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Mass Spectrometry-Based Metabolomics Reveals Occupational Exposure to Per- and Polyfluoroalkyl Substances Relates to Oxidative Stress, Fatty Acid β-Oxidation Disorder, and Kidney Injury in a Manufactory in China

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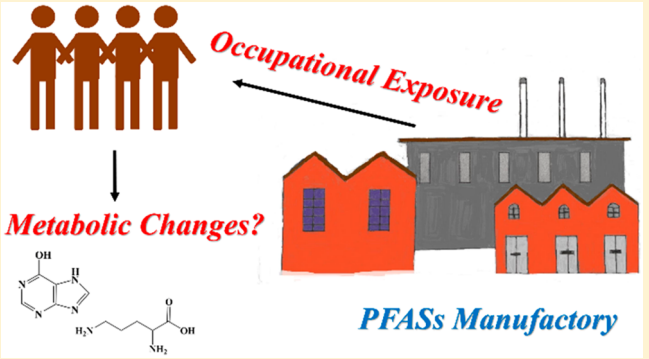
Supporting Information

ABSTRACT: Occupational workers are usually exposed to high levels of per- and polyfluoroalkyl substances (PFASs), placing them under greater health risks compared to the general population. Herein, 40 occupational workers from a factory in China and 52 control subjects from the general population were involved in an investigation on the potential health concerns of occupational exposure to PFASs by mass spectrometry-based metabolomics analysis. The PFAS levels in plasma from both groups were analyzed. Six PFAS congeners ($\sum_6$PFASs) were found to be the main components of the 13 detected PFASs, with a geometric mean of 1770 and 22.2 ng mL^{-1} in occupational workers and the general population, respectively. Metabolic profiles of the plasma samples were acquired using liquid chromatography coupled with orbitrap high-resolution mass spectrometry and gas chromatography−mass spectrometry. The partial least-squares-discriminant analysis model indicated that the plasma metabolic profiles of the two groups could be clearly separated. Differential and correlation analyses were applied to discover potential biomarkers. A total of 14 potential biomarkers were identified, and they were found to be associated with oxidative stress, fatty acid β-oxidation disorder, and kidney injury. The obtained results indicated that the health effects of occupational exposure to PFASs on workers should not be ignored.



INTRODUCTION

Per- and polyfluoroalkyl substances (PFASs) are a group of synthetic chemicals that have received extensive attention. In 2009, perfluorooctane sulfonic acid (PFOS), its salts, and perfluorooctane sulfonyl fluoride were listed in Annex B of the Stockholm Convention.[1] Subsequently, perfluorooctanoic acid (PFOA), its salts, and PFOA-related compounds in 2015[2] and perfluorohexane sulfonic acid (PFHxS), its salts, and PFHxS-related compounds in 2017[3] were submitted to the Persistent Organic Pollutants Review Committee as Persistent Organic Pollutants candidates.

The widespread presence of PFASs has led to the concern regarding the health effects of PFAS exposure on both wildlife and human beings. Occupational workers are under greater health risks than the general population given their higher PFAS exposure levels. Our previous studies have investigated the environmental fate, as well as the human exposure conditions, including the exposure pathway, half-lives, and accumulation and elimination behavior of PFASs on occupational workers in a factory in China.[4−7] Differential exposure patterns (both external and internal) between occupational

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workers and the general population were found. Therefore, we hypothesized that the differences in PFAS exposure would lead to discrepancies between the occupational workers and the general population at the molecular level, which may eventually indicate the corresponding health effects of occupational PFAS exposure on workers.

Metabolomics refers to the analysis of low-molecular weight compounds in cells, tissues, and biofluids of the biological system. Environmental metabolomics determines the changes in levels of low-mass chemicals in organisms after disturbances by environmental stressors, including both natural factors such as temperature and anthropogenic factors such as pollution.[8−11] With regard to the study on the environmental pollutants, metabolomics can provide new insights into the toxic effects and risk assessments of pollutants by revealing the changes in the organism at the metabolic level. It is a powerful complement to classical toxicological approaches.

Several studies have adopted the metabolomics tool to assess the PFAS-induced toxicity and health effects on experimental cell and animal models.[12−18] However, recent study demonstrated that known PFAS-induced toxicity mechanisms, as well as the associated health risks, might not be applicable to humans.[19] Nevertheless, very few PFAS-related metabolomics studies have chosen humans as study subjects. Moreover, none of these studies have focused on the high PFAS exposure levels groups: occupational workers.[20,21]

Herein, plasma samples of occupational workers from a PFAS manufacturing facility in China and control subjects from the general population were collected to detect the PFAS concentrations, to acquire the metabolic profiles, and to find the potential biomarkers that relate to occupational PFAS exposure. This study aims to assess the potential health risks of high PFAS exposure levels on occupational workers at the molecular level.

MATERIALS AND METHODS

Study Design and Sample Collection. A total of 108 plasma samples were collected in 2017. Occupational workers were employed in a fluorochemical manufacturing plant, whereas the control subjects were recruited from a city 80 km away from the target factory to ensure their lifestyle and living environment were similar to those of occupational workers. Information on demographics, health history, medical history, and lifestyles were obtained by self-reported questionnaires. Blood samples were collected in the morning after an overnight fasting and were divided into aliquots immediately after centrifugation before being stored at −80 °C. Samples of individuals with genetic diseases, clinical diseases and under long-term medication were excluded. After the exclusion, a number of 92 participants were included for further analysis, with 40 occupational workers and 52 control subjects from the general population.

