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Full length article

Meet-in-the-middle meets multi-omics identifying molecular signatures of environmental drivers of childhood overweight

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ABSTRACT

Background: Obesity is a multi-cause chronic disease recognized across the lifespan, with childhood obesity prevalence rising over the past decades. Although exposome-wide association studies have identified early-life environmental drivers of child obesity, and explored the multi-omics signatures of the exposome of children, it is understudied whether the combined effects of multiple exposures are potentially mediated by multi-omics. *Methods:* Within the Human Early Life Exposome (HELIX) project, 1041 mother–child pairs were surveyed for a wide range of environmental exposures including over 354 prenatal and childhood exposures. Multi-omics molecular features were measured during childhood, encompassing the blood methylome and transcriptome,

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MOFA
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plasma proteins and urinary and serum metabolites. Exposome and multi-omics features were integrated into latent factors by Multi-omics Factor Analysis, based on which structural equation modelling was used to assess whether multi-omics mediated associations between exposome and child body mass index (BMI).
Results: Key findings included: (i) prenatal nutrition, exercise, and passive smoking influencing BMI via DNA methylation of *HOXA5* and *Tenascin XB*; (ii) childhood exposure to PCBs and phenols linked with BMI through inflammation and coagulation pathways; and (iii) childhood PCB and dietary exposures associated with BMI via immune pathways.
Conclusions: This novel untargeted workflow elucidated biological mechanisms linking environmental exposures to child obesity, potentially supporting targeted public health interventions.

1. Introduction

Obesity is defined at the population level based on body mass index (BMI) and is described as a multi-causal chronic disease recognized across the lifespan (Jastreboff et al., 2019). At the global level, the prevalence of childhood obesity, in parallel with obesity in adults, has risen to alarming rates over the last four decades, shifting from less than 1 % in 1975 to almost 6 % and 8 % in 2016 in girls and boys, respectively. Children with obesity are five times more likely to be obese in adulthood (Simmonds et al., 2016) and have a higher risk of suffering from both short-term psychosocial and physical effects (including social stigma, depression, anxiety, shortness of breath and asthma) (Lang et al., 2018; Rankin et al., 2016) and long-term health consequences in adulthood, including cardiovascular diseases, type 2 diabetes and cancers (Lindberg et al., 2020; Reilly & Kelly, 2011).

Obesity results from an excess of calorie intake compared to expenditure, which finds its proximal determinants in the gene-environment interaction. Genetic factors acting in isolation are unlikely to explain the rapid increase in the prevalence of obesity that occurred during the past four decades, implying that gene-environment interactions likely drive the obesity epidemic (Eisenmann, 2006; Qasim et al., 2018; Rhee et al., 2012). Beginning of this millennium, a link between the surge in new industrial chemicals over the last four decades and the emergence of the obesity epidemic was suggested, (Baillie-Hamilton, 2002) claiming that these "obesogens" might have disrupted the body's natural weight-regulating mechanisms. Expanding on this notion and supported by experimental findings, the environmental obesogens hypothesis was introduced, which proposed that exposure to synthetic chemicals during prenatal or early life stages may predispose individuals to increased fat accumulation and excess weight (Grun & Blumberg, 2009). Obesogenic effects have been demonstrated in *meta*-analyses of persistent organic pollutants (POPs) for pesticides and their metabolites already banned decades ago, like hexachlorobenzene (HCB) and dichlorodiphenyldichloroethylene (DDE), a metabolite formed by dehydrohalogenation of dichlorodiphenyltrichloroethane (DDT) (Stratakis et al., 2022), and per- and polyfluorinated substances (PFAS), as previously reported for the childhood exposure to perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA) and perfluorohexane sulfonate (PFHxS) (Frangione et al., 2024).

Exposome-wide association studies (ExWAS) identified several early-life environmental drivers of BMI and obesity in children and adolescents (Haddad et al., 2022; Vrijheid et al., 2020). Nevertheless, the intercorrelation and joint pattern of the internal exposome through the study of multiple omics layers are underexplored. To our knowledge, only one environment- and epigenome-wide association study was conducted in parallel in Chinese adolescents to discover associations with BMI (Zhao et al., 2023).

In this study, in a subset of 1,041 mother–child pairs from the Human Early Life Exposome (HELIX) project, we evaluated whether multi-omics features, encompassing the blood methylome and transcriptome, plasma proteins and urinary and serum metabolites, mediated associations of a wide range of prenatal and childhood environmental exposures to child BMI and overweight (including obesity).

2. Methods

2.1. Study population

The population under study originates from the HELIX subcohort of 1301 mother child pairs established across six longitudinal birth cohorts in Europe (years of birth 2023–2008) (Maitre et al., 2018): Born in Bradford (BiB) in the UK (Wright et al., 2012), study of determinants of pre- and postnatal developmental (EDEN) in France (Heude et al., 2016), Infancia y Medio Ambiente, Environment and Childhood (INMA) in Spain (Guxens et al., 2012), Kaunas Cohort (KANC) in Lithuania (Grazuleviciene et al., 2009), Norwegian Mother, Father and Child Cohort Study (MoBa) in Norway (Magnus et al., 2016), and Mother-Child Cohort in Crete (Rhea) in Greece (Chatzi et al., 2017). All cohorts consisted of singletons and collected data on many variables, including prenatal exposures, during at least one follow-up point in pregnancy and between birth and five years. Additionally, urine and blood samples were also collected from mothers during pregnancy for biomarkers of chemical exposure assessment. The subcohort underwent follow-up examinations between December 2013 and February 2016, when children were six to 11 years of age, in order to i) assess child health outcomes, including BMI, ii) fully characterize the postnatal exposome, and iii) measure the blood and urine omics profiles. Among the subcohort participants, we selected only mother–child pairs that were of European ancestry determined from genome-wide genetic information (N = 1,041). The flowchart in Supplementary Fig. S1 depicts the participant inclusion procedure.

All studies were conducted according to the guidelines of the Declaration of Helsinki and received approval from local ethical committees of the centers involved, listed as follows. BiB: Bradford Teaching Hospitals NHS Foundation Trust. EDEN: Agence nationale de sécurité du médicament et des produits de santé. INMA: Comité Ético de Investicación Clínica Parc de Salut MAR. KANC: LIETUVOS BIOETIKOS KOMITETAS. MoBa: Regional komité for medisinsk og helsefaglig forskningsetikk. Rhea: Ethical committee of the general university hospital of Heraklion, Crete. Informed consent was obtained from a parent and/or legal guardian for all participants in the study. Further details of the protocols of the HELIX subcohort and of each of the six birth cohorts can be found in the respective references.

2.2. The measurement of environmental exposures

In this study, we used 349 environmental exposures assessed during pregnancy and childhood (N = 142 prenatal and N = 207 childhood exposures). We grouped these exposures each into two different categories as detailed in Supplementary Data S1: (i) the general external exposome, including air pollution, meteorological data, water disinfectant by-products (DBPs), natural spaces, traffic, noise, traffic and built environment data for a total of 67 prenatal exposures and 122 childhood exposures; (ii) the specific external exposome, including chemicals such as organochlorines (OCs), polybrominated diphenyl ethers (PBDEs), metals, persistent organic pollutants (POPs), PFAS, phthalates, phenols, and organophosphates (OPs), lifestyle and indoor air pollution data for a total of 75 prenatal exposures and 85 childhood exposures. In brief, air

pollution, meteorological variables, natural spaces, traffic, noise, and built environment were determined for the duration of pregnancy and for the year before childhood examination by environmental geographic information systems (GIS) (Nieuwenhuijsen et al., 2019; Robinson et al., 2018). DBPs were estimated from water company concentration data and habits were obtained from questionnaire data, only available during pregnancy. OCs, PBDEs, metals, POPs, PFASs, phenols, phthalates, OP and pesticides, were assessed via biomarkers in maternal blood and urine samples during pregnancy, and in children's blood or urine at the time of the childhood clinical examination (Haug et al., 2018). Lifestyle factors were evaluated through questionnaires, and indoor air pollution was estimated by a prediction model (Tamayo-Uria et al., 2019) combining indoor measurements from a subgroup of children with questionnaire data. When exposure variables for the HELIX project were available as both continuous and numeric variables (N = 455), the continuous variables were selected, with the exception of lifestyle parameters. Categorical variables with more than two levels were transformed into multiple dummy variables. Continuous lifestyle variables were transformed into categorical ones by tertile splitting in order to comply with the data format of the majority of the variables in the same group. We included variables with missing data, correlated with each other, and measured over multiple time windows. The correlations among the exposures have been described in detail elsewhere (Tamayo-Uria et al., 2019). Missing data per each exposure under study are reported in Supplementary Data S1 and Supplementary Fig. S3. No imputation was applied in the exposome data.

2.3. The multi-omics profiling

Molecular phenotypes were assessed in children between six and 11 years of age. Peripheral blood and urine samples were collected during the follow-up examination, as described in detail in Supplementary Method S1. DNA and RNA were extracted from the buffy coat and whole blood, respectively, in order to profile the whole-genome DNA methylation and gene expression, which is described in Supplementary Method S2. Untargeted methods were used to assess blood DNA methylation via the Illumina 450 K array and gene expression via the Affymetrix HTA v2.0 array; targeted methods were used to assess plasma proteins with 3 Luminex multiplex assays, serum metabolites with the targeted LC-MS/MS metabolomic assay Biocrates AbsoluteIDQ p180 kit and urinary metabolites with 1H nuclear magnetic resonance (NMR) spectroscopy. A full description of omics assays and data pre-processing of all five omics is provided in Supplementary Methods S3–S7. For the targeted assays, the lists of the measured plasma proteins, serum metabolites and urine metabolites can be found in Supplementary Data S2-S4, respectively. Methods for data denoising, outlier removal and probe filtering are summarized in Supplementary Table S1. Briefly, all omics layers were denoised for batch effects and winsorized to reduce the influence by extreme outliers. For blood cell-based measurements (DNA methylome and gene expression transcriptome), the blood cell type heterogeneity was also removed. Plasma proteins, serum and urine metabolites were denoised for omics layer-specific technical variables that were related to biological sample collection. Missing data pattern in the omics data are illustrated in Supplementary Fig. S3. No imputation was applied to the omics data.

