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Research Article

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Association of phthalates and early menarche in Korean adolescent girls from Korean National Environmental Health Survey (KoNEHS) 2015–2017

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Abbreviations
BMI: body mass index; CI: confidence interval; DEHP: di-2-ethylhexyl phthalate; EDC: endocrine-disrupting chemical; GM: geometric mean; IRB: Institutional Review Board; KoNEHS: Korean National Environmental Health Survey; LOD: limit of detection; MBzP: mono-benzyl phthalate; MCNP: mono-(carboxy-isononyl) phthalate;

ABSTRACT

Background: Phthalates are one of renowned endocrine-disrupting chemicals, although inconsistent results are present around their effect on onset of menarche. Our hypothesis is that pre-pubertal exposure to phthalates is associated with acceleration of menarche.
Methods: We analyzed a total of 236 middle school (7th to 9th grade) girls from Korean National Environmental Health Survey 2015–2017. We used multiple linear regression to investigate impact of eight phthalate metabolites on age of menarche. We also conducted logistic regression to evaluate association between phthalate metabolite concentrations and early onset of menarche, adjusting for grade, maternal age of menarche and body mass index (BMI).
Results: In linear regression analysis, no significant association was found for any phthalate metabolites. In logistic regression analysis, however, odds ratios (ORs) of early menarche were significantly increased for mono-n-butyl phthalate (MnBP) and for sum of all phthalates. When compared to group with the lowest level, high concentration group for MnBP presented significantly increased odds of early menarche (OR: 2.09; 95% confidence interval [CI]: 1.03, 4.23) after adjusting for grade, maternal age of menarche and BMI. Furthermore, high concentrations of sum of all phthalates were associated with significant increase of OR of early menarche (OR: 2.22; 95% CI: 1.10, 4.49) after adjustment, compared to the lowest concentration group.
Conclusions: Results of our study suggest that exposure to phthalates around puberty may be associated with increased risk of early menarche.

Keywords: Phthalate; Menarche; Early menarche; Puberty; Endocrine-disrupting chemicals

BACKGROUND

For the past few decades, average age of menarche has been consistently decreasing throughout the world [1,2]. South Korea was no exception in this trend, as average age at menarche of South Korean girls had been reduced to 12.7 years in 2011 from 13.4 years in 2001 and 14.2 years in 1980 [3,4]. Concerns arose around this worldwide phenomenon since early onset of menarche was found to be associated with increased risk of hypertension [5], type 2 diabetes [6], coronary heart disease [7], and breast cancer [8]. In addition to attributable factors such as race and increased adiposity, it was hypothesized that

MCOP: mono-carboxyoctyl phthalate; MCPP: mono-(3-carboxypropyl) phthalate; MECPP: mono-(2-ethyl-5-carboxypentyl) phthalate; MEHHP: mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP: mono-(2-ethyl-5-oxohexyl) phthalate; MEP: mono-ethyl phthalate; MMP: mono-methyl phthalate; MnBP: mono-n-butyl phthalate; OR: odds ratio; SG: specific gravity.

Competing interests
The authors declare that they have no competing interest.

Availability of data and materials
The data analyzed in this study are from Korean National Environmental Health Survey 2015–2017, which is open for any researchers upon request at National Institute of Environmental Research of Korea (https://www.nier.go.kr/NIER/kor/index.do).

Authors contributions
Conceptualization: Park O, Park JT, Kwak K; Data curation: Park O, Chi Y; Formal analysis: Park O, Kwak K; Supervision: Kwak K; Writing - original draft: Park O, Kwak K; Writing - review & editing: Park JT, Chi Y, Kwak K.

environmental factors such as exposure to endocrine-disrupting chemicals (EDCs) had also played a role in changing the onset of puberty [9].

Phthalates are a group of chemicals that have a variety of usage as plasticizer and solvents in producing building materials, adhesives, food-packaging, cosmetics, and other numerous consumer products [10]. Due to their non-covalent bond with original products, they are readily released and absorbed into human body by ingestion, inhalation, and dermal contact, making their exposure ubiquitous in everyday life [11]. Exposure of children and adolescents to phthalates could have more hazardous effect since they are in the critical period of sexual maturation [12].

