



Prenatal Exposure to Chemical Mixtures and Metabolic Syndrome Risk in Children

Nuria Güil-Oumrait, MSc; Nikos Stratakis, PhD; Léa Maitre, PhD; Augusto Anguita-Ruiz, PhD; Jose Urquiza, PhD; Lorenzo Fabbri, MSc; Xavier Basagaña, PhD; Barbara Heude, PhD; Line Småstuen Haug, PhD; Amrit Kaur Sakhi, PhD; Nina Iszatt, PhD; Hector C. Keun, PhD; John Wright, PhD; Leda Chatzi, PhD; Marina Vafeiadi, PhD; Mariona Bustamante, PhD; Regina Grazuleviciene, PhD; Sandra Andrušaitytė, PhD; Rémy Slama, PhD; Rosemary McEachan, PhD; Maribel Casas, PhD; Martine Vrijheid, PhD

Abstract

IMPORTANCE Prenatal exposure to ubiquitous endocrine-disrupting chemicals (EDCs) may increase the risk of metabolic syndrome (MetS) in children, but few studies have studied chemical mixtures or explored underlying protein and metabolic signatures.

OBJECTIVE To investigate associations of prenatal exposure to EDC mixtures with MetS risk score in children and identify associated proteins and metabolites.

DESIGN, SETTING, AND PARTICIPANTS This population-based, birth cohort study used data collected between April 1, 2003, and February 26, 2016, from the Human Early Life Exposome cohort based in France, Greece, Lithuania, Norway, Spain, and the UK. Eligible participants included mother-child pairs with measured prenatal EDC exposures and complete data on childhood MetS risk factors, proteins, and metabolites. Data were analyzed between October 2022 and July 2023.

EXPOSURES Nine metals, 3 organochlorine pesticides, 5 polychlorinated biphenyls, 2 polybrominated diphenyl ethers (PBDEs), 5 perfluoroalkyl substances (PFAS), 10 phthalate metabolites, 3 phenols, 4 parabens, and 4 organophosphate pesticide metabolites measured in urine and blood samples collected during pregnancy.

MAIN OUTCOMES AND MEASURES At 6 to 11 years of age, a composite MetS risk score was constructed using z scores of waist circumference, systolic and diastolic blood pressures, triglycerides, high-density lipoprotein cholesterol, and insulin levels. Childhood levels of 44 urinary metabolites, 177 serum metabolites, and 35 plasma proteins were quantified using targeted methods. Associations were assessed using bayesian weighted quantile sum regressions applied to mixtures for each chemical group.

RESULTS The study included 1134 mothers (mean [SD] age at birth, 30.7 [4.9] years) and their children (mean [SD] age, 7.8 [1.5] years; 617 male children [54.4%] and 517 female children [45.6%]; mean [SD] MetS risk score, -0.1 [2.3]). MetS score increased per 1-quartile increase of the mixture for metals ($\beta = 0.44$; 95% credible interval [CrI], 0.30 to 0.59), organochlorine pesticides ($\beta = 0.22$; 95% CrI, 0.15 to 0.29), PBDEs ($\beta = 0.17$; 95% CrI, 0.06 to 0.27), and PFAS ($\beta = 0.19$; 95% CrI, 0.14 to 0.24). High-molecular weight phthalate mixtures ($\beta = -0.07$; 95% CrI, -0.10 to -0.04) and low-molecular weight phthalate mixtures ($\beta = -0.13$; 95% CrI, -0.18 to -0.08) were associated with a decreased MetS score. Most EDC mixtures were associated with elevated proinflammatory proteins, amino acids, and altered glycerophospholipids, which in turn were associated with increased MetS score.

(continued)

Key Points

Question Is prenatal exposure to mixtures of endocrine-disrupting chemicals (EDC) associated with metabolic dysfunction in children?

Findings In this cohort study of 1134 mother-child pairs from 6 European countries, prenatal exposures to EDC mixtures, including metals, organochlorine pesticides, polybrominated diphenyl ethers, and perfluoroalkyl substances, were associated with increased metabolic syndrome risk score and altered proinflammatory proteins, amino acids, and glycerophospholipid levels in childhood.

Meaning These results suggest that exposure to widespread EDC mixtures in pregnancy may be associated with adverse metabolic health in children and contribute to the ongoing surge of metabolic syndrome across the life course.

+ Invited Commentary

+ Supplemental content

Author affiliations and article information are listed at the end of this article.

Abstract (continued)

CONCLUSIONS AND RELEVANCE This cohort study suggests that prenatal exposure to EDC mixtures may be associated with adverse metabolic health in children. Given the pervasive nature of EDCs and the increase in MetS, these findings hold substantial public health implications.

JAMA Network Open. 2024;7(5):e2412040. doi:10.1001/jamanetworkopen.2024.12040

Introduction

Metabolic Syndrome (MetS) represents a cluster of multiple factors associated with increased risk for cardiovascular diseases and type 2 diabetes (T2D), including abdominal obesity, hypertension, insulin resistance and dyslipidemia, affecting 1 in 4 adults worldwide.¹ Pediatric MetS prevalence ranges from 2% to 10%,² with individual risk factors on the rise.³⁻⁵ Childhood MetS has shown great utility in predicting adult MetS, T2D, and cardiovascular disease,⁶⁻⁸ even better than individual MetS components.⁹

Exposure to endocrine-disrupting chemicals (EDCs) during fetal development, a critical period of increased susceptibility and programming, may increase the risk of MetS later in life.¹⁰ EDCs are a class of environmental pollutants with the ability to cross the blood-placenta barrier and interfere with human metabolism and hormonal balance.¹⁰ These include pesticides, metals, plasticizers such as phthalates and phenols, and other widely used chemicals, including perfluoroalkyl substances (PFAS).¹⁰

While previous research has examined associations of prenatal EDC exposure with separate components of MetS in children,^{11,12} comprehensive studies on overall cardiometabolic risk remain limited.¹³⁻¹⁸ Additionally, only a few studies have assessed the mixture effects of selected classes of EDCs,¹⁶⁻¹⁸ despite widespread exposure to chemical mixtures. Finally, although some associations of prenatal EDC exposure with protein and metabolic signatures in childhood have been described,^{19,20} the internal phenotypes associated with EDC mixtures and underlying MetS pathogenesis are poorly understood.

We utilized a multicenter cohort of 1134 mothers and their children aged 6 to 11 years to conduct the most comprehensive study to date of the association of prenatal EDC mixtures from 9 chemical classes with child MetS risk score. Furthermore, we aimed to identify associated protein and metabolic signatures to unravel underlying mechanisms and altered metabolic pathways.

Methods

Study Population

This cohort study used data from the Human Early Life Exposome (HELIX) project and followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline. The HELIX Project is a collaboration between 6 ongoing European longitudinal population-based cohort studies²¹: Born in Bradford (BiB [UK]),²² Étude des Déterminants Pré et Postnataux du Développement et de la Santé de l'Enfant (EDEN [France]),²³ Infancia y Medio Ambiente (INMA [Spain]),²⁴ Kaunas Cohort (KANC [Lithuania]),²⁵ Norwegian Mother, Father, and Child Cohort Study (MoBa [Norway]),²⁶ and Mother and Child Cohort in Crete (RHEA [Greece]).²⁷ Approval for the HELIX project was obtained from local ethics committees in each country, and all participating families provided written informed consent. Pregnant women across cohorts were recruited between April 1, 2003, and January 30, 2009. From December 1, 2013, until February 26, 2016, a subcohort of 1301 mother-child pairs was followed-up when the children were aged 6 to 11 years using standardized protocols for clinical examination, interview, and biological sample collection.²⁸ Details about the protocol and subcohort inclusion criteria are described elsewhere.²⁸

This study included mother-child pairs with measured prenatal EDC exposures and complete data on childhood MetS risk factors, proteins, and metabolites (eFigure 1 in [Supplement 1](#)).

EDCs Exposure Assessment

EDC levels were measured in maternal serum, plasma, whole blood, and urine samples collected during pregnancy or cord blood at birth (eTable 1 in [Supplement 1](#)).²⁹ A total of 45 compounds from 9 chemical classes were analyzed: 9 metals; 3 organochlorine (OC) pesticides; 5 polychlorinated biphenyls (PCBs); 2 polybrominated diphenyl ethers (PBDEs); 5 PFASs; 7 high-molecular-weight phthalate metabolites (HMWPs), including 4 diethylhexyl phthalate (DEHP) metabolites, 2 di-isononylphthalate (DiNP) metabolites, and 1 metabolite of butyl benzyl phthalate; 3 low-molecular-weight phthalate metabolites (LMWPs); 4 parabens; 3 phenols; and 4 organophosphate (OP) pesticide metabolites. Persistent organic pollutants (OC pesticides, PCBs, PBDEs, and PFAS) and metals were determined in maternal blood except for total mercury which was measured in cord blood in the INMA cohort. Lipophilic compounds (OC pesticides, PCBs, and PBDEs) were corrected for plasma or serum lipid content and expressed in nanograms per gram of lipids. Nonpersistent EDCs (phthalates, parabens, phenols, and OP pesticides) were measured in a spot maternal urine sample, corrected for urine creatinine levels to account for urine dilution and expressed in micrograms per gram of creatinine. Details on laboratories and analytical methods are in eTable 2 in [Supplement 1](#). Quality control, interlaboratory comparison, and limit of detection for each laboratory are available elsewhere.²⁹ Values below the limit of detection (0%-30%) were singly imputed using a quantile regression approach for the imputation of left-censored missing data with the R statistical software version 4.3.2 rexposome package (R Project for Statistical Computing).³⁰ This fill-in estimation method offers the advantage of inserting values between 0 and the limit of detection while preserving the shape of a normal distribution.

