

Letter to the Editor

Bacterial metabolites of diet-derived lignans and isoflavones inversely associate with asthma and wheezing

To the Editor:

Lignans and isoflavones are plant-derived chemicals with potent anti-inflammatory and antioxidant effects.¹ Lignans are ubiquitous in Western diets, with flaxseeds containing the greatest amounts, while isoflavones are most abundant in soybeans (see Table E1 in this article's Online Repository at www.jacionline.org). The end product(s) of gut microbial metabolism of lignans is enterolactone²; metabolism of the isoflavone daidzein gives rise to equol and *O*-desmethylangolensin (*O*-DMA). Human studies demonstrate inverse associations between lignans and isoflavones and hormone-dependent cancers, cardiovascular disease, and osteoporosis,¹ and improvements in asthma control are reported in those with high soy consumption.³ Experimental studies suggest that bacterial metabolites of isoflavones attenuate allergic airway inflammation⁴; however, similar studies are unavailable for enterolactone.

Prospective human studies demonstrate that microbial exposures are important for asthma development and control,⁵ but the mechanism of this association is unclear. Given their anti-inflammatory and antioxidative properties, we hypothesized that bacterial metabolites of lignans and isoflavones may mediate the beneficial effects of bacteria on asthma and lower airway disease. To provide evidence supporting our hypothesis, we conducted a cross-sectional population-based study using data from the National Health and Nutrition Examination Survey (NHANES), which collected urinary levels of lignan and isoflavone metabolites and medical history of asthma and wheeze.

Data were obtained from the 2003-2004, 2005-2006, 2007-2008, and 2009-2010 NHANES. A total of 9633 subjects aged 6 to 85 years had complete data for the primary analyses of the association between urinary levels of lignan and isoflavone metabolites (eg, enterolactone, equol, and *O*-DMA) and physician-diagnosed current asthma and self-reported nonasthmatic wheeze, adjusting for age, sex, race/ethnicity, log-transformed urinary creatinine level, poverty index ratio (PIR), and body mass index (BMI). We performed 3 different sensitivity analyses: (1) adjusting for smoke exposure (we adjusted for the presence of household smoke exposure among subjects <20 years old and never, former, or current smoking status among subjects ≥20 years old); (2) adjusting for plant intake (using total dietary fiber intake); and (3) stratifying each analysis by atopic status (presence or absence of allergen-specific IgE level, available in 2005-2006 only). Statistical analyses were performed with STATA12.0 (StataCorp, College Station, Tex) using the sampling and weighting variables provided by NHANES. For additional details, see this article's Methods section in the Online Repository at www.jacionline.org.

Participants reporting current asthma were more likely to be female, younger, African American, aeroallergen sensitized, and have a PIR of less than 1. Those reporting nonasthmatic wheeze were more likely to be older and Caucasian. Current asthmatic subjects were more likely to report being former smokers, and those with nonasthmatic wheeze were more likely to be current smokers or have household smoke exposure. Asthma and wheeze

status was positively associated with BMI (see Table E2 in this article's Online Repository at www.jacionline.org). Urinary levels of bacterial metabolites by tertile are presented in Table E3 in this article's Online Repository at www.jacionline.org. These levels varied by race/ethnicity, PIR, age, BMI category, and tobacco exposure status (see Tables E4-E8 in this article's Online Repository at www.jacionline.org). NHANES participants were selected at random for urinary lignan and isoflavone analysis. The sample with data available on urinary bacterial metabolite measurements was similar to the general NHANES population, although more adults (82% vs 72%, $P < .001$) and more subjects with BMI greater than 30 (29% vs 26%, $P < .001$) composed the subsample (see Table E9 in this article's Online Repository at www.jacionline.org).

Urinary enterolactone levels were inversely associated with current asthma (Table 1; Fig 1). In logistic regression models adjusted for race/ethnicity, PIR, sex, age, creatinine, and BMI, the odds ratio (OR) for current asthma for the highest tertile of urinary enterolactone was 0.69 (95% CI, 0.56-0.85). Levels of urinary enterolactone and *O*-DMA were inversely associated with nonasthmatic wheeze. The OR for nonasthmatic wheeze for the highest tertile of urinary enterolactone was 0.53 (95% CI, 0.42-0.67). The OR for nonasthmatic wheeze for the highest tertile of urinary *O*-DMA was 0.64 (95% CI, 0.52-0.80). In a model including all metabolites, urinary enterolactone remained independently inversely associated with current asthma, while both enterolactone and *O*-DMA remained independently inversely associated with nonasthmatic wheeze (see Table E10 in this article's Online Repository at www.jacionline.org).

