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Associations of PFAS and phthalate/DINCH metabolites with metabolic regulation in teenagers from the HBM4EU aligned studies

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ABSTRACT

Background: Exposure to per- and polyfluoroalkyl substances (PFAS) and phthalates is widespread during adolescence, a critical developmental period for metabolic regulation.
Aim: To assess associations of serum PFAS and urinary phthalate/DINCH metabolites-individually and as mixtures-with a unified panel of metabolic biomarkers representing adipose-brain-liver cross-talk and oxidative stress in European teenagers.
Methodology: Serum PFAS and urinary phthalate/DINCH metabolites were measured in 1033 European teenagers (12–17 years) from the Human Biomonitoring Initiative for Europe (HBM4EU) Aligned Studies. Metabolic biomarkers representing adipose (HDL, LDL, cholesterol, and triglycerides)-brain (leptin, adiponectin, and kisspeptin)–liver (glucose, insulin) cross-talk and oxidative stress (8-hydroxy-2′-deoxyguanosine, 8OHdG) were measured. The Body Mass Index z-score (zBMI) was calculated. Single pollutant models, multivariate MANOVA, quantile g-computation, and BKMR models were fit, including interaction terms with sex.
Results: Single pollutant models showed positive associations of PFAS and phthalate/DINCH metabolites with 8OHdG. PFAS were associated with higher leptin, HDL, LDL, and cholesterol, while some phthalate/DINCH metabolites were associated with lower kisspeptin, HDL, triglycerides, cholesterol, zBMI, and higher adiponectin. We observed weak but statistically significant associations between PFAS and phthalate/DINCH metabolites with the entire set of metabolic biomarkers in the MANOVA. The PFAS mixture was associated with higher kisspeptin, LDL, HDL, cholesterol, and 8OHdG. The phthalate/DINCH mixture was associated with lower HDL.
Conclusions: Exposure to these contaminants may be related to dyslipidemia in teenagers. PFAS and phthalate/DINCH metabolites may exert opposite associations on metabolism, with the exception of increasing oxidative stress. Given the cross-sectional design and potential residual confounding, longitudinal studies are warranted.

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1. Introduction

Phthalates and per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals widely used in consumer goods, such as textiles, cookware, or food packaging materials (Sunderland et al., 2018). Additionally, diisononylcyclohexane-1,2-dicarboxylate (DINCH) is a commonly used alternative plasticizer to phthalates, and its primary metabolite, MINCH, is widely used in human biomonitoring studies (Engel et al., 2018; Mol et al., 2022; Schütze et al., 2017). Although many efforts have been made to regulate the production of these substances globally, they are still being manufactured in many regions (Katsikantami et al., 2016). Consequently, these environmental pollutants are detected worldwide in the human population (Katsikantami et al., 2016; Schwedler et al., 2020; Vogel et al., 2023). The HBM4EU (Human Biomonitoring Initiative for Europe) project found that teenagers across several European countries are widely exposed to these pollutants (Gilles et al., 2021; Govarts et al., 2023). Moreover, their potential health effects on body mass index, reproduction, and asthma/allergies are under investigation (Desalegn et al., 2024; Hassen et al., 2025; Richterová et al., 2023; Rodríguez-Carrillo et al., 2023; Schillemans et al., 2023).

Evidence from in vivo studies suggests that exposure to PFAS and phthalates may disrupt metabolic homeostasis through multiple interconnected pathways. These chemicals can activate nuclear receptors, such as the peroxisome proliferator-activated receptor (PPARα), a key regulator of lipid metabolism and energy balance, thus influencing adipose tissue function and systemic lipid profiles (Behr et al., 2020; Rosen et al., 2022). In addition, PFAS have been shown to interfere with bile acid metabolism and related gene expression, potentially altering cholesterol transport and hepatic lipid homeostasis (Bjork et al., 2021; Fletcher et al., 2013; Harada et al., 2007; Marques et al., 2022; Steenland et al., 2009). Phthalates may further contribute to metabolic disruption through endocrine-related mechanisms, including anti-androgenic, anti-thyroid, and the induction of oxidative stress (De Falco et al., 2015; Huang et al., 2022; Kasper-Sonnenberg et al., 2017; Pérez-Díaz et al., 2024; Taibl et al., 2022). To date, limited or controversial evidence is available regarding these effects during adolescence, a critical period of development, especially concerning metabolic function.

To comprehensively investigate the biological pathways underlying metabolic alterations associated with PFAS and phthalate exposure, it is necessary to adopt an integrated framework that captures the interplay between multiple physiological systems. Experimental and epidemiological evidence suggests that these chemicals may disrupt metabolic homeostasis through interconnected mechanisms involving adipose tissue signaling (e.g., adipokines), central neuroendocrine regulation, hepatic lipid and glucose metabolism, and oxidative stress (Gaillard et al., 2025; Pérez-Díaz et al., 2024). This “adipose–brain–liver crosstalk” provides a systems-level perspective in which alterations in one pathway may influence others, ultimately affecting overall metabolic regulation.

Within this framework, classical metabolic biomarkers (lipids, glucose, insulin, adipokines) reflect well-established pathways of metabolic dysfunction, while emerging biomarkers may provide additional insight into neuroendocrine regulation. In this context, kisspeptin, a peptide hormone primarily known for its role in reproductive function (Uenoyama et al., 2021), is increasingly recognized as a regulator of energy balance and metabolic homeostasis, with expression in both central and peripheral tissues (Hudson and Kauffman, 2022; Wolfe and Hussain, 2018). Although epidemiological evidence remains limited, experimental data and recent findings from HBM4EU adolescents suggest that kisspeptin may be sensitive to endocrine-disrupting chemicals (Rodríguez-Carrillo et al., 2024, 2023), supporting its inclusion as an exploratory biomarker within this integrated framework.

We hypothesize that higher exposure to PFAS and phthalate/DINCH metabolites may be associated with a dysregulated metabolic profile in adolescents, characterized by alterations in lipid metabolism, glucose homeostasis, adipokine signaling, oxidative stress, and neuroendocrine pathways. To test this hypothesis, we assessed associations of PFAS and phthalate/DINCH metabolites (individually and as mixtures) with a panel of metabolic and effect biomarkers, including total cholesterol, HDL, LDL, triglycerides, glucose, insulin, leptin, adiponectin, kisspeptin, urinary 8OHdG, and zBMI, within the HBM4EU adolescent population.

2. Material and methods

2.1. Study population

Teenagers from five studies aligned within the Human Biomonitoring Initiative for Europe (HBM4EU) were included in the present cross-sectional study (Gilles et al., 2022; Govarts et al., 2023). These studies adhered to two specific inclusion requirements: available biological samples collected between 2014 and 2021, and comparable analytical results for exposure biomarkers analyzed in laboratories that met the HBM4EU Quality Assurance/Quality Control (QA/QC) criteria (Esteban López et al., 2021; Gilles et al., 2021). Out of the initial eleven studies, ten were included in our previous analysis on urinary phthalate/DINCH metabolites, serum/plasma PFAS concentrations and zBMI (Schillemans et al. 2023, Desalegn et al. 2024); and of these, five provided data on serum/plasma metabolic biomarkers levels: FLEHS IV (*Flemish Environment and Health Study IV*; Belgium, n = 239 participants aged 14–15 years), PCB cohort (*Endocrine disruptors and health in children and teenagers in Slovakia*; Slovakia, n = 267 participants aged 15–17 years), BEA (*Biomonitorización en Adolescentes*; Spain, n = 285 participants aged 14–16 years), NEBII (*Norwegian Environmental Biobank II*; Norway, n = 152 participants aged 12–14 years), and SLO CRP (*Exposure of children and adolescents to selected chemicals through their habitat environment*; Slovenia, n = 90 participants aged 12–15 years). The final sample for the cross-sectional analysis consisted of 1033 teenagers (563 females and 470 males). Nevertheless, BEA had missing data for glucose (GLU); SLO CRP had no insulin (INS) measurements; and NEBII cohort had no data for 8-hydroxy-2′-deoxyguanosine (8OHdG), GLU, and total cholesterol (CHOL) levels. Thus, final sample sizes for these metabolic biomarkers were: 881 participants for CHOL; 877 for 8OHdG; 596 for GLU, and 924 for INS. Signed informed consent was obtained from the participating teenagers and at least one parent/guardian. The studies were approved by the local ethical committees and adhered to the Principles of the Declaration of Helsinki.