Phthalates are renowned for their endocrine-disrupting properties. Previous studies revealed that phthalates can influence thyroid hormone levels, lipogenesis, and male and female reproductive system [13-15]. In experimental studies, phthalates have shown both agonistic and antagonistic effects in hormonal receptors, interfering in hypothalamic-pituitary-gonadal axis and perturbing normal sexual development [16,17]. However, human epidemiological studies presented varying results in accordance with types of phthalates involved and the time of exposure. Some studies found that prenatal exposure to certain phthalates was associated with earlier onset of puberty [18]. In a study conducted with 201 girls in China, exposure to certain metabolites of di-2-ethylhexyl phthalate (DEHP) during puberty was also associated with earlier onset of menarche [19], whereas there were other studies that presented no association or rather delayed indices of puberty [20-23].

In this study, we retrieved data from Korean National Environmental Health Survey (KoNEHS) 2015–2017 and analyzed a sample of middle school girls to examine association between urinary phthalate metabolites and timing of menarche. We hypothesized that pre-pubertal exposure to higher concentration of phthalates is associated with earlier onset of menarche.

METHODS

Study subjects

KoNEHS, which started its first round in 2009, is a cross-sectional study that has two-stage, stratified, weighted sampling design to represent Korean population and evaluate exposure levels to various chemicals as well as related clinical and demographic features. The third round of KoNEHS (2015–2017) has expanded its study population into children and adolescents in addition to adult-only design of previous rounds.

The study sample was obtained from middle (junior high) and high school database of the KoNEHS 2015–2017. The database comprises of 430 boys and 492 girls from middle and high schools of urban and rural districts of Korea. Since KoNEHS is a school-based survey, age of subjects is only presented with their grade. The database has subjects from 7th grade to 12th grade, which is correspondent to age 12 to 17.

Due to cross-sectional design of KoNEHS and short half-life of phthalates in human body, levels of phthalate metabolites measured in high school students are less likely to be correlated with levels before or upon the time of menarche that occurred years ago. Therefore, the study sample was confined to only middle school students (i.e., 7th to 9th graders). Those without urinary phthalate metabolite data available were excluded. Finally, 236 girls were eligible for study subjects (**Fig. 1**).

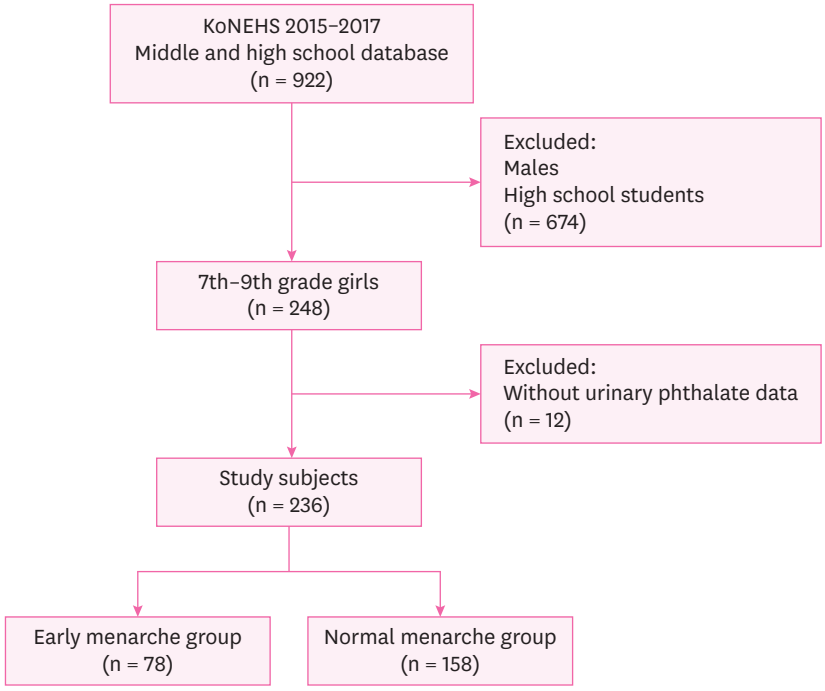

Fig. 1. Flow chart of the selection of study subjects.
KoNEHS: Korean National Environmental Health Survey.