In sensitivity analyses, the inverse association between isoflavone and lignan metabolites and current asthma and nonasthmatic wheeze remained significant after the addition of smoke exposure to the models, stratifying by age (see Tables E11 and E12 in this article's Online Repository at www.jacionline.org). Adjustment for total dietary fiber intake, a global measure of plant intake, led to minimal change (0% to 3%, data not shown) in the point estimates for the association between enterolactone and current asthma and the association between enterolactone and *O*-DMA and nonasthmatic wheeze. There were similar associations between enterolactone and *O*-DMA and current asthma and nonasthmatic wheeze when the outcomes were stratified by atopic status (data not shown).

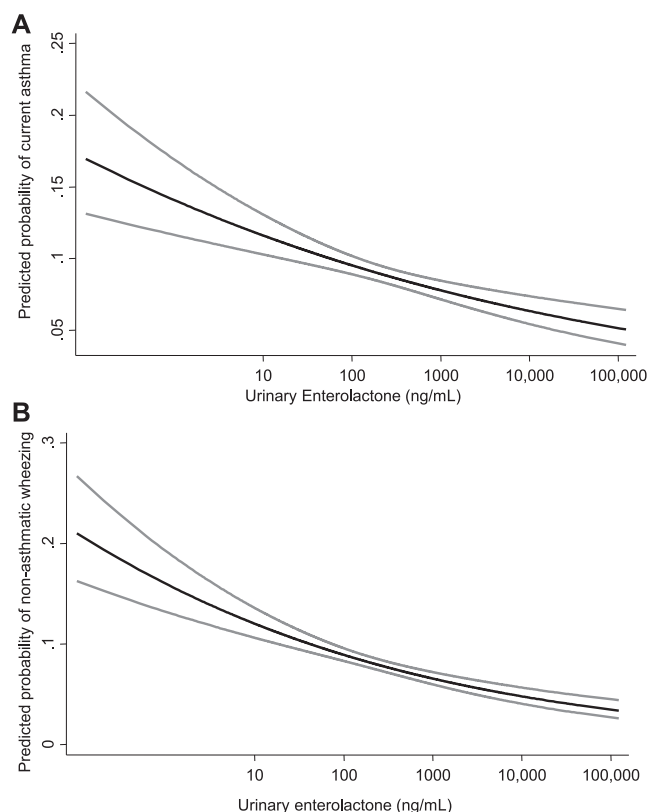
We examined the relationship between bacterial metabolites of dietary lignans and isoflavones and asthma and wheeze using a large, nationally representative sample. We hypothesized that these metabolites would be inversely associated with asthma and wheezing because of their effects on molecular pathways involved in allergic inflammation and oxidation and limited human studies supporting an association.⁴ We found that higher urinary levels of the lignan metabolite enterolactone were inversely associated with a physician diagnosis of current asthma, and both enterolactone and *O*-DMA were inversely associated with nonasthmatic wheezing within the last year. Our findings were similar among adults and children and robust to smoke exposure, fiber intake, and atopic status. These results suggest that interventions to increase levels of enterolactone and *O*-DMA may be beneficial in the prevention and treatment of asthma and nonasthmatic wheeze.

TABLE I. ORs (and 95% CI) for current asthma and nonasthmatic wheeze by level of urinary metabolites of lignans and isoflavones

Metabolite	Tertile	Current asthma		Nonasthmatic wheeze	
		Crude OR	Adjusted OR*	Crude OR	Adjusted OR*
Enterolactone	1	1.0 [REF]	1.0 [REF]	1.0 [REF]	1.0 [REF]
	2	0.59 (0.47-0.74)	0.61 (0.48-0.78)	0.64 (0.48-0.86)	0.67 (0.49-0.90)
	3	0.63 (0.52-0.76)	0.69 (0.56-0.85)	0.52 (0.34-0.65)	0.53 (0.42-0.67)
	Test for trend (<i>P</i>)	<.001	<.001	<.001	<.001
O-DMA	1	1.0 [REF]	1.0 [REF]	1.0 [REF]	1.0 [REF]
	2	1.03 (0.86-1.23)	1.06 (0.88-1.28)	0.79 (0.63-1.00)	0.79 (0.62-1.00)
	3	0.94 (0.77-1.23)	1.00 (0.80-1.26)	0.63 (0.51-0.79)	0.64 (0.52-0.80)
	Test for trend (<i>P</i>)	.82	.98	<.001	<.001
Equol	1	1.0 [REF]	1.0 [REF]	1.0 [REF]	1.0 [REF]
	2	0.97 (0.79-1.20)	1.01 (0.81-1.26)	1.15 (0.94-1.41)	1.12 (0.91-1.40)
	3	1.08 (0.90-1.30)	1.15 (0.93-1.43)	0.94 (0.76-1.16)	0.95 (0.76-1.18)
	Test for trend (<i>P</i>)	.39	.18	.56	.57