PFAS Analysis by HPLC-MS/MS. The plasma samples were preconcentrated and cleaned by online Turboflow solid phase extraction, and 21 PFAS congeners were analyzed by high performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS). The method has been described in detail in our previous study.[22] Briefly, 25 μL of plasma were directly injected into the online solid phase extraction column (Turboflow Cyclone-P, 1.0 × 50 mm, Thermo Scientific, U.S.A.) for preconcentration, cleanup, and elution. After HPLC separation (UltiMate 3000 system, Thermo Scientific, U.S.A.; Acclaim 120 C18, 4.6 × 150 mm, 5.0 μm, Thermo Scientific, U.S.A.), all analytes were further analyzed by TSQ Quantiva triple quadrupole mass spectrometry (Thermo Scientific, U.S.A.) coupled with electron spray ionization (ESI) source using the Multiple Reaction Monitoring transitions in the negative ion mode. The limits of detection of the 21 analyzed PFASs ranged from 0.008 to 0.19 ng mL^{-1}.

Metabolic Profiling Analyses Using LC-MS and GC-MS. Minimal sample preparation processing procedures were adopted to obtain as many metabolites as possible. For LC-MS-based metabolic profiling, 50 μL of thawed plasma were diluted with 200 μL of cold methanol for protein precipitation. The mixtures were vortexed for 30 s and then centrifuged at 15 000*g* for 15 min at 4 °C. The supernatant was collected and dried under nitrogen gas. The dried residues were stored at −80 °C until analysis and were reconstituted in 150 μL of a methanol and water mixture (v:v = 1:1) for instrumental analysis. For GC-MS-based metabolic profiling, 50 μL of plasma were extracted by 200 μL of cold methanol prior to vortexing for 1 min. After centrifugation at 15 000*g* for 15 min at 4 °C, the supernatants were collected and dried under nitrogen gas. The residues were derivatized before GC-MS analysis. A total of 30 μL of methoxyamine hydrochloride (20 mg mL^{-1} in pyridine) was first added to residues, vortexed for 1 min, and incubated for 1 h at 30 °C. Then, 50 μL of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide was added and vortexed for 1 min. The sample was incubated for 1 h at 60 °C and was further centrifugated at 15 000*g* for 10 min at room temperature. Finally, the supernatants were collected for injection.

Nontargeted LC-MS analysis was performed on a UPLC-Q Exactive Focus Orbitrap MS system (Thermo Scientific, U.S.A.) equipped with an ACQUITY UPLC HSS T3 column (1.8 μm, 2.1 mm × 30 mm; 1.7 μm, Waters Corp., U.S.A.). The mobile phases A and B were water and acetonitrile, both containing 0.1% formic acid. The injection volume was set at 10 μL, and the flow rate was constant at 0.3 mL min^{-1}. The gradient program was as follows: starting from 2% B, held for 1 min; changed to 100% B by 19 min, held for 2 min; returned to the initial ratio from 21 min, and maintained for 4 min to re-equilibrate the column. The capillary voltages were 3.5 kV in the positive mode and 2.5 kV in the negative mode. The capillary gas and sheath gas temperatures were 300 and 320 °C, respectively. The full-scan mode was applied, with a scan range from 70 to 1000 m/z; resolution was set at 35 000.

GC-MS metabolic profiling was performed on the Agilent 7890B/5977A Series Gas Chromatograph/Mass Selective Detector system, equipped with a DB-5MS capillary column (30 m × 0.25 mm I.D., 0.1 μm film thickness; Agilent, J&W Scientific., U.S.A.). A 1 μL derivatized aliquot was injected into the system in splitless mode carried by helium gas with a constant flow of 1 mL min^{-1}. The oven program was set as follows: the initial temperature was set at 70 °C and maintained for 2 min, followed by an increase to 190 °C at 6 °C min^{-1}, to 250 °C at 5 °C min^{-1} and to 300 °C at 25 °C min^{-1}, and finally isothermal heating at 300 °C for 9 min. Mass spectra was acquired in full-scan acquisition mode (from 30 to 600 m/z), with the solvent delay set at 6 min and the electron impact (EI) ionization voltage set at 70 eV. The quality control (QC) sample was a mixture of equal amount of each sample involved, set to monitor the stability of both the LC-MS and the GC-MS system and for signal correction. The blank samples were used to assess the cleanliness status of the

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instrument. Both QC and blank sample were analyzed at the beginning, at the end, and throughout the analytical process.

Data Analysis of Plasma Metabolomics. Data analysis for metabolomics has been described in a previous study.[23] Raw data files of LC-MS and GC-MS were converted into netCDF format before data analysis. Data pretreatment was conducted using the XCMS package, which is implemented in the freely available R language. The output file of the XCMS was a three-dimensional matrix containing peak inductions (retention time, m/z pairs), sample names (observations), and ion intensity information (variables). The quality control-based robust LOESS (locally estimated scatterplot smoothing) signal correction (QC-RLSC) method was used for signal correction in statTarget package in R statistical language.[24,25] The obtained data matrix was further introduced into SIMCA-P (version 13.0, Umetrics, Sweden), and following mean-centering and Pareto-scaling, the data set was further analyzed by multivariable statistical analysis.

