Letter

Is there an Association between Per- and Poly-Fluoroalkyl Substances and Serum Pepsinogens? Evidence from Linear Regression and Bayesian Kernel Machine Regression Analyses

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Gastric cancer is the third leading cause of cancer-related mortality and remains a major global health issue[1]. Annually, approximately 479,000 individuals in China are diagnosed with gastric cancer, accounting for almost 45% of all new cases worldwide[2]. Furthermore, gastric cancer is associated with a high mortality rate of approximately 78%[3], partly because patients typically exhibit non-specific symptoms in the early stages, complicating early detection until the cancer advances. In clinical setting, screening for gastric cancer involves a combination of patient history, physical examination, and relevant diagnostic tests. Gastric pepsinogen (PG), a precursor enzyme (zymogen) released by the chief cells of the gastric mucosa, reflect the morphology and function of the gastric mucosa. It is considered a promising biomarker for gastric cancer screening and for evaluating the presence of gastric atrophy. Abnormal proteasome activity, particularly a lower PG I/PG II ratio, is often associated with an increased risk of gastric cancer.

Many factors contribute to gastric mucosal lesions and abnormal proteasome activity, including Helicobacter pylori infection, alcohol consumption, autoimmune disorders, and exposure to environmental toxins. Recently, per- and poly-fluoroalkyl substances (PFAS), a class of synthetic organic chemicals that do not occur naturally, have been recognized as major environmental contaminants of serious concern because of their widespread and unrestricted use. Bioaccumulation of PFAS in the environment has led to detectable concentrations of these compounds in the blood samples of nearly the entire human population. Exposure to PFAS in humans can lead to various health problems including thyroid disruption, liver damage, reproductive toxicity, and increased cancer risk. Several studies have identified a correlation between PFAS exposure and an elevated risk of gastrointestinal diseases and cancer. For instance, higher PFAS concentrations have been associated with an increased likelihood of developing inflammatory bowel disease or colorectal cancer in exposed populations[4]. However, the relationship between PFAS exposure and serum pepsinogen levels has not yet been reported in general populations. In this study, we examined the relationship between PFAS and serum pepsinogen levels in the general population. Further, we explored the independent or combined effects of PFAS congeners, with the aim of revealing the potential role and mechanism by which PFAS may affect the occurrence of gastrointestinal diseases.

The participants were recruited from Shanghai between 2022 and 2023. Questionnaires, health checks, and blood sampling were conducted with the study participants. The study was performed by well-trained researchers responsible for providing the necessary explanations and guidance. A total of 335 participants were recruited for this study, all of whom provided written informed consent and received approval from the Medical Ethics Committee of Shanghai Zhoupu Hospital (Project No. 2022-C-068-E01).

Blood samples were collected to evaluate biochemical parameters and PFAS concentrations. The PFAS content was measured using ultra-performance liquid chromatography/tandem mass spectrometry (UPLC/MS-MS; Agilent1290 6495, Agilent Technologies Inc., USA), and seven PFAS were quantified in the serum samples. The full

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names of the PFASs are listed in Supplementary Table S1. The methods for measuring PFAS have been described in detail elsewhere[5]. Supplementary Table S2 summarizes the precursor and product ions, collision energies, and retention times for each targeted analyte. Supplementary Table S3 provides comprehensive data on the detection limits, recoveries, and precision of all targeted chemicals. Additionally, serum pepsinogen levels were quantified using an enzyme-linked immunosorbent assay (ELISA), with millimoles per liter used as the unit for these measurements.

For PFAS, this study reported the median, interquartile range (IQR), and geometric mean. Other continuous and classification variables were reported separately as means with standard deviations, numbers with percentages, and medians with interquartile ranges. The Shapiro Wilk and Bartlett's tests were employed to assess the normality and homogeneity of variance of continuous variables, respectively. Owing to the non-normal distribution of all PFAS concentrations, a natural log transformation was applied to obtain a normal distribution. Spearman correlation analysis was used to investigate the relationship between PFAS concentrations and pepsinogens. Additionally, multiple interpolations was used to interpolate the missing values between covariables; Supplementary Table S4 presents the rates of missing data for each variable, along with the distribution of both original and interpolated values.

