Association of phthalate exposure and airway dysfunction, with mediation by serum periostin

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Abstract

Background: Phthalates can cause respiratory and immunological disorders. However, little is known about the role of serum periostin and YKL-40 levels in mediating the effects of phthalates. We investigated the mediating role of these biomarkers in the relationship between phthalates and airway dysfunction. Methods: A total of 487 children (aged 10 to 12 years-old) were examined. Four high-molecular-weight phthalate (HMWP) [Σ4HMWP] metabolites and 3 low-molecular-weight phthalate (LMWP) [Σ3LMWP] metabolites in urine samples were measured. Serum periostin and YKL-40 levels were measured. Airway function was measured using impulse oscillometry. A mediation model was used to quantify the mediating effects of periostin and YKL-40 on airway dysfunction. Results: After adjustment for height, gender, BMI z-score, aeroallergen sensitization, secondary smoking, and vitamin D level, the level of urinary Σ3LMWP metabolites was significantly associated with respiratory system resistance at 5 Hz (Rrs5; adjusted β: 0.020, 95% CI: 0.005 to 0.034; P = .010). The levels of urinary Σ4HMWP and Σ3LMWP metabolites were significantly associated with periostin level, but not with YKL-40 level. In addition, the periostin level was associated with Rrs5 (adjusted β: 0.048, 95% CI: 0.015 to 0.081; P = .005) and Rrs20-5 (adjusted β: 0.040, 95% CI: 0.011 to 0.069; P =.007). Serum periostin level had a significant effect in mediating the relationship between Σ3LMWP and Rrs5 (13.9%, 95% CI: 10.7 to 77.0; P < .001). Conclusion: Exposure to LMWPs was significantly associated with airway dysfunction, and this effect was partially attributable to increased serum periostin level.

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a running title : **Phthalate, airway dysfunction and periostin**

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Conflicts of interest

All authors declare no conflict of interest.

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ABSTRACT

Background : Phthalates can cause respiratory and immunological disorders. However, little is known about the role of serum periostin and YKL-40 levels in mediating the effects of phthalates. We investigated the mediating role of these biomarkers in the relationship between phthalates and airway dysfunction.

Methods: A total of 487 children (aged 10 to 12 years-old) were examined. Four high-molecular-weight phthalate (HMWP) [Σ4HMWP] metabolites and 3 low-molecular-weight phthalate (LMWP) [Σ3LMWP] metabolites in urine samples were measured. Serum periostin and YKL-40 levels were measured. Airway function was measured using impulse oscillometry. A mediation model was used to quantify the mediating effects of periostin and YKL-40 on airway dysfunction.

Results: After adjustment for height, gender, BMI z-score, aeroallergen sensitization, secondary smoking, and vitamin D level, the level of urinary Σ3LMWP metabolites was significantly associated with respiratory system resistance at 5 Hz (Rrs5; adjusted β: 0.020, 95% CI: 0.005 to 0.034; P = .010). The levels of urinary Σ4HMWP and Σ3LMWP metabolites were significantly associated with periostin level, but not with YKL-40 level. In addition, the periostin level was associated with Rrs5 (adjusted β: 0.048, 95% CI: 0.015 to 0.081; P = .005) and Rrs20-5 (adjusted β: 0.040, 95% CI: 0.011 to 0.069; P =.007). Serum periostin level had a significant effect in mediating the relationship between Σ3LMWP and Rrs5 (13.9%, 95% CI: 10.7 to 77.0; P < .001).

Conclusion: Exposure to LMWPs was significantly associated with airway dysfunction, and this effect was partially attributable to increased serum periostin level.

