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DEHP and DINP Induce Tissue- and Gender-Specific Disturbances in Fatty Acid and Lipidomic Profiles in Neonatal Mice: A Comparative Study

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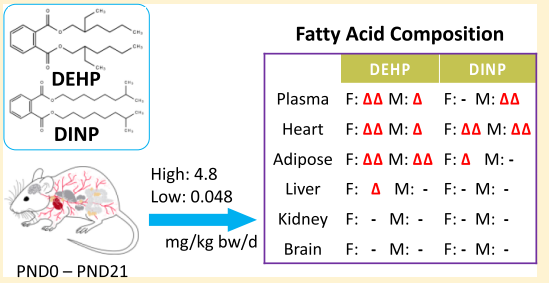
Supporting Information

ABSTRACT: Di-isononyl phthalate (DINP) is considered one of the main industrial alternatives to di(2-ethylhexyl)phthalate (DEHP), a well-known chemical with various toxic effects including the disruption with lipid metabolism. However, the potential effects of DINP on lipid metabolism have rarely been investigated in mammals. Our study demonstrated that exposure of neonatal mice to DEHP and DINP at a daily dose of 0.048 or 4.8 mg/kg from postnatal day 0 (PND0) to PND21 caused nonmonotonic as well as tissue- and gender-specific alterations of total fatty acid (FA) compositions in plasma, heart, and adipose tissues. However, the patterns of disruption differed between DEHP- and DINP-treated groups. On the basis of targeted lipidomic analyses, we further identified gender-specific alterations of eight lipid classes in plasma following DEHP or DINP exposure. At the higher dose, DEHP induced decreases in total phosphatidylcholines and phosphatidylinositol (PI) in females and increases in phosphatidylethanolamines (PEs) and triglycerides in males. By contrast, DINP at the higher dose caused alterations of PEs, PIs, phosphatidylserines, and cholesterols exclusively in male mice, but no changes were observed in female pups. Although the most significant dysregulation of lipid metabolism was often observed for the higher dose, the lower one could also disrupt lipid profiles and sometimes its effects may even be more significant than those induced by the higher dose. Our study for the first time identified tissue- and gender-specific disruptions of FA compositions and lipidomic profiles in mice neonatally exposed to DINP. These findings question the suitability of DINP as a safe DEHP substitute and lay a solid foundation for further elucidation of its effects on lipid metabolism and underlying mechanisms.



INTRODUCTION

As one of the major groups of environmental contaminants with global occurrences, phthalates have been ubiquitously found in human urine and blood.[1] Di(2-ethylhexyl)phthalate (DEHP) represents one of the most studied phthalates, mainly because of its high occurrence rate and reproductive and endocrine disrupting toxicities.[2] This had led to the use of alternative chemicals as DEHP substitutes, among which di-isononyl phthalate (DINP) represents a major replacement. The annual global production of DINP was estimated to be 1.5 million tons in 2013,[3] reaching approximately 75% of annual DEHP production volume.[4]

Although DINP has been suggested with decreased carcinogenic toxicity compared with DEHP, previous work has demonstrated that DINP can cause disturbances to the immune system[5,6] and liver and kidney damage at a daily dose of 20−200 mg/kg body weight.[7] However, compared with numerous DEHP studies, DINP's toxicities have been subjected to much less investigations. In particular, DINP's chronic effects in mammals, such as the interference with lipid synthesis and metabolism, remain largely uninvestigated.

Lipids, either endogenously synthesized through lipogenesis or originated from diet, are composed of saturated fatty acids (SFAs), monounsaturated fatty acids (Monos), trans-fatty acids (Trans), and polyunsaturated fatty acids (PUFAs). Fatty acids (FAs) not only provide sources of energy but also constitute significant components of cell membranes.[8] Metabolized lipids also serve as hormonal mediators, which regulate multiple functions including inflammation responses.[9] A large number of studies have demonstrated that some lipid molecules (e.g., plasma phospholipid FAs) can serve as important biomarkers in the development of chronic metabolic conditions at various disease stages.[10−12]

Some studies have reported that DEHP and its metabolite mono-(2-ethylhexyl)phthalate (MEHP) can disrupt lipid metabolism by altering total triglycerides (TG),[13,14] mainly through activation of the nuclear peroxisome proliferator-

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activated receptors (PPARs).[15−18] Transcriptomics and nontargeted global metabolomics both demonstrated the alteration of lipid metabolism as a consequence of DEHP exposure.[13] However, as a major DEHP substitute, DINP has received much less attention with regard to its potential impacts on lipid metabolism, particularly in mammals. To the best of our knowledge, the only data available were reported in aquatic organisms including zebrafish and seabream studies, which indicated that exposure to DINP causes disturbances in lipid-related metabolism, as well as the induction of oxidative stress and activation of immune responses.[19−22]

Consequently, the main goal of this study was to investigate and compare the disturbances of FA and lipidomic profiles in neonatal mice following exposure to DEHP and DINP. As a pilot study of a larger-scale project aiming to elucidate the potential effects of DEHP substitutes on lipid synthesis and metabolism, the specific objectives of the present work were to explore differential effects of DEHP and DINP on (1) the distributions of FA compositions in different tissues and genders and (2) gender-specific changes of lipidomic profiles in plasma. Findings from the present study will lay a solid foundation for further elucidation of the impacts of DINP on lipid metabolism homeostasis, underlying mechanisms, and associated biological consequences. The findings also contribute to a better elucidation of whether DINP is a safe substitute to DEHP.

MATERIALS AND METHODS

Chemicals and Reagents. Reference standards of DINP and DEHP were purchased from Sigma (St. Louis, USA). FA methyl ester (FAME) reference standard mixture (GLC-463) was obtained from NuChek (Prep Inc., USA). The internal standard mixture for lipidomic assay, containing bis-(monoacylglycero)phosphate 14:0/14:0; ceramide, Cer 18:1/17:0; phosphatidylcholine, PC 14:0/14:0; phosphatidylethanolamine, PE 17:0/17:0; phosphatidylglycerol, PG 14:0/14:0; and phosphatidylserines, PS 17:0/17:0, was purchased from Sigma (St. Louis, USA). Dihexosylceramide 18:1/16:0 (d3); glucosylceramide, GC 18:1/16:0 (d3); phosphatidylinositol, PI 16:0/16:0; and trihexosylceramide 18:1/17:0 were purchased from Matreya LLC (Pleasant Gap, USA). All solvents used were of high-performance liquid chromatography grade (Fisher Scientific, New Hampshire, USA), except for methanol and water (Optima, Fisher Scientific, USA).

Animal Experiment. Six male and 18 female-specific pathogen-free Kunming mice (8−10 weeks old) were obtained from Guangdong Medical Laboratory Animal Center (Guangzhou, China). Animals were acclimated for 10 days prior to experiments and were maintained under a 12 h light/12 h dark cycle at a room temperature of 25 °C. Glass water bottles and polypropylene cages were used in this study. After acclimation, each cage contained one male and two female mice at 7 pm for mating. Once confirmed pregnant, each female mouse was kept in individual cage until birth. All mice were fed ad libitum on a standard chow.

