Phthalate exposure and lipid disruption in Taiwanese children: Insights from serum lipidomic approach

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Abstract

Phthalates are ubiquitous environmental contaminants that raise particular concern for children, who generally experience higher exposure levels and are more susceptible to environmental pollutants. To understand potential molecular mechanisms, we conducted a cross-sectional study of 256 Taiwanese children aged 8−10 years from two population-based cohorts: the Taiwan Birth Panel Study and the Taiwan Early-Life Cohort. Serum phosphocholine-containing lipids were profiled by liquid chromatography coupled with triple quadrupole mass spectrometry and associated with urinary levels of 12 phthalate metabolites. Multiple linear regression models, adjusted for demographic and socioeconomic factors, revealed that mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), an oxidative metabolite of di(2-ethylhexyl) phthalate (DEHP), exhibited the strongest associations with lipid alterations. Specifically, higher urinary MEHHP was positively associated with several diacyl-phosphatidylcholines and lyso-phosphatidylcholines, while inversely related to sphingomyelins and ether-linked phosphatidylcholines. These lipid signatures suggest disturbances in lipoprotein metabolism, peroxisome proliferator-activated receptor-related signaling, and sphingolipid balance, offering mechanistic clues to previously observed links between phthalate exposure and hepatic, cardiovascular, and neurological outcomes. Overall, this study identifies lipid perturbations associated with metabolites of DEHP exposure in children and provides molecular insights into how phthalates may disrupt lipid metabolic homeostasis during early life.

Keywords: Environmental toxicology, Lipidomics, Phosphatidylcholine, Phthalate, Sphingomyelin

1. Introduction

Phthalate esters (PAEs) are a class of chemicals widely used in plastic products to enhance flexibility and elasticity [1]. Humans are exposed to PAEs through multiple routes, including ingestion, inhalation, and dermal contact during manufacturing processes and the use of plastic items, as well as through non-plastic sources such as indoor dust, personal care products, and cosmetics [2,3]. Furthermore, PAEs are considered among the most hazardous additives in plastics [2]. Their widespread use and potential adverse health effects have raised significant public health concerns.

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Previous studies have reported associations between PAE exposure and various health conditions, including endocrine disruption, reproductive toxicity, childhood asthma, neurodevelopmental disorders, and cardiovascular disease [1–3]. Epidemiological research has shown that PAE exposure may lead to decreased levels of testosterone, reduced semen quality in men, and shortened anogenital distance [2]. Longitudinal studies on the Taiwanese population have further indicated associations between PAE metabolite exposure and lower cognitive function in children [3]. Furthermore, studies have shown that patients with coronary heart disease have higher levels of PAE metabolites in urine. Increased carotid intima-media thickness has also been observed in Taiwanese adolescents with higher PAE exposure [1].

More recently, PAE exposure has been linked to dyslipidemia, suggesting that disruption of lipid metabolism may be a contributing factor in disease development [4]. Lipids are essential small molecules involved in regulating cell membrane properties, signal transduction, and energy homeostasis [5]. Understanding lipid composition is therefore crucial for elucidating disease mechanisms and toxicological pathways. However, current evidence on the relationship between PAE exposure and the human serum lipid profile remains limited, especially in children. To the best of our knowledge, only one prior publication has examined associations between the serum lipidome and PAE levels in children [6].

In recent years, lipidomics, a subfield of metabolomics, has been increasingly used to investigate the biological impacts of environmental stressors. In environmental toxicology, lipidomics can help identify key molecular events induced by chemical exposures and explore potential mechanisms of toxicity [7]. This approach also facilitates the linkage between exposure and disease, offering potential biomarkers and molecular-level evidence to support findings from environmental epidemiological studies [8]. Among the lipidomic strategies developed, phosphorylcholine-containing lipid (PC-CL; Fig. 1) analysis focuses on the most abundant lipid classes in cell membranes, phosphatidylcholine (PC) and sphingomyelin (SM), to assess chemical-induced perturbations in high-abundance lipids [9]. In addition to their abundance, PC and SM are important functional lipids that play key roles in biological processes such as regulating lipoprotein metabolism, inflammation, oxidative stress, and cell death. Emerging evidence has also linked these lipids to a range of health outcomes, including metabolic disorders, cardiovascular diseases, and neurological conditions [10–14]. PC-CL analysis has been recognized as a useful tool in environmental contaminant research and has been applied to studies on pollutants such as per- and polyfluoroalkyl substances and fine particle exposure [15,16].

Given the limited evidence on the relationship between PAE exposure and serum lipid profiles in children, this study aimed to address this knowledge gap to understand the potential biochemical effects of PAEs. We applied a lipidomic approach focusing on PC-CL analysis to characterize serum lipid alterations associated with the levels of eight common PAE metabolites in urine. This approach could provide molecular-level evidence that may help elucidate potential pathways linking environmental chemical exposure to adverse health outcomes in children.

