Environmental Exposures and Puberty in Inner-City Girls

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Abstract

Background—Hormonally active environmental exposures are suspected to alter onset of puberty in girls, but research on this question has been very limited.

Objective—We investigated pubertal status in relation to hormonally active environmental exposures among a multiethnic group of 192 healthy nine-year old girls residing in New York City.

Methods—Information was collected on breast and pubic hair stages, weight and height. Phytoestrogen intake was estimated from a food frequency questionnaire. Three phytoestrogens and *bis*-phenolA (BPA) were measured in urine. In a subset, 1,1′-dichloro-2,2′-*bis*(4-chlorophenyl)ethylene (DDE), polychlorinated biphenyls (PCBs) were measured in blood plasma and lead (Pb) in blood. Associations of exposures with pubertal stages (present=stage 2+ vs absent=stage 1) were examined using t-tests and Poisson multivariate regression to derive prevalence ratios (PR, 95%-confidence limits [CI]).

Results—Breast development was present in 53% of girls. DDE, Pb, and dietary intakes of phytoestrogens were not significantly associated with breast stage. Urinary phytoestrogen biomarker concentrations were lower among girls with breast development than with no development. In multivariate models, main effects were strongest for two urinary isoflavones, daidzein (PR 0.89 [0.83-0.96] per ln-μg/g creatinine) and genistein (0.94 [0.88-1.01]). Body mass index (BMI) is a hormonally relevant, strong risk factor for breast development. Therefore, BMI-modification of exposure effects was examined, and associations became stronger. Delayed breast development was observed among girls with below-median BMI and 3rd tertile (high exposure) of urinary daidzein (PR 0.46 [0.26-0.78]); a similar effect was seen with genistein, comparing to girls median BMI and lowest two tertiles (combined) of these isoflavones. With urinary enterolactone

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a phytoestrogen effect was seen only among girls with high BMI, where breast development was delayed among those with high urinary enterolactone (PR 0.55 [0.32 - 0.96] for the upper tertile *vs* lower two combined). There was no main effect of PCBs on breast stage, but girls with below-median BMI and median PCB levels had reduced risk for breast development (any *vs* none) compared with other BMI-PCB groups. No biomarkers were associated with hair development, which was present in 31% of girls.

Conclusions—Phytoestrogens and PCBs are environmental exposures that may delay breast development, especially in conjunction with BMI which governs the endogenous hormonal milieu. Further research to confirm these findings may improve our understanding of the role of early-life development in breast cancer risk and other chronic diseases related to obesity.

Keywords

puberty; environment; biomarkers; BMI; phytoestrogen; DDE; PCB; diet

Introduction

First breast development occurs at 9 years of age on average in Black and at 10 years in white girls in the U.S., while average age at menarche is 12.5-13 years (Herman-Giddens et al., 1997; Richards et al., 1992; Selevan et al., 2003; Wu et al., 2003). Racial/ethnic disparities in pubertal timing have been attributed to height and body mass index (BMI, m/kg^2) (Kaplowitz et al., 2001), but variability is not entirely explained by body size characteristics and other factors such as physical activity and genetics (Richardson et al., 1983). Breast development accompanies an upsurge in steroid hormones, chiefly estrogen (Jones et al., 2007). Based on knowledge of hormonal activity of environmental contaminants, exogenous exposures have come to be of interest as potential etiologic agents for sexual maturation.

Environmental exposures are known to alter pubertal onsent in experimental models. Experimental data support a delay with lead (Pb) exposure (Ronis et al., 1998), and advanced female development after exposure to hormonally active agents, including phytoestrogens (Whitten & Naftolin, 1992), PCBs (Gellert, 1978), bisphenol A (BPA) (Honma et al., 2002), and pesticides (Walters et al., 1993). Environmental and dietary factors have been investigated little with regard to pubertal onset or even menarche among girls, with inconsistent results (Blanck et al., 2000; de Ridder et al., 1991; Gladen et al., 2000; Karmaus et al., 2002; Kato et al., 1988; Koprowski et al., 1999) except for inorganic lead which was associated with delayed breast development (Denham et al., 2005; Selevan et al., 2003; Wu et al., 2003). No data have been reported on phytoestrogen biomarkers and pubertal maturation.