Dams were allowed to give birth naturally at term. Newborn mice were recorded as postnatal day 0 (PND0), and they remained with their natural mothers in the same cage until PND21. Each cage was randomly assigned to a treatment (DEHP or DINP) or control group. Each group contained three replicate cages. Both male and female pups were exposed to vehicle control (corn oil), DEHP (high dose 4.8 mg/kg bw/day or low dose 0.048 mg/kg bw/day), or DINP (high dose 4.8 mg/kg bw/day or low dose 0.048 mg/kg bw/day) through subcutaneous injection at 10−12 am daily from PND0 to PND21. The high dose chosen corresponds to the no-observed-adverse-effect level (NOAEL)[23] suggested for DEHP and DINP, while the low dose is slightly lower than the tolerable daily intake (TDI) dose of DEHP (0.05 mg/kg bw/day) and DINP (0.15 mg/kg bw/day) according to the European EU Risk Assessment Reports (EU RAR).[23] Dosages were adjusted daily for body weight change, and such an injection approach was chosen in order to maintain a consistent dosage, as well as considering its practicality for neonates. Pups were kept with their dams until PND21 and were fasted for 6 h prior to sampling on PND22. The exposure period of PND0−PND21 was employed because this is an important developmental stage as well as a sensitive window of time within which the pups are susceptible to environmental stresses.[24]

At PND22, 10 male and 10 female pups were randomly selected from three replicate cages per treatment group (three each from two cages and another four from the third cage). These pups were selected for phthalate metabolite residue, biochemical, and FA/lipidomic analyses. Urine samples were collected using the stainless metabolic cage method, and urine from two random pups was pooled to obtain sufficient volume for chemical analyses. Basic phenotype measurements, including body weight, abdominal circumference, and body length, were conducted before tissue collection. Blood samples were collected via cardiac puncture into an ethylenediaminetetraacetic acid-treated vacutainer and kept on ice prior to separation of plasma and erythrocytes at 2800*g* centrifugation. Brain, heart, kidney, liver, and reproductive white adipose tissues were subsequently sampled from each pup. Urine, plasma, and tissue samples for biochemical and oil red O staining were all snap frozen in liquid nitrogen and subsequently stored in −80 °C. Samples for mRNA analysis were placed in RNAlater solution (QIAGEN, Germany) and then stored in −80 °C. Urine samples for phthalate metabolite analysis were stored in −20 °C.

Plasma total glucose and liver total bile acid (TBA) were determined using a commercial kit (Nanjing Jiancheng Bioengineering Institute, China), performed according to the manufacturer's instructions. Frozen liver sections were stained with oil red O staining to examine if there was histopathological hepatic steatosis (see the Supporting Information for details). Animal experiments in this study were approved by the Laboratory Animal Ethics Committee of Jinan University (Guangzhou, China).

FA Analysis. An aliquot of 50 μL of plasma or ∼0.1 g of homogenized tissue samples was extracted using the Folch method with a mixture of methanol and chloroform (1:2, v/v).[25] Lipids were then transmethylated to FAMEs and then extracted with heptane. The final extracts were determined by gas chromatography−mass spectrometry. Detailed information on sample preparation and instrumental analysis is summarized in the Supporting Information. Quality assurance and control (QA/QC) procedures included the process of a solvent blank and a commercial plasma sample (BestBio, Shanghai) along with every batch of samples. The latter serves as QC samples to determine intra- and interassay coefficient of variations (CVs). Results were presented as weight percentages of total FAs. Our in-house QC data demonstrated intra- and interassay CVs of less than 8.5 and 9.8%, respectively, for major FAs (i.e., concentration > 0.1%).

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Table 1. Basic Phenotypes of Mice Exposed to Control, DEHP, and DINP at Two Doses (4.8 or 0.048 mg/kg bw/d) at PND22

	gender	control	DEHP-L	DEHP-H	DINP-L	DINP-H
body weight (g)	F	17.8 ± 0.9	19.1 ± 0.7*	15.4 ± 0.9*	18.4 ± 0.8	17.1 ± 1.1
	M	18.7 ± 0.8	20.2 ± 0.7*	16.6 ± 1.0*	19.2 ± 1.0	18.5 ± 1.3
body length (mm)	F	8.17 ± 0.38	8.51 ± 0.13	7.69 ± 0.38*	8.09 ± 0.07	7.95 ± 0.26
	M	8.29 ± 0.25	8.36 ± 0.04	8.03 ± 0.29	7.97 ± 0.35	8.28 ± 0.25
abdominal circumference (mm)	F	5.52 ± 0.28	6.10 ± 0.17*	5.00 ± 0.39*	6.17 ± 0.33*	5.38 ± 0.27
	M	5.52 ± 0.25	5.92 ± 0.11*	5.07 ± 0.23*	5.87 ± 0.23	5.53 ± 0.29
BMI ($kg{\cdot}m^{-2}$)	F	2.67 ± 0.21	2.65 ± 0.11	2.61 ± 0.15	2.81 ± 0.14	2.71 ± 0.13
	M	2.72 ± 0.18	2.90 ± 0.08	2.58 ± 0.19	3.04 ± 0.22*	2.70 ± 0.13
liver weight/bw (g/20 g)	F	0.789 ± 0.071	0.789 ± 0.033	0.807 ± 0.043	0.762 ± 0.039	0.813 ± 0.049
	M	0.856 ± 0.053	0.824 ± 0.029	0.862 ± 0.042	0.814 ± 0.028	0.855 ± 0.087
brain weight/bw (g/20 g)	F	0.466 ± 0.034	0.445 ± 0.014	0.498 ± 0.022	0.447 ± 0.017	0.481 ± 0.028
	M	0.464 ± 0.02	0.43 ± 0.017	0.483 ± 0.026	0.451 ± 0.019	0.465 ± 0.03
kidney weight/bw (g/20 g)	F	0.269 ± 0.027	0.256 ± 0.011	0.281 ± 0.019	0.251 ± 0.015	0.257 ± 0.015
	M	0.277 ± 0.015	0.267 ± 0.035	0.275 ± 0.021	0.258 ± 0.012*	0.258 ± 0.013
heart weight/bw (g/20 g)	F	0.123 ± 0.013	0.123 ± 0.004	0.121 ± 0.014	0.129 ± 0.012	0.115 ± 0.011
	M	0.123 ± 0.012	0.118 ± 0.006	0.123 ± 0.011	0.120 ± 0.008	0.110 ± 0.013
blood glucose (g)	F	7.61 ± 0.60	7.75 ± 0.81	9.95 ± 2.76	7.50 ± 1.03	6.90 ± 2.73
	M	7.43 ± 0.50	8.24 ± 0.66	9.36 ± 2.98	7.57 ± 0.57	6.52 ± 2.45
plasma total cholesterol (μM)	F	330.5 ± 84.0	485.1 ± 175.1*	326.4 ± 30.3	315.7 ± 37.2	300.3 ± 52.7
	M	245.9 ± 33.3	240.8 ± 80.6	258.1 ± 37.4	463.2 ± 325.8*	384.5 ± 122.2*
plasma total TG (μM)	F	79.3 ± 44.9	113.6 ± 36.4	30.1 ± 17.0*	70.6 ± 17.0	53.1 ± 29.8
	M	90.4 ± 65.1	104.2 ± 49.4	207.3 ± 51.8*	70.2 ± 21.2	47.4 ± 17.9
plasma total diglyceride (μM)	F	4.82 ± 0.72	1.77 ± 1.14*	2.53 ± 0.40*	1.71 ± 1.35*	4.34 ± 0.74
	M	5.12 ± 0.86	5.08 ± 2.11	5.26 ± 0.85	4.80 ± 1.25	5.07 ± 0.71
liver TBA (μ $mol{\cdot}gprot^{-1}$)	F	4.07 ± 0.42	4.62 ± 0.23*	4.08 ± 0.19	4.14 ± 0.21	4.18 ± 0.31
	M	3.99 ± 0.24	4.05 ± 0.26	4.34 ± 0.34	4.08 ± 0.19*	4.50 ± 0.35*

BMI, body mass index; TBA, total bile acids. Each measurement was replicated in 10 for both male and female mice. * Asterisks denote statistical significance ($p < 0.05$) between treatment and control groups based on nonparametric Kruskal–Wallis tests with Dunn's post-hoc test. Data are presented as mean ± standard deviation.