Measurement of urinary phthalate metabolites

Within 24 hours after collection, spot urine samples were transported at 2ºC–6ºC to laboratories and were stored at −20ºC before analysis. Analysis was conducted through ultra performance liquid chromatography-mass spectrometry with electrospray ionization. Details of transportation, storage, analysis and quality control procedures followed the manual issued by National Institute of Environmental Research [24].

Urinary phthalate metabolites included mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-n-butyl phthalate (MnBP), mono-benzyl phthalate (MBzP), mono-carboxyoctyl phthalate (MCOP), mono-(carboxy-isononyl) phthalate (MCNP), and mono-(3-carboxypropyl) phthalate (MCPP). Results under limit of detection (LOD) were replaced with method detection limit divided by square root of 2.

Variables of interest

Since actual age of girls was unavailable owing to school-based nature of the survey, we defined early menarche as "menarche occurring before 6th grade", which is a mixed group of 11- and 12-year-olds. Consequently, menarche occurring in or after 6th grade was defined as normal menarche.

We probed possible confounders previously suggested through literature review. Potential confounders included maternal age of menarche [25,26], BMI [2,27-29], and household income [30-32]. Maternal age of menarche was presented with grade as was the daughter's, and maternal early menarche was defined as the same (menarche reached before 6th grade). Body fat status was categorized as normal (BMI < 23), overweight (23 ≤ BMI < 25), and obese

(BMI ≥ 25). Monthly household income was allocated into 4 categories: I (under $1,660), II ($1,660–$2,490), III ($2,490–$4,150), and IV (over $4,150).

Statistical analysis

MEHHP, MEOHP, and MECPP are secondary metabolites of DEHP, so sum of these three were also calculated under the name of 'DEHP metabolites.' Total sum of eight metabolites measured were added as 'total phthalates.' Concentration of each metabolite was divided by its molar mass and added together for summation. For all phthalate metabolites, level above LOD was detected in more than 75% of subjects.

Due to the fact that significant portion of elimination of phthalates is processed through active tubular secretion of kidney, altogether with variable level of urinary creatinine affected by diet, muscularity, and other health status, it has been suggested that specific gravity (SG) is a better tool to adjust for urinary dilution [33]. Therefore, we employed formula Pc = P × [(1.024 − 1)/(SG − 1)] to calculate SG corrected urinary phthalate concentration (Pc is SG corrected phthalate metabolite concentration, P is phthalate metabolite concentration). We presented geometric means (GMs) and 95% confidence intervals (CI) of phthalate metabolite levels in accordance with categories of possible confounding characteristics.

Levels of urinary phthalate metabolites were positively skewed; therefore, natural log-transformation was applied to SG-corrected concentrations. We conducted linear regression analysis with and without covariates to evaluate association between urinary phthalate metabolite levels and menarche age. Nineteen girls who had not yet reached menarche at the time of survey were excluded from linear regression analysis (n = 217). Also, we divided study subjects into three groups according to concentration tertiles of each metabolite and conducted logistic regression. We compared the odds of early menarche of group with low concentrations to that of groups with moderate and high concentrations respectively. We included grade, maternal early menarche, and BMI group as covariates for adjustment. Data analysis was performed with SAS statistical software ver 9.4 (SAS Institute, Cary, NC, USA).

Ethics statement

This study was conducted after obtaining an approval from the Institutional Review Board (IRB) of Korea University Medical Center (IRB No. 2020AS0272).

RESULTS

Demographic and socioeconomic characteristics of 236 girls from 7th to 9th grade in KoNEHS (2015–2017) are described in **Table 1**. Seventy-eight girls were classified as early menarche group and 158 girls were classified as normal menarche group. Distributions of characteristics between groups were compared using χ^2 test for grade, Fisher's exact test for maternal early menarche, and Cochran-Armitage trend test for ordinal variables such as BMI and household income. None of the characteristics presented significant difference between groups.

