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Associations of bisphenol mixture exposure with glucose metabolism during mid-pregnancy: A focus on emerging substitutes in a Chinese birth cohort

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Abstract

With the widespread use of bisphenol A (BPA) substitutes such as BPS and BPF, pregnant women are commonly exposed to complex mixtures of these endocrine-disrupting chemicals. However, epidemiological evidence on the combined effects of bisphenol mixtures on gestational glucose metabolism remains limited. This study included 1,258 pregnant women from the Wuhu Birth Cohort in China. Serum concentrations of BPA, BPS, BPF, and BPAF were measured using UHPLC-MS/MS. Glucose metabolism was assessed by 75-g OGTT measuring fasting, 1-hour, and 2-hour plasma glucose. We employed multivariable linear regression for single-chemical analysis and three advanced mixture models—Weighted Quantile Sum (WQS) regression, Quantile-based g-computation (Qgcomp), and Bayesian Kernel Machine Regression (BKMR)—to evaluate the joint effects. All four bisphenols were frequently detected, with BPS showing 100% detection rate. Single-chemical models revealed that BPS and BPF were significantly associated with elevated glucose levels at all time points (e.g., for BPS: β=0.371, 95% CI: 0.218-0.524 for 1-hour glucose). **The mixture analysis consistently demonstrated significant joint effects:** WQS showed a positive association between the mixture index and all glucose measures (e.g., β=0.129, 95% CI: 0.058-0.200 for 1-hour glucose); Qgcomp indicated that a simultaneous quartile increase in all bisphenols was associated with a 0.343 mmol/L rise in 1-hour glucose (95% CI: 0.179-0.507); BKMR revealed an approximately linear increase in glucose levels with mixture exposure. **Across all mixture models, BPS consistently emerged as the primary contributor,** with the highest weights in WQS (up to 0.542) and posterior inclusion probabilities approaching 1.0 in BKMR. Our study demonstrates that exposure to bisphenol mixtures significantly impairs mid-pregnancy glucose metabolism, with BPS and BPF serving as the predominant risk contributors. These findings underscore the necessity of mixture-based exposure assessment and support the inclusion of BPA substitutes in future public health regulations.

Highlights

- Pervasive exposure to bisphenol mixtures, including substitutes like BPS and BPF, was documented in a Chinese birth cohort of 1,258 pregnant women, with BPS detected in all serum samples.
- The bisphenol mixture was significantly associated with elevated glucose levels at all OGTT time points (e.g., a quartile increase in the mixture raised 1-hour plasma glucose by 0.343 mmol/L).
- Across three advanced mixture models (WQS, Qgcomp, BKMR), BPS consistently emerged as the primary driver of the adverse effects on mid-pregnancy glucose metabolism.
- Single-chemical analyses revealed significant positive and J-shaped associations of BPS and BPF with impaired glucose tolerance.
- The findings highlight the necessity of mixture-based exposure assessment in environmental health and implicate BPA substitutes as emerging risk factors requiring regulatory attention.

Introduction

Bisphenols (BPs), a class of high-production-volume chemicals widely used in consumer plastics, resins, and thermal papers, are recognized as endocrine-disrupting chemicals (EDCs) with multiple routes of human exposure, including diet, dermal contact, and inhalation **(Lee, et al., 2018; Zhou, et al., 2022)**. Structurally, bisphenols are characterized by their hormone-like activity, primarily acting as estrogen receptor agonists or androgen receptor antagonists, thus disrupting endocrine homeostasis(Li, et al., 2020). Among them, bisphenol A (BPA)—the most historically produced and widely used analogue—has been extensively studied for its multi-system toxicities**(Braun, et al., 2014; Casas, et al., 2015; Lin, Lee, Chuang, & Shih, 2023; Pan, et al., 2019; Williams, et al., 2025).** In response to growing health concerns and regulatory restrictions on BPA in many regions, several structural analogues, including bisphenol S (BPS), bisphenol F (BPF), and bisphenol AF (BPAF), have been increasingly used as substitutes in a range of consumer and industrial products**(Czarny-Krzymińska, Krawczyk, & Szczukocki, 2023; Wang, et al., 2023).**

EDCs are increasingly implicated in the development of metabolic disorders such as type 2 diabetes mellitus. A meta-analysis of observational studies assessed the relationship between BPA levels and the risk of GDM, concluding that there was no significant association between BPA concentrations and GDM risk(Koushki, et al., 2024). A few recent studies have begun to explore the associations of individual replacement bisphenols (e.g., BPS, BPF) with GDM or glycemic indicators during pregnancy, yet findings are inconsistent and limited by single-chemical analytical approaches(Soomro, et al., 2024; Wenxin Zhang, et al., 2019).

Critically, humans are exposed to multiple bisphenols simultaneously, and these chemicals may interact additively, synergistically, or antagonistically, leading to combined effects that cannot be predicted by studying single compounds in isolation. Traditional regression models are inadequate to fully decipher the complex, potentially non-linear relationships inherent in mixture exposures. Advanced mixture analytical methods, such as weighted quantile sum (WQS) regression, quantile-based g-computation (Qgcomp), and Bayesian kernel machine regression (BKMR), have been developed to assess the overall effect of chemical mixtures and identify key contributors(Cheng, et al., 2025; Zhang, et al., 2025; Zhou, et al., 2022). To date, the application of these methods to evaluate the joint effect of bisphenol mixtures on gestational glucose metabolism is exceedingly rare.

To address these significant knowledge gaps, we utilized data from a well-established Chinese birth cohort to comprehensively assess the relationship between exposure to a mixture of bisphenols (BPA, BPS, BPF, and BPAF) and mid-pregnancy oral glucose tolerance test (OGTT) results. Specifically, this study aimed to: 1) characterize the internal exposure profiles of four major bisphenols among pregnant women; 2) investigate the associations of individual bisphenols with fasting, 1-hour, and 2-hour plasma glucose levels; and 3) employ advanced mixture analysis methods (WQS, Qgcomp, and BKMR) to evaluate the combined effect of the bisphenol mixture and identify the primary drivers within the mixture. Our findings are expected to provide novel epidemiological evidence on the metabolic health risks associated with co-exposure to emerging bisphenol substitutes during pregnancy, thereby informing more accurate risk assessment and the development of public health strategies aimed at reducing exposure to complex environmental chemical mixtures.