Earlier puberty is associated with breast cancer, insulin resistance, bone development, and cardiovascular disease, especially among African-American women (Morrison et al., 1999). Therefore understanding its determinants may offer preventive measures for later health effects.

We examined pubertal stages in relation to hormonally active environmental factors, including exposure biomarkers as well as dietary phytoestrogen intake, among nine-year-old girls from three ethnic groups in New York City.

Materials and methods

The population, previously described in detail (Britton et al., 2004), included 192 9 year-old girls recruited at Mount Sinai Hospital in New York City and in a nearby pediatric private

practice during 1996-1997. The participation rate was 89% (200/224 of those approached), and the final group for the environmental analyses was 186 girls with complete information on pubertal stages, age, race and BMI. Pediatric nurses measured the girls' heights and weights and obtained blood samples. Baseline data and dietary intake were collected by in-person interview. A food-frequency questionnaire was used (Harvard Youth/Adolescent Questionnaire YAQ (Rockett et al., 1995)) that has been validated in a multiethnic population and found to be reproducible over a year's time. We queried girls directly on each dietary item, with mothers or guardians also present. Responders were not aware of the results of the physical examination when they completed the interview. Pubertal stages were assessed by pediatricians using a form with standard drawings provided by Prof. J. Richard Udry (Morris, 1980), Carolina Population Center, Chapel Hill, NC 27516-3997 (with permission).

Methods for organochlorines in plasma, Pb, urinary phytoestrogen metabolites and creatinine (to monitor urine dilution) have been reported, including quality control measures (Berkowitz et al., 2003; Liu et al., 2005; Wolff et al., 2005). The organochlorines were measured in a randomly selected subset because of budget limitations (DDE, PCB #s 118, 153, 138, 180). As reported, limits of detection for the organochlorines were 0.07 µg/L, for Pb 0.1 µg/dL, and for for the urinary metabolites 0.79 µg/L for daidzein, 1.0 µg/L for enterolactone, 0.5 µg/L for genistein, and 0.5 µg/L for bisphenol A (defined as 3-times the blanks). There was a single non-positive non-zero value of one PCB congener which was replaced with the lowest positive value for that congener (Berkowitz et al., 2003); the four PCB congeners were added together to obtain the PCB-total variable. Because concentrations of the individual congeners were low, we did not investigate them individually.

A phytoestrogen database was compiled for 65 food items in the YAQ based on the literature and existing databases (Block et al., 1986; Horn-Ross et al., 2000; Pillow et al., 1999; usda, 2000). Dietary intake (mg/d) of phytoestrogens were grouped as isoflavones (genistein, daidzein, formononetin, biochanin-A, coumestrol); flavones (luteolin, apigenin), flavonols (quercetin, kaempherol, myricetin); phytosterols (beta-sitosterol, campesterol, stigmasterol); and lignans (enterolactone, enterodiol). In the interview, additional questions were asked about phytoestrogen foods consumed within the past 24 hr (any cruciferi, sesame, granola, bran, squash, beans and lentils, cherries, apples/apple juice, tofu, sprouts, garlic, tea, cola, onion).

In statistical analyses, biomarkers including creatinine were log-transformed. Urinary metabolites were examined both as µg/L and corrected for creatinine (µg/gC). Dietary phytoestrogens and urinary metabolites were also categorized as tertiles of intake (mg/day). We identified a minimum set of exposure covariates by fitting multiple regression models to predict the exposure variables. Covariates included those essential for the pubertal models (race, age, BMI, and height (Britton et al., 2004) as well as additional potential predictors identified in bivariate comparisons (p<0.2). The resulting minimal covariates were then used in the models predicting pubertal development from exposures. They were breast-fed for DDE models; race, breast-fed, maternal country of birth (non-US) for PCB; race, private clinic (*vs* hospital) for Pb; race and urinary creatinine for urinary phytoestrogens and BPA, adding maternal education for daidzein and BPA, adding BMI, maternal education for enterolactone; no covariates for dietary flavones; calories for flavonols and isoflavones; private clinic for lignans; private clinic, maternal U.S. birth for phytosterols.