Lipidomic Analysis. An aliquot of 50 μL of plasma was spiked with internal standard mixture (400 pmol each) prior to the Folch extraction.[25] After drying, the lipid extract was dissolved in 500 μL of a mixture of methanol, tetrahydrofuran, and water (5:2:3, v/v/v) and subjected to instrumental analysis which employed an ExionLC AD ultraperformance LC (UPLC, AB Sciex, Toronto, Canada) equipped with an Agilent Eclipse Plus C18 column (Agilent Technologies) and coupled to an AB Sciex Q TRAP 5500 mass spectrometer. Details on sample preparation and instrumental determination are given in the Supporting Information. QA/QC procedures included the process of solvent blanks, solvent spiked with internal standards, matrix blanks without spiking internal standards, and commercial plasma samples serving as QC samples to determine intra- and interassay CVs. Concentrations were calculated by relating peak areas of the analytes to peak areas of their corresponding internal standard. Our in-house QC data demonstrated intra- and interassay CVs of less than 14.7 and 16.8% for analytes above quantitation limits (i.e., 1.8–17.2 nmol/L), respectively.

Urinary Phthalate Metabolite Analyses. Urinary metabolites of DEHP and DINP were determined to confirm internal exposure in the pups following the method described by Asimakopoulos et al. with modifications.[26] In brief, 500 μL of urine was transferred into polypropylene tubes and spiked with 10 μL of surrogate standard (mono-benzyl phthalate-d_4). The mixture was then hydrolyzed with β-glucuronidase/sulfatase (Sigma-Aldrich, Munich, Germany), extracted with a mixture of methyl *tert*-butyl ether and ethyl acetate (4:1; v/v). The extract was dried under gentle nitrogen stream and then reconstituted with a mixture of acetonitrile and water (6:4; v/v) and spiked with $^{13}C_{12}$-bisphenol A. Instrumental analysis employed an ExionLC AD UPLC equipped with an Agilent Extend-C18 column (Narrow Bore RR 2.1 × 100 mm, 3.5 μm, 80 Å; Agilent Technologies) and coupled to an AB Sciex Q TRAP 5500 MS. The limit of quantification (LOQ), defined as an analyte response 10 times the standard deviation of the noise, was determined to be 0.5 ng/mL for MEHP, mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono(2-ethyl-5-carboxypentyl)phthalate (MECPP) and 1 ng/mL for monoisononyl phthalate (MINP) and mono carboxyoctyl phthalate (MCOP). Details on sample preparation, instrumental determination, and QA/QC practices are given in the Supporting Information.

Lipid Metabolism-Related Gene Expression Analysis. The expression of genes related to lipid metabolism[27] including peroxisome proliferator-activated receptors (*Pparα*, *Pparβ*, and *Pparγ*), lipoprotein lipase (*Lpl*), acetyl CoA carboxylase-1 (*Acc1*), FA synthase (*Fas*), stearoyl CoA desaturase-1 (*Scd1*), FA elongase-6 (*Elovl6*), Acyl-coenzyme A oxidase-1, palmitoyl (*Acox-1*), FA elongase-2 (*Elovl2*), and FA desaturase (*Fads1* and *Fads2*) were analyzed by quantitative real-time polymerase chain reaction (qRT-PCR) assay. Detailed information on qRT-PCR is described in the Supporting Information.

Statistical Analysis. All data are presented as mean ± standard deviation unless otherwise stated. FA percentage data were arc-sin converted prior to statistical analyses. For each type of control or treatments, we performed intragroup comparisons between replicate cages using nonparametric

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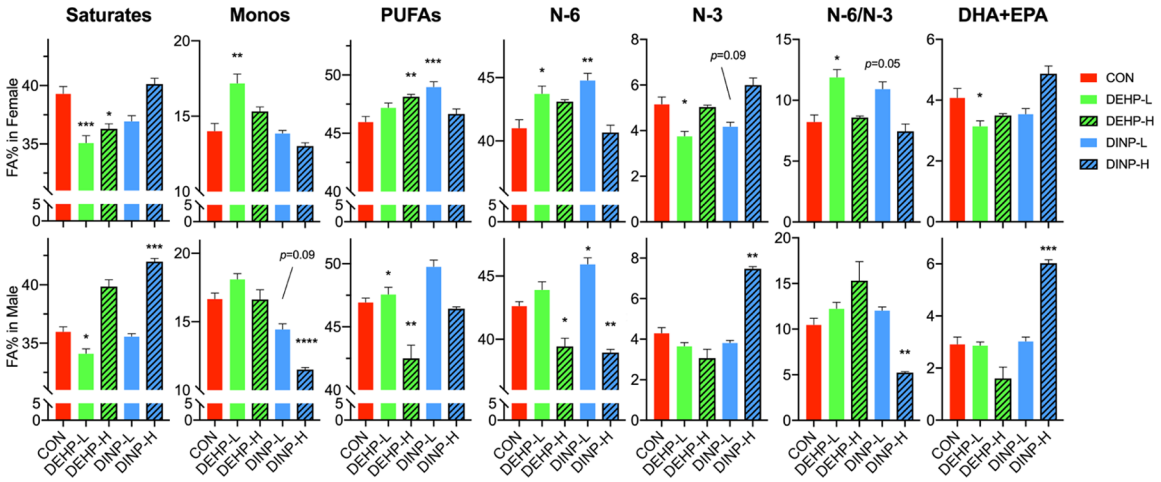

Figure 1. Plasma total FA compositions in mice exposed to vehicle (corn oil), DEHP-L (0.048 mg/kg bw/day), DEHP-H (4.8 mg/kg bw/day), DINP-L (0.048 mg/kg bw/day), or DINP-H (4.8 mg/kg bw/day) during PND0−PND21 (N = 10 for each group). (a−e) Plasma FA compositions in female mice; (f−j) FA compositions in male mice. Data are presented as mean with standard error of the mean. Statistical analyses were conducted using the Kruskal−Wallis test, followed by the Dunn's post-hoc test. *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.

Kruskal−Wallis test followed by Dunn's post-hoc test and found no significant differences in body weight and the compositions of major FA groups (SFAs, Monos, and PUFAs). This demonstrated minimal within-group variations. Therefore, each pup was considered as an individual sample for statistical analyses. Comparisons among control and treatment groups were conducted via Kruskal−Wallis with the Dunn's post-hoc test. Nonparametric Wilcoxon−Mann−Whitney test was performed to compare the differences between control and any treatment group. We also performed the analysis of variance (ANOVA) and heat map analysis for the illustration of lipidomic alterations using the online MetaboAnalyst4.0 platform containing R packages.[28] Other statistical analyses and figure illustrations were performed with GraphPad Prism v8.0 (GraphPad, USA). The level of significance was set at α = 0.05 throughout the study.