2.2. Assessment of serum metabolic biomarkers

The process for measuring serum kisspeptin (kiss54 isoform) protein levels using the human KISS-54 kit (Biotek Synergy HT, MyBiosource, San Diego, CA) is thoroughly detailed in (Rodriguez-Carrillo et al., 2023). This was performed at the Center of Biomedical Research (CIBM), University of Granada, Spain. The mean intra-coefficients of variation (intra-CV, %) and inter-coefficients of variation (inter-CV, %) were consistently below 5%, ensuring high quality and reproducibility of the measurements.

Except for the NEBII cohort, serum adipokines leptin (LEP) and adiponectin (ADIPO) were measured using the leptin Human ELISA kit, KAC2281 (Invitrogen by Thermo Fisher Scientific, Frederick, MD) and adiponectin ELISA kit (human) ALX-850-377 (ENZO Life Science, Farmingdale, NY), respectively. Samples were thawed, vortex-mixed, diluted (1:100), and analyzed according to the manufacturer's instructions at the facilities of the CIBM, University of Granada, Spain. The remaining metabolic serum biomarkers were measured at the laboratory of the Hospital Clinico San Cecilio, University of Granada, Granada (Spain) using Beckman® Coulter AU: glucose (GLU), triglycerides (TRIGL), total cholesterol (CHOL), high- and low-density lipoprotein (HDL, LDL), and insulin (INS). Concretely, CHOL, HDL, LDL, and TRIGL were determined with enzymatic colorimetric assays, GLU with

ultraviolet radiation, and INS with electrochemiluminescence-based assays (Fernandez et al., 2022).

In the NEB II cohort, LEP, ADIPO, HDL, LDL, and INS were measured in plasma samples at the facilities of the Finnish Institute for Health and Welfare (THL) in Helsinki, Finland (accredited T077 by the Finnish Accreditation Service, fulfilling the requirements of the standard SFS-EN ISO/IEC 17025:2017). The ARCHITECT® ci8200 System (Abbott Laboratories, Abbott Park, IL) was used for blood lipids. LDL cholesterol was calculated using the Friedewald formula. Plasma leptin was determined by using the Human Leptin Duoset ELISA (R&D Systems Europe Ltd, Abindgon, U.K) according to the manufacturer's instructions. Plasma adiponectin was measured by using the Human Adiponectin/Acrp30 DuoSet ELISA (R&D Systems Europe Ltd, Abingdon, U.K).

Finally, 8-hydroxy-2-deoxyguanosine (8OHdG) was measured in urine samples from all studies using rapid extraction coupled to LC-MS/MS as described previously (Bláhová et al., 2023) within the facilities of the Faculty of Science, Masaryk University, Czech Republic. Specific gravity (sg) was used to adjust for the urinary dilution by normalizing samples to a population average urinary concentration of 1.024 (Kuiper et al., 2021). Intra-CV and inter-CV were $< 5\%$ and $< 15\%$, respectively, for all effect biomarkers.

2.3. Assessment of PFAS

Serum (FLEHS IV, BEA, PCB cohort, and SLO CRP) and plasma (NEB II) were collected in fasting (PCB cohort) and non-fasting (FLEHS IV, BEA, NEB II, and SLO CRP studies) conditions and kept at −20 °C or colder. Chemical analyses were conducted in laboratories fulfilling HBM4EU QA/QC criteria, guaranteeing the quality and comparability of data (Esteban López et al., 2021). For the current study, twelve PFAS were ascertained using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Limits of quantification (LOQs) were set as cut-off values for reporting quantifiable data, since detection limits (LODs) and quantification limits (LOQs) varied among studies. PFAS with quantification rates $> 70\%$ were included in the statistical analysis: perfluorooctanoate (PFOA), perfluorononanoic acid (PFNA), and perfluorooctane sulfonate (PFOS). Additionally, the arithmetic sum of PFOA, PFNA, and PFOS concentrations was calculated ($\sum$PFAS = PFOA + PFNA + PFOS) (μg/L). Supplemental Table S1 provides the limits of quantification (LOQ) and frequencies of quantification (FOQ, %) of PFAS measurements per study.

2.4. Assessment of urinary phthalates/DINCH metabolites

First morning (BEA) and random spot urine samples (FLEHS IV, NEBII, PCB cohort, and SLO CRP) were collected and kept at −20 °C or colder until analysis. Phthalates and DINCH metabolites were analyzed using HPLC-MS/MS under the HBM4EU QA/QC program (Esteban López et al., 2021; Nübler et al., 2022). Ten phthalate monoester metabolites were measured: mono-ethyl phthalate (MEP) from diethyl phthalate (DEP), mono-benzyl phthalate (MBzP) from benzyl butyl phthalate (BBzP), mono-*iso*-butyl phthalate (MiBP) from diisobutyl phthalate (DiBP), and mono-n-butyl phthalate (MnBP) from di-n-butyl phthalate (DnBP). Additionally, the primary and secondary metabolites of diethylhexyl phthalate (DEHP) were measured, including mono (2-ethylhexyl) phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (OH-MEHP), mono-2-ethyl-5-oxohexyl phthalate (oxo-MEHP), mono-2-ethyl-5-carboxypentyl phthalate (cx-MEPP), and those of di-isononyl phthalate (DiNP): mono-hydroxyisononyl (MHNP) and mono-carboxyisooctyl phthalate (MCOP). One DINCH metabolite, the mono-hydroxyisononyl cyclohexane-1,2-dicarboxylate (MHNCH), also known as OH-MINCH, was also analyzed. Concentrations of phthalates/DINCH metabolites with quantification frequencies above 70% were included in statistical analysis (Table 2). The molar-weight (mw) sums of the primary urinary metabolites of DEHP ($\sum$DEHP) and DiNP ($\sum$DiNP) were calculated as follows: $\sum$DEHP (μmol/L) = (MEHP *1/mw + OH-MEHP *1/mw + oxo-MEHP *1/mw) and $\sum$DiNP (μmol/L) = (MHNP *1/mw + MCOP *1/mw). LOQ and FOQ (%) are provided in Supplemental Table S1. Since urinary phthalate/DINCH metabolites concentrations are influenced by dilution level, specific gravity (sg) was used to adjust for the urinary dilution by normalizing samples to a standard or a population average urinary concentration of 1.024 (Kuiper et al., 2021; O'Brien et al., 2015; Pearson et al., 2009).

2.5. Anthropometric values and covariates

Data from the HBM4EU Aligned Studies were harmonized according to centrally developed codebooks described in Govarts et al., 2023. Thus, covariates were collected using questionnaires, including variables such as teenager's age (years), sex (female/male), weight (kg), height (cm), and household educational level, following the International Standard Classification of Education (ISCED, 2011) guidelines [low education was defined as no secondary to lower secondary education (ISCED level 0–2), medium education as having attained upper secondary to post-secondary non-tertiary education (ISCED level 3–4), and high education as having attained tertiary education or higher (ISCED level $\geq$ 5)], country of the study (Belgium, Norway, Slovakia, Slovenia, Spain), alcohol consumption (yes/no), and passive smoking (yes/no). Body mass index z-score (zBMI), equivalent to the BMI standard deviation score (BMI-SDS), was computed following the World Health Organization (WHO) age- and sex-specific growth standards using the 'anthroplus' package in R (WHO, n.d.). It was included as a comparison to our previous analyses in HBM4EU Aligned Studies on these compounds and zBMI (Desalegn et al., 2024; Schillemans et al., 2023).

