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Article

Associations of Prenatal Exposure to Per- and Polyfluoroalkyl Substances with the Neonatal Birth Size and Hormones in the Growth Hormone/Insulin-Like Growth Factor Axis

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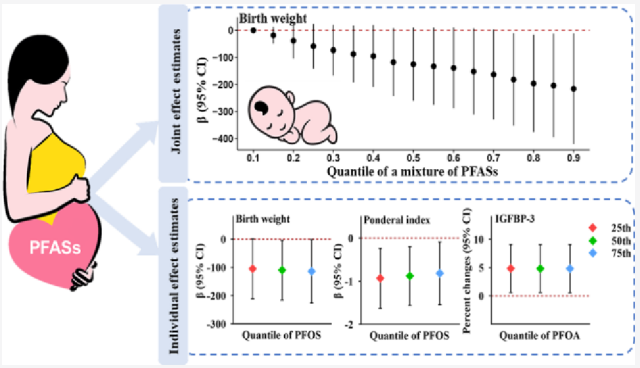
ABSTRACT: Toxicological data suggest a significant developmental toxicity of per- and polyfluoroalkyl substances (PFASs); however, evidence in humans remains inconclusive. Furthermore, the effects of prenatal exposure to PFASs on hormones in the growth hormone (GH)/insulin-like growth factor (IGF) axis of newborns remain largely unclear. We aimed to investigate the associations of prenatal exposure to PFASs with the neonatal birth size, GH, IGF-1, and IGF-binding protein 3 (IGFBP-3). The concentrations of 22 PFASs were measured in the plasma of 224 pregnant women collected within 3 days before delivery (39.3 weeks) in Guangzhou, China, and the anthropometric data were gathered from medical records. Paired cord blood was collected at delivery to determine GH, IGF-1, and IGFBP-3 levels. Multivariable linear regression models revealed the inverse associations of several long-chain PFASs with birth weight and ponderal index as well as the significant associations of perfluorobutanoic acid and perfluorooctanoic acid (PFOA) with IGFBP-3 levels. The Bayesian kernel machine regression confirmed the association of perfluorooctane sulfonate with birth weight and ponderal index and of PFOA with IGFBP-3 and identified an inverse joint effect of exposure to a mixture of multiple PFASs on birth weight. The findings provide the first comprehensive evidence on the individual and joint effects of multiple PFASs on the neonatal birth size and hormones in the GH/IGF axis, which requires further confirmation.



KEYWORDS: *perfluorinated compounds, fetal growth, GH/IGF axis, Bayesian kernel machine regression, joint effect*

INTRODUCTION

Per- and polyfluoroalkyl substances (PFASs), which comprise a group of synthetic compounds with strong carbon−fluorine bonds, have been mass-produced and extensively used in numerous industrial and commercial products since the 1950s.[1,2] Because of their special physicochemical properties, all PFASs exhibit persistence and many of the PFASs that have been studied exhibit bioaccumulation and toxicity. As a result, they can be frequently detected in various environmental media and biota samples.[1−3] The main legacy PFASs, the most studied long-chain perfluoroalkyl carboxylic acids (PFCAs) with ≥7 perfluorinated carbons and perfluoroalkyl sulfonic acids (PFSAs) with ≥6 perfluorinated carbons,[4] have attracted much attention because of their widespread exposure in wildlife and humans, especially perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). PFOS and PFOA, the two most widely used PFASs, are resistant to degradation and are estimated to have long half-lives of 4.8 and 3.5 years for human serum elimination, respectively.[5] With increasing concerns about the environmental risk and multiple toxic effects of PFASs, PFOS- and PFOA-related chemicals have been included in the Persistent Organic Pollutants list under the Stockholm Convention.[6,7] Meanwhile, great efforts have been made to develop more suitable alternatives to legacy PFASs. Chlorinated polyfluoroalkyl ether sulfonic acids (Cl-PFESAs), also known as F-53B (trade name), have been used as potential substitutes for PFOS. In recent years, these historically neglected PFAS alternatives, which have only been used in China,[8] have become ubiquitous and can be detected in multiple environments,[9−12] wildlife,[13−16] and even human specimens[17−22] worldwide. Cl-PFESAs can accumulate in humans for a long time with an estimated half-life of 15.3 years, making them the most persistent PFASs recorded so far,[17] and they have higher transplacental transfer efficien-

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cies[18,20] and a similar trophic transfer behavior[14] to that of PFOS. This implies that Cl-PFESAs may have similar or higher toxicity potential. Consequently, Cl-PFESAs are receiving increasing attention regarding health hazards.

It has been reported that the general population, pregnant women, and children have widespread exposure to PFASs.[23−25] However, more attention should be paid to the fact that PFASs can pass through the placenta to reach the developing fetus,[26] a population more vulnerable to environmental pollutants, which may lead to irreversible damage during fetal development. Substantial evidence from in vivo studies has indicated the significant developmental and/or reproductive toxicity of legacy PFASs, including reduced birth weight, compromised survival, delayed growth and development in offspring mice after PFAS exposure during pregnancy, and kidney injury.[27−29] In the in vivo zebrafish model, 6:2 Cl-PFESA exposure might delay hatching, induce the occurrence of malformations, decrease survival of zebrafish embryos, and cause damage to the reproductive system.[30,31] Human data on the effects of prenatal PFAS exposure on fetal growth have been well documented; however, the results of previous studies are inconsistent, as indicated in a recent review.[32] In this review of 18 studies of prenatal PFAS exposure in relation to fetal growth from different countries and regions, inverse correlations were reported for PFOS in seven studies and for PFOA in seven studies, while the other studies showed null correlations.[32] Similarly, emerging research from a prospective cohort study conducted in Japan and Sweden found that prenatal exposure to PFOS, PFOA, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and perfluoroundecanoic acid (PFUnDA) was inversely associated with birth weight,[33,34] whereas others have reported no significant associations.[35,36] These inconclusive findings might be attributed to the methodology they use for statistical analysis. We also note that most previous studies mainly focused on the toxic effects of traditional PFASs; the available epidemiological data on the developmental toxicity of PFAS alternatives are extremely limited with ambiguous results. To the best of our knowledge, only three recent human studies from China have assessed the adverse effect of Cl-PFESA exposure on fetal growth and obtained ambiguous results.[22,37,38] In addition, most studies used multivariable linear regression (MLR) models that could not address the multicollinearity to explore the relationships between individual PFAS and birth outcomes, and the joint effects of exposure to a PFAS mixture on the birth size are also unclear. Hence, there is an imperative need to evaluate the adverse effects of prenatal PFAS, especially their alternative exposure on fetal growth using a more advanced method, either estimated individually or as a mixture.

The potential mechanisms underlying PFAS-induced adverse outcomes remain uncertain. The growth hormone (GH)/insulin-like growth factor (IGF) axis is a key endocrine pathway regulating fetal growth in which GH and IGF-1 have been regarded as reliable biomarkers for monitoring the GH/IGF system. Most IGF-1 (>90%) are bound to IGF-binding protein 3 (IGFBP-3) to regulate the concentration balance of IGF-1 in the systemic circulation. Epidemiological studies have indicated that the levels of GH, IGF, and IGFBP-3 in the GH/IGF axis were closely linked with fetal growth.[39] Meanwhile, several toxicological studies in vivo provide a hint that PFASs might interfere with the homeostasis of the GH/IGF axis, resulting in abnormal growth and development. For instance, using Atlantic salmon as an animal model, Spachmo and Arukwe reported a time-dependent decrease in mRNA expression of GH and IGF-1 following PFOS exposure, and PFOA exposure also produced some alterations in the expression of the GH/IGF signaling pathway.[40] Another study conducted in zebrafish embryos showed that PFOS exposure might induce the dysregulation of mRNA expression of multiple genes involved in the GH/IGF axis and lead to a decrease in body length.[41] However, we note that only one epidemiological study conducted among young children has investigated the relationship between PFAS exposure and IGF1 levels, and this study found that PFOS and PFNA were negatively associated with IGF1 levels.[42] There is no available information on the effects of prenatal exposure to PFASs on hormones in the GH/IGF axis of newborns; thus, additional research is needed to determine if these relationships likely explain the developmental effects of PFASs in humans.