Key words

1. Phthalate
2. perisotin
3. chitinase-like proteins (YKL-40)
4. airway dysfunction

Key Messages

Phthalates have known adverse effects on the respiratory and immunological systems. However, the mechanisms by which they promote asthma, impaired lung function, and airway inflammation are unknown because few studies have thoroughly examined this topic. We found that exposure to low-molecular-weight phthalates was significantly associated with airway dysfunction, and this effect is partially attributable to increased serum periostin level. Our results suggest that periostin might stimulate Th2 cell-mediated inflammation and thereby cause pulmonary dysfunction in children exposed to phthalates.

INTRODUCTION

Previous studies reported that exposures to environmental pollutants are responsible for the increasing prevalence of allergic diseases.[1,2] Here, we focused on phthalates, a group of chemicals commonly used as plasticizers that commonly have higher concentrations in children than adults.[3] Phthalates can have adverse effects on the respiratory and immunological systems. Epidemiological studies reported positive associations between indicators of phthalate exposure and risk of asthma, and allergic diseases.[4,5] Other studies reported that phthalates also adversely affect pulmonary functions.[6,7]Experimental studies demonstrated that exposure to phthalates increased the levels of T helper 2 (Th2) cells and cytokines, and also increased

airway inflammation.[8,9] Several clinical studies found an association between phthalate exposure and fractional exhaled nitric oxide (FeNO).[5,8] The immunological mechanisms by which phthalate exposure leads to asthma and disruptions of lung function and airway inflammation are unknown. In addition, few studies have examined the association of phthalate exposure with allergic inflammation and lung function.

Recent studies identified periostin and chitinase-like proteins as biomarkers of asthma. Periostin promotes chronic allergic inflammation in response to Th2-associated cytokines by inducing proinflammatory cytokines.[10] Chitinase-3-like protein 1, also known as YKL-40, is a glycoprotein secreted by various cell types, including macrophages, neutrophils, and airway epithelial cells.[11] However, little is known about the levels or function of periostin and YKL-40 following exposure to environmental pollutants or other chemicals. We hypothesized that phthalate exposure may alter the serum levels of periostin and YKL-40.

Our primary objective was to determine the associations between phthalate exposure and pulmonary functions in a population-based sample of randomly selected sixth-grade students from 11 elementary schools in Korea. Our secondary objective was to examine the potential role of periostin and YKL-40 in mediating the relationship between phthalate exposure and airway dysfunction.

METHODS

Subjects and Protocol

A total of 620 fifth- and sixth-grade elementary school students (10–12 years old) who participated in the Seongnam Atopy Project (SAP) 2017 cohort were enrolled. This study was sponsored by the Seongnam City Government for the prevention and education of allergic diseases in Korean children and was conducted between January 2017 and October 2017.[12]

Demographic data were collected, and questionnaires completed by the parents were used to record symptoms of the children during the previous 12 months (wheezing, nasal symptoms, and eczema), based on the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire.[13]. Urine samples were collected in sterile cups and stored at -70°C for up to 3 months before analysis. Blood samples were stored at -70degC until determination of the levels of serum periostin, serum YKL-40, and total and allergen-specific immunoglobulin (Ig) E. FeNO levels were measured by a physician and impulse oscillometry (IOS) was performed by a well-trained technician. The study protocol was approved by the appropriate Institutional Review Board of CHA University (2017-04-049), and written informed consent documents were obtained from all parents or guardians of participating children.

Measurement of phthalate metabolites

Phthalate metabolite concentrations were determined by gas chromatography/tandem mass spectroscopy.[14] Reported phthalate concentrations were expressed relative to urinary creatinine (μg/g UCr) to control for urine dilution. A total of 455 urine samples were analyzed for the following 7 phthalate metabolites: mono-(iso-butyl) phthalate (MiBP), mono-isononyl phthalate (MNP), mono-benzyl phthalate (MBzP), mono-(3-carboxypropyl) phthalate (MCPP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP). (Appendix S1: Fig. E1). Phthalate metabolites were grouped based on molecular weight and reported as the sum of 4 major high-molecular weight phthalate (Σ_4HMWP) metabolites (MEHP, MEOHP, MECPP, and MCPP), or the sum of the 3 major low-molecular weight phthalate (Σ_3LMWP) metabolites (MiBP, MNP, and MBzP). They were further divided into quartiles according to concentration (lowest to highest,Q1 to Q4). Previous research indicated these two groups of metabolites have different physicochemical properties.[14,15]