Modified poisson regression with robust error variance was used to estimate adjusted prevalence ratios (relative risks) rather than odds-ratios because the outcome (pubertal development) was not rare (Zou, 2004). Prevalence ratios were computed using Proc

Genmod (SAS, Inc., Cary, NC). Models were fitted for pubertal stages (any pubertal signs *vs* none: B2+ *vs* B1 for breast and H2+ *vs* H1 for hair) by including exposure measures (continuous variables [ln] or tertiles as indicator variables), age, race, BMI, height, and relevant predictor variables for each exposure. Backward elimination was conducted to remove covariates that did not alter the coefficient for pubertal stage by more than 10%. We assumed that hormonal exposures were unlikely to operate independent of BMI, a strong endogenous hormonal factor in pubertal development. Therefore, the BMI-exposure joint effects were also explored using four-category indicator BMI-environmental exposure variables with BMI dichotomized at the median and the environmental exposure dichotomized at the top vs bottom two tertiles of Pb, urinary phytoestrogen, BPA metabolites and dietary intake; exposure dichotomized at the median for DDE and PCBs because the numbers were small.

Results

We recruited healthy girls for this study from our hospital or a nearby affiliated private clinic. They represented 3 ethnic groups and were 9 years old (average 9.5 years), an age that we chose to provide equal proportions of breast development (B1=none; B2+=any) based on a earlier pilot study at the private clinic. Breast development was present in 53% of girls (74/192 girls were B2, 21 were B3, and 6 were B4) and pubic hair development in 31%. Socioeconomic status was broad, with half of the mothers having less than 13 years' education; proportions were similar at the hospital and private clinic (Table 1). Mothers not born in the US were mainly from Europe (n=8, 4%) and Latin America (n=40, 21%). As previously reported, pubertal stage (any *vs* none) was more advanced for Black ethnicity and greater weight, BMI, or height in this population (Britton et al., 2004). Average BMI (18.8 kg/m^2) was close to the national 75th-percentile of 18.3 kg/m^2 among 9.5 year-old girls in the year 2000; the national norm (50th-percentile) was 16.5 kg/m^2 (CDC, 2000).

Plasma organochlorine (OCs) concentrations were low (Table 2), but the range was similar to that reported in other young populations during this time period, including the NHANES data for 12-19 year-olds in 1999-2000 (CDC, 2005). Median Pb was 2.4 µg/dL, typical of New York inner city children (Haley & Talbot, 2004) but higher than the CDC data for 6-11 year-olds in 1999-2000 (median 1.3 µg/dL) (CDC, 2005). The highest urinary phytoestrogen concentration was enterolactone, followed by daidzein and genistein, a pattern similar to that among children in the 1999-2000 NHANES. Median concentrations of urinary enterolactone (173 µg/L) and daidzein (70 µg/L) were somewhat lower than those found in NHANES children in 1999-2000 (353 and 101 µg/L, respectively); levels of daidzein (25 µg/L) were similar (32 µg/L for NHANES) (CDC, 2005). Our median creatinine-corrected concentrations were closer to those of NHANES children in 1999-2000 (260, 88, 28 µg/gC in our girls for enterolactone, daidzein, and genistein *vs* 384, 93, 28 µg/gC respectively for NHANES).

Phytoestrogen intake was highest for phytosterols and flavonols; quercetin comprised most of the flavonol intake (median 8.7 mg/d; data not shown), which is typical of Western diets (Hertog et al., 1993). As reported for other studies, quercetin came mainly from fruit (data not shown). Other dietary phytoestrogens, aside from the phytosterols, were derived mainly from fruit and vegetables (not shown). Phytosterols chiefly come from oils (including margarine and oil-containing foods such as bread or fried food). Urinary phytoestrogens were not correlated with phytoestrogen intakes from the YAQ; however urinary phytoestrogens were significantly correlated with having eaten fruit within the past 24 hr (apples, apple juice, or cherries; Spearman r p<0.05, not shown).

Girls with breast stage 1 (91/192) had higher mean urinary phytoestrogen levels compared with girls at B2+ (Table 2). Results were similar for creatinine-corrected concentrations (shown in Table 2) or for uncorrected values (as μg/L, not shown). Plasma OCs, Pb, and dietary phytoestrogens did not differ by breast stage, and no exposures varied by pubic hair stage.