2. Materials and methods

2.1. Study design and population

An overview of the study framework is presented in Fig. 2. This study employed a cross-sectional design within the Taiwan Birth Panel Study (TBPS) and the Taiwan Early-Life Cohort (TEC). Between June 2013 and January 2014, a total of 290 children aged 8–10 years were recruited, including 214 from TBPS (Taipei City) and 76 from TEC (Yunlin County) [17]. The study protocol was approved by the Institutional Review Board of National Taiwan University Hospital (Project Identification Code: 201112137RIC), and written informed consent was obtained from all participating children and their parents. Each participant completed a structured questionnaire to collect demographic and lifestyle information. Non-fasting urine and serum samples were collected in the morning and immediately kept in an insulated ice bucket. After accumulating a sufficient number of samples, the samples were aliquoted and stored at −20 °C for subsequent phthalate metabolite measurement and metabolomic analysis.

2.2. Quantification of urinary phthalate metabolites

Urinary concentrations of 12 phthalate metabolites including mono-methyl phthalate (MMP), mono-ethyl phthalate (MEP), mono-(3-carboxypropyl) phthalate (MCPP), mono-isobutyl phthalate (MiBP), mono-n-butyl phthalate (MnBP), mono-cyclohexyl phthalate (MCHP), mono-benzyl phthalate (MBzP), mono-octyl phthalate (MOP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP),

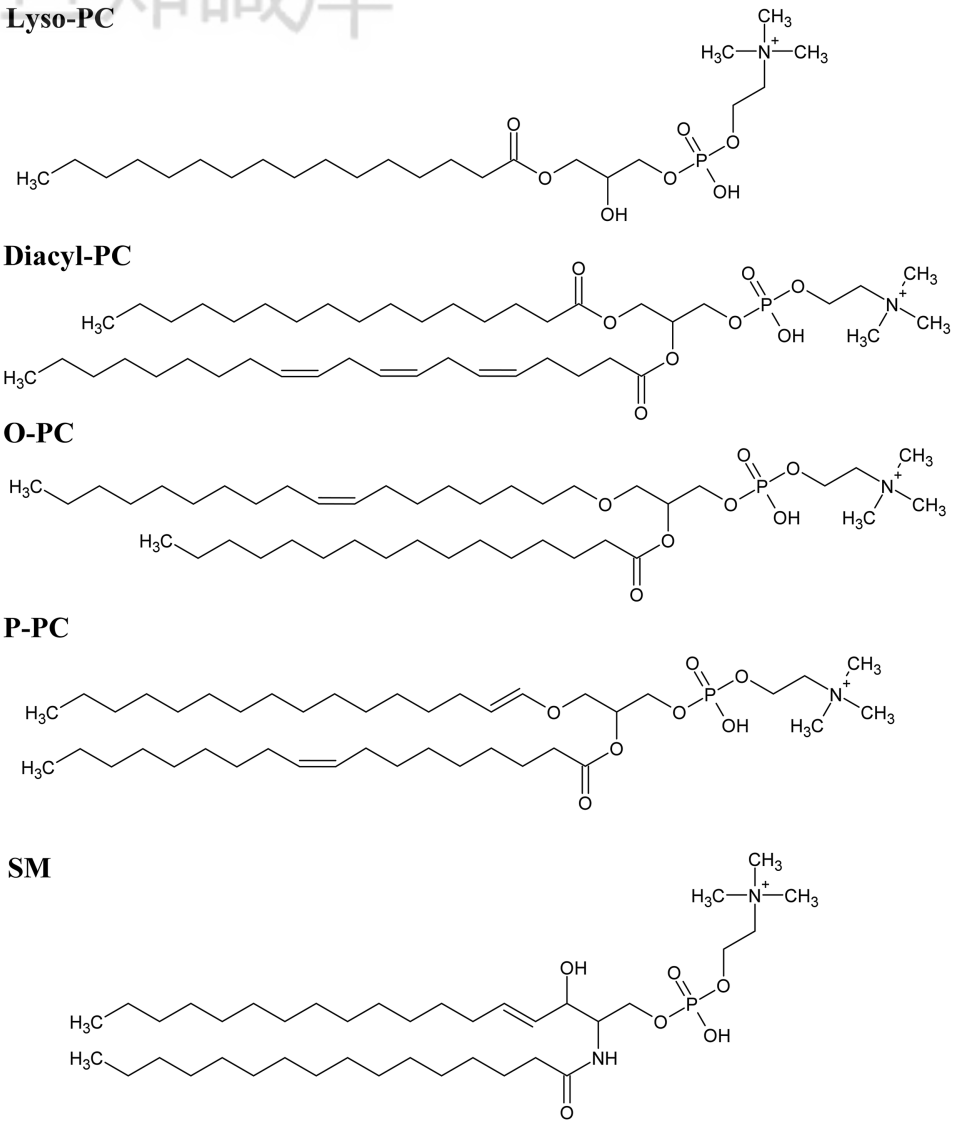

Fig. 1. Representative structures of phosphorylcholine-containing lipids, including lyso-phosphatidylcholine (lyso-PC), diacyl-PC, O-alkyl-acyl-PC (O-PC), O-alkenyl-acyl-PC (P-PC), and sphingomyelin (SM).

mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethylhexyl) phthalate (MEHP), and mono-isononyl phthalate (MiNP) were determined previously [18–20].