GMs and 95% CIs of SG adjusted concentrations of urinary phthalate metabolites are shown in **Table 2**. Categorized by possible confounding characteristics, concentrations within each characteristic were compared using analysis of variance test or Kruskal-Wallis test. Other than MBzP level among BMI groups (p = 0.017) and MECPP (p = 0.011) and MCPP (p = 0.041)

Table 1. Demographic and socioeconomic characteristics of the subjects

Characteristics	Category	All subjects (n = 236)	Early menarche (n = 78)	Normal menarche (n = 158)	*p*-value
Grade	7	79 (33.5)	31 (39.7)	48 (30.4)	0.358[a]
	8	77 (32.6)	23 (29.4)	54 (34.2)	
	9	80 (33.9)	24 (30.8)	56 (45.4)	
Maternal early menarche	No	207 (87.7)	66 (84.6)	141 (89.2)	0.394[b]
	Yes	5 (2.1)	3 (3.9)	2 (1.3)	
	Unknown	24 (10.2)	9 (11.5)	15 (9.5)	
BMI	< 23	192 (81.4)	60 (76.9)	132 (84.5)	0.158[c]
	23–25	20 (8.5)	7 (9.0)	13 (8.2)	
	> 25	24 (10.2)	11 (14.1)	13 (8.2)	
Household income	I (lowest)	36 (15.3)	12 (15.4)	24 (15.2)	0.444[c]
	II	29 (12.3)	14 (18.0)	15 (9.5)	
	III	73 (30.9)	20 (25.6)	53 (33.5)	
	IV (highest)	85 (36.0)	27 (34.6)	58 (36.7)	
	Unknown	13 (5.5)	5 (6.4)	8 (5.1)	

Values are presented as number (%).
BMI: body mass index.
[a]Analyzed by χ^2 test; [b]Analyzed by Fisher's exact test; [c]Analyzed by Cochran-Armitage trend test.

level among household income groups, all other phthalate metabolites did not present significant difference according to the characteristics.

Table 3 provides the result of multiple linear regression analysis between age of menarche and log-transformed concentrations of urinary phthalate metabolites. For both crude and adjusted regression, MECPP, DEHP metabolites, MnBP, MBzP, MCOP, MCNP, MCPP, and total phthalates showed negative estimate, which is in the direction of advancing the onset of menarche, but none were statistically significant.

Table 4 presents the result of crude and adjusted logistic regression analyses which compare early menarche of groups with moderate and high phthalate metabolite concentration respectively, with that of group with low concentration. High MnBP group showed crude odds ratio (OR) of 2.27 (95% CI: 1.14, 4.54) and adjusted OR of 2.09 (95% CI: 1.03, 4.23) after taking into account grade, maternal age of menarche and BMI. None of the other individual metabolites presented notable effect size. However, for total phthalates, group with the highest tertile had crude OR for early menarche of 2.27 (95% CI: 1.14, 4.54), and adjusted OR of 2.22 (95% CI: 1.10, 4.49).

DISCUSSION

We investigated the association between urinary phthalate metabolites and age of menarche, using data from KoNEHS 2015–2017. In linear regression analysis, none of the phthalates showed any significant association with either speeding up or delaying onset of menarche. In logistic analysis, however, the risk of early menarche was significantly increased in high concentration of MnBP compared to low concentration. The risk of early menarche was also significantly increased in high concentration of total phthalates. Our findings suggest that phthalates may be associated with early menarche.

We found 78 of 236 subjects (33.1%) to have experienced early menarche, definition of which was “menarche occurring before 6th grade.” Previous researches usually defined early menarche by using age as “menarche occurring before age of 12 years.” A study that analyzed Korean National Health and Nutrition Examination Surveys, another nationally representative

Table 2. Levels of urinary phthalate metabolites regarding characteristics of the subjects

