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Nutritional modulation of associations between prenatal exposure to persistent organic pollutants and childhood obesity: a prospective cohort study

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Abstract:	Background : Prenatal exposure to persistent organic pollutants (POPs) may contribute to the development of childhood obesity and metabolic disorders. However, little is known about whether the maternal nutritional status during pregnancy can modulate these associations. Objectives : The main objective was to characterize the joint associations and interactions between prenatal levels of POPs and nutrients on childhood obesity. Methods : We used data from to the Spanish INMA birth cohort, on POPs and nutritional biomarkers measured in maternal blood collected at first trimester of pregnancy and child anthropometric measurements at 7 years. Six organochlorine compounds (OCs) (dichlorodiphenyldichloroethylene, hexachlorobenzene [HCB], β-hexachlorocyclohexane [β-HCH] and polychlorinated biphenyls 138, 153, 180) and four

	per-/polyfluoroalkyl substances (PFAS) were measured. Nutrients included vitamins (D, B12 and folate), polyunsaturated fatty acids (PUFAs), and dietary carotenoids. Two POPs-nutrients mixtures datasets were established: (i) OCs, PFAS, vitamins, and carotenoids (n=660) and (ii) OCs, PUFAs, and vitamins (n=558). Cumulative effects of mixtures on obesity were characterized using Bayesian Kernel Machine Regression (BKMR). Relative importance of biomarkers and 2-way interactions were identified using Gradient Boosting Machine, hierarchical group-lasso regularization, and BKMR. Interactions were further characterized using multivariate regression models in the multiplicative and additive scale. Results : Forty percent of children were overweight or obese. We observed a positive overall cumulative effect of both POPs-nutrients mixtures on overweight/obesity risk, being HCB and vitamin B12 the biomarkers contributing the most. Recurrent interactions were found between HCB and vitamin B12 across screening models. Relative risk and 95% confidence interval (95% CI) for a log increase of HCB was 1.31 (1.11-1.54, p interaction =0.02) in the tertile 2 of vitamin B12 and in the additive scale a Relative Excess Risk due to Interaction of 0.11 (95% CI, 0.02-0.20) was found. Interaction between perfluorooctane sulfonate and b-cryptoxanthin suggested a protective effect of the antioxidant on overweight/obesity risk. Conclusion : These results support that maternal nutritional status may modulate the effect of prenatal exposure to POPs on childhood overweight/obesity. These findings may help to develop biological hypothesis for future toxicological studies and to better interpret inconsistent findings in epidemiological studies.
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Title: **Nutritional modulation of associations between prenatal exposure to persistent organic pollutants and childhood obesity: a prospective cohort study**

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Conflict of Interest

The authors declare that they have no conflict of interest

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Abstract

Background: Prenatal exposure to persistent organic pollutants (POPs) may contribute to the development of childhood obesity and metabolic disorders. However, little is known about whether the maternal nutritional status during pregnancy can modulate these associations.

Objectives: The main objective was to characterize the joint associations and interactions between prenatal levels of POPs and nutrients on childhood obesity.

Methods: We used data from to the Spanish INMA birth cohort, on POPs and nutritional biomarkers measured in maternal blood collected at first trimester of pregnancy and child anthropometric measurements at 7 years. Six organochlorine compounds (OCs) (dichlorodiphenyldichloroethylene, hexachlorobenzene [HCB], β-hexachlorocyclohexane [β-HCH] and polychlorinated biphenyls 138, 153, 180) and four per-/polyfluoroalkyl substances (PFAS) were measured. Nutrients included vitamins (D, B12 and folate), polyunsaturated fatty acids (PUFAs), and dietary carotenoids. Two POPs-nutrients mixtures datasets were established: (i) OCs, PFAS, vitamins, and carotenoids (n=660) and (ii) OCs, PUFAs, and vitamins (n=558). Cumulative effects of mixtures on obesity were characterized using Bayesian Kernel Machine Regression (BKMR). Relative importance of biomarkers and 2-way interactions were identified using Gradient Boosting Machine, hierarchical group-lasso regularization, and BKMR. Interactions were further characterized using multivariate regression models in the multiplicative and additive scale.

Results: Forty percent of children were overweight or obese. We observed a positive overall cumulative effect of both POPs-nutrients mixtures on overweight/obesity risk, being HCB and vitamin B12 the biomarkers contributing the most. Recurrent interactions were found between HCB and vitamin B12 across screening models. Relative risk and 95% confidence interval (95% CI) for a log increase of HCB was 1.31 (1.11-1.54, $p_{\text{interaction}}$=0.02) in the tertile 2 of vitamin B12 and in the additive scale a Relative Excess Risk due to Interaction of 0.11 (95%

CI, 0.02-0.20) was found. Interaction between perfluorooctane sulfonate and β-cryptoxanthin suggested a protective effect of the antioxidant on overweight/obesity risk.

Conclusion: These results support that maternal nutritional status may modulate the effect of prenatal exposure to POPs on childhood overweight/obesity. These findings may help to develop biological hypothesis for future toxicological studies and to better interpret inconsistent findings in epidemiological studies.

1. Introduction

Growing evidence supports that the environment and nutrition during the early stages of development may impact the subsequent health during childhood, including obesity and metabolic diseases (Gluckman and Hanson 2004; Inadera 2013). Childhood obesity remains a public health priority due to the high prevalence, associated risk of comorbidities and high societal costs (Lin and Li 2021; NCD-Risk 2017), which has been linked to a growing list of environmental factors including synthetic chemicals (Güil-Oumrait et al. 2021; Legler et al. 2015). Persistent organic pollutants (POPs) represent a vast family of chemicals characterized by their hydrophobicity, stability, and capacity to bioaccumulate across the trophic chains and widespread in fatty tissues of populations from across the globe (Jones and De Voogt 1999; UNEP 2017; WHO 2010). Some POPs like the pesticide dichlorodiphenyltrichloroethane (*p,p'*-DDT), its main metabolite dichlorodiphenyldichloroethane (*p,p'*-DDE), or hexachlorobenzene (HCB) have been associated with obesity or metabolic disruption in human prospective studies and supported by several experimental studies (Iszatt et al. 2015; Nadal et al. 2017; Ren et al. 2020; Stratakis et al. 2021; Valvi et al. 2012).

Such hydrophobic pollutants present physicochemical commonalities with some nutrients, sharing mechanisms of uptake, transport, and metabolism, and/or targeting similar molecular pathways, which results in a large potential to interact in multiple health outcomes (Cano-Sancho and Casas 2021). Specific dietary patterns and nutritional status can modulate the effect of toxicants, and thus it can become a source of heterogeneity in environmental epidemiological research, if not properly addressed (Cano-Sancho and Casas 2021; Hennig et al. 2012). For instance, in a seminal study where nutritional confounding was first reported, the neurotoxic effects of prenatal methyl-mercury exposure were strengthened when polyunsaturated fatty acids (PUFA) were included in the regression models (Choi et al. 2008). The interactive effect of POPs and nutritional compounds in metabolic disorders has been

observed in experimental studies; for instance, counteracting the beneficial effects of PUFA ω-3 on the prevention of insulin resistance and obesity (Ibrahim et al. 2011; Ruzzin et al. 2010). Previous studies within the Spanish longitudinal INMA (INfancia y Medio Ambiente – Environment and Childhood) birth cohort have reported positive associations between prenatal exposure to organochlorine compounds (OCs) with higher offspring obesity risk (Agay-Shay et al. 2015; Güil-Oumrait et al. 2021; Valvi et al. 2012; Valvi et al. 2014), but mild or null associations for per- and polyfluoroalkyl substances (PFAS) (Manzano-Salgado et al. 2017). To the best of our knowledge, no previous studies have been conducted to assess the joint effect of prenatal POPs and nutrients to date. Thus, built on the hypothesis that the health effects of toxicants may be modulated by the nutritional status (Cano-Sancho and Casas 2021), this study extends the previous INMA work to characterize joint effect of prenatal POPs and nutrients on childhood obesity risk. To this end, we conducted a comprehensive multi-step framework intended to answer major questions in epidemiological mixture analyses including (Barrera-Gómez et al. 2017; Braun et al. 2016; Knol and VanderWeele 2012; Lazarevic et al. 2019): 1) what is the effect of individual POPs when other POPs are considered in the model?; 2) what is the cumulative effect of the mixture of POPs and nutrients on the specific outcome?; 3) are there interactions between POPs and nutrients within the mixture? and 4) if exist, how these interactions affect the POPs-obesity effect estimates?

