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Prenatal exposure to di-(2-ethylhexyl) phthalate and decreased skeletal muscle mass in 6-year-old children: A prospective birth cohort study

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ABSTRACT

Background/Aim: Phthalate is a well-known endocrine-disrupting chemical that has anti-androgenic effects. Although there are several studies on the relationship between body composition and phthalate, studies that investigated the effects of phthalate on skeletal muscle during childhood are lacking.

Methods: We analyzed data from 481 mother-and-child pairs enrolled in the Environment and Development of Children cohort in South Korea. We examined the association between phthalate metabolites (mono [2-ethyl-5-hydroxyhexyl] phthalate [MEHHP], mono [2-ethyl-5-oxohexyl] phthalate [MEOHP], molar sum of MEHHP and MEOHP [Σ DEHP], and mono-n-butyl phthalate [MnBP]) in prenatal maternal urine and children's urine at the age of 6, and body composition indices (body mass index [BMI] z-score, percentage of fat mass, fat mass index, percentage of skeletal muscle, and the skeletal muscle index [SMI]) measured when the child was 6 years using a bioelectrical impedance analyzer.

Results: A 2-fold increase in Σ DEHP and MnBP in the prenatal maternal urine was significantly associated with a −0.07 unit (95% CI: −0.11, −0.03) and −0.09 unit (95% CI: −0.14, −0.03) change in SMI, respectively, in 6-year old girls alone. BMI z-score was also negatively associated with a 2-fold increase in MEHHP and MnBP in prenatal maternal urine as −0.11 unit (95% CI: −0.22, −0.01) and −0.15 unit (95% CI: −0.28, −0.02) change, respectively, only among girls. Among boys, phthalate metabolites in the prenatal and children's urine were not significantly associated with any body composition indices.

Conclusions: Our longitudinal study shows that high levels of prenatal exposure to phthalates are significantly associated with decreased SMI among girls. We can postulate that anti-androgenic effects of phthalates during pregnancy may affect girl offspring's muscle growth.

1. Introduction

Recently, secular declines in muscle mass and strengths of children have been reported in developed countries such as the U.S. (Sun et al., 2012), England (Cohen et al., 2011), Canada (Tremblay et al., 2010), and Spain (Moliner-Urdiales et al., 2010). These trends may be attributable to the decline in physical activities which affect muscular developments. However, the evidences for decline in physical activity of children were not only insufficient but inconsistent (Booth et al., 2015). Therefore, the secular decline in muscle mass and strengths of children need to be explained better.

Phthalate, a class of chemicals synthesized by esterification of

List of abbreviations: DBP, dibutyl phthalate; DEHP, di-(2-ethylhexyl) phthalate; EDC cohort, the Environment and Development of Children cohort; MEHHP, mono-(2-ethyl-5-hydroxy-hexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxo-hexyl) phthalate; MnBP, mono-n-butyl phthalate; MEHP, mono-2-ethylhexyl phthalate; LOD, limits of detection; BIA, bioelectrical impedance analysis; SMI, skeletal muscle index; FMI, fat mass index; GM, geometric mean; SD, standard deviation; IGF-1, insulin-like growth factor-1; PPAR, peroxisome-proliferator activated receptor

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phthalic acid, is widely used as plasticizers to promote flexibility and softness of plastics and in various personal care products. Phthalates can be classified into 2 groups, high-molecular weight phthalates (HMWPs) and low-molecular weight phthalates (LMWPs). HMWPs are used in the manufacturing of flexible plastics for a variety of products including building materials, medical devices, and paints, while LMWPs are usually used in the manufacturing process of cellulose acetate, used in the production of shampoos, cosmetics, lotions, and other personal hygiene products. As it was widely used, phthalate metabolites were detected in the urine of 75% of Americans in 1999–2000, and were detected in the urine of more than 95–100% of South Korean mothers and children in 2011–2012 (Ha et al., 2014; Silva et al., 2004; Song et al., 2013). As potential health risk of phthalates for children had been issued in South Korea, products for children are regulated to contain di-(2-ethylhexyl) phthalate (DEHP) and dibutyl phthalate (DBP) at a concentration of < 0.1% of the total content in products since 2012. Although some phthalates exposure have declined due to several legislations and activities (Zota et al., 2014), exposure to phthalates among children and females is still substantial. The risk assessment performed for phthalates in childcare facilities reported that 82–89% of children exceed tolerable levels of DBP exposure and 8–11% of children exceed tolerable levels of DEHP exposure (Gaspar et al., 2014).

Phthalate is a well-known endocrine-disrupting chemical which has anti-androgenic effects. Adverse health effects of phthalates have been studied such as abnormal sexual development, adverse birth outcomes, neurodevelopment, and hormonal disturbances (Borch et al., 2006; Kim et al., 2009; Lee et al., 2014, 2018; Moore et al., 2001; Swan, 2008). Recently, a rodent study reported that the prenatal exposure to DEHP could induce decreased muscle mass among females (Neier et al., 2019). A recent epidemiologic study was reported that decreased muscle mass was associated with increased urinary concentration of phthalates metabolites, but the study was limited by its cross-sectional study design (Corbasson et al., 2016). Other studies that investigated the relationship between human body composition and phthalates reported inconsistent results (Amin et al., 2018; Buckley et al., 2016b; Zhang et al., 2014). Therefore, further investigation is required to study the effects of phthalates on skeletal muscle growth in childhood and its association with anti-androgenic effects.