In multivariate models, only urinary daidzein remained significantly associated with breast stage (Table 3). Genistein was weakly protective for pubic hair stage, but not significantly. Results were almost identical for urinary phytoestrogen concentrations whether they were creatinine-corrected (Table 3) or not (μg/L with or without adjusting for ln-creatinine in the model, not shown; continuous ln-variables). Flavones had a suggestive inverse association with breast stage, but the trend was not linear and not significant. For other exposures, the estimates were near 1.0 and had wide confidence intervals. Combinations of exposures within types (all blood biomarkers, all urine, all diet) were also examined; effects did not change from the single exposure models (not shown).

Consideration of BMI as a modifier of hormonal exposures provided further information about relationships of the biomarkers with breast development. Table 4 shows results after stratifying the effects of urinary metabolites (top vs bottom two tertiles) by BMI (< vs median, or low- vs high-BMI), and Figure 1 depicts these findings. Our hypothesis was that girls with high BMI and low phytoestrogen levels would have higher breast stage (B2+). Indeed, in this subgroup, breast stage 2 or higher was more common than other subgroups for all four urinary biomarkers; they are the reference groups in Table 4 and Figure 1. In addition, girls having low BMI and highest urinary biomarker levels had delayed breast development for 3 of the 4 biomarkers. For urinary daidzein and genistein, there was a similar effect on delayed development in both BMI groups such that high exposure lowered the prevalence ratio by ~30% compared with the referents (high-BMI, low exposures). Still, low-BMI/high exposure girls had the most delayed development (PRs and 95% CI = 0.46 [0.26-0.78] and 0.44 [0.26-0.76] for daidzein and genistein, respectively). Without considering the joint effect of BMI, girls with higher urinary daidzein or genistein had delayed breast development (PR for both biomarkers was 0.66 CI 0.47-0.9, 3rd vs 1st and 2nd tertiles combined, adjusting for BMI in the model). Urinary enterolactone was associated with significantly delayed breast development only in high-BMI girls. However, without considering the joint effect of BMI, enterolactone had no association with breast development (PR 0.83 CI 0.62-1.19, adjusting for BMI in the model). BPA appeared to be unrelated to risk (with or without consideration of BMI) (Table 4). The trends were similar for phytoestrogen biomarkers without creatinine correction, with slight shifts in the confidence intervals (not shown). We also examined modification by BMI of DDE-, PCB-, and Pb-effects on development. DDE and Pb had no significant exposure modification. For PCBs, two subgroups had similar and null effects (low-BMI/low-PCB, high-BMI/high-PCB), but girls with low-BMI and   median PCB levels, were less likely to be B2+ than B1 (PR 0.60 [0.40-0.92]), all as compared with high-BMI/low-PCB (referent).

Discussion

Our main finding is that girls with high levels of urinary phytoestrogens are less likely to have experienced breast stage 2 than girls with low urinary metabolites. The protective effect of phytoestrogens may only be apparent when the joint BMI effect is considered. For instance, the effect of isoflavones (daidzein or genistein) was stronger in BMI strata, and the effect of urinary enterolactone was significant only among high-BMI girls. Of note, enterolactone is the urinary biomarker and the phytoestrogen with the highest exposure both in our population and in Western women. Girls with low BMI and high PCBs were also less likely to have breast stage 2+. We did not find significant associations of diet, plasma DDE,

or Pb exposures with development. We found no effects on hair development, but our study was designed to detect effects with breast development. In addition, hormonal effects on pubertal hair development may be more responsive to adrenal androgens whereas breast maturation requires estrogen (Jones et al., 2007).

For DDE and PCBs limitations include our sample size which was small, low-level exposures, and not having lipid measurements which might alter the estimates for PCBs toward the null; low-BMI girls with higher PCB levels might also have lower lipids. Pb levels were also very low, with a median of 2 mg/dL. Analyses of NHANES girls found delayed development with high Pb in a large sample (Selevan et al., 2003; Wu et al., 2003). These reports found effects among girls with Pb >3 or >5 mg/dL, respectively. The lower bound of the upper tertile of Pb among our girls was close to 3 mg/dL, but we had only 7 girls with >5 mg/dL; therefore we may have had too few girls to detect an effect of elevated exposures.