As mentioned above, it is also worth noting that when assessing the adverse birth outcomes caused by PFASs, the common practice has been to consider one PFAS at a time using the MLR model in previous epidemiological studies,[33,37] which may lead to inconclusive results. Although traditional models can provide more direct and simple associations between individual compounds and outcomes, this approach ignores the coexposure to multiple PFASs that occurs in real life. If multiple highly correlated compounds are simultaneously incorporated into the same linear model, biased conclusions may be drawn. Moreover, the MLR model has limited ability to evaluate the joint effects of multipollutant mixtures. The Bayesian kernel machine regression (BKMR), a novel statistical method that flexibly models the joint effects of a mixture of components, was introduced to assess the overall and nonlinear effects of multiple pollutants.[43] Considering the above research gaps that need to be urgently filled, we conducted this study using the MLR model and a more advanced method, the BKMR model, to (1) examine the associations of prenatal exposure to PFASs with the neonatal birth size, (2) investigate the effects of prenatal exposure to PFASs on the neonatal hormones in the GH/IGF axis, and (3) assess the joint effects of a mixture of multiple PFASs on the birth size and hormone levels. As far as we know, this is the first report that comprehensively evaluated the single and overall associations of prenatal exposure to multiple PFASs with the neonatal birth size and hormones in the GH/IGF axis.

MATERIALS AND METHODS

Study Population and Sample Collection. The pregnant women in our study were recruited from Zhujiang Hospital in Guangzhou (South China) between July 2017 and September 2019. In order to better evaluate the adverse effects of PFAS exposure, pregnant women with chronic or serious diseases (e.g., cardiovascular and pulmonary diseases, hepatic and renal diseases, thyroid dysfunction, and other genetic diseases) and newborns with congenital anomalies were excluded. A total of 302 mother−newborn pairs met our inclusion criteria; 31 mother−newborn pairs provided incomplete questionnaire information, and hemolysis occurred in 47 cord blood samples. Thus, 224 mother−newborn pairs were finally enrolled in the present analysis. Maternal blood during pregnancy was collected in anticoagulation tubes containing sodium heparin within 3 days before delivery (39.3 weeks), and the paired cord blood was collected immediately at delivery. The season of maternal and umbilical cord blood collection was defined according to the birthdate of

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the newborns as follows: spring (March−May), summer (June−August), autumn (September−November), and winter (December−February). All participants involved in our study signed informed consent to confirm their understanding of the project and completed the corresponding questionnaire survey. The ethics committee of Jinan University and the hospital approved this research protocol.

Sample Analysis of PFASs. A total of 22 target PFASs, including 10 PFCAs (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, and PFTrDA), four PFSAs (PFBS, PFHxS, PFOS, and PFDS), two chlorinated alternatives (6:2 Cl-PFESA and 8:2 Cl-PFESA), three perfluorooctane sulfonamidoacetic acids (FOSAA, N-MeFOSAA, and N-EtFOSAA), 6:2 FTS, HFPO-DA, and NaDONA, was measured in the maternal plasma in the current study. The corresponding isotopically labeled PFASs were used as internal standards for quantitative analysis. The detailed abbreviations and full name of PFASs are listed in Table S1. The simultaneous extraction of 22 PFASs from plasma samples was conducted using a solid-phase extraction method based on previous studies with some modifications.[44,45] Following this, 22 PFASs were determined by a high-performance liquid chromatography system coupled to a triple quadrupole mass spectrometer (HPLC-MS/MS, AB Sciex, USA). The transitions of the monitored ion pairs of PFASs and the optimized instrumental parameters are listed in Table S2. The results of method validation are presented in Table S3. Details of the sample analysis and quality assurance and quality control (QA/QC) are provided in the Supporting Information.

Measurements of Hormone Levels in the Umbilical Cord Blood. GH, IGF-1, and IGFBP-3 in the umbilical cord blood were measured using an IMMULITE 2000 analyzer (Siemens Healthcare Diagnostics, Germany) with commercial kits based on the solid-phase chemiluminescence immunoassay method. Samples were pretreated and analyzed according to the manufacturer's instructions. The method detection limits (MDLs) were 0.01 ng/mL for GH, 25 ng/mL for IGF-1, and 100 ng/mL for IGFBP-3. Internal QC included repeated measurements for QC samples at high and low concentrations (Bio-Rad, USA) in each batch of analysis. The inter- and intra-assay coefficient of variations (CVs) were 4.94−5.65% for GH, 5.29−8.63% for IGF-1, and 3.70−4.55% for IGFBP-3.

Assessment of the Birth Size and Covariates. The anthropometric data of newborns and basic information of mothers were obtained from medical records of the study hospital or face-to-face questionnaires conducted by experienced nurses. Once a newborn was born, obstetric nurses determined and recorded the birth weight (in grams) and length (in centimeters) of a newborn immediately. Ponderal index, an indicator describing body proportionality at birth and reflecting the potential of asymmetrical intrauterine growth retardation, was computed as birth weight (in kilograms) divided by the cube of length (in meters) in newborns (kg/m^3).[46] Gestational age at delivery, newborn sex, birthdate, history of gestation, and medical history were abstracted by obstetric nurses. Additionally, the information on maternal demographic characteristics (age, weight, height, education level, occupation, and household income) and personal habits during pregnancy (environmental tobacco smoke exposure and alcohol drinking) were investigated before delivery through a developed questionnaire. Prepregnancy body mass index (BMI) is the ratio of self-reported weight to the square of height before pregnancy.

Statistical Analysis. The demographic characteristics of the mother−newborn pairs are first summarized. Following the substitution of nondetectable PFASs and hormones (<MDL) for MDL/$\sqrt{2}$, we characterized the distribution profiles of maternal PFAS concentrations and umbilical hormone levels. Several PFASs with a low detection frequency (<30.0%) were excluded from the main statistical analysis. In view of the positively skewed distribution of PFASs and hormone levels confirmed using the Shapiro−Wilk test, these data were all transformed using the natural logarithm (ln) to approximate normality before the main analysis. The correlations between PFAS concentrations were estimated by calculating the Pearson correlation coefficients (r).

We constructed a separate MLR model to examine the associations of prenatal exposure to PFASs with the neonatal birth size and levels of hormones in the GH/IGF axis, among which the regression coefficients (β) and corresponding 95% confidence intervals (CIs) were calculated as effect estimates. We adjusted for potential covariates in the MLR model according to previous reports or statistical considerations. The change-in-estimate approach was applied to select the covariates included in the final analysis.[47] The final model was adjusted for the following covariates: maternal age, newborn sex (male/female), prepregnancy BMI (<18.5, 18.5−23.9, and ≥24.0 kg/m^2), maternal education (less than high school/high school or equivalent/college or higher), parity (primiparous/multiparous), environmental tobacco smoke exposure (yes/no), alcohol drinking (yes/no), and gestational age. In order to easily interpret the results of statistical analysis of PFAS−hormone associations, we converted the estimated β (95% CI) from the linear models to percent changes in hormone levels for each ln-transformed unit increase in PFAS concentrations using the following formula: percent change (%) = $(e^{\beta} - 1) \times 100$. Furthermore, a restricted cubic spline (RCS) model with a linear link approach was utilized to evaluate the potential nonlinearity visually and statistically in the dose−response associations of continuous PFAS concentrations with the birth size and hormone levels. There were three knots in the RCS model located at the 25th, 50th, and 75th percentiles, with the 10th percentile as the reference. In addition to the above main analysis, we also analyzed the relationship between hormone levels and birth size using the MLR model to establish a clear connection between them and further explored whether these hormones mediated PFAS−effect relationships by a causal mediation analysis. The mediation analysis developed by Valeri and VanderWeele was applied to assess the direct (adjusted for hormones), indirect (mediated by hormones), and total effects of prenatal exposure to PFASs on the birth size.[48]

Since the research participants were concurrently exposed to multiple PFASs during pregnancy, we also utilized the BKMR model to evaluate the joint effects of mixed PFAS exposure on the birth size and the levels of each hormone as well as the single-exposure effects of each PFAS. BKMR is a novel nonparametric method for effectively estimating the joint health impacts of multipollutant mixtures using a kernel function, and it allows for potential nonlinearity and interactions in exposure−response relationships without a priori assumption of parametric models.[43] We fitted the BKMR model using the Gaussian kernel that has been proven to perform well in simulation studies and obtained estimates of the exposure−response function and 95% CIs. Note that the CIs produced by BKMR contained uncertainties because of the

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estimation of high-dimensional exposures, which took multiple testing into account. A hierarchical variable selection approach with 25,000 iterations using a Markov chain Monte Carlo sampler was incorporated into the BKMR model to address the multicollinearity caused by high correlations between compounds, and the grouped posterior inclusion probabilities (PIPs) and conditional PIPs were calculated to identify measurements of the most important PFAS exposure. The predominant PFASs were divided into three groups, with seven PFCAs (PFBA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, and PFTrDA) grouped together (group 1), three PFSAs (PFBS, PFHxS, and PFOS) grouped together (group 2), and two Cl-PFESAs (6:2 Cl-PFESA and 8:2 Cl-PFESA) grouped into another group (group 3). The BKMR model is established as follows:

$$Y_i = h(x_i) + z_i^{T}\beta + e_i$$

where Y_i represents the birth size (birth weight, birth length, and ponderal index) or individual hormone levels in the umbilical cord blood (GH, IGF-1, and IGFBP-3), x_i = [group 1 = ($PFBA_i$, $PFOA_i$, $PFNA_i$, $PFDA_i$, $PFUnDA_i$, $PFDoDA_i$, and $PFTrDA_i$), group 2 = ($PFBS_i$, $PFHxS_i$, and $PFOS_i$), group 3 = (6:2 Cl-$PFESA_i$ and 8:2 Cl-$PFESA_i$)], h () is a Gaussian kernel exposure−response function, z_i contains a set of potential confounders, and e_i refers to the residuals.