MetS Risk Score

We calculated a continuous MetS risk score using the score previously validated for children by the European Multicenter Identification and Prevention of Dietary and Lifestyle-Induced Health Effects in Children and Infants study.³¹ Further details are in the eMethods in [Supplement 1](#). We applied the following formula to build the MetS risk score:

$$z\text{-waist circumference} + (-z\text{ high-density lipoprotein cholesterol} + z\text{-triglycerides})/2 + z\text{-insulin} + (z\text{-systolic blood pressure} + z\text{-diastolic blood pressure})/2,$$

where z refers to the standardized residuals. A higher score indicates a higher risk of developing MetS.

Child Metabolites and Proteins

We used targeted methods to assess metabolite and protein levels in child urine and blood samples collected at the same follow-up visit. Metabolites were assessed at the Imperial College of London (London, UK). A total of 44 urinary metabolites were characterized with ¹H nuclear magnetic resonance spectroscopy. Serum metabolites were quantified with the liquid chromatography-mass spectrometry metabolomic assay AbsoluteIDQ p180 kit (Biocrates), allowing for the analysis of 177 metabolites, including amino acids, biogenic amines, acylcarnitines, glycerophospholipids, sphingolipids, and sum of hexoses. A total of 35 plasma proteins were determined with 3 Luminex multiplex assays: Cytokines 30-plex, Apolipoprotein 5-plex, and Adipokine 15-plex (University Pompeu Fabra Centre for Genomic Regulation Proteomics Unit, Barcelona, Spain). Details about the assessment of children's metabolites and proteins are available in eAppendix 1 in [Supplement 1](#) and elsewhere.²⁰

Statistical Analysis

Maternal EDCs and child protein and metabolites were log₂-transformed to correct skewed distributions. Missing data for all exposures and covariates (0%-54%; eTable 3 in [Supplement 1](#)) were

imputed using multiple imputations by chained equations, generating 20 imputed data sets, which were combined using Rubin rules in all the subsequent analyses.³² Further imputation details are available elsewhere.³³

Adjusted generalized additive models confirmed no departures from linearity between individual EDCs and MetS risk score (eFigure 2 and eFigure 3 in Supplement 1). We used bayesian weighted quantile sum (BWQS) regressions to assess associations of mixtures of EDCs belonging to each chemical class with MetS score. BWQS estimates a single weighted index summarizing overall exposure to the mixture considering the relative contribution of each exposure within the group mixture using weights.³⁴ BWQS characteristics are available in eAppendix 2 in Supplement 1. BWQS regressions were stratified by sex due to potential sex-specific effects of some EDCs.³⁵⁻³⁷ We note that sex interactions were not tested due to the absence of interaction testing functions in the BWQS package. Sensitivity analyses to ensure results robustness included (1) single-exposure analyses using linear regressions, correcting for multiple testing with false discovery rate (FDR) and assessment of between-cohort heterogeneity using the I^2 statistic of association³⁸; (2) mixture analyses for lipophilic compounds, stratifying mothers by gestational weight gain according to the Institute of Medicine guidelines³⁹; (3) phthalate mixture analysis, incorporating molar sums of DEHP and DiNP metabolites; (4) metal and persistent mixture analysis, including sum of PCBs, and nonpersistent mixture analysis, incorporating molar sums of DEHP, DiNP, and parabens; and (5) testing main significant mixture associations with a binarized MetS risk outcome, using the 80th percentile as the cutoff.

To identify proteins and metabolites associated with both chemical mixtures and MetS, we first fitted generalized linear regression models between each molecular feature and the MetS risk score, correcting for multiple testing using FDR. Subsequently, we performed BWQS regressions between EDC classes and each associated molecular feature with an FDR P value < .05. BWQS model corrections for multiple testing were computed using P values derived from bayesian probability of direction, following Makowski et al.⁴⁰ All analyses were conducted in R version 4.3.2.

All statistical models were adjusted for the confounders selected based on previous knowledge and a directed acyclic graph (eFigure 4 in Supplement 1), including subcohort, parental country of birth (both parents native, none or 1 parent native), maternal age, self-reported prepregnancy body mass index (BMI; calculated as weight in kilograms divided by height in meters squared), maternal educational level, parity, maternal smoking in pregnancy, and fish intake in pregnancy. Statistical analysis occurred from October 2022 to July 2023.

Results

Our study comprised 1134 mother-child pairs (mean [SD] maternal age, 30.7 [4.9] years; 517 female children [45.6%] and 617 male children [54.4%]) (Table). Of all mothers, 574 (50.6%) were highly educated and 506 (44.6%) were nulliparous. The mean (SD) age of children was 7.8 (1.5) years at outcome assessment. The mean (SD) MetS score was -0.1 (2.3), with 341 children (30.1%) classified as high-risk. Maternal prepregnancy BMI was associated with higher child MetS score, and there was some variation in MetS score by cohort (eTable 4 in Supplement 1). Prenatal EDC concentrations are detailed in eTable 5 in Supplement 1. Pearson correlations indicated positive moderate to high correlation within each EDC class, with a few negative correlations within the metals class (eFigure 5 in Supplement 1).

Mixture analyses showed increased MetS risk score per 1-quartile increase in prenatal EDC mixture for metals ($\beta = 0.44$; 95% credible interval [CrI], 0.30 to 0.59), OC pesticides ($\beta = 0.22$; 95% CrI, 0.15 to 0.29), PBDEs ($\beta = 0.17$; 95% CrI, 0.06 to 0.27), and PFAS ($\beta = 0.19$; 95% CrI, 0.14 to 0.24); while HMWPs and LMWPs were associated with decreased MetS risk score (β for HMWPs = -0.07; 95% CrI, -0.10 to -0.04; β for LMWPs = -0.13; 95% CrI, -0.18 to -0.08) (Figure 1A and eTable 6 in Supplement 1). No association was observed for mixtures of PCBs, phenols, parabens, and OP pesticide metabolites (Figure 1A and eTable 6 in Supplement 1). The main contributor to the metal

mixture association was mercury (weight, 0.33). Hexachlorobenzene (HCB; weight, 0.51) and perfluorononanoic acid (PFNA; weight, 0.48) were the primary contributors in the OC pesticides and PFAS mixture associations. Within the LMWP mixture, mono-n-butyl phthalate (MnBP) showed the highest contribution (weight: 0.48), while chemicals within PBDEs and HMWPs had similar weights within each group (Figure 1B and eTable 7 in Supplement 1).

Table. Characteristics of the Human Early Life Exposome Subcohort Study Population