2. Methods

2.1. Study Population

Data from the Spanish INMA birth cohort were used for the present analysis, extensively described elsewhere (Guxens et al. 2012). Briefly, a total of 2,150 pregnant women from the regions of Gipuzkoa, Sabadell, and Valencia were recruited at first trimester of pregnancy (weeks 10-13 of gestation) from 2003 to 2008. To be eligible, women must be at least 16 years

143 old and present singleton pregnancy, no communication barrier, no reproductive assistance and
144 giving birth in the reference hospital (Guxens et al. 2012). The study was approved by the
145 ethical committees of the centers involved in the study. Written informed consent was obtained
146 from the parents of all children. In the present analysis, we included mother-child pairs with
147 information on blood biomarkers of POPs (OCs and PFAS) exposure and nutrient intake
148 (vitamins, PUFAs, and carotenoids) during pregnancy and child anthropometric measurements
149 at 7 years of age (Figure S1). A total of 1241 mothers had information on OCs and vitamins
150 and child obesity outcomes (Figure S1). However, since not all women had available
151 information of all POPs and nutrients, we generated two consolidated datasets for the mixture
152 analysis: the ANTIOX dataset with OCs, PFAS, vitamins and carotenoids (n=660 with 30
153 variables) and the PUFA dataset consisting of OCs, vitamins and PUFAs (n=558 with 14
154 variables), see details in Table 1. Among both datasets there is an overlap of 241 mother-child
155 pairs. The list of compounds included within each dataset is detailed in Table 1.

156 *2.2. Prenatal POPs determination*

157 Blood samples from mothers were collected at recruitment at the end of first trimester of
158 gestation (weeks 10-13 of gestation), aliquoted in 1.5 mL cryotubes and stored at −80 °C until
159 their analysis. Concentrations of HCB, β-hexachlorocyclohexane (β-HCH), *p,p'*-DDE, and
160 polychlorinated biphenyl (PCB) congeners 138, 153, and 180 were determined in serum
161 samples using gas chromatography equipped coupled to electron capture detector or mass
162 spectrometer as previously described (Goñi et al. 2007; Grimalt et al. 2010; Mendez et al. 2011;
163 Valvi et al. 2012). Concentrations of OCs were adjusted to total serum lipids calculated with
164 the reduced equation using cholesterol and triglycerides determined enzymatically (Phillips et
165 al. 1989). Concentrations of perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS),
166 perfluorohexane sulfonate (PFHxS), and perfluorononanoate (PFNA) were determined in
167 plasma samples by column-switching liquid chromatography coupled with tandem mass

spectrometry at the Institute for Occupational Medicine, RWTH Aachen University (Aachen, Germany), as described previously (Manzano-Salgado et al. 2015; Manzano-Salgado et al. 2017). Limits of detection (LOD) ranged between 0.01 and 0.07 ng/ml for OCs and between 0.05 and 0.20 ng/mL for PFAS.

2.3. Nutritional biomarkers determination

Biomarkers of vitamins, PUFAs, and carotenoids were determined in maternal blood using validated analytical methods described elsewhere (Montes et al. 2013; Morales et al. 2015; Vioque et al. 2013). Briefly, serum levels of vitamin B12 and folate were measured using a commercially available radioassay (Vioque et al. 2013). Levels of 25-hydroxyvitamin D3 were measured in plasma by high-performance liquid chromatography (HPLC) using a BIO-RAD kit (BIO-RAD Laboratories GmbH, Munchen, Germany) as measure of vitamin D status (Morales et al. 2015). Concentrations of long-chain PUFAs were determined in maternal plasma using fast-gas chromatography (Montes et al. 2013). The levels of carotenoids (α- and β-tocopherol, β-cryptoxanthin, α- and β-carotene, lutein, lycopene, zeaxanthin, and retinol) were measured in serum using HPLC with diode array detection and UV detection at 292 nm, in case of α-tocopherol (Vioque et al. 2013). All biomarkers showed coefficients of variation below 10% in inter-assays and 5% intra-assays.

2.4. Childhood obesity outcomes

Trained nurses measured weight and height from children at the follow-up visit at 7 years (mean: 7.7, standard deviation (SD): 0.23 - ANTIOX dataset), using standard protocols. Age- and sex-specific body mass index (BMI) z-scores (zBMI) were calculated based on the WHO standard reference curves (de Onis et al. 2007). Overweight and obesity were defined as the proportion of children with values zBMI over >1SDs and >2SDs, respectively (de Onis et al. 2010; Vrijheid et al. 2020).

2.5. Covariates

Information on socio-demographic (age, parity, education,) and lifestyle characteristics (smoking) of the mothers was collected by questionnaires administered to mothers during the first trimester of pregnancy. Measured maternal height and reported weight by the mother at the first trimester visit was used to calculate pre-pregnancy BMI (kg/m^2). Data regarding the maternal health status during pregnancy was directly collected from clinical records. Confounding variables were selected on the basis of published literature on established determinants of maternal levels of POPs and childhood obesity risk (Ibarluzea et al. 2011; Llop et al. 2010). All models were thus adjusted for maternal pre-pregnancy BMI (kg/m^2), age of the mother (years), education level (primary or without education, secondary, university), smoking during pregnancy (nonsmoking, any smoking during pregnancy), region of residence (Gipuzkoa, Sabadell, Valencia), and child's sex (female, male).

2.6. Data analysis

The multi-step workflow for data-analysis is illustrated in Figure 1 covering major questions about the effect of mixtures: an exploratory analysis (*step 1*) to identify the correlations between POPs and nutrients; a preliminary characterization of associations between individual biomarkers and obesity outcomes without accounting for the rest of biomarkers (*step 2*); a ranking of biomarker importance accounting for the co-exposure in multipollutant models (*step 3*); an estimation of the cumulative effect of the mixtures on obesity outcomes (*step 4*); a screening of 2-way interactions to select suspected pairs (*step 5*); and a refined characterization of those interactions using conventional regression methods to facilitate the interpretation in terms of risk estimation (*step 6*). To this end, we applied a battery of complementary algorithms developed to integrate multiple correlated exposure variables to identify joint-effects and interactions (Barrera-Gómez et al. 2017; Lazarevic et al. 2020).

2.6.1. Data pre-processing and unsupervised exploratory analysis

The distribution profiles of POPs and nutrients were explored in order to characterize the skewness and identify extreme values, left-censored data, and missing data. Biomarkers with detection frequencies below 75% were removed from the analysis (*p,p'*-DDT and PCB118); only complete exposure data was used in the statistical analysis. Multiple multivariate imputation procedure was applied to covariate variables with some missing data following the workflow described elsewhere for data missing at random (van Buuren and Groothuis-Oudshoorn 2011). Batches of 15 variables were considered in the multivariable imputation models using the R package mice, developed specifically for each dataset. Pre-processing of continuous data included log-transformation and scaling to the SD to improve model fit, if necessary. Exploratory analysis included Spearman's rank correlation analysis to identify correlation patterns between pairs of biomarkers and support the biomarker grouping in the Bayesian kernel machine regression (BKMR) (see section 2.5.3.) and interpretation of results.

2.5.2. Single-biomarker outcome associations

Multivariate linear and Poisson regression (MLR) with robust variance were used to characterize the associations between individual biomarkers (POPs and nutrients) and continuous (zBMI) or binary outcomes (normal weight vs overweight and obese combined), respectively. Confounding variables were included in the model as covariates, allowing a flexible estimation of marginal effects. Modified Poisson regression with robust variance was computed using the "sandwich" approach, which is considered to provide unbiased estimates of risk ratios under potential model misspecification (Chen et al. 2018). The method was implemented in R software using the *coeftest* function with 'sandwich' and 'lmtest' packages.

2.5.3. Biomarker importance from multipollutant models

Three different statistical methods to examine multipollutant associations were selected based on their complementarity to characterize linear and non-linear associations, with the capacity to manage confounding data and identify potential interactions. These models also allow the

characterization of rankings of associations for individual exposures, thus providing a measure of their relative importance within each model, while accounting for the rest of biomarker effects and the identification of interactions (see next sub-section). A summary of main features from each model supporting the complementarity are displayed in Table S2.

- *Group Lasso Interaction Network (Glinternet)* is a flexible regularization algorithm designed to identify pairwise interactions in regression models imposing the group-lasso (L1) penalties with strong hierarchy (Lim and Hastie 2015). Thus, if an interaction coefficient is estimated to be nonzero, then its two associated main associations also have nonzero estimated coefficients, controlled by the parameter λ. Optimal λ can be selected by cross validation in order to identify an adequate bias-variance trade-off. In the present study, 10-fold cross-validation was used to identify the λ exhibiting the lowest mean squared error, computed with the R package "*glinternet*". To increase the robustness of findings, the models were fitted to 100 bootstrap samples, as described elsewhere (Havard et al. 2019). This feature allowed the measurement of coefficient variability and the frequency of interaction detection across bootstrap samples. Averaged model coefficients were used as variable importance scores considering all of them were at the same scale. The approach is intuitive, may handle a large number of independent variables (e.g., up to 10^5) and their interactions, computationally efficient and the results are straightforward to interpret. The method may be limited to characterize non-linear associations, and the built-in package does not allow forcing confounding variables out of model penalties.

