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A high-resolution metabolomics analysis of the association between perfluoroalkyl substances (PFASs), body composition and related metabolic outcomes in an Atlanta cohort

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Abstract

A high-resolution metabolomics analysis of the association between perfluoroalkyl substances (PFASs), body composition and related metabolic outcomes in an Atlanta cohort

By Katherine M. Krasnodemski

Objective: To determine the association between plasma levels of perfluoroalkyl substances (PFASs), measured by high resolution metabolomics, body composition and related metabolic outcomes at the baseline visit in the Emory-Georgia Tech Predictive Health Institute's Center for Health Discovery and Well Being (CHDWB) cohort.

Methods: We performed a cross-sectional study on 179 adults who were enrolled in the CHDWB cohort who had baseline plasma high-resolution metabolomics data available. Multiple linear regression models were used to assess the association between plasma PFASs, body composition, and related metabolic outcomes.

Results: Males had significantly higher PFAS intensities compared to women. Age, race, income, and education were not significantly associated with PFAS intensity. Visceral adipose tissue mass was positively associated with plasma PFASs, but the relationship was no longer statistically significant after adjusting for sex. No significant associations were present between intensities of PFASs and other cardiometabolic outcomes (insulin resistance, fasting glucose, fasting insulin, triglycerides, cholesterol [total, HDL, and LDL], measures of oxidative stress, or proinflammatory cytokines).

Discussion: In this study, we found no significant associations between PFAS intensity, body composition, and related metabolic outcomes. Larger, longitudinal studies should be conducted in order to determine the true relationship between PFAS exposure, body composition, and related metabolic outcomes.

Acknowledgments

I had the pleasure of working with some wonderful people on my MPH thesis and I want to take this opportunity to recognize them. Specifically, thank you to: Jessica Alvarez, for her close guidance throughout this entire project; Thomas Ziegler, for his professional mentoring and support; Qiang Zhang, for being my committee chair and academic advisor; Douglas Walker, for providing his expertise in metabolomics and for his mentoring on the project.

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Introduction

There has been a marked increase in the global prevalence of both obesity and type II diabetes mellitus (T2DM) over the past three decades (Eckel et al., 2011). As obesity and T2DM are complex diseases, using the overly simplistic view of "calories in versus calories out" to address these important public health issues has not been successful. Addressing the global epidemic of obesity and associated T2DM by identifying new modifiable risk factors and mechanisms of effects is imperative.

Environmental endocrine disrupting chemicals (EDCs) that alter metabolic programming and adipose tissue (AT) differentiation and expansion may increase the risk of obesity and metabolic disease (Blumberg, Iguchi, & Odermatt, 2011; Heindel et al., 2017; Janesick & Blumberg, 2011; Thayer, Heindel, Bucher, & Gallo, 2012). Animal studies indicate that a broad spectrum of persistent organic pollutants (POPs) and other EDCs accumulate in adipose tissue, disrupt metabolic systems, and have obesogenic and diabetogenic effects (Blumberg et al., 2011; Hines et al., 2009; Thayer et al., 2012). Recent literature has shown that exposure to certain EDCs during critical stages of development predisposes the individual to weight gain and obesity through a variety of mechanisms that act during lineage specification, differentiation, and maintenance of adipocytes (Janesick & Blumberg, 2011). EDCs also have the ability to alter gene expression by modulating developmental signaling pathways and promoting epigenetic changes that produce stably inherited changes in gene expression (Janesick & Blumberg, 2011).

POPs are organic compounds that are resistant to environmental degradation through chemical, biological, and photolytic processes. They are capable of long-range transport, bioaccumulation in human and animal tissue, and biomagnification in food chains, and may have significant impacts on both human health and the environment. A recent comprehensive review

about the link between POPs, obesity, and diabetes concluded that human evidence on POPs and obesity remains insufficient, but evidence on POPs and T2DM is much stronger (Lee, Porta, Jacobs, & Vandenberg, 2014).

In 2001, the Stockholm Convention on Persistent Organic Pollutants identified 12 initial POPs including alderin, chlordane, dichlorodiphenyltrichloroethane (DDT), dieldrin, endrin, heptachlor, hexachlorobenzene (HCB), mirex, toxaphene, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) (Stockholm Convention, 2001). They recently identified 16 new POPs in June 2017 including: α- and β-hexachlorocyclohexane, chlordecone, decabromodiphenyl ether, hexabromobiphenyl, hexabromocyclododecane, hexabromodiphenyl ether, heptabromodiphenyl ether, hexachlorobutadiene, lindane, pentachlorobenzene, pentachlorophenol & its salts and esters, perfluorooctane sulfonic acid (PFOS), its salts and perfluorooctane sulfonyl fluoride, polychlorinated naphthalenes, short-chain chlorinated paraffins, technical endosulfan and its related isomers, tetrabromodiphenyl ether and pentabromobiphenyl ether (Stockholm Convention, 2017).