2.4. Child anthropometrics

During the sub cohort follow-up examinations, height and weight were respectively measured with a stadiometer and a digital weight scale both without shoes and with light clothing. These measurements served for determining the two primary outcomes of the study: standardized BMI and a binary overweight indicator. BMI was calculated by dividing weight (in kilograms) by the squared height (in meters), and then converted to age- and sex-adjusted z-scores (zBMI) with the LMS method in statistical package "zanthro" (Vidmar et al., 2004) in Stata, where an individual's anthropometric data is compared to reference populations, in this case the international World Health Organization (WHO) reference curves (de Onis et al., 2007). The binary overweight indicator (OW), including overweight and obesity, were defined as having a zBMI above the age-and-sex-specific 85th and 95th percentiles, respectively, as recommended by the WHO (https://www.who.int/mediacentre/factsheets/fs311/en/).

2.5. Statistical analysis

The overarching aim of the study was to assess the mediating role of omics markers in the association between the pre- and postnatal exposome and BMI/OW in children. To achieve this, our analysis was structured in four stages (Fig. 1).

2.5.1. Stage 1

At stage 1, we applied an unsupervised modelling method, Multi-Omics Factor Analysis (MOFA) (Argelaguet et al., 2020), to infer sparse latent factors from the external exposome. The input of the MOFA corresponded to a list of six matrices (sub-exposome), corresponding to: i) prenatal general external exposures, where exposure variables were all numeric; ii) postnatal general external exposures, which were all numeric; iii) prenatal specific external numeric exposures, including only numeric exposures; iv) postnatal specific external numeric exposures, including only numeric exposures; v) prenatal specific external categorical exposures, including dummy variables generated from the categorical exposures vi) postnatal specific external categorical exposures, including dummy variables generated from the categorical exposures. Gaussian likelihood was specified for the numeric exposure matrices and Bernoulli likelihood was specified for the dummy variable matrices. MOFA was trained with an initialization of $K = 20$ factors. In addition, we specified a variance threshold of 5 % which determined the number of latent factors to retain: factors that explained less than 5 % of the data variance were dropped.

Parallelly, MOFA was applied on multi-omics data, where the model input consisted of a list of five matrices, each matrix corresponding to one omic layer: i) DNA methylation, ii) gene expression, ii) proteins, iv) serum metabolites and v) urinary metabolites. Since the data dimensionality was relatively higher in DNA methylation and gene expression transcriptome, we filtered out CpGs that were reported to have low probe reliability in the Illumina 450 K array and further kept only the top 10 % most variable CpGs and transcripts (Argelaguet et al., 2018). We considered a Gaussian likelihood for all the five omics layers, and trained MOFA with an initialization of K = 20 factors with a variance threshold of 1 %.

No prior imputation was applied to the exposome data or the omics data, since MOFA model is able to handle missing values by ignoring them in update equations of the algorithm (Argelaguet et al., 2018). At this stage, we did not include covariates for model adjustment and we did not denoise the exposome and omics variables for cohorts. The potential confounding effects of the key covariates were accounted for in the regression analyses in the next stages.

2.5.2. Stage 2

At stage 2, we further reduced the dimensionality of multi-omics to align with the "meet in the middle" principle, by associating the multi-omics factors with both i) exposome factors and ii) BMI/OW. According to the meet in the middle concept (Chadeau-Hyam et al., 2011), only the multi-omics factors that were associated with both i) zBMI/OW and ii) external exposome factors were kept as the "selected multi-omics latent factors". Consequently, we kept as the "selected external exposome latent factors" only the external exposome factors associated with internal exposome factors. The selected internal exposome latent factors and the selected external exposome latent factors were used in the subsequent mediation analyses. Two types of models were applied for the two outcome variables: 1) Gaussian linear regression for zBMI, and

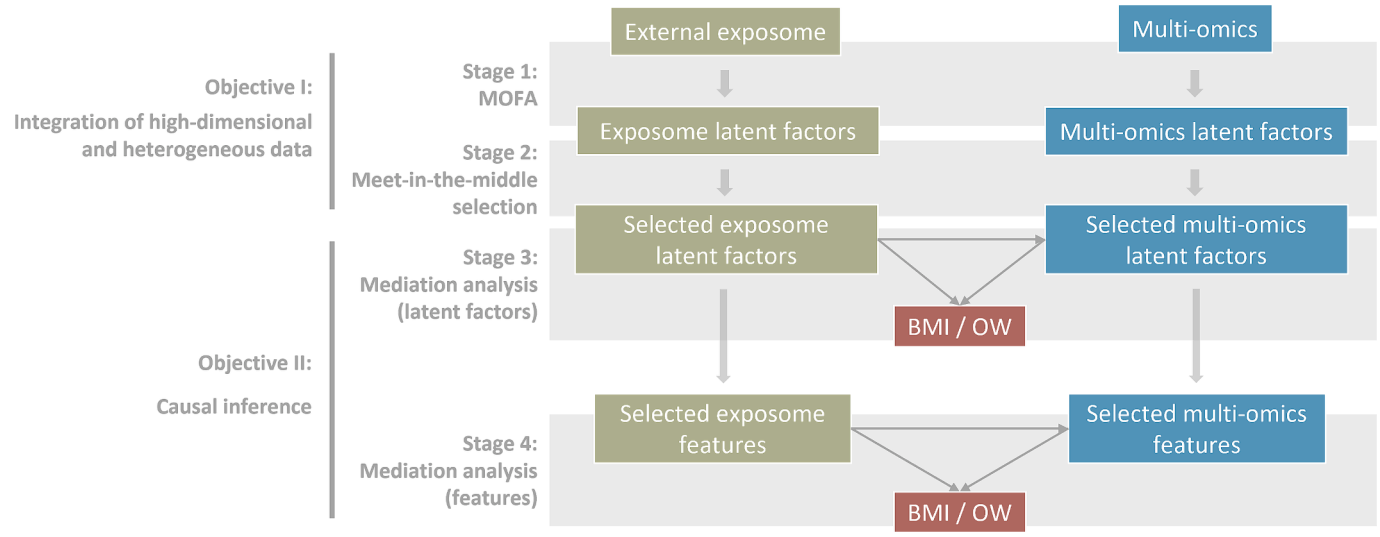

Fig. 1. Statistical analyses workflow. Multi-omics factor analysis (MOFA) was performed on external and internal exposome, respectively, for each of which a set of latent factors was extracted (stage 1). The internal exposome latent factors were further selected by regression analysis against i) child BMI/OW and ii) external exposome latent factors, which resulted in the selected internal exposome latent factors and external exposome latent factors (stage 2). The selected external and internal exposome latent factors were used to perform a factor-based structural equation modelling (stage 3). An additional feature-based mediation analysis was run for the individual features showing a relative stronger factor loading in the selected latent factors (stage 4).

2) binomial regression with a probit link for the OW. All regression models in this stage were adjusted for the key covariates (child age, sex, gestational age, and the first four principal components of the genetic data representing variance due to cohort affiliation and ethnicity). Associations with a p-value <0.05 were considered significant.

2.5.3. Stage 3

In the stage 3, structural equation modelling (SEM) was applied to test whether the selected internal exposome latent factors mediated the effect of the selected external exposome latent factors on zBMI/OW. For the structural equation modelling we analyzed potential mediating effects of the selected internal exposome factors in the association between the selected external exposome factors and childhood zBMI with the lavaan package (Rosseel, 2011). The set of key-covariates (child age, sex, gestational age, and the first four principal components of the genetic data) was also included in the statistical model. To avoid overcorrection, maternal anthropometrics and lifestyle were not included in the model. These variables were already included as features (weights) in the exposome factors which functioned as exogenous (independent) variables in the SEM model. To evaluate the robustness of this model we additionally performed a sensitivity analysis including a more extended set of covariates including maternal pre-pregnancy BMI, pregnancy complications, parity, maternal education, maternal sustained smoking and alcohol consumption during pregnancy, and breastfeeding at any point. Since the lavaan functionalities do not allow the use of a logit function for a binary outcome variable, we applied a probit model for the association with OW. In the probit model, the inverse standard normal distribution of the probability is modeled as a linear combination of the predictors. The probit regression coefficients express the change in the z-score or probit index for a one-unit change in the predictor. We fitted the models using the default maximum likelihood estimator for the continuous outcome of zBMI and the diagonally weighted least squares estimator for the outcome of OW as recommended for binary outcomes in lavaan and computed the bootstrap percentile confidence intervals (CIs) with the "standardizedSolution_boot_ci()" function from the semhelpinghands package (Cheung et al., 2023) with 5000 replications. Estimates of total (TE), direct (DE) and indirect effects (IE) were expressed as change in standardized unit of zBMI and change in standardized z-score units for OW, respectively, for a one-unit change in the predictors. To ensure that the assumptions of exogeneity and independence are not violated we performed a sensitivity analysis correcting for covariances between the external exposome and multi-omics factors. Furthermore, we also applied a sensitivity analysis, including the interaction terms between external exposome factors and multi-omics factors to evaluate their p-values and potential changes in the SEM outcome. Additionally, we performed a sex-stratified analysis to evaluate potential differences in the outcome due to sex-specific molecular mechanisms underlying the relationship between environmental exposures and obesity. To evaluate the SEM, we further used the Root Mean Square Error of Approximation (RMSEA) and the Comparative Fit Index (CFI). RMSEA assesses model fit per degree of freedom, with values ≤ 0.05 indicating close fit and ≤ 0.08 acceptable (Hu and Bentler, 1999). CFI compares the model to a baseline, with values ≥ 0.90 considered adequate and ≥ 0.95 good (Hu and Bentler, 1999). These indices ensure that the model appropriately represents the observed data.