- *Gradient boosting machine (GBM)* is one of the first approaches proposed to evaluate the joint associations of environmental exposures and their interactions (Lampa et al. 2014). Boosting machines combines a large number of simple regression tree models throughout an iterative process of simpler models' combination (boosting) to improve the overall model fit. We implemented the method using the R packages "*gbm*" and "*dismo*" (Elith et al. 2008). The

input settings used in the gbm.step function included a learning rate of 0.001, tree complexity of 4, bag fraction at 0.8, and 10-fold cross-validation. Learning rate is a weight applied to the parameter that minimizes the loss function; thus, slower learning rates (smaller values) require more iterations to achieve local minima. Tree complexity (interaction depth) is the number of nodes in a tree and should be sufficiently large to capture potential interactions (interactions between predictors are also evaluated after running a model). The bag fraction is the proportion of data randomly selected to propose the next expansion in a tree. In order to obtain robust estimates, we replicated the model 100 times and extracted the most frequently detected variables and their interactions across the replicates (>50 %). The relative contribution of biomarkers to the overall model fit were used as variable importance metric. Strengths of GBM includes the capacity to detect non-linear associations and high-order interactions, at a moderate computational cost, however the interpretability is often challenging, requiring a second modeling step.

- *Bayesian kernel machine regression (BKMR)*. The BKMR framework is a flexible non-parametric approach that allows the estimation of the overall effect estimate of multiple correlated exposures accounting for confounding variables (Bobb et al. 2015). The method was implemented with the R package "*bkmr"* using 10,000 iterations (Bobb et al. 2018). All variables were included in the model using the variable selection mode which allows the computation of posterior inclusion probabilities (PIPs) to support the selection of most relevant variables and rank the variables according to their probability to be included in the model as approximate measure of variable importance. Important assets of BKMR compared to the previous methods include the unique capacity to measure the cumulative effect of the mixture and the model structure specifically accounting for confounding variables. Despite the method can efficiently account for complex interactions, the identification process involves graphical inspection that can become sometimes conflicting.

293 *2.5.4. Cumulative effect of mixtures*

294 The joint effect of the mixture composed by those POPs and nutrients with higher PIPs was
295 characterized using BKMR with hierarchical variable selection, as described above and using
296 the OverallRiskSummaries function from the "*bkmr*" R package. The summary estimate
297 displayed the overall effect of biomarkers as the comparison of the predicted outcome when all
298 biomarkers are fixed at given percentiles with the predicted outcome when all biomarkers are
299 fixed at the 10^{th} percentile.

300 2.*5.5. Screening of 2-way interactions*

301 In this step the 2-way interactions included in the multipollutant models were identified. With
302 Glinternet, two-way interactions were identified through the inherent strong hierarchical fitting
303 process, together with those main-effects likely to be nonzero. In the case of GBM, as other
304 tree-based models, interactions between predictors are inherently included, as some of the
305 regression trees used are likely to be asymmetric (thus inducing interactions between
306 variables), where the response of one variable depends on the others higher in the tree. The
307 presence of interactions was assessed using the gbm.interactions routine from the "*dismo*"
308 package. Briefly, the interactions were identified if departures of model predictions for linear
309 combinations of pair of variables was elucidated (Elith et al. 2008). For BKMR, two-way
310 interactions were graphically explored using the cross-section plots depicting the exposure-
311 response function for a given exposure when another other exposure was fixed at the 25th,
312 50th, or 75th percentile fixing the rest of exposures to the median.

313 In order to develop a priority list of interactions for refined analysis, we selected those
314 chemicals pairs considering the criteria of being a pair of POPs and nutrients and either
315 exhibiting: the most influential or largest strength estimates in at least one approach (e.g.
316 contribution for GBM, coefficients for Glinternet, clear visual trends for BKMR) or
317 weak/moderate strength estimates in at least two approaches or obesity outcomes (i.e.

continuous or binary). For those methods involving bootstrapping samples (GBM and Glinternet), a frequency detection threshold was defined based on the number of interactions identified (e.g. 50% large number of interactions or 25% low number of interactions).

2.5.6. Characterization of interactions for selected pairs

In the latter step, we aimed to characterize the joint effect of pairs biomarkers (i.e. POPs and nutrients) identified in the previous screening phase to facilitate interpretation. We first built the generalized additive models (GAM) including the interaction product term for the selected pairs of variables, considering the POPs in continuous scale and nutrients in categorical scale (tertiles), adjusted for the abovementioned covariates. Interaction plots were inspected in order to characterize the shape of the exposure-response functions and refine the regression models. Unlike distributions of nutrients broken in tertiles for a better interpretation of interactions, POPs were categorized in quartiles if departure from linearity was graphically visualized. Risk estimates of POPs were then evaluated across the different tertiles of nutrients using linear or robust Poisson regression models for zBMI or overweight/obesity risk, respectively, as detailed above. In order to formally evaluate the departures from additive joint effects we also estimated the relative excess risk of overweight/obesity due to interaction (RERI) with the regression models' coefficients from Poisson regression (Knol et al. 2007; Knol and VanderWeele 2012). The additive interaction is present when the RERI (95% confidence interval) >0 if positive or <0 if negative.

3. Results

3.1. Unsupervised exploratory analysis

The prevalence of overweight including obesity was 43% and 41% in the ANTIOX and PUFA datasets, respectively (Table S1). The respective mean age of children ranged was 7.7 and 7.3 years old, having an equal proportion of girls and boys in both datasets (Table S1). Mothers

had a mean age of 30 years at recruitment with a presence of about 30% of smokers at some moment during pregnancy. Levels of OCs and vitamins were similar between datasets (Table 1). The correlation analysis showed high positive correlations ($\rho > 0.5$) between POPs within families (Figure 2A and 2B), and mild or no correlation between families. In turn, *p,p'*-DDE was not correlated with PFAS and moderately correlated (ρ: 0.3-0.2) with PCBs, HCB, and β-HCH. Regarding the fatty acids, arachidonic acid (AA) and docosahexaenoic acid (DHA), both showed positive correlations with PCBs and negative correlation with β-HCH (ρ: 0.3 and -0.1, respectively). Similar but weaker association profiles were shown for eicosapentaenoic acid (Figure 2B), and LA was negatively correlated with PCBs and the rest of PUFAs. Most OCs and PFAS congeners were not correlated with dietary carotenoids and vitamins with few exceptions. For instance PFHxS showed mild positive correlations with most nutrients (ρ: 0.1-0.2), whereas *p,p'*-DDE was negatively associated with retinol (ρ: -0.3) (Figure 2B).

3.2. Single-biomarkers outcome associations

The contributions and associations of individual biomarkers with overweight/obesity risk are summarized in Figure 3 (ANTIOX dataset) and Figure S3 (PUFA dataset); whereas the estimates for zBMI scores can be found in Figures 2 (ANTIOX dataset) and 4 (PUFA dataset). Numeric results are reported in Tables S3 and S4. Single-pollutant models showed consistent statistically significant associations of prenatal exposure to HCB and β-HCH with obesity outcomes in children (e.g. adjusted Relative Risk (RR): 1.18 [95% confidence interval (CI): 1.07, 1.31] per 1-SD increase in log HCB ANTIOX dataset, Figure 3). PFNA was positively associated with overweight/obesity risk (e.g., RR 1.10 [95% CI: 1.01, 1.20] per 1-SD increase in log PFNA p=0.03, Figure 3) but not with zBMI (Figures S2 and S4). Null associations were found for the rest of POPs, nonetheless PCB153, PCB180 and PFOA were positively associated with overweight/obesity risk but at limit of conventional statistical significance (p=0.06-0.10, Table S3, S4). Estimates for most nutrients were also null with the exception of β-

cryptoxanthin, zeaxanthin or α-tocopherol, positively associated with zBMI, whereas docosahexaenoic acid (DHA) showed a negative association.

3.3. Biomarker importance from multipollutant models

Variable selection methods (Glinternet, GBM, BKMR) consistently indicated that HCB and vitamin B12 had a high relative importance in the multipollutant models of overweight/obesity risk (Figures 3 and Figure S3) and zBMI (Figures S2 and 4). In turn, β-HCH, PFNA, PFOA and PFOS also scored high, but the ordering was less consistent across datasets, models, and obesity outcomes. Similarly, the ordering of PUFAs in the importance ranking was less consistent between obesity outcomes. For instance, AA was identified among most contributing variables on overweight/obesity risk (Figure S3), whereas DHA, LA and ALA appeared among the most important contributors on zBMI models (Figure S4). Among the carotenoids, β-cryptoxanthin was the most important biomarker across models and outcomes, followed by α-tocopherol and γ-tocopherol. In turn, folate showed one of the most inconsistent behaviors across datasets, models, and obesity outcomes, being among the top 5 PIPs from BKMR in one overweight/obesity model (ANTIOX dataset, Figure 3) but scoring low in the rest of the models.

3.4. Cumulative effect of mixtures

The profile of most relevant chemicals in the mixtures is depicted by the conditional PIPs discussed in the previous section. The hierarchical variable selection approach, built on groups of biomarker nature (i.e. OCs, PFAS, vitamin, PUFA, and antioxidant), resulted in a balanced composition of top ranked biomarkers with representatives from each family. The overall cumulative effect of the mixture was positive, mostly linear and significant, for overweight/obesity risk (Figure 4A) and zBMI (Figure 4B). Details of most relevant biomarkers in the mixtures can be found in Figures S9-12.