Lung function measurements and FeNO

IOS was performed in accordance with established guidelines.[16]The difference of the respiratory system resistance (Rrs) at 5 Hz and 20 Hz (Rrs5-20) has a high sensitivity for detecting small airway dysfunction.[17]Airway resistance was also measured at 1, 2, 3, 5, 10, 15, and 20 Hz for calculation of area of reactance (AX) as

a function of frequency. The signals were analyzed for 30 s at each frequency. Oscillometric tests were performed using a Jaeger MasterScreen device (Jaeger Co., Wurzburg, Germany).

The FeNO concentration in exhaled breath in parts per billion (ppb) was measured using a portable nitric oxide analyzer (NIOX MINO®; Aerocrine, Solna, Sweden) according to the American Thoracic Society (ATS) guidelines.[18]

Measurement of serum biomarkers

Serum total and specific IgE antibodies for common aeroallergens (*Dermatophagoides farinae* , cat dander, dog dander, birch,*Alternaria alternata* , and *Humulus japonicus*) were measured using the ImmunoCAP system (Phadia, Uppsala, Sweden). Atopy was defined by positive specific IgE antibodies to at least one common inhaled allergen. Serum periostin was determined using the Human Periostin/OSF-2 DuoSet ELISA Kit (catalog number DY3548B; R&D Systems, Inc., Minneapolis, MN). Serum YKL-40 was determined using the Human Chitinase 3-like 1 Quantikine ELISA Kit (catalog number DC3L10; R&D Systems, Inc., Minneapolis, MN).

Statistical analysis

The data were analyzed using SPSS version 21.0 (SPSS, Chicago, IL) and R software version 3.6.3. Continuous data are expressed as means with standard deviations or as medians with the interquartile ranges, depending on the data distribution. The relationships of the concentrations of different of urinary phthalates with serum periostin level, Rrs5, and Rrs5-20 were analyzed using linear regression with adjustment for height, gender, BMI z -score, aeroallergen sensitization, secondary smoking, and vitamin D level. The estimates were regression slopes for phthalate concentration *vs.* periostin level and Rrs5. Beta (β) and a 95% confidence interval (CI) were obtained from a generalized linear regression model with the gamma function. The R package, "Mediation"[19] was used to quantitatively estimate the mediating effect of periostin level in the association of phthalate concentration with Rrs5 and Rrs5-20. This package estimates confidence intervals using bootstrapping with 1000 resamples. A two-sided P value of .05 or less indicated statistical significance.

RESULTS

Characteristics of study subjects

We recruited 487 fifth- and sixth-grade students for this study (Table 1). The average age was 11.01 years (95% CI: 10.9 to 11.1) and the average BMI z- score was -0.02 (95% CI: -0.11 to 0.07). Fifty-six children (11.7%) had wheezing at some time, 28 (5.8%) had asthma, and 25 (10.9%) were born premature and/or had low birth weight. A total of 61.4% of the children had atopic sensitization. The median level of serum periostin was 35.7 (ng/mL) and the median level of YKL-40 was 21.47 (ng/mL). Table 1 shows the IOS parameters.

