



Published in final edited form as:

Environ Int. 2025 January ; 195: 109206. doi:10.1016/j.envint.2024.109206.

Associations of early life per- and polyfluoroalkyl substances (PFAS) exposure with body mass index and risk of overweight or obesity at age 2–18 years: Mixture analysis in the prospective Boston Birth Cohort

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Abstract

Background: Per- and polyfluoroalkyl substances (PFAS) are a class of widespread persistent chemicals, which may have obesogenic effects during the fetal period. This study investigated the

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Declaration of competing interest

Dr. Braun has been compensated for services as an expert witness to plaintiffs involved in litigation related to PFAS-contaminated drinking water.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2024.109206>.

long-term association of maternal plasma PFAS concentrations at delivery and their mixture with child body mass index (BMI) and the risk of Overweight or Obesity (OWO) at the age of 2–18 years.

Methods: The study included 1189 mother–child dyads from the prospective Boston Birth Cohort. Eight PFAS were measured in maternal plasma samples collected 24–72 h after delivery. Outcomes were BMI Z-score and OWO status of children at 2–18 years. The exposure-outcome associations were evaluated with linear and modified Poisson mixed-effects regression for individual PFAS and Bayesian kernel machine regression and quantile-based g-computation models for PFAS mixture. We explored the effect modification by maternal pre-pregnancy OWO, child age, sex, and race.

Results: Maternal plasma samples had PFAS detection frequencies from 87 % to 100 % and geometric means ranging from 0.11 to 3.67 ng/mL. PFHpS and PFHxS were associated with higher child BMI Z-score. Such associations were stronger in children aged 6–12 years and 13–18 years than in 2–5 years. Stratified by maternal pre-pregnancy OWO, significant associations of the PFAS mixture with child BMI Z-score were only found in children of non-OWO mothers. In children aged 13–18 years, children with both high maternal plasma PFDeA, PFNA, and PFOA concentrations and maternal OWO had the highest risks of OWO compared to children with either only. Such synergistic effects were not found in younger children.

Conclusions: Early life exposure to individual PFAS and their mixture were associated with a higher risk of childhood OWO, with stronger associations observed in older child age groups and in children of non-OWO mothers. Synergistic effects of PFAS exposures and maternal pre-pregnancy OWO became evident in adolescence.

Keywords

PFAS; Childhood overweight or obesity; BMI; Maternal overweight or obesity; DOHaD theory; Prenatal exposure

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a class of manufactured chemicals that have been widely applied in fire extinguishers, industrial detergents, water- or oil-resistant materials, and food packaging (Pan et al., 2024). Due to their physicochemical properties, PFAS are persistent and widespread in the environment, leading to bioaccumulation and biomagnification in the food chain (Jain & Ducatman, 2019), and once in the human body, they remain for a long time (half-lives of 1.5–8.5 years) (Rosato et al., 2024). Although the use of some PFAS has been restricted since the early 2000s in the United States (US) (Buck et al., 2011), they are still widely detected in human blood, breastmilk, and other bio-samples (Iszatt et al., 2019; Starling et al., 2024), indicating ubiquitous health risk on the general population. PFAS have been broadly reported to have adverse effects on endocrine, immune, nervous, digestive, and reproductive systems (Fenton et al., 2021). Besides the effects on adults, PFAS can also cross the placental barrier (Wang et al., 2019), with implications for irreversible long-term health threats to fetuses.

Childhood Overweight or Obesity (OWO) has increased substantially over the past decades and become a worldwide health and social issue (Di Cesare et al., 2019). In the US, approximately one in five (19.7 %) youth aged 2–19 years had obesity from 2017 to 2020 (Hu & Staiano, 2022). The negative impacts of childhood OWO not only include physical and psychological disorders of children (Liu et al., 2023) but also track into adulthood. As suggested by the Developmental Origins of Health and Diseases (DOHaD) theory (Barker & Osmond, 1986), adverse health events in early life may lead to worse health status across the lifespan. Children with OWO have higher risks of hypertension, type-2 diabetes, musculoskeletal disorders, cardiovascular disease, and cancers in adulthood (Gurnani et al., 2015), causing health burdens on both individuals and society. Therefore, identifying and preventing risk factors of childhood OWO are of great public health significance.

As a class of endocrine-disrupting chemicals (EDC), PFAS have been reported to be a group of potential obesogens, whose effects begin in the fetal period (Nappi et al., 2016). However, the previous studies between *in-utero* PFAS exposure and childhood OWO yielded inconsistent findings. While two systematic reviews concluded no significant associations between prenatal exposure to PFAS and child body mass index (BMI) (Frangione et al., 2024; Stratakis et al., 2022), another *meta*-analysis including 26 studies found that child BMI had a positive relation with prenatal exposure to perfluorononanoic acid (PFNA) but negative with perfluorooctanesulfonic acid (PFOS) (Frigerio et al., 2023). The Environmental influences on Child Health Outcomes (ECHO) Program reported that maternal gestational plasma/serum 2-(N-Methyl-perfluorooctane sulfonamido) acetic acid (Me-PFOSA-AcOH), perfluorodecanoic acid (PFDeA), perfluorohexanesulfonic acid (PFHxS), and the PFAS mixture were associated with BMI Z-score and OWO status between the ages of 2–5 years (Liu et al., 2023). The Healthy Start longitudinal cohort (US) and Shanghai-Minhang Birth Cohort Study (China) both observed associations between maternal gestational serum/plasma PFNA and higher BMI of preschool-aged children (Starling et al., 2024; Sun et al., 2024). The Sheyang Mini Birth Cohort Study (SMBCS, China) found that cord serum PFAS mixture was associated with higher obesity risk in only female children at 10 years (Wang et al., 2024b). On the other hand, the Health Outcomes and Measures of the Environment (HOME) Study suggested that *in-utero* perfluorooctanoic acid (PFOA) exposure was linked to lower BMI in early childhood but higher BMI at the age of 12 years, while PFOS and PFHxS exposure was related to lower BMI across the first 12 years (Braun et al., 2021). Laizhou Wan (Bay) Birth Cohort (China) concluded that PFAS mixture in maternal serum collected three days before delivery was associated with decreased fat mass and body fat in children aged 7 years, while the negative associations remained in male children but reversed to positive associations in females (Zhang et al., 2022b).

The current study sought to address important data gaps and provide new insight into the heterogeneity of the associations of PFAS with child BMI/OWO across the published literature. Most existing studies only included children under 13 years, were conducted in predominantly white populations or outside the US, focused on a few legacy PFAS, and only a few studies investigated associations with the PFAS mixture. Based on the Boston Birth Cohort (BBC), a US, urban, low-income, racially diverse prospective birth cohort, the current study aimed to investigate associations of maternal plasma concentrations of 8 PFAS

at delivery with child BMI and the risk of OWO at the age of 2–18 years with both single and mixture analyses. Additionally, we investigated effect modifiers including age, sex, race, and maternal pre-pregnancy OWO.