3.5. Screening of 2-way interactions

The screening of interactions relied in three computational methods (Glinternet, GBM, and BKMR) with complementary features to fit the data and identify interactions. For that reason and in order to increase the robustness of findings, we selected interactions with the largest strength present in at least a single model or weaker strength but selected by multiple models. Results from each model and outcome are summarized in tables and interaction network plots depicting the bivariate interactions in the ANTIOX (Table S5 and Figures S5-6) and PUFA datasets (Table S5 and Figures S7-8). Specific interactions outputs from GBM (bootstrap interaction contributions) and Glinternet (bootstrap interaction coefficients) can be found in Figures S13-14 for overweight/obesity risk and Figures S15-16 for zBMI. The frequency of interactions and the variability of strength attributed to each interaction across bootstrap samples is depicted with the raw shaded points and summarized with the means depicted with the white dot.

Overall, we observed larger uncertainty for interaction coefficients in Glinternet models than model contributions from GBM models. Detection frequency of interactions across bootstrap samples was higher among the findings from PUFA dataset (threshold set up 50%) than ANTIOX dataset (threshold set up 25%). Detailed graphical output of bivariate cross-section from BKMR can be found in Figures S9-12. For the present study we focused on interactions between POPs and nutrients; however, a list of POP-POP and nutrient-nutrient interactions were also automatically identified from GBM and Glinternet, and represented in the interaction network plots (Figures S5-8). In general, interactions between POPs and nutrients were weak and inconsistent across models, with presence of more but weaker interactions within the ANTIOX dataset, whereas in the PUFA dataset, we found less but stronger interactions.

In order to conduct a more refined analysis, ten interactions were discerned due to their largest strength/contribution in models, or because they were frequently detected across models (highlighted in bold in Table S5), as detailed in the prioritization criteria. For instance, the

interaction between HCB and vitamin B12 showed the largest contribution in the GBM models for overweight/obesity risk but also graphically suggested in the BKMR model (Table S5). Similarly, but to lesser extent, the interaction between β-HCH and vitamin B12 was found in the ANTIOX dataset (Table 2). The pesticide β-HCH appeared to interact with folate, recurrently detected by GBM in the ANTIOX dataset and Glinternet in the PUFA dataset (Table S5). In turn, β-HCH also showed frequently detected interactions with ALA and LA (Table S5). The interaction between PCB138 and LA also exhibited the highest coefficient and largest detection rates in the Glinternet model for overweight/obesity risk. The interactions between PFOA and vitamin B12, and between PFOS and γ–tocopherol, β–cryptoxanthin, and retinol were also considered of priority interest based on detection frequency and/or strength (Table S5).

3.6. Characterization of interactions for selected pairs of POPs and nutrients

For the selected ten interactions, we further conducted a regression analysis using GAM models and supported by graphical summaries (interaction plots) in order to identify non-linearity and the direction of interactions. This visualization allowed for instance to identify the potential synergic effect of HCB and vitamin B12 (Figure 5A) and the potential protective effect of β-cryptoxanthin on PFOS (Figure 5B). To further characterize the impact of the identified interactions between OCs and vitamins, we used the dataset with complete data on OCs and vitamins (n=1,241) as depicted in Figure S1.

The regression analysis confirmed those effects, being the associations between HCB and childhood overweight/obesity risk and zBMI strengthened at higher levels of vitamin B12 (Table 3). For instance, the associations between HCB and overweight/obesity risk at tertile 2 of vitamin B12 showed a RR 95%CI of 1.31 (1.11-1.54), whereas at tertile 1 were close to the null 0.99 (0.85-1.14) (p_{int} =0.02). Those trends were also observed in the additive scale, with corresponding RERIs 95%CI of 0.11 (0.02-0.20) for tertile 2 and 0.12 (0.03-0.21) for tertile 3.

443 A similar but weaker trend was also observed for the interaction between β-HCH and vitamin
444 B12 (Table S6) and between β-HCH and folate (Table 3). In the latter case, despite being
445 statistically not significant at the multiplicative scale, a synergism was suggested in the additive
446 scale between β-HCH and tertile 2 folate with a RERI 95%CI of 0.11 (0.01-0.21).
447 Synergistic interactions were also noticed between PFOS and retinol on the associations with
448 overweight/risk and zBMI, yet at the limit of statistical significance (p_{int}=0.069) at the
449 multiplicative scale (Table 3). For example, at the highest concentration of retinol, the RR
450 95%CI of overweight/obesity of PFOS was 1.42 (0.96-2.11) for quartile 3 vs 1. Likewise, in
451 the additive scale the estimates supported the synergism with a RERI of 0.58 (0.11-1.05) for
452 the same contrast. Similar associations were also observed between PFOS and γ-tocopherol,
453 with a RERI of 0.60 (0.06-1.15) at highest levels of PFOS and γ-tocopherol.
454 In the opposite direction, a negative interaction was observed between PFOS and β-
455 cryptoxanthin. In this case, the associations between PFOS and overweight/obesity or zBMI,
456 were substantially higher at the lowest tertile of β-cryptoxanthin, reaching a RR 95%CI of 1.59
457 (1.02-2.05) for quartile 4 vs 1 of PFOS. Whereas the interaction was not statistically significant
458 in the multiplicative scale (p_{int}>0.1), the RERIs supported antagonisms in the additive scale
459 (Table 3).
460 Finally, the synergistic interactions suggested between POPs and PUFAs appeared to be mostly
461 weak and non-statistically significant in both scales (Table S6) with the exception of PCB138
462 and linoleic acid (LA) that showed a RERI 95% of 0.18 (0.02-0.33) in the third tertile.

463 **4. Discussion**

464 In the present study we have attempted to develop and apply a comprehensive statistical
465 framework to evaluate the mixture effect of prenatal exposure to POPs and nutrients on
466 childhood overweight/obesity. This approach, applied to the population-based birth cohort
467 study INMA, confirmed findings from previous studies in this cohort, highlighting the role of

prenatal exposure to HCB (Agay-Shay et al. 2015; Güil-Oumrait et al. 2021; Valvi et al. 2012) and β-HCH (Agay-Shay et al. 2015) on childhood obesity risk and providing evidence of a positive cumulative effect of the mixture of POPs and nutrients. Screening for interactions using advanced approaches highlighted a number of potential combinations. Conventional regression models allowed to translate those interactions in more meaningful effect estimates in terms of inferential interpretation. Among the ten POPs-nutrient interactions retained in the screening step, HCB x vitamin B12 was the most consistent across models and outcomes, and the regression models suggested a potential synergistic effect. Interaction between PFOS and β-cryptoxanthin suggested a protective effect of the antioxidant on overweight/obesity risk, with a higher risk associated with PFOS exposure only being observed at lower concentrations of β-cryptoxanthin.

Despite the raise of multipollutant modelling approaches, few studies have considered mixtures of biomarkers others than pollutants (Lazarevic et al. 2019). In fact, some dietary nutrients have the potential to counterbalance the effects of environmental pollutants, highlighting the interest of accounting them in the mixture model (Cano-Sancho et al. 2020; Cano-Sancho and Casas 2021). The mixture analysis allowed the identification of an unexpected synergistic effect of vitamin B12, strengthening the associations of HCB. Vitamin B12 is an essential hydrophilic vitamin mainly found in animal food products, being dairy products and meat the major contributors exhibiting a short half-life in the body (Obeid et al. 2019). Despite the lack of international consensus on the optimal levels during pregnancy, there is some agreement that the range between 220 and 850 pmol/L would be adequate (Sukumar et al. 2016). Thus, considering that median levels of vitamin B12 within our population were 350 pmol/L (Interquartile range 279-435 pmol/L) most population would fall within that optimal range, with only 12 participants exceeding the upper threshold. Considering that sources of vitamin B12 in humans is exogenous, the blood levels may be determined either by the dietary intake