Values in boldface are statistically significant ($P < .05$).

*Adjusted for age, sex, race/ethnicity, log-transformed urinary creatinine, PIR, and BMI.

**FIG 1.** Predicted probability of current asthma (**A**) and nonasthmatic wheeze (**B**) with 95% CIs by urinary enterolactone levels.

Several epidemiologic and laboratory-based studies have demonstrated associations between bacterial flora and lower respiratory tract disease.⁶ The presence of specific organisms and the diversity of microflora are thought to be relevant in this relationship.⁵ Notably, bacteria of the genus *Bacteroides*, which are positively correlated with lignan metabolism, are inversely associated with the development of allergy, while the presence of *Clostridium*, which is negatively correlated with lignan metabolism, is positively associated with the development of allergy.^{7,8} These observations together with our findings suggest that the bacterial metabolites of lignans and isoflavones,

in particular enterolactone, may have a causal role in disease prevention and control.

Our study has several limitations including incomplete characterization of respiratory outcomes, and cross-sectional studies such as this are only a starting point for identifying causal relationships between exposures and disease. Further research will be necessary to determine whether our observations result from different dietary choices among those with and without lower respiratory tract disease, which we could not detect by adjusting for fiber. Although lignan and isoflavone metabolites have antioxidant, anti-inflammatory, and other biological activities, which we hypothesize underlie their association with lower respiratory tract disease, these may be biomarkers of another causal factor rather than themselves being causal in disease amelioration. Nonetheless, treatment with other (non-metabolite) lignans such as macelignan and honokiol decreases T_H2-mediated pulmonary inflammation in animal models of asthma, supporting our overall hypothesis.⁹

In summary, we have identified significant concentration-dependent associations between bacterial metabolites of diet-derived lignan and isoflavone metabolites and asthma and wheezing in a large, nationally representative population-based sample. To our knowledge, this is the first report of an association between biologically active bacterial metabolites and lower respiratory tract disease outcomes in a nationally representative population. Future clinical and laboratory-based studies are needed to support a causal relationship between lignan and isoflavone metabolites and lower respiratory tract disease. The potential diagnostic or therapeutic role for these bacterial metabolites, in particular enterolactone, warrants further study.

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METHODS

Study design

Data were obtained from the 2003–2004, 2005–2006, 2007–2008, and 2009–2010 NHANES, a US-based survey designed to sample the non-institutionalized US population.^{E14} Participation in the survey is voluntary. Sociodemographic data, respiratory disease and medical condition questionnaire data, body measurements, smoking history, aeroallergen-specific IgE levels, dietary records, and urinary levels of lignan and isoflavone metabolites were included in the data set. Aeroallergen-specific IgE levels were collected only in the 2005–2006 NHANES.

Body measurements and urine collection

Height and weight data were obtained for all subjects by trained health technicians. The urine collection took place within 1 to 3 weeks of answering the NHANES questionnaire (NHANES staff, personal communication, 2014).

Dietary history

A complete dietary history including 24-hour dietary recall and food frequency questionnaire is obtained by the NHANES staff at the same visit as the urine collection is completed. A second dietary history is obtained via a combination of telephone and self-completed questionnaires within 3 to 10 days of obtaining the first history. From these, NHANES calculates total nutrient intake, including total dietary fiber, per day. The average value of the 2 total dietary fiber measurements was used to generate quartiles of fiber intake. This variable was used as a categorical variable in sensitivity analyses of the relationship between enterolactone and *O*-DMA and current asthma and nonasthmatic wheeze, adjusting for fiber intake.