We hypothesized that environmental phthalates exposure during developmental period could result in disturbing muscular developments of children. The aim of our study was to clarify if prenatal- and postnatal-exposure to DEHP and DBP, the most widely used HMWP and LMWP that have well-known anti-androgenic effects, is associated with muscular mass and body composition indices in children (Lorz et al., 2000).

2. Materials & methods

2.1. Study participants

This prospective birth cohort study was based on the Environment and Development of Children cohort (EDC cohort), an on-going cohort study to observe the effects of exposure to environment disrupting chemicals on the development of children. The EDC cohort was constructed in 2012 by contacting a previously closed study, “The Congenital Anomaly Study”. In this study, we recruited 13,484 pregnant women between 2008 and 2011 in Seoul and Gyung-gi area of South Korea, gathered the information of parents, and collected maternal blood and urine during the second trimester of pregnancy, and followed-up until their delivery. Among these participants, we contacted 2085 mothers with offsprings without congenital anomalies to construct the EDC cohort and to follow-up longitudinally. In total, 726 mother-child pairs were enrolled. We have conducted follow-ups for all children and parents every 2 years. At the follow-up examination, parents completed a survey on demographic factors, medical history, recent illness of their children, and environmental factors. Urine and blood samples of the children were collected. More details are described in the published cohort profile paper (Kim et al., 2018). We obtained informed consent from all parents and children, and the study protocol was approved by the Institutional Review Board at the College of Medicine, Seoul National University (IRB No. 1201-010-392).

2.2. Phthalate measurement

Maternal phthalate metabolites were measured in maternal urine collected at the second trimester of pregnancy (a mean of 20.3 weeks of gestation with standard deviations (SDs) of 4.5, ranging 6.9–29.6 weeks). Maternal spot urine was collected from each participant between 9 a.m. and 11 a.m. and stored at −20 °C. Childhood phthalate metabolites were measured in the first morning urine of the offspring at 6 years of age. The first morning urine of children were collected in an urine collection cup (pre-screened for phthalate metabolites) by their mothers. We measured mono-(2-ethyl-5-hydroxy-hexyl) phthalate (MEHHP), and mono-(2-ethyl-5-oxo-hexyl) phthalate (MEOHP) for DEHP, and mono-n-butyl phthalate (MnBP) for DBP metabolites. MEHHP and MEOHP are secondary metabolites of DEHP which reflect the molar fraction of excretion to absorbed DEHP in the human body better than mono-2-ethylhexyl phthalate (MEHP), a primary metabolite of DEHP. We used MEHHP, MEOHP and molar sum of MEHHP and MEOHP as metabolites of DEHP. Molar sum of MEHHP and MEOHP was calculated by diving the concentrations of MEHHP and MEOHP by their molecular weights (294.34 g/mol and 292.33 g/mol, respectively) (Hauser et al., 2016).

All laboratory analyses were measured at Green Cross Laboratories, certified by the Korean Society for Laboratory Medicine. First, for measuring urinary concentrations of MEHHP, MEOHP and MnBP, thawed and vortexed urine samples were treated with 2M sodium acetate buffer solution (1.0 mL) and β-glucuronidase (20 μL) and were hydrolyzed at 37 °C for 16 h. After hydrolysis, 50 μL of internal standards and 4 mL of ethyl acetate were added. After 1 h of shaking and 5 min of centrifugation, the solution layers were removed, and the extract was dried with a nitrogen evaporator. The dried extract was treated with 300 μL of 60% acetonitrile and analyzed using high-performance liquid chromatography-tandem mass spectrometry (TQ4500; AB Sciex, USA). The limits of detection (LOD) for MEHHP, MEOHP and MnBP were 0.208, 0.487, and 0.724 μg/mL, respectively. The values below the limits of detection were substituted as $LOD/\sqrt{2}$. We used creatinine-adjusted phthalate metabolites (μg/g Cr) and used urinary creatinine level as covariates in statistical models for controlling measurement error bias caused by difference in urine dilutions (O'Brien et al., 2015). Creatinine was measured was performed using a kinetic colorimetric assay with a Hitachi 7600 machine (Hitachi®, Tokyo, Japan) and CREA reagent (Roche®, Indianapolis, IN, USA).

2.3. Body composition

Weight (kilogram) and height (centimeter) of the 6-year old children were measured. Body composition was assessed using an InBody® 770 body composition analyzer (Inbody®, Seoul, Korea), which uses the 4-electrode method. Bioelectrical impedance analysis (BIA) is a method to assess total fat mass and skeletal muscle mass in the body (Medici et al., 2005). InBody® 770 body composition analyzer, which measures body composition using BIA, has reasonable accuracy compared to dual X-ray absorptiometry measurements, a gold-standard method for measuring body composition (Jaffrin, 2009). Children were required to be fasting and were given the same instructions when measuring body composition. BMI z-score was calculated based on the reference data of Korean adolescents developed by the Korean pediatric society (Moon et al., 2008). Skeletal muscle index (SMI) was suggested by Baumgartner to quantitate muscle objectively relative to the height, and was calculated as skeletal muscle mass divided by height squared (kg/m^2) (Baumgartner et al., 1998). Percentages of fat mass and skeletal muscle

mass were calculated as body fat mass (kg) and skeletal muscle mass (kg) of children divided by the total body weight (kg). Fat mass index (FMI) was calculated as body fat mass divided by height squared (kg/m^2).