Materials and methods

Study Population

This study employed a cohort design, with participants drawn from the Wuhu Birth Cohort Study in Anhui Province, China(Dou, et al., 2024). Eligible participants were pregnant women with confirmed intrauterine pregnancy, who intended to continue the pregnancy to delivery, planned to deliver locally, and were willing to cooperate with long-term follow-up. Exclusion criteria comprised unwillingness to participate, a history of major mental illness or other severe organic diseases, occurrence of spontaneous abortion, stillbirth, or fetal death during the study period, or a pre-pregnancy diagnosis of diabetes mellitus or hypertension. The study protocol received approval from the Ethics Committee of Anhui Medical University (Approval No. 20180081) and the Ethics Committee of Wuhu Maternity and Child Health Care Hospital (Approval No. 20220003). The research was conducted in strict accordance with the principles of the Declaration of Helsinki. All participants provided written informed consent prior to enrollment.

Data Collection

During the second-trimester prenatal visit, trained research staff administered a standardized, interviewer-administered questionnaire to collect detailed information. Pre-pregnancy weight was self-reported. Pre-pregnancy body mass index (BMI) was calculated as self-reported pre-pregnancy weight (kg) divided by measured height squared (m^2). Pre-pregnancy BMI was categorized according to Chinese standards: underweight (BMI < 18.5 kg/m^2), normal weight (18.5 ≤ BMI < 24.0 kg/m^2), and overweight/obesity (BMI ≥ 24.0 kg/m^2).

Assessment of Bisphenol Exposure

Residual maternal serum samples, remaining after completion of routine clinical testing, were collected daily from the hospital laboratory. Samples were linked to cohort participants, aliquoted into 1.5 mL polypropylene cryovials, and stored at -20°C in a dedicated biobank until analysis. This approach optimized the use of existing clinical specimens. Serum concentrations of bisphenol A (BPA), bisphenol AF (BPAF), bisphenol S (BPS), and bisphenol F (BPF) were quantified using a validated method based on liquid-liquid extraction coupled with ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS), as previously described(Dou, et al., 2024). The method detection limits (MDL, defined as signal-to-noise ratio S/N=3) were 66.67 pg/mL for BPA, 0.83 pg/mL for BPAF, 1.67 pg/mL for BPS, and 33.33 pg/mL for BPF. Values below the MDL were imputed as MDL/√2(Kim, et al., 2022). Detailed methodology is described in the our prior paper(Huang, 2022).

OGTT Administration

Upon completion of the mid-pregnancy questionnaire, and after obtaining informed consent from each participant, a complimentary 75-g oral glucose tolerance test (OGTT) was offered to all pregnant women. The test included measurements of fasting plasma glucose (FPG), 1-hour plasma glucose (1-hour PG), and 2-hour plasma glucose (2-hour PG).

Statistical Analysis

Descriptive statistics were used to summarize participant characteristics and exposure distributions. Bisphenol concentrations were natural log-transformed to approximate normality and standardized (z-scores) for subsequent analyses. Spearman rank correlation coefficients were calculated to assess bivariate associations between individual bisphenol concentrations and OGTT outcomes.

To evaluate the association of each bisphenol analogue with glucose metrics, multivariable linear regression models were employed. Each bisphenol (ln-transformed) was entered individually as the independent variable, with FPG, 1-hour PG, and 2-hour PG as separate dependent variables. Models were adjusted for a priori selected potential **confounders**: maternal age (continuous), annual household income (categorical), educational attainment (categorical), and pre-pregnancy BMI (categorical). To explore potential non-linear dose-response relationships, we employed two approaches. First, bisphenol concentrations were categorized into quartiles, and the median value within each quartile was modeled as a continuous variable to test for trend. Second, restricted cubic splines (RCS) with three knots placed at the 5th, 35th, 65 th and 95th percentiles were fitted to visualize the shape of the exposure-response relationship for each significant bisphenol.

To investigate the joint effect of the bisphenol mixture on glucose metabolism, we applied three complementary mixture analysis methods: Weighted Quantile Sum (WQS) regression, Quantile-based g-computation (Qgcomp), and Bayesian Kernel Machine Regression (BKMR). For WQS, bisphenol concentrations were first transformed into quartiles. A weighted index was constructed in a training set (60% of data) by estimating weights that maximize the association between the index and the outcome, constrained to be positive and sum to 1. The index's association with the outcome was then validated in the remaining test set (40%). The derived weights indicate the relative contribution of each compound to the overall mixture effect. Qgcomp was used to estimate the joint effect of increasing all bisphenols by one quartile simultaneously, allowing for both positive and negative directional weights for individual components, thus identifying the overall mixture effect and the contribution direction of each analogue. Finally, BKMR was implemented to model the potentially complex, non-linear, and interactive effects of the mixture. A Gaussian kernel was used, and models were run with 20,000 Markov Chain Monte Carlo (MCMC) iterations after a burn-in of 5,000 iterations. The cumulative effect of the mixture was estimated by comparing the predicted outcome when all exposures were at a given percentile (e.g., 75th) to when all were at the 50th percentile. Posterior inclusion probabilities (PIPs) were calculated to identify the most important mixture components, with a PIP>0.5 considered indicative of a meaningful contribution. Univariate exposure-response functions and bivariate interaction plots were derived from the BKMR model to illustrate individual and interactive effects(Cheng, et al., 2025; Zhang, et al., 2025).All conventional regression analyses were performed using IBM SPSS Statistics (Version 22.0). Mixture analyses (WQS, Qgcomp, BKMR) and RCS modeling were conducted in R software (Version 4.3.3; R Foundation for Statistical Computing) utilizing the gWQS, qgcomp,bkmr, and rms packages, respectively. Statistical significance was defined as a two-sided P-value < 0.05.