RESULTS AND DISCUSSION

Major Phenotype Changes Following Exposure. Mice exposed to the low and high doses of DEHP exhibited urinary MEHP concentrations of 2.9 ± 0.6 and 123 ± 133 ng/mL at PND22, respectively, whereas urinary concentrations were below LOQ in control groups. In addition to MEHP, DEHP's secondary metabolites, including MECPP, MEOHP, and MEHHP, were also detected with a combined concentration of 69.7 ± 23.6 and 3426 ± 1140 ng/mL in mice exposed to the low and high doses of DEHP, respectively. In DINP-treated mice, concentrations of urinary MINP (a major metabolite of DINP) and MCOP (secondary metabolite) were detected to be 36.4 ± 5.4 and 3570 ± 196 ng/mL in the low- and high-dose groups, respectively. No significant differences were observed in the concentrations of any metabolites between genders. These results validated the occurrence of internal exposure.

Neonatal exposure to DEHP or DINP appeared to alter a number of phenotypes in mice, while the impacts differed between chemicals and dosages (Table 1). At PND22, both male and female pups exposed to the high dose of DEHP (referred to as DEHP-H) exhibited lower body weights compared with control groups, although the body mass index (BMI) was not changed. By contrast, exposure to the high dose of DINP (DINP-H) did not significantly change body weights or BMI of pups. Interestingly, exposure to low-dose DEHP (DHEP-L) increased body weight and abdominal circumferences in both genders, exhibiting obesogenic effects. Although no significant changes in body weight or body length were observed, exposure to low-dose DINP (DINP-L) did result in an increase of female abdominal circumferences and male BMI. These data suggest obesogenic effects of DEHP-L and DINP-L exposure, agreeing with the findings from previous studies.[14,29−31] Therefore, distinct mechanisms of actions could exist at different doses. The nonmonotonic dose−response curves of phthalates have been reported in both animal studies[32] and human cohort studies.[33] Exposure at relatively high levels could cause damages to cell differentiation and growth, thus hindering normal growth,[29,31] whereas low-level exposure could disrupt the endocrine system by disrupting hormonal functions.[14,29]

Exposure resulted in little changes in liver weight among most groups except for the DINP-L treatment which caused significant liver weight reduction in male mice. DEHP-H decreased kidney, heart, and brain weights exclusively in the males, while DINP-H treatments diminished male heart weight compared to the control pups. Biochemical assays showed inconsistent trends of changes in plasma total cholesterol, total TGs, diglycerides, and liver TBA in male and female pups (Table 1). Taken together, the above results clearly suggest gender- and/or tissue-specific effects, as well as the differences in biochemical consequences between DEHP and DINP exposure. Therefore, subsequent analyses were conducted to explore gender- and tissue-specific effects of DEHP and DINP on lipid metabolism.

Tissue- and Gender-Specific Effects on FA Composition. DEHP is known to interrupt FA metabolic pathways through PPAR regulation,[13] while little has been done to explore the potential interference of DINP with FA metabolism homeostasis in mammals and the underlying mechanisms. In this study, we observed for the first time that both DEHP-H and DINP-H could cause major alterations in FA compositions (i.e., more than 12 out of 33 FAs affected) in plasma (Figure 1), white adipose tissue, and heart (Tables S4 and S5) and some lesser alterations (i.e., 3−11 out of 33 FAs

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Table 2. Plasma Total FA Compositions in Mice Treated with Control, DEHP, or DINP at Two Doses (0.048 or 4.8 mg/kg bw/d)[a]