To enhance the interpretation of latent factors within significant indirect pathways, we focused on the exposures and molecular features that were found to have a substantial contribution to the latent factors. For this purpose, features with an absolute loading >0.25 were selected for each factor. The selected external exposures were grouped on their inherent definitions or nature (e.g., fish was grouped in the food group, smoking in the lifestyle group), while the selected molecular features were summarized via pathway analyses. Briefly, the genes annotated to the selected CpGs were identified as the closest gene at <1500 bp with the Bioconductor package IlluminaHumanMethylation450kanno.ilmn12.hg19, (Hansen, 2016) gene transcripts and plasma proteins were used to run a pathway enrichment analysis, with Reactome, gene ontology of biological processes and KEGG as the reference databases (Yu & He, 2016), using the clusterProfiler R package (Yu et al., 2012). Significance was defined as having an adjusted p-value <0.01, based on the Benjamini & Hochberg method (Benjamini & Hochberg, 1995), and at the same time with at least five genes belonging to the gene set of the pathway. In addition, the serum and urinary metabolites in the latent factors were used to run a metabolite over-representation analysis on their KEGG ID via the webtool (https://genap.metaboanalyst.ca/), with KEGG as the reference database. Significant metabolic pathways were those with an adjusted p-value <0.05 and at least two metabolite counts.

2.5.4. Stage 4

In stage 4, mediation analysis was conducted using the external exposures and molecular features that were found to have substantial contributions to the selected latent factors, instead of the latent factor themselves, to assess the indirect effect of single molecular features in the associations of single external exposures on zBMI/OW. We used 0.25 as the threshold for the absolute factor loadings to select exposures from the external exposome latent factors, and to select the molecular features from the multi-omics latent factors. Pair-wise mediation analysis was performed using the mediation R package (Tingley et al., 2014) on the selected external exposures and multi-omics features. Notably, this analysis was restricted to external exposure-molecular feature pairs belonging to pairs of factors laying in paths where a significant indirect effect was detected in the SEM analyses (stage 3). Gaussian model and binomial models were used for zBMI and OW, respectively. All models were adjusted for the key covariates (child age, sex, gestational age, and the first four principal components of the genetic data) and additional covariates (maternal pre-pregnancy BMI, pregnancy complications, parity, maternal education and alcohol consumption during pregnancy). Standardized TE, IE, and DE were estimated from the models and statistical inference was made with 1,000 bootstrap samples. In order to account for the large number of hypothesis testing, we applied Benjamini-Hochberg correction on the p-values generating the false discovery rate (FDR) and considered a significant mediation effect if the FDR was lower than 0.05. The exposures and molecular features involved in the significant mediations were visualized using a Sankey plot via the networkD3 R package (Allaire et al., 2017), where exposures and molecular features were represented by the nodes and standardized associations were shown as the links between nodes.

In parallel, in order to show the variation of effect estimates of TE, IE and DE, we calculated the CIs, where multiple testing was accounted for using an effective number of tests, represented by the multiplication between the number of exposome factors (seven) and the number of multi-omics factors (five) that were relevant for the individual features in the mediation analysis at this stage. In total, the effective number of tests was 35, and the confidence level was controlled as 1–0.05/35 = 99.86 %.

We performed additional analysis to evaluate the robustness of the mediation analysis with respect to model specifications. Specifically, we considered 1) potential interaction effects between individual exposure and molecular feature (as mediator), 2) additional model adjustments for breastfeeding (yes or no) and maternal sustained smoking during pregnancy (yes or no), as well as 3) sex-stratified mediation analysis. For analyses 1) and 2), estimated standardized IE and DE as well as their CIs were compared to those from the main analysis. For analysis 3), the estimated effects and CIs were compared between boys and girls.

2.6. Covariates

We considered different sets of covariates for model adjustment in the statistical analysis stages described in the above sections and Supplementary Fig. S2. We defined a set of key covariates used for the downstream modelling of the latent factors of multi-omics and exposome, with the following criteria. First, at the stage of meet-in-the-middle factor selection (stage 2), we chose the variables that showed a strong confounding effect for the exposome-multi-omics association (cohort, ethnicity and gestational age), and the multi-omics-BMI association (cohort, ethnicity, child age, sex, and gestational age). Second, at early analysis stages (stage 2 and stage 3), statistical models should ideally be fitted on as many observations as possible for a more generalizable selection. Covariates that contained a higher proportion of missing values were thus excluded from the key covariates. Especially, alcohol consumption during pregnancy would lead to an exclusion of two whole cohorts (BIB and MoBa) in which this information was not collected, leading to a loss of 326 observations. Therefore, the key covariates included the child's age, sex, gestational age, and the first four principal components of the genetic data which represented the variation due to cohort and ethnicity. For the mediation analysis based on the individual exposures and molecular features (stage 4), we used additional covariates related to maternal characteristics: pre-pregnancy BMI, pregnancy complications, parity, education and alcohol consumption during pregnancy. Maternal pre-pregnancy BMI, pregnancy complications and parity were accessed via medical records. Maternal education level was categorized into three groups (low, medium, and high) according to the International Standard Classification of Education (ISCED), an international reference classification for organizing education programs and related qualifications by levels and fields (EUROSTAT, 2023). Maternal alcohol consumption during pregnancy was evaluated as the average number of glasses of alcoholic beverages consumed per month.

3. Results

3.1. Study population characteristics

Our study population included 1,041 mother–child pairs from six cohorts, as detailed in Table 1**.** the sex distribution in children was nearly equal (46 % were girls) and gestational age was on average 39.2 weeks. At the examination, children were on average 8 years old. The combined prevalence of overweight and obesity was 20.7 % for the included children, with a mean BMI of 16.9 kg/m2. The mothers had an average pre-pregnancy BMI of 24.8 kg/m2, about 10.8 % of the mothers kept smoking during pregnancy, and the average alcohol consumption was 0.6 glasses per month during pregnancy. Eleven percent of the mothers suffered from complications of the pregnancy. Most had achieved a higher education level (54 %) and had their first child (47.2 %). The missing data patterns within each sub-exposome and each omics are illustrated in Supplementary Fig. S3. The distribution of omics features is shown in the density plots of Supplementary Fig. S4.

3.2. Dimension reduction of the external exposome and multi-omics

For the external exposome, a MOFA model of eleven latent factors was selected as the remaining nine factors did not explain >5 % variance in the exposome data. Fig. 2**A** depicts the variance explained by each factor within each sub-exposome. The largest part of the variance came from the general external exposures (both prenatal and postnatal) and was captured by exposome factor 1 (EF1). The variance in the rest sub-exposomes was captured by the other factors. Correspondingly, the MOFA model showed a better fit in the two general external sub-exposomes than in others (Fig. 2**C**), where more than 50 % of the variance was captured by the model.

For the multi-omics MOFA model, all 20 latent factors in the initiating model were kept since variance explained was higher than 1 % for each of them (Fig. 2**B**). The largest variance in common came from the serum metabolites, which was almost the only source of variance that was captured by multi-omics factor 1 (OF1). Plasma protein was the second largest source of variation and gene expression was the third. Correspondingly, the MOFA model fitted the serum metabolites data the best (Fig. 2**D**). Urine metabolites and DNA methylation did not contribute much to the common variance of multi-omics, although DNA methylation was the layer with the highest data dimension. Comparing Fig. 2C and Fig. 2D, the proportion of variance in multi-omics data explained by the multi-omics MOFA model was in general lower than the proportion of variance in external exposome data explained by the external exposome MOFA model.

As we did not correct the exposome and multi-omics data for cohort effects in this dimension reduction stage using MOFA, we performed an additional check on the relationship between cohorts and the extracted latent factors. As illustrated in Supplementary Fig. S5, the standardized factor scores of exposome displayed higher between-cohort variation than that of the multi-omics. Among the extracted exposome factors,

Table 1
Characteristics [N (%) or mean(±SD)] of the study population (n = 1,041) of the HELIX cohort study.

Characteristics	Missing	Study Population (N = 1,041)	BiB (N = 90; 8.6 %)	EDEN (N = 135; 13 %)	INMA (N = 198; 19 %)	KANC (N = 196;18.8 %)	MoBa (N = 236; 22.7 %)	RHEA (N = 186; 17.9 %)
Child								
Female	0	473 (45.5 %)	41 (45.6 %)	59 (43.7 %)	90 (45.5 %)	88 (44.9 %)	109 (46.2 %)	86 (46.2 %)
Gestational age, weeks	12	39.6 (1.7)	40 (1.7)	39.8 (1.8)	40.0 (1.4)	39.4 (1.3)	40.1 (1.8)	38.4 (1.5)
Age at examination, years	0	8.0 (1.5)	6.6 (0.25)	10.8 (0.55)	8.8 (0.56)	6.5 (0.49)	8.5 (0.49)	6.5 (0.28)
BMI at examination, kg/m^2	7	16.9 (2.6)	16.0 (1.4)	18 (2.9)	18.1 (3.1)	16.4 (2.3)	16.3 (1.8)	16.8 (2.6)
Overweight or obese	7	214 (20.7 %)	11 (12.2 %)	25 (18.7 %)	62 (31.6 %)	38 (19.3 %)	21 (8.9 %)	57 (30.6 %)
Mother								
Pre-pregnancy BMI, kg/m^2	16	24.8 (5.0)	29.2 (5.8)	23.3 (4.3)	24.0 (4.8)	27.8 (5.3)	22.6 (3.0)	24.0 (4.2)
Sustained smoking in pregnancy, yes	34	100 (10.8 %)	20 (28.2 %)	15 (12.9 %)	24 (14.3 %)	12 (6.3 %)	12 (6.6 %)	24 (14.3 %)
Maternal alcohol consumption pregnancy, glasses/month	381	0.61 (3.0)	/	1.4 (3.0)	0.44 (2.6)	0.086 (0.36)	/	0.82 (4.6)
Pregnancy complications	0	115 (11.0 %)	9 (0.1 %)	11 (8.1 %)	14 (7.1 %)	38 (19.4 %)	17 (7.2 %)	26 (14 %)
Parity, n	22							
1		481 (47.2 %)	46 (45.1 %)	66 (48.9 %)	107 (54.3 %)	83 (43.5 %)	107 (46.5 %)	72 (39.8 %)
2		374 (36.7 %)	23 (27.1 %)	47 (34.8 %)	82 (41.6 %)	56 (29.3 %)	90 (39.1 %)	76 (42.0 %)
>=3		164 (16.1 %)	16 (18.8 %)	22(16.3 %)	8(4.1 %)	52 (27.2 %)	33 (14.3 %)	33 (18.2 %)
Maternal education, ISCED classification*	26							
Low		115 (11.3 %)	36 (44.4 %)	10 (7.5 %)	49 (24.9 %)	11 (5.8 %)	0 (0.0 %)	9 (4.9 %)
Medium		352 (34.7 %)	15 (18.5 %)	48 (36.1 %)	79 (40.1 %)	68 (35.6 %)	41 (18.0 %)	101 (54.9 %)
High		548 (54.0 %)	30 (37.1 %)	75 (56.4 %)	69 (35.0 %)	112 (58.6 %)	188 (82.0 %)	74 (40.2 %)