2. Material and methods

2.1. Study participants

The current study was based on the BBC, a predominantly urban, low-income, Black prospective birth cohort study in the US, which has been registered on [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT03228875)(NCT03228875). The rationale and details of the study design were described in a previous publication (Pearson et al., 2022). Briefly, the BBC was initiated at Boston Medical Center (BMC) in 1998. Any woman who delivered a singleton live infant at BMC was eligible to be included. Research staff met with mothers 24–72 h after delivery to obtain written informed consent. Research staff administered a standardized postpartum questionnaire, collected a maternal venous blood sample, and abstracted electronic medical records. In a separate but linked Institutional Review Boards (IRB) protocol, the research team approached mother–child dyads during outpatient medical appointments at BMC to obtain consent for participation for longitudinal follow-up. Informed consent was obtained from the mother and the child at age-determined intervals as required by the BMC IRB. When children reached age 18 + years, they were reconsented as adults. A total of 3,416 mother–child dyads consented to be longitudinally followed and the median follow-up length was 14.5 years. Of those, 3,235 children had at least one height and weight measurement. In 2023, 1,333 maternal plasma samples were processed for PFAS measurement. Finally, we included 1,189 mother–child pairs who had both maternal plasma PFAS data and at least one child height and weight measure at the age of 2–18 years (Figure S1). The study protocol has been reviewed and approved by the IRB of the BMC and the Johns Hopkins Bloomberg School of Public Health.

2.2. PFAS measurement

Maternal venous blood samples were collected 24–72 h after delivery by trained research staff in ethylenediaminetetraacetic (EDTA) acid-coated test tubes, centrifuged within two hours of collection to separate plasma and red blood cells, and stored at –80 °C until measurement (Li et al., 2024; Zhang et al., 2022a).

In 2023, 1,333 maternal plasma samples, with the inclusion criteria of participants' enrollment in postnatal follow-ups and adequate volumes of plasma samples, were processed for PFAS measurement. The plasma samples were transported on dry ice to the Environmental and Chemical Laboratory Services (ECLS), Public Health and Environmental Laboratories (PHEL) at the New Jersey Department of Health (NJDOH), to measure 12 PFAS using a sensitive method optimized from a standard method of the Centers for Disease Control and Prevention (CDC) (#6304.04) (Yu et al., 2017). This method has been successfully employed in a statewide PFAS biomonitoring project for more than one thousand de-identified human samples (Yu et al., 2020). In the current study, we excluded 4 PFAS with a low detection frequency (range: 15 %-60 %) and included 8 PFAS with a detection frequency over 85 %: 1) Me-PFOSA-AcOH; 2) PFDeA; 3)

perfluoroheptanesulfonic acid (PFHpS); 4) PFHxS; 5) PFNA; 6) PFOA; 7) PFOS; 8) perfluoroundecanoic acid (PFUnA).

The detailed measurement methods of PFAS were described elsewhere (Graber et al., 2021; Li et al., 2024; Yu et al., 2017). In short, PFAS were measured using an online solid-phase extraction (SPE) unit (PICO, Spark Holland, Amsterdam, Netherlands) coupled to a high- or ultra-high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS SCIEX QTRAP™ 6500 or UHPLC-MS/MS SCIEX QTRAP™ 7500). 50 µL of plasma, spiked with 25 µL of mass-labeled internal standards and diluted to 600 µL with 0.1 M formic acid, were loaded onto the online SPE cartridge for the 6500 (Polaris C8-SE, 2 mm x 10 mm x 8µm, PEEK, bar) and for the 7500 (CHROspe Polymer Divinylbenzene, 25–35 µm, 10x1 mm ID, PEEK, 1000 bar) for extraction. The extract flowed onto the protective guard column (Agilent XDB-C₈, 4.6 mm x 12.5 mm x 5 µm) for the 6500 and (Agilent Zorbax SB-C18, 2.1 mm x 5 mm x 1.8 µm) for the 7500 to remove any impurities from reaching the analytical column. This extract then finally flowed onto the analytical column for the 6500 (Agilent Eclipse XDB C₈, 3 mm x 100 mm x 3.5 µm) and the 7500 (Agilent ZORBAX SB-C18 RRHD, 2.1 mm x 150 mm, 1.8 µm) for separation. Target analytes were eluted using an aqueous solution mobile phase A, for the 6500 (buffered with 10 mM NH₄Ac / ACN, 95:5, pH of 4.30 +/- 0.05, filtered with 0.2 µm nylon filter) and for the 7500 (buffered with 10 mM NH₄Ac / ACN, 95:5, pH = 4.00 ± 0.05, filtered with 0.2 µm nylon filter). The organic solution mobile phase B for the 6500 (ACN and Methanol, 1:1) and 7500 (ACN) was then identified and quantified by tandem mass spectrometry using the scheduled multiple reaction monitoring (SMRM) mode. The precursor and product ions were monitored to identify and quantify the target PFAS. Besides, additional product ions were used for PFHxS and PFOS for further confirmation of each species, as bile salts present in biological samples are known to interfere with proper quantification.

The analytical laboratory has maintained proficiency in the CDC's Biomonitoring Quality Assurance Support Program (BQASP) or the Arctic Monitoring and Assessment Program (AMAP) Ring Test for Persistent Organic Pollutants in Human Serum since 2016, achieving a 100 % score on all proficiency tests. All sample analyses and data underwent rigorous NJDOH Quality Assurance / Quality Control (QA/QC) procedures, including blank and QC checks for each analytical batch, and validation against established protocols prior to reporting. The limits of detection (LODs) for PFAS ranged from 0.005 ng/mL to 0.061 ng/mL, with corresponding limits of quantification (LOQs) from 0.015 ng/mL to 0.182 ng/mL. Routine correlations were performed to assess that both the 6500 and 7500 LC-MS/MS instruments are producing similar results.

2.3. Child BMI Z-score and OWO measurement

Child height and weight data were extracted from the electronic medical records, which were measured with light clothes and without shoes, and documented by trained medical staff with standard clinical protocols and equipment during child clinic visits. To date, the BBC has recorded 60,676 measures of height and body weight from 3,235 children. Each child had a median of 16 (interquartile range: 9, 24) measures. As BBC recruited participants in a rolling process, most of the children are still under 18 years; some

participants could only be followed until 18 years old unless they reconsented themselves. Therefore, only 49 of 3,235 children reached 18 years and had measurements after 18 years, of whom only 4 also had maternal PFAS data. We dropped the data collected after the age of 18 years to address potential selection bias. Child BMI was calculated as the child's body weight (kg) divided by the child's height (m). BMI Z-score and percentile were standardized by sex and age according to the US national reference values from the CDC (CDC, 2023). As this reference for calculating a child's BMI Z-score and percentile begins at age 2 years, we restricted our age range to 2–18 years. We categorized children as OWO if the BMI percentile was ≥ 85 percentile.