Association of phthalate concentration with lung function and FeNO level

Analysis of the association of the quartiles of phthalate metabolites with Rrs5 (Table 2, Figure 1) indicated the quartiles of urinary Σ_4HMWP metabolites were associated with greater Rrs5 (Crude β: 0.028; 95% CI: 0.011 to 0.046; $P = .001$), but these associations were not significant after adjusting for height, gender, BMI z- score, aeroallergen sensitization, secondary smoking, and vitamin D level. However, after adjusting for these same confounders, Σ_4HMWP was significantly associated with Rrs1 (Q1/Q4, adjusted β: 0.047, 95% CI: 0.009 to 0.084, $P = .015$), Rrs2 (Q1/Q4, adjusted β: 0.046, 95% CI: 0.007 to 0.084, $P = .021$), and Rrs3 (Q1/Q4, adjusted β: 0.057, 95% CI: 0.015 to 0.099, $P = 0.008$) (Figure 1A).

Children with higher quartiles of Σ_3LMWP metabolites (Table 2, Fig. 1) also had significantly greater Rrs5 (Crude β: 0.028, 95% CI: 0.011 to 0.045; $P = .001$) and this relationship remained significant after adjustment for confounding (adjusted β: 0.020, 95% CI: 0.005 to 0.034; $P = .010$). In addition, after adjusting for confounding, quartiles of Σ_3LMWP was also significantly associated with Rrs1 (Q1/Q4, adjusted β: 0.046, 95% CI: 0.008 to 0.083,$P = .018$), Rrs2 (Q1/Q4, adjusted β: 0.051, 95% CI: 0.012 to 0.089, $P = .010$), Rrs3

(Q1/Q4, adjusted β: 0.057, 95% CI: 0.015 to 0.099, P = .008), Rrs10 (Q1/Q4, adjusted β: 0.055, 95% CI: 0.010 to 0.099, P = .016), and Rrs15 (Q1/Q4, adjusted β: 0.055, 95% CI: 0.001 to 0.109, P = .044).

After adjustment for confounding, there was a significant association in the quartiles of urinary Σ_4HMWP metabolites with FeNO (adjusted β: 0.053, 95% CI: 0.007 to 0.099; P = 0.024), but no significant association in the quartiles of urinary Σ_3LMWP metabolites with FeNO (Table 2).

Associations of phthalate concentrations with serum periostin level, YKL-40 level, and lung function

Analysis of the relationships of urinary phthalate metabolites with serum periostin level and serum YKL-40 level indicated significant associations in the quartiles of urinary Σ_4HMWP and Σ_3LMWP metabolites with periostin (Table 3). After adjustment using a generalized linear regression model with the logit function, these relationships remained significant. After adjustment, the level of urinary Σ_4HMWP metabolites was also significantly associated with YKL-40 (adjusted β: 0.882, 95% CI: 0.687 to 0.983; P =.032), but level of urinary Σ_3LMWP metabolites was not significantly associated with YKL-40 level.

Analysis of the relationships of serum periostin and serum YKL-40 levels with pulmonary function indicated periostin level was significantly associated with Rrs5 (adjusted β: 0.048, 95% CI: 0.015 to 0.081;P = .005), but not with FeNO (Table 4). There was also a significant association between periostin level and Rrs5-20 (adjusted β: 0.040, 95% CI: 0.011 to 0.069; P = .007). The serum YKL-40 level was not significantly associated with Rrs5 or Rrs5-20.

Mediating effect of periostin on lung function

We performed an in-depth analysis of the potential role of periostin in mediating the association between the level of urinary phthalate metabolites and Rrs5 (Fig. 2). The results indicated that a higher Σ_3LMWP level had a significant and direct association with a higher Rrs5 (β: 0.084, 95% CI: 0.005 to 0.14, P = 0.04), and that some of the effect of Σ_3LMWP was mediated by an increased periostin level (β: 0.013, 95% CI: 0.002 to 0.030, P< 0.001). Periostin mediates the relationship between Σ_3LMWP level and Rrs5 (13.9% mediation, 95% CI: 10.7 to 77.0; P <.001).