When characterizing the overall association of prenatal exposure to the PFAS mixture with the birth size and hormone levels, we calculated the differences in hormone levels and birth size by comparing the effects of exposure to 12 PFASs simultaneously fixed at a specific percentile (from the 10th to 90th percentile increasing by 5%) and the effects of the exposures held at their 10th percentile. We also investigated the overall association of PFCA, PFSA, and Cl-PFESA mixture with health indicators. We assessed the single-exposure effect of each PFAS within the context of the joint exposure, where the change in the birth size and hormone levels was associated with a change in individual PFAS from its 25th to 75th percentile when fixing all remaining PFASs at a particular exposure (25th, 50th, or 75th percentile). We also further examined the univariate exposure−response relationships for individual PFASs in relation to each indicator with the other PFASs set at their median levels to explore possible nonlinearity. The BKMR model was adjusted for a set of identical covariates included in the MLR model for consistency. In addition, we carried out sensitivity analyses by additionally adjusting the season of sample collection or gestational diabetes mellitus in the MLR model to assess the robustness of the results.

Data analyses were carried out using SAS version 9.4 (SAS Institute, Cary, NC, USA) and R version 4.0.1 (R Foundation for Statistical Computing, Vienna, Austria). A two-tailed significance level for P value was set at 0.05 in all statistical analyses.

RESULTS

Participant Characteristics. The basic characteristics of the 224 mother−newborn pairs participating in this study are summarized in Table 1. The mothers were 29.0 (± 4.3) years old on average at delivery. Among all mothers, 70.5% had a normal prepregnancy BMI (18.5−23.9 kg/m^2), 57.6% received an education level of college or higher, and 58.9% reported a moderate annual household income in the range of 100,000−200,000 yuan per year. More than half of the mothers were primipara (58.0%). Approximately 15.6% of pregnant women suffered from environmental tobacco smoke exposure, and 5.4% reported alcohol drinking during pregnancy. A total of 36.2% of babies were born in summer and 27.7% of babies were born in spring. Among the newborns, the proportion of boys was slightly higher than that of girls (58.0 vs 42.0%). The mean birth weight, birth length, ponderal index, and gestational age of newborns were 3159.7 (± 334.7) g, 49.9 (± 1.6) cm, 25.5 (± 2.0) kg/m^3, and 39.3 (± 1.0) weeks, respectively.

Table 1. Basic Characteristics of the Mother−Newborn Pairs in the Study (N = 224)[a]

characteristics	N	mean ± SD or percent (%)
mothers		
age at delivery (years)	224	29.0 ± 4.3
prepregnancy BMI (kg/m^2)		
underweight (<18.5)	50	22.3
normal (18.5−23.9)	158	70.5
overweight (≥24)	16	7.1
maternal education		
less than high school	45	20.1
high school and equivalent	50	22.3
more than high school	129	57.6
annual household income (RMB, yuan)		
<100,000	35	15.6
100,000−200,000	132	58.9
>200,000	57	25.5
parity		
primiparous	130	58.0
multiparous	94	42.0
environmental tobacco smoke exposure		
yes	35	15.6
no	189	84.4
alcohol drinking		
yes	12	5.4
no	212	94.6
season of sample collection		
spring (march−may)	62	27.7
summer (june−august)	81	36.2
autumn (september−november)	54	24.1
winter (december−february)	27	12.1
newborns		
sex		
male	130	58.0
female	94	42.0
birth weight (g)	224	3159.7 ± 334.7
birth length (cm)	224	49.9 ± 1.6
ponderal index (kg/m^3)	224	25.5 ± 2.0
gestational age (weeks)	224	39.3 ± 1.0

[a]Abbreviations: SD, standard deviation and BMI, body mass index.

PFAS Concentrations in the Maternal Plasma and Hormone Levels in the Cord Blood. The distribution profiles of PFAS exposure during pregnancy and neonatal hormones are summarized in Table 2. Among the 22 PFASs detected, PFBA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFBS, PFHxS, PFOS, 6:2 Cl-PFESA, and 8:2 Cl-PFESA showed high detection rates in the range of 84.4−100%, while the remaining PFASs were not detectable or were detected in <30.0% of plasma samples. PFHxS, PFOS, and PFOA were the three most abundant analytes with high

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Table 2. Distribution of PFAS Concentrations (ng/mL) in Maternal Blood and Hormone Levels (ng/mL) in Umbilical Cord Blood (N = 224)[a]

			percentiles				
analytes	DR (%)	GM (95% CI)	5th	25th	50th	75th	95th
PFASs							
PFBA	100	0.30 (0.26, 0.34)	0.07	0.12	0.33	0.66	1.78
PFPeA	0.0						
PFHxA	0.0						
PFHpA	25.4						0.03
PFOA	100	3.32 (3.11, 3.54)	1.45	2.33	3.51	4.80	6.83
PFNA	100	0.51 (0.48, 0.54)	0.23	0.39	0.50	0.67	1.10
PFDA	100	0.47 (0.43, 0.50)	0.17	0.34	0.48	0.70	1.04
PFUnDA	100	0.53 (0.49, 0.57)	0.19	0.39	0.53	0.79	1.36
PFDoDA	84.4	0.06 (0.06, 0.07)	0.02	0.04	0.06	0.09	0.17
PFTrDA	100	0.06 (0.05, 0.06)	0.02	0.04	0.06	0.08	0.17
PFBS	93.3	0.02 (0.02, 0.02)	0.01	0.02	0.02	0.03	0.05
PFHxS	100	10.11 (9.53, 10.73)	5.14	7.67	10.36	13.44	19.41
PFOS	100	5.01 (4.59, 5.47)	1.75	3.32	5.01	7.62	12.65
PFDS	9.8						0.01
6:2 Cl-PFESA	100	1.70 (1.57, 1.84)	0.55	1.14	1.74	2.58	4.60
8:2 Cl-PFESA	99.6	0.02 (0.02, 0.02)	0.00	0.01	0.02	0.03	0.05
FOSAA	4.9						
N-MeFOSAA	24.6						0.00
N-EtFOSAA	11.2						0.00
6:2 FTS	4.5						
HFPO-DA	0.0						
NaDONA	0.0						
hormones							
GH	100	12.70 (11.77, 13.71)	4.60	8.71	12.90	19.40	34.20
IGF-1	85.7	42.91 (40.34, 45.65)	17.70	33.40	46.55	60.70	83.50
IGFBP-3	100	1260.1 (1223.6, 1297.8)	879.0	1080.0	1270.0	1460.0	1870.0

[a]Abbreviations: DR, detection rate; GM, geometric mean; and CI, confidence interval.

median concentrations (PFHxS: 10.36 ng/mL; PFOS: 5.01 ng/mL; and PFOA: 3.51 ng/mL) in all samples followed by 6:2 Cl-PFESA (1.74 ng/mL), PFUnDA (0.53 ng/mL), PFNA (0.50 ng/mL), and PFDA (0.48 ng/mL). Other PFASs had relatively low geometric concentrations. Emerging PFAS alternatives HFPO-DA, NaDONA, and 6:2 FTS were at an undetectable concentration. GH, IGF-1, and IGFBP-3 in newborns could be detected in most samples (>85.0%), and the corresponding geometric concentrations in the umbilical cord blood were 12.90, 46.55, and 1270.0 ng/mL, respectively. In the Pearson correlation analyses, significant and positive correlations among most PFASs, with r ranging from 0.15 to 0.93, were generally observed except for PFBA and PFHxS (Figure 1).