2.4. Covariates

Information on covariates was extracted from medical records and the maternal postpartum questionnaire of BBC. We incorporated covariates based on confounders included in previous studies and directed acyclic graphs (DAGs; Figure S2). The following covariates were included in the fully adjusted model: maternal age at delivery (years), maternal educational level (high school or below versus college or above), maternal race (Black versus non-Black), maternal pre-pregnancy BMI (kg/m^2), maternal diabetes (either Gestational Diabetes Mellitus or Diabetes Mellitus), child's biological sex (male versus female), birthweight-for-gestational age [appropriate for gestational age (AGA), small for gestational age (SGA), or large for gestational age (LGA)], year of birth (1999–2006 versus 2007–2016), delivery mode (vaginal delivery versus cesarean section), and parity (nulliparous versus multiparous). Maternal pre-pregnancy BMI was calculated as maternal pre-pregnancy body weight (kg) divided by maternal height (m), which were both obtained from the maternal postpartum questionnaire. Maternal pre-pregnancy OWO was defined as a pre-pregnancy BMI $\geq 25 \text{ kg}/\text{m}^2$. Maternal diabetes and birthweight-for-gestational age were adjusted in all main models because they are strongly associated with childhood obesity. We adjusted for the year of birth because our samples were collected over a long period during which the PFAS exposure levels may change. The variable of year of birth was included as a binary variable cut by 2006, because eight major companies in the PFAS industry in the US agreed to phase out PFOA and PFOA-related production in 2006, before when PFOS was phased out in 2002 (Viberg & Eriksson, 2011). We observed a turning point around 2006 on the smooth plots between birth year and maternal plasma PFAS concentrations. Previous studies also suggested significant changes in population exposure levels around 2006 (Pan et al., 2024). We adjusted for delivery mode since more blood loss during cesarean section may result in lower PFAS concentrations in maternal plasma at delivery (Ansari et al., 2022; Khan et al., 2006). Preterm birth was not included as a covariate because we did not observe a significant difference in gestational age or proportion of preterm between children with or without childhood OWO (Table 1), and preterm is more likely a potential mediator than a confounder. We conducted multivariate imputation by chained equations (*mice* package for R) for missing values of covariates (fewer than 5 %).

2.5. Statistical analysis

Mean, standard deviation, frequency, and percentages were applied to describe the general characteristics of included mother–child pairs. Characteristics were described among all children, children with childhood OWO, and children without OWO. We compared the

characteristics between included and excluded mother–child dyads to check for potential selection bias. The distribution of PFAS in maternal plasma samples at delivery was described with geometric mean, minimum, maximum, and quartiles. The correlation among PFAS was tested with Spearman’s rank order correlation coefficients. The concentrations of PFAS below the LODs were imputed with $\text{LOD}/\sqrt{2}$.

To evaluate associations between individual PFAS and repeated measures of child BMI Z-score and child OWO status, we conducted linear and modified Poisson mixed-effects models adjusting for covariates. Log2-transformed PFAS concentrations were included as exposure variables. To address the predominant effect of maternal OWO on childhood OWO suggested by our previous work (Si et al., 2023), we conducted stratified analyses by maternal OWO. Also, we combined maternal OWO (no versus yes) with quartiles (Q1–Q3 versus Q4) of each PFAS as a 4-level categorical variable as the exposure, to evaluate the joint association of PFAS with maternal OWO on child BMI Z-score and OWO status. To explore other effect modifiers, we conducted secondary analyses stratified by child age group, sex, and race. To further examine the age-dependent effects of PFAS, we used linear spline mixed-effects models to predict child BMI trajectories at 2–18 years stratified by quartiles of maternal PFAS concentrations. We created child age splines with 2 knots at 6 and 13 years and fixed the other covariates at their means in the trajectory analysis.

We conducted mixture analyses to investigate associations between the PFAS mixture and the last measure of child BMI Z-score and OWO. We used Bayesian kernel machine regression (BKMR) models (*bkmr* package for R) with a Markov Chain Monte Carlo (MCMC) algorithm of 50,000 iterations (Bobb et al., 2015). We tested the convergence of models with effective sample size for bulk and tail quantities and potential scale reduction factor on rank normalized split chains. Log2-transformed PFAS were set as exposure variables to examine their relations with either the child BMI Z-score or the child OWO status. Models among all children, children with maternal OWO, and children without maternal OWO were fitted. Besides, we further fitted BKMR with 8 PFAS and maternal pre-pregnancy BMI as exposure and examined bivariate exposure–effect relations to investigate the potential effect modification of maternal OWO. Additionally, we conducted quantile-based g-computation models (*qgcomp* package for R) (Keil et al., 2020). Analyses were run using package default settings to estimate associations of the PFAS mixture and two outcomes, overall and stratified by maternal OWO. We used *qgcompint* package for R to assess if the effects of the PFAS mixture on child BMI Z-score and child OWO varies by maternal OWO (Keil et al., 2020). While BKMR uses Gaussian process regression to fit a flexible, nonparametric function to estimate the individual, joint, and cumulative effects of the mixture allowing for non-linearity and interactions among mixture components, quantile-based g-computation provides a more interpretable, single dose–response parameter for the association and has increased statistical power compared to BKMR. Therefore, the application of both models complements each other and helps cross check the robustness of results.

We conducted sensitivity analyses as follows: First, as diet is the primary source of PFAS exposure in most populations (especially fish intake) (Wang et al., 2024a), we further adjusted for fish intake during pregnancy and Mediterranean Diet Score (Che et al., 2023).

Second, to address potential concerns that maternal diabetes and birthweight-for-gestational age could be causal intermediates between prenatal PFAS exposures and childhood obesity, we explored the associations excluding these two variables as confounders. Third, we examined if maternal smoking during pregnancy could affect PFAS-OWO association, since our previous study found that maternal smoking may increase childhood risk of OWO (Hou et al., 2022). Fourth, we additionally adjusted for breastfeeding as breastmilk is a potential source of elevated PFAS exposures in infancy (Zheng et al., 2021).

We conducted the statistical analyses using R version 4.4.0 (R Core Team, 2024), RStudio 2024.04.0 (Posit team, 2024), and Stata version 18.0 (StataCorp, 2023).

3. Results

The average delivery age of included mothers was 28.8 ± 6.5 years, 59 % were Black, 65 % received education of high school or lower, and 54 % had pre-pregnancy OWO (Table 1). Of the children, 49 % were male and 38 % were born with cesarean section. Compared with normal-weight children, OWO children were more likely born to mothers who were older at delivery, had OWO before pregnancy, had diabetes, and delivered via cesarean section. Compared to all participants in the BBC, although the included participants had a lower proportion of preterm (23 % versus 29 %), the other characteristics were comparable (Table S1). The numbers of children with at least one BMI measured at 2–5 years, 6–12 years, and 13–18 years were 1,136, 964, and 472, respectively (Figure S1).

The concentration distributions of PFAS in maternal plasma are reported in Table 2. The detection frequencies of PFAS in study participants ranged from 87.22 % (Me-PFOSA-AcOH) to 100 % (PFNA and PFOS). Among 8 PFAS, PFOS had the highest geometric mean of 3.67 ng/mL, while Me-PFOSA-AcOH and PFHpS had the lowest geometric mean of 0.11 ng/mL. The Spearman correlation coefficients among PFAS ranged from 0.02 (Me-PFOSA-AcOH with PFUnA) to 0.80 (PFDeA with PFUnA) (Figure S3).

Fig. 1 and Table S2 present the associations between individual PFAS and repeated measures of child BMI Z-score and OWO examined by linear and modified Poisson mixed-effects models. Maternal plasma PFHxS concentrations were associated with higher child BMI Z-score ($\beta = 0.07$; 95 % CI: 0.02, 0.12). Stratified by child age group, the associations between PFHxS and child BMI Z-score were stronger in older child age groups (2–5 years: $\beta = 0.04$; 95 % CI: -0.01, 0.09; 6–12 years: $\beta = 0.08$; 95 % CI: 0.03, 0.13; 13–18 years: $\beta = 0.09$; 95 % CI: 0.02, 0.15). Similar positive associations and age-dependent associations were also observed between PFHpS and child BMI Z-score. We did not observe a significant difference in child BMI trajectories by maternal plasma PFAS concentration quartiles (Figure S4). PFDeA concentration was associated with a 10 % (95 % CI: 1.00, 1.20) higher risk of child OWO (Table S2).