2.4. Covariates

The hospital delivery records of participating mothers were acquired, including maternal age at birth, maternal pre-pregnancy BMI, birth weight of their offsprings, gestational age at delivery. Data on demographic, socioeconomic status, and health-related issues were collected from participating mothers through self-assessed questionnaires and were reviewed by trained interviewers. Collected questionnaires included household income per month (< 4,000,000 KRW [≒ 3333 US$], 4,000,000 KRW – 6,000,000 KRW [≒ 5000 US$] and ≥6,000,000 KRW) and maternal education level (≤high school graduate, college graduate, and above college). Frequency of strength exercise of children was obtained by following question: "What is the frequency of muscle strengthening exercise performed by your children per week?". Energy intake per day was assessed using the Computer Aided Nutritional Analysis Program 4.0 for Professionals (Korean Society of Nutrition, Seoul, Republic of Korea) with food frequency questionnaires collected from the children's mothers.

2.5. Statistical analysis

Demographic characteristics of the study participants were presented, and differences in SMI according to the characteristics were tested using Student's t-test or analysis of variance. Next, we presented mean values of body composition indices of the study participants, including height, weight, BMI z-score, fat mass %, FMI, skeletal muscle %, and SMI. Geometric means (GMs) and SDs of metabolites of phthalates were also presented. Correlation analysis was performed for constructing correlation matrix for phthalate metabolites with Spearman correlation coefficients (SCC). Next, we performed simple linear regressions between phthalate metabolites as independent variables and fat-related body composition parameters (BMI z-score, percentage of fat mass, and FMI) and muscle-related body composition parameters (percentage of skeletal muscle mass, and SMI) as dependent variables. We used $\log_2$-transformed values of phthalate metabolites for analyses (maternal MEHHP, maternal MEOHP, molar sum of maternal MEHHP and maternal MEOHP, maternal MnBP, children MEHHP, children MEOHP, molar sum of children MEHHP and children MEOHP and children MnBP) as they were not normally distributed. The assumption of normality of the residuals was violated among some linear regression models for $\log_2$-transformed phthalates metabolites and body composition indices. However, we considered $\log_2$-transformed phthalate as a continuous independent variable in the linear regression model as we believe that the number of observations were sufficient (n ≥ 100) to overcome moderate non-normality (Lumley et al., 2002). The estimates of linear regression model were interpreted as differences in the body composition by a two-fold increase in phthalate metabolites. In the multivariate linear regression model between phthalate metabolites and body composition, potential confounders were selected a priori using directed acyclic graphs (Williamson et al., 2014). For the association between prenatal maternal phthalate exposure and body composition of children at 6 years, maternal age as continuous variable, maternal education (≤high school graduate, college graduate, and above college), and household income (< 4,000,000 KRW [≒ 3333 US $], 4,000,000 KRW – 6,000,000 KRW [≒ 5000 US$] and ≥6,000,000 KRW) were used as covariates in the model. For the association between phthalate exposure in children and their body composition, maternal education, household income, energy intake per day, and sex were used as covariates in the model (Supplementary Figs. S1–2).

All analyses were performed both among the total participants and after stratification according to sex. Statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC, USA). Two-tailed p-values < 0.05 were considered statistically significant.

3. Results

Among the 726 mother-child pairs, mothers whose urine were not analyzed for phthalate metabolites (n = 88), and children who were not examined at 6 years of age (n = 64), participants with missing information with phthalate metabolites (n = 74), extreme values (above and below mean ± 3SD) of phthalate metabolites (n = 13), and missing values for body composition and covariates (n = 6) were excluded. Finally, 481 mother-child pairs (66.3%) were included as subjects in our study. The mean age of the study participants was 5.9 years (± 0.1). There were no significant differences in demographic variables and body composition indices between the mother-child pairs not included in the analysis (n = 245) and mother-child pairs included in the analysis, except for differences in maternal pre-pregnancy BMI and birthweight Supplementary Tables S1–2). Phthalate metabolites in the prenatal maternal urine and children's urine measured at 6 years of age were not significantly different in the included and excluded group, except for MnBP in the prenatal maternal and children's urine (Supplementary Table S3). Most of excluded participants due to extreme values of phthalate metabolites (over mean ± 3SD) were due to outlying MnBP values in prenatal maternal urine (n = 13) and children's urine (n = 6).

Table 1 shows the demographic and anthropometric characteristics of the study participants. There were 255 (53.0%) and 226 (47.0%) boys and girls, respectively. Most mothers were college-educated (72.4%), had normal BMI before pregnancy (65.7%), and gave birth to their offspring at the age of 31–35 years (48.7%). Most children had normal birth weight (90.6%), were born in full-term (92.9%), and did no strength exercise (69.0%). SMI was significantly higher for the male sex, obesity, and higher energy intake in children.

Body composition indices of children are presented in Table 2. Mean values of body compositions were as follows: mean height of 115.6 cm (SD ± 4.4), mean weight of 21.2 kg (SD ± 3.2), mean BMI z-score of −0.10 (SD ± 1.04), fat mass percentage of 18.3% (SD ± 6.3), FMI of 2.94 kg/m^2 (SD ± 1.37), skeletal muscle percentage of 38.5% (SD ± 2.9), and SMI of 6.00 kg/m^2 (SD ± 0.48). Mean SMI was 6.13 kg/m^2 (SD ± 0.48) in boys and 5.84 kg/m^2 (SD ± 0.44) in girls.