Briefly, 100 μL of thawed urine was mixed with β-glucuronidase solution and 1 M ammonium acetate buffer (pH 6.5) and incubated at 37 °C for 120 min. Internal standards ($13C_4$-MMP, $13C_4$-MBP, and $13C_4$-MEHP), 0.1 M sodium acetate buffer (pH 4.5), and milli-Q water (resistivity: 18.2 MΩcm) were then added to a final volume of 200 μL. After mixing, aliquots were kept in the autosampler at 4 °C and analyzed within 24 h using ultra-high-performance liquid chromatography coupled with triple quadrupole mass spectrometry (TQMS).

Analyses were performed using an Ekspert™ ultraLC 100 system (SCIEX, Framingham, MA, USA) coupled with a SCIEX QTRAP 6500 mass spectrometer. Chromatographic separation was achieved on a Thermo Hypersil Gold column (50 mm × 2.1 mm; 1.9 μm) with a guard column (5 mm × 2.1 mm; 0.2 μm) at 40 °C. The mobile phase consisted of acetonitrile and 0.04% acetic acid in water with a flow rate of 0.3 mL/min. The mobile phase started at 0% acetonitrile and was linearly increased to 100% within 10 min, held for 2 min, and then re-equilibrated to the initial condition for 2.5 min before the next run. The MS was operated in negative electrospray ionization (ESI) mode with multiple reaction monitoring. To ensure reliability and account for urinary dilution,

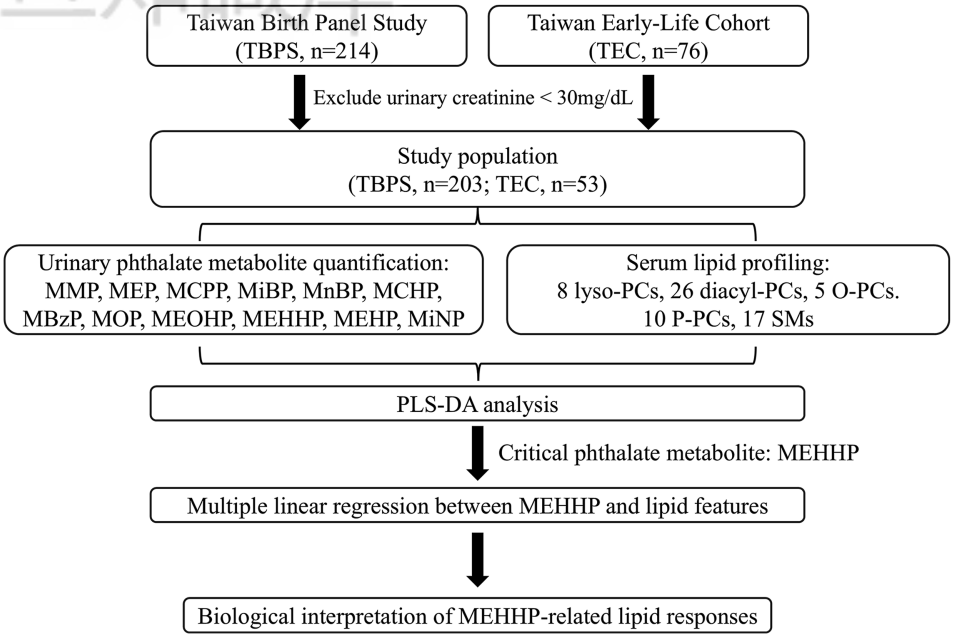

Fig. 2. Study framework for investigating associations between urinary phthalate metabolites and the serum lipidome in Taiwanese children. MMP: mono-methyl phthalate; MEP: mono-ethyl phthalate; MCPP: mono-(3-carboxypropyl) phthalate; MiBP: mono-isobutyl phthalate; MnBP: mono-n-butyl phthalate; MCHP: mono-cyclohexyl phthalate; MBzP: mono-benzyl phthalate; MOP: mono-octyl phthalate; MEOHP: mono-(2-ethyl-5-oxohexyl) phthalate; MEHHP: mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP: mono-(2-ethylhexyl) phthalate; MiNP: mono-isononyl phthalate; PC: phosphatidylcholine; SM: sphingomyelin; PLSDA: partial least squares discriminant analysis.

metabolite concentrations were normalized to creatinine (μg/g creatinine). Method validation details can be found in Supplementary Table S1 (https://doi.org/10.38212/2224-6614.3582). According to the World Health Organization's recommended range for urinary creatinine (30–300 mg/dL), samples with creatinine concentrations below 30 mg/dL (n = 34) were excluded. In total, 256 paired urine and serum samples were included in the final analysis. Creatinine measurements were completed previously in the same batch of urine samples described in a previous publication [18]. Following previous studies [19], exposure groups were defined based on creatinine-adjusted concentrations.