2.6. Statistical analyses

We applied complementary statistical approaches to evaluate individual and combined associations of exposures. Single-pollutant models were used to assess exposure–outcome associations for each contaminant separately. Multivariate models (MANOVA) were then applied to evaluate whether each exposure was associated with the overall metabolic profile, accounting for correlations among biomarkers. In addition, mixture models (quantile g-computation and BKMR) were used to estimate the joint effect of multiple exposures. To reduce the effect of outliers, all exposure (PFAS and phthalate/DINCH metabolites) and effect (metabolic) biomarkers were natural log-transformed. Exposure and effect biomarkers below LOQ and LOD were imputed by single random imputation from a truncated lognormal distribution within each study (Govarts et al., 2020; Schillemans et al., 2023). Pairwise Spearman's correlation was used to assess correlations between PFAS and phthalates/DINCH metabolites and among effect biomarkers.

Exposure-effect associations were modelled with separate linear regression models for each exposure-outcome combination in R. MANOVA was fitted to evaluate whether each exposure was associated with the overall metabolic profile (kisspeptin, adiponectin, leptin, HDL, LDL, total cholesterol, triglycerides, glucose, insulin, and 8OHdG), accounting for correlations among biomarkers. MANOVA is based on a set of simultaneous linear regressions —one for each dependent variable— sharing the same predictors. The coefficients obtained from this analysis correspond to those from the individual regressions, which we report in the Supplementary Material. Wilks' Λ test then evaluates the joint significance of these coefficients across all outcomes. Wilks' Λ ranges from 0 to 1, where values closer to 0 indicate stronger multivariate effects (greater overall differences in the metabolic profile across exposure levels), and values near 1 suggest weaker or no multivariate association. This approach allows assessing whether exposures are associated with changes across multiple metabolic pathways rather than isolated effects on individual biomarkers. This provides a system-level perspective on metabolic regulation, aligned with the adipose-brain-liver cross-talk framework (Rencher and William, 2012).

We then used two multi-pollutant models to estimate the total mixture effect: quantile G-computation (Keil et al., 2020) as the main mixture analysis and Bayesian Kernel Machine Regression (BKMR) (Bobb et al., 2015) as a secondary analysis. These approaches were applied to exploit their complementary aims and methodological strengths (e.g., BKMR is able to assess non-linearity, while g-computation is easier to interpret and widely implemented in standard software). To improve the interpretation of results, mixtures were assessed separately according to the chemical family due to their physicochemical differences and half-life in the human body: PFAS mixture (PFOA, PFNA, and PFOS) and phthalates/DINCH metabolites mixture (MEP, MBzP, MiBP, MnBP, ΣDEHP, cx-MEPP, ΣDINP, and MINCH).

The selection of mixture components was guided by a priori considerations related to shared parent compounds, correlation structure, and model stability. For phthalates/DINCH metabolites, several compounds derived from the same parent chemicals showed high correlations ($\rho > 0.7$), and including all metabolites simultaneously led to unstable estimates and convergence issues. Therefore, metabolites sharing a common parent compound were grouped into summed biomarkers (e.g., ΣDEHP, ΣDINP) to reduce collinearity and better reflect overall exposure to parent compounds. For PFAS, only compounds with a detection frequency ≥ 70% were included to ensure robustness of the estimates, as compounds with lower detection frequencies may introduce bias due to a high proportion of imputed or censored values.

Quantile G-computation was performed using exposure quartiles and 1000 bootstraps in the 'gcomp' package in R (Keil et al., 2020). BKMR is a non-parametric method that models the exposure response using a kernel function that considers potential interactions between exposures and a possible nonlinear association between exposure and outcome. We used component-wise variable selection and a Gaussian kernel function. The model was fit by running the Markov chain Monte Carlo sampler for 100,000 iterations with the 'bkmr' and 'bkmrhat' packages in R (Bobb et al., 2015). All BKMR analyses showed Rhat values below 1.01, indicating good model convergence and reliability.

To adjust for potential confounders, covariates were selected based on their toxicological relevance and their previous use in other epidemiological studies. All models were adjusted for age (continuous) as indicator of the growth stage (Fassler et al., 2019; Shih et al., 2022); sex, to account for sex-dependent differences of the metabolic function (Mauvais-Jarvis, 2023); country of study (Belgium, Norway, Slovakia, Slovenia, Spain); and household educational level [low (ISCED 0–2), medium (ISCED 3–4), and high (ISCED 5–8)] as indicator for socioeconomic status (Buekers et al., 2018). Although phthalate/DINCH metabolites were standardized by urinary specific gravity, we additionally adjusted for natural log-transformed specific gravity (Pearson et al., 2009) to account for residual variability in urinary dilution (Kuiper et al., 2021; O'Brien et al., 2016).

Sensitivity analyses were conducted to assess the robustness of results, including models excluding specific gravity and models restricted to cohorts with complete biomarker data (FLEHS IV and PCB cohorts). These sensitivity analyses were conducted in the main models (linear regression and quantile g-computation), and results are presented in the Supplementary Materials. Finally, the sex-interaction (biomarkers of exposure*sex) was included in a secondary model to test for potential differences due to sex. Statistically significant interaction terms (p-interaction < 0.05) were interpreted as evidence of effect modification by sex. Other potentially relevant covariates, such as alcohol intake and passive smoking, were not considered due to high missing rates.

Regression estimates were expressed as the percentage change in the effect biomarker (e.g., metabolic biomarkers) for each p75/p25 factor increase in PFAS and phthalate/DINCH metabolites exposure with corresponding 95% confidence intervals (CIs), and credible intervals (CrIs) for BKMR models. G-computation results were expressed as the percentage change in the effect biomarker when increasing all PFAS or phthalate/DINCH metabolites by one-quartile simultaneously (Fu et al., 2023). The threshold for statistical significance in the exposure-effect associations was set at $p < 0.05$. No formal correction for multiple testing was applied; instead, results were interpreted based on overall patterns, consistency across models, and coherence with prior toxicological and epidemiological evidence (Amrhein et al., 2019).

3. Results

3.1. Characteristics of the study population

Table 1 shows the characteristics of European teenagers included in the present study. Teenagers aged 12–17 years, and 54.5% were females. The mean (SD) zBMI was 0.29 (1.11), which is close to the expected standard normal distribution. Mean (SD) urinary specific gravity was 1.02 (0.01), also within normal ranges (1.01 to 1.03), and 50.15%, 40.08%, and 9.78% of participants reported high, medium, and low household education respectively.

Tables 2 presents the concentrations of metabolic biomarkers and exposure markers (PFAS and phthalate/DINCH metabolites), respectively. Median levels of metabolic biomarkers were within non-fasting reference values (ADIPO: 1.9–17 μg/mL; LEP: 3–18 ng/mL; HDL: >50 mg/dL; LDL: <129 mg/dL; CHOL: <200 mg/dL; TRIGL: <130 mg/dL; GLU: 80–130 mg/dL; and INS: <50 mU/L, (Flynn et al., 2017). No reference values are available for kisspeptin nor 8OHdG.

Among serum PFAS, PFOS showed the highest median concentration (P50 = 1.80 ng/mL), while PFNA had the lowest (P50 = 0.28 ng/mL) (Table 2). For urinary phthalate/DINCH metabolites, MEP exhibited the highest concentration (P50 = 47.19 μg/L), and MHNCH the lowest (P50 = 1.96 μg/L) (Table 2). In the Spearman correlation analysis (Fig. 1), serum PFAS were positively and significantly correlated with each other, with Rho values exceeding 0.60. A similar pattern was observed among phthalate/DINCH metabolites: strong correlations (Rho > 0.60) were found between MiBP and MnBP, MnBP and OH-MEHP, cx-MEPP and oxo-MEHP, MEHP and oxo-MEHP, and MHNP and MCOP. In contrast, serum PFAS and urinary phthalate metabolites were weakly but significantly negatively correlated (Fig. 1). Finally, among the effect biomarkers, only LDL showed a strong and significant correlation with CHOL levels (Rho = 0.86; Fig. 1).