Table 3 shows geometric means (GMs) and percentiles of concentration of phthalate metabolites in prenatal maternal urine and children's urine. Only 4 samples (0.8%) for MEHHP and 2 samples (0.4%) for MnBP were below the LODs among prenatal maternal urine. GMs and SDs of creatinine-adjusted MEHHP, MEOHP, molar sum of MEHHP and MEOHP (Σ DEHP), and MnBP of prenatal maternal urine were 15.36 μg/g Cr (SD ± 2.42), 15.92 μg/g Cr (SD ± 2.06), 0.11 nmol/g Cr (SD ± 2.15), and 39.68 μg/g Cr (SD ± 1.99), respectively. Phthalate metabolites in prenatal maternal urine were moderately to highly correlated (SCC: 0.51–0.99), and metabolites in children urine were also moderately to highly correlated (SCC: 0.38–0.99). There was no significant correlation between prenatal phthalates and phthalates in children (Supplementary Table S4).

Table 4 shows the association between the concentrations of urinary phthalate metabolites (prenatal and postnatal) and body composition indices. SMI and BMI were significantly associated with phthalate metabolites in prenatal maternal urine, but the percentage of fat mass, FMI, and percentage of skeletal muscle mass were not associated. There was no association between phthalate metabolites in children's urine and body composition indices. Two-fold increases in phthalate metabolites in maternal urine were associated with decreased SMI, including MEHHP (−0.04, 95% CI: −0.08, −0.01, p = 0.007), MEOHP (−0.06, 95% CI: −0.10, −0.02, p = 0.007), and Σ DEHP (−0.05, 95% CI: −0.09, −0.005, p = 0.032) in the adjusted model. In the analyses with stratification based on the sex of the children, concentrations of

Table 1
Demographic of the study participants and skeletal muscle index.

Variable	N (%)	SMI (kg/m^2) mean ± SD	*p*-value[a]
Total	481 (100.0)	6.00 ± 0.48	
Sex			
Boy	255 (53.0)	6.14 ± 0.48	< 0.001
Girls	226 (47.0)	5.85 ± 0.44	
Household income (KRW)			
< 4000 K (≒ 3333 US$)	149 (31.0)	6.05 ± 0.48	0.301
4000 K–6,000K (≒ 5000 US $)	186 (38.7)	5.98 ± 0.47	
≥ 6000 K	146 (30.3)	5.98 ± 0.49	
Maternal education level			
≤ High school graduate	77 (16.0)	5.97 ± 0.49	0.387
College graduate	348 (72.4)	6.02 ± 0.47	
Above college	56 (11.6)	5.93 ± 0.51	
Maternal age at birth (years)			
18–25	19 (4.0)	5.93 ± 0.42	0.565
26–30	176 (36.6)	6.02 ± 0.47	
31–35	234 (48.7)	5.98 ± 0.48	
36–45	52 (10.8)	6.06 ± 0.53	
Parity			
Nulliparous	286 (59.5)	6.02 ± 0.47	0.324
Multiparous	195 (40.5)	5.98 ± 0.49	
Delivery type			
Vaginal	304 (63.2)	5.98 ± 0.47	0.310
C-section	177,936.8)	6.03 ± 0.50	
Maternal pre-pregnancy BMI (kg/m^2)			
< 23	316 (65.7)	5.95 ± 0.46	0.002
23–25	93 (19.3)	6.11 ± 0.53	
≥ 25	72 (15.0)	6.10 ± 0.48	
Birth weight (g)			
< 2500	31 (6.4)	5.90 ± 0.41	0.205
2500–3999	436 (90.6)	6.00 ± 0.49	
≥ 4000	14 (2.9)	6.18 ± 0.43	
Gestational age at delivery (weeks)			
≥ 37	447 (92.9)	6.00 ± 0.49	0.397
< 37	34 (7.1)	6.07 ± 0.4	
Energy intake per day of children (kcal)			
1Q (683–1227)	120 (25.0)	5.86 ± 0.47	< 0.001
2Q (1229–1424)	120 (25.0)	5.92 ± 0.44	
3Q (1424–1694)	121 (25.0)	6.11 ± 0.51	
4Q (1694–3295)	120 (25.0)	6.10 ± 0.45	
Frequency of strength exercise			
No	332 (69.0)	5.98 ± 0.49	0.262
1–2 times/week	100 (20.8)	6.01 ± 0.47	
≥ 3 times/week	49 (10.2)	6.10 ± 0.47	

SMI, skeletal muscle index, SD, standard deviation; BMI, body mass index.
[a] T-test or ANOVA was performed to test the differences of SMI by demographic variables.

Table 2
Body composition indices of the study participants.