2.3. Serum PC-CL analysis

Serum PC-CL were extracted and measured previously [15]. Serum lipids were extracted using the modified Folch method [21]. Briefly, 20 μL of serum was mixed with 750 μL of chloroform, methanol, and saline (8:4:3, v/v/v), vortexed for 10 min, and centrifuged at 11,200 g for 10 min at 10 °C. A total of 380 μL of the bottom phase was collected, dried using a centrifugal evaporator (1500 g), and reconstituted in 400 μL of methanol containing one ppm of internal standards PC(17:0/0:0), PC(13:0/13:0), and SM(d18:1/17:0). The solution was filtered through a 0.22 μm PTFE membrane and subjected to PC-CL analysis. In addition, a small aliquot of each sample was pooled to prepare a quality control (QC) sample for analysis.

PC-CL analysis was performed previously according to published protocols [9,15]. The analytical platform consisted of a Waters ACQUITY UPLC system coupled with a Quattro Premier XE triple quadrupole mass spectrometer. LC separation was achieved on a Waters BEH C18 column (1.7 μm, 2.1 mm × 100 mm). The mobile phase consisted of solvent A (10 mM ammonium acetate in water) and solvent B (acetonitrile: methanol, 65:35, v/v) containing 1% of 1.0 M ammonium acetate. The flow rate was 0.7 mL/min, the column temperature was maintained at 70 °C, and the injection volume was 10 μL. The gradient program was as follows: 35% B at 0 min, increased to 70% B at 0.1 min, ramped to 100% B at 1.5 min, held for 4.5 min, decreased to 35% B at 6.0 min, and equilibrated for 2 min, with a total run time of 9 min. The TQMS was operated in positive ESI mode at a source temperature of 120 °C, with capillary voltage of 2.5 kV, cone voltage of 35 V, and collision energy of 30 eV. Precursor ion scanning was performed using *m/z* 184 as the diagnostic fragment. To ensure data quality, pool

QC samples were analyzed at regular intervals, and the stability of QC signals was monitored to evaluate reproducibility.

Lipid structure identification was performed at a confidence level based on diagnostic ion patterns acquired in multiple MS experiments [9]. According to the Metabolomics Standards Initiative guidelines, the lipid identification performed using this method is classified as level 2 [22]. Based on the nitrogen rule, the measured signals belonging to PCs can be briefly distinguished from those of SMs. The signals of lyso-PCs can be clearly differentiated from PCs by the retention time. The linking type and position of the fatty acid chain in lyso-PCs can be informed by the product ion spectrum of $[M+H]^+$. The fragmentation pattern of $[M+Na]^+$ was employed to determine the subclasses of PCs, enabling discrimination among diacyl-, alkyl-acyl-, and alkenyl-acyl-PCs. The product ion spectrum of $[M-Me]^-$ was then used for determining the fatty chain composition and the linking positions (sn-1 or sn-2) in the PC molecular structure. According to the recommendations of the Lipid Metabolites and Pathways Strategy [23], the lipids were designated/abbreviated in this study. If both fatty acid chains could not be resolved, the PC species was denoted by the estimated total carbon numbers and double bond equivalents according to the determined subclass, such as PC(38:5), PC(O-38:5) or PC(P-38:5). When the signal intensity was insufficient to determine the subclass, the PC species was simply annotated with its nominal mass, such as PC(835).

2.4. Statistical analysis

Four phthalate metabolites with detection rates below 10% were excluded from further statistical analyses. Concentrations of phthalate metabolites below the limit of quantification (S/N ratio = 10) were replaced with 0.5 × limit of quantification. Exposure classification was based on creatinine-adjusted concentrations of phthalate metabolites. For metabolites with detection rates > 66%, participants were categorized into low-, medium-, and high-exposure groups according to tertiles. For metabolites with detection rates < 66%, concentrations below the detection limit were assigned to the low-exposure group, while detectable values were split at the median into medium- and high-exposure groups. This strategy ensured appropriate grouping for both frequently and less frequently detected phthalate metabolites and was applied in subsequent analyses.

Preprocessed lipidomic data were imported into SIMCA 13.0 software, log-transformed, and unit variance scaled prior to analysis. Partial least squares discriminant analysis (PLS-DA) was used to investigate group differences. Model performance was evaluated using R^2X, R^2Y, and Q^2 values, reflecting explanatory power, group separation, and predictive ability. To avoid model overfitting, results were validated using cross-validation analysis of variance (CV-ANOVA) at a significance level of $p < 0.05$.

In addition, to adjust for potential confounding by demographic characteristics, multiple linear regression models were performed in SAS 9.3 (SAS Institute, Cary, NC, USA), including sex, age, BMI, and household income as covariates. To further minimize bias from regional heterogeneity, analyses were also conducted separately within TBPS and TEC to assess associations between individual phthalate metabolite exposures and serum lipid alterations. Normalized lipid intensity was assessed using the Shapiro–Wilk test, and non-normal variables were transformed by Box–Cox. Multicollinearity was examined using variance inflation factors (VIFs), with VIF < 10 considered acceptable.