Fig. 2 shows the associations between the PFAS mixture and the last measure of child BMI Z-score and OWO. In overall models of BKMR and quantile-based g-computation, the PFAS mixture was not significantly associated with child BMI Z-score and OWO. However, after stratifying by maternal OWO in quantile-based g-computation models, the

PFAS mixture was associated with higher child BMI Z-score in children of non-OWO mothers only ($\beta = 0.21$; 95 % CI: 0.05, 0.36) (Table S4). The effect measure modification *P* value of maternal OWO between PFAS and child BMI Z-score was 0.03 (Table S4). The bivariate exposure-effect relations between PFAS and maternal pre-pregnancy BMI in BKMR also suggested the modifying role of maternal OWO (Figure S5). In models of individual PFAS, associations of Me-PFOSA-AcOH, PFDeA, and PFHxS with child BMI Z-score or OWO were also stronger in children of non-OWO mothers (Table S2).

Fig. 3 and Table S7–S10 report the joint effects of maternal PFAS and maternal OWO. In overall models (Table S7), compared to the reference group of children with PFAS in Q1–Q3 and non-OWO mothers, children with high maternal PFNA concentrations only (RR = 1.41; 95 % CI: 1.06, 1.87) and children with maternal OWO only (RR = 1.67; 95 % CI: 1.40, 1.99) both had higher risks of child OWO, and those with both high maternal PFNA concentrations and maternal OWO showed the highest risk of child OWO (RR = 1.99; 95 % CI: 1.54, 2.56). Notably, synergistic joint effects of PFDeA, PFNA, and PFOA with maternal OWO were observed in the age group of 13–18 years but not in younger children (Fig. 3).

Secondary analyses stratified by sex and race are reported in Table S2, Table S9, and Table S10. Stratified by child sex, Me-PFOSA-AcOH, PFDeA, and PFHxS showed stronger associations with child OWO in females than males. In the race-stratified analysis, associations of PFDeA, PFHpS, PFHxS, and PFOS with child BMI Z-score or OWO tended to be stronger among non-Black children compared to Black children.

In the sensitivity analyses, additional adjustment of fish intake and Mediterranean diet score (Table S11), excluded adjustment of maternal diabetes and birthweight-for-gestational age (Table S12), additional adjustment of maternal smoking (Table S13), and additional adjustment of breastfeeding (Table S14), respectively, did not substantially change our results.

4. Discussion

The current study investigated the associations of maternal plasma PFAS concentrations at delivery with childhood BMI and OWO risk at 2–18 years. We observed that PFHpS and PFHxS were associated with higher child BMI Z-score. The associations were stronger in older child age groups. In both single and mixture analyses, associations of PFAS with child BMI Z-score were stronger in children of non-OWO mothers. We observed synergistic joint effects of PFAS and maternal pre-pregnancy OWO only in the age group of 13–18 years. This study investigated the effects of more types of PFAS in a longer time scale compared with prior research, provided new insights that maternal OWO may modify the relation between PFAS mixture and child OWO, and reported differential joint effects of PFAS and maternal OWO by child's age group. Our findings may contribute to explaining the inconsistent exposure-effect relations reported by previous studies and indicate a new perspective for health effect identification and prevention of PFAS.

We found that PFHxS was associated with higher child BMI Z-score, which is consistent with previous studies. The ECHO cohorts observed a subtle positive association of BMI

Z-score with PFHxS ($\beta = 0.07$; 95 % CI: 0.01, 0.12) (Liu et al., 2023). The SMBCS observed that PFHxS was associated with being in the high BMI trajectory group (OR: 1.43; 95 % CI: 1.02, 2.01; $P = 0.04$) (Dai et al., 2023). As we found a 10 % higher risk of child OWO at 2–18 years associated with PFDeA, the SMBCS suggested that PFDeA ($\beta = 0.19$; 95 % CI: 0.06, 0.31; $P < 0.01$) was linked to increased BMI from birth to 10 years in linear mixed-effects models (Dai et al., 2023). The Healthy Start longitudinal cohort reported a non-significant association of the mixture of PFDeA, PFHxS, PFNA, PFOA, and PFOS in maternal gestational serum with the BMI of children (median age: 4.6 years) (Starling et al., 2024), which were observed in our study as well. Such positive relations between PFAS and child BMI/OWO were also reported by the Scandinavian Successive Small-for-Gestational Age births study and the HOME Study (Lauritzen et al., 2018; Liu et al., 2020). However, two systematic reviews observed conflicting results in previous studies and concluded that prenatal PFAS exposures were not statistically associated with child BMI (Frangione et al., 2024; Frigerio et al., 2023). The inconsistency may be attributable to different PFAS types tested, exposure levels, follow-up years (age of children), and source populations.

We observed stronger associations of PFHpS and PFHxS with child OWO in children aged 6–12 years and 13–18 years than children aged 2–5 years. Such findings suggested that the effects of prenatal PFAS exposures may have latent effects that become evident in later child life stages, which may help explain the null associations reported by previous studies (Frangione et al., 2024; Frigerio et al., 2023), as most studies only included children under 13 years. The age-dependent associations between prenatal PFAS exposures and child BMI were also found in other studies. The Project Viva observed associations of PFAS mixture in maternal early pregnancy plasma with higher BMI ($\beta = 0.52$; 95 % CI: -0.02 , 1.06) and higher obesity risk at 16–20 years (by quantile-based g-computation models, estimate per quartile: 1.52; 95 % CI: 1.03, 2.25); notably, they found a more rapid increase in child BMI starting from 9–11 years in children with higher exposures of PFOS, Me-PFOA-AcOH, and 2-(N-ethyl-perfluorooctane sulfonamido) acetate (Zhang et al., 2023). In the HOME Study, maternal first trimester serum PFOS and PFHxS were associated with child adiposity at age 8 years (Braun et al., 2016). However, when they followed the children up until age 12 years, PFOS and PFHxS were monotonically associated with lower child BMI (Braun et al., 2021). They also found PFOA was associated with lower BMI in infancy and early childhood, but accelerated BMI gains later, and then higher BMI at 12 years (Braun et al., 2021). Taiwan Birth Panel Study concluded cord blood PFOS concentration was associated with a lower BMI in female children at 6 months to 2 years, but was associated with a higher BMI at 5–9 years (Chen et al., 2017). So far, most studies that investigated the effects of prenatal exposure to PFAS were limited to the age below 13 years, the latent effects of prenatal PFAS exposures remained to be more explored.

Our mixture analyses stratified by maternal OWO suggested that the trend between PFAS mixture differed substantially among children with or without OWO mothers. In quantile-based g-computation models, we observed a significant association between the PFAS mixture and a higher child BMI Z-score in children of non-OWO mothers only. Using BKMR, we also found a more prominent positive trend in children of non-OWO mothers. To our knowledge, although most studies adjusted for maternal pre-pregnancy BMI as a covariate, our study was the first to consider maternal OWO as an effect modifier when

exploring the association between PFAS and childhood OWO. As mentioned above, almost all studies that investigated the mixture effects of PFAS found a weak or non-significant relation, even though they reported a significant association with individual PFAS (Liu et al., 2023; Starling et al., 2024). Our exploration incorporating maternal OWO may provide a clue to clarify underlying associations or explain subtle findings in mixture analyses, that is, the effects of the PFAS mixture may be masked by the effect of maternal OWO. Different proportions of OWO mothers might also partly explain the incoherent results in published studies. Future studies on prenatal PFAS exposures and child obesity may conduct secondary analyses stratifying by maternal OWO to address potential effect masking. Additionally, we found the synergistic effects of PFAS and maternal OWO only in children aged 13–18 years. Maternal OWO may influence the nutrient and inflammatory biomarkers transportation to the fetus, cause epigenetic changes and reprogram organ and tissue structure of the fetus, and alter the metabolic function and microbiome of offspring, predisposing the fetus more sensitive to *in-utero* exposures to environmental obesogens, such as PFAS (Catalano & Shankar, 2017). However, such effects may not be observed in early childhood, as we found stronger associations between PFAS and child BMI in older child age groups. In adolescence, the effects of prenatal PFAS became stronger and presented synergistic effects with maternal OWO. However, we did not find an animal study that investigated the joint effects of maternal OWO and prenatal PFAS exposures, the biological mechanisms of our findings remain further exploration.