Laboratory evaluations

Levels of urinary lignan and isoflavone metabolites (enterolactone, *O*-DMA, equol) were measured in a randomly selected one-third subsample of participants in NHANES aged 6 years and older. The method for detection used in 2003–2004 was HPLC-MS/MS with electrospray ionization, modified in 2005–2006 with the use of atmospheric pressure photoionization. Crossover studies comparing samples analyzed by using the former method and the latter method demonstrated high correlation coefficients ($r > 0.99$), with regression slopes approximately equal to 1 and intercepts close to 0. The lower limits of detections for all 4 assays were 0.03 to 0.2 ng/mL, with all samples tested yielding values greater than the lower limit of detection of the assays.^{E15} Repeated measurements of these metabolites demonstrate a moderately high intraclass correlation coefficient for enterolactone (>0.55),^{E16–E18} while it is lower for the isoflavone metabolites (<0.30). These compounds are stable for many years at temperatures of -20° and -80°C ^{E16} and in general can tolerate up to 3 freeze-thaw cycles without compromising detection.^{E19}

Serum aeroallergen-specific IgE levels were measured with the ImmunoCAP system (Phadia, Uppsala, Sweden) in subjects 6 years and older. Serum specific IgE level was measured to cat, dog, mouse, rat, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, cockroach, ragweed, thistle, rye, Bermuda, oak, birch, *Alternaria* species, and *Aspergillus* species.

During the years 2003–2006, urinary creatinine was quantified with a Beckman Synchron CX3 (Beckman Coulter, Inc, Brea, Calif) after subjecting creatinine to a Jaffé rate reaction with picrate.^{E20} Since 2007, creatinine has been quantified by enzymatic conversion using a Roche/Hitachi Modular P Chemistry Analyzer (Roche Diagnostics Corp, Indianapolis, Ind). Therefore, we adjusted 2003–2006 urinary creatinine measurements to 2009–2010 equivalents.^{E20}

Variable definitions

A participant was considered to have current asthma if he or she responded affirmatively to the following question “Has a doctor or other health professional ever told you that you have asthma?” and to the question

“Do you still have asthma?” A participant was considered to have nonasthmatic wheeze if he or she responded affirmatively to the following question: “In the past 12 months, have you had wheezing or whistling in your chest?” but did not have current asthma. A participant was considered to not have nonasthmatic wheeze if he or she responded negatively to this question about wheezing.

The PIR, the ratio of family income to the poverty threshold, was used as a measure of socioeconomic status. The BMI is weight in kilograms divided by the squared height in meters. Sensitization to environmental aeroallergens was defined as having at least 1 positive (≥ 0.35 kU/L) aeroallergen-specific IgE level. Subjects aged 20 years and older were designated as never, former, or current smokers. Current smokers responded affirmatively to the question “Have you smoked at least 100 cigarettes in your life?” and responded “all days” or “some days” to the question “Do you now smoke cigarettes?” Former smokers answered affirmatively to the question “Have you smoked at least 100 cigarettes in your life?” and answered “not at all” to the question “Do you now smoke cigarettes?” Never smokers were defined by a negative response to the question “Have you smoked at least 100 cigarettes in your life?” Subjects aged 19 years and younger were not asked questions about their lifetime cigarette use but were considered to have household smoke exposure if the household respondent responded affirmatively to the question “Does anyone who lives [in this house] smoke cigarettes, cigars, or pipes anywhere inside this home?”

Statistical analyses

Statistical analyses were performed with STATA 12.0 software (Stata-Corp). Primary sampling units and strata variables provided in the NHANES data set were used in the analyses to account for the complexity of the survey design. Sampling weights corresponding to each NHANES survey year were used to generate estimates representative of the US non-institutionalized population. Urinary levels of lignan and isoflavone metabolites had non-normal distributions, and levels were divided into tertiles and/or log-transformed for analysis. BMI was divided into categories (<25 , 25–30, >30 kg/m²), as defined by pediatric obesity screening recommendation proposed by the US Preventive Services Task Force,^{E21} which overlap with the adult BMI overweight and obese categories.

The chi-square test was used to determine whether subjects with and without current asthma and nonasthmatic wheeze differed by sociodemographic factors or measures of IgE and whether the subsample with available data on urinary lignan and isoflavone metabolite levels was comparable to the subsample without these data in terms of demographic characteristics and respiratory outcomes.