	Total Mean ± SD	Boys Mean ± SD	Girls Mean ± SD
Height (cm)	115.6 ± 4.4	116.0 ± 4.6	115.1 ± 4.1
Weight (kg)	21.2 ± 3.2	21.3 ± 3.2	21.0 ± 3.1
BMI (kg/m^2)	15.6 ± 1.7	15.6 ± 1.6	15.7 ± 1.8
BMI z-score	−0.10 ± 1.04	−0.18 ± 1.02	−0.01 ± 1.07
Fat mass %[a]	18.3 ± 6.3	17.0 ± 5.9	19.5 ± 6.4
FMI (kg/m^2)	2.94 ± 1.37	2.75 ± 1.31	3.16 ± 1.40
Skeletal muscle %[a]	38.5 ± 2.9	39.4 ± 2.7	37.6 ± 2.9
SMI (kg/m^2)	6.00 ± 0.48	6.13 ± 0.48	5.84 ± 0.44

SD, standard deviation; BMI, body mass index; FMI, fat mass index; SMI, skeletal muscle index.
[a] Percentages of body mass.

phthalate metabolites in maternal urine were significantly associated with SMI among females and were not significantly associated with SMI among males. Among girls, two-fold increases in MEHHP, MEOHP, Σ DEHP, and MnBP in prenatal maternal urine were associated with −0.07 (95% CI: −0.11, −0.03, $p < 0.001$), −0.09 (95% CI: −0.13, −0.04, $p < 0.001$), −0.08 (95% CI: −0.13, −0.04, $p < 0.001$), and −0.09 (95% CI: −0.14, −0.03, $p = 0.002$) unit changes of SMI, respectively. In addition, among females, two-fold increase of MEHHP in maternal urine was associated with −0.11 unit change in BMI z-score (95% CI: −0.22, −0.01, $p = 0.029$), and two-fold increase of MnBP in maternal urine was associated with −0.15 unit change in BMI z-score (95% CI: −0.28, −0.02, $p = 0.025$).

4. Discussion

Increased DEHP metabolites and DBP metabolites in prenatal maternal urine were associated with decreased SMI of their offspring measured at 6 years of age, after adjusting for maternal age, body mass index, household income level, and maternal education level. Furthermore, concentration of MEHHP in prenatal maternal urine was negatively associated with BMI z-score of children. These associations were more prominent among girls than among boys. Phthalate metabolites and body composition at 6 years of age, measured simultaneously, were not associated with body composition indices including SMI and BMI z-score. To the best of our knowledge, this is the first longitudinal study that clarified the association between prenatal phthalate exposure and skeletal muscle mass of children, although few studies cross-sectionally investigated the association between phthalate and skeletal muscle mass.

In our study, the mean concentrations of phthalate metabolites in the prenatal maternal urine were similar with those in previous studies, which reported mean concentrations ranging from 7.1 to 22.5 μg/g Cr, 6.9–18.7 μg/g Cr, 0.10–0.30 nmol/gCr, and 21.1–39.68 μg/g Cr for MEHHP, MEOHP, Σ DEHP, and MnBP, respectively (Buckley et al., 2016a; Harley et al., 2017; Vafeiadi et al., 2018). These metabolites were relatively higher in children's urine than in prenatal maternal urine. The children included in our study had higher concentration of urinary phthalate metabolites than those included in studies in the U.S., which ranged from 14.0 to 25.8 μg/g Cr, 9.8–10.0 μg/g Cr, 0.38 nmol/gCr, 16.3–21.7 μg/g Cr for MEHHP, MEOHP, Σ DEHP, and MnBP, respectively (Meeker and Ferguson, 2014; Shoaff et al., 2017; Trasande et al., 2013). Other studies also reported that phthalate levels in South Korean children were higher than those in U.S. children (Jung Koo and Mu Lee, 2005). The difference could be partially contributed by frequencies of consumption of dairy products and meat using plastic packaging (Kim et al., 2014). However, phthalate metabolites in children's urine in our results were similar with those of studies in Korea, Greece, and Denmark (Boas et al., 2010; Song et al., 2013; Vafeiadi et al., 2018).

In our study, we could not find a positive association between phthalate metabolites in children's urine and obesity. Although previous studies reported inconsistent results for this association, several studies also argued that phthalates exposure in children could be related with obesity. A cross-sectional study with 845 Danish children aged 4–9 years reported that urinary phthalate metabolites are negatively associated with height and weight (Boas et al., 2010). The National Health and Nutrition Examination Survey (NHANES) data shows that LMWP could be associated with increased BMI z-score (Trasande et al., 2013), and a longitudinal study in the U.S. also reported that phthalates exposure at 5 years of age are associated with obesity at 8 years of age (Shoaff et al., 2017). These studies suggested that the role of peroxisome-proliferator activated receptors (PPARs) is important to induce obesity. PPARs are nuclear hormone receptors that have regulator roles in adipogenesis and lipid storage, and could be affected by DEHP to induce adipogenesis (Desvergne et al., 2009; Hao et al., 2013; Janesick and Blumberg, 2011). However, our results showed that obesity is not associated with neither prenatal nor postnatal exposure to phthalate.

We found that the increased prenatal exposure to phthalates was associated with decreased SMI, which means relative lower muscle

Table 3
Distribution of the creatinine-adjusted urinary concentration of phthalate metabolites.