Associations of Prenatal Exposure to PFASs with the Neonatal Birth Size and Hormone Levels in MLR Models. The results of the MLR analyses for the associations between prenatal exposure to PFASs and the neonatal birth size are summarized in Table 3. In the adjusted linear models, the concentrations of all PFASs in the maternal plasma showed inverse correlations with birth weight, and most of these correlations reached a statistically significant level. For instance, each ln-unit increase in maternal PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFOS, and 8:2 Cl-PFESA concentrations was significantly associated with a decrease of 123.57 g (95% CI: −214.41, −32.74), 96.76 g (95% CI: −178.01, −15.50), 104.20 g (95% CI: −179.58, −28.83), 85.05 g (95% CI: −155.88, −14.23), 90.07 g (95% CI: −162.75, −17.38), 93.34 g (95% CI: −157.92, −28.75), and 59.95 g (95% CI: −119.72, −0.19) in birth weight, respectively. In addition, for one ln-unit increase in the maternal concentrations of PFOA, PFUnDA, and PFOS, the ponderal index decreased by 0.61 kg/m^3 (95% CI: −1.15, −0.06), 0.55 kg/m^3 (95% CI: −1.03, −0.07), and 0.67 kg/m^3 (95% CI: −1.08, −0.26), respectively. There was no significant correlation between prenatal exposure to PFASs and birth length.

Table 4 presents the associations between prenatal exposure to PFASs and neonatal hormone levels. After adjusting for confounders, each ln-unit increase in the maternal concentrations of PFBA was significantly related to a 3.20% (95% CI: −5.83, −0.49%) decrease in the levels of IGFBP-3 in the umbilical cord blood; however, there was a 9.36% (95% CI: 2.95, 16.16%) increase in IGFBP-3 levels linked with an increase in maternal PFOA concentrations. No significant correlation was observed between prenatal PFAS exposure and neonatal GH or IGF-1 levels. Furthermore, the results from the RCS models are shown in Figure S1, suggesting linear dose−response relationships of prenatal PFAS exposure with the neonatal birth size and hormone levels (all P for nonlinearity >0.05).

The results of the relationships between hormone levels and birth size are shown in Table S4. The GH levels were negatively associated with birth weight and birth length; IGF-1 levels were positively associated with birth weight, birth length, and ponderal index; and IGFBP-3 levels were positively associated with birth weight and birth length. Table S5 presents the mediation effects of IGFBP-3 on the associations

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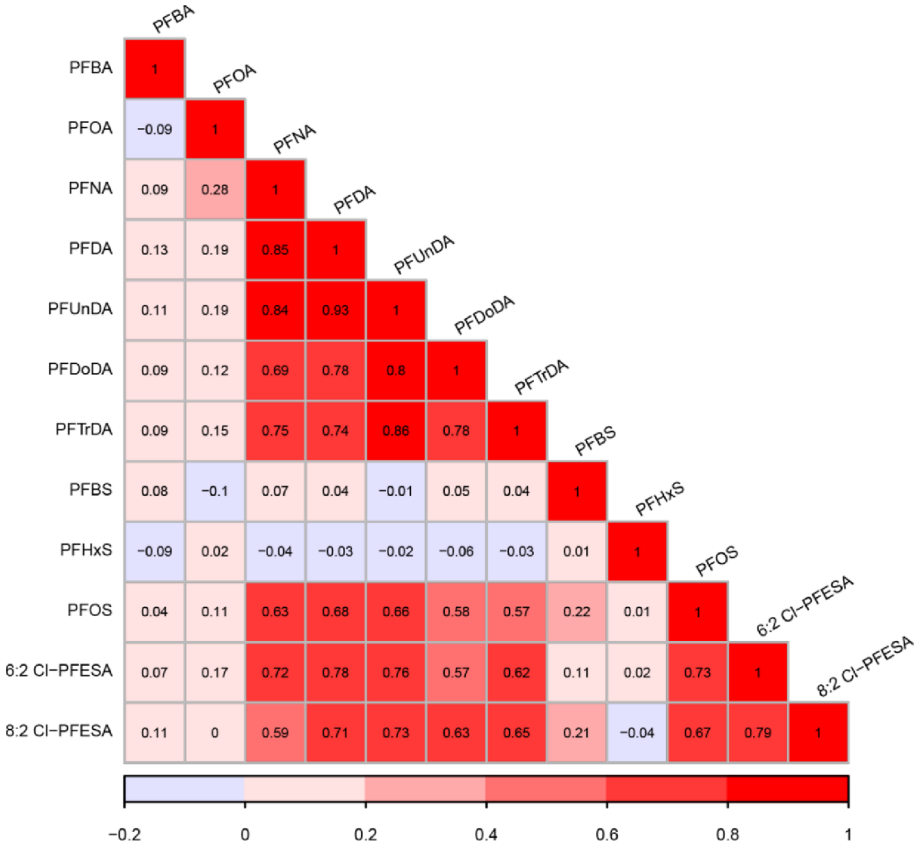

Figure 1. Heatmap of Pearson correlation coefficients among all PFASs.

Table 3. Regression Coefficients (95% CI) for the Associations between ln-Transformed PFAS Concentrations (ng/mL) in Maternal Blood and Birth Size (N = 224)[a]

	birth weight (g)	birth length (cm)	ponderal index (kg/m^3)
analytes	β (95% CI)	β (95% CI)	β (95% CI)
PFBA	−12.30 (−51.83, 27.24)	−0.15 (−0.35, 0.05)	0.12 (−0.13, 0.38)
PFOA	−62.37 (−149.08, 24.35)	0.08 (−0.36, 0.52)	−0.61 (−1.15, −0.06)*
PFNA	−123.57 (−214.41, −32.74)*	−0.37 (−0.83, 0.09)	−0.42 (−1.00, 0.17)
PFDA	−96.76 (−178.01, −15.50)*	−0.23 (−0.64, 0.19)	−0.45 (−0.96, 0.07)
PFUnDA	−104.20 (−179.58, −28.83)*	−0.20 (−0.59, 0.18)	−0.55 (−1.03, −0.07)*
PFDoDA	−85.05 (−155.88, −14.23)*	−0.27 (−0.63, 0.09)	−0.29 (−0.74, 0.16)
PFTrDA	−90.07 (−162.75, −17.38)*	−0.21 (−0.58, 0.17)	−0.43 (−0.89, 0.03)
PFBS	−31.62 (−99.92, 36.68)	−0.19 (−0.53, 0.16)	0.02 (−0.41, 0.46)
PFHxS	−12.50 (−106.78, 81.78)	0.04 (−0.43, 0.52)	−0.29 (−0.89, 0.31)
PFOS	−93.34 (−157.92, −28.75)*	−0.05 (−0.38, 0.28)	−0.67 (−1.08, −0.26)*
6:2 Cl-PFESA	−55.67 (−127.21, 15.87)	−0.09 (−0.45, 0.27)	−0.30 (−0.75, 0.15)
8:2 Cl-PFESA	−59.95 (−119.72, −0.19)*	−0.11 (−0.41, 0.19)	−0.33 (−0.71, 0.05)

[a]Abbreviation: CI, confidence interval. The models were adjusted for maternal age, prepregnancy BMI, education, parity, environmental tobacco smoke exposure, alcohol drinking, gestational age, and newborn sex. *P < 0.05.

between PFBA or PFOA and the birth size. IGFBP-3 positively mediated the effects of PFOA on birth weight and length (indirect effect); the negative association between PFOA and birth weight was identified after adjusting for IGFBP-3 (direct effect). We additionally adjusted for the season of sample collection or gestational diabetes mellitus in the MLR model, and the results did not change substantially (Tables S6 and S7).

Joint/Individual Effects of Prenatal Exposure to PFASs on the Neonatal Birth Size and Hormone Levels in BKMR Models. The grouped PIPs and conditional PIPs for PFASs calculated using BKMR are summarized in Table S8. In the BKMR model for birth weight, the grouped PIP of the group 1 was similar to that of the group 2, both of which were close to the 0.5 threshold. Furthermore, there were no PIPs of PFASs within the group 1 for birth weight greater than the 0.5 threshold, but the conditional PIP of PFOS within the group 2 was estimated to be 0.85, indicating that PFOS exposure within the context of coexposure mainly drives the effect of the group 2 on birth weight. Similarly, the grouped PIP value of the group 2 for the ponderal index exceeded the 0.5 threshold level, and PFOS showed the highest conditional PIP within the group 2, supporting that PFOS within the group 2 was the most important contributor to the decreased ponderal index. The grouped PIPs for other health outcomes did not reach the 0.5 threshold although some PFASs showed relatively high conditional PIPs.