Characteristics	Category	Geometric mean (μg/L) (95% CI)							
		MEHHP	MEOHP	MECPP	MnBP	MBzP	MCOP	MCNP	MCPP
All subjects		16.19 (14.76, 17.76)	10.33 (9.19, 11.62)	34.62 (32.56, 36.82)	37.86 (34.09, 42.05)	2.90 (2.45, 3.43)	2.09 (1.90, 2.31)	0.56 (0.52, 0.61)	1.67 (1.58, 1.77)
Early menarche	No	16.13 (14.36, 18.12)	10.06 (8.64, 11.71)	34.15 (31.57, 36.94)	35.63 (31.21, 40.66)	2.72 (2.24, 3.32)	2.07 (1.82, 2.35)	0.55 (0.51, 0.61)	1.65 (1.54, 1.78)
	Yes	16.32 (14.06, 18.95)	10.91 (9.16, 12.99)	35.61 (32.35, 39.20)	42.83 (36.22, 50.65)	3.29 (2.41, 4.50)	2.14 (1.87, 2.46)	0.58 (0.51, 0.67)	1.71 (1.55, 1.89)
	p-value[a]	0.906	0.524	0.528	0.104	0.294	0.734	0.542	0.565
Grade	7	16.50 (14.37, 18.94)	10.38 (8.67, 12.42)	34.58 (31.55, 37.91)	40.25 (33.58, 48.24)	3.14 (2.49, 3.96)	2.19 (1.92, 2.51)	0.56 (0.49, 0.64)	1.74 (1.58, 1.93)
	8	15.18 (12.54, 18.39)	9.95 (7.79, 12.72)	33.45 (29.84, 37.50)	35.75 (29.82, 42.86)	2.47 (1.77, 3.46)	1.91 (1.55, 2.34)	0.57 (0.51, 0.65)	1.67 (1.51, 1.86)
	9	16.91 (14.62, 19.56)	10.66 (8.90, 12.77)	35.83 (32.05, 40.06)	37.67 (31.39, 45.20)	3.13 (2.36, 4.19)	2.18 (1.86, 2.56)	0.56 (0.49, 0.63)	1.60 (1.44, 1.78)
	p-value[a]	0.621	0.895	0.670	0.665	0.428	0.425	0.941	0.500
Maternal early menarche	No	16.05 (14.58, 17.67)	10.16 (8.98, 11.49)	34.42 (32.28, 36.69)	36.42 (32.66, 40.62)	2.79 (2.33, 3.34)	2.05 (1.84, 2.28)	0.56 (0.52, 0.61)	1.65 (1.55, 1.75)
	Yes	23.27 (12.56, 43.12)	16.53 (10.26, 26.63)	36.75 (22.62, 59.72)	44.11 (17.61, 110.49)	4.20 (2.37, 7.44)	2.57 (1.67, 3.96)	0.53 (0.26, 1.15)	1.70 (1.25, 2.33)
	Unknown	16.16 (11.49, 22.72)	10.83 (7.10, 16.54)	36.02 (28.83, 44.99)	51.21 (35.81, 73.25)	3.73 (2.13, 6.51)	2.38 (1.88, 2.99)	0.57 (0.46, 0.70)	1.89 (1.57, 2.28)
	p-value[b]	0.413	0.265	0.529	0.184	0.346	0.510	0.811	0.295
BMI	< 23	16.03 (14.47, 17.75)	10.30 (9.03, 11.75)	34.36 (32.10, 36.79)	38.07 (33.79, 42.89)	2.71 (2.26, 3.26)	2.12 (1.92, 2.34)	0.57 (0.52, 0.62)	1.67 (1.57, 1.77)
	23–25	20.12 (15.99, 25.31)	12.58 (9.54, 16.60)	36.65 (29.91, 44.90)	34.65 (25.66, 46.80)	2.38 (1.31, 4.35)	1.92 (1.21, 3.05)	0.52 (0.40, 0.68)	1.82 (1.36, 2.42)
	> 25	14.66 (10.48, 20.51)	9.01 (6.02, 13.47)	35.08 (28.93, 42.54)	39.03 (28.82, 52.87)	5.83 (3.61, 9.42)	2.00 (1.36, 2.94)	0.56 (0.44, 0.71)	1.61 (1.35, 1.92)
	p-value[b]	0.544	0.749	0.766	0.882	0.017[c]	0.787	0.821	0.943
Household income	I	21.42 (17.00, 27.00)	14.11 (10.79, 18.46)	44.97 (38.25, 52.88)	49.02 (37.13, 64.72)	4.13 (2.69, 6.35)	2.30 (1.65, 3.20)	0.67 (0.55, 0.82)	1.99 (1.70, 2.34)
	II	15.40 (11.69, 20.29)	10.20 (7.17, 14.53)	35.85 (30.23, 42.51)	45.27 (35.00, 58.57)	3.38 (1.91, 5.99)	2.09 (1.70, 2.58)	0.53 (0.43, 0.65)	1.85 (1.56, 2.19)
	III	15.01 (12.55, 17.96)	8.94 (6.99, 11.43)	33.38 (30.18, 36.92)	35.54 (30.18, 41.86)	2.63 (2.00, 3.45)	2.28 (2.01, 2.60)	0.58 (0.50, 0.66)	1.60 (1.43, 1.80)
	IV	15.41 (13.41, 17.70)	10.15 (8.68, 11.87)	31.08 (28.60, 33.77)	33.67 (27.75, 40.85)	2.59 (1.97, 3.41)	1.87 (1.56, 2.24)	0.54 (0.48, 0.60	1.57 (1.44, 1.71)
	Unknown	17.67 (12.90, 24.21)	11.33 (6.56, 19.57)	38.65 (24.82, 60.20)	38.16 (25.56, 56.97)	2.81 (1.40, 5.65)	2.06 (1.44, 2.94)	0.48 (0.34, 0.68)	1.59 (1.28, 1.97)
	p-value[b]	0.076	0.076	0.011[c]	0.235	0.356	0.092	0.390	0.041[c]