DISCUSSION

The role of environmental phthalate exposure on allergic airway diseases has been a topic of great interest. However, very few studies have examined the association of phthalate exposure with allergic inflammation and lung function. Our measurements of IOS in a population-based sample of children showed that an increased level of urinary phthalate metabolites was significantly associated with airway dysfunction, and that this association was partially attributable to an increased serum periostin level. Moreover, the associations we identified persisted after adjusting for multiple covariates (height, gender, BMI z -score, aeroallergen sensitization, secondary smoking, and vitamin D level).

Phthalate exposure may occur from ingestion, inhalation, dermal absorption, and parenteral administration.[20,21] LMWPs are used in a variety of personal-hygiene and cosmetic products, such as nail polish and fragrances, as scent stabilizers.[22]HMWPs are used in plastic tubing, food packaging, containers, vinyl toys, vinyl floor coverings, and building products.[21,22] Koch et al.[14] showed that exposure to HMWPs was mostly due to dietary intake, and that exposure to LMWPs was mainly from non-dietary exposures, such as from personal care products, dust, and indoor air.[14] In the present study, we showed that children with higher urinary levels of LMWPs had significantly greater airway resistance after adjusting for confounding variables.

Experimental studies demonstrated that exposure to phthalates increases the levels of Th2 cells and multiple cytokines, and thereby enhances airway inflammation.[8,9] Clinical studies found an association between phthalate exposure and FeNO.[5,8]Serum periostin is a biomarker of type-2 inflammation in asthma.[23] The exact function of YKL-40 remains unclear, but it consistently correlates with airway obstruction in studies of patients with asthma,[24-26] and with measures of airway remodeling, such as bronchial wall thickness and

subepithelial fibrosis.[24,25] Little is known about the function of periostin and YKL-40 in patients with allergic inflammation related to environmental pollutants. We initially hypothesized that there may be some differences in serum levels of periostin and YKL-40 following phthalate exposure because type-2 and non–type-2-induced airway inflammation are involved in phthalate-related airway inflammation. We therefore evaluated the relationships of serum periostin and YKL-40 levels in children with phthalate exposure, and investigated their relevance to clinical characteristics and other type-2 biomarkers, including blood eosinophil counts, serum total IgE, and FeNO. We found that FeNO level was significantly associated with the quartiles of urinary Σ_4HMWP metabolites, but not quartiles of urinary Σ_3LMWP metabolites. Our multivariate linear regression analysis indicated that urinary Σ_4HMWP and Σ_3LMWP metabolites were both significantly associated with serum periostin level. These findings are clinically significant because they demonstrate an association between phthalate exposures with serum periostin, an established marker of Th2 inflammation. However, we found no significant associations in the levels of urinary Σ_4HMWP and Σ_3LMWP metabolites with serum YKL-40 level. These results suggest that serum periostin may be used as a biomarker for type-2 inflammation in children following phthalate exposure, but serum YKL-40 has less value for this assessment.

We found that serum periostin level was significantly associated with Rrs5 and Rrs20-5. This outcome is similar to that of a recent study which reported the relationship between periostin level and pulmonary function in asthma patients. This previous study found that patients with high periostin levels had lower FEV_1/FVC values.[27] Although there are reports of associations between pulmonary function and periostin level, there are only limited data on the relationships of periostin with small airway function. We therefore used an objective method — IOS — to evaluate small airway dysfunction our patients.

We found that periostin had a significant effect in mediating the relationship between urinary LMWP metabolites and airway resistance. To quantify this mediating effect, we performed a model-based mediation analysis by using the mediation package in R software.[19] The algorithm uses a quasi-Bayesian Monte Carlo method to estimate the presence of mediation (average causal mediation effect/indirect effect) and the proportion of the link between phthalate exposure and airway dysfunction that is mediated by periostin.[19]

There are several limitations to the present study. First, because this was a cross-sectional study, we did not obtain any direct evidence for cause-and-effect relationships. Second, use of periostin as a dependable biomarker in growing children may be questionable because it is an extracellular matrix protein that is secreted by osteoblasts. However, the levels in our study subjects (10-12 years-old) were not higher than published values for adults[28] and were not significantly associated with age (data not shown).