493 or as result of their metabolism. Thus, we draw different hypothesis that could help to explain
494 the interaction with HCB on obesity risk. First, there is the possibility that vitamin B12 is
495 confounding the true effect of some other concomitant nutrients of animal origin (e.g. total fat,
496 saturated fat, branched amino acids), or specific food items contributing to poor diet quality
497 (e.g. meat) associated with childhood obesity (Chen et al. 2021; Fernández-Barrés et al. 2016).
498 In turn, some authors have suggested that meat could be a determinant of HCB intake (Gasull
499 et al. 2011); however, our previous analysis rule out that hypothesis in the INMA population
500 (Ibarluzea et al. 2011; Llop et al. 2010). The second hypothesis is the actual joint effects,
501 considering that vitamin B12 intake during pregnancy could also be due to supplementation,
502 often found in multivitamin complexes, as previously reported in the same cohort (Navarrete-
503 Muñoz et al. 2015). Despite the excessive use of supplements as upstream source of highest
504 levels of vitamins remains to be explored, several mechanistic hypotheses can be developed to
505 explain the synergistic effects with HCB on fetal metabolic programming and increasing the
506 risk of obesity later in childhood. We observed a similar trend for folate on the associations
507 between β-HCH and higher overweight/obesity risk and zBMI, being the associations
508 strengthened among women with higher levels of folate. Interestingly, studies in mice showed
509 that supplementation with high doses of folate during pregnancy was associated with offspring
510 metabolic disruption and obesity related phenotypes (Huang et al. 2014; Kintaka et al. 2020).
511 Modes of action supporting the joint effect of OCs and vitamin B12 or folate on overweight
512 risk can involve epigenetic programming (McKay et al. 2012; Ouidir et al. 2020). A maternal
513 intake of methyl-group donors (e.g. folates, vitamin B12) could also alter the DNA methylation
514 profiles of offspring's metabolic genes (Pauwels et al. 2017). In turn, HCB and vitamin B12
515 can both individually impact the metabolic programing of adipocytes during differentiation, or
516 their DNA methylation profiles (Bastos Sales et al. 2013; Green et al. 2016). Indeed, vitamin
517 B12 plays a crucial role in humans as a cofactor of methionine synthase, which is actively

involved in methionine biosynthesis via the re-methylation of total homocysteine. Interestingly, two enzymes involved on s-adenosyl methionine synthesis (phosphatidylethanolamine N-methyltransferase and glycine N-methyltransferase), are transcriptional target of the aryl hydrocarbon receptor (Kim et al. 2018), which is activated by HCB (Chiappini et al. 2022). Thus, we may hypothesize that a maternal intake of methyl-group donors (i.e. vitamin B12), together with a higher HCB exposure contribute to an increased lipogenesis. Considering the active research to establish more accurate recommendations and thresholds of vitamin supplementation during pregnancy (Maruvada et al. 2020), future studies should consider the concomitant presence of environmental pollutants. A third hypothesis could be developed around the fact that higher levels of vitamin B12 may also reflect a metabolic alteration of one-carbon metabolism, as shown in some hepatic disorders (Ermens et al. 2003). Under this scenario, our findings could reflect an effect of HCB on maternal mitochondrial dysfunction (Park et al. 2021), having a direct impact on one-carbon metabolism pathways due to their coupling to the respiratory chains (Bao et al. 2016). This can be manifested by an alteration of cell uptake and utilization of vitamins resulting in imbalanced blood levels of vitamin B12 (Lyon et al. 2020). High levels of vitamin B12 and folate among pregnant women have been associated with metabolic disruption and gestational diabetes probably due to a mild liver dysfunction (Chen et al. 2021).

The protective effect of β-cryptoxanthin on the association between PFOS and childhood obesity also deserve attention. β-cryptoxanthin is a naturally occurring carotenoid. It is found in many foods of plant and animal origin (e.g. oranges, apples, egg yolk). It is closely related to ß-carotene and has antioxidant properties. Conversely, PFOS is known for its prooxidative activity (Chen et al. 2014) and increase adipogenesis in vitro (Modaresi et al. 2022), but epidemiological studies are globally inconsistent. A study in the same INMA cohort showed mild or null associations (Manzano-Salgado et al. 2017); other previous studies have generally

shown inconsistent findings (Lee et al. 2021) and even proposed as anti-obesogens by some authors (Di Gregorio et al. 2019). For the first time, we were able to observe a higher risk of obesity among children exposed to higher levels of PFOS and lower levels of this antioxidant during gestation. Current evidence with adult women has shown that concentrations of carotenoids, including β-cryptoxanthin, are inversely associated with BMI and waist circumference, with major effect modification by exposure to toxicants like smoking (Kabat et al. 2016). In experimental studies, β-cryptoxanthin exerted and anti-obesogenic effect reducing the body fat of mice and increasing the expression of uncoupling protein 1 (UCP1) in adipose tissue via the retinoic acid receptor (RAR) (Hara et al. 2019). In turn, PFOA and PFOS has been shown also to activate UCP1 in brown adipose tissue, which can modulate the food intake and body weight (Di Gregorio et al. 2019), but our findings suggest the presence of other potential mechanisms to explain the obesogenic effects of PFOS. For instance, PFOS and PFOA are activators of the Peroxisome Proliferator-Activated Receptor-alpha (PPAR-α) in humans. PPAR-α and RAR share a common dimerization partner, the Retinoid X Receptor (RXR). The activation of both receptors (PPAR-α by PFOS) and (RAR by ß-cryptoxanthin) could lead to a competitive effect towards this partner (RXR).

Biomonitoring studies during perinatal periods supports the fact that women are exposed to multiple environmental chemicals during pregnancy and lactation periods, as critical windows for offspring development (Cano-Sancho et al. 2020; Haug et al. 2018). This exposure paradigm has stimulated the increasing interest in characterizing the joint effect of environmental chemicals during pregnancy on offspring's health outcomes, raising the development and implementation of statistical approaches to address mixture related questions (Lazarevic et al. 2019). Whereas the apparel of algorithms and statistical methods has been growing during the last few years, there is no specific method consistently outperforming the others as assessed in simulation studies (Agier et al. 2016; Barrera-Gómez et al. 2017;

568 Lazarevic et al. 2020). For this reason, we have conceived an approach combining multiple
569 models in order to strengthen the robustness of our findings supported by the specific features
570 from each algorithm. The method selection included Glinternet, GBM, and BKMR, based on
571 previous literature supporting their relatively high statistical performance and capacity to
572 characterize the joint associations of correlated variables accounting for their interactions
573 (Barrera-Gómez et al. 2017; Lampa et al. 2014). A simulation study showed that BKMR in
574 case of non-monotonic exposure-response relationships, may outperform penalized regression
575 methods that assume linearity (Lazarevic et al. 2020), a group of methods that includes
576 Glinternet. Identifying relevant components of the mixtures remains a major question in terms
577 of public health but also regulatory decision-making. Statistically, this is commonly
578 accomplished by using variable selection, a process that becomes specially challenging as
579 correlation between variables increases (Lenters and Vermeulen 2018). Whereas data-driven
580 approaches such as BKMR may improve the predictive performance of models, those may fail
581 to attribute the true effect to right candidates within the correlated cluster (Braun et al. 2016).
582 Probably, this fact together with the different nature of variable selection method (e.g.
583 Glinternet and GBM), may help to explain some inconsistencies in the variable importance
584 rankings between models. An alternative way to leverage this issue would be to benefit of *a*
585 *priori* toxicological knowledge to inform the variable selection process, especially in
586 exploratory contexts where improving predictive performance falls out of scope. Selection of
587 relevant interactions follows a similar process than main effects, based on likelihood
588 penalization in case of Glinternet or 'spike-and-slab' priors in case of BKMR (Bobb et al. 2015;
589 Lim and Hastie 2015). We noticed that detection of interactions becomes specially challenging
590 and inconsistent between models when the interaction is weak (Brookes et al. 2004), supported
591 by the fact that low powered studies prone to false positive detection (Christley 2010). For that
592 reason, the agreement criteria across screening methods could be a solution to attenuate the

false positive as observed in the case of the strongest interaction between HCB and vitamin B12. In order to increase the robustness of findings from Glinternet and GBM we applied a bootstrapping approach with 100 replicates, allowing the identification of interactions in terms of frequency of detection and relative contribution or strength. The findings also highlighted the presence of other interactions (i.e. pollutant:pollutant or nutrient:nutrient) not discussed in the present manuscript, which may help to illustrate the complex interplay of chemicals within the mixture.

The present study should be considered with caution in the light of some study limitations. First, the sample size is relatively small (n=558-1241) for the exploration and characterization of interactions, which might have resulted in low power to detect interacting effects. Second, we applied a data-driven approach to explore potential interactions with biological meaning. The high correlation between some pollutants and our lack of congener-specific knowledge about their obesogenic potential, may increase the risk of exposure misclassification, thus attributing the interactive effect to the wrong chemical within the clusters of highly correlated variables. Current *in vitro* and *in vivo* studies about obesogenic effects of POPs are relatively limited to few congeners, which in turn, can be highly correlated in biological matrices. For simplicity and due to the limited sample size, we have focused the study to characterize 2-way interactions; however, higher order interactions cannot be neglected, either between pollutants and nutrients but also, with other individual characteristics like maternal smoking or child's sex, as previously observed (Casas et al. 2015). We may have also failed to accurately measure vitamin levels representative of all pregnancy as these nutrient biomarkers are reflecting the current intakes or relatively short time-frames (Burri et al. 2001; Gregory et al. 1998). However, for POPs, a single spot blood measurement is considered to be indicative of long-term exposure, due to their long elimination half-lives as proved also in the INMA cohort (Lopez-Espinosa et al. 2016; Manzano-Salgado et al. 2015). The exploration of interactions is

an emerging and active field of methodological research, and other novel approaches accommodating the complexities of pollutant datasets could be considered in future studies (Ferrari and Dunson 2020, 2021). Finally, the biological interpretation of statistical interactions should be considered with caution in part due to the different implication of interaction scales or the definitions used in different fields (Howard and Webster 2013). Following current recommendations, we reported the interactions in multiplicative and additive scale (Knol and VanderWeele 2012), and we believe that our findings may help to develop biological hypothesis for future toxicological studies and better interpret inconsistent findings in epidemiological studies.