All concentrations were corrected with urinary specific gravity.
BMI: body mass index; CI: confidence interval; MEHHP: mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP: mono-(2-ethyl-5-oxohexyl) phthalate; MECPP: mono-(2-ethyl-5-carboxypentyl) phthalate; MnBP: mono-n-butyl phthalate; MBzP: mono-benzyl phthalate; MCOP: mono-carboxyoctyl phthalate; MCNP: mono-(carboxy-isononyl) phthalate; MCPP: mono-(3-carboxypropyl) phthalate.
[a]Analyzed by analysis of variance test; [b]Analyzed by Kruskal-Wallis test; [c]$p < 0.05$.

cross-sectional survey of Korea, reported proportion of early menarche in girls aged 10–18 years to be 21.4% in 2001 and 34.6% in 2011 [4]. Another study from the United States that analyzed National Health and Nutrition Examination Surveys 2003-2010 found early menarche in 27.8% of girls aged 12–19 years [34]. Prevalence of early menarche in our study, despite the minor difference in definition, appear to be consistent with previous reports.

Previous epidemiological researches presented fairly inconsistent results as association with accelerated puberty, delayed puberty, and no association at all coexisted. A recent meta-analysis estimated the effect of six phthalates (mono-ethyl phthalate [MEP], mono-methyl phthalate [MMP], MnBP, mono-(2-ethylhexyl)-phthalate, MEHHP, and MEOHP) on menarche as well as thelarche and pubarche [35]. In pooled analysis, none of the phthalates exhibited significant association with either precocious or delayed menarche. Some amount

Table 3. Crude and adjusted association of continuous urinary phthalate metabolites with age at menarche

Phthalates	Crude		Adjusted[a]	
	β (95% CI)	*p*-value	β (95% CI)	*p*-value
MEHHP	0.054 (−0.130, 0.239)	0.562	0.065 (−0.118, 0.247)	0.485
MEOHP	0.037 (−0.107, 0.182)	0.611	0.049 (−0.093, 0.191)	0.500
MECPP	−0.116 (−0.392, 0.160)	0.407	−0.113 (−0.384, 0.157)	0.409
DEHP metabolites[b]	−0.046 (−0.311, 0.219)	0.732	−0.034 (−0.293, 0.226)	0.799
MnBP	−0.092 (−0.255, 0.072)	0.270	−0.066 (−0.228, 0.095)	0.418
MBzP	−0.029 (−0.130, 0.071)	0.568	−0.009 (−0.110, 0.091)	0.853
MCOP	−0.111 (−0.283, 0.062)	0.207	−0.102 (−0.272, 0.067)	0.237
MCNP	−0.156 (−0.384, 0.072)	0.179	−0.164 (−0.387, 0.058)	0.147
MCPP	−0.165 (−0.453, 0.123)	0.260	−0.107 (−0.392, 0.177)	0.459
Total phthalates[c]	−0.089 (−0.348, 0.170)	0.501	−0.064 (−0.309, 0.181)	0.608