We found stronger positive associations of Me-PFOA-AcOH, PFDeA, and PFHxS with child OWO in females. Among existing studies examining the sex-specific effects of PFAS, the HOME Study found PFOA and PFHxS were consistently associated with higher adiposity in females but not in males aged 12 years (Liu et al., 2020). The Hamamatsu Birth Cohort for Mothers and Children (HBC) study observed a negative association between PFOA and BMI Z-score only among females aged 0–5.5 years (Horikoshi et al., 2021). As more research concluded more pronounced associations among females (Sun et al., 2024; Svensson et al., 2023), null sex-differential associations were also reported (Liu et al., 2023; Starling et al., 2024). However, given the synergistic effects of PFAS and maternal OWO in children aged 13–18 years we observed, these studies likely missed the differential effects by sex because the children were not followed long enough.

The biological mechanisms by which early life PFAS exposure affects childhood OWO are not fully understood, but some pathways have been suggested by previous studies. First, PFAS can simulate free fatty acids and activate the peroxisome proliferator-activated receptors (PPARs), facilitating the proliferation of 3 T3-L1 pre-adipocytes, influencing fatty acid metabolism, and causing adipogenesis, therefore increasing the risk of obesity (Behr et al., 2020). Moreover, an animal study reported that exposure to PFAS in the embryonic period may reduce the PPAR gene expression, leading to life-long effects on obesity (Sant et al., 2021). Second, as a class of EDC, PFAS may change the thyroid hormones and cortisol levels, reprogram the hypothalamic–pituitary–adrenal (HPA) axis, and then influence the body weight (Lopez-Espinosa et al., 2012; Zhao et al., 2011). Third, PFAS were reported to have estrogenic properties, which may help explain the sex-specific effects (Rosen et al., 2017). Fourth, PFAS may be able to interrupt the lipid metabolism process by impacting the transport, storage, oxidation, and synthesis of lipids and fatty acids (Kaye et al., 2024).

Another BBC study also observed associations of individual PFAS and PFAS mixture in maternal plasma with multiple lipids in cord plasma (Li et al., 2024). Finally, mitochondrial dysfunction (Starkov & Wallace, 2002), change in DNA methylation (Starling et al., 2020), and gut microbiome dysbiosis (Sen et al., 2024) were suggested to be potential mechanisms through which PFAS impact childhood obesity risks. Additionally, the only significant individual association with PFHxS observed in our study may be attributable to its highest placental transfer rate compared to other PFAS (Kang et al., 2021).

Some strengths of this study should be highlighted. The prospective cohort study design guaranteed the temporal relationship between exposures and outcomes; the large sample size offered relatively high statistical power; the unique composition of predominantly urban, low-income, Black populations suggested insights for under-represented populations. Most importantly, we followed the children for 18 years, longer than most existing studies, which allowed us to identify synergistic effects of PFAS and maternal OWO that become evident in adolescence and highlight the latent, long-term effects of early life PFAS exposure. Second, we included 8 legacy PFAS, especially some rarely measured PFAS such as Me-PFOSA-AcOH, PFDeA, and PFUnA, allowing a more thorough scene of exposure-effect relations between multiple types of PFAS and childhood obesity. We conducted both single and mixture analyses to investigate the impacts of both individual and combined PFAS exposures. Finally, benefiting from the high proportion of OWO mothers in the BBC (52 %), we were able to conduct stratified analyses by maternal OWO and evaluated joint effects of early life exposure to PFAS and maternal pre-pregnancy OWO, proposing new insights and informing future studies between PFAS and childhood obesity.

Nevertheless, several limitations of the study cannot be ignored. First, we observed a smaller proportion of preterm-born children in the included participants than all participants in the BBC, indicating potential selection bias in this study. However, the other characteristics were generally comparable, and we adjusted for most of these characteristics in models to account for differences in these factors. Second, a single maternal plasma sample at delivery was used to represent PFAS exposure throughout the gestational period. Although PFAS levels may change from pregnancy to postpartum, a previous study reported that PFAS concentrations in maternal serum at birth were highly correlated with concentrations in infant cord serum ($r = 0.79\text{--}0.92$) (Kato et al., 2014). Therefore, maternal PFAS concentrations in samples collected only 24–72 h after delivery may be a reliable proxy for fetal exposure in this study. Third, BMI Z-score and OWO status identified by BMI percentile were applied as the outcomes, which were both not direct indexes for body fat, leading to potential misclassification of child OWO. Fourth, although we observed joint effects of maternal OWO and early life PFAS exposures, we did not have animal studies to explain the underlying biological mechanisms. Fifth, Children in our study spanned a wide age range and were in different stages of puberty. While we did not adjust for pubertal status as it could be a causal intermediate (Lee et al., 2021), future studies could consider the role of pubertal development in the relations between early life PFAS exposure and body composition. Finally, we were unable to control unmeasured confounding, such as child PFAS exposures, and residual confounding, although we adjusted for fish intake, Mediterranean diet score, maternal smoking history, and breastfeeding in sensitivity analyses and found our estimates were robust to potential confounding.

PFOS and PFOA were voluntarily phased out by major companies in 2002 and 2006 (Viberg & Eriksson, 2011), and these PFAS were designated as hazardous substances under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) by the U.S. Environmental Protection Agency (EPA) in April 2024 (US EPA, 2024). The PFAS Strategic Roadmap of EPA (US EPA, 2021) emphasizes the need for continuing research on toxicity and human health effects of other PFAS to inform future actions to protect public health. Thus, the current study contributes to the evidence of long-term health effects of early-life exposure to multiple PFAS, provides new insights in explaining inconsistent findings of previous studies, and informs future studies to better elucidate the health effects of PFAS and develop effective interventions for childhood obesity.

5. Conclusion

In this prospective birth cohort of US understudied, high-risk mother–child dyads, the study demonstrated individual and mixture effects of early life PFAS exposures on childhood OWO. PFHpS and PFHxS concentrations in maternal plasma at delivery were associated with a greater child BMI Z-score. Such associations were stronger in older child age groups (6–12 and 13–18 years). Maternal OWO acted as an effect modifier, with associations generally stronger among children of non-OWO mothers. However, the synergistic effects of prenatal PFAS exposure and maternal pre-pregnancy OWO became evident in adolescence. These findings underscore the complexity of the PFAS-BMI (or OWO) associations, help explain inconsistent findings reported by previous studies, and inform future research design, analyses, and comparisons across studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We sincerely appreciate all study participants in the Boston Birth Cohort. We also thank the Boston Medical Center nursing staff and the Boston Birth Cohort field team for their support and help with the study.