3. Results

After excluding urinary samples with creatinine concentrations below 30 mg/dL (n = 34), 256 paired urine and serum samples were included in the further analysis. Descriptive statistics of the study population have been reported in our previous publications [19]. In brief, the study included 256 children, with 203 from the TBPS (Taipei) and 53 from the TEC (Yunlin). Compared with TBPS participants, children from the TEC were slightly older (9.3 vs. 9.2 years, $p < 0.001$), had higher BMI (19.3 vs. 17.5 kg/m^2, $p = 0.026$), and were more likely to come from lower-income households (< 600,000 NT dollars: 54.9% vs. 17.2%, $p < 0.001$). No significant difference was observed in gender distribution between the two cohorts.

Urinary phthalate metabolite concentrations are shown in Table 1. Phthalate metabolites with low detection rates (< 10%) are not included in this table. The metabolites of di(2-ethylhexyl) phthalate (DEHP), MEOHP and MEHHP, showed the highest detection rates (97.9% and 100.0%, respectively) with median concentrations of 12.5 and 17.7 μg/g creatinine. The diethyl phthalate metabolite, MEP, had a higher median concentration (30.86 μg/g creatinine), whereas MMP and MBzP, the metabolites of dimethyl phthalate and butyl benzyl phthalate, showed lower detection rates and median levels in the urine of Taiwanese children.

Table 1. Concentrations of phthalate metabolites in participating children's urine (n = 256).

Phthalate metabolites[a]	Detection rate (%)	Median[b]	Low[b]	Medium[b]	High[b]	LOQ[c]
MMP	40.5	0.08	0.02–0.05	0.05–2.37	2.45–39.29	0.100
MEP	96.9	30.86	0.09–20.60	20.71–47.55	47.74–2611.63	0.500
MiBP	87.9	14.17	0.15–7.89	7.97–22.39	22.58–135.70	0.500
MnBP	85.5	16.54	0.18–8.65	8.69–25.78	26.04–282.92	0.500
MBzP	52.1	0.27	0.01–0.04	0.04–0.96	0.97–66.10	0.050
MEOHP	97.9	12.51	0.02–7.88	8.05–17.76	17.77–191.28	0.050
MEHHP	100.0	17.70	1.12–10.03	10.03–23.83	23.98–294.67	0.050
MEHP	80.0	4.18	0.18–2.72	2.74–6.79	6.81–575.75	0.500

MMP: mono-methyl phthalate; MEP: mono-ethyl phthalate; MiBP: mono-isobutyl phthalate; MnBP: mono-n-butyl phthalate; MBzP: mono-benzyl phthalate; MEOHP: mono-(2-ethyl-5-oxohexyl) phthalate; MEHHP: mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP: mono-(2-ethylhexyl) phthalate; LOQ: limit of quantitation.

[a] Phthalate metabolites with detection rates below 10% were not listed in this table.

[b] (μg/g creatinine).

[c] (μg/L), values below the LOQ were given a value of the LOQ divided by two.

Exposure assessment results were modified from a previous publication [19].

3.1. Associations between serum lipidome and phthalate metabolites

Using the PC-CL analytical platform, a total of 67 lipid species met the criteria of CV < 25% in the QC samples, including eight lyso-PCs, twenty-six diacyl-PCs, five O-alkyl-acyl-PCs (O-PCs), ten O-alkenyl-acyl-PCs (P-PCs), one unclassified PC, and seventeen SMs. The complete list of lipid species is presented in Supplementary Table 1 (https://doi.org/10.38212/2224-6614.3582).

Multivariate modeling of phthalate metabolite concentrations and serum lipid profiles was performed using PLS-DA (Table 2). Urinary concentrations of MMP, MEP, MiBP, MnBP, MBzP, and MEHP did not yield valid PLS-DA models. MEOHP could establish a model with the serum lipidome; however, the CV-ANOVA test was not significant, indicating potential overfitting. MEHHP was the only PAE metabolite that successfully built a PLS-DA model and passed the CV-ANOVA validation, suggesting a significant association between MEHHP exposure and lipid alterations.

To minimize bias from regional heterogeneity, further multiple linear regression analyses were conducted separately for TBPS and TEC. In the TBPS cohort, multiple lipids were significantly associated with MEHHP concentrations (Fig. 3). When comparing the medium- and low-exposure groups (Fig. 3a), five lipids showed significant differences: PC(18:0/22:4) was significantly elevated, while PC(O-18:1/16:0) and three SMs were significantly decreased. When comparing the high- and low-exposure groups (Fig. 3b), 23 lipids were significantly different, including five lyso-PCs, seven diacyl-PCs, three O-PCs, two P-PCs, and six SMs. Lipids with significant changes ($p < 0.05$) in the medium- or high-dose groups and their directional trends are illustrated in a heat map (Fig. 3c).