Statistical Analysis. All statistical analyses were performed on SPSS 18.0 (SPSS Inc., USA) and Origin 8.0 (OriginLab Corp., USA). The Mann−Whitney U test was used to find significant differences between the two groups. Spearman correlation analysis was utilized to explore the relationship among levels of the detected PFASs. The relationship between the metabolic features and $\sum_6$PFASs concentration were investigated by partial correlation analysis after adjusting for age, BMI, gender, smoking, and drinking status. Linear fit of $\sum_6$PFASs and peak intensity of potential biomarkers was processed by the linear regression analysis. Partial correlation analysis and linear regression analysis were based on log-transformed data. A p value of less than 0.05 was considered as a significant difference.

Biomarker Identification. Metabolic features meeting the following three criteria were regarded as potential biomarkers: (1) variable importance in the projection (VIP) score > 1 in the partial least-squares-discriminant analysis (PLS-DA) model; (2) the peak intensities between the two groups were significantly different with a p value < 0.05 in Mann−Whitney U test; and (3) the variation of peak intensity was significantly correlated to the $\sum_6$PFASs level after log-transformation with a p value < 0.05 in partial correlation analysis. Discriminated features from LC-MS were further identified by comparing the accurate mass and MS/MS fragment patterns of the sample in parallel reaction monitoring mode to databases (METLIN (https://metlin.scripps.edu) and Human Metabolome Database (http://www.hmdb.ca)). Features from GC-MS were searched in NIST (National Institute of Standards and Technology) mass spectra library, and of those similarity indexes (Match and R. Match) more than 750 were selected for further analysis. Metabolites with commercial standards available were finally identified using authentic standards. Pathway analysis was performed on the KEGG database (http://www.genome.jp/kegg/).

RESULTS

Demographic Characteristics. Demographic characteristics of the study population are listed in Table 1.

PFAS Profiles. Twenty-one PFASs in plasma were analyzed, and eight of them were under the limit of detection in all samples. Among the detected 13 PFASs ($\sum_{13}$PFASs), six PFAS congeners ($\sum_6$PFASs), namely, perfluorobutanoic acid (PFBA), PFOA, perfluorobutanesulfonic acid (PFBS), PFHxS, PFOS, and 6:2 chlorinated polyfluorinated ether sulfonate (6:2 Cl-PFESA) were the main components in each sample. The concentration of $\sum_6$PFASs accounted for 99.7 ± 0.6% of the $\sum_{13}$PFASs in the workers and for 88.6 ± 18.2% in the general population. Thus, only the 6 main congeners were included in the following data analysis and further discussion.

Table 1. Demographic Characteristics of the Participants

characteristics[a]	occupational workers	general population	p
no. of subjects	40	52	
gender, male:female	27:13	13:39	
age, years (mean ± SD)	45.2 ± 8.1	50.0 ± 9.3	0.004
height, cm (mean ± SD)	166.5 ± 6.5	163.5 ± 6.5	0.032
weight, kg (mean ± SD)	66.5 ± 10.1	62.2 ± 9.5	0.013
BMI, kg/m^2 (mean ± SD)	23.9 ± 3.0	23.2 ± 3.0	0.143
smoker, n (%)	14 (35.0%)	6 (11.5%)	
drinker, n (%)	19 (47.5%)	9 (17.3%)	

[a]SD: standard deviation.

The Mann−Whitney U test indicated that each of these main six congeners showed significant difference in concentration between the two groups (p < 0.001). Based on the fact that PFBA, PFOA, PFBS, PFHxS, PFOS, and 6:2 Cl-PFESA are the main components of the detected PFASs in all samples and their concentrations were significantly different in the two groups, we presumed them to be the key factors leading to the potential health effects following occupational exposure. Detailed information of the concentration of these six PFASs is provided in Table 2.

Plasma Metabolome Profiles and Potential Biomarkers. Occupational exposure resulted in higher plasma PFAS concentrations in workers than in the general population, likely leading to endogenous metabolite changes in workers. Following data extraction and signal correction in R statistical language, the data matrix was introduced into SIMCA-P for further analysis. The PLS-DA model was established to differentiate the metabolic features of occupational workers from those of the general population. The PLS-DA model was first validated by R^2Y and Q^2 (cum) values for the goodness of fit and prediction of the model and then further validated by the permutation test (n = 200). The PLS-DA scatter plot of LC-MS in the positive-ion mode (Figure 1A) showed that the metabolic features of occupational workers and the general population clearly separated. Using two latent variables, the R^2Y = 0.91 and Q^2 (cum) = 0.719, which indicated the goodness of fit and prediction of the PLS-DA model. The permutation test showed the PLS-DA model was not overfitting (see Supporting Information Figure S2-A).

One outlier in LC-MS negative-ion mode and four outliers in GC-MS resulting from improper experimental operation were excluded, and the remaining samples were used for further analysis. The separation between the two groups was clear on the scatter plot of PLS-DA models, with R^2Y = 0.934 and Q^2 (cum) = 0.746 for LC-MS negative-ion mode using three latent variables (Figure 1B) and R^2Y = 0.677 and Q^2 (cum) = 0.395 for GC-MS using two latent variables (Figure 1C). Both models were further validated by a permutation test, and the results demonstrated that the two models were not overfitting (see Supporting Information Figure S2-B,C).

Features with the VIP score > 1 in PLS-DA models were selected for further identification, and the Mann−Whitney U test was applied to search for the feature that demonstrated a significant difference in peak intensity between the two groups.