Results

Basic characteristics of participants

Among 1,258 enrolled participants, 50.1% were under 30 years of age, 37.3% were 30–34 years, and 12.6% were ≥ 35 years. Educational attainment included college diploma (35.2%), bachelor's degree or higher (26.5%), high school/technical secondary school (19.4%), and junior high school or below (18.9%). Household annual income was distributed across five categories (<50,000 to > 150,000 CNY), each representing 16.4%–24.6% of participants. By pre-pregnancy BMI, 67.2% were normal weight, 21.4% overweight or obese, and 11.4% underweight.

Bisphenol exposure was ubiquitous. BPS was detected in all samples, BPAF in 98.9%, BPF in 84.0%, and BPA in 85.1%. Median concentrations were highest for BPA (238.8 ng/g; max 7796.0 ng/g), followed by BPF (108.2 ng/g), BPS (20.5 ng/g), and BPAF (8.5 ng/g). The sum of bisphenols (ΣBPs) ranged from 84.4 to 7947.9 ng/g (median 445.8 ng/g).

Regarding glycemic measures, FPG ranged from 3.4 to 8.5 mmol/L, with a mean of 4.573 mmol/L (SD = 0.4200). 1h PG levels ranged widely from 1.3 to 15.2 mmol/L (mean = 7.942, SD = 1.7226). 2hPG **levels ra**nged from 3.6 to 14.0 mmol/L (mean = 6.873, SD = 1.3234).

Association between single bisphenol and OGTT results

As shown in Table 1, after adjustment for covariates, BPS demonstrated significant positive associations with FPG (β = 0.088, 95% CI: 0.050–0.126), 1h PG (β = 0.371, 95% *CI*: 0.218–0.524), and 2hPG (β = 0.275, 95% *CI*: 0.157–0.393) (all $P < 0.001$). Similarly, BPF was positively associated with FPG (β = 0.020, 95% CI: 0.000–0.039; $P = 0.048$), 1h-PG (β = 0.118, 95% CI: 0.039–0.196; P = 0.003), and 2h-PG (β = 0.072, 95% CI: 0.012–0.133; $P = 0.019$).

Table 1
Associations of bisphenol Compound with OGTT

Bisphenols[a]		FPG		1h PG		2h PG	
		β(95%CI)[b]	P value	β(95%CI)[b]	P value	β(95%CI)[b]	P value
BPA	Continuous	0.001(-0.016,0.018)	0.913	0.05(-0.021,0.120)	0.165	0.036(-0.018,0.09)	0.191
	Q1	0(reference)		0(reference)		0(reference)	
	Q2	0.024(-0.041,0.089)	0.468	-0.068(-0.33,0.194)	0.612	-0.014(-0.217,0.188)	0.889
	Q3	-0.007(-0.072,0.059)	0.842	-0.119(-0.382,0.144)	0.375	0.001(-0.202,0.204)	0.995
	Q4	0.028(-0.036,0.093)	0.390	0.146(-0.116,0.407)	0.274	0.076(-0.125,0.278)	0.458
	P for trend	0.005(-0.015,0.026)	0.599	0.039(-0.044,0.121)	0.360	0.024(-0.039,0.088)	0.453
BPAF	Continuous	0.016(-0.009,0.042)	0.218	0.063(-0.04,0.166)	0.227	0.038(-0.042,0.117)	0.354
	Q1	0(reference)		0(reference)		0(reference)	
	Q2	0.027(-0.038,0.092)	0.412	0.169(-0.093,0.43)	0.207	0.085(-0.117,0.287)	0.408
	Q3	0.053(-0.012,0.118)	0.107	0.261(-0.002,0.523)	0.051	0.047(-0.156,0.249)	0.652
	Q4	0.032(-0.033,0.097)	0.336	0.129(-0.133,0.39)	0.334	0.047(-0.155,0.249)	0.650
	P for trend	0.012(-0.008,0.033)	0.244	0.048(-0.035,0.131)	0.256	0.01(-0.054,0.074)	0.753
BPS	Continuous	**0.088(0.05,0.126)**	**< 0.001**	**0.371(0.218,0.524)**	**< 0.001**	**0.275(0.157,0.393)**	**< 0.001**
	Q1	0(reference)		0(reference)		0(reference)	
	Q2	0.056(-0.009,0.121)	0.091	0.097(-0.165,0.359)	0.468	0.128(-0.074,0.331)	0.213
	Q3	0.022(-0.043,0.087)	0.506	0.019(-0.243,0.281)	0.887	0.061(-0.141,0.263)	0.552
	Q4	**0.108(0.043,0.172)**	**0.001**	**0.444(0.183,0.706)**	**0.001**	**0.312(0.111,0.514)**	**0.002**
	P for trend	**0.029(0.008,0.049)**	**0.006**	**0.126(0.043,0.209)**	**0.003**	**0.087(0.023,0.151)**	**0.008**
BPF	Continuous	**0.02(0,0.039)**	**0.048**	**0.118(0.039,0.196)**	**0.003**	**0.072(0.012,0.133)**	**0.019**
	Q1	0(reference)		0(reference)		0(reference)	
	Q2	-0.001(-0.066,0.063)	0.965	-0.045(-0.307,0.217)	0.736	-0.036(-0.238,0.165)	0.723
	Q3	0.025(-0.04,0.09)	0.45	0.143(-0.118,0.404)	0.284	-0.051(-0.252,0.151)	0.622
	Q4	**0.086(0.021,0.151)**	**0.009**	**0.323(0.062,0.584)**	**0.015**	**0.267(0.066,0.468)**	**0.009**
	P for trend	**0.028(0.008,0.049)**	**0.007**	**0.116(0.033,0.198)**	**0.006**	**0.079(0.015,0.142)**	**0.016**
ΣBPs	Continuous	0.027(-0.002,0.055)	0.064	**0.206(0.092,0.321)**	**< 0.001**	**0.139(0.051,0.227)**	**0.002**
	Q1	0(reference)		0(reference)		0(reference)	
	Q2	**0.085(0.02,0.15)**	**0.010**	0.083(-0.179,0.345)	0.534	0.085(-0.116,0.287)	0.407
	Q3	**0.1(0.035,0.164)**	**0.003**	0.218(-0.044,0.479)	0.103	**0.273(0.071,0.474)**	**0.008**