	LOD (μg/mL)	Samples below LOD	GM (± SD)	Percentiles						
				5th	10th	25th	50th	75th	90th	95th
MEHHP (M)	0.208	4 (0.8%)	15.36 ± 2.42	3.9	5.6	9.4	16.2	26.5	41.7	54.3
MEOHP (M)	0.487	0 (0%)	15.92 ± 2.06	4.6	6.6	10.4	16.0	24.7	38.3	49.4
Σ DEHP (M)	–	–	0.11 ± 2.15	0.03	0.04	0.06	0.11	0.17	0.27	0.36
MnBP (M)	0.724	2 (0.4%)	39.68 ± 1.99	12.5	18.3	26.8	39.0	57.3	97.8	134.1
MEHHP (C)	0.208	0 (0%)	57.40 ± 1.76	23.8	29.3	39.8	57.1	80.3	113.6	154.6
MEOHP (C)	0.487	0 (0%)	39.04 ± 1.79	15.2	19.3	26.3	38.6	55.7	78.8	107.2
Σ DEHP (C)	–	–	0.33 ± 1.77	0.13	0.17	0.23	0.33	0.47	0.65	0.89
MnBP (C)	0.724	0 (0%)	70.00 ± 1.65	31.2	38.5	48.4	68.9	95.7	136.7	170.4

GM, geometric mean; SD, standard deviation; (M), Measured from maternal urine at second trimester; (C), Measured from children urine at 6 years old; MEHHP, mono-(2-ethyl-5-hydroxy-hexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxo-hexyl) phthalate; ΣDEHP, molar sum of MEHHP + MEOHP; MnBP, mono-n-butyl phthalate (MnBP).

mass considering the height of children. It implies that phthalates could disturb adequate muscle development rather than change body adiposity. Previous studies on prenatal exposure to phthalate and body compositions have focused on obesity, and the results were inconsistent. The CHAMACOS study in the US reported that prenatal exposure to DEHP and DBP were associated with increased obesity outcomes at the ages of 5–12 years (Harley et al., 2017). Vafeiadi et al. examined 500 mother-child pairs in Greece and reported that prenatal exposure to phthalates were not associated with obesity at the age 4–6 years (Vafeiadi et al., 2018). Another study involving 707 children in the US reported that prenatal exposure to DEHP could be associated with decreased BMI z-scores in girls aged 4–7 years alone (Buckley et al., 2016a). Although a cross-sectional study in the U.S. reported that decreased lean mass is associated with increased urinary concentration of phthalate metabolites (Corbasson et al., 2016), studies that clarified the association of prenatal phthalates exposure with skeletal muscle development in children are lacking. SMI could be independent from body fat percentage or fat mass index because it measures different portions in body composition, but SMI is inevitably associated with BMI as it includes both fat mass and lean mass. However, SMI is relatively weakly associated with BMI (R^2 = 0.112), especially for girls (Van Der Werf et al., 2018). If phthalates are more selectively associated with muscle mass than fat mass, it could explain the inconsistencies reported in previous studies about the association between prenatal exposure to phthalates and childhood BMI.

The mechanism between phthalate exposure and decreased SMI could be plausibly explained by the anti-androgenic effects of phthalate in muscle development, because phthalates are well-known for their endocrine disrupting effects (Borch et al., 2006; Stroheker et al., 2005). Androgens have an important role in muscle development. In the animal study, androgen withdrawal in mice resulted in decreased myofibrillar protein synthesis, and it was reversed by anabolic steroid administration (White et al., 2013). Another study using mice reported that testosterone have positive effects on the mass and ultrastructure of muscles (Sinha et al., 2014). Epidemiologic studies also consistently reported that androgen is positively associated with muscle growth. The study which followed up 50 boys and 50 girls from the age of 8–17 years reported that increased testosterone level was significantly associated with muscle strength (Round et al., 1999). Another study involving hysterectomized women reported that testosterone is associated with muscle mass and strength in women and there is a dose-response relationship between them (Huang et al., 2014). Prenatal phthalate exposures are associated with decreased anogenital distance, which is positively related with androgenic properties (Bornehag et al., 2014; Swan et al., 2005). In an animal study, prenatal DEHP exposure could induce a decrease in the offspring's testosterone production both in the fetal period and postnatal period (Parks et al., 2000). Another study involving human participants also reported that the increased phthalate metabolites were associated with the decreased level of serum testosterone (Meeker and Ferguson, 2014). These anti-androgenic properties of phthalates could be the important link between prenatal exposure to phthalates and decreased SMI.

Insulin-like growth factor-1 (IGF-1) could be another plausible explanation for the negative association between phthalate exposure and decreased muscle mass. IGF-1 pathway acts as a positive regulator of muscle growth processes that takes place after birth (Schiaffino et al., 2013). Some epidemiologic studies have reported that urinary phthalate metabolites are negatively associated with IGF-1. Cross-sectional studies have reported the associations between phthalates and decreased level of IGF-1 among children (Boas et al., 2010; Tsai et al., 2016; Wu et al., 2017). These epidemiologic studies imply that phthalates could induce decreased level of IGF-1, disturbing the normal muscle growth in children.

We found that the association between prenatal phthalate exposure and SMI at 6 years of age is significant among girls. The association was not statistically significant among boys, although the direction of the association was consistent. It was consistent with a recent study using mice which showed that perinatal exposure to DEHP was associated with decreased muscle mass percent only in female offsprings (Neier, 2019). Further researches on the mechanism of this association are needed to fully understand this phenomenon. The differences in hormones and epigenetics between boys and girls have been suggested as an explanation for the sex-specific association of phthalates (McCabe et al., 2017; Teitelbaum et al., 2012). Thyroid-hormone, closely related with IGF-1, homeostasis could be disturbed by phthalate exposure (Boas et al., 2010; Wu et al., 2017), and the fact that thyroid hormones are only associated with girls suggest that phthalate exposure could affect muscle development more selectively in a sex-specific manner (Morgenstern et al., 2017).