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Table 2. Detailed Concentration of the Six PFASs in the Two Groups (ng mL^{-1})[a]

	PFBA	PFOA	PFBS	PFHxS	PFOS	6:2 Cl-PFESA	$\sum_6$PFASs	$\sum_6$PFASs/$\sum_{13}$PFASs × 100%
occupational workers								
mean	43.0	570.3	222.3	2153	3183	10.7	6183	99.7
geomean	6.7	136.6	68.1	454.0	753.0	6.9	1770	99.7
median	17.4	164.6	76.4	785.2	909.3	8.9	2420	99.9
SD	50.2	1209	411.3	2931	7226	8.3	10178	0.6
range	<LOD−189.6	2.0−7214	0.8−2449	<LOD−1226	9.6−43299	<LOD−43.4	43.7−54790	96.8−100
general population								
mean	0.5	4.7	1.0	8.5	17.5	0.7	33.0	88.6
geomean	0.1	2.7	0.4	3.8	8.7	0.3	22.2	85.3
median	0.1	3.8	0.5	6.5	15.9	0.4	32.3	95.0
SD	2.0	4.4	1.2	7.9	14.9	0.8	20.9	18.2
range	<LOD−14.5	<LOD−23.3	<LOD−5.3	<LOD−39.1	<LOD−67.1	<LOD−3.5	0.47−99.1	22.0−99.6

[a]PFAS, per- and polyfluoroalkyl substances; PFBA, perfluorobutanoic acid; PFOA, perfluorooctanoic acid; PFBS, perfluorobutanesulfonic acid; PFHxS, perfluorohexane sulfonic acid, PFOS, perfluorooctane sulfonic acid; 6:2 Cl-PFESA, 6:2 chlorinated polyfluorinated ether sulfonate; LOD, limits of detection; SD, standard deviation.

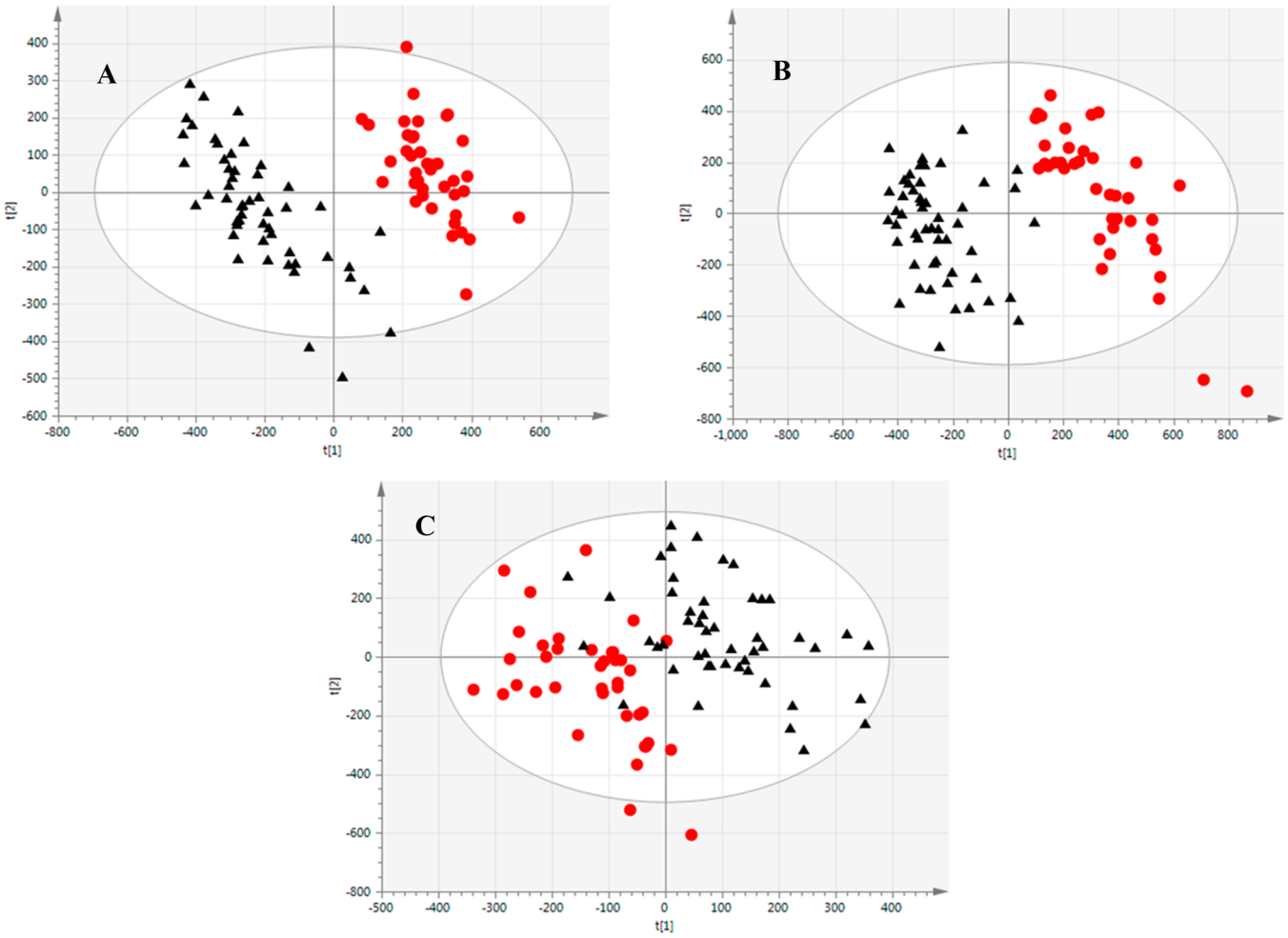

Figure 1. Scatter plots obtained from PLS-DA models: LC-MS-ESI positive-ion mode (A), LC-MS-ESI negative-ion mode (B), and GC-MS (C). The black triangles and red circles indicate for general population and occupational workers, respectively.