To sum up, the present study supports the hypothesis that nutritional status during pregnancy can modify the effect of environmental pollutants on child health. Specifically, we have found that high levels of vitamin B12 may strengthen the associations between prenatal exposure to HCB and childhood obesity. In the opposite direction, the dietary antioxidant β-cryptoxanthin might have a protective effect against the obesogenic effects of PFOS. Our findings suggest that independent models may fail to identify weak interactions between pollutants and nutrients; thus combining complementary models may be a more powerful approach to consider. In the light of the public health implications of these findings, further observational and experimental research will be required for confirmation and gaining insight on the complex interplay between pollutants and nutrients during pregnancy on the metabolic programing of the offspring. As highlighted, these interactions may uncover sub-populations at risk for specific chemicals under regulatory policies; but also support more accurate nutritional guidelines during pregnancy.

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Table 1. Distribution (median [25th – 75th percentile]) of persistent organic pollutants and nutrients in maternal blood for each dataset.

Maternal Biomarkers (units)	ANTIOX N=660	PUFA N=558
Organochlorine Compounds		
HCB (ng/g lipid)	55.2 [31.3 - 91.0]	39.0 [29.2 - 78.7]
β-HCH (ng/g lipid)	23.6 [6.71 - 35.1]	30.5 [15.3 - 38.4]
p,p'-DDE (ng/g lipid)	134.0 [85.5 - 234]	122.0 [79.7 - 202]
PCB138 (ng/g lipid)	33.5 [23.5 - 45.6]	25.5 [16.0 - 37.6]
PCB153 (ng/g lipid)	52.5 [37.3 - 71.6]	42.9 [29.0 - 61.8]
PCB180 (ng/g lipid)	39.2 [26.7 - 55.6]	30.2 [20.2 - 43.1]
Per- and polyfluoroalkyl substances		
PFOA (ng/mL)	2.10 [1.47 - 2.91]	-
PFOS (ng/mL)	5.88 [4.49 - 7.66]	-
PFHxS (ng/mL)	0.483 [0.37 - 0.66]	-
PFNA (ng/mL)	0.582 [0.43 - 0.76]	-
Vitamins		
Vitamin B12 (pmol/L)	397 [310 - 519]	341 [271 - 428]
Vitamin D (mmol/L)	30.3 [22.9 - 37.6]	29.7 [21.2 - 37.5]
Folate (mmol/L)	17.9 [12.7 - 25.7]	14.2 [9.70 - 23.1]
Poly-unsaturated fatty acids		
LA (% of fatty acids)	-	30.9 [25.2 - 34.2]
ALA (% of fatty acids)	-	0.3 [0.2 - 0.3]
EPA (% of fatty acids)	-	0.3 [0.2 - 0.5]
DHA (% of fatty acids)	-	2.9 [2.3 - 4.1]
AA (% of fatty acids)	-	8.0 [6.8 - 9.6]
Carotenoids		
γ-tocopherol (μmol/L)	1.49 [1.24 - 1.87]	-
α-tocopherol (μmol/L)	30.9 [26.1 - 35.6]	-
β-cryptoxanthin (μmol/L)	0.17 [0.111 - 0.25]	-
α-carotene (μmol/L)	0.11 [0.07 - 0.18]	-
β-carotene (μmol/L)	0.278 [0.17 - 0.43]	-
Lutein (μmol/L)	0.22 [0.17 - 0.28]	-
Lycopene (μmol/L)	0.408 [0.23 - 0.77]	-
Zeaxanthin (μmol/L)	0.06 [0.05 - 0.08]	-
Retinol (μmol/L)	1.95 [1.52 - 2.55]	-

Abbreviations: AA, arachidonic acid; ALA, alpha linolenic acid; β-HCH, β-hexachlorocyclohexane; DHA, docosahexaenoic acid; EPA, eicosopentaenoic acid; Folate, Folic acid; HCB, hexachlorobenzene; LA, linoleic acid; PCB138, 2,2',3,4,4',5'-hexachlorobiphenyl; PCB153, 2,2',4,4',5,5'-hexachlorobiphenyl; PCB180, 2,2',3,4,4',5,5'-Heptachlorobiphenyl; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; PFNA, perfluorononanoate; PFHxS, perfluorohexane sulfonate; *p,p'*-DDE, dichlorodiphenyldichloroethylene.

Table 2. Summary estimates for the associations between selected POPs across tertiles of nutrients with obesity outcomes from Poisson/lineal regression models with a cross-product interaction term. Additive interactions on overweight/obesity risk are depicted by the relative excess risk due to interaction (RERI) and respective 95% confidence intervals (95%CI). All models were adjusted for maternal age, pre-pregnancy body mass index, smoking during pregnancy, education and region of residence, in addition models on overweight/obesity risk were further adjusted for child sex and age.

		Overweight/obesity					zBMI		
POP	***Nutrient tertiles***	***RR***	***95% CI***	p_{int}	***RERI***	***95% CI***	β	***95% CI***	p_{int}
HCB (log) [a]	Vitamin B12 (<302 pmol/L)	0.99	(0.85; 1.14)	0.022			-0.07	(-0.21; 0.07)	0.002
	Vitamin B12 (302-339 pmol/L)	1.31	(1.11; 1.54)		0.11	(0.02; 0.20)	0.26	(0.12; 0.40)	
	Vitamin B12 (>339 pmol/L)	1.21	(1.06; 1.37)		0.12	(0.03; 0.21)	0.16	(0.05; 0.28)	
β-HCH (log) [a]	Folate (<11.4 mmol/dl)	1.00	(0.88; 1.15)	0.260			0.00	(-0.12; 0.12)	0.110
	Folate (11.4-19.0 mmol/dl)	1.16	(1.02; 1.32)		0.11	(0.01; 0.21)	0.16	(0.06; 0.27)	
	Folate (>19.0 mmol/dl)	1.13	(1.02; 1.25)		0.09	(-0.02; 0.20)	0.12	(0.03; 0.21)	
PFOS Q2 [b]	Retinol (<1.7 μmol/L)	0.90	(0.59; 1.38)	0.069			-0.34	(-0.80; 0.13)	0.076
Q3		0.77	(0.48; 1.22)				-0.26	(-0.73; 0.20)	
Q4		0.97	(0.63; 1.49)				-0.13	(-0.59; 0.33)	
PFOS Q2	Retinol (1.7-2.3 μmol/L)	1.20	(0.83; 1.74)		0.12	(-0.23; 0.81)	0.37	(-0.05; 0.78)	
Q3		1.09	(0.74; 1.61)		0.32	(-0.19; 0.84)	0.26	(-0.17; 0.69)	
Q4		1.16	(0.79; 1.69)		0.18	(-0.36; 0.72)	0.23	(-0.19; 0.64)	
PFOS Q2	Retinol (>2.3 μmol/L)	0.62	(0.35; 1.10)		0.12	(-0.80; 0.38)	-0.30	(-0.72; 0.12)	
Q3		1.42	(0.96; 2.11)		0.58	(0.11; 1.05)	0.35	(-0.06; 0.76)	
Q4		1.10	(0.69; 1.74)		0.11	(-0.45; 0.67)	0.07	(-0.35; 0.49)	
PFOS Q2 [b]	β-cryptoxanthin (<0.1 μmol/L)	1.32	(0.82; 2.11)	0.244			0.11	(-0.30; 0.52)	0.367
Q3		1.34	(0.85; 2.13)				0.38	(-0.07; 0.82)	
Q4		1.59	(1.02; 2.50)				0.26	(-0.17; 0.69)	
PFOS Q2	β-cryptoxanthin (0.1-0.2 μmol/L)	0.79	(0.49; 1.28)		0.12	(-1.48; 0.30)	-0.02	(-0.45; 0.41)	
Q3		1.05	(0.70; 1.58)		-0.28	(-1.09; 0.54)	0.12	(-0.31; 0.55)	
Q4		1.12	(0.74; 1.69)		-0.44	(-1.32; 0.45)	0.28	(-0.14; 0.70)	
PFOS Q2	β-cryptoxanthin (>0.2 μmol/L)	0.75	(0.50; 1.11)		0.12	(-1.75; 0.20)	-0.32	(-0.77; 0.12)	
Q3		0.86	(0.60; 1.23)		-0.59	(-1.50; 0.32)	-0.19	(-0.61; 0.24)	
Q4		0.71	(0.47; 1.07)		-1.12	(-2.19; -0.05)	-0.35	(-0.79; 0.09)	
PCB138 (log) [c]	Linoleic acid (<27.6% fatty acids)	0.96	(0.75; 1.24)	0.297			-0.09	(-0.35; 0.16)	0.372
	Linoleic acid (27.6-33.4% fatty acids)	1.10	(0.86; 1.41)		0.10	(-0.13; 0.33)	0.03	(-0.21; 0.27)	
	Linoleic acid (>33.4% fatty acids)	1.25	(0.99; 1.58)		0.18	(0.02; 0.33)	0.14	(-0.08; 0.36)	

Abbreviations: $p_{int,}$ p-value from interaction testing; RERI, relative excess risk due to interaction; RR, adjusted relative risks; SD, Standard Deviation; zBMI, child body mass index z-score. Details of chemical abbreviations are provided in Table 1.