Note: /, not applicable; BMI, body mass index; BiB, Born in Bradford, United Kingdom; EDEN, Étude des Déterminants pré et postnatals du développement et de la santé de l'ENfant, France; INMA, INfancia y Medio Ambiente, Spain; KANC, Kaunas birth cohort, Lithuania; MoBa, The Norwegian Mother, Father and Child cohort study, Norway; RHEA, The Rhea Mother-Child Cohort in Crete, Greece; N, number of observations; SD, standard deviation. "/" = not assessed.
* International Standard Classification of Education (ISCED).

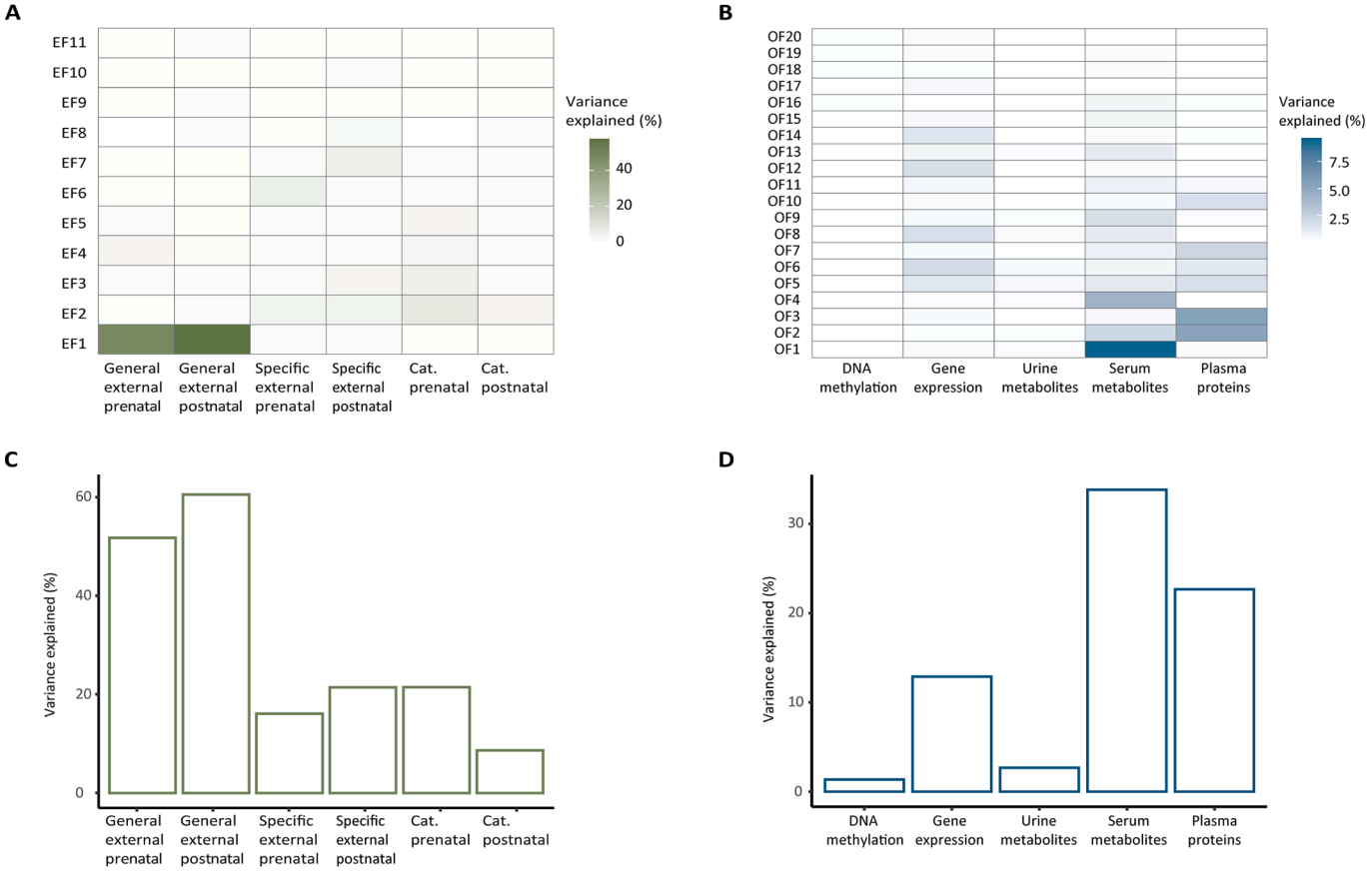

Fig. 2. Variance explained by factors from multi-omics factor analysis (MOFA) of the external exposome and the multi-omics data. Percentage of variance explained by each factor across each sub-exposome (A) and by each factor across each omics layer (B). Total variance explained in each sub-exposome (C) and in each omics layer (D). Cat.: categorical.

factors 2 – 6 showed a relatively stronger variation. Cohort effects were considered as one of the model adjustments for the subsequent regression analyses, where the extracted latent factors were investigated.

In the meet-in-the-middle selection analysis, five OFs (selected multi-omics) were associated (raw p-value <0.05) with both i) zBMI and OW, and ii) seven EFs (selected external exposome) (Fig. 3**A**). The path from the selected external exposome to anthropometric outcomes, possibly mediated by these five OFs, has been depicted in Fig. 3**B**.

3.3. Multi-omics factors mediating the effect of the external exposome factors on childhood anthropometrics

3.3.1. Mediation analysis by structural equation modelling

The graphical representation of the SEM for the investigated mediations is shown in Supplementary Fig. S6 for the zBMI, and Supplementary Fig. S7 for OW. The SEM for zBMI showed an acceptable model fit with an RMSEA of 0.04 and a CFI of 0.92. The model performance for OW was relatively weaker, with an RMSEA = 0.04 and CFI = 0.30. Three multi-omics factors (OF5, OF6 and F16) were mediating the association between four external exposome factors (EF2, EF3, EF7 and EF8) and zBMI or OW. In detail, the SEMs suggested a significant mediation on both zBMI and OW of: i) EF2 through OF16 (TE = -0.15 for zBMI and −0.04 for OW, IE = −0.02 for both zBMI and OW); ii) EF3 through OF6 (TE = 0.03 for zBMI and 0.11 for OW, IE = 0. 06 for both zBMI and OW); iii) EF7 through OF5 and OF6 (TE = 0.38 for zBMI and 0.43 for OW, IE = 0.02 via OF5 as well as OF6, for zBMI and 0.01 and 0.02, via OF5 and OF6 respectively for OW); iv) EF8 through OF5 (TE = -0.07 for zBMI and −0.19 for OW, IE = 0.02 for both zBMI and OW) (Table 2).

In the sex-stratified analysis we generally observed comparable results (Supplementary Tables S2 and S3). Because the sample size was about half, the CIs of the indirect associations were sometimes crossing zero while the estimates stayed the same. We observed no additional indirect effects that were statistically significant in the stratified analysis compared to the main analysis. When the covariance matrices for external exposome factors and multi-omics factors were incorporated into the SEM model, the estimates and CIs remained largely unchanged (Supplementary Table S4). In the sensitivity analysis including the interaction terms between all exposome factors and all multi-omics factors (Supplementary Table S5), we observed one additional statistically significant path for the assessment of zBMI (EF6 with OF9) and in the assessment of OW between EF8 and OF11. In the latter analysis three other paths (EF2 with OF16, EF3 with OF6, and EF7 with OF5) were not significant anymore. The interaction term p-value for EF6 with OF9 in the assessment of zBMI was additionally significant (β = -0.08, p = 0.011) (Supplementary Table S6).

The results from the sensitivity analysis including an extended set of covariates showed differences between the assessment of zBMI and OW (Supplementary Table S7). For the outcome OW the indirect effect of EF7 via OF6 remained significant, whereas in the analysis of zBMI, two of the five indirect paths that were significant in the main analysis remained significant, and one additional indirect path from EF1 via OF5 was also identified with statistical significance.

3.3.2. Contribution of features to mediating factors

To facilitate the interpretation, we examined features with absolute loadings exceeding 0.25 within each of the factors belonging to a significant path in the SEM (namely, EF2, EF3, EF7 and EF8, and OF5, OF6, and OF16, factors labelled in black in Fig. 4). The number of features listed per each factor is reported in Supplementary Tables S8 and S9. The proportion of the features belonging to each group (for the external exposome factors) or molecular layer (for the multi-omics factors) within each factor is summarized in Fig. 4. For the external exposome factors (Fig. 4**A**), EF2 and EF3 consisted mainly of lifestyle, tobacco smoke and phenols exposures, EF7 of OCs and PFASs, while EF8 was almost purely made of phenols and phthalates. For the multi-omics factors (Fig. 4**B**), gene expression transcripts contributed to all but OF16, which contained mostly DNA methylation. OF5 was the most diverse in terms of the source of features. Urine metabolites contributed to OF5, OF6, and serum metabolites contributed to all but OF6.