Linear regression was used to determine whether levels of lignan and isoflavone metabolites varied by race/ethnicity, PIR, sex, age, BMI, and tobacco smoke exposure categories. Logistic regression was used to determine the association between urinary levels of lignan and isoflavone metabolites and current asthma and nonasthmatic wheeze. Multivariate models were adjusted for age, sex, race/ethnicity, log-transformed urinary creatinine level, PIR, and BMI. Sensitivity analyses were performed adjusting for household smoke exposure (age <20 years), smoking status (age ≥ 20 years), and fiber intake and by stratifying by the presence or absence of aeroallergen-specific IgE levels. Missing data were considered missing at random.

The Hosmer-Lemeshow goodness-of-fit test was used to confirm good model fit.

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TABLE E1. Sources and effects of lignans and isoflavones

	Dietary source	Precursors	Intestinal bacterial metabolites	<i>In vitro</i> observation	<i>In vivo</i> observation
Lignans	● Flaxseeds* ● Wine ● Coffee ● Tea ● Sesame ● Wheat ● Rye^{E1-E3}	● Secoisolariciresinol ● Matairesinol ● Pinoresinol ● Medioresinol ● Lariciresinol ● Syringaresinol ● Artigenin ● Esamin	● Enterolactone	● Scavenging of hydroxyl radicals^{E4,E5} ● Inhibition of fatty acid peroxidation^{E4}	● No animal studies address the <i>in vivo</i> effects of enterodiols, enterolactone, or their precursors on asthma
Isoflavones	● Soybeans* ● Clover ● Mung beans^{E1-E3}	● Daidzein	● Equol ● O-DMA	● Inhibition of function and maturation of dendritic cells^{E6,E7} ● Decrease in natural killer cell lytic activity and proliferation of PBMCs^{E8} ● Inhibition of NF-κB, STAT1, and iNOS^{E9} ● Inhibition of cAMP phosphodiesterases^{E10}	● Decrease in eosinophilic lung inflammation, airway hyperresponsiveness, and cytokine transcription and expression^{E11} ● Decrease in lung eosinophilic inflammation with increase in BAL fluid eosinophilia^{E12} ● Decreased mast cell degranulation, anaphylaxis, food-specific antibodies, dendritic cell activation and function^{E13}

BAL, Bronchoalveolar lavage; cAMP, cyclic adenosine monophosphate; iNOS, inducible nitric oxide synthase; NF-κB, nuclear factor kappa B.

*Contain highest precursor concentrations.

TABLE E2. Prevalence of current asthma and nonasthmatic wheezing by subject characteristics*

Characteristic	Current asthma			Nonasthmatic wheeze†		
	Yes (n = 848)	No (n = 8785)	P value	Yes (n = 722)	No (n = 8356)	P value
Sex						
Female (%)	59	50	.001	49	51	.42
Age (y)						
≥18	76	82	.001	91	81	<.001
Ethnicity						
Caucasian	69	69	.005	76	68	.005
African American	16	12		11	12	
Hispanic	11	14		9	14	
Other	4	6		5	6	
BMI (kg/m ²)						
<25	37	43	<.001	35	44	<.001
≥25-30	24	28		28	28	
>30	39	28		37	27	
PIR						
≥1	80	85	.002	84	85	.33
Sensitization to aeroallergens						
Yes‡	63	41	<.001	44	40	.66
Smoking status (age ≥20 y)						
Never	44	52	.02	32	54	<.001
Former	30	25		21	25	
Current	26	24		47	21	
Household smoke exposure (age <20 y)						
Yes	21	17	.27	27	17	.01

*Includes subjects with complete data for sex, age, race/ethnicity, BMI, PIR, and urinary metabolites.

†Subjects with current asthma reporting wheezing are excluded from this group.

‡Data on sensitization to aeroallergens were obtained during the 2005-2006 NHANES only. There are 2226 subjects with complete data for this analysis.

TABLE E3. Urinary levels of bacterial metabolites by tertile (ng/mL)

Metabolite	Class	Tertile 1	Tertile 2	Tertile 3
Enterolactone	Lignan	0.1-173	174-640	641-122,000
<i>O</i> -DMA	Isoflavone	0.1-1.4	1.5-12.5	12.6-21,600
Equol	Isoflavone	0.04-4.44	4.45-12.4	12.5-20,700

TABLE E4. Mean (95% CI) levels (ng/mL) of urinary metabolites of lignans and isoflavones by race/ethnicity

Metabolite	Race/ethnicity			
	Caucasian	African American	Hispanic	Other
Enterolactone	866.6 (757.9-975.3)	663.9 (613.9-714.1)*	751.7 (595.7-907.6)	895.9 (556.8-1235.1)
<i>O</i> -DMA	83.6 (66.5-100.7)	91.4 (59.3-123.4)	75.8 (44.4-107.1)	102.9 (57.7-148.0)
Equol	64.4 (43.7-85.2)	35.5 (22.6-48.3)*	45.9 (26.3-65.4)	86.9 (19.4-154.3)

*Values are significantly different ($P < .05$) from those for the Caucasian reference group.