[a] Population sample size n=1241 (see details in the study flowchart in Figure S1)

[b] Population sample size n=660 (ANTIOX Dataset)

[c] Population sample size n=558 (PUFA Dataset)

FIGURE LEGENDS

Figure 1. Statistical Workflow. Abbreviations: BKMR; Bayesian Kernel Machine Regression; GAM, Generalized Additive Models; GBM, Gradient Boosting Machine; MLR, multivariate linear/Poisson regression analysis; relative excess risk due to interaction (RERI)

Figure 2. Spearman's correlation plots depicting the association strength between POPs and nutrients in the ANTIOX (Panel B, n=660) and PUFA (Panel A, n=558) datasets. Details of chemical abbreviations are provided in Table 1.

Figure 3. Associations between persistent organic pollutants and nutrients with childhood overweight/obesity within the ANTIOX dataset (n=660). The forest plots depict the associations between individual prenatal exposures (log scaled) and risk of childhood overweight/obesity (dashed panel). Summary estimates from single-biomarker models based on multivariate robust Poisson regression are depicted by adjusted relative risk (RR) and respective 95% confidence intervals (95% CI). Variable importance plots (non-dashed panels) depict the rank of variables based on their relative importance in multipollutant models using the absolute coefficients for Glinternet; model contribution for gradient boosting machine regression (GBM); and posterior inclusion probabilities (PIPs) for Bayesian kernel machine regression (BKMR). All models were adjusted for maternal age, pre-pregnancy body mass index, smoking during pregnancy, region of residence, education, child sex, and age. Details of chemical abbreviations are provided in Table 1.

Figure 4. Overall effect estimates from Bayesian kernel machine regression (BKMR) on the association between mixtures of POPs and nutrients and childhood overweight/obesity risk (Panel A) and body mass index z-score (zBMI) (panel B) for the mixtures of chemicals selected with the hierarchical procedure from ANTIOX dataset (gray) and PUFA dataset (black). Details of most relevant chemicals in the mixtures are depicted in Figures S9-S12. Graphs show the difference in the effect estimates when all exposures are at a particular quantile compared to when all are at the 10th quantile as reference. All models were adjusted for maternal age, pre-pregnancy body mass index, smoking during pregnancy, region of residence and education, child sex and age were also included in overweight/obesity models.

Figure 5. Interaction plots on the associations between hexachlorobenzene (HCB, log increase) and tertiles of vitamin B12 (Panel A) and between perfluorooctane sulfonate (PFOS, log increase) and tertiles of β-cryptoxanthin (Panel B) on overweight/obesity risk. All models were adjusted for maternal age, pre-pregnancy body mass index, smoking during pregnancy, region of residence, education, child sex and age.