The joint effects of prenatal exposure to a mixture of PFASs on neonatal birth size and hormone levels from BKMR models are given in the form of a graph in Figure 2. We found that there was an inverse overall association between exposure to a mixture of multiple PFASs during pregnancy and birth weight,

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Table 4. Percent Changes (95% CI) in Neonatal Hormone Levels (ng/mL) Associated with ln-Transformed PFAS Concentrations (ng/mL) in Maternal Blood (N = 224)[a]

	GH	IGF-1	IGFBP-3
analytes	percent changes (95% CI)	percent changes (95% CI)	percent changes (95% CI)
PFBA	−3.19% (−9.85, 3.96%)	−3.26% (−8.56, 2.34%)	−3.20% (−5.83, −0.49%)*
PFOA	8.87% (−6.93, 27.36%)	−2.64% (−14.01, 10.24%)	9.36% (2.95, 16.16%)*
PFNA	3.03% (−12.78, 21.71%)	−0.33% (−12.64, 13.70%)	1.43% (−4.98, 8.27%)
PFDA	0.70% (−13.20, 16.82%)	−0.10% (−11.16, 12.35%)	0.51% (−5.17, 6.53%)
PFUnDA	1.06% (−12.00, 16.05%)	−2.29% (−12.41, 9.00%)	1.44% (−3.91, 7.09%)
PFDoDA	6.36% (−6.53, 21.03%)	−4.71% (−13.96, 5.54%)	−1.79% (−6.64, 3.32%)
PFTrDA	−0.78% (−13.14, 13.33%)	−1.78% (−11.58, 9.11%)	1.78% (−3.38, 7.22%)
PFBS	0.15% (−11.49, 13.31%)	−4.77% (−13.61, 4.97%)	−0.53% (−5.23, 4.40%)
PFHxS	−2.37% (−17.65, 15.73%)	−11.08% (−22.20, 1.63%)	−0.53% (−6.94, 6.33%)
PFOS	−5.78% (−16.31, 6.07%)	−3.03% (−11.71, 6.51%)	−0.02% (−4.57, 4.74%)
6:2 Cl-PFESA	−2.61% (−14.46, 10.88%)	1.78% (−8.14, 12.78%)	1.60% (−3.43, 6.89%)
8:2 Cl-PFESA	−1.55% (−11.70, 9.76%)	−1.19% (−9.34, 7.69%)	0.83% (−3.38, 5.22%)

[a]Abbreviation: CI, confidence interval. The models were adjusted for maternal age, prepregnancy BMI, education, parity, environmental tobacco smoke exposure, alcohol drinking, gestational age, and newborn sex. *P < 0.05.

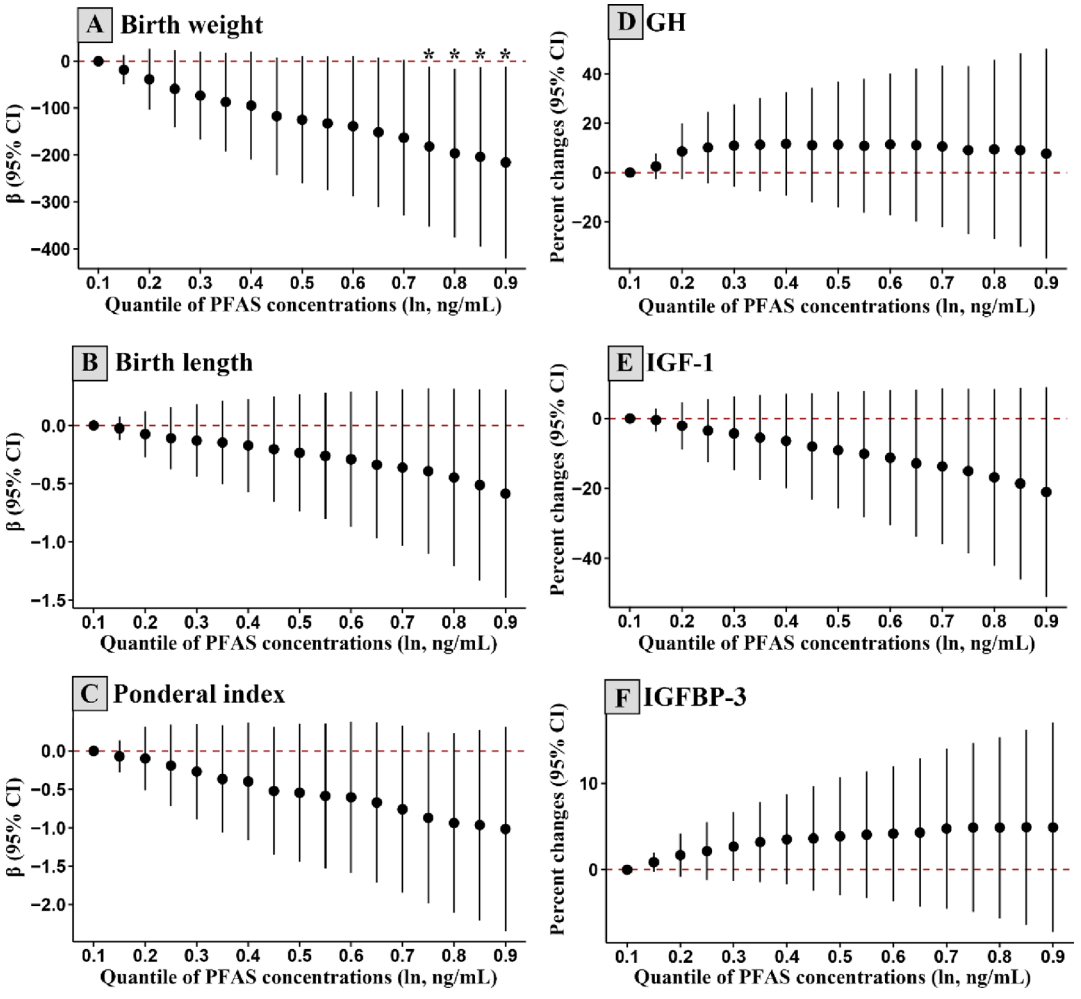

Figure 2. Joint effects of prenatal exposure to a mixture of multiple PFASs on neonatal birth weight (g), birth length (cm), ponderal index (kg/m^3), GH (ng/mL), IGF-1 (ng/mL), and IGFBP-3 (ng/mL) in BKMR models. The results were derived from the differences in health outcomes between the effects of exposure to12 PFASs simultaneously at a specific percentile (from the 10th to 90th percentile) and the effects of the exposures fixed at their 10th percentile. Circles indicate effect estimates, black vertical lines represent 95% CIs, and red horizontal dotted lines represent the null. The models were adjusted for maternal age, prepregnancy BMI, education, parity, environmental tobacco smoke exposure, alcohol drinking, gestational age, and newborn sex. BKMR, Bayesian kernel machine regression; BMI, body mass index; GH, growth hormone; IGF-1, insulin-like growth factor 1; and IGFBP-3, insulin-like growth factor binding protein 3. * P < 0.05.