Abbreviations: FPG, fasting plasma glucose; 1-hour PG, 1-hour plasma glucose;1-hour PG, 2-hour plasma glucose; BPA, bisphenol A; BPAF, bisphenol AF; BPS, bisphenol S; BPF, bisphenol F; Q1, the first quartile; Q2, the second quartile; Q3, the third quartile; Q4, the fourth quartile; CI, confidence interval. Bolded values were considered statistically significant. a Bisphenol levels were ln-transformed and standardized. b: Adjusted for maternal age, household income, educational level, and pre-pregnancy BMI.

Bisphenols[a]	FPG		1h PG		2h PG	
	β(95%CI)[b]	P value	β(95%CI)[b]	P value	β(95%CI)[b]	P value
Q4	0.06(-0.005,0.125)	0.069	**0.365(0.105,0.626)**	**0.006**	**0.248(0.047,0.449)**	**0.016**
P for trend	0.02(-0.001,0.04)	0.061	**0.123(0.04,0.206)**	**0.004**	**0.093(0.029,0.157)**	**0.004**

Abbreviations: FPG, fasting plasma glucose; 1-hour PG, 1-hour plasma glucose;1-hour PG, 2-hour plasma glucose; BPA, bisphenol A; BPAF, bisphenol AF; BPS, bisphenol S; BPF, bisphenol F; Q1, the first quartile; Q2, the second quartile; Q3, the third quartile; Q4, the fourth quartile; CI, confidence interval. Bolded values were considered statistically significant. a Bisphenol levels were ln-transformed and standardized. b: Adjusted for maternal age, household income, educational level, and pre-pregnancy BMI.

Quartile-based analyses revealed J-shaped exposure-response relationships. For BPS, compared with the lowest quartile (Q1), the highest quartile (Q4) was associated with significantly increased FPG (β = 0.108, 95% *CI*: 0.043–0.172; *P* = 0.001), 1-hour PG (β = 0.444, 95% *CI*: 0.183–0.706; *P* = 0.001), and 2-hour PG (β = 0.312, 95% *CI*: 0.111–0.514; *P* = 0.002), whereas Q2 and Q3 showed no significant associations. A similar pattern was observed for BPF, with Q4 demonstrating elevated FPG (β = 0.086, 95% *CI*: 0.021–0.151; *P* = 0.009), 1-hour PG (β = 0.323, 95% *CI*: 0.062–0.584; P = 0.015), and 2-hour PG (β = 0.267, 95% *CI*: 0.066–0.468; *P* = 0.009). Significant positive trends across quartiles were observed for both compounds (all *P* for trend < 0.05). The visualization results are shown in Fig. 1.

Association between the bisphenol mixture and OGTT results

Figure 2 presents the findings of the WQS regression analyses investigating the associations between the bisphenol mixture and glucose metabolism indicators. Adjusted for covariates, a one-quartile increase in the WQS index of the bisphenol mixture was positively associated with FPG (β = 0.023, 95% *CI*: 0.005 to 0.041), 1-hour PG (β = 0.129, 95% CI: 0.058 to 0.200), and 2-hour PG (β = 0.081, 95% CI: 0.030 to 0.132). Within the mixture, BPS consistently demonstrated the highest contribution across all three endpoints, with weights ranging from 0.425 (1h PG) to 0.542 (2h PG). BPF ranked as the second major contributor for FPG (0.266) and 2-hour PG (0.280), while for 1-hour PG, BPA (0.140) and BPAF (0.062) showed increased relative contributions compared to other endpoints.

The bisphenol mixture was significantly associated with elevated glucose levels across all time points. Specifically, a simultaneous quartile increase in all bisphenols was associated with an increase of 0.079 mmol/L in FPG (95% CI: 0.038–0.120; *P* < 0.001), 0.343 mmol/L in 1-hour PG (95% CI: 0.179–0.507; *P* < 0.001), and 0.207 mmol/L in 2-hour PG (95% CI: 0.081–0.334; *P* = 0.001). Within the mixture, BPS and BPF consistently demonstrated the strongest positive contributions across all glycemic parameters. The mixture composition contained exclusively positive weights for both fasting and 1-hour postprandial glucose. For 2-hour PG, BPAF exhibited a marginal negative weight (− 0.001) while other bisphenols showed positive contributions (Fig. 3).

In the BKMR models, FPG, 1-hour PG, and 2-hour PG all showed an approximately linear increase as the bisphenol mixture rose from the 10th to the 90th percentile (Fig. 4A–C). Among the components of the mixture, BPS exhibited the highest contribution to FPG (posterior inclusion probability, PIP = 0.978), 1-hour PG (PIP = 1.000), and 2-hour PG (PIP = 1.000). As shown in Fig. S1–S6, the univariate exposure–response relationships between individual bisphenols and FPG, 1-hour PG, and 2-hour PG are illustrated, with the remaining non-exposure mixture components fixed at their median concentrations. Notably, BPS displayed a positive linear association with FPG, 1-hour PG, and 2-hour PG.

Interactions between different bisphenol mixture

Figures S7–9 illustrate the interactions between different bisphenol compounds. Fig. S7 shows no significant interaction among bisphenol compounds in their association with FPG. Figs. S8–S9, however, suggest potential synergistic interactions between BPS and BPAF on 1-hour and 2-hour PG.

Discussion

This prospective cohort study provides novel epidemiological evidence on the associations between exposure to bisphenol mixtures and mid-pregnancy glucose metabolism among Chinese women. By employing both traditional single-pollutant and advanced mixture analytical methods, our findings reinforce the concern that emerging BPA substitutes (BPS and BPF) may pose independent and joint risks to glycemic control during a sensitive life stage.