Analyzed by multiple linear regression model.
Natural log-transformed, specific gravity corrected concentrations of urinary phthalate metabolites were used.
CI: confidence interval; MEHHP: mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP: mono-(2-ethyl-5-oxohexyl) phthalate; MECPP: mono-(2-ethyl-5-carboxypentyl) phthalate; DEHP: di-2-ethylhexyl phthalate; MnBP: mono-n-butyl phthalate; MBzP: mono-benzyl phthalate; MCOP: mono-carboxyoctyl phthalate; MCNP: mono-(carboxy-isononyl) phthalate; MCPP: mono-(3-carboxypropyl) phthalate.
[a]Model was adjusted for grade, maternal early menarche, and body mass index group; [b]Molar sum of MEHHP, MEOHP, and MECPP; [c]Molar sum of MEHHP, MEOHP, MECPP, MnBP, MBzP, MCOP, MCNP, and MCPP.

Table 4. Crude and adjusted OR for early menarche based on levels of urinary phthalate metabolites

Phthalates	Moderate		High	
	Crude OR (95% CI)	Adjusted OR (95% CI)[a]	Crude OR (95% CI)	Adjusted OR (95% CI)[a]
MEHHP	0.78 (0.40, 1.52)	0.78 (0.39, 1.54)	1.04 (0.54, 2.00)	0.99 (0.51, 1.93)
MEOHP	0.92 (0.47, 1.83)	0.90 (0.45, 1.81)	1.45 (0.75, 2.81)	1.44 (0.74, 2.83)
MECPP	1.48 (0.75, 2.89)	1.52 (0.77, 3.02)	1.32 (0.67, 2.60)	1.26 (0.63, 2.53)
DEHP metabolites[b]	1.32 (0.67, 2.60)	1.35 (0.68, 2.69)	1.48 (0.75, 2.89)	1.44 (0.72, 2.85)
MnBP	1.83 (0.91, 3.69)	1.77 (0.87, 3.59)	2.27 (1.14, 4.54)	2.09 (1.03, 4.23)
MBzP	1.49 (0.76, 2.94)	1.41 (0.71, 2.82)	1.57 (0.80, 3.10)	1.37 (0.68, 2.76)
MCOP	0.69 (0.36, 1.35)	0.69 (0.35, 1.36)	0.83 (0.43, 1.60)	0.80 (0.41, 1.56)
MCNP	1.04 (0.53, 2.03)	1.20 (0.60, 2.40)	1.10 (0.57, 2.14)	1.19 (0.60, 2.36)
MCPP	1.24 (0.63, 2.43)	1.22 (0.62, 2.44)	1.31 (0.67, 2.58)	1.26 (0.63, 2.49)
Total phthalates[c]	1.83 (0.91, 3.69)	1.90 (0.93, 3.88)	2.27 (1.14, 4.54)	2.22 (1.10, 4.49)

Analyzed by multiple logistic regression model.
Groups were determined by tertile of each metabolite (low, moderate, and high); group with the lowest tertile set as reference group.
OR: odds ratio; CI: confidence interval; MEHHP: mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP: mono-(2-ethyl-5-oxohexyl) phthalate; MECPP: mono-(2-ethyl-5-carboxypentyl) phthalate; DEHP: di-2-ethylhexyl phthalate; MnBP: mono-n-butyl phthalate; MBzP: mono-benzyl phthalate; MCOP: mono-carboxyoctyl phthalate; MCNP: mono-(carboxy-isononyl) phthalate; MCPP: mono-(3-carboxypropyl) phthalate.
[a]Model was adjusted for grade, maternal early menarche, and body mass index group; [b]Molar sum of MEHHP, MEOHP, and MECPP; [c]Molar sum of MEHHP, MEOHP, MECPP, MnBP, MBzP, MCOP, MCNP, and MCPP.

of inconsistency might be inherent because multiple factors including genes, race, adiposity, nutrition, and environmental exposure act together in regulating hormones and orchestrating sexual development [13,27,36,37]. Furthermore, another study showed that phthalate exposure at Tanner stage B1 was associated with delayed menarche, whereas exposure at stage B4 was associated with earlier menarche [25]. This indicates that the impact phthalates have on onset of menarche might not be linearly dose-dependent, and that they might be capable of changing pubertal onset in either directions depending on the time of exposure.