Funding sources

The Boston Birth Cohort is funded by the National Institutes of Health (2R01HD041702, R01HD098232, R01ES031272, R01ES031521, and U01ES034983) and the Maternal and Child Health Bureau (UT7MC45949). Dr. Choi is supported by the National Institutes of Health (K99ES035464). Drs. Yu and Fan are supported by the CDC State Biomonitoring Grant (U88EH001151) and the New Jersey State Government. Dr. Buckley is supported by the National Institutes of Health (R01ES030078, R01ES033252). The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies.

Data availability

Data will be made available on request.

Abbreviations:

AGA appropriate for gestational age

BBC	Boston Birth Cohort
BMC	Boston Medical Center
BKMR	Bayesian kernel machine regression
BMI	body mass index
CDC	Centers for Disease Control and Prevention
CI	confidence interval
DOHaD	Developmental Origins of Health and Diseases theory
EDC	endocrine-disrupting chemical
HPLC-MS/MS	high-performance liquid chromatography-tandem mass spectrometry
IRB	Institutional Review Boards
LGA	large for gestational age
LOD	limit of detection
LOQ	limit of quantification
Me-PFOSA-AcOH	2-(N-Methyl-perfluorooctane sulfonamido) acetic acid
OWO	overweight or obesity
PFAS	per- and polyfluoroalkyl substances
PFDeA	perfluorodecanoic acid
PFHpS	perfluoroheptane sulfonic acid
PFHxS	perfluorohexanesulfonic acid
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctanesulfonic acid
PFUnA	perfluoroundecanoic acid
OR	odds ratio
QA	Quality Assurance
QC	Quality Control
RR	risk ratio
SGA	small for gestational age

SPE solid-phase extraction

US the United States

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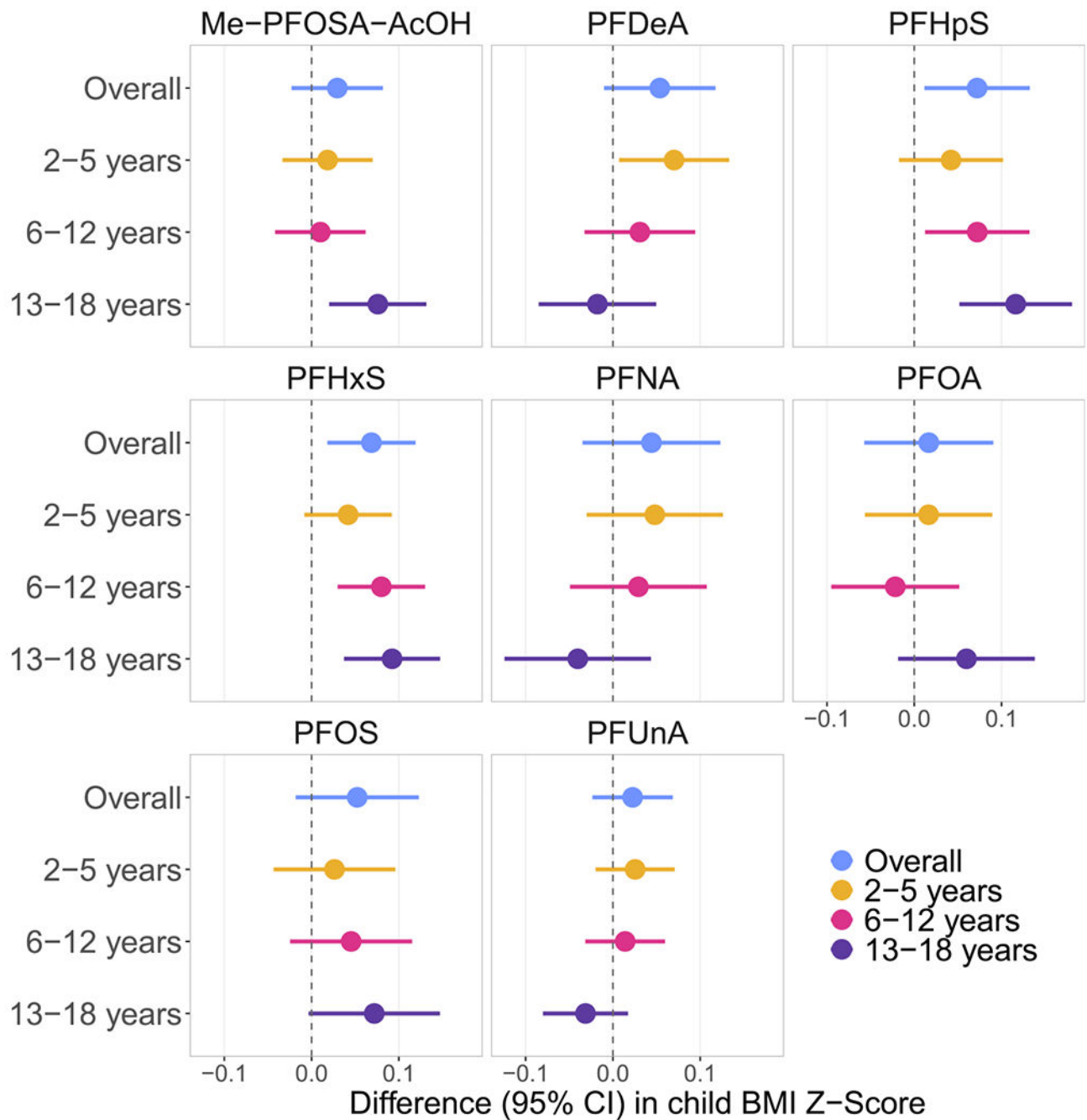
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**Fig. 1.**

Association of maternal plasma PFAS with repeated child BMI Z-score at the age of 2–18 years examined by linear and modified Poisson mixed-effects models among 1,189 mother–child dyads in the Boston Birth Cohort. Note: Abbreviations: PFAS – per- and polyfluoroalkyl substances; BMI – body mass index; CI – confidence interval; Me-PFOSA-AcOH – 2-(N-Methyl-perfluorooctane sulfonamido) acetic acid; PFDeA – perfluorodecanoic acid; PFHpS – perfluoroheptanesulfonic acid; PFHxS – perfluorohexanesulfonic acid; PFNA – perfluorononanoic acid; PFOA – perfluorooctanoic

acid; PFOS – perfluorooctanesulfonic acid; PFUnA – perfluoroundecanoic acid. Models were adjusted for maternal age, education, race, maternal pre-pregnancy BMI, diabetes, child's sex, birthweight-for-gestational age (ie. AGA, SGA, LGA), year of birth, delivery mode, and parity. The estimates and 95 % CI of associations of PFAS with child BMI Z-score and child OWO are reported in Table S2.