Our results demonstrate ubiquitous co-exposure to multiple bisphenols, with BPS and BPF being the primary drivers of the observed mixture effect on elevated OGTT levels. The single-pollutant analyses revealed significant positive associations, particularly for BPS and BPF, across all three glycemic time points. Notably, the exposure-response relationships for these two compounds exhibited J-shaped patterns, suggesting that adverse effects on glucose homeostasis may become more pronounced only after a certain exposure threshold is exceeded. This non-linearity aligns with the concept of endocrine disruptors having complex dose-response curves that are not always monotonic(Stavros, et al., 2025; Yadav, et al., 2023). The strong and consistent signal from BPS is of particular public health relevance. As a primary replacement for BPA, BPS is increasingly prevalent in consumer products. Our findings, showing its dominant contribution (PIPs approaching 1.0 in BKMR models) to the mixture effect, challenge the assumption of its safety and underscore the "regrettable substitution" paradigm, where a banned chemical is replaced by a structurally similar one with potentially comparable or unknown toxicity(Bousoumah, et al., 2021; Tillotson, et al., 2026).

The application of three complementary mixture models (WQS, Qgcomp, BKMR) represents a major methodological strength of this study, allowing for a more robust assessment of the joint effect than any single method alone. The convergent results across these models strengthen the inference that co-exposure to bisphenols, at levels found in this general pregnant population, is adversely associated with glucose regulation. The overall mixture effect estimates from Qgcomp indicate that a simultaneous quartile increase in all bisphenols is associated with clinically meaningful elevations in plasma glucose (e.g., 0.343 mmol/L for 1-hour PG). While the observed interaction between BPS and BPAF on post-load glucose requires further confirmation, it highlights the potential for complex inter-chemical dynamics that single-chemical models cannot capture(Taylor, et al., 2016). The largely additive effects observed for the mixture align with a shared mechanism of action, likely through disruption of pancreatic β-cell function and induction of peripheral insulin resistance. Experimental studies indicate that BPA, a xenoestrogen, mimics 17β-estradiol effects in vivo, altering pancreatic β-cell insulin storage and release via genomic and nongenomic estrogen receptor signaling pathways. In vitro and in vivo models further demonstrate that BPA exposure disrupts glucose homeostasis and β-cell metabolism, including altered gene expression and mitochondrial morphology, while also contributing to insulin resistance developmen(Alonso-Magdalena, Morimoto, Ripoll, Fuentes, & Nadal, 2006; Chevalier & Fénichel, 2015; Farrugia, Aquilina, Vassallo, & Pace, 2021). The emerging BPA substitutes (BPS, BPF, BPAF) share a similar phenolic structure and likely exhibit comparable estrogenic activities, potentially mediating adverse metabolic outcomes through analogous pathways—especially at low, environmentally relevant doses where estrogenic potency may be significant. Consequently, the cumulative impact of these structurally related bisphenols may result in predominantly additive effects on glucose regulation, reflecting their common ability to interfere with insulin secretion and sensitivity.

Our findings must be interpreted within the context of the existing literature. While a prior meta-analysis found no significant association between BPA and GDM risk(Koushki, et al., 2024). our single-chemical models also did not find a strong, consistent signal for BPA across all outcomes. This may reflect a true difference in the metabolic toxicity of BPA compared to its substitutes in our study population, differences in exposure assessment (serum vs. urine), or the possibility that BPA's effect is more modest and discernible only in mixture analyses. The pronounced effect of BPF and BPS corroborates some recent studies suggesting these substitutes may be potent metabolic disruptors(Tang, et al., 2023; W. Zhang, et al., 2019), but contrasts with others, highlighting the need for further replication in diverse populations. Importantly, our study advances the field by moving beyond single chemicals, providing some of the first epidemiological evidence that the combined burden of these commonly co-occurring chemicals is associated with impaired glucose tolerance.

This study has several limitations. First, the cross-sectional assessment of bisphenol exposure and OGTT outcomes during the second trimester precludes the establishment of temporal causality. Although we adjusted for key confounders, residual confounding from unmeasured factors (e.g., detailed diet, physical activity, exposure to other non-persistent chemicals) cannot be ruled out. Second, we utilized a single serum measurement to assess exposure, which may not fully capture the variability of these non-persistent chemicals over time and could lead to exposure misclassification, likely biasing results toward the null. Third,

while our mixture methods are state-of-the-art, they are not without assumptions, and the results can be sensitive to model specifications and correlations between exposures (Bobb et al., 2015). Fourth, the generalizability of our findings may be limited to similar Chinese pregnant populations, and external validation in other ethnic and geographic settings is warranted. Finally, the clinical significance of the observed glucose elevations, though statistically significant, in relation to the diagnosis of GDM requires further investigation in studies with larger numbers of clinical endpoints.

Conclusion

In summary, this study demonstrates that exposure to bisphenol mixtures, rather than to a single compound in isolation, is associated with elevated plasma glucose levels during mid-pregnancy among Chinese women. BPS and BPF emerged as the primary contributors to this mixture effect. These findings underscore the critical importance of adopting a mixture exposure framework in environmental epidemiology and risk assessment, particularly as humans are constantly exposed to complex chemical cocktails. From a public health perspective, our results suggest that regulatory and intervention strategies aimed at protecting pregnant women's metabolic health should consider the cumulative impact of bisphenol co-exposure, moving beyond a sole focus on BPA.

Declarations

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Author contribution

ZHH, AHZ: Investigation, Formal analysis, Data curation, Writing – original draft, review & editing.

MZ: Investigation, Methodology, Data curation.

QZ, LJD: Validation, Investigation, Methodology.

JYM: Conceptualization, Supervision, Resources, Funding acquisition, Project administration, Writing – review & editing.

Data availability All data supporting the fndings of the present study is available from the corresponding author on reasonable request.

Ethical approval and consent to participate The authors declare that they have read and approved the manuscript and agree that the work is ready for submission.

Consent for publication The authors gave consent for the publications of the manuscript.