Features with both VIP score > 1 and $p < 0.05$ in the Mann−Whitney U test were regarded as differential features between two groups and were further identified using the commercial standards. Eventually, a total of 26 differential metabolites between the two groups were identified (see Supporting Information Tables S1 and S2). The brief biological pathways of these 26 altered metabolites were provided (see Supporting Information Figure S3).

To discover the potential biomarkers related to PFAS exposure level, partial correlation analysis was adopted to investigate the relationship between the peak intensities of the 26 metabolites and the value of $\sum_6$PFAS concentration. After adjusting for BMI, age, gender, and smoking and drinking

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Table 3. Brief Summary of the 14 Potential Biomarkers

	metabolites	VIP	FC[a]	class
LC-MS	acylcarnitine 18:2 (C18:2-CN)	1.67	1.43	acyl carnitines
	acylcarnitine 18:1 (C18:1-CN)	1.63	1.40	acyl carnitines
	methionine sulfoxide	1.14	0.81	amino acids
	ornithine	1.69	1.41	amino acids
	glycerophosphocholine (GPC)	1.36	0.70	glycerophosphocholines
	DL-2-aminooctanoic acid	1.91	1.61	amino acids
	hypoxanthine	2.57	2.19	hypoxanthines
	pyroglutamic acid	1.30	1.26	amino acids and derivatives.
	piperine	2.71	0.45	alkaloids and derivatives
	gamma-carboxyethyl hydroxychroman (γ-cehc)	1.33	1.38	1-benzopyrans
	sebacic acid	1.39	1.47	medium-chain fatty acids.
	azelaic acid	1.26	0.78	medium-chain fatty acids
	3-hydroxyoctanoic acid	1.27	1.45	hydroxy acids and derivatives
GC-MS	ornithine	1.94	1.30	amino acids
	myo-inositol	1.55	0.84	cyclohexanols

[a]FC, the ratio of peak intensity of occupational workers to general population.

status, 14 potential biomarkers were found to be significantly correlated with $\sum_6$PFAS concentration ($p < 0.05$). Furthermore, the peak intensities of these 14 potential biomarkers were also found to be correlated with the concentration of most of the PFAS congeners and categories, including PFBA, PFBS, PFHxS, PFOS, PFOA, 6:2 Cl-PFESA, short-chain PFASs (PFBA + PFBS), C8-PFASs (PFOS + PFOA), perfluorocarboxylic acids (PFCAs), perfluorosulfonates (PFSAs), and perfluoroalkyl acids (PFAAs) ($p < 0.05$) (Supporting Information Table S3).

Regarding the biological significance of the 14 potential biomarkers, the perturbed pathways they were involved in included lipid metabolism (3-hydroxyoctanoic acid, azelaic acid, sebacic acid, gamma-CHEC, C18:1-CN, and C18:2-CN), amino acids metabolism (pyroglutamic acid, ornithine, methionine sulfoxide, and DL-2-aminooctanoic acid), purine metabolism (hypoxanthine), inositol metabolism (myo-inositol), retinol metabolism (glycerophosphocholines), and metabolism of alkaloids and their derivatives (piperine). The brief information of these metabolites is shown in Table 3. The linear fit results between the $\sum_6$PFASs level and the peak intensity of potential biomarkers can be found in Figure 2, and the box charts of peak intensity of the two groups can be found in Figure 3.

DISCUSSION

PFAS Exposure Characteristics in the Study Population. Herein, the physical conditions of the participants were provided by self-reported questionnaires. Individuals with illness, long-term medication, and a family history of genetic disease were excluded. Only healthy subjects were involved for the purpose of obtaining a more accurate and goal-specific result. Six PFASs (PFBA, PFOA, PFBS, PFHxS, PFOS, and 6:2 Cl-PFESA) were the principal components of the 21 congeners analyzed, which is consistent with the product structure and the product volume of the studied factory.[7] The concentrations of PFBA, PFBS, and 6:2 Cl-PFESA in human blood, especially for occupational workers, were very limited so far worldwide. This is the first study to report the comprehensive occurrence of these six PFASs in occupational workers and the general population. Significant correlations of the levels of the six PFASs were observed in the workers by Spearman correlation analysis ($p < 0.05$), indicating that the six PFASs may have the same source, while not all of these six PFASs showed this trend in the general population (Supporting Information Tables S4 and S5). The geometric mean concentrations of plasma $\sum_6$PFASs were 1770 and 22.2 ng mL^{-1} in occupational workers and the general population, respectively, which is comparable to the biomonitoring results concluded from other PFAS detection studies worldwide.[26−28]