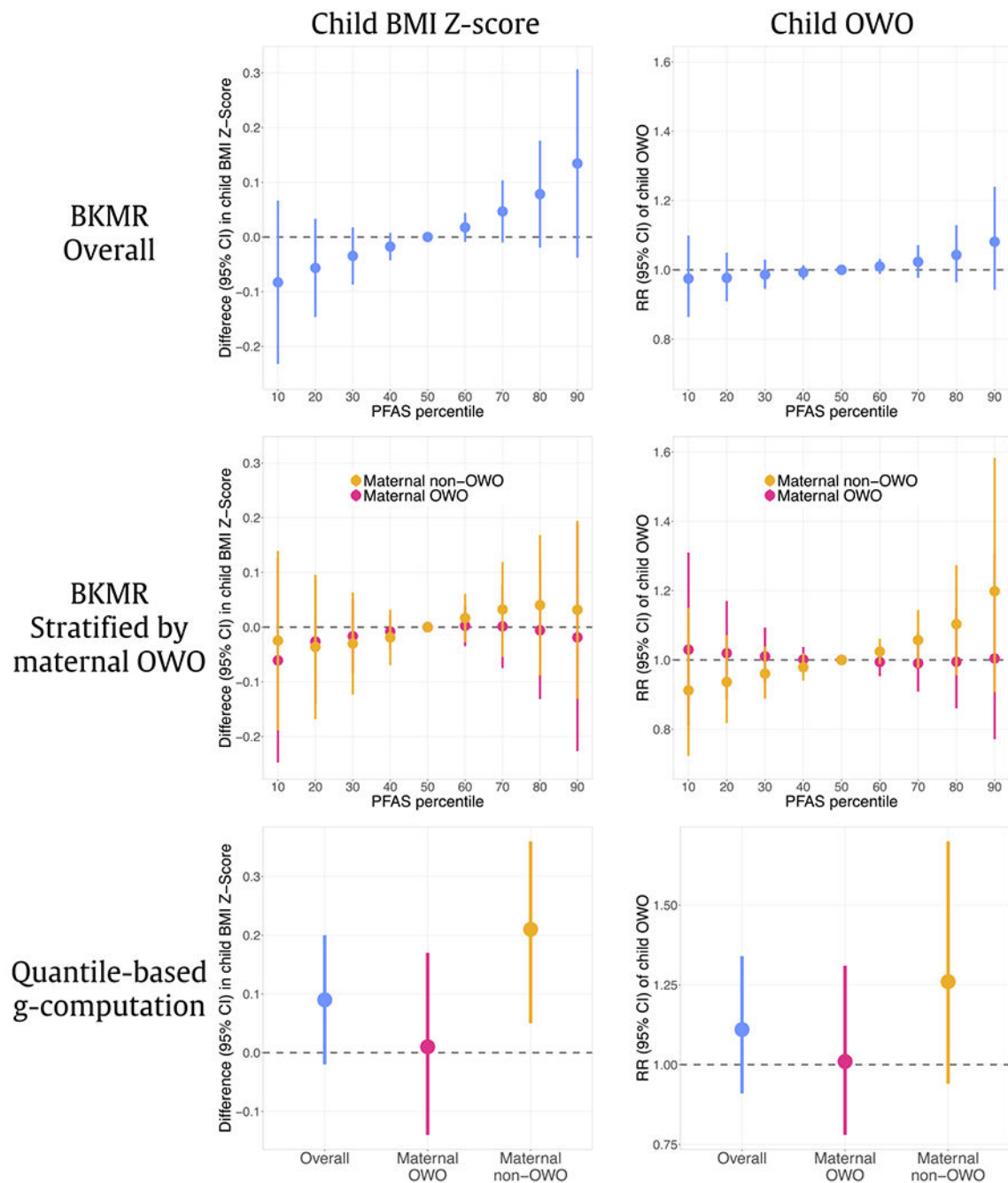
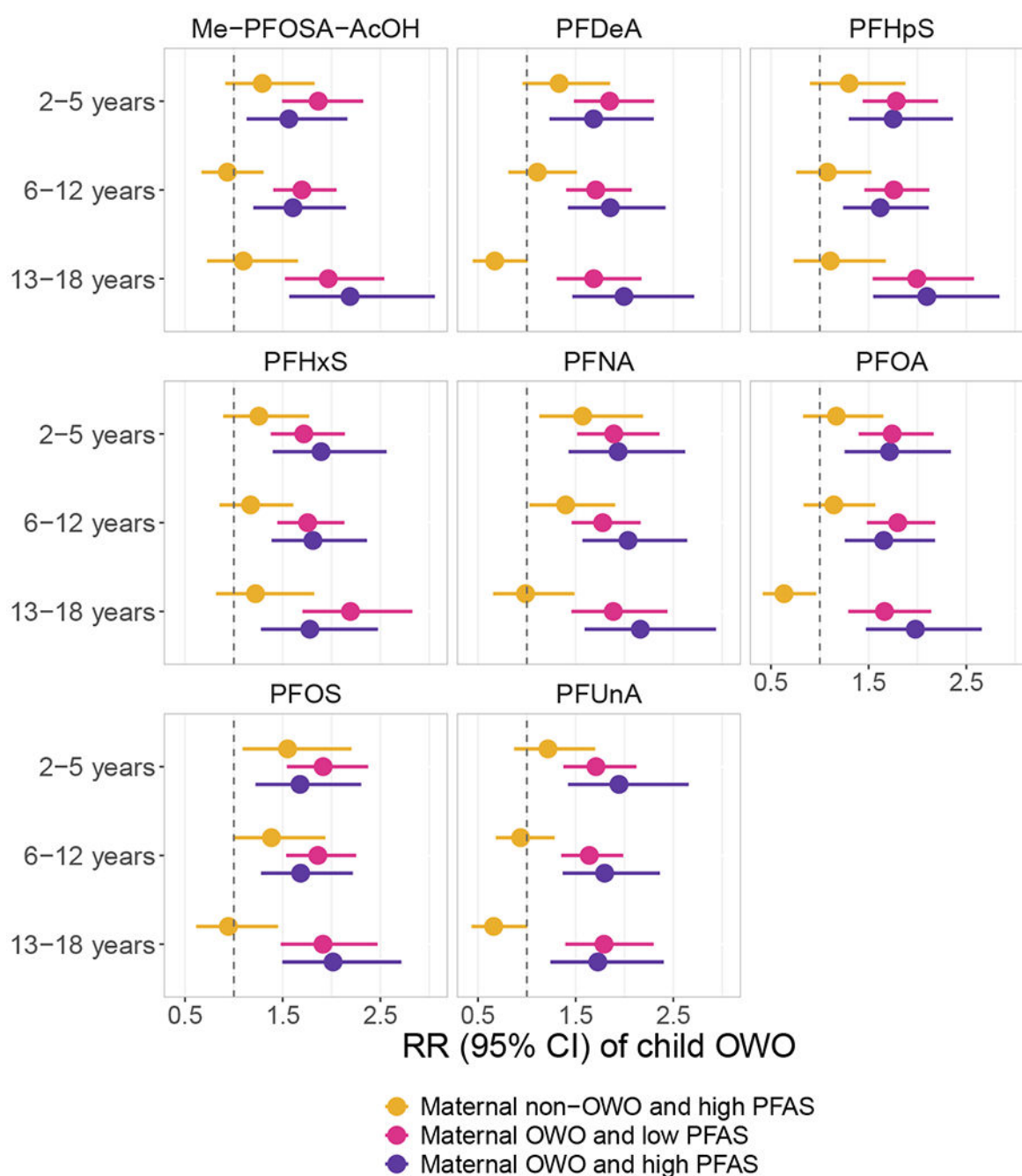


Fig. 2.