This study used multivariate linear regression models to assess the relationship between PFAS concentrations and pepsinogen levels. A restricted cubic spline (RCS) was employed to evaluate the linear relationship between them, and sensitivity analyses were conducted across sex and age subgroups. Additionally, a Bayesian Kernel Machine Regression (BKMR) model was used to estimate the association between PFAS mixture exposure and PG concentration. This relatively novel statistical method allows the use of nonparametric methods to model the correlation between chemical exposures and health outcomes without assuming the form of these associations. In this study, all BKMR models were fitted with 25,000 iterations using a Markov Chain Monte Carlo (MCMC) sampler. The results of the BKMR are presented as a) mixing effects of PFAS mixtures, b) exposure-response cross-sections for single variables and outcomes, c) effects of an individual PFAS, and d) posterior inclusion probabilities (PIP). The covariates for the analysis were selected based on a priori knowledge guided by a directed acyclic graph (DAG) (Supplementary Figure S1). The statistical analysis was performed using SPSS27.0 and RStudio. $P < 0.05$ was considered statistically significant.

Supplementary Table S5 presents the demographic characteristics of the study population. The mean age of the 335 participants was 37.52 years, with an average BMI of 23.50 kg/m^2, and a mean waist circumference of 79.56 cm. The majority of the participants were female (61.5%), had a university education (83.6%), and were employed as administrators (43.3%). Most participants were nonsmokers (84.5%), nondrinkers (87.5%), and engaged in mild physical activities (64.8%). The median concentrations of PG I, PG II, and PG I/II were 61.10, 7.53, and 8.14 ng/mL, respectively. The detection rates of HFPO-DA, PFDA, n-PFHxS, br-PFHxS, PFNA, and PFOA were > 95% (Supplementary Table S6). The highest median serum concentration was observed for PFNA (13.061 ng/mL), followed by PFOA (11.770 ng/mL), n-PFHxS (2.283 ng/mL), PFDA (1.532 ng/mL), br-PFHxS (0.039 ng/mL), PFDoA (0.011 ng/mL), and HFPO-DA (0.0107 ng/mL (Supplementary Table S6). Our study population was exposed to a range of PFAS, among which PFNA, PFOA, and n-PFHxS exhibited the highest serum concentrations. Notably, we observed higher median serum concentrations of PFNA and PFOA, but lower concentrations of PFDoA compared to a previous study on breast cancer conducted in Tianjin, China[6]. Thus, the differences in the types and levels of PFAS exposure may be attributed to variations between populations and regions.

Supplementary Table S7 presents the correlations between the seven PFASs and pepsinogens. The results of the multiple linear regression analysis, adjusted for variables including sex, age, BMI, smoking, and drinking habits, are presented in Table 1. The β estimates for the associations between PG II and PFDA, n-PFHxS, br-PFHxS, and PFNA were 0.642 (95% *CI*: 0.049, 1.235), 0.696 (95% *CI*: 0.092, 1.484), 0.595 (95% *CI*: 0.207, 0.983), and 0.338 (95% *CI*: 0.087, 0.590), respectively. For PG I/II, the β estimates associated with HFPO-DA, PFDoA, br-PFHxS, and PFNA were 0.426 (95% *CI*: 0.145, 0.708), 0.560 (95% *CI*: 0.064, 1.184), −0.526 (95% *CI*: −0.840, −0.211) and −0.334 (95% *CI*: −0.537, −0.131), respectively. However, none of the PFASs were significantly associated with PG I. To our knowledge, this is the first studyto investigate the relationship between PFAS exposure and serum pepsinogen levels. Previous research has demonstrated that exposure to environmental

pollutants (e.g., PFAS, PAHs, and asbestos) is associated with an increased risk of gastrointestinal cancer[7]. For instance, PFAS exposure has been linked to a higher prevalence of colorectal cancer among occupational workers[4]. However, studies examining the association between PFAS and gastric diseases remain scarce. Therefore, our study provides preliminary evidence regarding a potential relationship between PFAS exposure and pepsinogen levels. The results of the RCS and sensitivity analyses are presented in Supplementary Table S8 to 10 of the Supplementary Material.

Nonetheless, it is possible that various environmental pollutants exert diverse effects on health outcomes, thereby displaying directional heterogeneity. Additionally, we performed a BKMR analysis to explore potential heterogeneity. Correlation coefficients between pollutants ranged from −0.41 to 0.77 (Supplementary Figure S2). BKMR was used to estimate the combined exposure-response function across all PFAS analyzed in this study. First, we investigated the relationship between the cumulative mixture dose response and pepsinogen levels. The results indicated that the PFAS mixtures were positively correlated with PG I and PG II and negatively correlated with the PG I/II ratio (Figure 1); however, the CI for PG I and PG I/II overlapped with zero correlation (Figure 1A and 1C). In contrast, exposure to the PFAS mixtures concentrations at or above the 55th percentile was significantly associated with increased PG II levels (Figure 1B).