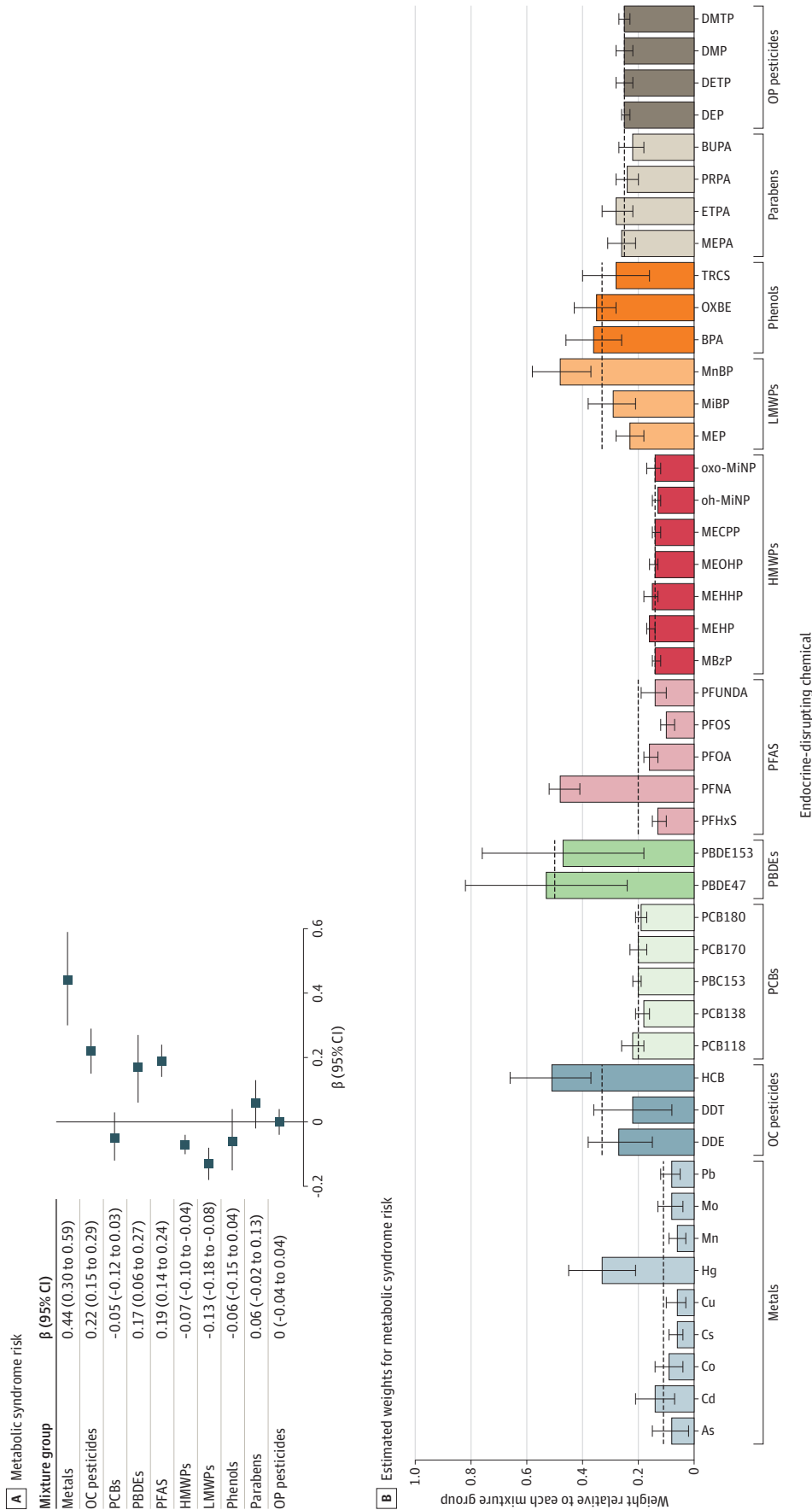
Characteristic	Participants, No. (%) (N = 1134)
Parental characteristics	
Subcohort	
BiB (UK)	193 (17.0)
EDEN (France)	144 (12.7)
INMA (Spain)	206 (18.2)
KANC (Lithuania)	196 (17.3)
MoBa (Norway)	205 (18.1)
RHEA (Greece)	190 (16.8)
Family native from the country of the cohort	
At least 1 native parent	1010 (89.1)
No native parent	124 (10.9)
Maternal age at birth, mean (SD), y	30.7 (4.9)
Maternal prepregnancy body mass index, mean (SD) ^a	25.1 (5.0)
Gestational weight gain status (Institute of Medicine criteria)	
Low or adequate	503 (44.4)
Excessive	631 (55.6)
Maternal educational level	
Low	167 (14.7)
Medium	393 (34.7)
High	574 (50.6)
Parity	
Nulliparous	506 (44.6)
Primiparous	421 (37.1)
Multiparous	207 (18.3)
Maternal smoking in pregnancy	
No	966 (85.2)
Yes	168 (14.8)
Maternal fish intake in pregnancy	
<2 times/wk	468 (41.3)
2-4 times/wk	342 (30.2)
>4 times/wk	324 (28.6)
Child characteristics	
Sex	
Female	517 (45.6)
Male	617 (54.4)
Age at assessment, mean (SD), y	7.8 (1.5)
Waist circumference, mean (SD), cm	58.5 (7.6)
Systolic blood pressure, mean (SD), mm Hg	99.1 (11.1)
Diastolic blood pressure, mean (SD), mm Hg	58.3 (9.6)
High-density lipoprotein cholesterol, mean (SD), mg/dL	59.4 (12.4)
Triglycerides, median (IQR), mg/dL	75.3 (59.3-101.0)
Insulin, median (IQR), μIU/mL	317.6 (217.7-552.5)
Metabolic syndrome score, mean (SD)	-0.1 (2.3)
Metabolic syndrome risk group (80th percentile: 1.7 cutoff score)	
Low-risk	793 (69.9)
High-risk	341 (30.1)

Abbreviations: BiB, Born in Bradford; EDEN, Étude des Déterminants Pré et Postnatals du Développement et de la Santé de l'Enfant; INMA, Infancia y Medio Ambiente; KANC, Kaunas cohort; MoBa, Norwegian Mother, Father, and Child Cohort Study; RHEA, RHEA Mother Child Cohort.

SI conversion factors: To convert high-density lipoprotein cholesterol to millimoles per liter, multiply by 0.0259; insulin to picomoles per liter, multiply by 6.945; triglycerides to millimoles per liter, multiply by 0.0113.

^a Body mass index was calculated as weight in kilograms divided by height in meters squared.

Figure 1. Metabolic Syndrome (MetS) Risk and Estimated Posterior Weights of Exposure Mixture Groups on MetS Risk Score Using the Bayesian Weighted Quantile Sum Regression



Panel A shows β coefficient and 95% credible intervals for child MetS per quartile increase in prenatal chemical mixtures. Panel B shows the estimated posterior weights with 95% credible intervals (Cris; presented as error bars) for MetS risk. Weights represent the relative contribution of each chemical to the overall group association. Within a chemical mixture group, the estimated weights total 1. Dotted horizontal lines indicate expected weights if all chemicals within a group contributed equally to the mixture. All models were adjusted for subcohort, parental country of birth, maternal age, maternal education level, maternal prepregnancy body mass index, parity, maternal smoking in pregnancy, and maternal fish intake in pregnancy.

As indicates: inorganic arsenic; BPA, bisphenol A; BUPA, N-butyl paraben; Cd, cadmium; Co, cobalt; Cs, caesium; Cu, copper; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; DEP, diethyl phthalate; DETP, diethylthiophosphate; DMP, dimethyl phthalate; DMTP, dimethylthiophosphate; ETPA, ethyl paraben; HCB, hexachlorobenzene; Hg, total mercury; HMWPs, high-molecular weight phthalates; LMWPs, low-molecular weight phthalates; MiBzP, monobenzylphthalate; MECPP, Mono-(2-ethyl-5-carboxypentyl) phthalate cyclodiphosphate; MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, mono-2-ethylhexyl phthalate; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate; MEP, monoethyl phthalate; MEPA, methyl paraben; MiBP, mono-iso-butyl phthalate; Mn, manganese; MnBP, mono-n-butyl phthalate; Mo, molybdenum; OC, organochlorine; oh-MiNP, hydroxy-isononyl phthalate; OP, organophosphate; OXBE, oxybenzone; oxo-MiNP, mono-oxo-isononyl phthalate; Pb, lead; PBDEs, polybrominated diphenyl ethers; PCBs, polychlorinated biphenyls; PFAS, perfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoic acid; PFOA, perfluoro-octanoic acid; PFOS, perfluoro-octane sulfonate; PFUNDA, perfluoroundecanoic acid; PRPA, propyl paraben; and TRCS, triclosan.

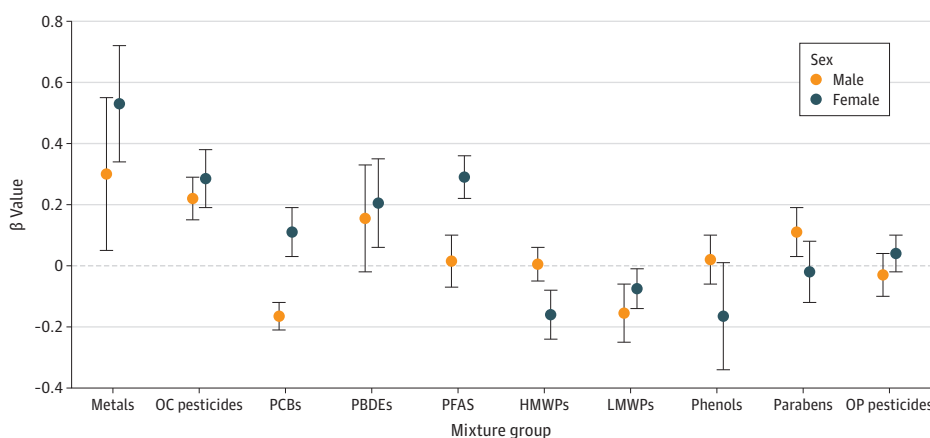
When stratified by sex, nonoverlapping 95% CrIs were observed for PCB, PFAS, and HMWP mixtures between both groups (Figure 2). The PCB mixture was associated with higher MetS score in female children ($\beta = 0.11$; 95% CrI, 0.03 to 0.19) and lower MetS score in male children ($\beta = -0.17$; 95% CrI, -0.21 to -0.12) (Figure 2 and eTable 6 in Supplement 1). Associations of PFAS and HMWPs with MetS score were observed only in female children.

A total of 14 plasma proteins, 110 serum metabolites and 10 urine metabolites were cross-sectionally associated with child MetS risk score (eTables 8-10 in Supplement 1). Figure 3 depicts associations of these metabolites and proteins with the prenatal chemical mixtures (for visualization purpose, only those with a percent change >5% are shown) and child MetS risk score. The number of molecular markers related to both MetS score and the mixture ranged from 43 for the LMWP mixture to 109 for the PFAS mixture (eTables 11-13 in Supplement 1). All prenatal mixtures were associated with elevated serum levels of C-reactive protein (CRP) and at least 2 of the following proteins, which were all associated with higher MetS score: interleukin (IL)-1 β , IL-6, IL-1RA, and leptin (Figure 3, eTable 8, and eTable 11 in Supplement 1). Metals and persistent chemicals were associated with increased serum levels of α -aminoadipic acid (α -AAA), leucine, isoleucine, and valine, which also were associated with an increased MetS score. The same set of mixtures was associated with altered diacyl chain phosphatidylcholine levels, primarily associated with higher MetS score. Metals and PFAS were associated with decreased acylcarnitines, which were associated with lower MetS score (Figure 3, eTable 9, and eTable 12 in Supplement 1). Metals and persistent chemicals (except for PBDEs) were associated with higher urine concentrations of 4-deoxyerythronic acid and 3-hydroxisobutyrate, which were also associated with higher MetS score. Conversely, all mixtures, except for LMWPs, were associated with lower child urine hippurate, which was associated with lower MetS score (Figure 3, eTable 10, and eTable 13 in Supplement 1).