In our study, only MnBP exposure displayed association with early menarche as an individual phthalate metabolite. Specifically, group with high MnBP level showed significantly elevated OR of early menarche compared to low level group. In previous studies, MnBP exposure has presented no or only suggestive association with menarche and other pubertal indices [20]. In a cohort study conducted in China, a one-unit increase in natural logarithmic concentration of MnBP was related with 80% increase in the odds of menarche, though the

result was not significant [19]. Another study reported that high pre-pubertal concentration of MnBP was related with both breast and pubic hair development [38].

A research conducted in US couldn't find association between summed concentrations of 11 phthalate metabolites and age at menarche [39], which is comparable with our data where no linear association was detected. However, our data also suggest that group with high total phthalates level showed significantly elevated OR of early menarche compared to low level group, and moderate concentration group, although not statistically significant, showed similar effect size in dose-dependent trend. This indicates when compared to low concentration group, higher concentration of total phthalates might increase risks of early menarche. The mechanism that phthalates and other EDCs intervene in human hormonal system is complex. They engage in hypothalamic-pituitary-gonadal system by mimicking sex and steroid hormones, preventing their synthesis, and thus interfering with reproductive development, insulin resistance, and adipose tissue production [16,40,41]. Phthalates are reported to show mainly anti-androgenic features in experimental and animal studies [42,43], which can in turn exhibit as delay of pubarche in females. In addition, animal studies using rodents have shown that exposure to relatively low dose of DEHP, which is comparable to environmental dose in humans, can accelerate sexual maturation, thus making the onset of puberty earlier [44,45]. Although detailed effects of each phthalate are not yet fully understood, our findings suggest that overall pre-pubertal exposure to phthalates might play a role in advancing menarche.

Since most study subjects had already reached menarche at the time of survey, urinary phthalate metabolites were measured after the onset of menarche. This temporal distance, along with short half-life of phthalates, complicates the representability for the exposure that occurred beforehand. There were three studies that investigated reliability of phthalate level in a single urine sample on average of long-term exposure, with period of one, three, and six months respectively [33,46,47]. They employed the same method where subjects were allocated into three groups by concentration tertiles (low, moderate, and high) of each sampling result. Then, average concentration of all samples in the follow-up period was compared between the groups. For each sampling, it was checked if there was monotonic increase of the average concentration of all samples from lower to higher groups, which would indicate its predictability for long-term exposure. Results from these studies presented consistent predictability for MnBP, MBzP, MEP, and MMP. DEHP metabolites did not demonstrate monotonic increase in the study with one-month follow-up, although the average level for the highest tertile group was greater than two times of that for the lowest tertile group in every sampling [46]. These researches suggest that subjects with higher level of phthalates from a single sample tend to stay at higher level in the long-term as long as six months. While actual concentrations might have variability, levels from single urine samples have certain predictive value for long-term exposure of phthalates.

Our study can generalize the results by adopting representative samples and directly measuring exposure metabolites. As a result, early menarche was higher at higher concentrations than at lower concentrations in total phthalates and some phthalate metabolites, which supported previous studies abroad. This implies that phthalate exposure in daily life is related to early menarche in adolescent women.

There are several limitations in our research. First, cross-sectional design of the study limits causal association of the results. Second, all questionnaires were conducted by parents,

which makes the study susceptible to recall bias, although restriction of study subjects to middle school girls would make it less troubling for parents to remember the age of menarche. Lastly, as previously discussed, urinary metabolite concentrations might not represent exposure that occurred before the onset of menarche. Even though we confined our study population to middle school girls, temporal distance persists in hindering the inference of direct association. However, given concentrations from single urine samples have predictive value for long-term exposure, our study can still suggest possibility of relation between higher level of phthalate exposure and early menarche.

CONCLUSIONS

In summary, our study was the first one to analyze samples from nationwide survey of South Korea and investigate association between phthalate exposure of adolescents and age of menarche. There were heterogeneous results, similar to previous studies. No linear association was notable between phthalate exposure and menarche age. On the other hand, our results present that high concentrations of MnBP and total phthalates may be associated with early menarche. This incongruity might derive from inherent complex mechanism of phthalates in endocrine system, and also from cross-sectional design of our study. Further investigations with larger scale samples are required, and longitudinal studies are also warranted to assess the effect of phthalate exposure in each critical point of development.

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