3.2. Associations of PFAS and Phthalate/DINCH metabolites exposure with metabolic biomarkers and zBMI in teenagers

3.2.1. Results from single-pollutant models

PFAS were associated with higher levels of lipid biomarkers (HDL, LDL, and total cholesterol), leptin, and 8OHdG (Fig. 2, Table S2). Estimates represent the percent change in each metabolic biomarker for a p75/p25 increase in exposure concentrations. Thus, PFOA was associated with higher LDL (10.59%; 95% CI: 6.16, 15.21), HDL (6.05%; 2.46, 9.77), total cholesterol (3.71%; 2.27, 5.16), leptin (34.54%; 5.05, 72.31), and 8OHdG (19.42%; 7.11, 33.15). PFNA showed similar patterns, with higher HDL (3.19%; 1.07, 5.37), leptin (19.76%; 3.08, 39.14), and total cholesterol (1.37%; 0.13, 2.62), and inverse associations with insulin (−6.44%; −12.30, −0.18) and 8OHdG (−5.27%; −10.11, −0.17). PFOS and ∑PFAS were also associated with higher HDL, total cholesterol, and 8OHdG (Fig. 2, Table S2).

In contrast, phthalate/DINCH metabolites were associated with lower HDL, zBMI, and kisspeptin levels, and higher adiponectin and 8OHdG levels. MnBP, MEHP, and OH-MEHP were associated with lower kisspeptin levels. MiBP, MEHP, oxo-MEHP, cx-MEPP, and MCOP, were associated with lower total cholesterol, while MnBP with lower HDL. MiBP was also associated with lower zBMI, and MEP with higher triglycerides. Most phthalate/DINCH metabolites showed positive associations with 8OHdG, except for MEP and MCOP (Fig. 2, Table S2).

Significant sex interactions were observed for PFAS in relation to leptin (Table S2). Stratified analyses showed that associations with higher leptin were observed only in females for PFOA and PFNA, whereas no significant associations were observed in males (Table S3).

Table 1
Characteristics of study population (n = 1033).

	Age (years) Range	Sex (%) (f/m)	sg Mean (SD)	Household education (%) Low	Medium	High	Sample collection (year)
All studies	12—17	55/45	1.02 (0.01)	9.78	40.08	50.15	2016–2020
BEA	14—16	57/43	1.02 (0.01)	17.89	28.77	53.33	2017–2018
FLEHS IV	14—15	53/47	1.02 (0.01)	4.18	34.31	61.51	2017–2018
NEB II	13—14	55/45	1.03 (0.01)	0	7.24	92.76	2016–2017
PCB cohort	15—17	57/43	1.02 (0.01)	7.49	77.53	14.98	2019–2020
SLO CRP	12—15	46/54	1.03 (0.01)	22.22	35.56	42.22	2018

BEA: Biomonitorización en Adolescentes, Spain; FLEHS IV; Flemish Environment and Health Study IV, Belgium; NEBII: Norwegian Environmental Biobank II, Norway; PCB cohort: Endocrine disruptors and health in children and teenagers in Slovakia, Slovakia; SLO CRP: Exposure of children and adolescents to selected chemicals through their habitat environment, Slovenia.

These findings were consistent across all sensitivity analyses, including models excluding specific gravity and those restricted to cohorts with complete biomarker data (Supplementary Tables S4 and S5).

3.2.2. *Results from single-pollutant multivariate models from MANOVA*

Results from MANOVA (Table S6.A) were comparable to those from single-pollutant models. PFAS associations remained unchanged, while several phthalate/DINCH metabolites showed stronger statistical evidence. Thus, MEP was associated with higher kisspeptin levels [1.51% (0.30; 2.72)]. MnBP and MHNP were associated with higher adiponectin levels [5.57% (1.86; 9.41) and 3.90% (0.32; 7.60), respectively], while MEHP and MHNCH were associated with lower leptin levels [21.15% (−35.14; −4.14) and 16.39% (−28.5; −2.22)]. Oxo-MEHP and ∑DEHP were associated with lower triglycerides levels [6.49% (−10.02; −2.83) and 5.21% (−9.52; −0.70)] (Table S6.A).

MANOVA further indicated that several pollutants were associated with the combined set of metabolic biomarkers (Table S6.B) with modest but statistically significant multivariate effects (Wilks' Λ ranging from 0.79 to 0.92), ($p < 0.001$). MCOP (Wilks' $\Lambda = 0.79$, $p < 0.001$) and OH-MEHP (Wilks' $\Lambda = 0.80$, $p < 0.001$) showed the strongest multivariate associations, whereas MHNCH did not reach statistical significance (Wilks' $\Lambda = 0.98$, $p = 0.07$).

3.3. *PFAS and Phthalate/DINCH metabolites mixture effect on metabolic biomarkers*

In quantile g-computation models, the PFAS mixture was significantly associated with higher lipids (HDL, LDL, and CHOL), kisspeptin, and 8OHdG (Fig. 3, Table S7). A one-quartile increase in PFAS mixture was associated with higher HDL (4.33%; 95% CI: 2.44, 6.26), LDL (2.87%; 0.15, 5.66), total cholesterol (2.46%; 0.89, 4.05), and 8OHdG (17.02%; 11.28, 23.05). PFOA was the main contributor to most of these associations, whereas PFNA contributed more strongly to kisspeptin levels (Fig. S1). Regarding the phthalate/DINCH mixture, a significant association with lower HDL (−2.84; −5.62, −0.04) was observed, with ∑DEHP as the main contributor (Fig. S2). In addition, inverse associations with leptin (−17.79%; −33.09, 0.99) and positive associations with 8OHdG (7.58%; −0.94, 16.84) were observed, although not statistically significant (Fig. 3, Table S7).

In BKMR models, the PFAS mixture was positively associated with HDL, LDL, CHOL, and 8OHdG (Fig. S3, S4, and S5), with PFOA showing the highest posterior inclusion probabilities (PIP ≥ 0.68; Table S8), may suggesting non-linear relationships (Fig. S4). In contrast, the phthalate/DINCH mixture showed inverse associations with ADIPO and HDL levels (Fig. S3, S4, and S6). ∑DEHP was the main contributor to HDL (PIP = 0.67), while ∑DINP contributed most to adiponectin (PIP = 0.34; Table S8).

No significant associations were found with zBMI in any multipollutant model (Fig. S3, S4, S5, and S6). These findings were consistent in sensitivity analyses excluding urinary dilution adjustment and restricting the sample to those studies with complete biomarkers data (*Table S7*).

4. Discussion

This cross-sectional study with European teenagers is, to our knowledge, the first to explore associations between PFAS and phthalate/DINCH metabolites and a comprehensive set of effect biomarkers reflecting interconnected metabolic pathways. Our findings suggest that PFAS and phthalate/DINCH metabolites were associated with distinct and, in some cases, opposing patterns across metabolic biomarkers, particularly in relation to lipid metabolism while both chemical families showed consistent positive associations with oxidative stress. In line with this, MANOVA results supported the presence of correlated changes across the metabolic profile, indicating system-level alterations rather than isolated effects on individual biomarkers.

Serum PFOA, PFNA, and PFOS, three of the four most commonly detected PFAS in human blood samples at the international level (CDC, 2022), exhibited varying concentrations across included studies. The BEA and PCB studies showed the lowest PFAS concentrations, whereas NEBII and FLEHS IV showed the highest, as reported previously by Richterová et al., (2023). These differences in PFAS concentrations across studies may be explained by variability in exposure sources, including dietary habits, use of consumer products, and environmental contamination (e.g., drinking water). Additionally, variations in industrial activity and temporal trends in PFAS production across countries, as well as cohort-specific characteristics and sampling periods, may have contributed to the observed differences in exposure.

In this regard, teenagers from this study showed lower PFAS concentrations than teenagers from the US (Sonnenberg et al., 2023) and comparable concentrations to Canadian teenagers of similar age (Caron-Beaudoin et al., 2019). Urinary phthalate/DINCH metabolites showed the highest concentrations in the PCB cohort and lowest in FLEHS IV (Vogel et al., 2023). When comparing with other studies, European teenagers showed comparable concentrations with those reported in teenagers from the US (Xiang et al., 2022) and higher than some metabolites (e.g., MnBP and MBzP) reported for Korean teenagers aged 13–17 years (Lee et al., 2021).