Competing interests The authors declare no competing interests.

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Figures

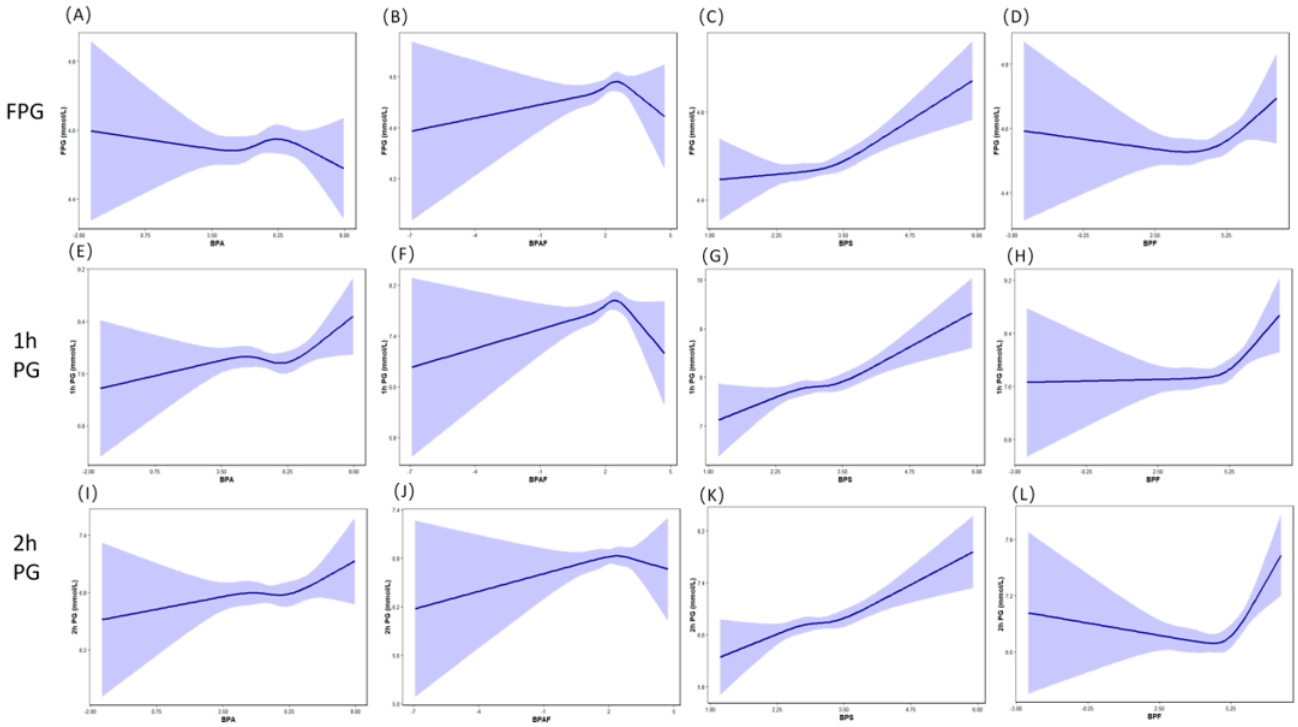

Figure 1

The dose-response associations between the single bisphenol and OGTT results using linear regression models. (A) BPA and FPG; (B) BPAF and FPG; (C) BPS and FPG; (D) BPF and FPG; (E) BPA and 1-hour PG; (F) BPAF and 1-hour PG; (G) BPS and 1-hour PG; (H) BPF and 1-hour PG; (I) BPA and 2-hour PG; (J) BPAF and 2-hour PG; (K) BPS and 2-hour PG; (L) BPF and 2-hour PG. blue dashed lines, reference values; dark blue shades, 95% conffdence intervals. Bisphenol levels were ln-transformed and standardized. Covariates in the model included maternal age, household income, educational level, and pre-pregnancy BMI.

Abbreviations: FPG, fasting plasma glucose (mmol/L); 1-hour PG, 1-hour plasma glucose (mmol/L); 2-hour PG, 2-hour plasma glucose (mmol/L); BPA, bisphenol A; BPAF, bisphenol AF; BPS, bisphenol S; BPF, bisphenol F.