In contrast, in the TEC cohort, only a few lipids were significantly associated with MEHHP levels (Table 3.). Specifically, PC(16:0/16:0) and PC(P-33:2) were significantly higher in the medium-exposure group compared with the low-exposure group,

Table 2. PLS-DA models demonstrating associations between serum lipidome and urinary PAE metabolites.

Model	Y variable	Number of LVs	n	R^2X	R^2Y	Q^2	CV-ANOVA (*p*-value)
PLS-DA	MMP	0	256	–	–	–	–
PLS-DA	MEP	0	256	–	–	–	–
PLS-DA	MiBP	0	256	–	–	–	–
PLS-DA	MnBP	0	256	–	–	–	–
PLS-DA	MBzP	0	256	–	–	–	–
PLS-DA	MEOHP	1	256	0.163	0.038	0.006	0.201
PLS-DA	MEHHP	1	256	0.167	0.051	0.018	**0.003**
PLS-DA	MEHP	0	256	–	–	–	–

MMP: mono-methyl phthalate; MEP: mono-ethyl phthalate; MiBP: mono-isobutyl phthalate; MnBP: mono-n-butyl phthalate; MBzP: mono-benzyl phthalate; MEOHP: mono-(2-ethyl-5-oxohexyl) phthalate; MEHHP: mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP: mono-(2-ethylhexyl) phthalate.