	female					male				
metabolite	control	DEHP-L[b]	DEHP-H[c]	DINP-L[b]	DINP-H[c]	control	DEHP-L	DEHP-H	DINP-L	DINP-H
C14_0	1.39 ± 0.27	0.91 ± 0.55	1.29 ± 0.05	1.11 ± 0.26*	2.33 ± 0.71**	1.04 ± 0.22	0.86 ± 0.23	1.46 ± 0.20****	0.92 ± 0.12	2.33 ± 0.21****
C15_0	0.22 ± 0.03	0.14 ± 0.03***	0.21 ± 0.01	0.14 ± 0.03***	0.19 ± 0.03*	0.18 ± 0.03	0.13 ± 0.03**	0.21 ± 0.01**	0.13 ± 0.02**	0.20 ± 0.02
C16_0	26.2 ± 0.5	25.0 ± 0.8**	23.6 ± 0.9****	25.5 ± 1.4	26.2 ± 1.1	24.6 ± 0.74	24.4 ± 0.8	26.1 ± 1.2***	25.0 ± 0.8	26.6 ± 1.4**
C16_1/n-7	1.32 ± 0.18	0.82 ± 0.22****	1.13 ± 0.08**	0.77 ± 0.20****	1.33 ± 0.48	1.19 ± 0.21	0.86 ± 0.22**	1.14 ± 0.13	0.76 ± 0.14****	1.43 ± 0.35*
C18_0	11.0 ± 1.1	8.83 ± 0.95**	10.7 ± 0.5	10.0 ± 0.6*	11.0 ± 1.2	9.76 ± 0.99	8.57 ± 0.65**	11.5 ± 0.8***	9.33 ± 0.47	12.4 ± 0.8****
t18_1/n-7	0.27 ± 0.16	0.10 ± 0.11**	0.18 ± 0.03	0.09 ± 0.087**	0.12 ± 0.12**	0.18 ± 0.08	0.08 ± 0.05**	0.70 ± 0.41**	0.08 ± 0.05**	0.05 ± 0.01****
t18_1/n-9	0.16 ± 0.15	0.06 ± 0.08*	0.07 ± 0.01	0.05 ± 0.07*	0.07 ± 0.11	0.18 ± 0.11	0.06 ± 0.04***	0.33 ± 0.13**	0.09 ± 0.08*	0.05 ± 0.02***
C18_1/n-9	9.81 ± 1.95	14.0 ± 2.1***	11.5 ± 0.8*	10.9 ± 0.9	9.81 ± 0.73	12.8 ± 1.6	14.9 ± 1.2**	13.3 ± 2.1	11.7 ± 0.9*	8.34 ± 0.58****
C18_1/n-7	1.47 ± 0.16	1.85 ± 0.22**	1.56 ± 0.06*	1.56 ± 0.12*	1.41 ± 0.11	1.70 ± 0.19	1.84 ± 0.15*	1.63 ± 0.19	1.55 ± 0.12*	1.30 ± 0.04****
C18_2/n-6	27.4 ± 3.0	32.5 ± 2.3***	32.2 ± 1.0***	30.9 ± 2.7*	27.5 ± 1.8	32.2 ± 2.4	33.1 ± 1.9	31.9 ± 1.3	33.8 ± 1.5	25.2 ± 1.2****
C18_3/n-6	0.35 ± 0.07	0.20 ± 0.06****	0.31 ± 0.08	0.23 ± 0.05***	0.41 ± 0.19	0.24 ± 0.05	0.20 ± 0.03*	0.28 ± 0.07	0.21 ± 0.02*	0.44 ± 0.16**
C20_0	0.18 ± 0.04	0.10 ± 0.03**	0.27 ± 0.05***	0.10 ± 0.03****	0.14 ± 0.07*	0.24 ± 0.07	0.13 ± 0.06***	0.26 ± 0.05	0.13 ± 0.05***	0.17 ± 0.03***
C18_3/n-3	0.59 ± 0.19	0.37 ± 0.13**	0.94 ± 0.09***	0.34 ± 0.12**	0.55 ± 0.10	0.91 ± 0.28	0.50 ± 0.19***	0.92 ± 0.12	0.48 ± 0.22***	0.61 ± 0.04***
C20_1/n-9	0.31 ± 0.08	0.24 ± 0.05*	0.52 ± 0.12***	0.23 ± 0.06*	0.25 ± 0.10*	0.49 ± 0.17	0.30 ± 0.12**	0.25 ± 0.06****	0.29 ± 0.09**	0.24 ± 0.05****
C20_2/n-6	0.26 ± 0.03	0.23 ± 0.02*	0.36 ± 0.01****	0.29 ± 0.02*	0.39 ± 0.06****	0.25 ± 0.04	0.21 ± 0.03**	0.30 ± 0.06*	0.24 ± 0.03	0.44 ± 0.01****
C20_3/n-6	0.91 ± 0.20	0.65 ± 0.14**	0.84 ± 0.06	0.68 ± 0.13**	0.91 ± 0.22	0.68 ± 0.09	0.59 ± 0.06**	0.66 ± 0.15	0.62 ± 0.05	1.10 ± 0.26****
C22_0	0.15 ± 0.05	0.06 ± 0.04**	0.10 ± 0.01*	0.07 ± 0.04***	0.09 ± 0.02**	0.09 ± 0.04	0.04 ± 0.01***	0.11 ± 0.02	0.06 ± 0.03*	0.11 ± 0.02*
C20_4/n-6	11.9 ± 2.1	10.1 ± 1.3*	9.16 ± 0.52**	12.6 ± 1.5	11.3 ± 0.9	9.09 ± 1.94	9.82 ± 1.49	6.19 ± 2.27**	11.4 ± 1.5**	11.4 ± 0.7***
C22_1/n-9	1.06 ± 0.52	0.27 ± 0.35**	0.52 ± 0.11*	0.33 ± 0.35**	0.18 ± 0.08***	0.43 ± 0.16	0.18 ± 0.14**	0.25 ± 0.10**	0.16 ± 0.09****	0.16 ± 0.02****
C20_5/n-3	0.50 ± 0.10	0.25 ± 0.09***	0.60 ± 0.04*	0.30 ± 0.08***	0.57 ± 0.16	0.48 ± 0.13	0.29 ± 0.10***	0.55 ± 0.20	0.32 ± 0.05***	0.84 ± 0.08****
C24_0	0.11 ± 0.04	0.05 ± 0.03***	0.08 ± 0.01*	0.06 ± 0.02**	0.08 ± 0.02*	0.07 ± 0.03	0.04 ± 0.02*	0.13 ± 0.07*	0.05 ± 0.02*	0.07 ± 0.02
C22_4/n-6	0.21 ± 0.07	0.05 ± 0.06***	0.23 ± 0.02	0.05 ± 0.06***	0.20 ± 0.07	0.17 ± 0.06	0.06 ± 0.06**	0.12 ± 0.05*	0.05 ± 0.04****	0.33 ± 0.05****
C24_1/n-9	0.06 ± 0.01	0.05 ± 0.01	0.04 ± 0.00***	0.06 ± 0.01	0.04 ± 0.01**	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.05 ± 0.01*	0.03 ± 0.01
C22_5/n-3	0.68 ± 0.21	0.34 ± 0.14**	0.73 ± 0.08	0.48 ± 0.12*	0.97 ± 0.21*	0.43 ± 0.16	0.31 ± 0.09*	0.41 ± 0.37	0.36 ± 0.05	1.16 ± 0.11****
C22_6/n-3	3.40 ± 0.76	2.81 ± 0.44*	2.77 ± 0.16*	3.07 ± 0.43	3.90 ± 0.64	2.49 ± 0.92	2.56 ± 0.36	1.19 ± 1.01**	2.68 ± 0.45	4.86 ± 0.35****
total SFAs	39.3 ± 1.9	35.1 ± 1.9****	36.3 ± 1.4***	36.97 ± 1.41**	40.1 ± 1.6	36.0 ± 1.6	34.1 ± 1.2**	39.8 ± 1.9****	35.6 ± 0.7	42.0 ± 0.9****
total Monos	14.0 ± 1.7	17.2 ± 1.9****	15.3 ± 0.9*	13.9 ± 0.6	13.0 ± 0.7	16.7 ± 1.7	18.1 ± 1.2*	16.6 ± 2.3	14.5 ± 1.2**	11.5 ± 0.5****
total PUFAs	46.2 ± 1.8	47.5 ± 1.9	48.1 ± 0.7**	49.0 ± 1.35**	46.7 ± 1.3	47.0 ± 1.3	47.6 ± 1.7	42.5 ± 3.3**	49.8 ± 1.6***	46.4 ± 0.5
total Trans	0.43 ± 0.31	0.16 ± 0.19*	0.25 ± 0.04	0.14 ± 0.16**	0.19 ± 0.23**	0.36 ± 0.18	0.14 ± 0.09***	1.03 ± 0.50**	0.17 ± 0.13**	0.10 ± 0.03****
total N-3	5.17 ± 0.96	3.76 ± 0.64**	5.04 ± 0.26	4.19 ± 0.58*	6.00 ± 0.99	4.31 ± 1.04	3.66 ± 0.54	3.07 ± 1.37*	3.83 ± 0.33	7.47 ± 0.34****
total N-6	41.0 ± 2.0	43.7 ± 1.9*	43.1 ± 0.5*	44.8 ± 1.7**	40.7 ± 1.8	42.6 ± 1.3	43.9 ± 2.0	39.4 ± 2.1****	45.9 ± 1.5****	39.0 ± 0.8****
N6/N3	8.24 ± 1.75	11.9 ± 1.9***	8.58 ± 0.39	10.9 ± 1.8**	7.45 ± 1.89	10.5 ± 2.8	12.3 ± 2.1	15.3 ± 6.6	12.1 ± 1.1	5.23 ± 0.36****
DHA + EPA	4.08 ± 0.96	3.15 ± 0.55*	3.50 ± 0.20	3.55 ± 0.54	4.87 ± 0.81	2.92 ± 1.07	2.87 ± 0.41	1.60 ± 1.38*	3.03 ± 0.49	6.03 ± 0.42****

[a]Nonparametric Wilcoxon−Mann−Whitney test was performed to compare the differences between control and treatment groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$. Data are presented as mean ± standard deviation. SFAs, saturated FAs; Monos, monounsaturated FAs; PUFAs, polyunsaturated FA; Trans, trans FAs. [b]Low dose = 0.048 mg/kg bw/d. [c]High dose = 4.8 mg/kg bw/d.

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affected) in liver (Table S6), whereas no significant changes were observed in kidney and brain (Figure S1). The alterations also exhibited a dose- and gender-specific manner which differs between chemicals.

Plasma. DEHP and DINP induced significant disturbances in plasma total FA compositions at both the high and low doses, but the effects differed between genders and doses (Figure 1). DEHP-H significantly decreased SFA composition but increased the PUFA content in female pups, while it significantly decreased the PUFA content and increased the Trans level in males. By contrast, DINP-H did not change FA compositions in female pups with the exception for Trans but significantly altered the compositions of SFAs, PUFAs, and Trans in male pups. In particular, DINP-H significantly altered the N-6/N-3 ratios and DHA + EPA (long-chain N-3) percentages in male pups only, whereas no change was observed in pups of either sex during DEHP-H treatments. It has been clear that the long-chain N-3 FAs are protective in cardiovascular diseases, cancers (including breast, colorectal, prostate, and others), asthma, and inflammatory diseases in the bowel and joints.[34] Maintaining a balanced plasma ratio of total N-6/N-3 has been demonstrated to be essential in modulating inflammatory activities, cancers, and rheumatoid arthritis.[35] Although increased N-6 can be beneficial in reducing low-density lipoprotein cholesterol (LDL-C, a major risk factor for coronary heart diseases),[36] the elevation in N-6 would compete with N-3 for essential enzymes including elongase and desaturase and consequently result in diminished N-3. Our results clearly demonstrate that male mice could be more effectively impacted by DINP than females when exposed to a high dose, whereas both genders could be affected by DEHP with respect to FA compositions. Gender-specific alteration of FA compositions, particularly the more selective effects on males following DINP-H exposure, implies that DINP could potentially cause stronger antiandrogenic or estrogenic effect on the endocrine system than the commonly recognized endocrine disrupting chemical DEHP.