The exact external exposome features contributing the most to the latent factors are depicted in Supplementary Data S5 with the corresponding factor loadings and summarized in Supplementary Table S10, together with a feature-based labeling of the latent factors. EF2 and EF3 had similar compositions, where the exposures with the highest absolute loadings (the top 10) were maternal cereals intake during pregnancy, child bread intake, childhood bleach use, passive smoking during pregnancy, a group of phenols (BPA, BUPA, OXBE) in the mothers during pregnancy, and DDT, TRCS and MEP in the children in EF2, and

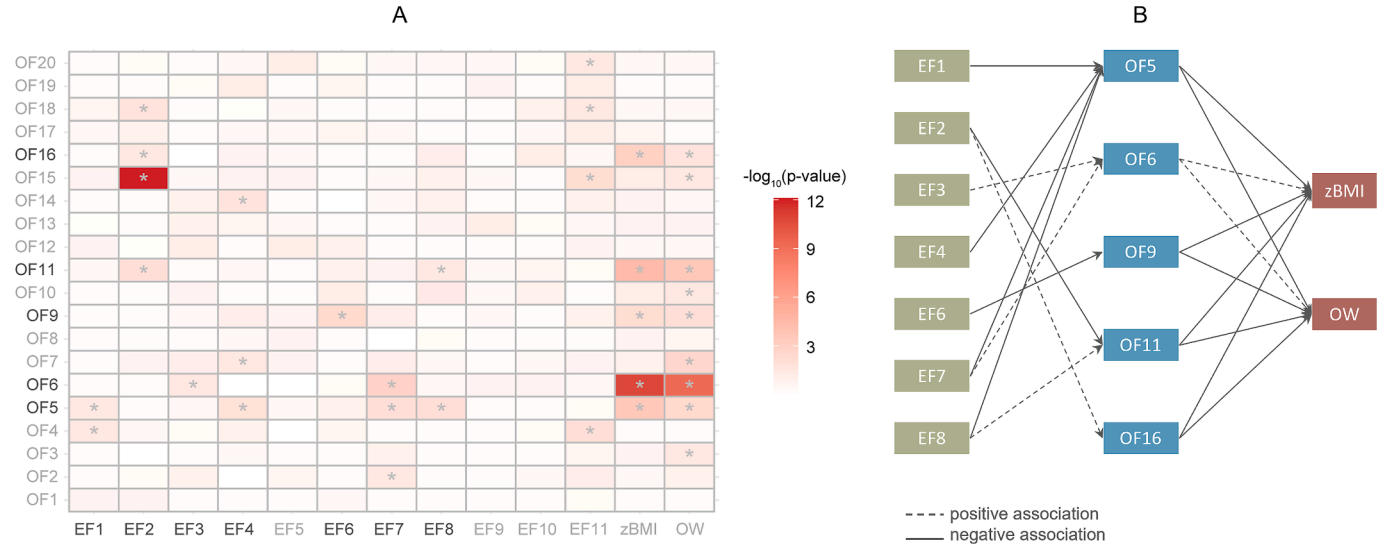

Fig. 3. Association of the omics factors with the exposome factors and the child anthropometric outcomes. (**A**) In the meet-in-the-middle selection analysis of the extracted latent factors, each of the extracted multi-omics factors (OF) was tested i) against each of the extracted external exposome factors (EF) and ii) the outcome variables, BMI z-score (zBMI) and being overweight (OW). Regression models (a Gaussian model for zBMI and logistic regression model for OW) were adjusted for child age, sex and first four principal components of the genetic data representing variance due to cohort affiliation and ethnicity. Associations with a raw p-value <0.05 were marked with a star in the heatmap. Multi-omics and external exposome factors selected are colored in black. (**B**) The selected EFs and the selected OFs, as well as the detected associations, are displayed with directional paths that were identified with the meet-in-the-middle method.

Table 2
Results from the structural equation modeling (SEM) analyzing the mediation via selected multi-omics factors of the association (95% bootstrapped confidence interval) between selected exposome factors and i) childhood overweight (OW), using a binomial probit model, and ii) childhood BMI z-score (zBMI), using a Gaussian model.

Outcome	External exposome Factor	Direct Effect	Multi-omics Factor	Indirect Effect	Total Effect
Overweight [a] (OW)	EF1	0.04 (−0.06, 0.14)	OF5	3.2e-03 (−5.3e-03, 0.02)	0.05 (−0.05, 0.14)
	EF2	−0.037 (−0.28, 0.24)	OF11	0.01 (−0.02, 0.07)	−0.04 (−0.30, 0.21)
			OF16	**−0.035 (−0.14, −2.1e-03)**	
	EF3	0.05 (−0.25, 0.29)	OF6	**0.06 (0.02, 0.29)**	0.11 (−0.14, 0.36)
	EF4	0.30 (−0.03, 0.61)	OF5	−0.01 (−0.06, 0.010)	0.29 (−0.04, 0.59)
	EF6	0.03 (−0.09, 0.15)	OF9	9.2e-03 (−1.8e-03, 0.11)	0.05 (−0.06, 0.16)
	EF7	**0.40 (0.28, 0.49)**	OF5	**9.0e-03 (5.8e-04, 0.05)**	**0.43 (0.34, 0.52)**
			OF6	**0.02 (3.8e-03, 0.09)**	
	EF8	**−0.20 (−0.36, −0.03)**	OF5	**0.02 (2.6e-03, 0.14)**	−0.19 (−0.33, −0.03)
			OF11	−0.01 (−0.09, 1.5e-03)	
BMI z-score [b] (zBMI)	EF1	0.05 (−0.01, 0.12)	OF5	4.0e-03 (−6.8e-03, 0.02)	0.068 (−0.01, 0.12)
	EF2	−0.14 (−0.29, 0.02)	OF11	0.01 (−0.02, 0.04)	−0.15 (−0.31, 0.02)
			OF16	**−0.02 (−0.04, −3.3e-03)**	
	EF3	−0.03 (−0.20, 0.14)	OF6	**0.06 (0.03, 0.11)**	0.03 (−0.14, 0.21)
	EF4	0.04 (−0.17, 0.25)	OF5	−0.02 (−0.05, 0.01)	0.03 (−0.19, 0.23)
	EF6	0.04 (−0.03, 0.12)	OF9	5.1e-03 (−2.7e-04, 0.01)	0.05 (−0.025, 0.13)
	EF7	**0.35 (0.29, 0.41)**	OF5	**0.02 (5.8e-03, 0.03)**	**0.38 (0.32, 0.43)**
			OF6	**0.02 (2.0e-03, 0.03)**	
	EF8	−0.08 (−0.18, 0.03)	OF5	**0.02 (5.2e-03 0.04)**	−0.07 (−0.17, 0.04)
			OF11	−6.8e-03 (−0.02, 3.9e-03)	

The models included the following set of covariates: child age, child sex, gestational age, and the first four principal components of the genetic data.
[a] Estimates represent the change in standardized z-score units for a one-unit change in the predictor in the associations with the binary outcome overweight.
[b] Estimates represent standardized regression coefficients in the association with BMI z-scores.

were childhood presence of pets at home, child yogurt intake, passive smoking during pregnancy, as well as a group of OCs (DDT, DDE), a group of phenols (PRPA, TRCS) and MEP in the children in EF3. In EF7, the main exposures were the absence of breastfeeding and OCs in the children (DDE and variants of PCBs). Finally, phenols and phthalates in the children constructed EF8.

The multi-omics features with main contribution to the latent factors are shown in Supplementary Data S6 and summarized in Supplementary Table S11**,** together with a feature-based labeling of the multi-omics latent factors. The genes in OF5 and OF6 showed significant over-representation of gene sets of pathways, while the other three multi-omics factors did not identify significant pathways. Additionally, none of the multi-omics factors showed significant metabolic pathways. As illustrated in Supplementary Fig. S8, the 43 gene transcripts and six genes corresponding to the plasma proteins in OF5 suggested mainly cellular pathways related to blood coagulation, JAK-STAT/MAPK signalling and inflammation. The only metabolic pathway identified in multi-omics factor 5 was the metabolism of alanine, aspartate and glutamate (adjusted p-value = 0.02, metabolite count = 3). Pathways suggested by 55 genes in OF6 (54 suggested by gene transcripts and one plasma protein) were mainly related to antimicrobial immune response (Supplementary Fig. S9), and its metabolic pathway was phenylalanine metabolism (adjusted p-value = 0.032 and metabolite count = 2). For OF16, ten genes were annotated to the 58 contributing CpGs (*TNXB*, *HOXA1*, *HOXA2*, *HOXA4*, *HOXA5*, *HOXA6*, *C6orf27*, *RYR1*, *CRTAC1* and *MOG*) which did not identify pathways. From these CpGs, the most frequently identified genomic regions were the gene body of *TNXB* and the upstream of transcription starting site (TSS) of the *HOXA5* and *HOXA4* gene.

3.4. Omics features mediating the associations between exposures and childhood anthropometrics

The aforementioned external exposures and molecular features (Supplementary Tables S8 and S9), with absolute loadings >0.25, belonging to the factors laying on significant path in SEM analyses, were used to perform feature-based mediation analyses. With the restriction imposed by the mediation paths illustrated in Fig. 3**B**, there were in total 12,938 mediation models fitted. The features involved in the FDR significant mediations are illustrated in Fig. 5. Eight omics features, including IL-1beta (IL-1β), four phosphatidylcholines (PC), gene expression of carcinoembryonic antigen-related cell adhesion molecule 8 (*CEACAM8*) and 4-deoxyerythronic acid, were mediating the effect of 20 external exposures (three prenatal exposures: maternal legume intake and maternal exposure to mercury and arsenic; 17 postnatal exposures: child fish intake, child exposure to mercury and arsenic, three perfluoroalkyl substances (PFAS), ten organochlorine compounds (OCs) and one pesticides) on child anthropometrics.