Phytoestrogen and dietary intakes in our study were consistent with other reports (Hertog et al., 1993; Horn-Ross et al., 2000; Pillow et al., 1999) including children this age (Munoz et al., 1997; Rockett et al., 1995). Like our negative findings, other studies about dietary intake have been inconclusive (Koprowski et al., 1999b; Pedersen et al., 1991; Persky et al., 1992; Roberts et al., 1977), although two longitudinal studies have found fiber intake to have a protective effect for age at menarche (de Ridder et al., 1991; Koo et al., 2002). High fiber foods are also often high in phytoestrogens (Grace et al., 2004). Phytoestrogens have been found to perturb female reproductive function including cycle characteristics (Phipps et al., 1993) and endometriosis (Tsuchiya et al., 2007). A soy formula trial found no relationship with pubertal maturation (Strom et al., 2001). A potential limitation of our study is that pubertal onset alters dietary patterns, or that girls in puberty alter their dietary reporting. The dietary assessment may inadequately measure some phytoestrogen-rich ethnic-specific foods. On the other hand, it is likely that Hispanic girls in New York City eat a more Western than traditional Hispanic diet; this would make the YAQ an appropriate instrument. Further indication that the YAQ was not inappropriate for our population are the correlations seen between cruciferous vegetables in the 24-hr checklist and in the YAQ, though modest, are consistent with annual repeated assessments reported for this questionnaire (Rockett et al., 1995). However, questionnaire estimates like the YAQ are known to miss occult sources of phytoestrogens such as soy-fortified meat patties (Lampe et al., 1999), although our exposure categories should be relatively insensitive to underreporting because we ranked each intake as high or low. A limitation of the cross-sectional design is that phytoestrogen intake at the age of 9 years may not be relevant to pubertal stage at the same age; for example, in animal models exposures early in life are found to influence onset of puberty (Whitten et al., 1995), and diet preceding puberty was found to be associated with the growth spurt (Berkey et al., 2000). We did not have data on earlier diet. Urinary metabolites represent only recent exposure because clearance of a single dose occurs within days; however, a few studies, including one in children this age (Teitelbaum et al., 2008), have shown that phytoestrogen biomarkers are reasonably stable over a range of months to a year (Zeleniuch-Jacquotte et al., 1998), suggesting that intake of phytoestrogen-containing foods is fairly consistent. Therefore, in our study, the urinary metabolites may be more representative of the past year's phytoestrogen intake than dietary recall, as suggested for isoflavones (Atkinson et al., 2002). Additional limitations of our study include the relatively small sample size, the cross-sectional design, and possibly inadequate adjustment for socioeconomic status. There is also possible residual confounding among the urinary biomarkers, BMI, and creatinine (Barr et al., 2005). Although our findings were hypothesis-driven, we cannot eliminate the possibility of chance associations among the multiple comparisons undertaken.

Differences in the biomarker associations with development could be explained by differences in concentrations combined with varying biological potency and endogenous hormones. The proposed anti-estrogenic effects of phytoestrogens are well known (Adlercreutz, 2002). These agents may alter estrogen's effectiveness (block CYP19, reduce cell proliferation, bind to the estrogen receptor, stimulate SHBG production) (Adlercreutz, 2002). Weak estrogens may be protective by competing with endogenous hormones, and hormonal *vs* anti-hormonal effects of such agents may depend on the hormonal milieu (Paris et al., 2002, Sun et al., 2006). This supports our findings of stronger effects when incorporating obesity as a modifier of exposure. The PRs were around 0.5 for the group with highest concentration phytoestrogen biomarkers if considered together with their BMI.

BPA levels were much lower than phytoestrogens in our study, but BPA may may act more as hormone-agonist than antagonist. BPA has not been widely studied in humans, but it has been associated with PCOS (a hyperandrogenic condition) (Takeuchi et al., 2004). PCBs may have dioxin-like activity, and it has been reported that PCBs delay puberty in rats (Faqi et al., 1998; Lundkvist, 1990); this is consistent with our finding of delayed breast development, seen only among girls with lower BMI who had higher PCB levels compared with other BMI-PCB groups. Similar delays in maturation were reported for both boys and girls with dioxin-like exposures (Den Hond et al., 2002). In contrast, Warner reported that exposure to dioxin (the quintessential environmental anti-estrogen) in childhood was related to earlier age at menarche (Warner et al., 2004). Relatively high in utero exposures to DDE or Pb but not PCB were associated with earlier menarche (Blanck et al., 2000; Vasiliu et al., 2004), while in North Carolina no relationships were seen between perinatal DDE or PCB exposure and pubertal development (Gladen et al., 2000).