Sensitivity Analyses

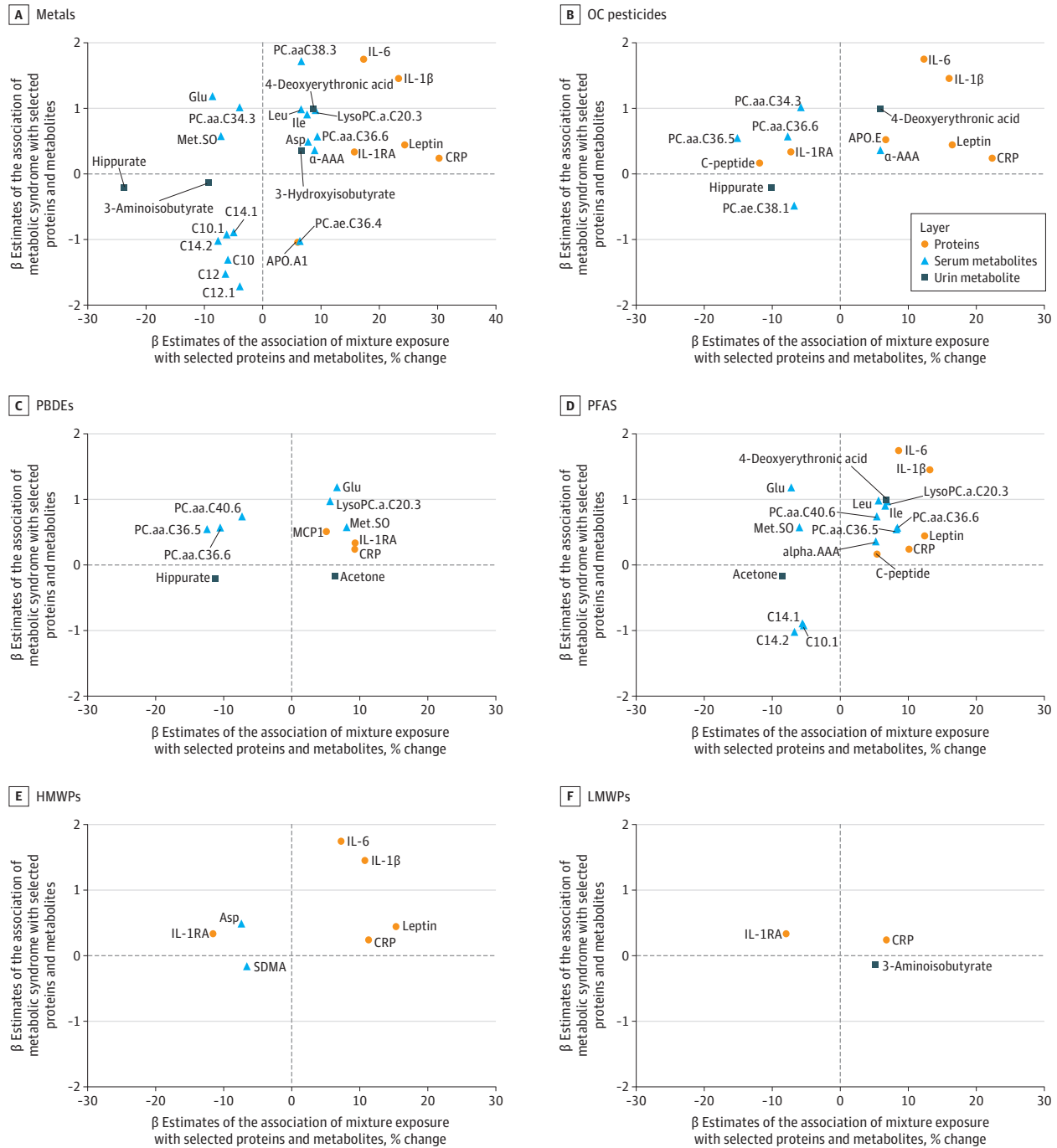
Single-exposure analyses showed an association of prenatal MnBP levels with decreased child MetS risk score ($\beta = -0.17$; 95% CrI, -0.34 to -0.01), but no other associations were observed (eTable 14 in Supplement 1). Between-cohort heterogeneity was not observed (I^2 values close to 0%). The association of OC pesticide mixtures with child MetS risk was notable only in children whose mothers had low or adequate weight gain during pregnancy ($\beta = 0.32$; 95% CrI, 0.23 to 0.40), whereas no clear differences were observed by weight gain categories for other classes of lipophilic chemicals (eTable 15 in Supplement 1). Associations of whole phthalate mixture, nonpersistent chemical mixture, and child MetS risk were comparable to those found with the LMWPs mixture (eTable 16 in Supplement 1). Notably, the metals and persistent chemical mixture exhibited larger associations than separate chemical groups ($\beta = 0.63$; 95% CrI, 0.47 to 0.78) (eTable 17 in Supplement 1).

Figure 2. Associations of Prenatal Chemical Mixtures With Metabolic Syndrome (MetS) Score Stratified by Sex



The dots denote the β estimate for MetS score per quartile increase in prenatal endocrine-disrupting chemical mixture exposure and the bars denote the 95% credible intervals from bayesian weighted quantile sum regression models. The horizontal dashed line at 0 line indicates the null. All models were adjusted for subcohort, parental country of birth, maternal age, maternal education level, maternal prepregnancy body mass index, parity, maternal smoking in pregnancy, and maternal fish intake in pregnancy. HMWPs indicate high-molecular-weight phthalates; LMWPs, low-molecular-weight phthalates; PBDEs, polybrominated diphenyl ethers; PCBs, polychlorinated biphenyls; PFASs, perfluoroalkyl substances; OC, organochlorine; OP, organophosphate.

Figure 3. Scatterplot of Selected Proteins and Metabolites Associated With at Least 1 Prenatal Chemical Mixture and Child Metabolic Syndrome (MetS) Risk Score



Each point corresponds to a protein or serum or urine metabolites. The x-axis shows the β coefficient of the associations of prenatal mixture with child omics expressed as percent change of omics levels per quartile increase of the exposure mixture (only associations with a % change >5% are shown). The y-axis shows the β coefficient of the associations of child omics with child MetS risk score expressed per doubling of omics levels. This analysis has been restricted to chemical mixtures significantly associated with MetS risk. Dotted vertical and horizontal lines denote the null. All models were adjusted for subcohort, parental country of birth, maternal age, maternal education level, maternal prepregnancy body mass index, parity, maternal smoking in pregnancy, and maternal fish intake in pregnancy. α -AAA indicates alpha-amino adipic acid; APO, apolipoprotein; Asp, aspartate; C, acylcarnitines; CRP, C reactive protein; HMWPs, high-molecular-weight phthalates; Glu, glutamate; IL, interleukin; Ile, isoleucine; IL-1RA, interleukin 1 receptor antagonist; Leu, leucine; LMWPs, low-molecular-weight phthalates; LysoPC, lysophosphatidylcholines; MCP1, monocyte chemoattractant protein-1; Met.SO, methionine sulfoxide; OCs, organochlorines; PBDEs, polybrominated diphenyl ethers; PC, phosphatidylcholine; PFASs, perfluoroalkyl substances; SDMA, symmetric dimethylarginine.

Associations remained consistent when using the dichotomous MetS risk outcome (eTable 18 in Supplement 1).

Discussion

In this multicenter cohort study of European mothers and their children, maternal exposure to mixtures of metals, OC pesticides, PBDEs, and PFAS during pregnancy was associated with an increased MetS risk score in childhood, while phthalate mixtures were associated with a lower MetS score. Our results suggest sex-specific associations for certain chemicals and identify molecular signatures in childhood associated with both prenatal EDC exposure and child MetS risk. Notably, associations of metals and persistent chemicals with MetS closely resemble those previously observed with nonalcoholic fatty liver disease risk,⁴¹ which contributes to increasing evidence supporting the relationship between both disorders.

To our knowledge, this study represents the first comprehensive evaluation of associations of prenatal exposure to mixtures of a wide range of EDC classes with MetS risk, and protein and metabolite profiles in childhood. Our use of state of the art mixture methods revealed associations not evident in single exposure models, highlighting the importance of evaluating health risks associated with EDC mixtures. The use of an aggregate MetS score offered a more comprehensive approach compared with isolated risk factors, capturing the overall metabolic effect better. By identifying child molecular phenotypes associated with EDC mixtures and underlying MetS, this study may support future early identification of EDC-exposed pediatric populations at risk for MetS development. Furthermore, the sample size of more than 1000 mother-child pairs enabled stratification and comparison of associations between male and female children. Previously, only 1 study¹⁸ assessed the association of a prenatal metal mixture with child MetS risk, observing a null association. However, this mixture did not include mercury,¹⁸ a high priority pollutant that has been suggested to elicit oxidative stress and inflammation.^{42,43} We found that mercury was the metal with the highest contribution to increasing child MetS. Our results corroborate the adverse metabolic health associated with prenatal mercury exposure previously found in the HELIX project¹⁵ and other epidemiological studies.⁴³

The obesogenic effects of in utero exposure to persistent chemicals, including OC pesticides, PBDEs, and PFAS have been extensively reported.¹¹ However, only a few studies^{13,14,16} have examined their association with MetS risk in childhood. We observed HCB to be the main contributor of the OC pesticides mixture association and PFNA to be the main contributors of the PFASs mixture association. These findings align with earlier studies using the Spanish INMA cohort,^{13,14} which found that prenatal HCB and PFNA was associated with higher MetS risk in adolescence and childhood. Of interest, PFNA was also the main contributor to prenatal PFAS mixture associations related to higher liver enzymes and liver injury in children in HELIX.^{41,44}