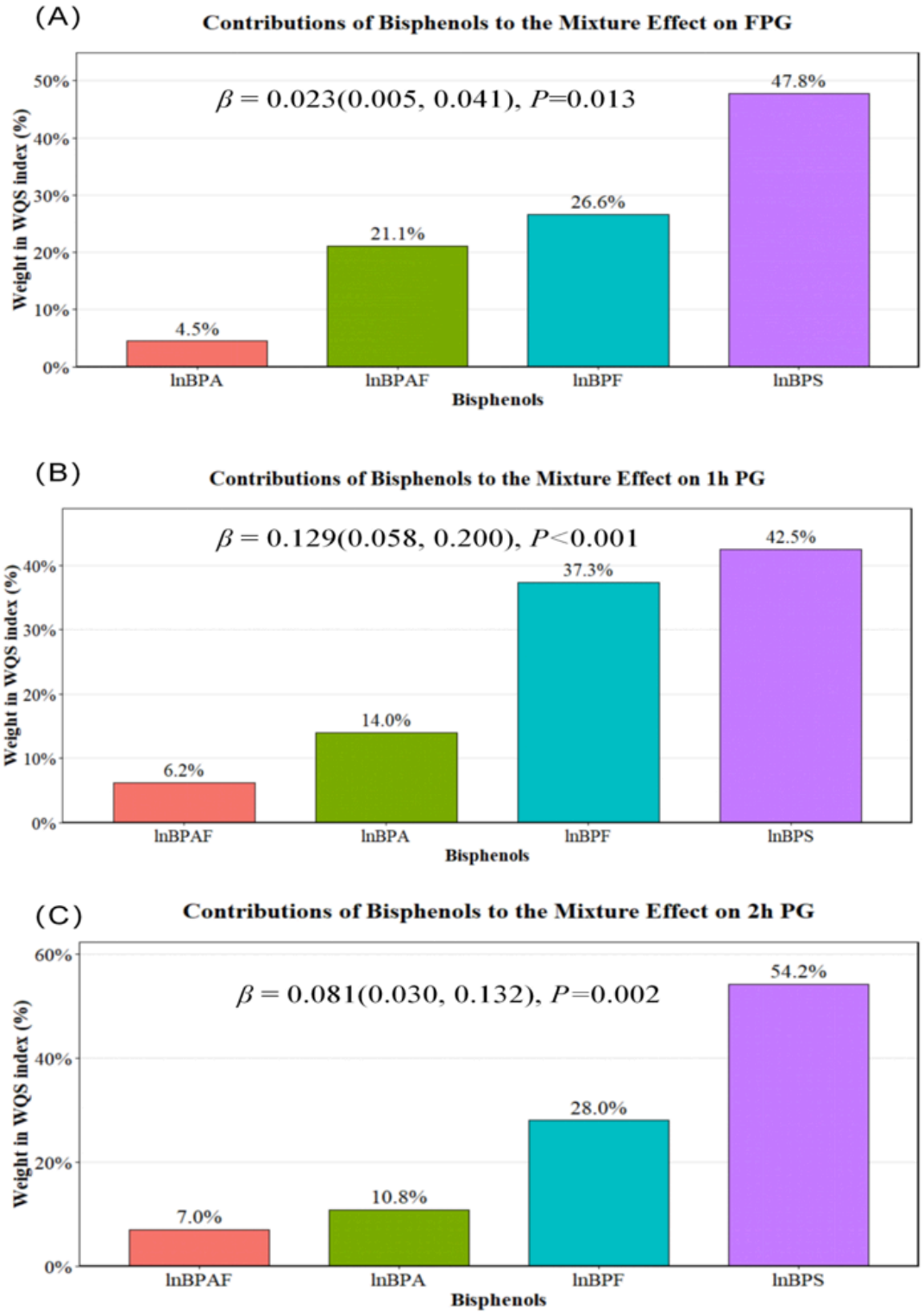

Figure 2

Analysis of the combined effects of bisphenol compounds on FPG (A), 1-hour PG (B), and 2-hour PG (C) using weighted quantile sum (WQS) regression models. Bisphenol levels were ln-transformed and standardized. Covariates in the model included maternal age, household income, educational level, and pre-pregnancy BMI. The bar charts display the estimated weights for each bisphenol within the mixture.

Abbreviations: FPG, fasting plasma glucose (mmol/L); 1-hour PG, 1-hour plasma glucose (mmol/L); 2hPG, 2-hour plasma glucose (mmol/L); BPA, bisphenol A; BPAF, bisphenol AF; BPS, bisphenol S; BPF, bisphenol F.