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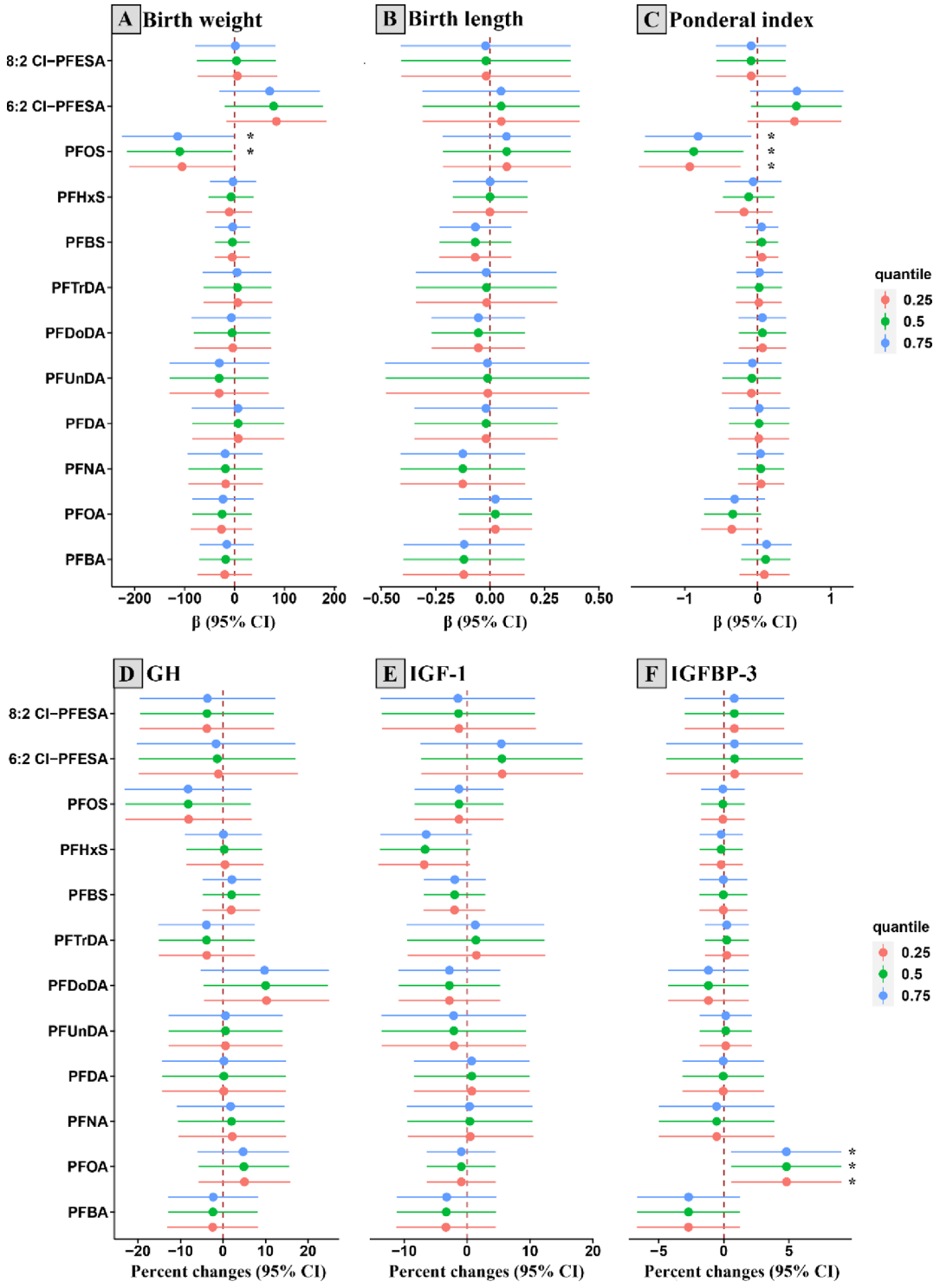

Figure 3. Individual effects of PFASs on neonatal birth weight (g), birth length (cm), ponderal index (kg/m^3), GH (ng/mL), IGF-1 (ng/mL), and IGFBP-3 (ng/mL) in BKMR models. This figure was plotted by comparing health effects caused by individual PFASs from its 25th to 75th percentile with all other PFASs held at the corresponding quantiles (25th, 50th, or 75th percentile). Circles indicate effect estimates, colored horizontal lines represent 95% CIs, and red vertical dotted lines represent the null. The models were adjusted for maternal age, prepregnancy BMI, education, parity, environmental tobacco smoke exposure, alcohol drinking, gestational age, and newborn sex. BKMR, Bayesian kernel machine regression; BMI, body mass index; GH, growth hormone; IGF-1, insulin-like growth factor 1; and IGFBP-3, insulin-like growth factor binding protein 3. * $P < 0.05$.

especially when the concentrations of 12 PFASs were concurrently fixed at or above the 75th percentile compared with when their concentrations were held at the 10th percentile (Figure 2A). The BKMR models also suggested inverse overall associations of the PFAS mixture with the birth length and ponderal index, but these associations were not statistically significant (Figure 2B, C). The associations of exposure to the PFAS mixture with neonatal GH, IGFBP-3,

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and IGF-1 levels did not reach statistical significance (Figure 2D−F). Figures S2−S4 show the joint effects of PFCA, PFSA, and Cl-PFESA mixtures on health indicators from BKMR models. Only an inverse overall association of a mixture of seven PFCAs with birth weight was observed in Figure S2 when holding the concentrations of all PFCAs at or above the 25th percentile; similarly, the mixture of three PFSAs showed an inverse overall association with the birth weight when fixing all PFSAs at or above the 55th percentile (Figure S3); however, no significant overall association of the mixture of two Cl-PFESAs with the birth weight was identified (Figure S4). The overall associations of PFCA, PFSA, and Cl-PFESA mixtures with birth length, ponderal index GH, IGFBP-3, and IGF-1 levels were not significant (Figures S2−S4).

We further explored the single-exposure effects of individual PFASs by assessing the change in the neonatal birth size and hormone levels linked with a change in individual PFAS from the 25th to 75th percentile when maintaining other PFASs at varying quantiles (25th, 50th, or 75th percentile) (Figure 3). A change in maternal PFOS concentrations from the 25th to 75th percentile was associated with a decrease of 105.09 g (95% CI: −210.91, 0.74), 109.85 g (95% CI: −215.25, −4.45), and 113.83 g (95% CI: −225.97, −1.69) in birth weight as well as a significant reduction of 0.93 kg/m^3 (95% CI: −1.63, −0.24), 0.88 kg/m^3 (95% CI: −1.56, −0.20), and 0.82 kg/m^3 (95% CI: −1.54, −0.09) in ponderal index, when other PFASs were assigned to the corresponding 25th, 50th, and 75th percentiles of combined exposure (Figure 3A, C). There was 4.82% (95% CI: 0.59, 9.06%), 4.82% (95% CI: 0.59, 9.05%), and 4.81% (95% CI: 0.57, 9.05%) increases in IGFBP-3 levels in relation to prenatal PFOA exposure ranging from the 25th to 75th percentile when holding other PFASs at the corresponding 25th, 50th, and 75th percentiles (Figure 3F). Additionally, we plotted the univariate exposure−response relationships between individual PFAS and the neonatal birth size and hormone levels when fixing the rest of the PFASs at their median levels. The results generated by the BKMR models indicated that most of the exposure−response functions were approximately linear, with some exceptions (Figures S5−S10). Even if there were nonlinear exposure−response curves for PFOS exposure associated with birth weight and ponderal index when fixing other PFASs at their median levels, it should be noted that the effect estimates of PFOS with high concentrations on the curves had considerable variability (Figures S5 and S7).

DISCUSSION

In the present study from Guangzhou, China, using traditional MLR models, we observed significant inverse associations of several long-chain PFASs (PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, and PFOS) and 8:2 Cl-PFESA, an alternative compound, in the maternal plasma with the birth weight as well as inverse associations of maternal PFOA, PFUnDA, and PFOS concentrations with the ponderal index. Additionally, PFBA concentrations in the maternal plasma were inversely related to IGFBP-3 levels in the umbilical cord blood, and PFOA in the maternal plasma had a positive correlation with IGFBP-3 levels in MLR models. The BKMR models further confirmed the associations of PFOS with the birth weight and ponderal index and of PFOA with IGFBP-3 and identified an inverse joint effect of prenatal exposure to a mixture of multiple PFASs on the birth weight. For the first time, we provide comprehensive evidence on the individual and joint effects of multiple PFASs on the neonatal birth size and hormones in the GH/IGF axis.