A central motivation for investigating relationships of environmental exposures with pubertal development is the idea that earlier puberty confers later risk for chronic disease, including breast cancer. Phytoestrogens have long been considered as strong candidates for ameliorating hormonally related disorders. One reason is the low breast cancer rates in Asia and the high isoflavone content of their diet (Adlercreutz, 2002). A growing body of epidemiologic research supports a protective effect of isoflavones on breast cancer risk, including adolescent soy consumption (Shu et al., 2001; Thanos et al., 2006), although the early epidemiologic studies were equivocal (Adlercreutz, 2002). Studies of dietary intake of other phytoestrogens have also found reduced breast cancer risk (Fink et al., 2007; Touillaud et al., 2005; Verheus et al., 2007), but the data are not consistent (Adebamowo et al., 2005). Measurement of phytoestrogen biomarkers has also shown some protection for breast cancer as well as heart disease (Hertog et al., 1993; Ingram et al., 1997; Liang et al., 2006; Teede et al., 2001; Zheng et al., 1999), but again there have also been many studies that found no such effects (Grace et al., 2004; Zeleniuch-Jacquotte et al., 2004). However, no studies have examined phytoestrogen biomarkers and puberty or menarche which may be the relevant window of exposure.

Conclusions

Our results support a possible effect of environmental agents on breast development, which is dependent on BMI. In particular, the delayed breast development associated with higher levels of urinary phytoestrogens is more pronounced among girls with high BMI. Further research is needed to confirm this in larger samples and in studies with a longitudinal design. Also, it may be important to study dietary intake earlier in life and genetic factors or other exposures that may be more relevant to puberty than current eating habits. Further investigation of environmental and lifestyle factors, including more accurate dietary assessments, may suggest approaches to prevention of early puberty.

Acknowledgments

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Abbreviations

BMI	body mass index
YAQ	Youth/Adolescent Questionnaire
DDE	1,1′-dichloro-2,2′-bis(4-chlorophenyl)ethylene
PCB	polychlorinated biphenyls
Pb	
CI	
PR	
CDC	Centers for Disease Control
μg/gC	micrograms-per-gram creatinine
NHANES	National Health and Nutrition Examination Survey
mg/d	milligrams per day
r_S	Spearman correlation coefficient
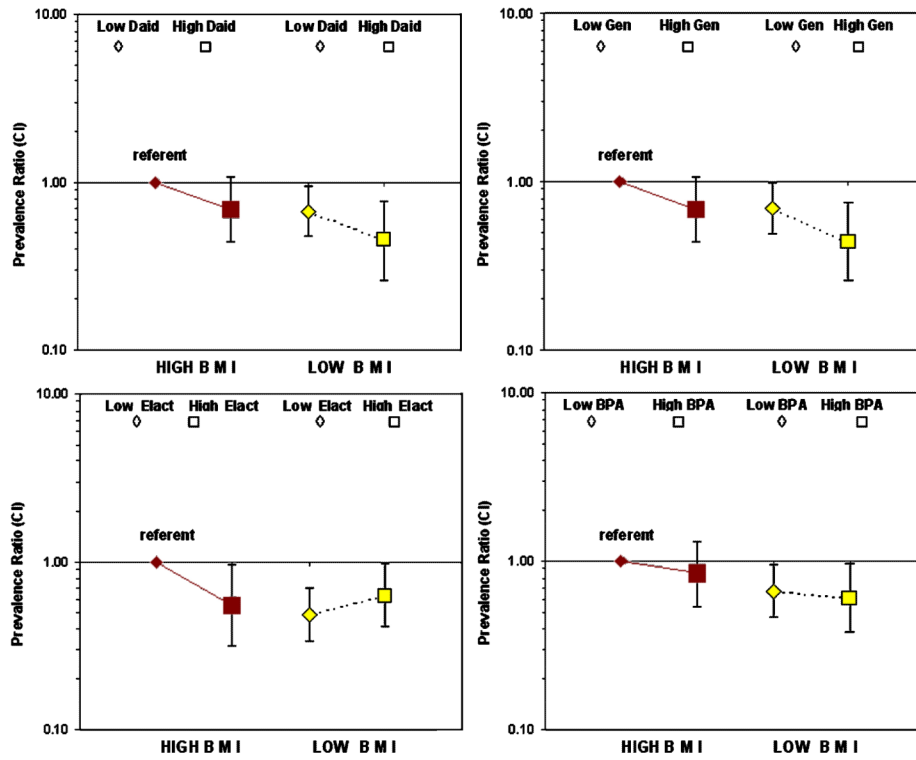
Figure 1.
BMI, urinary metabolites and risk for breast stage 2+ vs 1. BMI is quantiled at the median; urinary metabolites are the 3rd (top) tertile (high exposure) vs the $1^{st}+2^{nd}$ (☐ low exposure) tertiles. Prevalence ratios and CIs are shown; models parameters are given in Table 4.