Perfluoroalkyl substances (PFASs), such as PFOS, perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), and perfluorohexanesulfonic acid (PFHxS), were newly listed as POPs in 2017 even though they have been produced since the 1950s. PFASs are used in many industrial and manufacturing applications including production of nonstick cookware, waterproof and breathable textiles, and protective coatings for paper, food packing materials, and carpets (Corsini, Luebke, Germolec, & DeWitt, 2014). Unlike other POPs which accumulate in adipose tissue, the target tissue of PFASs is the liver, where they bind to proteins such as albumin (Han, Snow, Kemper, & Jepson, 2003; P. D. Jones, Hu, De Coen, Newsted, & Giesy, 2003). PFASs

are persistent in the environment and have substantial bioaccumulation and biomagnifying properties. Potential routes of exposure to PFASs include ingestion (food and contaminated drinking water), dermal contact (textiles, carpeting) and inhalation of indoor dust. Known serum half-lives of PFASs in humans range from 3.5 to 8.5 years, indicating bioaccumulation in the body (EPA, 2009; Olsen et al., 2007). As they are detected in the serum of almost every person in the United States, PFASs are an important public health issue (Kato, Wong, Jia, Kuklenyik, & Calafat, 2011).

Although humans are highly and widely exposed to these PFASs daily, their metabolic effects on humans are not well understood. Experimental animal studies have found that PFASs activate peroxisome proliferator-activated receptors (PPARs) (Rosen et al., 2008; Wolf, Schmid, Lau, & Abbott, 2012), which regulate energy homeostasis, lipid and glucose metabolism, and adipocyte differentiation and function (Berger & Moller, 2002). Some cross-sectional studies involving adults from the National Health and Nutrition Examination Survey (NHANES) support observations from animal studies, and showed that increased serum PFOA and PFOS levels were associated with metabolic outcomes such as increased fasting glucose (Liu, Wen, Chu, & Lin, 2018), increased insulin, and insulin resistance (assessed using the homeostatic model assessment for insulin resistance [HOMA-IR]) (Lin, Chen, Lin, & Lin, 2009).

The detrimental metabolic effects of obesity are predominantly driven by the accumulation of visceral adipose tissue (VAT), which is adipose tissue that accumulates within the abdominal cavity and surrounds internal organs, including the liver. VAT is more metabolically active than subcutaneous adipose tissue (SAT), and invokes changes to metabolism and other cellular processes, such as increased oxidative stress and inflammatory biomarkers (Kershaw & Flier, 2004). Detailed analysis of the linkage between PFASs and VAT

is warranted given the widespread negative health effects associated with both. In vitro or in vivo studies looking at obesogenic effects of PFASs are scarce and inconsistencies exist in the studies that are available (de Cock & van de Bor, 2014). Whether PFASs interfere with body weight regulation in humans is largely unknown. Recent literature suggests that prenatal exposure to PFASs may be associated with increased adiposity among children (Braun et al., 2016; Mora et al., 2016). Another recent study found that in women who are undergoing diet-induced weight loss trials, higher baseline PFAS concentrations were associated with greater weight regain (G. Liu et al., 2018).

The primary goal of this investigation was to determine if there is an association between plasma levels of PFOS, PFNA, and PFHxS, measured by high-resolution metabolomics, and VAT mass at the baseline visit in the Emory-Georgia Tech Predictive Health Institute's Center for Health Discovery and Well Being (CHDWB) cohort. We also aimed to determine the relationship between baseline plasma levels of these PFASs and concomitantly measured SAT mass, total body fat mass, indexes of glucose tolerance and insulin sensitivity (fasting blood glucose, fasting insulin, HOMA-IR), plasma proinflammatory markers (interleukin [IL]-6, IL-8, and TNF-α, c-reactive protein [CRP]), and measures of oxidative stress (glutathione [GSH], glutathione disulfide [GSSG] and cysteine [CyS]/CySS aminothiol redox biomarkers).

We hypothesized that higher levels of plasma PFASs would be correlated with higher VAT mass, given its proximity to the liver. We further hypothesized that higher plasma PFASs would be associated with increased SAT mass, higher insulin resistance, higher fasting glucose, higher plasma concentrations of pro-inflammatory cytokines, and greater oxidative stress.