Phthalates, as nonpersistent EDCs quickly metabolized and excreted in urine, have been shown in experimental studies^{45,46} to disrupt fetal programming of cardiovascular function and adipogenesis, predisposing to offspring MetS pathogenesis.¹² Surprisingly, we found that prenatal exposure to phthalates was associated with a decreased child MetS risk score. Similarly, a study¹⁷ with over 2000 Chinese mother-child pairs reported inconsistent associations of phthalate mixture exposure with MetS risk in childhood, depending on the exposure timing in pregnancy. We found no associations with MetS score for the phenols, parabens, and OP pesticide metabolite mixtures, even though single exposure studies have documented associations with components of MetS, especially for bisphenol A.^{10,11,47} The reason for such inconsistencies may be potential measurement error given the exposure assessment in a single urine sample.⁴⁸

Our results suggest sex-specific metabolic disruption, with females being more susceptible to PFAS and PCBs exposure. This could be due to their interference with sex steroid hormone pathways,⁴⁹⁻⁵¹ which in the case of PFAS, has been observed in human fetuses.⁵²

Our analysis of molecular markers unveiled proteins and metabolites associated with both prenatal EDC exposure and MetS development. Most mixtures were associated with upregulated cytokines, CRP, and leptin, all of them being proinflammatory proteins produced and released by the adipose tissue relevant to MetS pathogenesis.^{53,54} CRP has also shown to be associated with increased risk of MetS, T2D, and cardiovascular disease in healthy adults.⁵⁵ Our findings, using an exposure-mixture approach in a sensitive time-window as pregnancy, corroborate prior *in vitro*, *in vivo*, and epidemiological studies⁵⁶⁻⁶² that showed similar associations with individual metals, persistent chemicals, and phthalate exposure, including earlier HELIX studies^{15,63} assessing prenatal mercury and PFAS exposure.

At the metabolite level, we observed elevated levels of serum branched chained amino acids, α -AAA, urinary 4-deoxyerythronic acid, 3-hydroxyisobutyrate, and dysregulation in diacyl chain phosphatidylcholines associated with prenatal metals and persistent chemicals mixtures and increased MetS score. Branched chained amino acids, well-known regulators of glucose and lipid metabolism,⁶⁴ and α -AAA have been shown to be associated with MetS risk components in healthy adults before disease onset.⁶⁵⁻⁶⁸ Consistently, 4-deoxyerythronic levels were associated with a higher BMI in childhood in the HELIX study.⁶⁹ 3-Hydroxyisobutyrate, whose fermentation by gut bacteria may lead to the production of short-chain fatty acids regulating overall metabolic activity,⁷⁰ has also been associated with obesity, insulin resistance, and T2D.⁷¹ Perturbations in lipid metabolites associated with OC pesticides and PFAS exposure were noted in previous studies with children,^{72,73} and with prenatal exposure in children.⁴⁴ Diacyl chain phosphatidylcholines are crucial for the liver's release of triglyceride-rich, very low density lipoprotein particles and high-density lipoprotein,⁷⁴ consistent with our findings and with metabolomics studies in patients with T2D⁷⁵ and obesity.⁷⁶ Acylcarnitines transport fatty acids to mitochondria for oxidation,⁷⁷ and elevated levels may be associated with T2D and MetS risk.^{78,79} Odd-chain acylcarnitines, whose main source is circulating fatty acids from diet,⁸⁰ were associated with decreased MetS risk score in childhood. We speculate that these results may be partly due to differences in diet and fasting status.⁸⁰ Chemical mixtures were associated with lower urine hippurate levels. Hippurate, originating from dietary polyphenols metabolism,⁸¹ has been associated with increased gut microbiome diversity and reduced MetS risk,⁸² aligning with our findings.

Limitations

Our study has several limitations. First, potential measurement error in assessing highly variable nonpersistent chemicals⁸³ with attenuation bias reached as high as 80%.⁸⁴ Second, lipids and insulin were analyzed from blood taken after only 3 hours of fasting, which may have reduced the precision of the MetS risk score. Third, although we used a longitudinal study design, proteins and metabolomic biomarkers in children were assessed at the same time point as the MetS score, limiting the ability to disentangle any mediating effect of the observed features of the EDC-MetS associations. Fourth, no multiordered chemical-chemical interactions were considered in this study, hindering the possibility of detecting potential toxicological interactions among EDCs.⁸⁵ Fifth, because our focus was on EDCs exposure during fetal programming, childhood exposures were not considered, rendering it challenging to discern effects across exposure windows. For some of our EDC classes, further caution is warranted because exposure data were missing (ie, the chemicals were not analyzed) for a relatively large proportion of subjects; for example, PBDEs were missing in 51% to 54% of participants. To address this, we used multiple imputations to minimize bias that would result from deleting participants with missing data, thereby avoiding loss of power.^{86,87} We opted not to conduct complete-case analyses due to the limited sample size (less than 400 participants), which would not allow for a meaningful comparison with our imputed data sets. Future studies incorporating repeated urine samples during pregnancy,⁸⁸ and novel statistical tools combining mixtures with mediation and interaction analysis are needed for a deeper understanding of EDC metabolic effects.

Conclusions

This large, population-based cohort study suggests that prenatal exposure to EDCs mixtures, particularly metals, OC pesticides, PBDEs, and PFASs, may be associated with adverse metabolic health in childhood. These findings advance our limited understanding of metabolic effects of EDC mixtures in early life and can inform more efficient early-life prevention and intervention strategies to address rising trends in MetS across the life course.

ARTICLE INFORMATION

Accepted for Publication: February 21, 2024.

Published: May 23, 2024. doi:10.1001/jamanetworkopen.2024.12040

Open Access: This is an open access article distributed under the terms of the [CC-BY License](#). © 2024 Güil-Oumrait N et al. *JAMA Network Open*.

Corresponding Author: Martine Vrijheid, PhD, Barcelona Institute for Global Health (ISGlobal), 88 Dr Aiguader St, 08003 Barcelona, Spain (martine.vrijheid@isglobal.org).

Author Affiliations: Barcelona Institute for Global Health (ISGlobal), Barcelona, Spain (Güil-Oumrait, Stratakis, Maitre, Anguita-Ruiz, Urquiza, Fabbri, Basagaña, Bustamante, Casas, Vrijheid); Pompeu Fabra University, Barcelona, Spain (Güil-Oumrait, Maitre, Anguita-Ruiz, Fabbri, Basagaña, Bustamante, Casas, Vrijheid); Centro de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP), Madrid, Spain (Güil-Oumrait, Stratakis, Maitre, Anguita-Ruiz, Urquiza, Fabbri, Basagaña, Bustamante, Casas, Vrijheid); Université Paris Cité and Université Sorbonne Paris Nord, National Institute of Health and Medical Research (INSERM), National Institute for Agriculture, Food and the Environment (INRAE), Center for Research in Epidemiology and Statistics (CRESS), Paris, France (Heude); Department of Food Safety, Norwegian Institute of Public Health, Oslo, Norway (Haug, Sakhi); Division of Climate and Environmental Health, Norwegian Institute of Public Health, Oslo, Norway (Iszatt); Cancer Metabolism & Systems Toxicology Group, Imperial College London, Hammersmith Hospital Campus, London, United Kingdom (Keun); Bradford Institute for Health Research, Bradford Teaching Hospitals National Health Service Foundation Trust, Bradford, United Kingdom (Wright, McEachan); Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles (Chatzi); Department of Social Medicine, University of Crete, Heraklion, Crete, Greece (Vafeiadi); Department of Environmental Sciences, Vytautas Magnus University, Kaunas, Lithuania (Gražulevičienė, Andrušaitytė); Department of Prevention and Treatment of Chronic Diseases, Institute for Advanced Biosciences (IAB; INSERM U1209, CNRS UMR 5309), Université Grenoble Alpes, Grenoble, France (Slama).

Author Contributions: Ms Güil-Oumrait and Dr Vrijheid had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Concept and design: Güil-Oumrait, Stratakis, Maitre, Fabbri, Slama, McEachan, Casas, Vrijheid.

Acquisition, analysis, or interpretation of data: Güil-Oumrait, Maitre, Anguita, Urquiza, Fabbri, Basagaña, Heude, Haug, Sakhi, Iszatt, Keun, Wright, Chatzi, Vafeiadi, Bustamante, Gražulevičienė, Andrušaitytė, McEachan, Vrijheid.

Drafting of the manuscript: Güil-Oumrait, Urquiza, Vrijheid.

Critical review of the manuscript for important intellectual content: Stratakis, Maitre, Anguita, Fabbri, Basagaña, Heude, Haug, Sakhi, Iszatt, Keun, Wright, Chatzi, Vafeiadi, Bustamante, Gražulevičienė, Andrušaitytė, Slama, McEachan, Casas, Vrijheid.

Statistical analysis: Güil-Oumrait, Stratakis, Maitre, Anguita, Urquiza, Fabbri, Basagaña.

Obtained funding: Keun, Slama, McEachan, Vrijheid.

Administrative, technical, or material support: Sakhi, Wright, Andrušaitytė, Slama.

Supervision: Maitre, Urquiza, Chatzi, Vafeiadi, Gražulevičienė, Casas, Vrijheid.

Conflict of Interest Disclosures: None reported.