This study also quantified exposure-response cross sections for individual PFAS and associated outcomes, with all other PFAS concentrations held at their median values, to assess potential nonlinearity in the mixtures (Supplementary Figure S3A and S3C). Additionally, we examined the individual PFAS exposure response functions of pepsinogens (Figure 2A and 2C). However, almost no significant differences between any of them were observed. The results for the PIPs are detailed in Supplementary Table S11. Our findings revealed that an increase in PFAS concentrations from the 55th percentile was significantly associated with a proportional increase in the PG II concentration. Additionally, elevated PG II concentrations have been linked to atrophy of the fundic glandular ducts, gastric epithelial chemotaxis, and pseudo-pyloric glandular chemotaxis[8]. These findings provide novel insight into the potential contribution of higher PFAS concentrations to the incidence of certain gastric diseases. Furthermore, we speculated that PFAS exposure may not directly cause changes in gastric pepsinogen levels; that is, the quantitative correlations between PFAS and gastric pepsinogens may be due to indirect associations. Recent advances in modern manufacturing, particularly the widespread use of processed foods, have significantly increased the risk of human exposure to various environmental toxins. Studies have linked frequent consumption of processed foods to an increased risk of developing gastrointestinal disorders, such as gastric ulcers and gastric cancer[9]. Moreover, our study population primarily consisted of young and middle-aged professionals who frequently consumed processed and takeaway foods because of their demanding work schedules. PFAS

Table 1. Multiple linear regression of individual PFAS with pepsinogens

Ln-PFASs (ng/mL)	PG I		PG II		PG I/II	
	Adjusted β (95% *CI*)	*P*	Adjusted β (95% *CI*)	*P*	Adjusted β (95% *CI*)	*P*
HFPO-DA	1.132 (−1.220, 3.284)	0.788	−0.277 (−0.626, 0.073)	0.121	0.426 (0.145, 0.708)	0.003
PFDA	3.868 (−0.091, 6.222)	0.055	0.642 (0.049, 1.235)	0.034	−0.015 (−0.501, 0.471)	0.651
PFDoA	2.997 (−2.408, 7.363)	0.450	0.234 (−0.055, 0.536)	0.150	0.560 (0.064, 1.184)	0.039
n-PFHxS	−0.934 (−5.471, 4.603)	0.740	0.696 (0.092, 1.484)	0.043	−0.290 (−0.932, 0.235)	0.134
br-PFHxS	−0.369 (−2.122, 2.183)	0.692	0.595 (0.207, 0.983)	0.003	−0.526 (−0.840, −0.211)	0.001
PFNA	−0.206 (−1.683, 1.572)	0.720	0.338 (0.087, 0.590)	0.009	−0.334 (−0.537, −0.131)	0.000
PFOA	1.035 (−5.228, 6.298)	0.779	0.897 (−0.1134, 1.933)	0.088	−0.327 (−1.171, 0.517)	0.446

Note. Adjusted for sex, age, BMI, smoking and alcohol status. PFAS, per- and polyfluoroalkyl substances; HFPO-DA, hexafluoropropylene oxide-dimer acid; PFDA, Perfluoro-n-decanoic acid; PFDoA, Perfluoro-n-dodecanoic acid; n-PFHxS, Potassium perfluorohexanesulfonate; br-PFHxS, Sum of all branched isomers PFHxS; PFNA, Perfluoro-n-nonanoic acid; PFOA, perfluorooctanoic acid; PG I, pepsinogen I; PG II, pepsinogen II; PG I/II, pepsinogen I/II.

are found in these processed and takeaway foods owing to their extensive use in packaging materials[10], thereby increasing their serum concentrations in humans. Therefore, the combined effects of these factors may lead to an imbalance in gastric proenzyme secretion, that can explain the observed association between PG II and PFAS mixtures.

Our study has several limitations. First, this was a cross-sectional study; hence, nothing was known about the causal relationship between PFAS mixtures and health outcomes. Second, despite the inclusion of some covariates, many confounders remained that could not be excluded. Furthermore, the sample size was relatively small, which may explain the low rate of positive results. Taken together, our study is the first to examine the association between PFAS and gastrointestinal health, thereby providing new epidemiological evidence in the field. Our study employs both BKMR and multiple interpolation techniques; BKMR accounts for nonlinear and non-additive effects, whereas multiple interpolations minimize the effect of confounding factors.