Effects on FA compositions are also dose-dependent for each chemical (Table 2). Unlike DINP-H which appeared to exhibit more selective effects on the males, DINP-L altered FA composition in both genders. DINP-L altered the compositions of Monos, PUFAs, and Trans in males and SFAs, PUFAs, and Trans in female pups. Similarly, DEHP-L also exhibited impacts on both genders, but the alteration pattern differed between the high and low doses. Compared with DEHP-H, DEHP-L caused more significant changes in SFAs, Monos, and Trans, but no change was observed in PUFAs in female pups. In particular, the N-6/N-3 ratios were significantly increased by DEHP-L, suggesting that following exposure the high levels of N-6 out-competed N-3 for essential enzymes required for PUFA elongation and desaturation,[35] whereas such an alteration was not observed under DEHP-H exposure. In male pups, DEHP-L and DEHP-H even caused opposite alterations of PUFAs (i.e., increase under the low dose but decrease under the high-dose exposure). The different sensitivities to low and high doses of DINP have also been reported previously in zebrafish models.[21,22] For example, Forner-Piquer et al. reported that exposure to the low and medium doses of DINP (0.42 and 4.2 μg/L, respectively) significantly disrupted the expression of selected genes (e.g., *Fasn* and *Agpat*-4) related to lipid metabolism, whereas the high dose (42 μg/L) did not influence their expressions compared with the controls.[21] For other genes (e.g., *Acat-2*, *Hnf4a*, and *Lepr*) in the same experiments, their expressions were downregulated by all three doses.[21] These findings, along with ours, indicated that DINP and DEHP could induce nonmonotonic disruption of lipid metabolism homeostasis in different model species.

Plasma FA composition alteration is typically attributed to the change of dietary intake,[37] as well as lipid transportation or metabolic processes.[38] Because diet was controlled under the same conditions in our experiments, the change of plasma total FA composition was more likely due to the alteration of FA's incorporation into lipoproteins and FA metabolism. Changes in plasma FA compositions could have important clinical implications.[39,40] Increased levels of SFAs and decreased levels of Monos and/or PUFAs in plasma, as observed as a result of DINP-H and DEHP-H exposure in males, have been demonstrated to be associated with heightened risks of many metabolic syndromes, including obesity, type II diabetes, and CHDs.[9,40] Elevation in Monos and PUFAs, however, has been linked to protective effects including improved cardiovascular and metabolic functions.[9] Increase in the N-6/N-3 ratio, as observed following DEHP-L or DINP-L exposure in female pups, has been well demonstrated to be a risk factor for obesity through the mechanism of adipogenesis, brain−gut−adipose tissue axis, and overall inflammation.[35] Indeed, increases in body weights or abdominal circumferences have been observed in female pups following exposure to DEHP-L or DINP-L in the present study.

Heart and White Adipose Tissues. In the heart tissue, DEHP-H caused increase in total unsaturated FAs in female pups but decrease in males, whereas DINP-H induced elevation in total unsaturated FAs in both genders. DEHP-L showed the same trend of desaturation as that of DEHP-H in females but had little influence on male pups. Compared with DEHP-L, DINP-L posed a more potent effect on the majority of FA, inducing FA desaturation in both genders (Table S5). Increases in PUFAs and decreases in SFAs are associated with an elevation of LDL-C but reduction in the high-density lipoprotein cholesterol (HDL-C, the antiatherogenic lipoprotein).[40] Long-term stress with elevated LDL-C and reduced HDL-C constitutes a major risk factor for cardiovascular events.[41] Therefore, the long-term effect of early-life stage exposure to DEHP and DINP on cardiovascular risks merits further evaluations.

DINP exposure failed to induce major alterations of FA composition in reproductive white adipose tissues of male pups under both doses but caused FA desaturation in females, although at a lesser extent than that observed in the heart. This is opposite to the more selective effects on males occurring in plasma. Pups exposed to DEHP-H contained significantly reduced levels of SFAs but heightened Monos and/or PUFAs, primarily N-6 FAs, in white adipose tissues of both genders. By contrast, DEHP-L did not cause changes in major FA compositions in both genders (Table S4). FA composition in the white adipose tissue is dominantly affected by diet and also affected by endogenous synthesis of SFAs and Monos, as well as the catabolism of PUFAs at a lesser degree.[42] These FA changes could result in the promotion of adipose tissue fat storage and suppression of insulin-mediated fat mobilization, leading to increased energy storage and risks of insulin resistence.[43]

Liver, Kidney, and Brain. DINP and DEHP exhibited very little impact on liver's major FA compositions in both genders under the two investigated doses, except that DEHP-H

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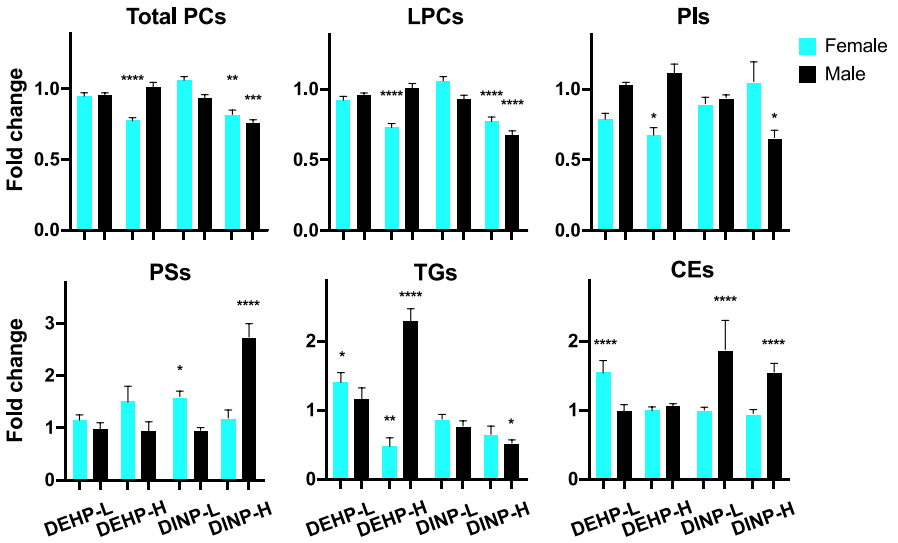

Figure 2. Changes in major plasma lipid classes in male and female mice neonatally exposed to low (0.048 mg/kg bw/day) or high (4.8 mg/kg bw/day) doses of DEHP or DINP compared with the control groups (N = 10 for each group). Data are presented as mean with standard error of the mean. The vertical axis shows the concentration fold changes to the mean values of control groups for their respective gender. Statistical analyses were conducted using the Kruskal–Wallis test, followed by the Dun's post-hoc test to compare each with its control. Asterisks denote statistical significance: *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001. Abbreviations for lipid classes: phosphatidylcholine (PC), lyso PC (LPC), phosphatidylinositol (PI), phosphatidylserines (PS), triglycerides (TG), and cholesterols (CE).