TABLE E5. Mean (95% CI) levels (ng/mL) of urinary metabolites of lignans and isoflavones by PIR

Metabolite	PIR	
	<1	≥1
Enterolactone	674.8 (565.0-784.5)	856.3 (762.4-950.2)*
<i>O</i> -DMA	62.7 (40.2-85.2)	88 (72.7-104.3)*
Equol	38.5 (23.7-53.2)	63.6 (46.8-80.5)*

*Values are significantly different ($P < .05$) from the <1 PIR reference group.

TABLE E6. Mean (95% CI) levels (ng/mL) of urinary metabolites of lignans and isoflavones by age

Metabolite	Age	
	≤18 y	>18 y
Enterolactone	645.6 (581.0-710.1)	871.1 (775.7-966.5)*
<i>O</i> -DMA	74.3 (52.6-96.0)	87.0 (69.3-104.7)
Equol	60.0 (32.7-87.4)	59.8 (45.6-74.0)

*Values are significantly different ($P < .05$) from the ≤18 y reference group.

TABLE E7. Mean (95% CI) levels (ng/mL) of urinary metabolites of lignans and isoflavones by BMI

Metabolite	BMI category		
	<25	≥25-30	>30
Enterolactone	953.3 (815.8-1090.8)	829.2 (717.7-940.7)	643.4 (717.7-940.7)*
O-DMA	95.8 (72.8-118.8)	79.2 (55.3-103.2)	73.2 (55.9-90.5)
Equol	77.3 (46.2-108.5)	52.9 (29.8-75.9)	40.5 (27.0-54.1)

*Values are significantly different ($P < .05$) from the <25 BMI reference group.

TABLE E8. Mean (95% CI) levels (ng/mL) of urinary metabolites of lignans and isoflavones by level of tobacco exposure, stratified by age

Age ≥ 20 y	Metabolite	Smoking status		
		Never	Former	Current
	Enterolactone	908.8 (787.7-1029.9)	1077.5 (826-1328.4)	565.5 (474.2-656.8)*
	<i>O</i> -DMA	100.9 (72.5-129.4)	84.4 (56.1-112.7)	59.5 (32.9-86.2)*
	Equol	61.8 (43.1-80.5)	74.3 (34.6-113.9)	42.1 (20.3-63.9)

Age < 20 y	Metabolite	Household smoke exposure	
		No	Yes
	Enterolactone	705.9 (611.3-800.5)	560.1 (469.4-650.8)
	<i>O</i> -DMA	79.7 (57.3-102.2)	60.1 (36.1-84.1)
	Equol	56.8 (29.4-84.1)	65.0 (15.0-115.0)

*Values are significantly different ($P < .05$) from the “never smoked” and “no household smoke exposure” reference groups.

TABLE E9. Demographic characteristics and outcomes in subsample with data available for urinary lignans and isoflavones and subsample with data not available for urinary lignans and isoflavones

Characteristic	Data available on lignan and isoflavone urinary levels (%)		P value
	Yes (N = 10,338)	No (N = 30,818)	
Sex			
Female	52	51	.14
Age (y)			
≥18	82	72	<.001
Ethnicity			
Caucasian	67	66	.64
African American	12	12	
Hispanic	15	15	
Other	6	7	
BMI (kg/m ²)			
<25	43	46	<.001
≥25-30	28	28	
>30	29	26	
PIR			
≥1	84	83	.07
Current asthma			
Yes	8	8	.27
Nonasthmatic wheeze			
Yes	9	9	.58
Sensitization to environmental aeroallergens			
Yes*	43	44	.44

*Data on sensitization were collected in 2005-2006 only. There are 2337 subjects with sensitization data and urinary measurements and 5196 subjects with sensitization data without urinary measurements.

