanner Stage 1 for breast development ; B2+: Tanner Stage 2 or higher for breast development

PR1: Adjusted for age, BMI, and year of urine sample

PR2: Further adjusted for total isoflavone intake in mg/1000 kcal (three-day average).