In detail, plasma IL-1β and urinary 4-deoxyerythronic acid were mediating the association between 7 OCs (HCB, DDE, DDT, PCB138, PCB153, PCB180, sum of PCBs) and zBMI (TE ranged from −0.91 to −0.42 for IL-1β and ranged from −1.21 to −0.84 for 4-deoxyerythronic acid; IE ranged from −0.64 to −0.30 for IL-1β and ranged from −0.12 to −0.04 for 4-deoxyerythronic acid), with an average mediated proportion (calculated as the ratio of IE on the TE) of 70.7 % and 8.0 % for IL-1β and 4-deoxyerythronic acid, respectively (Supplementary Data S7). In addition, an IE was identified for expression of carcinoembryonic antigen-related cell adhesion molecule 8 (*CEACAM8*) gene (IE = −0.04) and p-cresol sulfate (IE = 0.04) in the association between PCB180 and zBMI (TE = −1.00).

The association between PFASs and child BMI was identified to be mediated by three serum metabolites, PC aa C40:6, PC aa C36:5 and PC aa C38:6, through an inconsistent mediation (TE ranged from −0.32 to −0.24; IE ranged from 0.04 to 0.12). Serum PC aa C40:6 was also detected to mediate the association between maternal exposure during pregnancy and child exposure to arsenic and mercury and child zBMI (TE ranged from −0.06 to 0.09, insignificant; IE ranged from 0.04 to 0.18). Child exposure to both metals was additionally mediated by PC aa C36:5 (TE ranged from −0.07 to −0.03, insignificant; IE ranged from 0.09 to 0.10), and the association between child arsenic exposure and zBMI was also mediated by serum PC aa C38:1 (TE = −0.02, insignificant; IE = −0.04).

Two serum PCs, PC aa C40:6, PC aa C36:5, were mediating the association between child fish intake and zBMI (TE = 0.19, insignificant, and IE = 0.14 for PC aa C40:6; TE = 0.18, insignificant, and IE = 0.10 for PC aa C36:5). In addition, an indirect effect through PC aa C36:5 was detected for the effect of maternal legume intake on child zBMI (TE = 0.33, insignificant; IE = −0.15). The binomial models of the OW

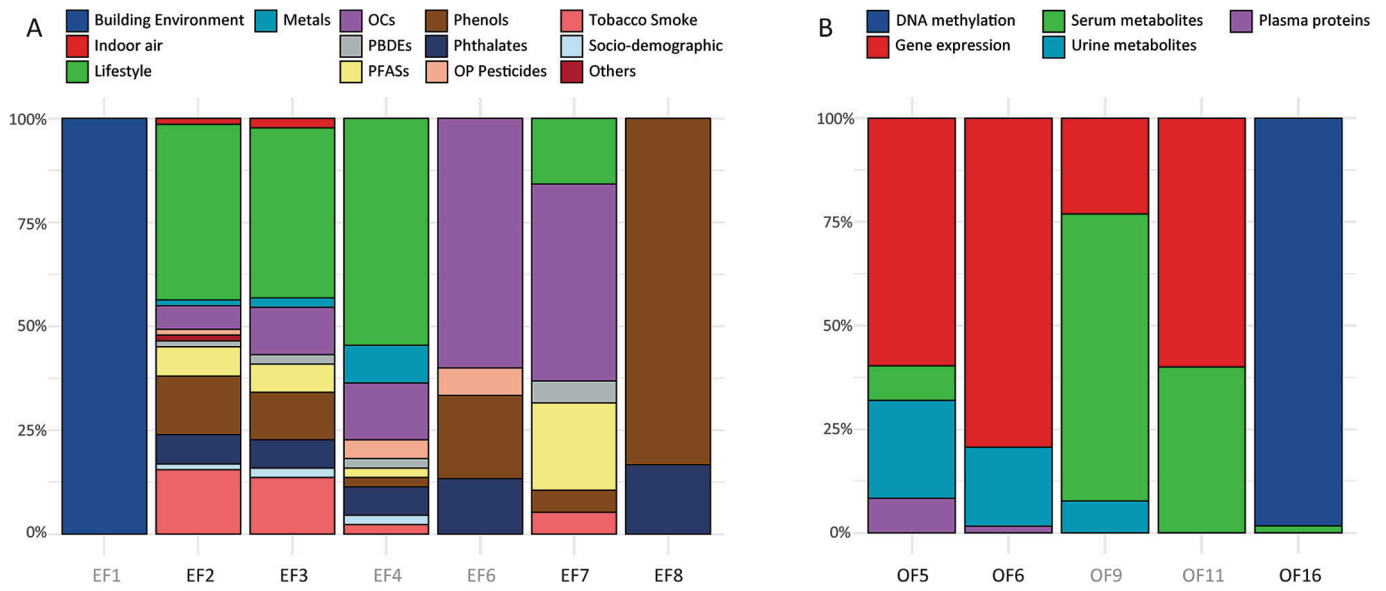

Fig. 4. Source of features contributing to the external exposome latent factors (A) and the multi-omics latent factors (B) in the mediation analyses. For each latent factor, only features with an absolute loading >0.25 were counted. The proportions represent the count of features from a specific source (view) relative to the total number of features associated with the same factor. Factors laying on significant indirect paths are labelled in black and the others are in grey. The loadings for each factor summarized by group of exposures or multi-omics layer are shown in Supplementary Data S5 and S6 respectively.

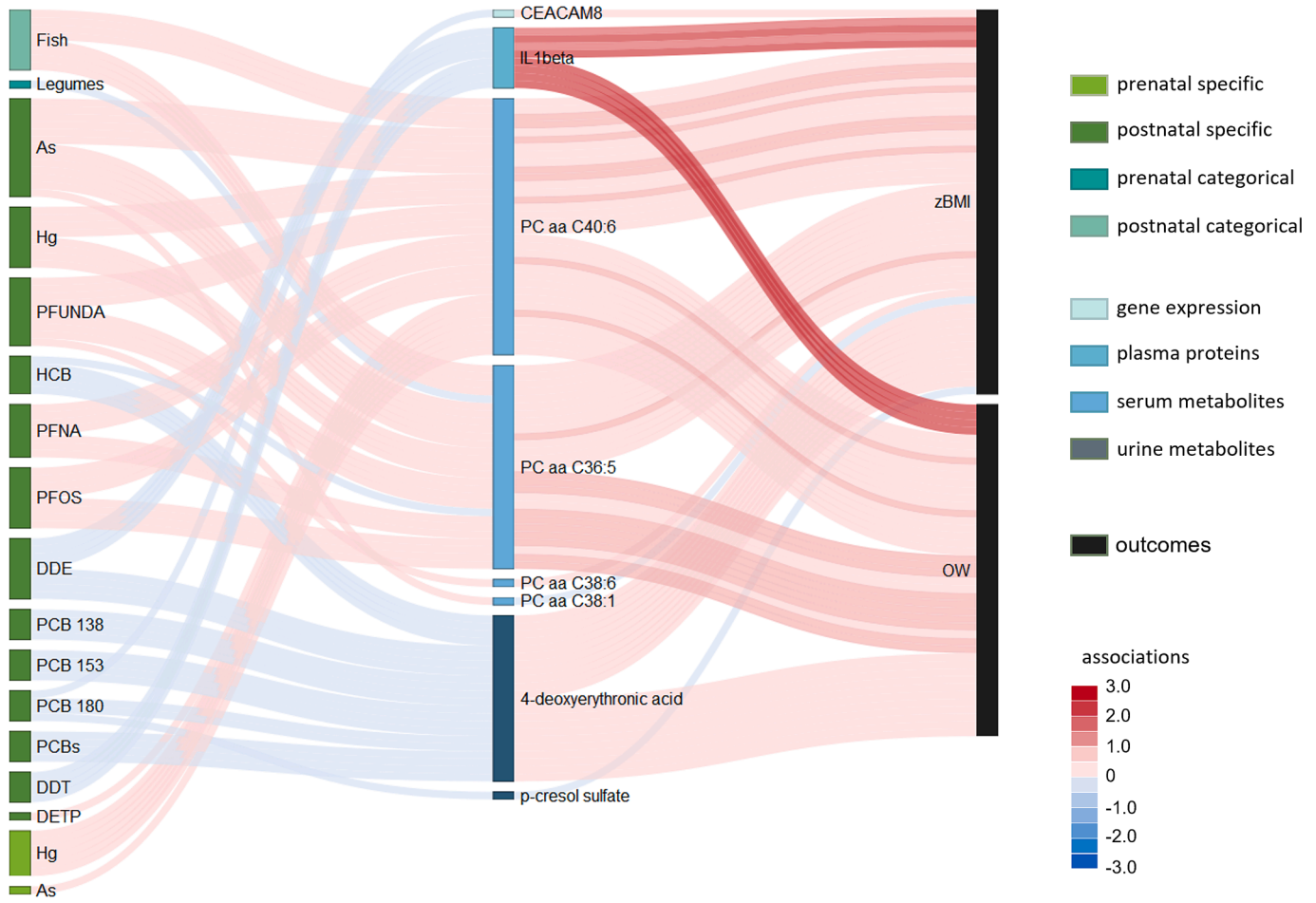

Fig. 5. Sankey diagram of the relationships of exposures, multi-omics features and child anthropometrics. Exposure (left column) and molecular feature (middle column) shown are involved in significant mediations (false discovery rate <0.05), for either child body mass index (BMI) z-score or childhood overweight (OW). The links between exposures and molecular features and the links between molecular features and child BMI represented the standardized regression coefficients.

(Supplementary Data S8) identified different types of OCs whose association with OW was mediated by 4-deoxyerythronic acid but did not show a mediation regarding maternal legume intake. The other associations were consistent between the binomial model of OW and the Gaussian model of the zBMI.