Table 1

Characteristics of a Multiethnic Cohort of Girls, Mount Sinai Hospital 1997-1998

		N (%)	Mean ± SD	range	N
Mother was US -born		138 (73%)			190
Maternal education (13+ yr)		91 (49%)			185
Parity (Nonparous before this child)		63 (48%)			130
Girl was breastfed		79 (42%)			187
Girl's race:	Black	54 (28%)			
	Hispanic	72 (38%)			192
	White	66 (34%)			
Breast stage	B2+ (vs B1)	101 (53%)			192
Pubic Hair stage	H2+ (vs H1)	59 (31%)			192
Recruited from private clinic (vs hospital clinic)		84 (44%)			192
Maternal age (yr)			28 ± 6.9	14-43	116
Girl's age (yr)			9.5 ± 0.3	9.002-9.998	192
Girl's BMI (kg/m^2)			18.8 ± 4.2	10.2-34.7	186

Table 2

Biomarker Levels and Phytoestrogen Intake Means by Breast and Hair Stages in a Multiethnic Cohort of Girls, Mount Sinai Hospital 1997-1998

	Breast stage = 1		Breast stage = 2+		Hair stage = 1		Hair stage = 2+	
	GM	GSD	GM	GSD	GM	GSD	GM	GSD
Exposure biomarkers[a]								
DDE (ug/L plasma)	0.43	2.0	0.39	2.1	0.42	2.1	0.39	1.9
PCB (ug/L plasma)	0.83	1.9	0.83	1.8	0.79	1.9	0.90	1.7
N	36		51		54		33	
Pb (ug/dL whole blood)	2.1	1.8	2.3	1.6	2.0	1.7	2.4	1.7
N	63		78		93		48	
Daidzein (ug/gC)	3.1	6.6	1.8	5.3	2.8	4.9	1.5	8.4
Genistein (ug/gC)	11.8	3.4	5.6	5.1 *	8.4	3.7	7.1	6.2
Enterolactone (ug/gC)	32.3	2.8	19.6	3.1 *	25.3	3.0	24.1	3.0
BPA (ug/gC)	0.24	10.3	0.11	12.9 *	0.19	10.1	0.10	16.1
N	81		88		117		52	
Phytoestrogen intake (mg/d)	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Flavones	0.7	1.2	0.6	1.0	0.7	1.1	0.6	1.1
Flavonols	11.7	6.1	11.9	6.5	11.4	6.1	12.6	6.8
Isoflavones	1.7	2.4	2.0	3.4	1.7	2.2	2.2	4.0
Lignans	0.8	0.5	0.8	0.6	0.8	0.5	0.9	0.6
Phytosterols	252	200	283	214	252	199	304	224
Calorie intake (cal/d)	3,185	1197	2,893	1107	3060	1241	2958	956
N	81		96		120		57	

[a] Biomarkers are presented as geometric means (GM) and standard deviations (GSD); dietary intakes are means and standard deviations. Starred rows had statistically significantly different geometric means by t-test (p<.05).

*
p<0.05 for t-test of means of log-transformed variables.