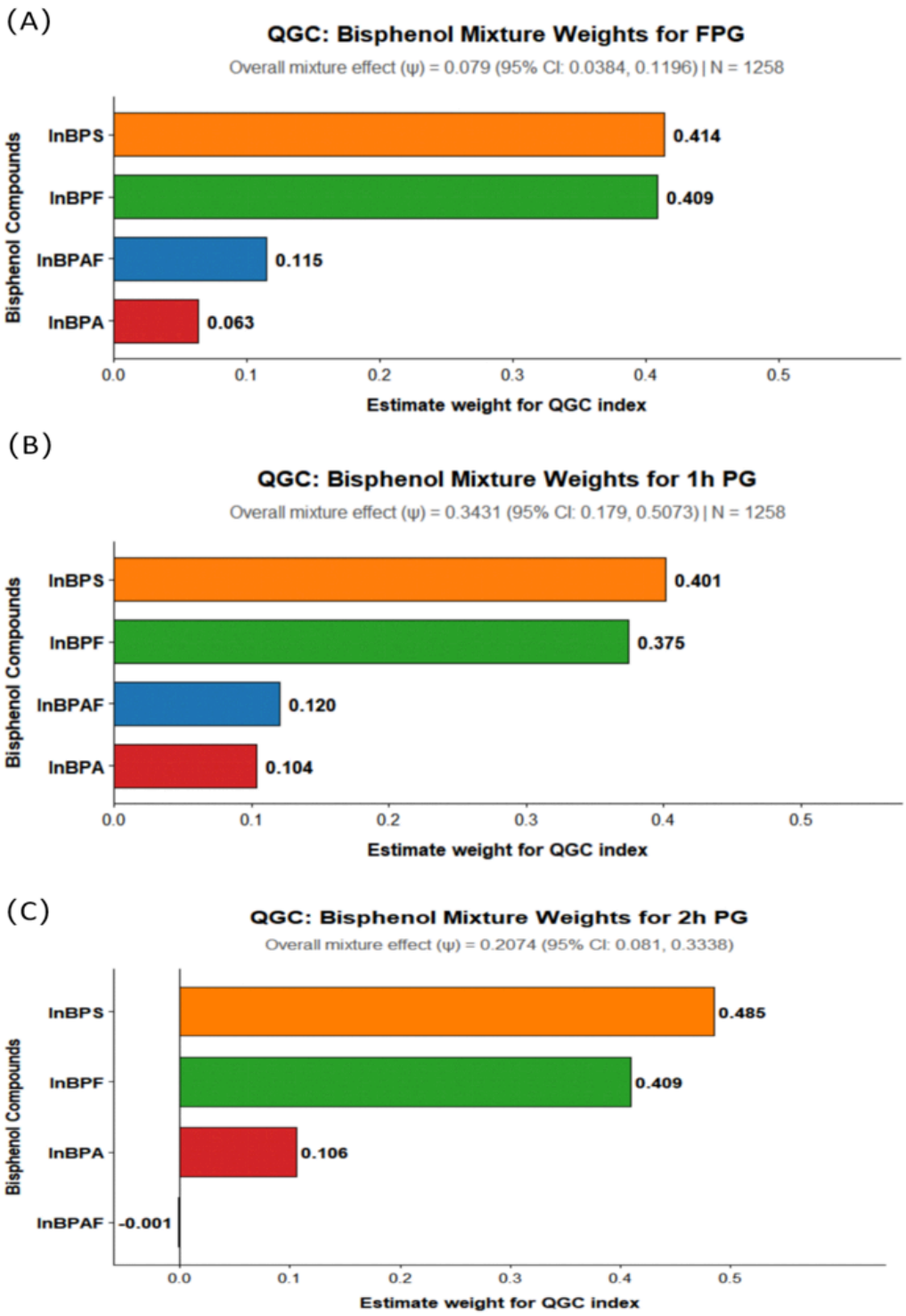

Figure 3

Estimated weights of bisphenol compounds on FPG (A), 1-hour PG (B), and 2-hour PG (C) using using quantile g-computation (QGC) models. Bisphenol levels were ln-transformed and standardized. Covariates in the model included maternal age, household income, educational level, and pre-pregnancy BMI. The bar charts display the estimated weights for each bisphenol within the mixture.

Abbreviations: FPG, fasting plasma glucose (mmol/L); 1-hour PG, 1-hour plasma glucose (mmol/L); 2-hourPG, 2-hour plasma glucose (mmol/L); BPA, bisphenol A; BPAF, bisphenol AF; BPS, bisphenol S; BPF, bisphenol F.

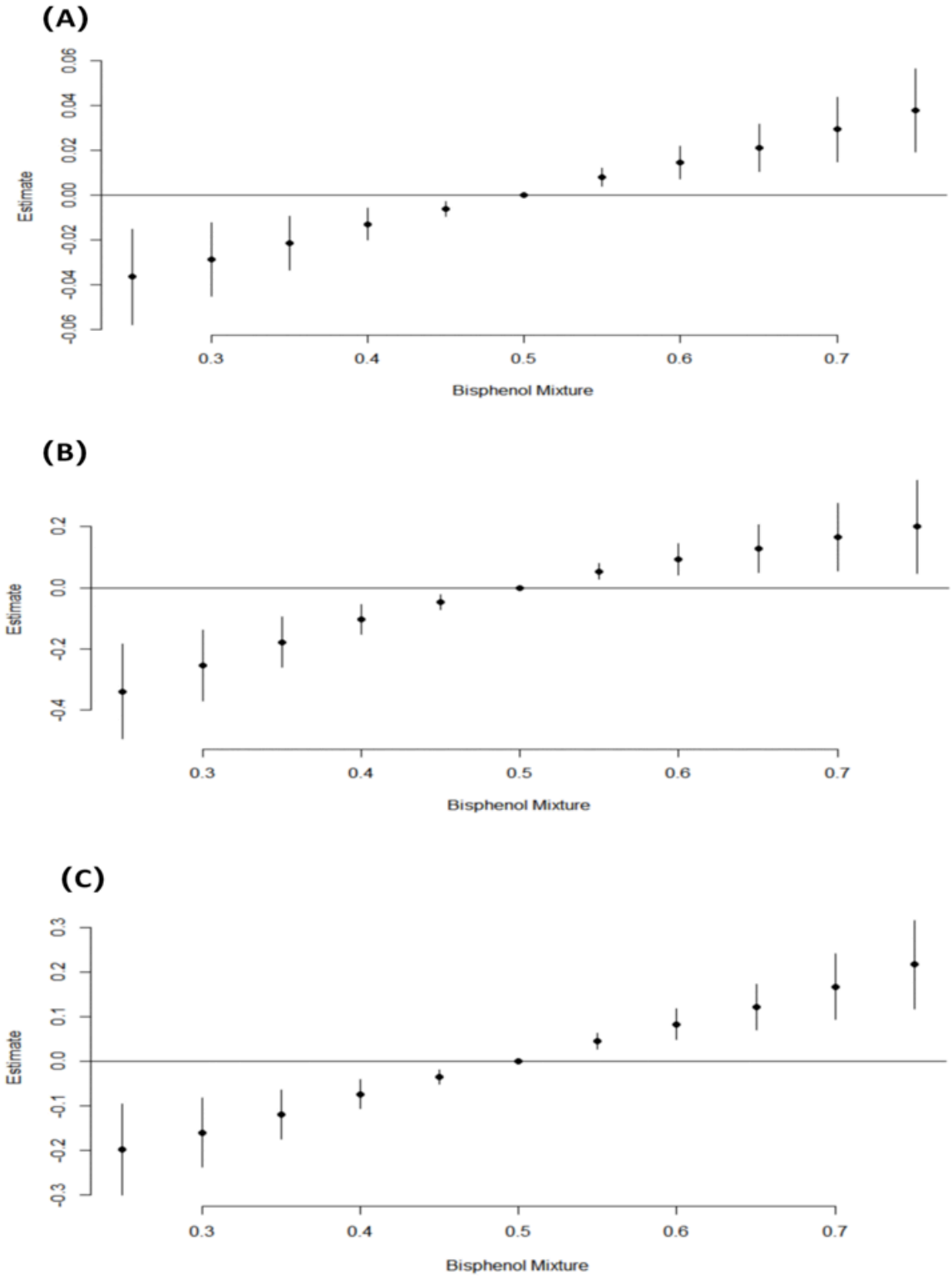

Figure 4

Joint effect of the bisphenol compounds on FPG (A), 1-hour PG (B), and 2-hour PG (C) using Bayesian kernel machine regression (BKMR) model. The joint effect of the mixture and 95% confidence intervals (CIs), obtained by comparing specific quantiles of bisphenol compounds (from the 10th to the 90th percentiles) with their respective median values. Bisphenol levels were ln-transformed and standardized. Covariates in the model included maternal age, household income, educational level, and pre-pregnancy BMI.

Abbreviations: FPG, fasting plasma glucose (mmol/L); 1-hour PG, 1-hour plasma glucose (mmol/L); 2-hour PG, 2-hour plasma glucose (mmol/L); BPA, bisphenol A; BPAF, bisphenol AF; BPS, bisphenol S; BPF, bisphenol F.

Supplementary Files

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