Epidemiological evidence of PFAS and metabolic regulators

Serum/plasma PFOS, PFNA, and PFOA concentrations showed positive associations with blood lipids HDL, LDL, and total cholesterol, which seems to be supported by the current evidence. Thus, previous studies showed cross-sectional and longitudinal associations with higher cholesterol, LDL, and HDL levels in teenagers from Norway (Averina et al., 2021) and China (Shih et al., 2022). Moreover, a meta-analysis comprising 12 studies with children and teenagers reported that several PFAS, including PFNA, PFOS, and PFOA, were also associated with higher LDL and total cholesterol (Zheng et al., 2024). Interestingly, this may suggest a potential role of PFAS in lipid dysregulation, a risk factor for developing cardiovascular disease in the future (Lin et al., 2025).

Regarding the cross-talk between adipose tissue and the brain, our study found associations between PFOA and PFNA and higher leptin levels, which remained significant only in females after splitting by sex, suggesting an important role of sex in these associations. However, null

Table 2
Metabolic effect biomarkers (n = 1033, except for GLU, n = 596; INS, n = 924; CHOL, n = 881; 8OHdG, n = 877; zBMI = 988), PFAS (ng/mL), and sg-normalized urinary phthalate metabolites (μg/L) concentrations in European teenagers (n = 1033).

		Metabolic Effect Biomarkers and zBMI															
		KISS	ADIPO	LEP		HDL		LDL		GLU		INS		CHOL		TRIGL	8OHdG	zBMI
All studies	P25	20.63	8.26	2.24		41.00		80.66		79.00		5.77		136.00		56.00	4.55	−0.41
	P50	22.31	11.55	7.00		48.00		95.00		88.00		9.10		156.00		73.00	6.93	0.24
	P75	23.70	15.73	16.71		56.00		110.00		97.00		16.20		183.00		101.00	12.73	1.02
BEA	P25	20.10	9.23	1.63		38.67		79.36				4.79		127.00		46.00	4.73	−0.40
	P50	22.08	12.67	6.44		44.36		92.00				7.44		143.00		59.00	5.92	0.31
	P75	23.60	16.71	15.51		50.87		107.00				15.63		160.00		80.00	8.13	1.02
FLEHS IV	P25	20.03	11.71	2.35		44.00		87.00		78.00		6.10		140.00		62.00	13.31	−0.38
	P50	21.43	14.90	7.25		51.00		101.00		89.00		9.70		157.00		82.00	17.24	0.24
	P75	23.18	18.70	15.11		59.00		115.00		97.00		17.03		173.00		110.00	21.36	0.32
NEB II	P25	21.73	4.60	3.79		53.56		78.21				8.50				56.49		−0.50
	P50	22.65	5.40	6.25		60.33		93.00				12.20				70.21		0.01
	P75	23.41	6.80	13.08		65.74		109.73				22.28				102.70		0.58
PCB cohort*	P25	20.99	9.39	2.15		38.00		84.20		77.00		5.95		136.00		63.00	3.54	−0.41
	P50	23.04	11.86	10.08		44.00		98.00		85.00		8.39		153.00		79.00	5.44	0.33
	P75	24.60	15.30	21.89		52.00		113.00		93.00		12.39		174.00		104.00	7.93	1.17
SLO CRP	P25	20.43	7.93	1.31		39.00		68.10		87.00				131.46		70.83	1.07	−0.42
	P50	22.24	9.19	5.10		45.00		79.50		96.50				148.86		88.54	2.40	0.19
	P75	23.29	11.75	10.71		52.00		95.45		105.25				162.39		126.17	6.11	1.13
	Exposure Biomarkers																	
		PFOA	PFNA	PFOS	∑PFAS	MEP	MBzP	MiBP	MnBP	MEHP	OH-MEHP	oxo-MEHP	∑DEHP	cx-MEPP	MHNP	MCOP	∑DINP	MHNCH
All studies	P25	0.63	0.19	1.10	0.69	20.82	1.26	16.44	15.12	1.14	6.55	3.88	0.04	9.15	3.54	3.34	0.02	1.12
	P50	0.85	0.28	1.80	0.99	47.19	2.40	26.50	25.31	2.09	11.20	6.23	0.07	13.71	6.28	6.41	0.04	1.96
	P75	1.16	0.40	2.81	1.45	114.62	4.99	48.37	58.69	3.98	23.72	10.62	0.13	20.28	14.27	10.89	0.08	3.47
BEA	P25	0.52	0.21	0.93	0.60	50.47	1.17	16.14	11.94	1.54	6.90	4.91	0.05	9.33	3.27	5.06	0.03	1.27
	P50	0.66	0.28	1.38	0.78	93.54	2.08	23.46	18.66	2.34	10.12	7.08	0.07	12.99	4.88	7.77	0.04	2.16
	P75	0.79	0.38	1.86	0.99	197.24	3.48	38.26	28.01	3.65	15.69	10.60	0.10	19.51	8.27	11.79	0.07	3.51
FLEHS IV	P25	0.87	0.22	1.50	0.92	14.60	1.28	13.83	11.86	0.83	4.03	2.51	0.03	11.86	2.79	1.31	0.01	0.76
	P50	1.10	0.31	2.20	1.23	24.51	2.18	22.43	17.67	1.32	6.26	4.06	0.04	16.13	4.23	1.90	0.02	1.25
	P75	1.40	0.44	3.40	1.71	62.01	5.35	40.51	27.38	2.14	10.01	6.47	0.06	22.26	6.32	2.88	0.03	2.37
NEB II	P25	1.05	0.33	2.27	1.28	9.63	2.85	15.22	16.35	0.67	5.43	3.19	0.03	9.24	3.48	6.31	0.03	1.63
	P50	1.32	0.45	2.81	1.57	16.07	4.95	26.56	24.91	1.25	8.12	4.89	0.05	14.42	5.44	8.46	0.05	2.42
	P75	1.63	0.66	3.85	1.96	28.46	8.48	46.04	38.95	2.31	13.35	7.37	0.08	21.86	9.86	13.46	0.08	5.23
PCB	P25	0.50	0.10	0.85	0.55	33.68	0.83	21.70	64.42	2.63	24.09	5.34	0.12	6.98	12.60	5.42	0.06	1.08
	P50	0.73	0.18	1.37	0.78	68.77	1.73	42.08	112.67	4.85	43.48	9.16	0.20	11.40	20.46	8.67	0.10	2.21
	P75	0.96	0.26	2.47	1.25	136.80	3.67	79.73	240.00	8.34	74.53	14.40	0.33	17.89	35.78	15.45	0.16	3.70
SLO CRP	P25	0.74	0.20	1.16	0.77	19.96	1.92	17.63	15.49	0.85	7.46	5.40	0.05	8.65	2.56	4.00	0.02	1.21
	P50	0.88	0.25	1.67	0.93	39.56	3.39	25.78	21.24	1.72	11.49	8.18	0.07	12.88	3.99	5.55	0.03	1.97
	P75	1.08	0.32	2.86	1.38	86.76	9.22	35.81	31.18	2.72	17.59	12.05	0.11	20.56	6.30	8.53	0.05	3.49

BEA: Biomonitorización en Adolescentes, Spain; FLEHS IV; Flemish Environment and Health Study IV, Belgium; NEBII: Norwegian Environmental Biobank II, Norway; PCB cohort follow-up: Endocrine disruptors and health in children and teenagers in Slovakia, Slovakia; SLO CRP: Exposure of children and adolescents to selected chemicals through their habitat environment, Slovenia. PFOA: perfluorooctanoate; PFNA: perfluorononanoic acid; PFOS: perfluorooctane sulfonate; ∑PFAS = sum of PFAS; mono-ethyl phthalate (MEP); mono-benzyl phthalate (MBzP); mono-*iso*-butyl phthalate (MiBP); MnBP: mono-n-butyl phthalate; MEHP: mono(2-ethylhexyl) phthalate; OH-MEHP: mono-2-ethyl-5-hydroxyhexyl phthalate; oxo-MEHP: mono-2-ethyl-5-oxohexyl phthalate; ∑DEHP: sum of diethylhexyl phthalate metabolites (MEHP, OH-MEHP, oxo-MEHP); cx-MEPP: mono-2-ethyl-5-carboxypentyl phthalate; MHNP: mono-hydroxyisononyl; MCOP: mono-carboxyisooctyl phthalate; ∑DiNP: Sum of di-isononyl phthalate metabolites (MHNP, MCOP); MHNCH: mono-hydroxyisononyl cyclohexane-1,2-dicarboxylate. KISS: kisspeptin 54 (ng/mL); ADIPO: adiponectin (μg/mL); LEP: leptin (ng/mL); HDL: high-density lipoprotein (mg/dL); LDL: low-density lipoprotein (mg/dL); GLU: glucose (mg/dL); INS: insulin (mU/L); CHOL: total cholesterol (mg/dL); TRIGL: triglycerides (mg/dL); 8OHdG: 8-hydroxy-2′-deoxyguanosine (μg/L); zBMI: Body mass index z-score. *Fasting samples were collected in PCB cohort.