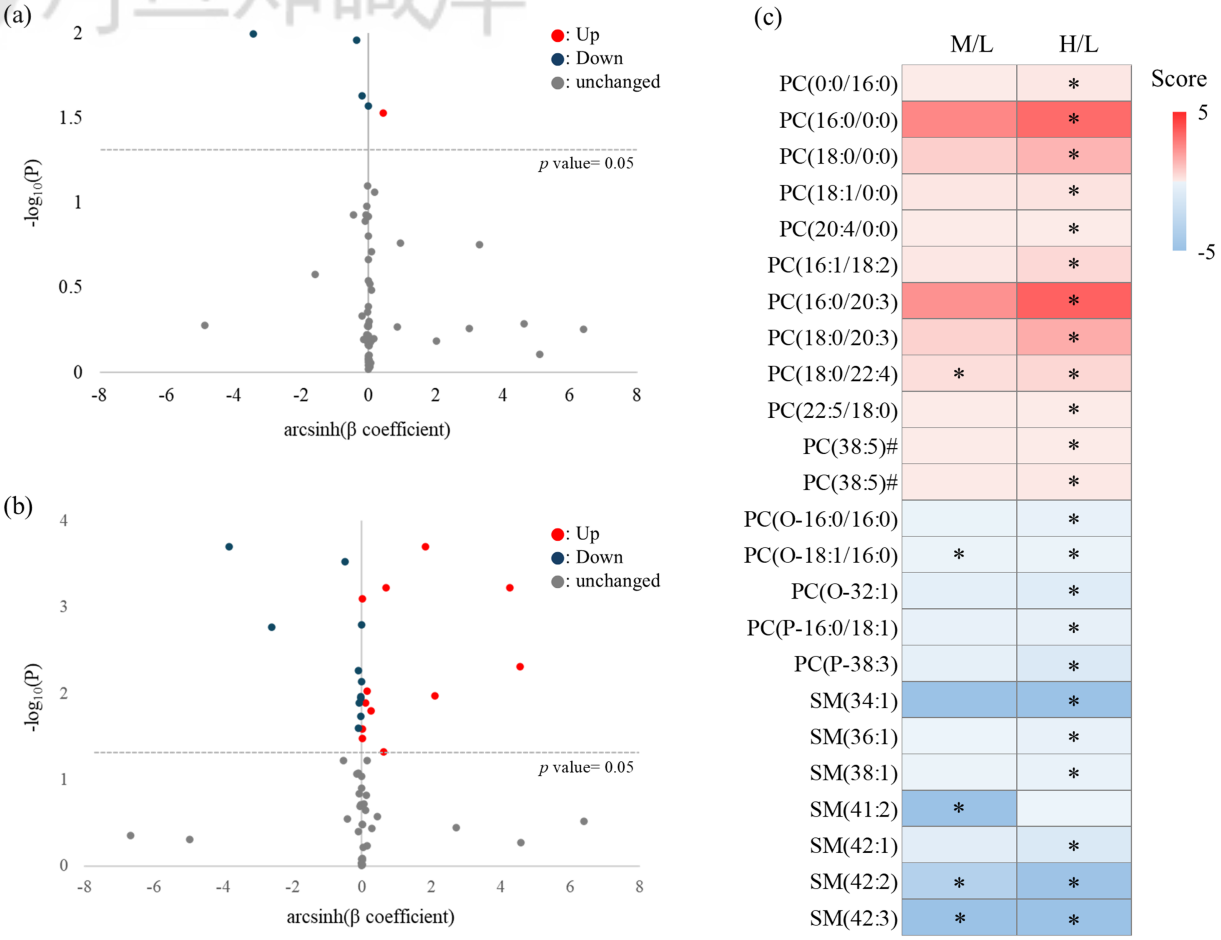

*Fig. 3. Associations between urinary MEHHP and serum lipid species identified by multiple linear regression in Taiwan Birth Panel Study. Volcano plots compare (a) medium vs. low MEHHP exposure and (b) high vs. low MEHHP exposure. The X-axis represents regression coefficients transformed using the inverse hyperbolic sine (arcsinh) function, and the Y-axis represents statistical significance as −log10 (p-values). (c) The heatmap illustrates the changes in differential lipids; red indicates higher levels, and blue indicates lower levels relative to the low-exposure group. Effect sizes and p-values were obtained from multiple linear regression models adjusted for age, sex, BMI, and household income. #: lipid species with isomeric forms. *: lipid alterations were statistically significant ($p < 0.05$).*

while PC(38:5) was significantly lower in the high-exposure group compared with the low-exposure group.

4. Discussion

PAE exposure is an important public health issue, and its higher levels in children make it especially relevant to child health [3]. In our study, we also observed that Taiwanese children exhibited relatively higher DEHP exposure levels compared with those reported in other countries, such as China and the United States [19]. Although PAE exposure has been associated with a wide range of adverse health outcomes, the molecular events and possible toxicological mechanisms remain incompletely

Table 3. Differential serum lipid species associated with urinary MEHHP exposure in the Taiwan Early-Life Cohort (TEC). β-coefficients and p-values from multiple linear regression analyses are reported for comparisons between medium and low (M/L) exposure, and high and low (H/L) exposure.

Lipid species	*m/z*	RT (min)	M/L		H/L	
			β	*p*-value	β	*p*-value
PC(16:0/16:0)	734.7	2.47	0.09675	0.010*	0.05182	0.133
PC(38:5)	806.7	2.19	−0.00001	0.694	−0.0001	0.048*
PC(P-32:1)	744.7	2.22	0.03096	0.028*	0.00794	0.538

*: lipid alterations were statistically significant ($p < 0.05$).

understood. To improve our understanding of these molecular events, our earlier nuclear magnetic resonance (NMR)-based metabolomics study reported significant associations between urinary PAE metabolite levels and alterations in serum metabolites in Taiwanese children [19]. However, NMR has inherent limitations for lipid analysis, particularly its lower sensitivity and limited structural specificity. In the present study, we further applied an MS–based lipidomic approach to analyze serum lipidome in Taiwanese children, aiming to provide additional mechanistic insights.

Multivariate analyses showed that urinary MEHHP, an oxidative metabolite of DEHP, was most strongly associated with the serum lipidome of Taiwanese children, indicating a prominent role in lipid metabolic alterations. Some PAE metabolites with relatively high exposure levels, such as MEP and MnBP, showed no significant associations with the serum lipidome in this study. These discrepancies likely reflect differences in the biological pathways through which individual PAEs influence lipid metabolism. Recent epidemiological evidence indicated that MEHHP contributed most strongly to the increased risk of metabolic dysfunction–associated steatotic liver disease among multiple PAE exposures [24], suggesting that MEHHP may possess greater biochemical activity or more direct effects on lipid metabolism. Our analytical platform focuses on PC-CL, such as phosphatidylcholines, which are known to be closely associated with metabolic diseases involving the liver and cardiovascular system [10,11]. Therefore, the strong association observed between urinary MEHHP and serum lipid responses in this study is biologically plausible. In contrast, other PAEs may exert weaker lipid-related effects or predominantly perturb non-lipid metabolic pathways. Previous metabolomic work demonstrated that MiBP and MnBP were more strongly associated with perturbation of hydrophilic metabolites, such as metabolites in glycolysis and the Krebs cycle, whereas MEHHP showed less associations with hydrophilic metabolic patterns [19,25], further supporting that MEHHP may mainly affect lipid metabolism.

This observation aligns with toxicological evidence from animal models. DEHP is among the most concerning PAEs [26]. DEHP affects multiple toxicity endpoints, including hepatic injury and reproductive toxicity, and activates peroxisome proliferator-activated receptors (PPARs) signaling [26,27]. Given the critical role of PPARs activation in lipid metabolism [28], this may underlie the observed associations between MEHHP and lipid alterations. Although some other phthalates can also modulate PPARs activity, DEHP appears to do so more robustly and strongly, implying more pronounced effects on lipid metabolism than other PAEs [29]. The following sections further explore the biological implications of these lipid changes.

Our study showed that the levels of multiple diacyl-PC species were positively associated with urinary MEHHP levels. This alteration may relate to the effects of PAE exposure on circulating lipoproteins. Although epidemiological evidence on lipoproteins has been inconsistent, studies have shown that PAE exposure can perturb lipoprotein homeostasis [1]. Because PC is an essential structural component of lipoproteins, changes in circulating lipoproteins may lead to altered serum PC levels [11]. Our results therefore support the hypothesis that PAEs influence lipoprotein metabolism.