The results of our study indicated that pregnant women from Guangzhou, China, are extensively exposed to multiple PFASs, especially PFOS and PFOA. The comparison of PFAS concentrations in different study locations is summarized in Table S9. For the most abundant PFASs, PFOS and PFOA were detected in 100% of participants, which was consistent with other studies.[34,35,49] PFOS concentrations in this study (median: 5.01 ng/mL) were higher than those measured in participants from the USA (3.9 ng/mL),[35] France (3.07 ng/mL),[49] Japan (3.4 ng/mL)[33] and comparable to those reported in Sweden (5.38 ng/mL)[34] and Canada (4.7 ng/mL),[50] but lower than those observed in Denmark (30.1 ng/mL).[51] The levels of PFOA in our study (median: 3.51 ng/mL) were also generally higher than those measured in pregnant women from the USA (1.4 ng/mL),[35] France (1.05 ng/mL),[49] Sweden (1.61 ng/mL),[34] Canada (1.7 ng/mL),[50] and Japan (2.0 ng/mL)[33] and slightly lower than those reported in Denmark (4.6 ng/mL).[51] When compared with other Chinese cities, the concentrations of PFOS and PFOA in our population were higher than those in participants from Beijing[22] but comparable to those reported in Tianjin.[52] The concentrations of other PFASs (e.g., PFNA, PFDA, PFUnDA, and PFHxS) were higher than those found in other studies or comparable to those observed in other studies.[34,35,49,51] As for PFAS alternatives, the median value of 6:2 Cl-PFESA in our study (1.74 ng/mL) was slightly lower than that reported in the previous study of Guangzhou (2.41 ng/mL)[37] and comparable to that found in Wuhan (1.89 ng/mL)[19] but higher than those determined in Hangzhou (0.73 ng/mL)[38] and Beijing (0.09 ng/mL);[22] our 8:2 Cl-PFESA levels were higher than those measured in Guangzhou (0.001 ng/mL)[37] and were comparable to those reported in Hangzhou (0.021 ng/mL)[38] and Wuhan (0.05 ng/mL).[19] Overall, the concentrations of main PFASs in our study were slightly higher than those in pregnant women in developed countries in recent years. The discrepancies in PFAS concentrations among different countries might be mainly attributed to the regulatory measures against the usage of some PFASs in the USA and the European Union[53] and dietary habits.[54]

Although there is an increasing body of research examining the effects of exposure to legacy PFASs during pregnancy on fetal growth, the findings are ambiguous.[32] PFOS and PFOA are the two most representative traditional PFASs. The inverse associations of prenatal PFOS exposure with the birth weight observed in both MLR and BKMR models in our study are similar to previous findings. For instance, according to an updated report from the Danish National Birth Cohort (n = 3507), higher in utero PFOS exposure was significantly associated with a lower birth weight, with a median of 30.1 ng/mL for maternal plasma PFOS in 1996−2002.[51] Wikström et al. analyzed the related data extracted from 1533 mother−infants pairs in Sweden and observed an inverse relationship between PFOS concentrations in the maternal serum during early pregnancy and birth weight (median: 5.38 ng/mL, 2007−2010).[34] Similarly, cohort studies from the USA (n = 1630) and China (n = 372) showed consistent findings.[37,55] On the contrary, conflicting conclusions have been proposed by other research teams. A large-scale cohort study conducted by Kashino et al. in Japan (n = 1985) reported a null association between PFOS exposure and birth size.[33] Furthermore, a nonsignificant relationship between PFOS concentrations and

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birth outcomes was identified in 506 pregnant women in San Francisco.[36] We inferred that this discrepancy might be partly due to differences in exposure levels. As a result of the large amount of PFOS consumption in China, we have relatively higher PFOS concentrations (median: 5.01 ng/mL, 2017−2019) in the maternal plasma in the third trimester than those reported in the Japanese plasma in the third trimester (median: 3.40 ng/mL, 2003−2009)[33] and in the maternal serum from the USA in the second trimester (median: 1.93 ng/mL, 2014−2018).[36] In addition, differences in exposure windows, study population, sample size, and confounders might contribute to inconsistent results. More research needs to be performed to examine these relationships.

We note that the ponderal index as an indicator of asymmetrical intrauterine growth retardation has been found to be related to PFOA, PFUnDA, and PFOS exposure in MLR models; however, only the association of PFOS with the ponderal index remained significant in BKMR. Although there is limited evidence on the effects of PFAS exposure on the ponderal index, similar findings regarding PFOS have been reported by other studies.[38,56,57] A cross-sectional study (n = 293) in Maryland revealed significant negative associations of PFOS and PFOA in the cord serum collected in 2004−2005 with the ponderal index.[56] In another investigation of 177 mother−infant pairs from the Hokkaido study in Japan, Kobayashi et al. observed that only higher PFOS levels in the maternal serum were significantly associated with a lower ponderal index in fully adjusted models.[57] Additionally, a recent study with a small sample size of 98 from Hangzhou, China, also pointed out that prenatal PFOS exposure resulted in a significant decrease in the ponderal index.[38] Unfortunately, an earlier Chinese study in Zhoukou (n = 337) did not identify such a significant association, possibly due to the sample type (cord serum) with lower PFOS levels (median: 1.01 ng/mL, 2013−2015).[58] The latter three studies did not find a significant association between PFOA and the ponderal index, contrary to the result from our traditional MLR models. Perhaps, PFOA concentrations, sample types, sample size, and confounders have contributed in part to this difference. For example, the median of PFOA in the maternal plasma in our study (3.51 ng/mL) was generally higher than that reported in the Japanese serum collected at 24−41 weeks of gestation (median: 1.40 ng/mL, 2002−2005);[57] the samples used in Hangzhou (2016−2017)[38] and Zhoukou (2013−2015)[58] were cord serum, which was inconsistent with our samples.

In addition to PFOS and PFOA, human data on the developmental toxicity of several other PFASs were also investigated in this study. The MLR models identified significant associations of higher exposure to PFNA, PFDA, PFUnDA, PFDoDA, and PFTrDA during pregnancy with a lower birth weight in this study. Consistent with our results, the study carried out in Sweden (n = 1533) concluded that prenatal exposure to PFNA and PFDA could significantly reduce the birth weight.[34] Similarly, in a Japanese study (n = 1985), PFNA and PFDA were found to have inverse associations with birth weight of all infants, and an inverse association between PFTrDA levels and birth weight was suggested among girls.[33] However, other studies did not find these obvious correlations for birth weight.[35,58] Cao et al. used cord serum samples collected in 2013−2015 to determine the concentrations of PFASs among 377 women, and the majority of the population from the research by Gardener et al. (n = 433) was white with different genetic backgrounds from Chinese population, both of which might have led to the varied results among these studies.[35,58] We found that in the single-exposure module of BKMR, the relationship of the birth size in relation to long-chain PFASs observed in MLR models disappeared, except for PFOS. This exactly gives a hint that the results of MLR may not be reliable because of the high correlations between multiple PFASs in our study. Thus, further research should be conducted using more appropriate statistical methods to confirm our findings.

Although Cl-PFESAs, as alternatives to PFOS, have been widely used in the Chinese plating industry in the past decades, research on their environmental occurrence and health hazards has just begun. Several toxicological studies reported that 6:2 Cl-PFESA exposure could delay hatching, induce the occurrence of malformations, decrease the survival of zebrafish embryos, and cause damage to the reproductive system.[30,31] Our study using the traditional MLR model showed that maternal 8:2 Cl-PFESA exposure had a significant correlation with decreased birth weight. Nevertheless, to our knowledge, only three available epidemiological studies conducted in China have explored the relationships between Cl-PFESA exposure and birth outcomes, and the findings were contradictory. A cohort study in Guangzhou by Chu et al. measured PFAS concentrations in the maternal serum among 372 pregnant women recruited in 2013 and reported that higher levels of 6:2 Cl-PFESA and 8:2 Cl-PFESA were associated with a lower birth weight.[37] In a cross-sectional study of 98 newborns in Hangzhou in 2016−2017, cord serum 6:2 Cl-PFESA and 8:2 Cl-PFESA in relation to birth outcomes did not reach a statistically significant level.[38] Meanwhile, another investigation in Beijing in 2015−2016 also showed non-significant associations between maternal 6:2 Cl-PFESA levels and birth outcomes (n = 109) but a positive correlation between cord serum 6:2 Cl-PFESA and birth length (n = 90).[22] The levels of Cl-PFESAs might be responsible for these differences. Compared with the concentrations of Cl-PFESAs in our study, higher 6:2 Cl-PFESA (2.41 ng/mL) and lower 8:2 Cl-PFESA concentrations (0.001 ng/mL) in the maternal serum in the third trimester were determined in the previous study of Guangzhou.[37] Additionally, the discrepancies in sample types, confounders, and sample size might explain the varied findings. It was not surprising that the association for 8:2 Cl-PFESA observed in our MLR models became insignificant in BKMR due to the high correlation with other PFASs as mentioned above. Our results require further confirmation.