Funding/Support: This project was supported by the European Union Horizon 2020 research and innovation programme (Grant Agreement No. 874583, Advancing Tools for Human Early Lifecourse Exposome Research and Translation [ATHLETE]). Data collection received support from the European Community Horizon 2020 programme (Grant Agreement No. 825712 [the Oberon project]) and the European Union 7th Framework Programme (Grant Agreement No. 308333 [the Human Early Lifecourse Exposome [HELIX] project]). The study was also supported by the Secretaria d'Universitats i Recerca de la Generalitat de Catalunya (AGAUR; fellowship to Ms Güil-Oumrait), and Fons Social Europeu (FSE; fellowship to Ms Güil-Oumrait), the Strategic Plan for Research

and Innovation in Health (PERIS) Catalan program (grant No. SLT017/20/000119 by the Departament de Salut de la Generalitat de Catalunya, Spain to Dr Urquiza), the European Commission (HORIZON-MSCA-2021-PF-01-01 - 2021 LIVER-X 101059245 to Dr Stratakis), and the Spanish Ministry of Science and Innovation (MCIN) and Research State Agency/(AEI; grant No. /10.13039/501100011033 to Dr Maitre). The Institute for Global Health (ISGlobal) acknowledges support from the Spanish Ministry of Science and Innovation through the Centro de Excelencia Severo Ochoa 2019-2023 Program (grant No. CEX2018-000806-S) and support from the Generalitat de Catalunya through the Research Centres of Catalonia (CERCA) Program. The Born in Bradford [BiB] study was supported by the Wellcome Trust (grant No. WT101597MA) and the UK Medical Research Council (MRC) and Economic and Social Science Research Council (ESRC) (grant No. MR/NO24397/1). The Infancia y Medio Ambiente [INMA] study was supported by the Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP), and the Generalitat de Catalunya-Catalan National Science Foundation (CIRIT). The Kanaus cohort [KANC] study was supported by the Lithuanian Agency for Science Innovation and Technology (grant No. 6-04-2014_3IV-66). The Norwegian Mother, Father, and Child Cohort [MoBa] study was supported by the Norwegian Ministry of Health and Care Services and the Ministry of Education and Research. The Rhea Mother Child cohort [RHEA] study was supported by European Projects (grant Nos. EU FP6-2003-Food-3-NewGeneris, EU FP6. STREP Hiwate, EU FP7 ENV.2007.1.2.2.2. [Project No 211250 Escape], EU FP7-2008-ENV-1.2.1.4 Envirogenomarkers, EU FP7-HEALTH-2009-single stage CHICOS, EU FP7 ENV.2008.1.2.1.6. [Proposal No 226285 ENRIECO], and EU- FP7- HEALTH-2012 [Proposal No 308333 HELIX]), and the Greek Ministry of Health, Program of Prevention of Obesity and Neurodevelopmental Disorders in Preschool Children, in Heraklion district, Crete, Greece (2011-2014) and the Rhea Plus: Primary Prevention Program of Environmental Risk Factors for Reproductive Health, and Child Health (2012-2015).

Role of the Funder/Sponsor: The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Data Sharing Statement: See Supplement 2.

Additional Contributions: The authors thank the participating families in the 6 cohorts (BiB, EDEN, INMA, KANC, MoBa, and RHEA) who took part in this research, as well as all the fieldworkers for their work and dedication in this study, and the contributions of the health professionals and researchers. Additionally, the authors thank the University of Málaga Picasso supercomputing facilities for providing computational resources to run the analyses conducted in this study.

REFERENCES

1. Saklayen MG. The Global Epidemic of the Metabolic Syndrome. *Curr Hypertens Rep.* 2018;20(2):12. doi:10.1007/s11906-018-0812-z
2. Reisinger C, Nkeh-Chungag BN, Fredriksen PM, Goswami N. The prevalence of pediatric metabolic syndrome—a critical look on the discrepancies between definitions and its clinical importance. *Int J Obes (Lond).* 2021;45(1):12-24. doi:10.1038/s41366-020-00713-1
3. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. *Lancet.* 2016;387(10027):1513-1530. doi:10.1016/S0140-6736(16)00618-8
4. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. *Lancet.* 2017;390(10113):2627-2642. doi:10.1016/S0140-6736(17)32129-3
5. Song P, Zhang Y, Yu J, et al. Global prevalence of hypertension in children: a systematic review and meta-analysis. *JAMA Pediatr.* 2019;173(12):1154-1163. doi:10.1001/jamapediatrics.2019.3310
6. DeBoer MD, Gurka MJ, Woo JG, Morrison JA. Severity of the metabolic syndrome as a predictor of type 2 diabetes between childhood and adulthood: the Princeton Lipid Research Cohort Study. *Diabetologia.* 2015;58(12):2745-2752. doi:10.1007/s00125-015-3759-5
7. Morrison JA, Friedman LA, Wang P, Glueck CJ. Metabolic syndrome in childhood predicts adult metabolic syndrome and type 2 diabetes mellitus 25 to 30 years later. *J Pediatr.* 2008;152(2):201-206. doi:10.1016/j.jpeds.2007.09.010
8. Morrison JA, Friedman LA, Gray-McGuire C. Metabolic syndrome in childhood predicts adult cardiovascular disease 25 years later: the Princeton Lipid Research Clinics Follow-up Study. *Pediatrics.* 2007;120(2):340-345. doi:10.1542/peds.2006-1699
9. Pandit D, Chipplonkar S, Khadilkar A, Kinare A, Khadilkar V. Efficacy of a continuous metabolic syndrome score in Indian children for detecting subclinical atherosclerotic risk. *Int J Obes (Lond).* 2011;35(10):1318-1324. doi:10.1038/ijo.2011.138

10. Heindel JJ, Blumberg B, Cave M, et al. Metabolism disrupting chemicals and metabolic disorders. *Reprod Toxicol*. 2017;68:3-33. doi:10.1016/j.reprotox.2016.10.001
11. Heindel JJ, Howard S, Agay-Shay K, et al. Obesity II: Establishing causal links between chemical exposures and obesity. *Biochem Pharmacol*. 2022;199:115015. doi:10.1016/j.bcp.2022.115015
12. Haverinen E, Fernandez MF, Mustieles V, Tolonen H. Metabolic syndrome and endocrine disrupting chemicals: an overview of exposure and health effects. *Int J Environ Res Public Health*. 2021;18(24):13047. doi:10.3390/ijerph182413047
13. Manzano-Salgado CB, Casas M, Lopez-Espinosa MJ, et al. Prenatal exposure to perfluoroalkyl substances and cardiometabolic risk in children from the Spanish INMA Birth Cohort Study. *Environ Health Perspect*. 2017;125(9):097018. doi:10.1289/EHP1330
14. Güll-Oumrait N, Valvi D, Garcia-Esteban R, et al. Prenatal exposure to persistent organic pollutants and markers of obesity and cardiometabolic risk in Spanish adolescents. *Environ Int*. 2021;151:106469. doi:10.1016/j.envint.2021.106469
15. Stratakis N, Conti DV, Borrás E, et al. Association of fish consumption and mercury exposure during pregnancy with metabolic health and inflammatory biomarkers in children. *JAMA Netw Open*. 2020;3(3):e201007. doi:10.1001/jamanetworkopen.2020.1007
16. Li N, Liu Y, Papandonatos GD, et al. Gestational and childhood exposure to per- and polyfluoroalkyl substances and cardiometabolic risk at age 12 years. *Environ Int*. 2021;147:106344. doi:10.1016/j.envint.2020.106344
17. Gao H, Geng ML, Tong J, et al. Combined effects of prenatal phthalate exposure on cardiometabolic risk score among 4- to 7-year-old children: MABC study. *Chemosphere*. 2023;311(Pt 2):137135. doi:10.1016/j.chemosphere.2022.137135
18. Kupscó A, Kioumourtzoglou MA, Just AC, et al. Prenatal metal concentrations and childhood cardiometabolic risk using bayesian kernel machine regression to assess mixture and interaction effects. *Epidemiology*. 2019;30(2):263-273. doi:10.1097/EDE.0000000000000962
19. Maitre L, Jedynek P, Gallego M, et al. Integrating -omics approaches into population-based studies of endocrine disrupting chemicals: A scoping review. *Environ Res*. 2023;228:115788. doi:10.1016/j.envres.2023.115788
20. Maitre L, Bustamante M, Hernández-Ferrer C, et al. Multi-omics signatures of the human early life exposome. *Nat Commun*. 2022;13(1):7024. doi:10.1038/s41467-022-34422-2
21. Vrijheid M, Slama R, Robinson O, et al. The human early-life exposome (HELIX): project rationale and design. *Environ Health Perspect*. 2014;122(6):535-544. doi:10.1289/ehp.1307204
22. Wright J, Small N, Raynor P, et al; Born in Bradford Scientific Collaborators Group. Cohort profile: the Born in Bradford multi-ethnic family cohort study. *Int J Epidemiol*. 2013;42(4):978-991. doi:10.1093/ije/dys112
23. Heude B, Forhan A, Slama R, et al; EDEN mother-child cohort study group. Cohort Profile: The EDEN mother-child cohort on the prenatal and early postnatal determinants of child health and development. *Int J Epidemiol*. 2016;45(2):353-363. doi:10.1093/ije/dyv151
24. Guxens M, Ballester F, Espada M, et al; INMA Project. Cohort profile: the INMA-Infancia y Medio Ambiente- (environment and childhood) project. *Int J Epidemiol*. 2012;41(4):930-940. doi:10.1093/ije/dyr054
25. Grazuleviciene R, Danileviciute A, Nadisauskiene R, Vencloviene J. Maternal smoking, GSTM1 and GSTT1 polymorphism and susceptibility to adverse pregnancy outcomes. *Int J Environ Res Public Health*. 2009;6(3):1282-1297. doi:10.3390/ijerph6031282
26. Magnus P, Irgens LM, Haug K, Nystad W, Skjaerven R, Stoltenberg C; MoBa Study Group. Cohort profile: the Norwegian Mother and Child Cohort Study (MoBa). *Int J Epidemiol*. 2006;35(5):1146-1150. doi:10.1093/ije/dyl170
27. Chatzi L, Plana E, Daraki V, et al. Metabolic syndrome in early pregnancy and risk of preterm birth. *Am J Epidemiol*. 2009;170(7):829-836. doi:10.1093/aje/kwp211
28. Maitre L, de Bont J, Casas M, et al. Human Early Life Exposome (HELIX) study: a European population-based exposome cohort. *BMJ Open*. 2018;8(9):e021311. doi:10.1136/bmjopen-2017-021311
29. Haug LS, Sakhi AK, Cequier E, et al. In-utero and childhood chemical exposome in six European mother-child cohorts. *Environ Int*. 2018;121(Pt 1):751-763. doi:10.1016/j.envint.2018.09.056
30. Jin Y, Hein MJ, Deddens JA, Hines CJ. Analysis of lognormally distributed exposure data with repeated measures and values below the limit of detection using SAS. *Ann Occup Hyg*. 2011;55(1):97-112.
31. Ahrens W, Moreno LA, Mårild S, et al; IDEFICS consortium. Metabolic syndrome in young children: definitions and results of the IDEFICS study. *Int J Obes (Lond)*. 2014;38(suppl 2):S4-S14. doi:10.1038/ijo.2014.130