To the best of our knowledge, this is the first large-sample study of urban children to comprehensively investigate the role of periostin in mediating the relationship between phthalate exposure and airway dysfunction. Previous studies have investigated the association of phthalate exposure with other inflammation markers, but no previous studies investigated the role of periostin and YKL-40 on airway dysfunction in children exposed to phthalates. Another merit of this study is that we assessed small airway function using IOS.

In conclusion, we found that exposure to low-molecular-weight phthalates was significantly associated with airway dysfunction, and this effect is partially attributable to increased serum periostin level. Periostin appears to function in the Th2 cell-mediated inflammation that causes pulmonary dysfunction in children exposed to phthalates, but further studies are required to clarify this relationship.

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Tables

Table 1. Demographic and clinical characteristics of study subjects (N=487)

Variable	Value	Value
	Mean	95% CI
Age, years	11.0	10.9 to 11.1
BMI *z*-score	-0.02	-0.11 to 0.07
Height, cm	149.4	148.7 to 150.1
	n*	%
Wheezing (ever)	56/479	11.7
Asthma, physician diagnosis	28/486	5.8
Passive smoking	211/483	43.6
Atopic sensitization+	289/479	61.4
	Median	IQR
Blood periostin,ng/mL	35.7	27.3 to 43.6
Blood YKL-40, ng/mL	21.4	17.4 to 27,4
Total IgE, IU/mL[3]	103.0	40.9 to 276.0
Blood eosinophil, 10^9 cells/L[4]	170.2	107.5 to 277.3
Vitamin D, ng/ml[5]	21.0	17.1 to 25.4
FeNO, ppb[6]	15.0	11.0 to 22.0
	Median	IQR
Rrs5, hPa/L/s	4.92	4.32 to 5.60
Rrs5-20, hPa/L/s	1.74	1.33 to 2.29
AX, hPa/L/sc	10.90	7.87 to 14.93

IQR, interquartile range; BMI, body mass index; IgE: immunoglobulin E; FeNO, fractional nitric oxide; Rrs5, resistance of respiratory system at 5 Hz; Rrs5-20, the difference of the Rrs at 5 Hz and 20 Hz; AX, reactance area

*Number of exposed or cases.

+Inhalant allergen-specific IgE > 0.35 kU/L for at least 1 of 6 allergens (*Alternaria* , birch, cat dander, dog dander, *Dermatophagoides farina* , Japanese hop).

Table 2. Association of low and high MWP metabolites with Rrs5 and FeNO.

Quartiles of urinary phthalate	Rrs5	Rrs5	Rrs5	Rrs5	Rrs5	FeNO	FeNO	FeNO	FeNO
	Crude β (95% CI)	*P* value		Adjusted β (95% CI)	*P* value	Crude β (95% CI)	*P* value		Adjusted β (95% CI)
High MWP									
Q1	Ref	-		Ref	-	Ref	-		Ref
Q2	0.039 (-0.015 to 0.093)	0.160		0.017 (-0.030 to 0.063)	0.490	-0.062 (-0.215 to 0.090)	0.425		-0.07 (-0.153 to 0.138)
Q3	0.036 (-0.018 to 0.090)	0.195		0.017 (-0.029 to 0.064)	0.466	-0.074 (-0.226 to 0.077)	0.334		-0.043 (-0.188 to 0.102)
Q4	**0.096 (0.042 to 0.150)**	**0.001**		0.043 (-0.004 to 0.090)	0.076	0.144 (-0.008 to -.295)	0.064		**0.185 (0.038 to 0.332)**
P for trend	**0.028 (0.011 to 0.046)**	**0.001**		0.013 (-0.002 to 0.028)	0.090	0.042 (-0.005 to 0090)	0.077		**0.053 (0.007 to 0.099)**
Low MWP									
Q1	Ref	-		Ref	-	Ref	-		Ref
Q2	**0.070 (0.014 to 0.124)**	**0.012**		0.033 (-0.014 to 0.080)	0.167	0.002 (-0150 to 0.154)	0.980		0.015 (-0.133 to 0.164)
Q3	**0.086 (0.032 to 0.140)**	**0.002**		0.047 (-0.000 to 0.094)	0.050	-0.124 (-0.276 to 0.028)	0.110		-0.108 (-0.255 to 0.039)
Q4	**0.088 (0.034 to 0.142)**	**0.001**		**0.061 (0.014 to 0.108)**	**0.011**	0.145 (-0.006 to 0.295)	0.059		0.137 (-0.008 to 0.283)
P for trend	**0.028 (0.011 to 0.045)**	**0.001**		**0.020 (0.005 to 0.034)**	**0.010**	0.034 (-0.013 to 0.081)	0.158		0.032 (-0.014 to 0.077)