induced minor but significant changes of SFAs, Monos, and PUFAs in female pup liver. This is in agreement with our oil red O stained histological analysis in liver tissues which did not exhibit hepatic steatosis (Figure S4). Moreover, liver mRNA expression data indicated that transcription factors and key rate-limiting enzymes regulating metabolism, including *Pparα*, *Pparβ*, *Pparγ*, *Lpl*, *Fas*, *Scd1*, *Elovl6*, *Acox-1*, *Elovl2*, *Fads1*, and *Fads2*, were altered and the alteration varied between chemicals or doses (Figure S5). Specifically, the upregulation of *Pparα* and *Acox-1* in both DEHP-H and DINP-H treatments agreed with our observation on decreased body weights, as both enzymes could promote lipid catabolism including β-oxidation, resulting in increased energy expenditure. Interestingly, a downregulation of *Pparγ* expression, a transcription factor regulating cell differentiation and adipogenesis,[44] was observed in the DINP-L treatments, while no alteration was observed in any other treatments. The nonactivation of *Pparγ* may partially explain the lack of hepatic steatosis as observed in the oil red O staining analysis because no cell differentiation or adipogenesis was upregulated. The downregulation of the expressions of *Fas* and *Elovl6* is in line with the reduction in Monos observed in DINP-H pups (Figure 1), while the upregulation of *Fads1* and *Fads2* expressions was seen in DEHP-treated pups where an elevation of PUFA compositions was observed. These data provide further evidence on the alterations in FA compositions determined in treated mice.

Additionally, we found no changes in major FAs in kidney and brain (Figure S1). Previous studies reported kidney and liver damage in Kunming mice following DINP exposure, but the histological toxicities were only observed under much higher daily doses (i.e., 20–200 mg/kg bw/d) than our study.[7] The study also reported increases in oxidative stress, which resulted in lipid perioxidation in both liver and kidney tissues, suggesting that exposure to high doses of DINP could cause increased FA oxidation. This is in agreement with the report on zebrafish that hepatic *Pparα* expression, not *Pparγ*, was disrupted as a result of DINP exposure.[21] Therefore, it is deduced that DINP could cause FA composition alteration through catabolism but only at relatively high doses.

Although minor but significant reductions in male brain weight was observed during DEHP-H treatment, there were no significant differences in brain FA compositions following DEHP or DINP exposure (Figure S1). This suggests that DEHP or DINP unlikely affects FA metabolism or uptake in brain under the investigated doses. However, one should not overextrapolate the null effect on neurodevelopment or cognitive function. Previous data suggested that gestational exposure to DEHP caused impaired cognitive function in neonatal Sprague Dawley rats at a dose twice the high dose used in our study.[45] More work is needed to understand the causations of these phthalate chemicals, if any, on neurodegeneration.

Gender-Specific Effects on Plasma Lipidome. Different FAs would compete for the incorporation in individual plasma lipid classes, lipoproteins, and red blood cells in blood, a feature that determines some of the characteristics of FA distribution in lipid pools.[46] Understanding such lipidomic profiles has important biological significance as each of these lipid classes is involved in different biological functions and pathogenesis of diseases. For example, PE plays an important physiological role in mediating multiple enzymes, including ethanolamine-phosphate cytidylyltransferase[47] and phosphatidylethanolamine *N*-methyltransferase,[48] while imbalances of PE can cause mitochondrial malfunctions and nonalcoholic fatty liver diseases (NAFLD).[11] PS are also important in the pathogenesis of NAFLD, while disruption in ceramide (Cer) and glucosylceramide equilibrium could induce insulin resistance and NAFLD.[49,50] Moreover, it has been demonstrated that TG and cholesterols (CE) are the major contributors to dysfunctional lipid metabolism, which may lead to many metabolic disorders.[11]

Given that both DEHP and DINP could induce gender-specific changes in plasma total FA composition, we further

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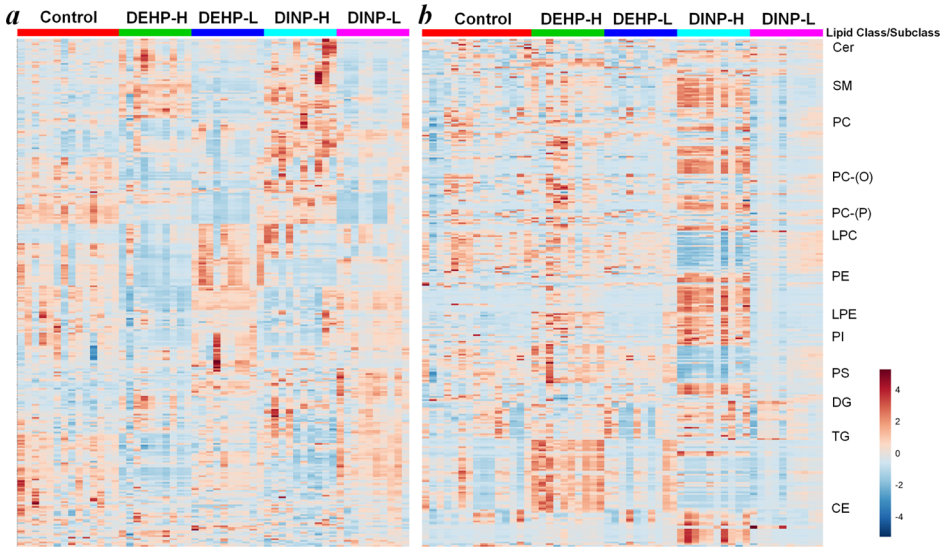

Figure 3. Heat map of plasma lipidomic metabolites in the groups treated with control, low (0.048 mg/kg bw/day), or high (4.8 mg/kg bw/day) doses of DEHP or DINP based on the *z* scores determined in both female (a) and male pups (b). Abbreviations for lipid classes: ceramide (Cer), sphingomyelin (SM), phosphatidylcholine (PC), phosphatidylcholine alkyl ether (PC-(O)), phosphatidylcholine plasmalogen (PC-(P)), lyso PC (LPC), phosphatidylethanolamines (PE), lyso PE (LPE), phosphatidylinositol (PI), phosphatidylserines (PS), diglyceride (DG), triglycerides (TG), and cholesterols (CE).

explored plasma lipidomic alterations using the targeted metabolomic approach, which measured 24 lipid classes (and subclasses) with a total of 363 lipid metabolites (details are given in Figure S2 and Tables S2 and S3). These lipid classes included Cer, sphingomyelin, PC, PC alkyl ether (PC-(O)), PC plasmalogen (PC-(P)), lyso PC (LPC), PE, lyso PE, PI, PS, PG, diglyceride, TG, and CE, as well as other groups.