32. White IR, Royston P, Wood AM. Multiple imputation using chained equations: Issues and guidance for practice. *Stat Med*. 2011;30(4):377-399. doi:10.1002/sim.4067
33. Tamayo-Uria I, Maitre L, Thomsen C, et al. The early-life exposome: description and patterns in six European countries. *Environ Int*. 2019;123:189-200. doi:10.1016/j.envint.2018.11.067
34. Colicino E, Pedretti NF, Busgang SA, Gennings C. Per- and poly-fluoroalkyl substances and bone mineral density: results from the bayesian weighted quantile sum regression. *Environ Epidemiol*. 2020;4(3):e092. doi:10.1097/EE9.0000000000000092
35. Zhang S, Lei X, Zhang Y, et al. Prenatal exposure to per- and polyfluoroalkyl substances and childhood adiposity at 7 years of age. *Chemosphere*. 2022;307(Pt 4):136077. doi:10.1016/j.chemosphere.2022.136077
36. Güll-Oumrait N, Cano-Sancho G, Montazeri P, et al. Prenatal exposure to mixtures of phthalates and phenols and body mass index and blood pressure in Spanish preadolescents. *Environ Int*. 2022;169:107527. doi:10.1016/j.envint.2022.107527
37. Berghuis SA, Bos AF, Sauer PJJ, Bocca G. Prenatal environmental exposure to persistent organic pollutants and indices of overweight and cardiovascular risk in Dutch adolescents. *Nutrients*. 2022;14(11):2269. doi:10.3390/nu14112269
38. Higgins JPT, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med*. 2002;21(11):1539-1558. doi:10.1002/sim.1186
39. National Research Council. Rasmussen KM, Yaktine AL. (eds). Weight gain during pregnancy: reexamining the guidelines. National Academies. 2009. Accessed April 11, 2024. <https://nap.nationalacademies.org/catalog/12584/weight-gain-during-pregnancy-reexamining-the-guidelines>
40. Makowski D, Ben-Shachar MS, Chen SHA, Lüdtke D. Indices of effect existence and significance in the bayesian framework. *Front Psychol*. 2019;10:2767. doi:10.3389/fpsyg.2019.02767
41. Midya V, Colicino E, Conti DV, et al. Association of prenatal exposure to endocrine-disrupting chemicals with liver injury in children. *JAMA Netw Open*. 2022;5(7):e2220176. doi:10.1001/jamanetworkopen.2022.20176
42. Tinkov AA, Ajsuvakova OP, Skalnaya MG, et al. Mercury and metabolic syndrome: a review of experimental and clinical observations. *Biometals*. 2015;28(2):231-254. doi:10.1007/s10534-015-9823-2
43. Roy C, Tremblay PY, Ayotte P. Is mercury exposure causing diabetes, metabolic syndrome and insulin resistance? a systematic review of the literature. *Environ Res*. 2017;156:747-760. doi:10.1016/j.envres.2017.04.038
44. Stratakis N, V Conti D, Jin R, et al. Prenatal exposure to perfluoroalkyl substances associated with increased susceptibility to liver injury in children. *Hepatology*. 2020;72(5):1758-1770. doi:10.1002/hep.31483
45. Hao C, Cheng X, Guo J, Xia H, Ma X. Perinatal exposure to diethyl-hexyl-phthalate induces obesity in mice. *Front Biosci (Elite Ed)*. 2013;5(2):725-733. doi:10.2741/E653
46. Shen O, Du G, Sun H, et al. Comparison of in vitro hormone activities of selected phthalates using reporter gene assays. *Toxicol Lett*. 2009;191(1):9-14. doi:10.1016/j.toxlet.2009.07.019
47. Sanders AP, Saland JM, Wright RO, Satlin L. Perinatal and childhood exposure to environmental chemicals and blood pressure in children: a review of literature 2007-2017. *Pediatr Res*. 2018;84(2):165-180. doi:10.1038/s41390-018-0055-3
48. Casas M, Basagaña X, Sakhi AK, et al. Variability of urinary concentrations of non-persistent chemicals in pregnant women and school-aged children. *Environ Int*. 2018;121(Pt 1):561-573. doi:10.1016/j.envint.2018.09.046
49. Kjeldsen LS, Bonefeld-Jørgensen EC. Perfluorinated compounds affect the function of sex hormone receptors. *Environ Sci Pollut Res Int*. 2013;20(11):8031-8044. doi:10.1007/s11356-013-1753-3
50. Li J, Li N, Ma M, Giesy JP, Wang Z. In vitro profiling of the endocrine disrupting potency of organochlorine pesticides. *Toxicol Lett*. 2008;183(1-3):65-71. doi:10.1016/j.toxlet.2008.10.002
51. Agusa T, Kunito T, Iwata H, et al. Mercury in hair and blood from residents of Phnom Penh (Cambodia) and possible effect on serum hormone levels. *Chemosphere*. 2007;68(3):590-596. doi:10.1016/j.chemosphere.2007.01.003
52. Rivera-Núñez Z, Kinkade CW, Khoury L, et al. Prenatal perfluoroalkyl substances exposure and maternal sex steroid hormones across pregnancy. *Environ Res*. 2023;220:115233. doi:10.1016/j.envres.2023.115233
53. Ballak DB, Stienstra R, Tack CJ, Dinarello CA, van Diepen JA. IL-1 family members in the pathogenesis and treatment of metabolic disease: Focus on adipose tissue inflammation and insulin resistance. *Cytokine*. 2015;75(2):280-290. doi:10.1016/j.cyto.2015.05.005
54. Ghadge AA, Khaire AA. Leptin as a predictive marker for metabolic syndrome. *Cytokine*. 2019;121:154735. doi:10.1016/j.cyto.2019.154735

55. Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. *Circulation*. 2003;107(3):391-397. doi:10.1161/01.CIR.0000055014.62083.05
56. Liu Z, Lu Y, Zhong K, Wang C, Xu X. The associations between endocrine disrupting chemicals and markers of inflammation and immune responses: a systematic review and meta-analysis. *Ecotoxicol Environ Saf*. 2022;234:113382. doi:10.1016/j.ecoenv.2022.113382
57. Zota AR, Geller RJ, Romano LE, et al. Association between persistent endocrine-disrupting chemicals (PBDEs, OH-PBDEs, PCBs, and PFASs) and biomarkers of inflammation and cellular aging during pregnancy and postpartum. *Environ Int*. 2018;115:9-20. doi:10.1016/j.envint.2018.02.044
58. Peinado FM, Artacho-Cordón F, Barrios-Rodríguez R, Arrebola JP. Influence of polychlorinated biphenyls and organochlorine pesticides on the inflammatory milieu: a systematic review of in vitro, in vivo and epidemiological studies. *Environ Res*. 2020;186:109561. doi:10.1016/j.envres.2020.109561
59. Campioli E, Martínez-Argüelles DB, Papadopoulou V. In utero exposure to the endocrine disruptor di-(2-ethylhexyl) phthalate promotes local adipose and systemic inflammation in adult male offspring. *Nutr Diabetes*. 2014;4(5):e115. doi:10.1038/nutd.2014.13
60. Howell G III, Mangum L. Exposure to bioaccumulative organochlorine compounds alters adipogenesis, fatty acid uptake, and adipokine production in NIH3T3-L1 cells. *Toxicol In Vitro*. 2011;25(1):394-402. doi:10.1016/j.tiv.2010.10.015
61. Ding N, Karvonen-Gutierrez CA, Herman WH, Calafat AM, Mukherjee B, Park SK. Associations of perfluoroalkyl and polyfluoroalkyl substances (PFAS) and PFAS mixtures with adipokines in midlife women. *Int J Hyg Environ Health*. 2021;235:113777. doi:10.1016/j.ijheh.2021.113777
62. Trim A, Hankinson SE, Liu S, et al. Biomarkers of phthalates and inflammation: findings from a subgroup of Women's Health Initiative participants. *Int J Hyg Environ Health*. 2021;234:113743. doi:10.1016/j.ijheh.2021.113743
63. Papadopoulou E, Stratakis N, Basagaña X, et al. Prenatal and postnatal exposure to PFAS and cardiometabolic factors and inflammation status in children from six European cohorts. *Environ Int*. 2021;157:106853. doi:10.1016/j.envint.2021.106853
64. Yoshizawa F. New therapeutic strategy for amino acid medicine: notable functions of branched chain amino acids as biological regulators. *J Pharmacol Sci*. 2012;118(2):149-155. doi:10.1254/jphs.11R05FM
65. Magnusson M, Lewis GD, Ericson U, et al. A diabetes-predictive amino acid score and future cardiovascular disease. *Eur Heart J*. 2013;34(26):1982-1989. doi:10.1093/eurheartj/ehs424
66. Yamakado M, Nagao K, Imaizumi A, et al. Plasma free amino acid profiles predict four-year risk of developing diabetes, metabolic syndrome, dyslipidemia, and hypertension in Japanese population. *Sci Rep*. 2015;5:11918. doi:10.1038/srep11918
67. Guasch-Ferré M, Hruby A, Toledo E, et al. Metabolomics in prediabetes and diabetes: a systematic review and meta-analysis. *Diabetes Care*. 2016;39(5):833-846. doi:10.2337/dc15-2251
68. Desine S, Gabriel CL, Smith HM, et al. Association of alpha-amino adipic acid with cardiometabolic risk factors in healthy and high-risk individuals. *Front Endocrinol (Lausanne)*. 2023;14(September):1122391. doi:10.3389/fendo.2023.1122391
69. Lau CE, Siskos AP, Maitre L, et al. Determinants of the urinary and serum metabolome in children from six European populations. *BMC Med*. 2018;16(1):202. doi:10.1186/s12916-018-1190-8
70. Mierziak J, Burgberger M, Wojtasik W. 3-hydroxybutyrate as a metabolite and a signal molecule regulating processes of living organisms. *Biomolecules*. 2021;11(3):402. doi:10.3390/biom11030402
71. Nilsen MS, Jersin RÅ, Ulvik A, et al. 3-Hydroxyisobutyrate, a strong marker of insulin resistance in type 2 diabetes and obesity that modulates white and brown adipocyte metabolism. *Diabetes*. 2020;69(9):1903-1916. doi:10.2337/db19-1174
72. Alderete TL, Jin R, Walker DI, et al. Perfluoroalkyl substances, metabolomic profiling, and alterations in glucose homeostasis among overweight and obese Hispanic children: a proof-of-concept analysis. *Environ Int*. 2019;126:445-453. doi:10.1016/j.envint.2019.02.047
73. Jin R, McConnell R, Catherine C, et al. Perfluoroalkyl substances and severity of nonalcoholic fatty liver in Children: an untargeted metabolomics approach. *Environ Int*. 2020;134:105220. doi:10.1016/j.envint.2019.105220
74. Cole LK, Vance JE, Vance DE. Phosphatidylcholine biosynthesis and lipoprotein metabolism. *Biochim Biophys Acta*. 2012;1821(5):754-761. doi:10.1016/j.bbali.2011.09.009
75. Floegel A, Stefan N, Yu Z, et al. Identification of serum metabolites associated with risk of type 2 diabetes using a targeted metabolomic approach. *Diabetes*. 2013;62(2):639-648. doi:10.2337/db12-0495

76. Pietiläinen KH, Sysi-Aho M, Rissanen A, et al. Acquired obesity is associated with changes in the serum lipidomic profile independent of genetic effects—a monozygotic twin study. *PLoS One*. 2007;2(2):e218. doi:10.1371/journal.pone.0000218
77. Schooneman MG, Vaz FM, Houten SM, Soeters MR. Acylcarnitines: reflecting or inflicting insulin resistance? *Diabetes*. 2013;62(1):1-8. doi:10.2337/db12-0466
78. Sun L, Liang L, Gao X, et al. Early prediction of developing type 2 diabetes by plasma acylcarnitines: a population-based study. *Diabetes Care*. 2016;39(9):1563-1570. doi:10.2337/dc16-0232
79. Libert DM, Nowacki AS, Natowicz MR. Metabolomic analysis of obesity, metabolic syndrome, and type 2 diabetes: amino acid and acylcarnitine levels change along a spectrum of metabolic wellness. *PeerJ*. 2018; 6:e5410. doi:10.7717/peerj.5410
80. Wedekind R, Rothwell JA, Viallon V, et al. Determinants of blood acylcarnitine concentrations in healthy individuals of the European Prospective Investigation into Cancer and Nutrition. *Clin Nutr*. 2022;41(8):1735-1745. doi:10.1016/j.clnu.2022.05.020
81. Walsh MC, Brennan L, Pujos-Guillot E, et al. Influence of acute phytochemical intake on human urinary metabolomic profiles. *Am J Clin Nutr*. 2007;86(6):1687-1693. doi:10.1093/ajcn/86.5.1687
82. Pallister T, Jackson MA, Martin TC, et al. Hippurate as a metabolomic marker of gut microbiome diversity: Modulation by diet and relationship to metabolic syndrome. *Sci Rep*. 2017;7(1):13670. doi:10.1038/s41598-017-13722-4
83. Vernet C, Philippat C, Calafat AM, et al. Within-day, between-day, and between-week variability of urinary concentrations of phenol biomarkers in pregnant women. *Environ Health Perspect*. 2018;126(3):037005. doi:10.1289/EHP1994
84. Perrier F, Giorgis-Allemand L, Slama R, Philippat C. Within-subject pooling of biological samples to reduce exposure misclassification in biomarker-based studies. *Epidemiology*. 2016;27(3):378-388. doi:10.1097/EDE.0000000000000460
85. Midya V, Gennings C. Detecting shape-based interactions among environmental chemicals using an ensemble of exposure-mixture regression and interpretable machine learning tools. *Stat Biosci*. Published online November 23, 2023. doi:10.1007/s12561-023-09405-6
86. Donders ART, van der Heijden GJM, Stijnen T, Moons KGM. Review: a gentle introduction to imputation of missing values. *J Clin Epidemiol*. 2006;59(10):1087-1091. doi:10.1016/j.jclinepi.2006.01.014
87. Schafer JL. Multiple imputation: a primer. *Stat Methods Med Res*. 1999;8(1):3-15. doi:10.1177/096228029900800102
88. Philippat C, Rolland M, Lyon-Caen S, et al. Pre- and early post-natal exposure to phthalates and DINCH in a new type of mother-child cohort relying on within-subject pools of repeated urine samples. *Environ Pollut*. 2021; 287:117650. doi:10.1016/j.envpol.2021.117650

SUPPLEMENT 1.

eFigure 1. Flow Chart Describing Population Included in the Study

eTable 1. Biological Matrices and Time of Collection During Pregnancy for EDC Assessment

eTable 2. Analytical Methods and Laboratories for Prenatal EDCs, Lipids and Creatinine Assessment

eMethods. MetS Risk Score

eAppendix 1. Measurement of Metabolites and Protein Levels in Childhood

eTable 3. Percentage of Missings in the Chemicals' Exposures and Covariates

eFigure 2. Generalized Additive Models of Prenatal Exposure to Metals and Persistent Chemicals and Child Metabolic Syndrome Risk Score to Test Linearity Prior Using BWQS

eFigure 3. Generalized Additive Models of Prenatal Exposure to Nonpersistent Chemicals and Child Metabolic Syndrome Risk Score to Test Linearity Prior Using BWQS

eAppendix 2. Bayesian Weighted Quantile Sum (BWQS) Regression Characteristics

eFigure 4. Directed Acyclic Graph (DAG) Elucidating Exposure-Outcome Association

eTable 4. Cofounder-Only Model for MetS Score

eTable 5. Summary Statistics of Log₂-Transformed Prenatal EDC Concentrations

eFigure 5. Correlation Plot Between EDC Exposures

eTable 6. BWQS Models for MetS for All the Study Population and Stratified by Sex

eTable 7. Estimated Posterior Weights of Exposure Mixture Groups on MetS Using BWQS Models for All the Study Population and Stratified by Sex

eTable 8. Adjusted Associations Between Childhood Proteins and Child MetS Risk Score

eTable 9. Adjusted Associations Between Childhood Serum Metabolites and Child MetS Risk Score

eTable 10. Adjusted Associations Between Childhood Urine Metabolites and Child MetS Risk Score

eTable 11. Adjusted Associations Between Prenatal EDC Mixtures and Child Proteins Using BWQS

eTable 12. Adjusted Associations Between Prenatal EDC Mixtures and Child Serum Metabolites Using BWQS

eTable 13. Adjusted Associations Between Prenatal EDC Mixtures and Child Urine Metabolites Using BWQS

eTable 14. Environmental-Wide Association Study (ExWAS) of Prenatal Individual EDCs and Child MetS

eTable 15. Sensitivity Analysis. BWQS Models of Lipophilic Mixture Groups and MetS Stratified by Gestational Weight Gain Category

eTable 16. Sensitivity Analyses. BWQS Models of Phthalate and Non-Persistent Chemical Mixtures

eTable 17. Sensitivity Analyses. BWQS Models of Metals and Persistent Chemicals Mixture

eTable 18. Sensitivity Analyses. BWQS Models for Dichotomous MetS Risk (Low vs High) for All the Study Population

eReferences

SUPPLEMENT 2.

Data Sharing Statement