Biological Significance of the Biomarkers. A number of 14 abnormal metabolites resulting from high PFAS exposure levels were identified in the occupational workers. Of the altered metabolites, pyroglutamic acid and ornithine were both up-regulated. Pyroglutamic acid, as the cyclic lactam of glutamic acid, is the potential precursor and reservoir of glutamate. Increased pyroglutamic acid may relate to oxidative damage in the body.[29] Ornithine is an amino acid generated from arginine during the process of excreting urea in humans. Both pyroglutamic acid and ornithine are involved in the glutathione metabolism (GSH metabolism), wherein glutathione is oxidized to glutathione disulfide (GSSG) when scavenging free radicals and other reactive species through enzymatic reactions, and GSH/GSSG is the most crucial redox couple toward oxidative stress. Therefore, the disturbance of GSH metabolism indicates that the body may be under oxidative stress.[30]When methionine residues are oxidized to methionine sulfoxide by reactive oxygen species, reactive species are scavenged and the cells are protected from oxidative damage. However, this mechanism is reversible by the regulation of peptide methionine sulfoxide reductase, which catalyzes the reaction of methionine sulfoxide back to methionine.[31,32] In the present study, the balance between methionine and methionine sulfoxide was broken, as indicated by the down-regulation of methionine sulfoxide in the workers. Gamma-carboxyethyl hydroxychroman (γ-CEHC) is a potent antioxidant, and it was up regulated in the workers.[33] Therefore, we speculated that high PFAS exposure level could induce oxidative stress and may activate the antioxidant defense in workers, which have also been found in in vivo and in vitro studies.[34]

The levels of the three medium-chain fatty acids (3-hydroxyoctanoic acid, azelaic acid, and sebacic acid, with carbon length C8−C10) changed significantly in the workers' blood. Interestingly, 3-hydroxyoctanoic acid (C8) and sebacic acid (C10), both of which have even-numbered carbon chains,

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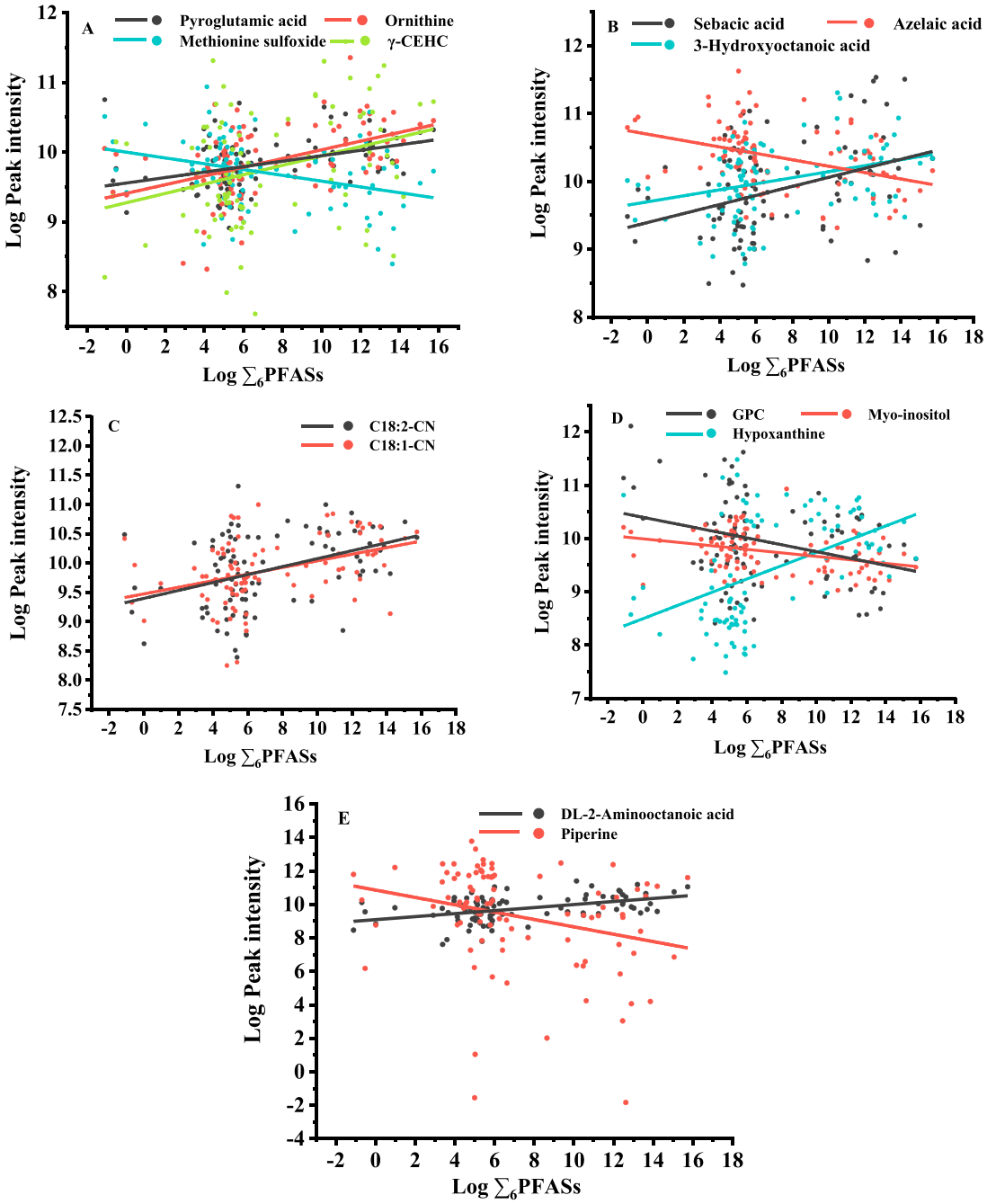

Figure 2. Linear fit graphs of log $\sum_6$PFASs and log peak intensity of the 14 potential biomarkers.