Our study has unique strengths. First, prospective cohort data were used to derive the result of our study. Biological markers measured in prenatal maternal urine and body compositions measured at 6 years of their offspring's age provide the temporal relationship to infer causality. Second, we estimated associations between phthalate exposure and body composition indices with the adjustment for energy intake per day to avoid confounding by food consumption in addition to potential confounders including maternal body mass index, household income level, maternal education level, and frequency of strength exercise per week. However, there are several limitations in our study. First, we measured metabolites of phthalate at one spot urine of participants and measured only MEHHP and MEOHP for HMWP and MnBP for LMWP. Metabolites of phthalates only reflect recent exposure because the excretion half-lives of the metabolite is short, 0.5–3.0 days (Koch et al., 2005). Although it has been reported that phthalate metabolites in a spot urine sample could predict 3-month average levels of metabolites with sensitivity of 0.56 and specificity of 0.83 (Hauser et al., 2004), more repetitive methods such as using mean levels of various phthalate metabolites assessed at multiple time points could be more precise and

Table 4
Associations between phthalate exposure and body composition indices.

	Unit increase by 2-fold increase of metabolites (95% CI)		
	Total (n = 481)	Boy (n = 255)	Girl (n = 226)
Body mass index z-score			
MEHHP (M)	−0.07 (−0.14–0.01)	−0.02 (−0.13–0.09)	−0.11 (−0.22 to −0.01)*
MEOHP (M)	−0.07 (−0.16–0.03)	−0.03 (−0.18–0.11)	−0.11 (−0.25–0.02)
Σ DEHP (M)	−0.07 (−0.16–0.02)	−0.03 (−0.17–0.11)	−0.12 (−0.24–0.01)
MnBP (M)	−0.07 (−0.17–0.03)	0.01 (−0.15–0.17)	−0.15 (−0.28 to −0.02)*
MEHHP (C)	0.04 (−0.08–0.15)	0.13 (−0.02–0.28)	−0.07 (−0.25–0.11)
MEOHP (C)	0.01 (−0.11–0.12)	0.09 (−0.07–0.24)	−0.08 (−0.25–0.09)
Σ DEHP (C)	0.03 (−0.09–0.14)	0.11 (−0.04–0.27)	−0.07 (−0.25–0.1)
MnBP (C)	−0.03 (−0.15–0.1)	−0.10 (−0.26–0.06)	0.04 (−0.16–0.24)
Percentage of fat mass (% of total body mass)			
MEHHP (M)	−0.05 (−0.59–0.48)	0.17 (−0.59–0.93)	−0.18 (−0.90–0.53)
MEOHP (M)	−0.04 (−0.68–0.61)	0.09 (−0.88–1.07)	−0.06 (−0.95–0.82)
Σ DEHP (M)	−0.04 (−0.66–0.58)	0.14 (−0.80–1.07)	−0.11 (−0.95–0.73)
MnBP (M)	−0.23 (−0.91–0.45)	0.21 (−0.98–1.40)	−0.57 (−1.40–0.26)
MEHHP (C)	0.15 (−0.57–0.88)	0.72 (−0.28–1.72)	−0.48 (−1.54–0.58)
MEOHP (C)	−0.08 (−0.79–0.62)	0.40 (−0.60–1.40)	−0.62 (−1.64–0.40)
Σ DEHP (C)	0.06 (−0.66–0.78)	0.60 (−0.41–1.61)	−0.54 (−1.58–0.51)
MnBP (C)	0.02 (−0.72–0.75)	−0.32 (−1.22–0.58)	0.37 (−0.79–1.52)
Fat mass index (kg/m^2)			
MEHHP (M)	−0.02 (−0.14–0.09)	0.04 (−0.14–0.22)	−0.08 (−0.23–0.07)
MEOHP (M)	−0.03 (−0.17–0.12)	0.03 (−0.21–0.26)	−0.07 (−0.26–0.12)
Σ DEHP (M)	−0.03 (−0.17–0.12)	0.04 (−0.19–0.26)	−0.07 (−0.25–0.10)
MnBP (M)	−0.06 (−0.22–0.10)	0.05 (−0.23–0.33)	−0.16 (−0.34–0.02)
MEHHP (C)	0.03 (−0.13–0.19)	0.17 (−0.05–0.39)	−0.12 (−0.36–0.11)
MEOHP (C)	−0.01 (−0.17–0.14)	0.11 (−0.11–0.33)	−0.15 (−0.37–0.07)
Σ DEHP (C)	0.01 (−0.15–0.17)	0.15 (−0.08–0.37)	−0.13 (−0.37–0.10)
MnBP (C)	−0.01 (−0.17–0.14)	−0.09 (−0.28–0.10)	0.07 (−0.18–0.31)
Percentage of skeletal muscle mass (% of total body mass)			
MEHHP (M)	−0.06 (−0.31–0.19)	−0.17 (−0.52–0.18)	−0.03 (−0.36–0.31)
MEOHP (M)	−0.10 (−0.40–0.20)	−0.18 (−0.62–0.27)	−0.11 (−0.51–0.29)
Σ DEHP (M)	−0.09 (−0.38–0.2)	−0.18 (−0.61–0.25)	−0.09 (−0.47–0.30)
MnBP (M)	0.01 (−0.31–0.33)	−0.20 (−0.76–0.37)	0.13 (−0.26–0.53)
MEHHP (C)	−0.11 (−0.44–0.22)	−0.35 (−0.81–0.12)	0.16 (−0.31–0.63)
MEOHP (C)	0.02 (−0.30–0.34)	−0.18 (−0.65–0.28)	0.25 (−0.20–0.70)
Σ DEHP (C)	−0.06 (−0.39–0.27)	−0.28 (−0.75–0.18)	0.20 (−0.27–0.66)
MnBP (C)	−0.14 (−0.49–0.2)	−0.01 (−0.46–0.43)	−0.28 (−0.81–0.24)
Skeletal muscle index (kg/m^2)			
MEHHP (M)	−0.04 (−0.08 to −0.01)*	−0.03 (−0.08–0.02)	−0.07 (−0.11 to −0.03)*
MEOHP (M)	−0.06 (−0.10 to −0.02)*	−0.05 (−0.11–0.01)	−0.09 (−0.13 to −0.04)*
Σ DEHP (M)	−0.05 (−0.09 to −0.02)*	−0.04 (−0.1–0.02)	−0.08 (−0.13 to −0.04)*
MnBP (M)	−0.05 (−0.09 to −0.005)*	−0.03 (−0.1–0.04)	−0.09 (−0.14 to −0.03)*
MEHHP (C)	0.0001 (−0.05–0.05)	0.02 (−0.05–0.09)	−0.02 (−0.09–0.05)
MEOHP (C)	0.01 (−0.04–0.06)	0.03 (−0.05–0.10)	−0.01 (−0.08–0.05)
Σ DEHP (C)	0.003 (−0.05–0.053)	0.02 (−0.05–0.10)	−0.02 (−0.09–0.05)
MnBP (C)	−0.04 (−0.10–0.01)	−0.07 (−0.15–0.02)	−0.02 (−0.10–0.05)