Both DEHP and DINP caused sexually dimorphic changes in a significant number of lipid species but with different patterns (Figures 2 and 3, see Table S7 for detailed comparisons). DEHP-H exposure induced decreases in PC, PC-(O), PC-(P), LPC, and PIs in female pups, whereas no effects on these lipid classes were observed in males. Conversely, there were significant increases in PE and TG in males but none in female pups. By contrast, DINP-H caused alterations of PE, PI, PS, and CE only in male mice, but no changes were observed in their female counterparts. This result is in good agreement with the above plasma FA composition data, which showed that at the higher dose DINP selectively induced significant FA composition change in male mice but lesser effects on the females. In low-dose treatment groups, DEHP-L prompted lipid changes predominantly in TG and CE for female pups only, whereas DINP-L induced PS elevation only in female pups and higher concentrations of CE exclusively in male mice. The increases in TG and CE provide additional evidence for the obesogenic effect of DEHP-L and DINP-L observed in our work along with other studies.[14,29−31] This is because dyslipidemia, including increased plasma TG, particularly the very-low-density lipoprotein TG (VLDL-TG), is associated with the change of plasma LDL and HDL lipid composition, which further leads to abdominal obesity.[14,30,31] Elevated plasma LDL-C and reduced HDL-C can also be linked to the aggregation of atherogenic remnants and overabundance of liver apolipoprotein B containing lipoproteins. This could further provoke proinflammatory responses, insulin resistance, and subsequent metabolic diseases.[51−53]

The increased ratios of PE/PC, as observed following DEHP-H and DINP-H exposure, are important features of NAFLD,[54] while changes in PS are linked to mitochondrial dysfunctions.[47] Although we did not observe NAFLD in both treatment groups, DINP-treated mice did show heightened hepatic TBA concentrations in the males (Table 1), further indicating possible occurrence of metabolic dysfunctions.

In our endeavor to identify important biomarkers responsive to phthalate exposure, we identified a number of specific lipids by partial least squares-discriminant analysis (PLS-DA), among which five lipid species had variable importance in projection scores greater than five. LPC 18:0 and TG 18:2/18:2/18:2 were responsive to both DEHP and DINP, and higher doses tend to cause more potent alterations. The CE 20:1 demonstrated responses only in low-dose groups, whereas CE 18:1 and PI 38:4 showed phthalate-specific alterations for DINP and DEHP, respectively. Following the same rationale, we also identified other lipid molecules with the biomarker potential, including PUFAs, DHA + EPA, total N-3, and C22_6/n-3 in adipose tissues and Monos, SFAs, C14_0, and N6/N3 in heart tissues. These lipid species could potentially serve as biomarkers for investigating lipid-related diseases following exposure to DEHP, DINP, or other related endocrine disrupting chemicals. However, additional studies are needed to verify their biomarker roles.

We performed two-way ANOVA for FA data in order to investigate the interactions between gender and treatment groups. These results suggested that gender could play some roles in the effects of DEHP and DINP on plasma FA compositions. In addition to the effect of different treatments on FAs, gender alone seemed to have effect on some plasma FAs including Monos and PUFAs but not on SFAs. Such analyses provide further indication that the disruptions in FAs induced by different phthalate treatments may also be affected by gender. It remains unclear on the mechanisms underlying the differences observed in lipidomic profile changes between male and female pups following neonatal exposure. It is possible that the gender-specific effects may be a consequence

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of disruption in estrogen receptor α (ERα) and proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α). Both receptors have been demonstrated to be essential part of the hypothalamic-signaling network.[55] ERα, as a nuclear receptor activated by the sex hormone estrogen, is critical for the regulation of lipid metabolism in males through the modulation of acetyl-CoA carboxylase (*Acc1*) and FA synthase (*Fas*) and the activation of *ESR1* binding to *Pck-1*, *G6Pase*, *Fas*, and *Acc1* promoters.[56] It is plausible to speculate that the above-mentioned receptors and promoters could be disrupted because of the strong estrogenic effects of the investigated phthalates on male mice over a wide range of doses.[2] This can further result in dysregulated gene expression on lipid metabolism and subsequently the observed discrepancies in lipidomic perturbation predominantly in male pups. Further work is needed to elucidate the dysregulation of key hormonal pathways, particularly in relation to lipid metabolism.

We would also like to point out the limitations of the present study. First, although urinary analysis revealed no difference in metabolite concentrations between genders, the potential influence of gender-specific accumulation, distribution, metabolism, and elimination (ADME) patterns on gender- and tissue-specific lipid metabolism cannot be completely excluded. The ADME should be taken into consideration for future elucidation of lipid metabolism imbalance following exposure. Second, only two doses were considered in our study, whereas lower but more environmentally relevant doses should be considered in future studies, particularly for the exploration of long-term exposure. Third, only plasma was investigated for lipidomic analysis. Other tissues should also be utilized in order to reveal more useful information and potential biomarkers.

Health Implications. Lipids play essential roles in the etiology of most, if not all, of the metabolic diseases. By conducting detailed assessment of FA composition in different tissues and plasma lipidomic profiles, our study provides an important basis in the understanding of the long-term toxicity of DEHP and DINP through the modulation of lipid biosynthesis and metabolism. Several important implications from the present study are summarized below.

In this study, we have identified that DEHP and DINP exposure could cause nonmonotonic dysregulation of lipid metabolism in a gender-specific and tissue-specific pattern. The nonmonotonic action indicates that low doses could still disrupt lipid profiles, and sometimes, the effects may be more harmful in certain tissues than those induced by high doses. Our findings on the gender- and tissue-specific patterns are also significant in that previous investigations have mainly focused on the reproductive function aspect of gender specificity, whereas other organs and tissues have been deemed of having unisexual functions. Our data revealed the gender-specific alteration of FA compositions in plasma, heart, and white adipose tissues following DEHP or DINP exposure, indicating gender-dependent health risks. In particular, our findings on the gender-specific alteration of lipidomic profiles upon DINP exposure suggest that compared with the females, males are subjected to greater risks of lipid metabolism perturbation following early-life stage exposure to DINP and increased chances of developing health conditions, such as insulin resistance, type II diabetes, obesity, and cardiovascular events, in later lives.[40] Therefore, future studies should take gender and tissue into consideration in order to better characterize the risks associated with lipid metabolism dysregulation following exposure to anthropogenic chemicals.

Our findings also reveal that neonatal exposure to DEHP or DINP at a dose lower than TDI could still exert adverse effects on lipid metabolism. This can be significant because these effects on lipid dysregulation could become risk factors for developing chronic metabolic diseases including obesity (as evidenced in our study), type II diabetes, and cardiovascular diseases. Indeed, the high occurrence of phthalate exposure is concurrent with the growing epidemic of chronic metabolic syndromes.[33,57] Further investigations are critically needed to better elucidate the long-term health effects of low-dose phthalate exposure and underlying mechanisms via both animal and human cohort studies.

Thorough lipidomic analyses may facilitate the identification of certain lipid species potentially as biomarkers of exposure to endocrine disrupting chemicals. Given that some of these lipid biomarkers may be tightly associated with many diseases such as metabolic syndromes, changes in these biomarkers can be used to predict potential risks of developing these diseases.

Additionally, our data raise questions on the suitability of using DINP as an industrial substitute to DEHP. Similar to DEHP, DINP can also disrupt FA composition and lipidomic profiles following neonatal exposure, although the two chemicals differ in disruption patterns. Compared with DEHP, DINP also exhibits more selective and stronger impacts on male pups than on the females, indicating the more susceptibility of males to DINP exposure. Thus, compared with DEHP, DINP exposure may induce comparable or even greater risks associated with lipid metabolism in males, such as hormonal dysregulation, cell signaling and mitochondrial disruption, and even metabolic diseases. These findings indicate that DINP is not a safe substitute for DEHP. Its nonmonotonic action, as well as tissue- and gender-specific effects, on lipid regulation provides an important message to both consumers and decision-makers when evaluating its environmental and human health risks. Future studies are needed to explore the underlying mechanisms of lipid metabolism disruption from DINP exposure and potential long-term effects on metabolic syndromes following the interruption of lipid metabolism homeostasis.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.9b04369.

Detailed method description for FA and lipidomic analyses (PDF)

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