were upregulated. While azelaic acid, with an uneven-numbered carbon (C9) chain, was down regulated in the workers. Two acylcarnitines, acylcarnitine C18:1 (C18:1-CN) and acylcarnitine C18:2 (C18:2-CN), both of which are attached to C-18 unsaturated fatty acids, presented an upward trend. Fatty acids and acyl carnitines are closely associated with fatty acids metabolism. Mitochondrial fatty acids β-oxidation (FAO) is the most common and important metabolic pathway for fatty acids in human beings. Prior to the FAO process, fatty acids are first activated by binding to coenzyme A (CoA) to form acyl CoA, which later generates acyl carnitines by combining with carnitines. Acyl carnitines are finally transferred to the inner mitochondrial membrane to release to CoA and free fatty acids by corresponding enzymes. Fatty acids are oxidized in the mitochondria, and the final product acetyl CoA enters into the tricarboxylic acid cycle to generate energy.[35] Accumulation of acyl carnitine metabolites in the blood is commonly used for diagnosis of FAO disorders.[36] On the basis of the up- or down-regulation of three fatty acids and the up-regulation of two acyl carnitines in the workers' plasma samples, we conjectured that the mitochondrial FAO processes were disturbed by high PFAS exposure levels. Furthermore, fatty acids, glucose, and proteins are three substrates for organisms to generate energy. FAO is pivotal for energy homeostasis in various organisms, especially for cardiac and skeletal muscle. Other tissues, in particular, the liver, use the FAO process product to form ketone bodies to provide energy for other tissues.[37] Due to the disturbances in the FAO process, we speculate that the energy homeostasis of the workers is perturbed by occupational PFAS exposure. Previous

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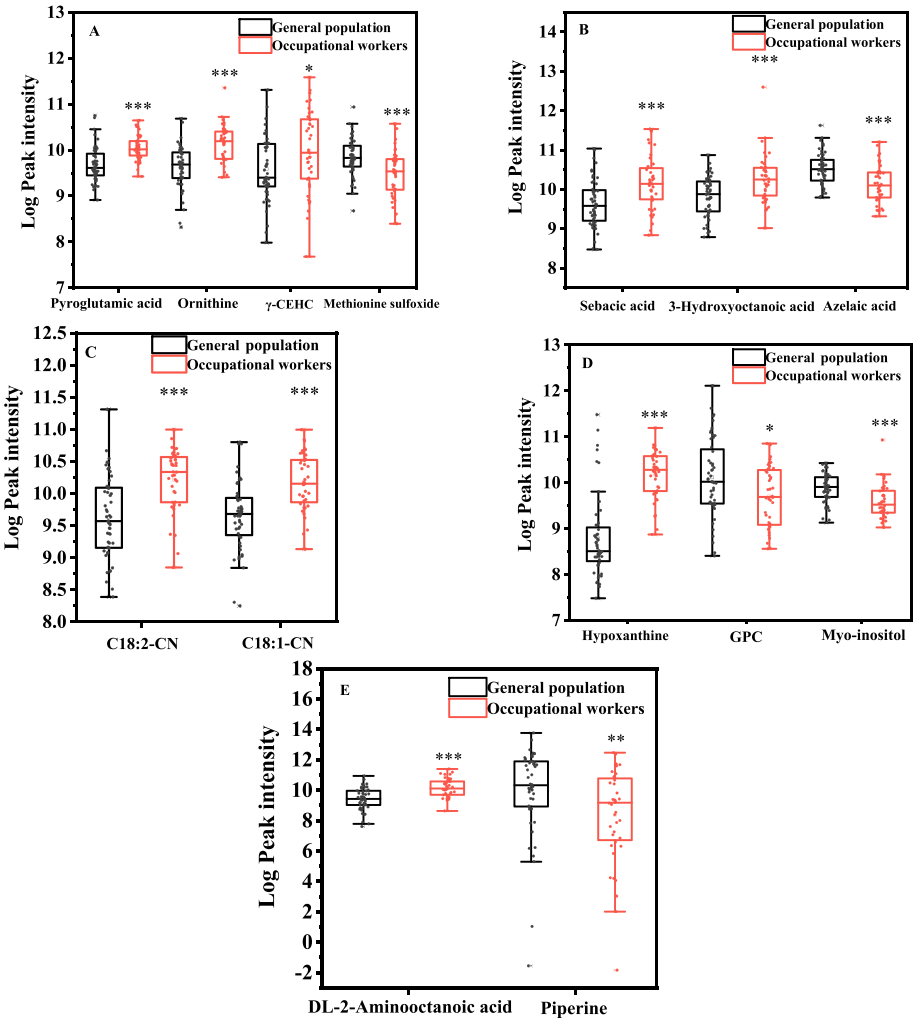

Figure 3. Box charts of log peak intensity between two groups of the 14 potential biomarkers. *p < 0.05; **p < 0.01; ***p < 0.001.

studies on animal models also proved that the exposure to PFAS might disturb the fatty acids metabolism, which is consistent with the phenomenon in the occupational exposed workers in this study.[38]