*p value < 0.05.
† Adjusted for maternal age as continuous variable, maternal education, and household income for the association between maternal phthalates, body composition indices of their children and urinary creatinine, and adjusted for maternal education, household income, energy intake per day, sex of the children, and urinary creatinine for the association between children phthalates and their body composition indices.
CI, Confidence interval; (M), Measured from maternal urine at second trimester; (C), Measured from children urine at 6 years old; MEHHP, mono-(2-ethyl-5-hydroxy-hexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxo-hexyl) phthalate; Σ DEHP, molar sum of MEHHP + MEOHP; MnBP, mono-n-butyl phthalate (MnBP).

accurate for predicting exposure to phthalates (Factor-Litvak et al., 2014). Second, relatively small sample size is a limitation for the interpretation of results. For example, the associations between SMI and prenatal urinary concentrations of phthalates metabolites among boys showed smaller sizes of association than among girls, as Σ DEHP (β = −0.04 [95% CI: −0.10, 0.02] vs. −0.08 [95% CI: −0.13, −0.04], respectively) and MnBP (β = −0.03 [95% CI: −0.10, 0.04] vs. −0.09 [95% CI: −0.14, −0.03], respectively). Because these associations were marginally significant in case of exposure to Σ DEHP and MnBP among boys, there is a possibility that inclusion of more study participants can reveal a significant association even among boys. Third, there was differences in maternal pre-pregnancy BMI, birthweight and MnBP in the prenatal urine between included participants and excluded participants. However, excluding data because of missing information and/or extreme values of phthalate metabolites may have not made the association biased significantly. Fourth, we performed multivariate linear regression analyses for various exposures to phthalates in maternal and children's samples (MEHHP, MEOHP, Σ DEHP, and MnBP), and various outcomes of interest (BMI z-score, percentage fat mass, FMI, percentage skeletal muscle, and SMI). Therefore, false positives could be derived from multiple comparisons since we performed 40 hypotheses simultaneously. However, multivariate linear regression analysis reported the p-value for the association between SMI at 6 years of age and phthalates in prenatal maternal urine as below 0.001, which suggests the reliability of our results despite the issue with multiple comparison. Finally, our results were derived from the EDC cohort dataset, which included Korean participants alone. Therefore, the findings from our study cannot be generalized to populations of other countries.

5. Conclusion

The results of our study support the hypothesis that environmental exposure to phthalates is associated with muscle development among children, especially among girls. To promote physical health of children, more efforts to reduce phthalate in environment are necessary in addition to promoting physical activity.

Declarations

Ethics approval and consent to participate

Approvals was obtained from the Institutional Review Board at the College of Medicine, Seoul National University (IRB No. 1201-010-392). We obtained informed consent from all parents and children according to the study protocol.

Consent for publication

Not applicable.

Availability of data and material

The datasets of The Environment and Development of Children cohort analyzed during the current study are only available with the approval of the board of the Environment and Development of Children cohort (EDC cohort) research team, including the corresponding author.

Authors' contributions

Conceived and designed the study: Dong-Wook Lee, Yun-Chul Hong.

Constructing cohort and collecting data: Youn-Hee Lim, Choong-Ho Shin, Young-Ah Lee, Bung-Nyun Kim, Johanna Inhyang Kim, Yun-Chul Hong.

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Wrote the paper: Dong-Wook Lee, Yun-Chul Hong.

Critically revised the paper: Youn-Hee Lim, Choong-Ho Shin, Young-Ah Lee, Bung-Nyun Kim, Johanna Inhyang Kim, Yun-Chul Hong.

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Declaration of competing interest

All authors declare that: (i) no support, financial or otherwise, has been received from any organization that may have an interest in the submitted work; and (ii) there are no other relationship or activities that could appear to have influenced the submitted work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2019.109020.

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