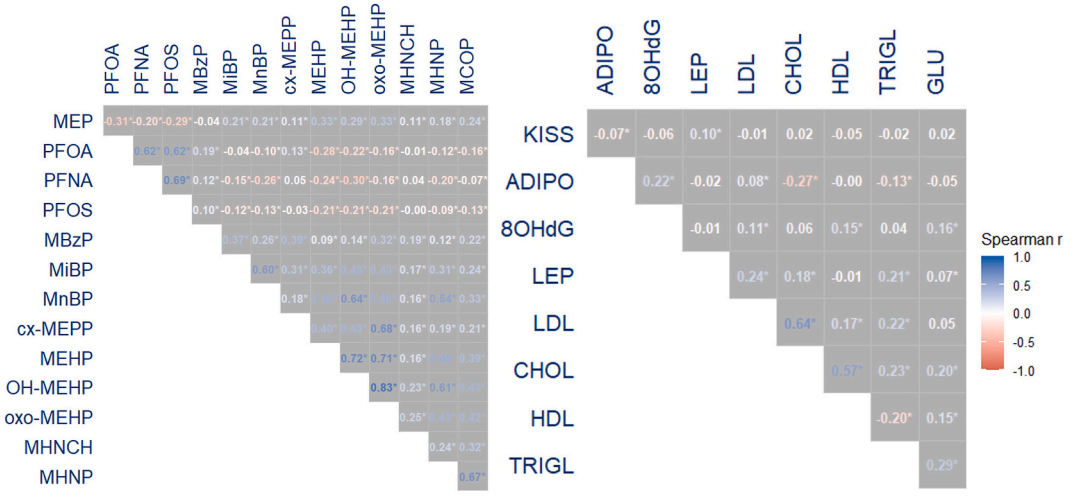

Fig. 1. Spearman's correlations among PFAS and urinary phthalate/DINCH metabolites, and among metabolic biomarkers in teenagers from HBM4EU Aligned Studies (n = 1033, except for CHOL, n = 881; GLU, n = 596; INS, n = 924; 8OHdG, n = 877). PFOA: perfluorooctanoate; PFNA: perfluorononanoic acid; PFOS: perfluorooctane sulfonate; ∑PFAS = sum of PFAS; mono-ethyl phthalate (MEP); mono-benzyl phthalate (MBzP); mono-*iso*-butyl phthalate (MiBP); MnBP: mono-n-butyl phthalate; MEHP: mono(2-ethylhexyl) phthalate; OH-MEHP: mono-2-ethyl-5-hydroxyhexyl phthalate; oxo-MEHP: mono-2-ethyl-5-oxohexyl phthalate; ∑DEHP: sum of diethylhexyl phthalate metabolites (MEHP, OH-MEHP, oxo-MEHP); cx-MEPP: mono-2-ethyl-5-carboxypentyl phthalate; MHNP: mono-hydroxyisononyl; MCOP: mono-carboxyisooctyl phthalate; ∑DiNP: Sum of di-isononyl phthalate metabolites (MHNP, MCOP); MHNCH: mono-hydroxyisononyl cyclohexane-1,2-dicarboxylate. KISS = kisspeptin, ADIPO = adiponectin, HDL = high density lipoprotein, LDL = low-density lipoprotein, CHOL = total cholesterol, TRIGL = triglycerides, GLU = glucose; INS = insulin; 8OHdG = 8-hydroxy-2′-deoxyguanosine.

results were found in adolescents from the HOME study (Arzu et al., 2024), and null or negative associations were found in Danish children at 9 years (Domazet et al., 2020; Grandjean et al., 2023), which highlight a lack of coherence and knowledge in this relationship. Regarding 8OHdG, few studies in teenagers were found, from which no significant associations were reported (Franken et al., 2017; Hassen et al., 2025). Nevertheless, prenatal exposure to PFOA and PFNA was associated with higher 8OHdG in newborns (Siwakoti et al., 2024), which is in line with our findings. This lack of consistency across results may be due to differences in study designs (longitudinal vs cross-sectional), the period of exposure (prenatal vs childhood or adolescence), and the period of development assessed (childhood vs adolescence).

G-computation and BKMR models showed that PFAS mixture was associated with higher kisspeptin, blood lipids, and 8OHdG, which is consistent with our findings from single-pollutant analyses and further supports the MANOVA results indicating correlated metabolic alterations. Moreover, PFOA was the main driver of these associations in both models as well, showing a common pattern. Supporting our results, a PFAS mixture including PFNA, PFOA, and PFOS showed associations with higher CHOL, HDL, and LDL in Chinese teenagers and with higher fatty acid metabolism in US teenagers (Goodrich et al., 2023; Wu et al., 2023). Moreover, a pilot study from our research group with Spanish males (n = 133, 15–17 years) found positive associations between PFAS mixture and serum kisspeptin and HDL levels (Rodriguez-Carrillo et al., 2025). However, evidence regarding mixtures is still lacking, and caution should be taken when interpreting these results.

Epidemiologic evidence of phthalate/DINCH metabolites and metabolic regulators

Evidence of phthalate/DINCH metabolites exposure and metabolism, concretely obesogenic factors, has increased in the last decade. Although there is evidence that these metabolites (individually and as a mixture) may have potential risk for developing metabolic alterations (Mérida et al., 2023; Perez-Diaz et al., 2024), there is still little information for kisspeptin, blood lipids, adipokines, and oxidative stress levels, particularly when assessing DINP or DINCH metabolites.

In line with our observations, three studies found that DEHP metabolites were associated with lower HDL, LDL, and higher 8OHdG levels in Chinese and US adolescents (12–19 years) (Ding et al., 2021; Etzel et al., 2023; Lin et al., 2017). However, null results were found when assessing phthalate/DINCH metabolites with biomarkers from the adipose tissue-cerebral metabolic axis (e.g., HDL, LDL, triglycerides, and leptin) in Dutch teenagers; although urinary mono-hydroxy-*iso*-nonyl phthalate (MHiNP) concentrations were associated with lower HDL and higher leptin, triglycerides, and insulin levels in girls (Berghuis et al., 2024). These results may suggest sex-dependent associations of phthalate/DINCH metabolites. Regarding this, our study found significant sex-interaction terms with leptin; however, after splitting by sex, only a significant association with MiBP was found in males. Nevertheless, these results may be plausible, first, because sex can influence the metabolic function, highlighting different susceptibilities according to sex (Krishnan et al., 2018); and second, because health effects of primary and secondary phthalate metabolites may differ according to their parent compounds (Zhang et al., 2021), which may explain the observations between studies.

The quantile g-computation model showed results in line with our single-pollutant models, since they both highlight associations with lower leptin, HDL, and higher 8OHdG levels. BKMR models, on the other hand, showed associations with lower adiponectin and HDL levels. There is very limited information on the effects of phthalate/DINCH mixtures on metabolic biomarkers during childhood or adolescence, and results in adults have been inconsistent. A multi-cohort study of children aged 6–11 years prenatally exposed to phthalate mixtures reported increased leptin levels (Güil-Oumrait et al., 2024). In Chinese adults, negative associations with LDL and total cholesterol were observed, whereas positive associations were reported in another study (Huang et al., 2024; Zhu et al., 2020). In contrast, a study in pregnant women in the US found no significant associations with LDL or HDL (Shen et al., 2024). Due to heterogeneities among studies—including participant age, study design (longitudinal vs. cross-sectional), and the specific phthalate/DINCH metabolites analyzed—these findings should be interpreted with caution. Overall, there is a clear need for longitudinal epidemiological studies that investigate the impact of phthalate mixtures on metabolic pathways, particularly during critical windows of development such as childhood and adolescence.