Although the mechanisms underlying the developmental toxicity of PFASs remain unclear, toxicological data have provided several potential pathways of action. Evidence showed that PFAS-induced adverse effects might be partly driven by activating peroxisome proliferator-activated receptors (PPARs), which regulate lipid and glucose metabolism and placental functions.[59] In cell assays of structure-based binding and activation of PFASs toward human PPARs, human PPARγ and PPARβ/δ binding affinities were closely related to the terminal functional groups of PFASs (sulfonate > carboxylate) in which PFOS has a stronger binding affinity than PFOA.[60,61] Most PFASs can activate human PPARγ and PPARβ/δ signaling pathways in a dose-dependent manner, and PFOS exerted similar or higher PPARγ and PPARβ/δ transcriptional activities than PFOA in accordance with the PPAR binding affinity.[60,61] In addition to human PPARs, the stronger binding affinity of PFOS with the human liver fatty acid binding

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protein might also account for the PFAS-mediated adverse effects on growth.[62] Thyroid function, known as a critical factor affecting fetal growth and development, is generally regarded as a potential mechanism. In vivo and vitro assays indicated that PFOS could directly bind to human thyroid hormone receptors (TR) with stronger binding potency than other PFASs (e.g., PFHxS, PFOA, PFNA, and PFUnDA) and activate the TR pathway with the highest potency.[63] Also, the binding affinity of PFOS with transthyretin was comparable to that of thyroxine (T4) and was stronger than that of other PFASs (e.g., PFHxS, PFOA, PFNA, PFDA, PFUnDA, and PFDoDA) in the T4 competitive binding assay.[64] It was reported that the regulation of glucose and lipid metabolisms by PFASs might be another reason for this. PFOS-treated rodents exhibited reduced serum cholesterol and triglyceride levels, while PFOA exposure might lead to insulin hypersensitivity and glucose tolerance in mice.[65,66] More experimental studies should be performed to understand the mechanisms of the detrimental effects caused by PFASs.

The GH/IGF system is a key endocrine pathway regulating fetal and postnatal growth. Not surprisingly, the significant effects of GH, IGF-1, and IGFBP-3 on the birth size were identified in the present study. Previous studies have also highlighted the importance of the GH/IGF axis in fetal growth and development. Consistent with our results, a prospective study conducted by Chiesa et al. in Italy reported the negative associations of cord GH with birth weight and length and the positive associations of cord IGF-1 and IGFBP-3 with birth weight, body length, and ponderal index among 153 mother−offspring pairs.[39] Similar findings from a birth cohort of Irish children supported the significant and positive relationships between IGF-1 levels and weight and length at birth.[67] As a key endocrine pathway, the GH/IGF axis is a feasible target of endocrine disruptors, and several studies have authenticated the role of the GH/IGF axis in PFAS-induced developmental toxicity. In a study on Atlantic salmon, both PFOS and PFOA exposure showed significant alterations in the expression of GH, IGF-1, and IGF-1 receptors in a time- and tissue-related pattern.[40] Exposure to PFOS at various levels was observed to downregulate the mRNA expression of genes related to GH receptors, IGF-1 receptors, and IGFBPs, which might contribute to the growth retardation induced by PFOS exposure in zebrafish.[41] A Japanese study found that prenatal PFOA exposure could decrease the methylation levels of IGF-2 in the cord blood, and the alteration of IGF-2 methylation explained approximately 21% of the effect of PFOA exposure on the ponderal index.[57] However, we did not observe significant associations of PFASs with GH and IGF-1 in the present study; instead, a positive association between PFOA and IGFBP-3 was suggested. To date, there has been only one study from the USA, by Lopez-Espinosa et al., exploring the associations between PFASs and IGF-1 levels.[42] They observed that higher PFOS and PFNA exposure had significant associations with lower IGF-1 levels among children aged 6−9 years.[42] Of note, this study was conducted among a childhood population and employed a cross-sectional design; consequently, whether prenatal exposure to PFASs exerts adverse effects on the GH/IGF axis in the offspring remains unknown. Given the enormous differences in exposure patterns and ethnic characteristics among different countries, it is important to carry out the present study in China. Certainly, our findings also need further verification.

Assessing the joint effects of environmental pollutants has been drawing more attention from the scientific community in environmental epidemiology. We noticed that most previous epidemiological studies used MLR models to evaluate the relationships between in utero individual PFAS exposure and birth outcomes.[33,37] Traditional MLR models can identify a simple and direct association between individual PFASs and health indicators more easily; however, human beings are commonly exposed to a variety of environmental pollutants simultaneously in the real-life scenario, and these concurrent exposures should be taken into account in conjunction. Additionally, when using MLR models to evaluate the health effects of combined exposure to PFASs, we face the key challenge of high correlations between multiple PFASs. Thus, a novel statistical method, the BKMR model, was introduced to explore the individual and joint effects of multiple PFASs on neonatal health in the present study. Several long-chain PFASs were observed to be associated with the birth size in MLR models in our study, but these associations tended to be null in BKMR models with the exception of PFOS. As a more conservative and stable method, the BMKR models can not only determine the joint effects of the PFAS mixture but also flexibly fit the PFAS-specific effects and exposure−response functions with other PFASs fixed at particular levels in either a linear or nonlinear way. Using BMKR models with a hierarchical variable selection approach to evaluate the health impacts of PFASs, we can address the multicollinearity caused by high correlations between compounds and explore the potential nonlinearity in the exposure−response functions as well as the control for multiple testing,[68] which may provide us with more stable and reliable results compared with the MLR models.

Our work has several strengths. First, we used a prospective study design and measured PFAS concentrations in the maternal blood and levels of hormones in the GH/IGF axis in the cord blood and collected anthropometric data from newborns, which allowed us to assess the potential causal associations of prenatal exposure to PFASs with the neonatal birth size and hormones. Second, the novel and flexible BMKR model that can overcome multicollinearity was used to quantify and visualize the joint and individual effects of multiple PFASs on health indicators, and it can also explore the potential nonlinearity in the exposure−response functions in the present study. Third, we determined a variety of PFASs, including legacy PFASs and their alternatives, and collected basic information from mother−newborn pairs using face-to-face questionnaires to systematically estimate the adverse effects of exposure to multiple PFASs after adjusting for potential confounders.

There were several limitations that should be noted in our study. First, this study population comprised only 224 mother−newborn pairs, which might limit the statistical power to identify weak effects and gain reliable and stable conclusions. Considering the availability of maternal and cord blood samples as well as the measurement costs, it is acceptable to conduct the present study in an exploratory nature and provide important evidence for the health effects of multiple PFASs. Second, one measurement of PFAS concentrations in the maternal plasma at one time point during pregnancy was used in this study to reflect the overall exposure to PFASs during the entire pregnancy period, which might lead to the misclassification of exposure. However, since PFASs are biopersistent with long half-lives, they have

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demonstrated good reproducibility across three trimesters.[19] Third, both the glomerular filtration rate (GFR) and the plasma volume increase during the process of pregnancy, which may lead to elevated PFAS excretion and dilution, affecting the fetal growth.[69] Unfortunately, GFR was not adjusted for in the models due to the lack of available information, and thus, the potential effect of hemodynamics on the PFAS−outcome associations cannot be ruled out in our study. Nevertheless, evidence suggests that the adjustment of GFR has little effect on the association between PFAS exposure and fetal growth.[70] Fourth, although major confounders have been controlled for in this study, the potential effects of other unavailable confounders (e.g., dietary intake, nutritional supplements, and other pollutants) might be unavoidable. Humans are commonly exposed to various environmental pollutants; thus, we could not accurately distinguish the effects of PFASs on health outcomes. Finally, a large proportion of mothers in this study had high education levels and good economic status, limiting the generalizability of our conclusions. Additional prospective studies on a larger scale are warranted to confirm our findings and uncover the underlying mechanisms.

In summary, we observed a significant inverse association of PFOS concentrations in the maternal blood with the birth weight and ponderal index of newborns as well as a positive association between maternal PFOA exposure and IGFBP-3 levels in the cord blood in both MLR and BKMR models. Additionally, in utero exposure to a mixture of multiple PFASs showed an inverse joint effect on the birth weight. These findings provide strong support for the detrimental effects of PFASs on the developing fetus. Further prospective research with large sample sizes is warranted to validate the findings.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c02670.

> Plasma PFAS analysis, abbreviations and full name of 22 PFASs (Table S1), MRM instrumental parameters (Table S2), methodology validation (Table S3), associations between hormones and birth size (Table S4), mediation analysis (Table S5), sensitivity analysis (Tables S6,S7), PIPs of BKMR models (Table S8), summary of PFAS median concentrations (Table S9), dose−response relationships of PFASs with the neonatal birth size and hormone levels by RCS models (Figure S1), joint effects of PFCAs, PFSAs, and Cl-PFESAs (Figures S2−S4), univariate exposure−response functions of each PFAS with birth size and hormone levels (Figures S5−S10) (PDF)

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Notes

The authors declare no competing financial interest.

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