Up-regulation of plasma hypoxanthine resulting from PFAS exposure was observed. Hypoxanthine is generated during the degradation of purine, is transformed to xanthine, and finally is oxidized to uric acid in human beings. Epidemiological research has demonstrated that the PFASs exposure may be correlated with the uric acid concentration.[39] It was reported that increasing serum uric acid concentration could cause renal injury, with higher concentrations of hypoxanthine and uric acid being observed in the end-stage renal disease patients.[40,41] Glycerophosphocholine, which can be generated by the removal of fatty acids from phosphatidylcholine, is a major choline storage molecule in human beings. Myo-inositol is the most common form of natural inositol, and it is synthesized from glucose. Glycerophosphocholine and myo-inositol are both osmolyte, which accumulate to counteract with hypertonicity or release to counteract with hypotonicity in renal medullary cells. Considering the down-regulation of these two organic osmolytes was observed, we surmised that an imbalance between intracellular and extracellular stress was present in the renal medullary cells.[42−44] Previous studies, especially PFAS-related epidemiological studies, have shown that PFAS might interface with kidney function in the general population. Our results indicated that exposure to PFAS might also disturb the kidney function on PFAS occupationally exposed workers.[45]

Piperine and DL-2-aminooctanoic acid were found to be associated with PFAS exposure. However, they could not be classified to any three categories as discussed above. Piperine belongs to alkaloids and their derivatives, and it can be found in natural plants such as herbs, spices, and peppers. Piperine has been found to have many biological functions including antiasthmatic, anti-inflammatory, antioxidant, and immunomodulatory properties,[46] which makes it difficult to correlate the down-regulation of piperine to specific health effects. DL-2-aminooctanoic acid is an alpha amino acid found to be upregulated in the workers. Nevertheless, few studies have illustrated the biological functions of this amino acid, and therefore, only the trend of concentration changes can be discussed herein.

Potential Health Risks of Occupational Exposure to PFASs. Four important oxidative/antioxidative biomarkers were observed to be up- or down-regulated in the workers' plasma, which indicated that the workers were under oxidative stress induced by high PFAS exposure levels. Oxidative stress plays a crucial role in the pathogenesis of many diseases, including cancer, inflammation, liver disease, HIV, AIDS,

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infection, diabetes, etc.[30] Although participants in this study self-reported as being healthy, long-term oxidative stress may induce the potential health risks to occupational workers. In addition, the mitochondrial FAO metabolic pathway was found to be disturbed. FAO plays a key role in the energy homeostasis in organs such as liver, heart, and skeletal muscle. Since FAO metabolism is tightly connected with energy homeostasis in biological systems as discussed above, we surmised that the energy homeostasis in workers might be disturbed by occupational PFAS exposure. Moreover, when the FAO process is impaired, serious clinical consequences may occur, including hypoglycemic seizures, muscle damage, cardiomyopathy, metabolic acidosis, and liver dysfunction.[36] Furthermore, three metabolites associated with kidney function were identified, which may be regarded as indicators that the kidney is under attack by PFAS exposure, ultimately leading to potential kidney injury. Epidemiological studies focusing on PFAS-related kidney health mainly draw conclusions based on the value of estimated glomerular filtration rate and the prevalence of chronic kidney disease.[47] In one meta-study investigating PFAS-induced health effects on the general population, the altered pathways were found to be directly linked to chronic kidney disease pathogenesis.[20,47] Our results, for the first time, reveal the potential kidney health effects of high PFAS exposure level on occupational workers based on the metabolic findings.

Limitations and Environmental Implications . The potential health risks of occupational PFAS exposure on human beings at the molecular level were elucidated for the first time using the integrated mass spectrometry-based metabolomics. The obtained results indicate that occupational PFAS exposure might induce oxidative stress, FAO disorder, and kidney injury in workers. We conjectured that, though no specific disease has been diagnosed clinically in occupational workers, the potential health risks of occupational exposure to workers could not be ignored. However, this study has limitations. Only 92 participants were involved in the present study due to the small number of employees in the factory, and the two groups were not perfectly demographically matched. Besides, the biomarkers related to oxidative stress were too general, and they were not quantitatively assessed. Moreover, a meta-study could not illustrate the mechanism of the interaction relationship between the altered metabolites and the corresponding pollutants, and it could only provide the downstream information of the metabolism. Considering the limitations, more demographically matched samples are needed for the purpose of obtaining stronger evidence. Besides, in order to verify the effect of oxidative stress, some indexes of oxidative stress in urine, such as malondialdehyde and 8-hydroxy-2′-deoxyguanosine, are recommended to be determined. Additionally, other omics-techniques, especially proteomics, can be adopted to complete the meta-study data. Finally, the molecular mechanism of the PFAS-induced toxicity, especially in the population under high PFAS exposure levels, should be further addressed. Molecular biology research methods are recommended to reveal the toxicity mechanisms of high PFAS exposure levels on human beings.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.9b01608.

Information about the target factory and the sampling details; the brief summary of the 21 PFASs analyzed; the stability of the method; the permutation tests of the PLS-DA models; detailed information of metabolites identified by differential analysis (VIP score > 1 in PLS-DA model and $p < 0.05$ in two group by the Mann−Whitney U test) in LC-MS; detailed information of metabolites identified by differential analysis (VIP score > 1 in PLS-DA model and $p < 0.05$ in two group by the Mann−Whitney U test) in GC-MS; the brief pathway plot of the 26 metabolites identified by differential analysis; the relationship between the peak intensity of the 14 potential biomarkers and $\sum_6$PFASs level in each sample after log-transformation by partial correlation analysis; the relationship of the PFAS levels in the occupational workers by Spearman correlation analysis; and the relationship of PFAS levels in the general population by Spearman correlation analysis (PDF)

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