Plausible mechanisms of action

In the present study, PFAS and phthalate/DINCH metabolites were associated with higher oxidative stress and DNA damage, as measured by 8OHdG, suggesting a potential shared mechanism of action. Both

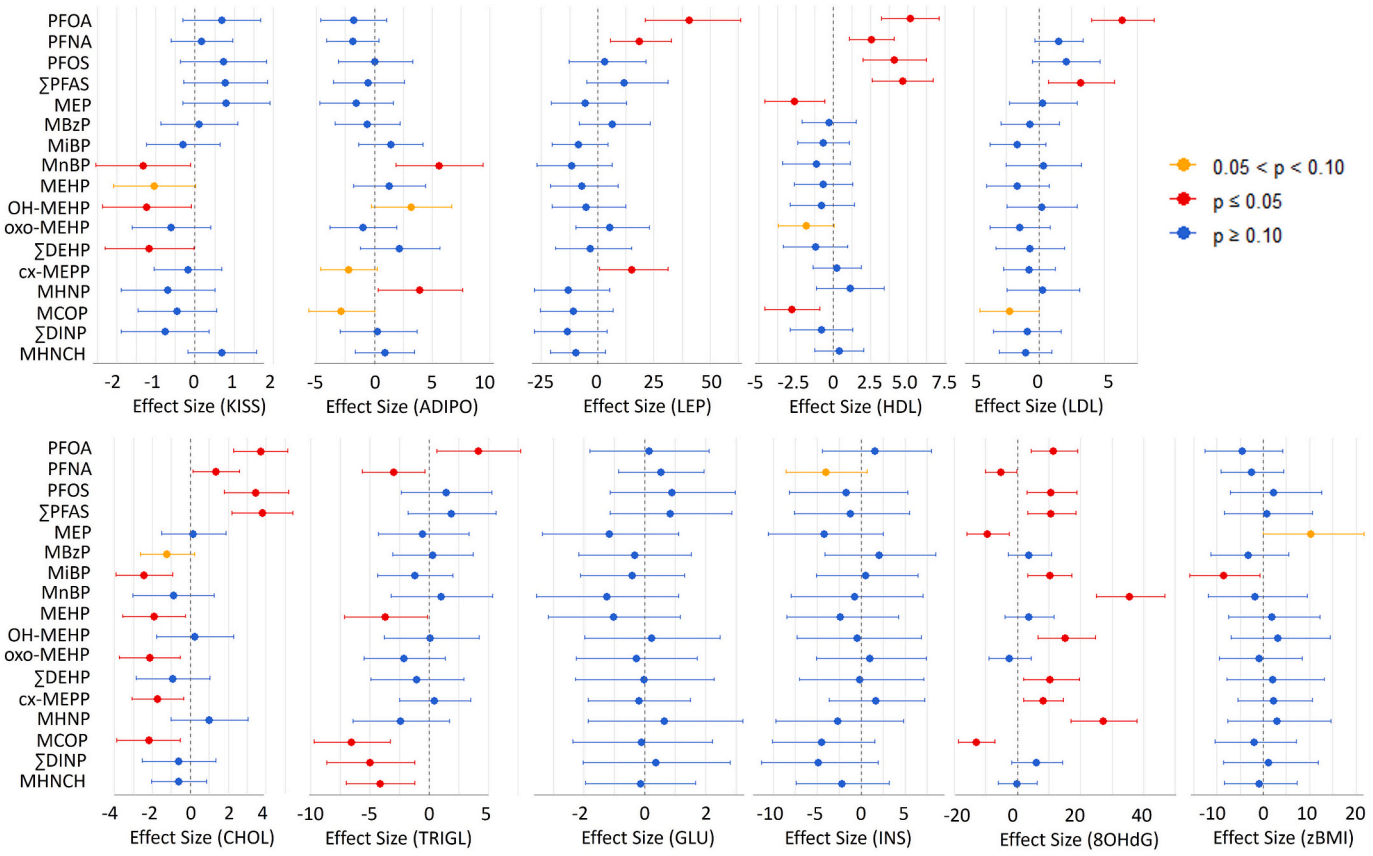

Fig. 2. Associations of serum PFAS and urinary phthalate/DINCH metabolites with metabolic biomarkers and zBMI in teenagers from the HBM4EU Aligned Studies (n = 1033, except for CHOL, n = 881; GLU, n = 596; INS, n = 924; 8OHdG, n = 877; zBMI = 988), estimated by linear regression models adjusted for age, sex, household income, country, and specific gravity (last one only for phthalate/DINCH metabolites). Estimates represent the percent change in the effect biomarker for each p75/p25 factor increase in PFAS and phthalate/DINCH exposure (circles) with corresponding 95% confidence intervals (CIs) (horizontal lines). PFOA: perfluorooctanoate; PFNA: perfluorononanoic acid; PFOS: perfluooroctane sulfonate; ∑PFAS = sum of PFAS; mono-ethyl phthalate (MEP); mono-benzyl phthalate (MBzP); mono-*iso*-butyl phthalate (MiBP); MnBP: mono-n-butyl phthalate; MEHP: mono(2-ethylhexyl) phthalate; OH-MEHP: mono-2-ethyl-5-hydroxyhexyl phthalate; oxo-MEHP: mono-2-ethyl-5-oxohexyl phthalate; ∑DEHP: sum of diethylhexyl phthalate metabolites (MEHP, OH-MEHP, oxo-MEHP); cx-MEPP: mono-2-ethyl-5-carboxypentyl phthalate; MHNP: mono-hydroxyisononyl; MCOP: mono-carboxyisooctyl phthalate; ∑DiNP: Sum of di-isononyl phthalate metabolites (MHNP, MCOP); MHNCH: mono-hydroxyisononyl cyclohexane-1,2-dicarboxylate. KISS = kisspeptin, ADIPO = adiponectin, HDL = high density lipoprotein, LDL = low-density lipoprotein, CHOL = total cholesterol, TRIGL = triglycerides, GLU = glucose; INS = insulin; 8OHdG = 8-hydroxy-2′-deoxyguanosine; zBMI = body mass index z-score. Red color: p-value < 0.05; Yellow color: p-value > 0.05 and < 0.10; Blue color: p-value > 0.10. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

pollutants have been shown to disrupt the redox balance by increasing ROS, leading to mitochondrial dysfunction, lipid peroxidation, and DNA damage (Vekic et al., 2023; Wielsøe et al., 2015; Yavaşoğlu et al., 2014). Experimental studies support these findings, showing increased oxidative stress markers for both PFAS and phthalates/DINCH metabolites in vitro and in vivo (Brassea-Pérez et al., 2022; Jiao et al., 2021; Shono and Taguchi, 2014). However, differences in toxicokinetics may partly explain the divergent patterns observed, as PFAS are persistent and reflect long-term exposure, whereas phthalate/DINCH metabolites are rapidly metabolized and may capture more transient metabolic changes.

Another common mechanism is the activation of peroxisome proliferator-activated receptors (PPAR). (Baken et al., 2019; Behr et al., 2020; Perez-Diaz et al., 2024; Rosenmai et al., 2017; Søderstrøm et al., 2022). PFAS can activate PPAR pathways, altering the expression of genes involved in lipid transport, metabolism, and storage, thereby contributing to increased circulating lipid levels and related metabolic alterations (Almeida et al., 2021; Attema et al., 2022; Beale et al., 2022; Roth et al., 2021). Furthermore, PFAS may impair LDL-cholesterol uptake independently of PCSK9 (Sabovic et al., 2023), providing a plausible mechanistic explanation for increased LDL and total cholesterol levels. Supporting this, longitudinal evidence suggests that reductions in PFAS concentrations are accompanied by parallel decreases in circulating lipids (Batzella et al., 2024).