Our study demonstrated an association between exposure to various PFAS and elevated serum pepsinogen II levels. As PFAS are ubiquitously present in the environment and gastrointestinal diseases, along with gastric cancer, have become increasingly prevalent in recent years, our findings have significant public health implications. Further epidemiological and clinical research is necessary to clarify the effects of PFAS

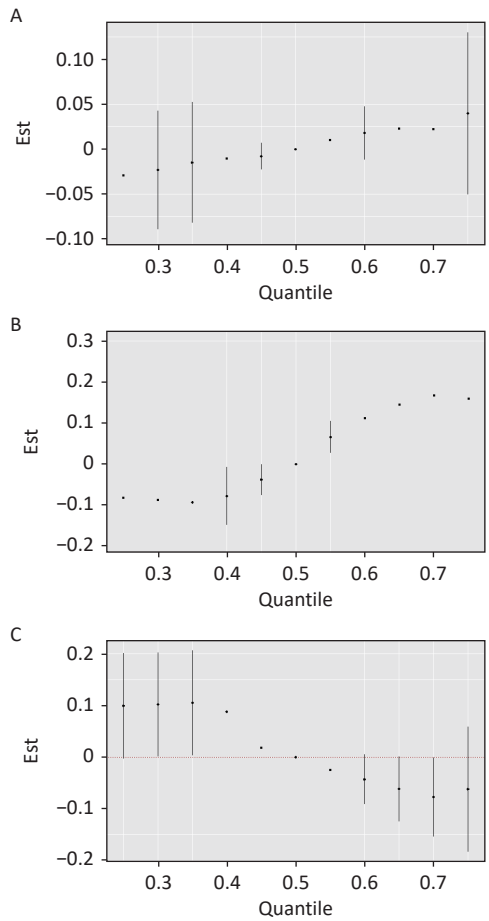

Figure 1. Estimated effects of PFAS mixtures on pepsinogens (*n* = 335).

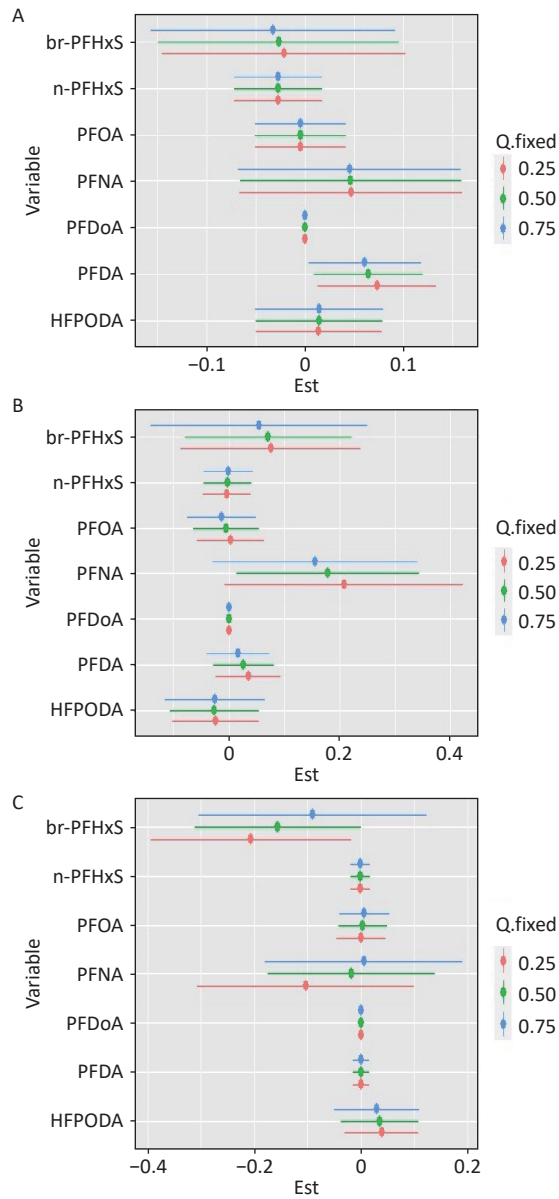

Figure 2. Estimated effects of individual PFAS on pepsinogens (*n* = 335).

exposure on gastrointestinal diseases and gastric cancer.

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Competing Interests The authors declare no competing interests.

Ethics This study was reviewed and approved by the Medical Ethics Committee of the Shanghai Zhoupu Hospital (Project No.2022-C-068-E01). All participants willingly participated in the study and provided written informed consent.

Authors' Contributions Conceptualization, Funding acquisition, Investigation, Validation, Roles/Writing original draft, Writing review & editing: Jing Wu. Data curation, Formal analysis, Methodology, Software, Visualization, Roles/Writing original draft: Shenglan Yang. Data curation, Formal analysis, Methodology: Yiyan Wang. Investigation, Supervision, Project administration: Yuzhong Yan. Conceptualization, Funding acquisition, Project administration, Resources: Ming Li.

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Data Sharing Data will be made available on request. The supplementary materials will be available in www.besjournal.com.

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