Table 3

Prevalence ratios and 95%-confidence intervals for any vs no development in relation to Environmental Exposures

	PR (CI) for Breast stage 2+ vs stage 1		PR (CI) for Pubic hair stage 2+ vs stage 1	
Blood biomarkers				
DDE (log_e ug/L plasma; N=86)	1.008 (0.72-1.40)		1.07 (0.71-1.62)	
PCB (log_e ug/L plasma)	0.92 (0.68-1.22)		1.12 (0.73-1.73)	
Pb (log_e ug/dL whole blood) N=139	1.01 (0.79-1.30)		1.25 (0.83-1.88)	
Urinary metabolites N=164				
Daidzein (log_e ug/gC urine)	0.89 (0.83-0.96)[*]		0.96 (0.84-1.14)	
Genistein (log_e ug/gC urine)	0.94 (0.88-1.01)		0.90 (0.80-1.02)	
Enterolactone (log_e ug/gC urine) n=152	0.92 (0.81-1.14)		1.12 (0.91-1.38)	
BPA log_e ug/gC urine)	0.96 (0.92-1.01)		0.98 (0.89-1.08)	
Phytoestrogen intake, n=172				
1st tertile=referent	2nd tertile	3rd tertile	2nd tertile	3rd tertile
Flavonols	1.36	1.18	1.67	1.50
	(0.98-1.89)	(0.81-1.72)	(0.99-2.82)	(0.89-2.50)
Flavones	0.76	0.75	1.26	0.91
	(0.56-1.02)	(0.54-1.06)	(0.77-2.06)	(0.52-1.59)
Isoflavones	1.14	1.14	1.33	1.18
	(0.83-1.54)	(0.80-1.63)	(0.80-2.22)	(0.68-2.04)
Lignans	0.95	1.19	1.04	1.26
	(0.68-1.32)	(0.86-1.64)	(0.61-1.77)	(0.79-2.03)
Phytosterols	0.86	1.10	1.04	1.52
	(0.62-1.20)	(0.79-1.53)	(0.59-1.85)	(0.91-2.56)

[*] $p < 0.05$

Models adjusted for DDE (Breast) age, BMI, height, Black race, breastfed; (Hair) BMI, height, black race, breastfed; PCB (breast) age, height, Black race, maternal country of birth (US/PR vs other); (Hair) height, Black race, maternal country of birth; Pb (breast) age, BMI, Black race; (Hair) height, Black race, private clinic.

Urinary phytoestrogen models for Breast Stage with daidzein and genistein included BMI, height, and Black race. The enterolactone model was adjusted for height, Black race, maternal education (13 yr vs 13+) and excluded urine samples with creatinine <10 mg/dL, yielding N = 152. For BPA, the model included height and Black race. For Hair Stage, models included BMI, height, and Black race, except for enterolactone which was adjusted for height, Black race, maternal education (13 yr vs 13+) and excluded urine samples with creatinine <10 mg/dL.

Phytoestrogen intake (mg/d) models for breast stage were adjusted for BMI, height, Black race, and calories; the lignan model was also adjusted for Hispanic race and private clinic enrolment; that for phytosterols added private clinic enrolment. The models for Hair stage adjusted for height and Black race; in addition the model for flavonols added age; lignans and phytosterols both added age and private clinic enrolment.

Table 4

Prevalence ratios and 95%-confidence intervals for joint BMI and urinary biomarker exposure in relation to breast development

Urinary metabolites, ug/gC n=164[*]	High BMI (median)		Low BMI (< median)	
	Tertiles 1+2	Tertile 3	Tertiles 1+2	Tertile 3
Daidzein	1.0 (ref)	0.69 (0.44 - 1.08)	0.67 (0.48 - 0.94) [*]	0.46 (0.26 - 0.78) [**]
Genistein		0.69 (0.44 - 1.07)	0.70 (0.49 - 0.98)[*]	0.44 (0.26 - 0.76)[**]
Enterolactone		0.55 (0.32 - 0.96)[*]	0.49 (0.34 - 0.70)[**]	0.63 (0.41 - 0.98)[*]
BPA		0.85 (0.54 - 1.33)	0.67 (0.47 - 0.96)[*]	0.60 (0.38 - 0.96)[*]

[*] n (from left to right)=56, 27, 54, 27 (daidzein ug/gC); 60, 23, 50, 31 (genistein ug/gC); 57, 26, 53, 28 (enterolactone ug/gC); 58, 25, 52, 29 (BPA ug/gC).

[*] p<0.05

[**] p<0.01