Association of maternal plasma PFAS mixture with child BMI Z-score and child OWO status at the age of 2–18 years examined by Bayesian kernel machine regression and quantile-base g-computation models among 1,189 mother–child dyads in the Boston Birth Cohort. Note: The points in BKMR figures mean the estimates of outcome as all the PFAS exposure are fixed at the same quantiles (from 10th to 90th) compared to when all PFAS are held at their medians, and the vertical lines mean the 95 % confidence interval of the estimate. The figures of overall models suggest increased child BMI Z-score and risk of

child OWO as cumulative levels across all PFAS exposures increase. Abbreviations: PFAS – per- and polyfluoroalkyl substances; BMI – body mass index; OWO – overweight or obesity. Models were adjusted for maternal age, education, race, maternal pre-pregnancy BMI (only in overall models), diabetes, child’s sex, birthweight-for-gestational age (ie. AGA, SGA, LGA), year of birth, delivery mode, and parity. The estimates, 95 % CI, and weights of each exposure in quantile-based g-computation models are reported in Table S4.

**Fig. 3.**

Joint association stratified by child age group of maternal plasma PFAS and maternal OWO status with child BMI Z-score and child OWO status among 1,189 mother-child dyads in the Boston Birth Cohort. Note: Abbreviations: PFAS – per- and polyfluoroalkyl substances; BMI – body mass index; CI – confidence interval; Me-PFOSA-AcOH – 2-(N-Methyl-perfluorooctane sulfonamido) acetic acid; PFDeA – perfluorodecanoic acid; PFHpS – perfluoroheptanesulfonic acid; PFHxS – perfluorohexanesulfonic acid; PFNA – perfluorononanoic acid; PFOA – perfluorooctanoic acid; PFOS – perfluorooctanesulfonic

acid; PFUnA – perfluoroundecanoic acid. Models were adjusted for maternal age, education, race, diabetes, child’s sex, birthweight-for-gestational age (ie. AGA, SGA, LGA), year of birth, delivery mode, and parity. The reference group is children of non-OWO mother and low PFAS exposures. Low PFAS exposures were defined as maternal plasma PFAS concentrations in Quartile 1–3, and high PFAS exposures were defined as maternal plasma PFAS concentrations in Quartile 4. We classified low/high as Quartile 1–3/Quartile 4 because we observed similar estimates of associations in Quartile 1–3 in Table S6. The estimates and 95 % CI of associations presented in this figure are reported in Table S8.

Table 1General characteristics of 1189 mother–child dyads in the Boston Birth Cohort. ^a.

Characteristics	No. (%) / Mean $\pm$ SD			P value ^e
	Total (n = 1189)	Child non-OWO ^b (n = 624)	Child OWO ^b (n = 565)	
Maternal age at delivery (years)	28.8 $\pm$ 6.5	28.1 $\pm$ 6.4	29.6 $\pm$ 6.4	< 0.01
Maternal race				
Black	705 (59)	365 (58)	340 (60)	0.60
Non-Black	484 (41)	259 (42)	225 (40)	
Maternal education ^c				
High school or below	764 (65)	397 (64)	367 (65)	0.61
College or above	419 (35)	225 (36)	194 (35)	
Maternal pre-pregnancy BMI (kg/m ²) ^c	27.0 $\pm$ 6.6	25.2 $\pm$ 5.6	29.0 $\pm$ 6.9	< 0.01
Maternal pre-pregnancy OWO ^d	616 (54)	250 (42)	366 (69)	< 0.01
Maternal pregestational or gestational diabetes	202 (17)	79 (13)	123 (22)	< 0.01
Maternal smoking history during pregnancy ^c	195 (16)	92 (15)	103 (18)	0.12
Parity				
Nulliparous	505 (42)	277 (44)	228 (40)	0.18
Multiparous	684 (58)	347 (56)	337 (60)	
Year of delivery				
1999-2006	360 (30)	203 (33)	157 (28)	0.09
2007-2016	829 (70)	421 (67)	408 (72)	
Delivery mode				
Vaginal delivery	741 (62)	423 (68)	318 (56)	< 0.01
Cesarean section	448 (38)	201 (32)	247 (44)	
Child's sex				
Male	585 (49)	318 (51)	267 (47)	0.22
Female	604 (51)	306 (49)	298 (53)	
Gestational age (weeks)	38.0 $\pm$ 3.1	38.0 $\pm$ 3.1	38.0 $\pm$ 3.1	0.94
Preterm				
< 37 weeks	277 (23)	148 (24)	129 (23)	0.77
$\geq$ 37 weeks	912 (77)	476 (76)	436 (77)	
Birth weight (g)	2983.8 $\pm$ 781.2	2914.3 $\pm$ 745.7	3060.5 $\pm$ 812.5	< 0.01
Birthweight-for-gestational age				
AGA	937 (79)	503 (81)	434 (77)	< 0.01
SGA	128 (11)	80 (13)	48 (8)	
LGA	123 (10)	41 (6)	82 (15)	

Note:

^a Abbreviations: No. – frequency; % – percentage; SD – standard deviation; OWO – overweight or obesity; BMI – body mass index; AGA – appropriate for gestational age; SGA – small for gestational age; LGA – large for gestational age.

^b Child OWO status is defined as the BMI percentile at the last visit at the age of 2–18 years was $\geq$ 85 percentile.

^cThe number of missing values: maternal education: 6; maternal pre-pregnancy BMI: 57; maternal smoking history during pregnancy: 7. The missing values were imputed with multivariate imputation by chained equations (*mice* package for R) in the following analyses.

^dMaternal pre-pregnancy OWO is defined as a maternal pre-pregnancy BMI ≥ 25 kg/m².

^eCovariate differences in children with and without childhood OWO were tested by *t*-test for continuous variables and chi-square test for categorical variables.

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Table 2

Distribution of PFAS in maternal postpartum plasma samples among 1189 mother–child dyads in the Boston Birth Cohort. ^a.

PFAS (ng/mL)	LOD ^b		DF (%)	GM	Min	P25	P50	P75	Max
	6500	7500							
Me-PFOSA-AcOH	0.061	0.019	87.22	0.11	< LOD	0.06	0.10	0.19	3.77
PFDeA	0.049	0.024	95.04	0.20	< LOD	0.13	0.20	0.31	2.18
PFHpS	0.015	0.005	97.22	0.11	< LOD	0.08	0.12	0.19	1.20
PFHxS	0.038	0.012	99.92	0.75	< LOD	0.41	0.69	1.33	24.61
PFNA	0.049	0.010	100.00	0.66	0.08	0.46	0.66	0.94	11.38
PFOA	0.046	0.025	99.92	1.50	< LOD	1.00	1.50	2.32	13.32
PFOS	0.046	0.008	100.00	3.67	0.45	2.27	3.57	6.01	36.00
PFUnA	0.047	0.006	95.37	0.14	< LOD	0.09	0.16	0.27	3.61

Note:

^a Abbreviation: PFAS – per- and polyfluoroalkyl substances; LOD – limit of detection; DF – detection frequency; GM – geometric mean; Min – minimum; Max – maximum; Me-PFOSA-AcOH – 2-(N-Methyl-perfluorooctane sulfonamido) acetic acid; PFDeA – perfluorodecanoic acid; PFHpS – perfluoroheptanesulfonic acid; PFHxS – perfluorohexanesulfonic acid; PFNA – perfluorononanoic acid; PFOA – perfluorooctanoic acid; PFOS – perfluorooctanesulfonic acid; PFUnA – perfluoroundecanoic acid.

^b PFAS were measured using high-or ultra high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS SCIEX QTRAP TM 6500 or UHPLC-MS/MS SCIEX QTRAP TM 7500).