MWP, molecular weight phthalate; Rrs5, resistance of respiratory system at 5 Hz; FeNO, fractional nitric

oxide; CI, confidence interval; Ref, reference

Rrs5 evaluations adjusted for height, gender, BMI *z* score, secondary smoking, aeroallergen sensitization, and vitamin D level. FeNO evaluations adjusted for age, gender, BMI *z* score, secondary smoking, aeroallergen sensitization, and vitamin D level. *P* values are from a generalized linear regression with a gamma function.

Table 3. Association of serum periostin and YKL-40 levels with high and low MWP metabolites.

Quartiles of urinary phthalate	Periostin (high *vs.* low)	Periostin (high *vs.* low)	Periostin (high *vs.* low)	Periostin (high *vs.* low)	Periostin (high *vs.* low)	YKL-40 (high *vs.* low)	YKL-40 (high *vs.* low)	YKL-40 (high *vs.* low)	YKL-40 (high *vs.* low)
	OR (95% CI)	*P* value		aOR (95% CI)	*P* value	OR (95% CI)	*P* value		aOR (95% CI)
High MWP									
Q1	Ref	-		Ref	-	Ref	-		Ref
Q2	**2.322 (1.349 to 3.998)**	**0.002**		**2.134 (1.232 to 3.696)**	**0.007**	0.926 (0.543 to 1.581)	0.778		1.055 (0.604 to 1.842)
Q3	**2.259 (1.306 to 3.906)**	**0.004**		**2.022 (1.166 to 3.508)**	**0.012**	0.617 (0.360 to 1.056)	0.078		0.593 (0.339 to 1.038)
Q4	**2.259 (1.306 to 3.906)**	**0.004**		**1.994 (1.141 to 3.484)**	**0.015**	0.655 (0.380 to 1.130)	0.128		0.634 (0.360 to 1.118)
P for trend	**1.270 (1.070 to 1.507)**	**0.006**		**1.222 (1.026 to 1.455)**	**0.025**	0.845 (0.711 to 1.003)	0.054		**0.882 (0.687 to 0.983)**
Low MWP									
Q1	Ref	-		Ref	-	Ref	-		Ref
Q2	1.538 (0.897 to 2.639)	0.118		1.679 (0.972 to 2.899)	0.063	1.505 (0.880 to 2.573)	0.135		1.468 (0.838 to 2.573)
Q3	1.481 (0.868 to2.528)	0.150		1.342 (0.774 to 2.329)	0.295	1.056 (0.619 to 1.801)	0.843		0.929 (0.530 to 1.626)
Q4	**2.436 (1.408 to 4.216)**	**0.001**		**2.313 (1.322 to 4.048)**	**0.003**	1.050 (0.612 to 1.800)	0.843		0.891 (0.508 to 1.563)