Figure 1

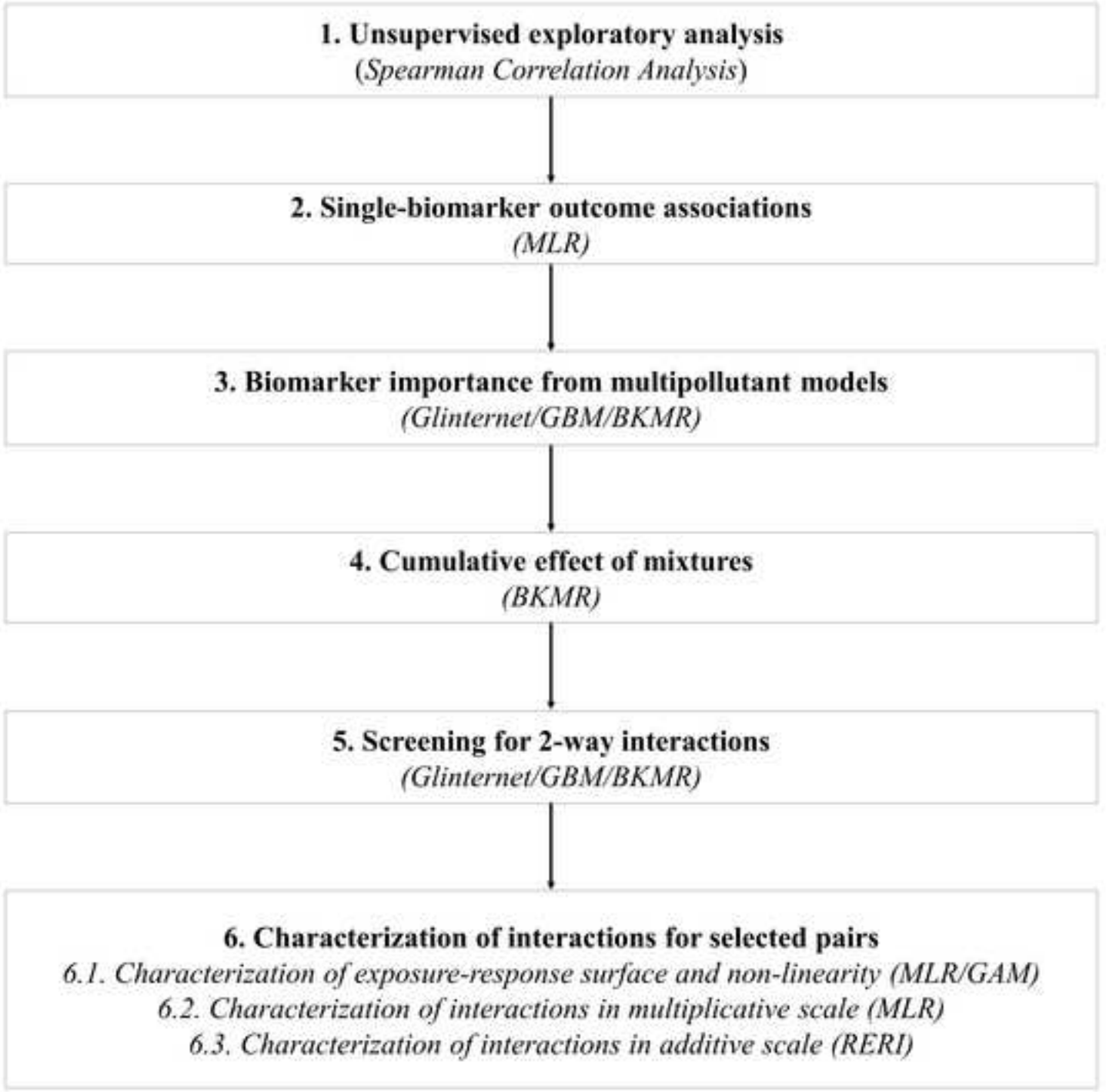

Figure 2

Click here to access/download;Figure;Fig 2. Corrplot.tif

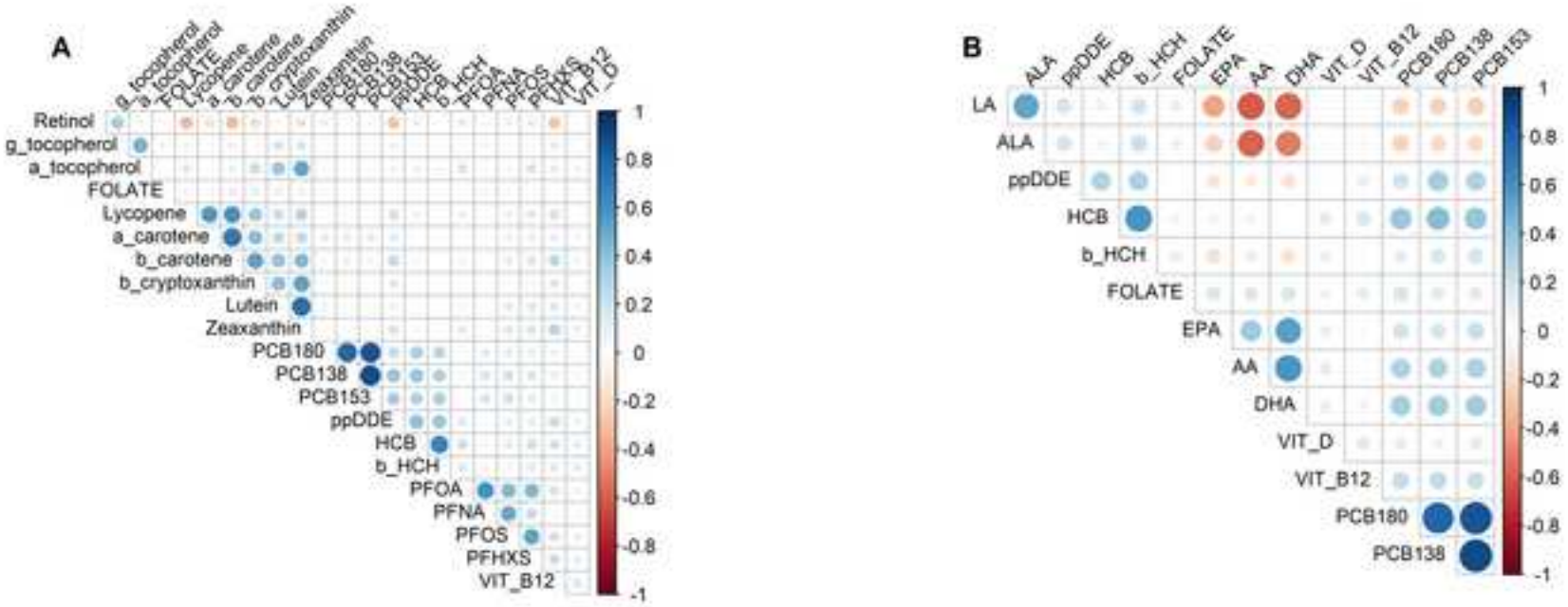

Figure 3

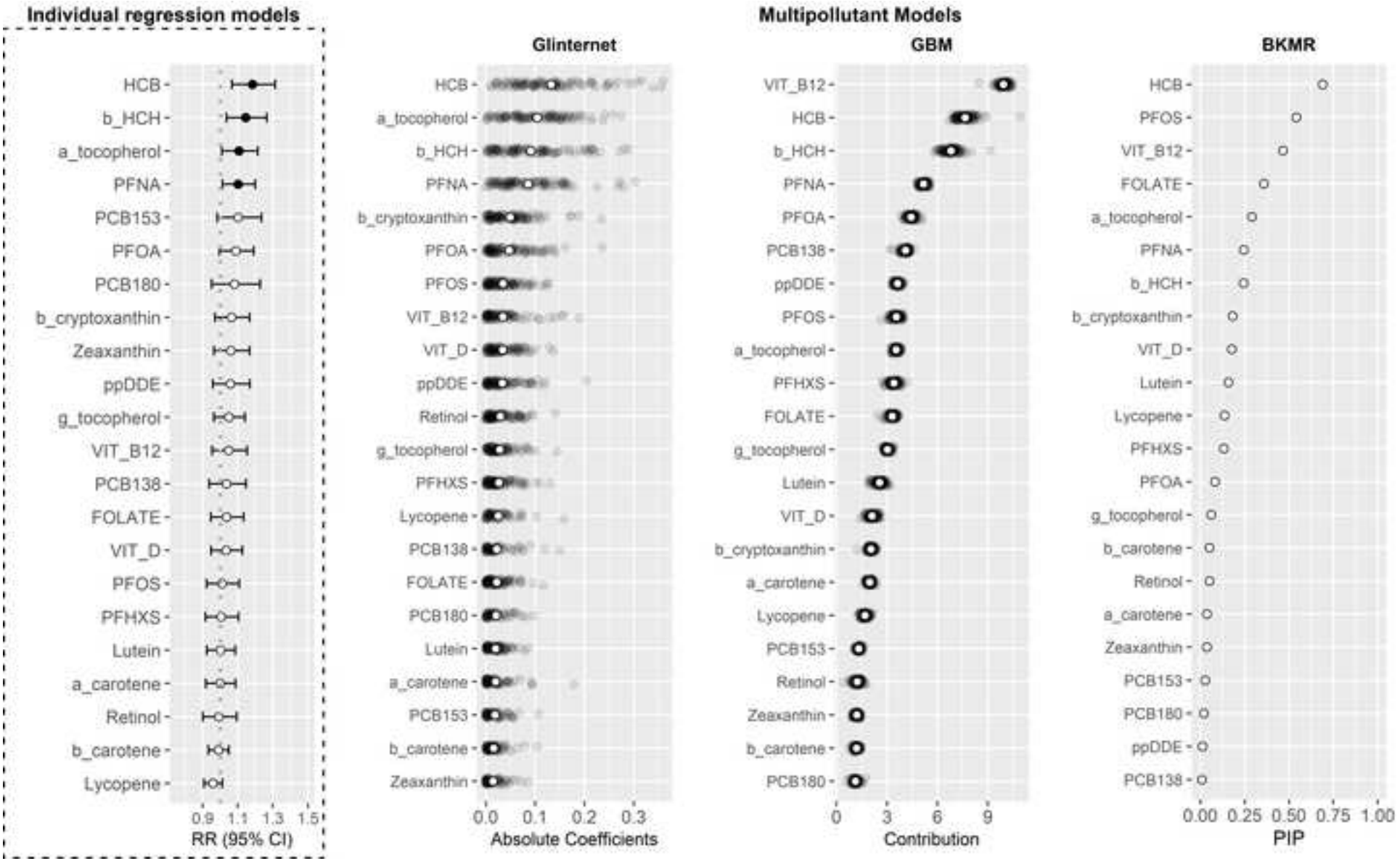

Figure 4

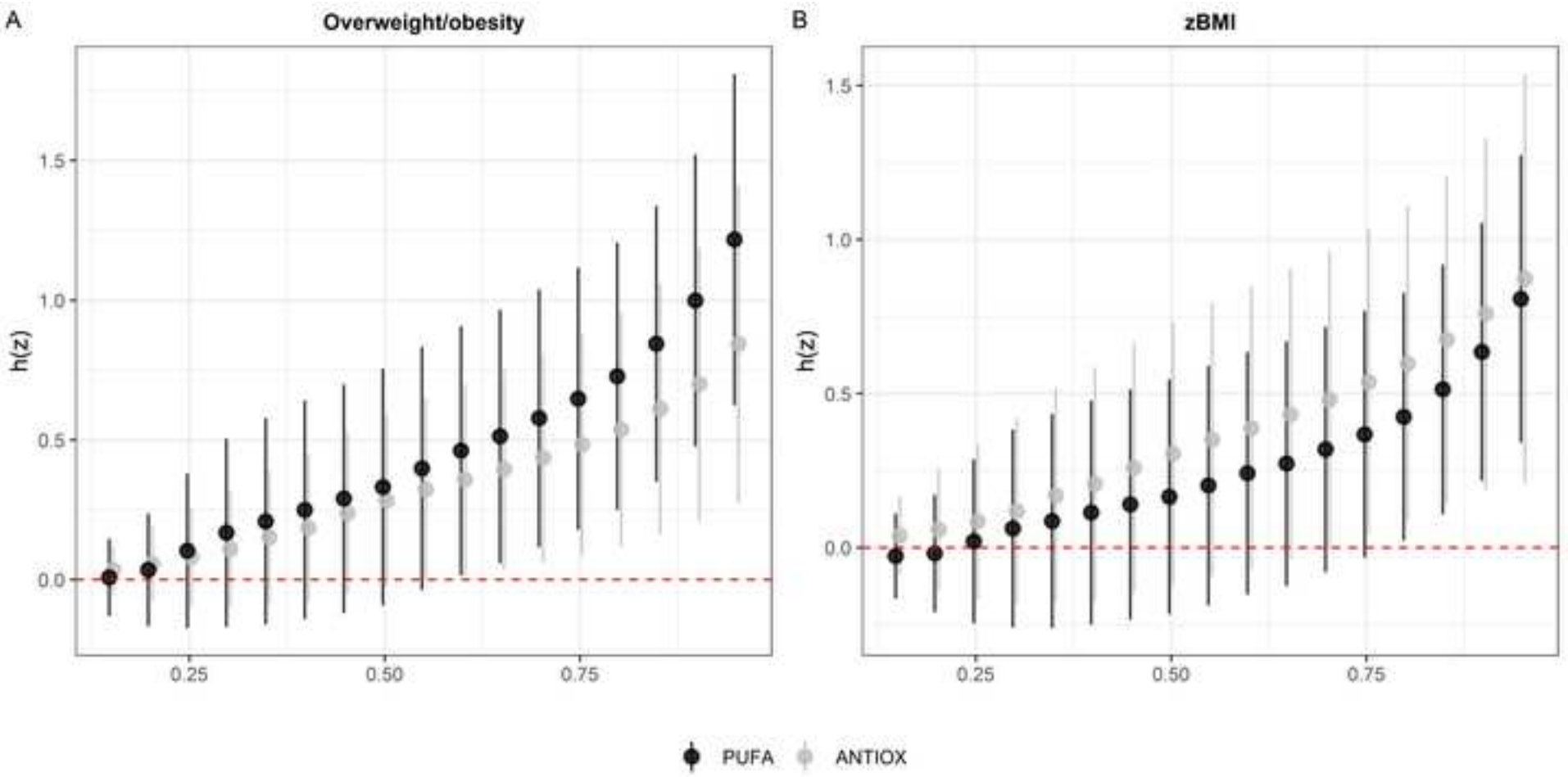

Figure 5