Interestingly, our study observed an increasing trend in serum lyso-PC. Literature has reported increased circulating lyso-PC levels as a potential biomarker of inflammation, and PAE exposure has also been linked to inflammatory responses [1]. In addition, lyso-PC is closely related to lipoprotein composition. Previous studies have shown that oxidized low-density lipoprotein (oxLDL) contains higher proportions of lyso-PC, and oxLDL is considered a more atherogenic form of LDL [30]. Our results, therefore, supported the link between DEHP exposure and adverse cardiovascular health.

We also observed decreasing levels of circulating ether-linked PCs, O-PCs and P-PCs, which may reflect impaired ether-linked lipid biosynthesis [12]. As ether-linked lipid synthesis occurs mainly in peroxisomes and DEHP is known to activate the PPARs pathway [31], this decrease may be related to the altered peroxisomal function. Notably, ether-linked lipids are also regarded as endogenous ligands of PPARs [12]. Thus, DEHP-mediated exogenous activation of PPARs may feed back to affect the synthesis of endogenous ligands, thereby disrupting ether-linked lipid homeostasis. Furthermore, studies have reported associations between reduced ether-linked lipid levels and hypertension [12]. Accordingly, our observation of lower ether-linked PC levels further supported that DEHP may interfere with peroxisomal lipid metabolism and PPARs signaling, thereby enhancing cardiovascular risk.

Finally, we found that the levels of multiple serum SM species were negatively associated with urinary MEHHP levels. This result is consistent with a previous lipidomics study in pregnant women in Puerto Rico, where DEHP exposure was positively associated with plasma ceramides (the

hydrolysis products of SM), suggesting that DEHP may promote the hydrolysis of SM to ceramide [13,32]. Such alterations are linked to multiple biological processes, including cell death and autophagy induction. Moreover, disruption of the sphingolipid pathway has been implicated in diseases such as neurological diseases, kidney disorders, and cancer [13,14]. Neurotoxicity is considered one of the most critical toxic effects of DEHP, and nephrotoxicity has also been reported [3,33]. DEHP has been classified by the International Agency for Research on Cancer as a Group 2B carcinogen [31]. Therefore, the significant alterations in SM observed in our study may suggest that the sphingolipid pathway may play a role in DEHP-induced neurotoxicity, nephrotoxicity, and carcinogenesis.

However, few lipid responses to urinary MEHHP levels in TEC. A total of 24 differential lipids were identified in the TBPS cohort, whereas three differential lipids were observed in TEC. The smaller response observed in TEC is likely attributable in part to its limited sample size ($n = 53$). To evaluate this possibility, we performed a post hoc power analysis (see Supplementary Results for details), which showed that the median power for the 24 differential lipids identified in TBPS was 79%, while the median power for those lipids was only 27% in TEC (Fig. S1 (https://doi.org/10.38212/2224-6614.3582)). Nevertheless, we still noted a decreasing trend in P-PC species in TEC, consistent with the lipid pattern observed in TBPS, although these changes have not reached statistical significance due to the limited sample size. However, low statistical power alone may be insufficient to explain the observed discrepancies between the two cohorts. Additional unadjusted confounders, such as differences in diet, lifestyle, or co-exposure to other chemicals, may also have contributed to the divergent results in TEC. In particular, we emphasize the potential impact of chemical co-exposure: our previous work demonstrated that per- and polyfluoroalkyl substance levels in TEC were substantially higher than those in TBPS [15], which have interfered with the association between MEHHP levels and PC-CL responses in TEC.

This study has certain limitations. First, our lipidomic analysis was restricted to a subset of PC- and SM-containing species, which may not capture the full complexity of the lipidome. Second, the cross-sectional design of the study precludes causal inference, and longitudinal or experimental studies will be needed to validate these associations. In addition, residual confounding by unmeasured factors may not be fully excluded. Notably, the substantial differences in lipid responses of TBPC and TEC further suggested that unadjusted confounders have influenced the findings, highlighting the need for replication in larger and more diverse populations. Finally, fasting was not required because of the young age of participants, and recent food intake may therefore have influenced the observed results.

In conclusion, our study identified PAE-associated systemic alterations in serum lipidome in Taiwanese children, including increases in diacyl-PC and lyso-PC, and decreases in ether-linked PC and SM. These findings suggest that DEHP exposure may perturb lipoprotein metabolism, PPARs-related pathways, and sphingolipid balance. As these pathways are closely linked to cardiovascular, neurological, renal, and cancer risks, our results provide possible molecular-level evidence connecting DEHP exposure to lipid dysregulation, underscoring its relevance to public health and children's health. Further mechanistic studies and epidemiological investigations are needed to clarify the causal relationships underlying these associations.

Ethics statement

All study procedures involving human participants were conducted in accordance with the ethical standards of the Institutional Review Board of National Taiwan University Hospital and with the 1964 Declaration of Helsinki and its later amendments. The study protocol was approved by the National Taiwan University Hospital Research Ethics Committee (Project Identification Code: 201112137RIC). Written informed consent was obtained from all participating children and their parents or legal guardians prior to enrollment.

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