Phthalates and alternative plasticizers may also interfere with PPAR signaling, particularly PPARγ, promoting adipocyte differentiation and lipid accumulation, and disrupting adipose tissue homeostasis through alterations in adipokine secretion and insulin sensitivity (Baken et al., 2019; Perez-Diaz et al., 2024; Radke et al., 2019; Rosenmai et al., 2017). These mechanisms may contribute to changes in lipid metabolism and energy balance and are supported by experimental evidence linking phthalate exposure to metabolic dysfunction (Radke et al., 2019).

At the organ level, PFAS may disrupt hepatic cholesterol clearance through interference with bile acid metabolism and alter adipocyte differentiation, potentially affecting leptin secretion and downstream neuroendocrine pathways, including kisspeptin regulation and the hypothalamic–pituitary–gonadal axis (Attema et al., 2022; Liu et al., 2019; Luo et al., 2016; Modaresi et al., 2022; Roth et al., 2021; Sanchez-Garrido and Tena-Sempere, 2013; Skorupskaite et al., 2014; Xie et al., 2022). Experimental evidence further suggests that exposure to plasticizers during critical developmental periods, including perinatal and pubertal stages, may induce persistent alterations in adipogenesis, lipid metabolism, and insulin signaling (Perez-Diaz et al., 2024; Radke et al., 2019). Given the rapid hormonal, neuroendocrine, and metabolic changes occurring during adolescence, this period may represent a

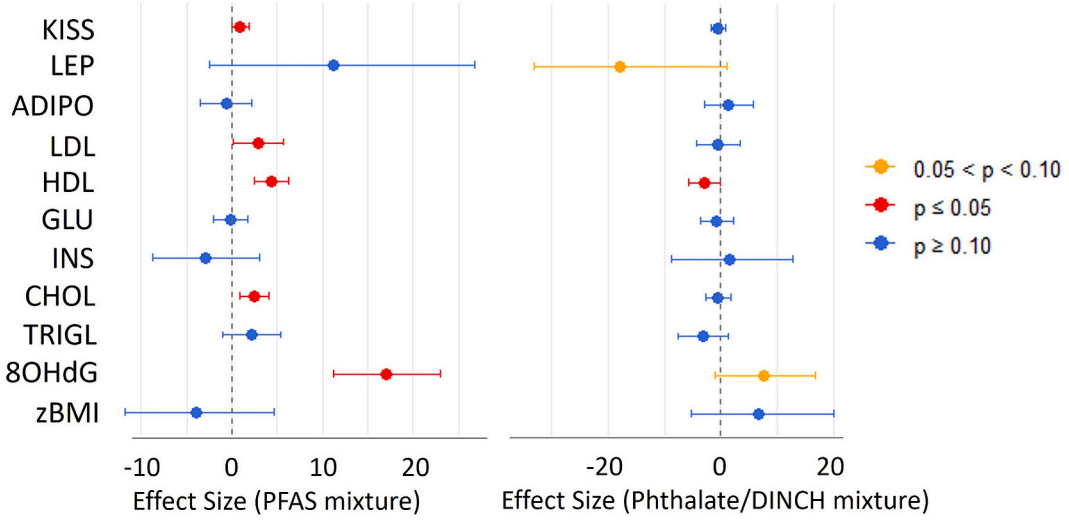

Fig. 3. PFAS mixture and Phthalate/DINCH metabolites mixture effect on metabolic biomarkers and zBMI of teenagers from the HBM4EU Aligned Studies (n = 1033, except for CHOL, n = 881; GLU, n = 596; INS, n = 924; 8OHdG, n = 877; zBMI = 988), estimated by quantile G-computation and representing the total effect of a quartile increase in all PFAS substances or phthalate/DINCH metabolites. KISS = kisspeptin, LEP = leptin, ADIPO = adiponectin, HDL = high density lipoprotein, LDL = low-density lipoprotein, CHOL = total cholesterol, TRIGL = triglycerides, 8OHdG = 8-hydroxy-2′-deoxyguanosine; zBMI = body mass index z-score. Model was adjusted by age, sex, household income, country, and specific gravity (last one only for phthalate/DINCH metabolites). Red color: p-value < 0.05; Yellow color: p-value > 0.05 and < 0.10; Blue color: p-value > 0.10. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

particularly susceptible window for the metabolic effects of environmental contaminants.

Strengths and limitations

This cross-sectional study has several limitations that should be taken into account. First, its cross-sectional design cannot establish causality or reverse causality. Second, the absence of repeated samples for the assessment of phthalates, since single-spot urine samples may lead to exposure misclassification. Nevertheless, non-differential exposure misclassification is likely to bias results toward the null, potentially underestimating rather than overestimating our observations. Third, metabolic biomarkers were not consistently measured across cohorts (e.g., participants assessed under fasting and non-fasting conditions), which may have attenuated some associations and contributed to inter-cohort variability. Furthermore, other relevant metabolic markers such as HOMA-IR or HbA1c were not available, limiting the assessment of insulin resistance and long-term glycemic control. Fourth, residual confounding due to unmeasured factors such as dietary intake, physical activity, and pubertal status cannot be ruled out. In particular, the lack of harmonized information on pubertal maturation (e.g., Tanner stages). This is particularly relevant for biomarkers such as leptin and kisspeptin, which are strongly influenced by pubertal maturation. Although the country of study was included as a covariate, residual heterogeneity across cohorts cannot be excluded. However, sensitivity analyses restricted to cohorts with a complete set of metabolic biomarkers showed similar patterns of association, suggesting that heterogeneity in biomarker availability and measurement protocols did not materially affect the main findings. Finally, given the large number of exposure-outcome comparisons performed, it is not possible to rule out that some findings may reflect chance. No formal correction for multiple testing was applied; therefore, results should be interpreted in the context of overall patterns and consistency across models rather than isolated statistically significant associations.

Among the strengths, first, the large sample size provides relatively high statistical power, enhancing the reliability of the findings. Second, the use of different mixture models allows for a more comprehensive interpretation of mixture effects, given their complementary objectives and analytical strengths. Third, combining studies from different European countries strengthens the assessment of the potential health impact of these contaminants across diverse populations. Fourth, the use of exposure biomarkers measured under a strict quality assurance/quality control (QA/QC) program, which ensured consistency and minimized measurement variability. Finally, a key strength of this study is its novelty, as it represents the first international epidemiological investigation in adolescents to integrate multiple metabolic pathways while assessing their associations with two major groups of environmental pollutants.

5. Conclusion

In this large, multi-country study of European adolescents, exposure to PFAS and phthalate/DINCH metabolites was associated with distinct patterns of metabolic biomarker variation, particularly involving lipid metabolism and oxidative stress. While PFAS and phthalates showed opposing associations for several metabolic markers, both chemical families were associated with higher levels of oxidative DNA damage, suggesting a potentially shared pathway related to redox imbalance. Although these findings cannot establish causality, they provide population-based evidence supporting the biological plausibility of early metabolic perturbations during adolescence. Longitudinal studies incorporating repeated exposure assessment and clinical metabolic outcomes are needed to clarify temporal relationships and long-term health implications.

CRediT authorship contribution statement

Andrea Rodríguez-Carrillo: Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **Bianca Cox:** Writing – review & editing, Methodology. **Hamid Y. Hassen:** Writing – review & editing, Methodology. **Eva Govarts:** Writing – review & editing, Project administration, Methodology, Conceptualization. **Nina Iszatt:** Writing – review & editing, Methodology. **Alicia Olivas-Martínez:** Writing – review & editing, Methodology. **Elly den Hond:** Writing – review & editing, Methodology. **Veerle J. Verheyen:** Writing – review & editing, Methodology. **Lucia Fábelová:** Writing – review & editing, Resources. **Lubica Palkovicova Murinova:** Writing – review & editing, Resources. **Susana Pedraza-Díaz:** Writing – review & editing, Resources. **Marta Esteban-López:** Writing – review & editing, Resources. **Line S. Haug:** Writing – review & editing, Resources.

Amrit K. Sakhi: Writing – review & editing, Resources. **Tina Kosjek:** Writing – original draft, Resources. **Žiga Tkalec:** Writing – review & editing, Resources. **Mariana F. Fernández:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation. **Sylvie Remy:** Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2026.110302.

Data availability

Data will be made available on request.

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