We performed additional analysis to evaluate the robustness of the mediation analysis with respect to model specification. When the interaction between individual exposure and molecular feature (as mediator) was considered, the estimated direct effect and mediated effects did not show much difference between exposure levels (Supplementary Fig. S10). When models were additionally adjusted for breastfeeding and maternal sustained smoking during pregnancy (Supplementary Fig. S11), the direct effects were in general slightly reduced in values (negative effects became more prominent, while positive effects became less strong), and the mediated effects slightly increased in absolute value (both negative and positive effects became more prominent). However, the wider CIs in Supplementary Fig. S11 suggested that the precision of estimation was lower when more covariates were included. When stratified by child sex (Supplementary Fig. S12), the direct effects and mediated effects showed an overall consistency between boys and girls, while the precision of effect estimates differed due to the sample sizes of subgroups. Notably, Supplementary Fig. S12D suggested slightly stronger mediation effects in girls compared to boys, especially regarding the mediated effects of DDE/DDT via IL-1β and the mediated effect of fish intake via PC aa C40:6. In general, these additional analyses did not change the conclusions regarding the exposure-molecular feature pairs that showed the strongest mediation effects, although indeed revealed slight deviations between boys and girls.

4. Discussion

To our knowledge, this study is the first to comprehensively integrate diverse factors from the external exposome, encompassing broad parameters such as air quality and urbanicity, alongside individual factors including nutrition, exercise, and smoking, with the internal exposome, represented by different omics-layers, in the analysis of childhood obesity. Previously, in the HELIX cohort, the link between the external and internal exposome has already been demonstrated in an ExWAS of >30 M exposure-omics associations (Maitre et al., 2022) and in a non-targeted network analysis of groups of non-persistent chemicals and multi-omics (Fabbri et al., 2023). The association between the external exposome and childhood obesity (Vrijheid et al., 2020), and the link between single pollutants and their mixtures, including persistent and non-persistent chemicals, and childhood trajectories of zBMI (Montazeri et al., 2023), have also been reported in the HELIX children. By applying a novel method, MOFA, as a dimensional reduction technique to streamline the external and internal exposome data and coupled it with a "meet in the middle" strategy to investigate the mediation role of the multi omics in the relationship between the external exposome and childhood obesity. In addition to replicating the associations between external exposome and childhood obesity previously identified in the HELIX children, this approach revealed the combined effects of multiple external exposures on childhood obesity and the potential mediated effect of multi-omics in these associations. Despite the large number of exposures tested, we were able to discover three combinations of multi-omics mediating the associations between four groups of external exposures with childhood anthropometrics: i) DNA methylation at multiple CpGs of *HOXA* and tenascin XB (*TNXB*) genes (OF16) mediated the associations between prenatal lifestyle factors (including prenatal nutrition, exercise, and passive smoking) (EF2) and childhood zBMI/OW, ii) inflammation and blood coagulation-related gene expression (OF5) mediated the association between breastfeeding and postnatal exposure to OCs (EF7) and phenols and phthalate, EF8) with childhood zBMI/OW and iii) antimicrobial immune pathways (OF6) mediated the association between childhood exposures to OCs and breastfeeding (EF7) and pre- and post-natal lifestyle (EF3) with childhood zBMI/OW. By looking at the effects of single omic features we were able to identify six metabolites (four different serum PCs, urinary 4-deoxyerythronic acid and p-creole sulfate), one transcript (*CEACAM8* gene expression) and one plasma protein (IL-1β) mediating the association between childhood exposure to chemicals (PCBs and metals) and food items (maternal intake of legumes and child fish intake) with childhood zBMI/OW. Below we discuss several key findings.

4.1. Combined effect of prenatal lifestyle exposures on child BMI is mediated by changes in integrated methylome

In our mediation analysis using SEM, we found that the combined effect of healthier prenatal nutrition (e.g., lower intake of cereals and of meat), higher passive smoking during pregnancy and lower moderate exercise during pregnancy on lower childhood BMI could be mediated by children's DNA methylation (OF16). In the HELIX cohort, these prenatal exposures were previously associated primarily with the methylome (Maitre et al., 2022). In our study, CpGs within OF16 that had the highest absolute loadings were frequently annotated with *HOXA5* located in the TSS region and tenascin XB (*TNXB*) genes located in the gene body. In line with our findings of *TNXB* hypomethylation being a mediator of the negative effects we detected, child methylation of *TNXB* has been associated with maternal diet (Sasaki et al., 2023), PFAS (Perng et al., 2023) and childhood anthropometrics (Alfano et al., 2022). Additionally, changes in *HOXA5* methylation patterns have also been associated previously with metabolic changes (Alfano et al., 2022; Reimann et al., 2019), in newborns, and with obesity and type 2 diabetes (T2D) in adults (Parrillo et al., 2023). Both maternal active and passive smoking during pregnancy have been reported to be positively associated with BMI or elevated risk of obesity in childhood (Braun et al., 2010; Oken et al., 2008). The association with child BMI has been found stronger for pregnancy active smoking than for pregnancy passive smoking in the INMA cohort of HELIX (Robinson et al., 2016), and in 1203 HELIX children, maternal active smoking, instead of maternal secondhand smoking during pregnancy, was found to be significantly associated with DNA methylation changes at 18 loci (Vives-Usano et al., 2020). In comparison, the strongest contribution observed using our approach was pregnancy passive smoking. This might be due to the fact that the MOFA model works in its nature to maximize the variance that can be captured by the latent factors (maternal pregnancy passive smoking was more heterogenous than maternal pregnancy active smoking) in an unsupervised way where the exposome's covariation with the multi-omics or child BMI was not taken into account.

In the sex-stratified analysis the association between the exposome factor mainly representing the absence of breastfeeding and postnatal exposure to OCs (EF7) via the alanine, aspartate and glutamate metabolism (OF5) remained stable but with comparatively higher estimates in boys than in girls. This might point in the direction of sex-specific influences of OCs on body weight through amino acid metabolism during critical windows of development which have already been demonstrated in animal models (Gao et al., 2019). The significant inverse interaction term between maternal OC exposure (EF6) and absence/low levels of certain offspring serum metabolites (OF9) in the assessment of zBMI could indicate a potential pathway of obesogenic effects in OC toxicity, as sphingolipids are known to be a modulator of insulin resistance (Catalano et al., 1991; Juchnicka et al., 2022). In the sensitivity analysis with an extended set of covariates only the indirect path between breastfeeding and childhood OC exposure (EF7) with the phenylalanine metabolism (OF6) showed a robust significant association compared to the main analysis with key-covariates. The absence of significant indirect paths may be a result of missing covariates for some of the cohorts, reducing the sample size in this analysis significantly. Interestingly, one additional indirect path reached statistical significance in the association with zBMI with the extended covariate-set, namely between building density (EF1) and the metabolism of

alanine, aspartate and glutamate (OF5).

4.2. Combined associations between childhood chemical exposures on BMI are mediated by inflammation and blood coagulation-related genes

Our results for the factor mediation analysis indicated that inflammation and blood coagulation could be a molecular mechanism underlying the association between postnatal exposure to PCBs and phenols and childhood obesity. In the SEM, genes involved in blood coagulation, JAK-STAT/MAPK signaling and inflammation-related cellular pathways (OF5) mediated the association between breastfeeding duration and organochlorine compounds, in particular PCBs and DDT (EF7), and phenols and phthalates levels of children (EF8) on childhood anthropometrics. Breastfeeding may expose infants to OCs like PCBs (Mekonen et al., 2021; Ribas-Fitó et al., 2003), which can accumulate due to their long half-lives and potentially influence childhood obesity. Several studies showed links between PCBs and effects on blood coagulation (Bouwman et al., 1999), disruption of key signalling pathways such as JAK-STAT/MAPK (Ferrante et al., 2014), and activation of inflammatory responses (Gupta et al., 2018; Mohammadi & Ashari, 2021; Peinado et al., 2023; Rahman et al., 2021; Trim et al., 2021; Wang et al., 2023), all of which have a large body of evidence suggesting their relevance for obesity (de Heredia et al., 2012; de Laat-Kremers et al., 2022; Ellulu et al., 2017; Kornblith et al., 2015; Monteiro & Azevedo, 2010).

4.3. Combined associations between childhood chemical exposures and prenatal and childhood lifestyle on BMI are mediated by antimicrobial immune pathways

Adipocytes release adipokines that modulate innate and adaptive immune responses, eliciting pathophysiological effects that can be independent of infection (Ellulu et al., 2017). In addition to immune pathways (OF5), we also identified specifically antimicrobial immune pathways that are over-represented by the gene transcripts contributing to OF6 mediating the association between breastfeeding duration and organochlorine compounds in children (EF7) and prenatal and childhood lifestyle (EF3) on childhood anthropometrics. Obesity tends to increase susceptibility to various infections (Karlsson & Beck, 2010; Pugliese et al., 2022), and certain infections could promote the development of obesity (Genoni et al., 2014; Hegde & Dhurandhar, 2013). Lifestyle factors indicated by EF3, such as prenatal maternal passive smoking and child diet, might have an effect on child BMI that is mediated by antimicrobial immune pathways (Hashimoto et al., 2023).

Our study validated previous findings in the literature on the influence of unhealthy maternal lifestyles during pregnancy and children's own behaviours on childhood anthropometric changes and extends the analysis by assessing the mediation effect through omics. Previous studies primarily focused on establishing associations between exposures and multi-omics or multi-omics and childhood anthropometrics. Our method delved deeper by examining the mediating mechanisms involved in these relationships. This novel approach provides a starting point for analysing disease etiology in future research and allows for causal inference of the observed health effects associated with environmental exposures. This comprehensive workflow incorporating both the integration of multi-omics and mediation analysis could serve as a valuable toolbox for future multi-omics cohort studies.

4.4. Mediation by individual metabolites and plasma proteins

Since latent factors represented the unobserved underlying mechanisms of a combination of the observed measurements, the effect of a latent factor is not completely equivalent to the effect of the contributing features. Therefore, by additionally conducting a mediation analysis of individual targeted molecular features, we validated findings from the latent factors- and SEM-based mediation analyses, and on the other hand, identified mediations of single features that were not as clearly explicit from the SEM, which focused on the mixture of exposures or the mixture of molecular features.