TABLE E10. ORs (and 95% CI) for asthma and wheeze by level of urinary metabolites of lignans and isoflavones, adjusting for all metabolites

Metabolite	Tertile	Current asthma	Nonasthmatic wheeze
		Adjusted OR*	Adjusted OR*
Enterolactone	1	1 [REF]	1 [REF]
	2	0.58 (0.45-0.75)	0.69 (0.51-0.94)
	3	0.64 (0.62-0.79)	0.56 (0.43-0.72)
O-DMA	1	1 [REF]	1 [REF]
	2	1.14 (0.94-1.40)	0.88 (0.69-1.11)
	3	0.10 (0.88-1.39)	0.75 (0.59-0.95)
Equol	1	1 [REF]	1 [REF]
	2	1.05 (0.85-1.31)	1.22 (0.98-1.52)
	3	1.25 (1.00-1.57)	1.13 (0.90-1.45)

Values in boldface are statistically significant ($P < .05$).

*Adjusted for age, sex, race/ethnicity, log-transformed urinary creatinine, PIR, and BMI.

TABLE E11. ORs (and 95% CI) for current asthma by level of enterolactone adjusted for smoke exposure, stratified by age

Age category (y)	Metabolite	Tertile	Current asthma		
			OR, crude	OR, adjusted, model 1*	OR, adjusted, model 2†
≥20	Enterolactone	1	1.0 [REF]	1.0 [REF]	1.0 [REF]
		2	0.56 (0.43-0.73)	0.57 (0.44-0.76)	0.59 (0.45-0.78)
		3	0.67 (0.54-0.84)	0.74 (0.58-0.94)	0.76 (0.59-0.97)
		Test for trend (<i>P</i>)	<.001	.008	.01
<20	Enterolactone	1	1.0 [REF]	1.0 [REF]	1.0 [REF]
		2	0.58 (0.39-0.86)	0.57 (0.38-0.86)	0.57 (0.38-0.86)
		3	0.50 (0.34-0.73)	0.50 (0.32-0.77)	0.50 (0.32-0.77)
		Test for trend (<i>P</i>)	.001	.002	.003

Values in boldface are statistically significant ($P < .05$).

*Adjusted for age, sex, race/ethnicity, log-transformed urinary creatinine, PIR, and BMI.

†Model 1, additionally adjusted for smoking status (never, former, or current smoker), age ≥20 y, and household smoke exposure (present or absent) for age < 20 y.

TABLE E12. ORs (and 95% CI) of nonasthmatic wheeze by level of enterolactone and *O*-DMA adjusted for smoke exposure, stratified by age

Age category (y)	Metabolite	Tertile	Nonasthmatic wheeze		
			OR, crude	OR, adjusted, model 1*	OR, adjusted, model 2†
≥20	Enterolactone	1	1.0 [REF]	1.0 [REF]	1.0 [REF]
		2	0.65 (0.46-0.92)	0.67 (0.47-0.96)	0.74 (0.51-1.06)
		3	0.53 (0.41-0.67)	0.54 (0.42-0.70)	0.63 (0.48-0.83)
		Test for trend (<i>P</i> value)	<.001	<.001	.002
	<i>O</i> -DMA	1	1.0 [REF]	1.0 [REF]	1.0 [REF]
		2	0.84 (0.34-1.11)	0.83 (0.63-1.10)	0.86 (0.65-1.14)
		3	0.67 (0.53-0.86)	0.67 (0.53-0.85)	0.76 (0.60-0.97)
		Test for trend (<i>P</i> value)	.002	.002	.03
<20	Enterolactone	1	1.0 [REF]	1.0 [REF]	1.0 [REF]
		2	0.77 (0.46-1.29)	0.76 (0.45-1.29)	0.76 (0.45-1.30)
		3	0.52 (0.30-0.91)	0.50 (0.26-0.95)	0.51 (0.27-0.99)
		Test for trend (<i>P</i> value)	.02	.04	.046
	<i>O</i> -DMA	1	1.0 [REF]	1.0 [REF]	1.0 [REF]
		2	0.70 (0.42-1.14)	0.67 (0.41-1.10)	0.65 (0.40-1.07)
		3	0.59 (0.36-0.99)	0.58 (0.34-1.00)	0.57 (0.33-0.98)
		Test for trend (<i>P</i> value)	.05	.06	.05

Values in boldface are statistically significant ($P < .05$).

*Adjusted for age, sex, race/ethnicity, log-transformed urinary creatinine, PIR, and BMI.

†Model 1, additionally adjusted for smoking status (never, former, or current smoker) for adults and household smoke exposure (present or absent) for children.