Quartiles of urinary phthalate	Periostin (high *vs.* low)	Periostin (high *vs.* low)	Periostin (high *vs.* low)	Periostin (high *vs.* low)	Periostin (high *vs.* low)	YKL-40 (high *vs.* low)	YKL-40 (high *vs.* low)	YKL-40 (high *vs.* low)	YKL-40 (high *vs.* low)
P for trend	**1.299 (1.094 to 1.542)**	**0.003**		**1.257 (1.054 to 1.498)**	**0.011**	0.980 (0.827 to 1.162)	0.820		0.924 (0.773 to 1.103)

YKL-40, chitinase-like proteins; MWP, molecular weight phthalate; OR, odds ratio; CI, confidence interval

Periostin and YKL-40 (high *vs.* low) adjusted for age, gender, BMI*z* score, aeroallergen sensitization, and secondary smoking.*P* values are from a generalized linear regression with the logit function.

Table 4. Relationship of periostin and YKL-40 levels with lung function (Rrs5 and FeNO).

	Rrs5	Rrs5	Rrs5	Rrs5	Rrs5	FeNO	F
	Crude β (95% CI)	*P* value*		Adjusted β (95% CI)	*P* value	Crude β (95% CI)	*P*
Periostin							
Low level	Ref	-		Ref	-	Ref	-
High level	**0.053 (0.013 to 0.092)**	**0.008**		**0.048 (0.015 to 0.081)**	**0.005**	0.055 (-0.057 to 0.168)	0
Chitinase							
Low level	Ref	-		Ref	-	Ref	-
High level	0.024 (-0.016 to 0.064)	0.240		0.007 (-0.031 to 0.045)	0.731	0.031 (-0.082 to 0.143)	0

YKL-40, chitinase-like proteins; Rrs5, resistance of respiratory system at 5 Hz; FeNO, fractional nitric oxide

*Adjusted for gender, height, BMI z score, aeroallergen sensitization, secondary smoking, and vitamin D level using a generalized linear regression with the gamma function.

Figure legends

Figure 1. Effect of frequency on impulse oscillometry resistance in children with different levels of Σ_4HMWP metabolites (A) and Σ_3LMWP metabolites (B). Lines were determined by locally weighted smoothing functions and shaded areas indicate 95% confidence intervals. The level of Σ_4HMWP metabolites (A) was significantly associated with Rrs1, Rrs2, and Rrs3 after adjusting for height, gender, BMI *z*- score, aeroallergen sensitization, secondary smoking, and vitamin D level. The level of Σ_3LMWP metabolites (B) was significantly associated with Rrs1, Rrs2, Rrs3, Rrs5, Rrs10, and Rrs15 after adjusting for these same factors. LMHP, low molecular weight phthalate; HMWP, high molecular weight phthalate; Rrs, respiratory system resistance.

Figure 2. Mediation model used to investigate the effect of serum periostin on the relationship between LMWP metabolites and lung dysfunction (Rrs5). The level of Σ_3LMWP metabolites was significantly and directly associated with Rrs5 (β: 0.084, 95% CI: 0.005 to 0.14, $P = 0.04$), and periostin (β: 0.013, 95% CI: 0.002 to 0.030, $P < 0.001$) mediated 13.8% of this effect (95% CI: 10.7 to 77.0, $P < 0.001$). The analysis adjusted for height, gender, BMI *z*- score, aeroallergen sensitization, secondary smoking, and vitamin D level. LMWP, low molecular weight phthalate; Rrs5, respiratory system resistance at 5 Hz.

Supplementary Figure E1. Concentrations (μg/g creatinine) of LMWP metabolites (top) and HMWP metabolites (bottom) in 487 urine samples. MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MCPP,

mono-(3-carboxypropyl) phthalate; MECPP, mono-(2-ethyl-5-carboxypentyl) phthalate; MiBP, mono-(isobutyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MNP, mono-isononyl phthalate; MBzP), mono-benzyl phthalate. Horizontal lines separate the different quartiles.

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