We found the by far the largest proportions of indirect effect for the association of the i) mercury concentration in maternal blood (74.64 %) and ii) child fish intake (73.18 %) with child zBMI, mediated by serum PC aa C40:6. The association found for maternal blood mercury levels might be in fact a result of the diet fish intake of the mothers during pregnancy, as the two entities were often highly related (Notario Barandiaran et al., 2024; Vergara et al., 2024) and that the maternal pregnancy fish intake was not shown in our results was solely due to multiple testing correction. With a proof analysis, we found a significant moderate association between the maternal fish intake and maternal mercury level during pregnancy, and observed a borderline-significant association between child's fish intake and child's blood mercury level (Supplementary Fig. S13). In addition, the mediation of the association in fish intake (either of the mother or the child) through serum PC aa C40:6 should also be interpreted with caution, as several phosphatidylcholine, including serum PC aa C40:6, have been proposed to be biomarkers of fish (oil) consumption (Cuparencu et al., 2019; Floegel et al., 2013; Hodson et al., 2018). While maternal mercury level has been shown to positively associate with the risk of child overweight (Wang et al., 2019), there is little reported regarding the association between serum PC AA C40:6 and BMI.

The second largest proportion of indirect effects was observed for DDT (72.15 %) and DDE (69.78 %), mediated by plasma IL-1β. Our study found an inverse association between postnatal DDT/DDE levels and IL-1β, possibly due to their accumulation in fat tissue, which contrasts with previous findings of positive associations (Martin et al., 2019; Shah et al., 2020). DDT/DDE are highly lipophilic and bioaccumulate in animals and humans (La Merrill & Birnbaum, 2011). Previous inverse associations between DDT/DDE and weight indices found in some cross-sectional studies have therefore been explained by reversed causality due to a "fat-trapping" of these substances with lower measured blood values linked to higher fat-stores (Cadiou et al., 2020). We also found a positive association between interleukin-1 beta (IL-1β) and zBMI/OW. On one hand, obesity is associated with a low-grade inflammation of white adipose tissue resulting from chronic activation of the innate immune system as IL-1β (Bing, 2015) and certain allele carriers of the IL-1β + 3953C/T polymorphism have been shown to have higher odds of being overweight/obese (Manica-Cattani et al., 2010). On the other hand, cytokines, including IL-1β are produced in adipocytes (Ghanbari et al., 2021) and obesity has been shown to upregulate transcript levels of IL-1β in visceral adipose tissue of colon carcinoma patients and increased expression of IL-1β a rat model of diet-induced obesity decreased after weight loss (Neira et al., 2024). Taking the above evidence into account, we could hypothesize multiple theoretical causal models between the DDE/DDT exposure identified in children, child plasma IL-1β, and child BMI (Supplementary Fig. S14), and we acknowledge that the estimated mediation effects could not provide direct confirmation of the theoretical models, as the data was only a snapshot of the measurements at one single time point. Following the assumption that IL-1β promotes overweight/obesity, the inverse association between DDE/DDT and IL-1β may describe an additional biological pathway underlying the inverse associations observed previously for DDT/DDE and weight measures.

We observed an association between higher childhood PCBs (PCB180, PCB138 and PCB153) mediated through child 4-deoxyerythronic acid. This result has been previously documented for this cohort (Vrijheid et al., 2020), where authors speculated that it may be explained by a sequestration mechanism of PCBs within adipose tissue. Other studies with a prospective design also reported positive associations between PCBs and obesity and features of the metabolic syndrome (Donat-Vargas et al., 2014; Valvi et al., 2012), which may indicate that the inverse associations found here in a cross-sectional approach could represent the effect of reversed causality. By lowering 4-deoxyerythronic acid, increased PCB concentrations may mitigate the positive

association between 4-deoxyerythronic acid concentrations and weight identified in our study. 4-deoxyerythronic acid is a catabolite of threonine metabolism. Previously, it has been positively correlated with BMI (Lau et al., 2020) and has been reported as a potential biomarker for type 1 diabetes (Appiah-Amponsah et al., 2009). A novel finding from the feature-based mediation analysis, with public health relevance, was that p-cresol sulfate, a microbial metabolite, that elicits inflammatory responses and oxidative stress *in vivo* (Sun et al., 2020) and *in vitro* (Koppe et al., 2021), mediates the association between PCB180 with childhood zBMI and overweight. In line with our findings, lower levels of p-cresol sulfate in urine have previously been associated with adult obesity (Yu et al., 2018). We also found in the feature mediation an inverse indirect effect of PCB180 on zBMI via *CEACAM8* expression, contributing to the weight-lowering effect of PCB180 observed in this study. In line with our findings, *CEACAM8* expression has been reported to be increased in obese subjects and significantly decreased after bariatric surgery (Berisha et al., 2011). Furthermore, we found statistically significant mediating effects between different PFAS congeners and serum metabolites in the association with zBMI (Fig. 5**,** Supplementary Table S7). The associations between PFAS and these diacylphosphatidylcholines (PC AA C36:5 and PC aa 38:6) have previously been described (Salihovic et al., 2019), as well as the association between PC aa C36:5 and PC aa C38.6 (Wallace et al., 2014) with obesity. In our study, we complemented the findings in literature by identifying an inconsistent mediation, where the indirect association between PFAS and zBMI through upregulation of these metabolites increased, while the direct effect between PFAS and zBMI was inverse.

4.5. Strengths and limitations

Our study has multiple strengths. The considerable sample size of this study contributes to the robustness and generalizability of our findings. We applied the novel stepwise procedure built upon MOFA, where we extended its application to the external exposome and distributed into different exposure categories. By reducing the high-dimensional and heterogeneous data to uncorrelated factors, this approach enabled us to combine the advantages of an untargeted approach akin to ExWAS studies with the modeling of complex exposure mixtures like in multi-pollutant studies. Moreover, integrating multi-omics and the external exposome in a comprehensive analysis provided a more realistic exposure scenario by acknowledging the correlation structures between different variables and reducing multiple testing bias. We used a meet-in-the-middle approach, which proved an efficient workflow to uncover mechanistic explanations and causal inference linking environmental exposures with anthropometric outcomes. In parallel with the mediation analysis of the latent factors, we also performed an additional mediation analysis of the individual exposures and molecular features. The two methods provided varied perspectives showed consistencies in some findings, and uncovered different potential mediation relationships.

We also acknowledge the limitations of the current study. First, multi-omics measurements have a coverage bias, as described previously for the multi-omics analysis in this cohort (Maitre et al., 2022). Omics were profiled in different tissues and on different platforms. In addition, DNA methylation and gene expression were measured in microarrays, whereas the metabolites and proteins were quantified in a targeted manner. The exposome was also heterogeneous in terms of the between-cohort variation, because data collection was not always accessed uniformly across the cohorts and had to be harmonized afterwards. Second, the interpretations of the factors extracted by MOFA might not be straightforward. Especially in the exposome, the factors were not fully orthogonal to each other, which brought in information overlap when we tried to label the factors with the most highly loaded exposures. Third, since MOFA is an unsupervised method, and we applied a pre-filtering of omics data based on features' variability, pieces of information relevant to child BMI, but not as variable, might have been lost. Fourth, another challenge of our approach was the relatively small variation found for many variables of the (general) external exposures compared to those of the multi-omics. This may partly result from the necessity to dichotomize certain categorical variables to fit them in the Gaussian or Bernoulli distribution required by the MOFA approach. Finally, mediation is built upon causality. Causal inference from exposures to multi-omics / child anthropometrics could stand since the exposures temporally preceded the measurements of omics profiles. However, we acknowledge that omics could also be biomarkers of exposure and in this case mediated effects could be overestimated (Valeri et al., 2017). Further, the causal inference from omics to child anthropometrics needs cautious interpretation.

5. Conclusions

This study presents for the first time a comprehensive integration of the early-life exposome with different multi-omics layers to investigate the underlying mechanisms of environmental influences on childhood weight. By applying this "meet in the middle" approach, we showed in replicable consecutive steps how to combine the advantages of unsupervised analytical methods for the internal as well as the external exposome. Our findings replicate known associations and uncover new combined effects of multiple exposures, mediated by DNA methylation, gene expression, and immune pathways, highlighting the complex interplay between prenatal and postnatal exposures, biological responses, and obesity. With our findings, we hope to promote future research and advances in public health policies regarding the effects of environmental exposures on metabolic health.

Resource availability

Reasonable requests for further information and resources should be directed to and will be fulfilled by the correspondence author, Rossella Alfano (rossella.alfano@uhasselt.be).

CRediT authorship contribution statement

Congrong Wang: Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis. **Brigitte Reimann:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis. **Tim S Nawrot:** Writing – review & editing, Supervision, Methodology. **Dries S Martens:** Writing – review & editing, Validation, Methodology. **John Wright:** Writing – review & editing, Project administration, Methodology. **Rosemary McEachan:** Writing – review & editing, Investigation. **Johanna Lepeule:** Writing – review & editing, Investigation. **Wenlun Yuan:** Writing – review & editing, Investigation. **Leda Chatzi:** Writing – review & editing, Project administration, Methodology. **Marina Vafeiadi:** Writing – review & editing, Project administration, Methodology, Investigation. **Regina Grazuleviciene:** Writing – review & editing, Investigation. **Sandra Andrusaityte:** Writing – review & editing, Investigation. **Oliver Robinson:** Writing – review & editing, Methodology. **Jordi Sunyer:** Writing – review & editing, Methodology. **Hector Keun:** Writing – review & editing, Methodology. **Chung-Ho E. Lau:** Writing – review & editing, Investigation. **Alexandros P. Siskos:** Writing – review & editing, Investigation. **Muireann Coen:** Writing – review & editing,

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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 material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2025.109630.

Data availability

Data will be made available on request.

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