Methods

Study Design, Data Collection and Protocol

This was a cross-sectional study of 179 adults who were enrolled in the Emory-Georgia Tech Predictive Health Institute's CHDWB cohort (Brigham, 2010) and who had baseline plasma high-resolution metabolomics data available. Participants for the CHDWB cohort were recruited between January 2008 and February 2013 and included male or female employees aged 18 and older with an absence of hospitalizations in the previous year (except for accidents). Exclusion criteria for the cohort comprised of the following: hospitalization for acute or chronic disease within the previous year; history of severe psychosocial disorder within the previous year; addition of new prescription medications to treat a chronic condition within the previous year (with the exception of changes in antihypertensive or antidiabetic agents); history of substance/drug abuse or alcoholism within the previous year; current active malignant neoplasm; history of malignancy other than localized basal cell cancer of skin during the previous 5 years; uncontrolled or poorly controlled autoimmune, cardiovascular, endocrine, gastrointestinal, haematologic, infectious, inflammatory, musculoskeletal, neurologic, psychiatric or respiratory disease; and any acute illness (such as viral infection) in the previous 12 weeks before baseline visits. The study was approved by the Emory University Institutional Review Board, and all participants provided informed consent.

Testing and data collection was performed over two baseline visits, each within three weeks of the other. The first visit occurred after the participant fasted overnight, and included blood draws and questionnaires regarding demographic information, health history and current status, tobacco use, and medication and supplement use. Body composition testing was performed on either the 1st or 2nd visit and assessed by dual energy X-ray densitometry (DEXA,

GE Lunar Densitometry iDXA, GE Healthcare, Waukesha, WI). Blood pressure was measured with an automated machine (Omron, Kyoto, Japan). Blood was collected in ethylenediaminetetraacetic acid (EDTA)-containing tubes (BD Vacutainer®, Franklin Lakes, NJ, USA), centrifuged at 1300 RCF for 10 min, and plasma was stored at −80°C until analysis. Serum fasting glucose, fasting insulin, and high-sensitivity CRP was measured commercially (Quest Diagnostics Nichols Valencia, Valencia, CA). Serum IL-6, IL-8 and TNF-α were measured using a Fluorokine® MultiAnalyte Profiling multiplex kit (R&D Systems, Minneapolis, MN) with a Bioplex analyzer (Bio-Rad, Hercules, CA). Serum GSH, GSSG, Cys, and CySS were measured by high performance liquid chromatography, as previously described (D. P. Jones & Liang, 2009), as indicators of oxidative stress and redox balance.

High-Resolution Metabolomics

Blood samples were analyzed in triplicate with high-performance liquid chromatography coupled to ultra-high-resolution mass spectrometry (LC-MS; LTQ–Velos Orbitrap, Thermo Scientific, San Diego, CA, USA) using C18 chromatography and electrospray ionization in negative ion mode. Samples were treated with acetonitrile (2:1, v/v), an internal standard mixture was added, and the samples were centrifuged at 4°C, using established procedures (Frediani et al., 2014; D. P. Jones & Liang, 2009). Each batch of 20 samples was preceded and followed by analysis of pooled reference samples to support quality control and quality assurance.

Raw data files were extracted using apLCMS (Yu, Park, Johnson, & Jones, 2009) with modifications by xMSanalyzer (Uppal et al., 2013). A feature table was generated that contained m/z (mass-to-charge ratio) features, where a feature is defined by its m/z, retention time and ion

intensity. Prior to data analysis, m/z features were batch-corrected using ComBat (Johnson, Li, & Rabinovic, 2007) and filtered to remove those with coefficient of variation (CV) ≥ 100% and >10% non-detected values. To identify potential PFASs in the dataset, analyte identification was confirmed by matching precursor m/z to authentic reference standards.

Statistical Analyses

Descriptive statistics were performed among all subjects and by sex. Normality of all dependent variables was assed and log transformation was performed on variables that visibly deviated from a normal distribution before further analyses were completed. Intensities were similar across age, race, BMI, household income, and number of years of education. Therefore, we only adjusted for sex in our linear regression analyses for the primary and secondary endpoints. Multiple linear regression analyses were performed to investigate the relationship between plasma PFAS intensities (independent variable) and VAT mass (primary dependent variable), adjusting for sex. Additional analyses included plasma PFAS intensities and the following secondary dependent variables: SAT mass, total body fat mass, fasting blood glucose, fasting insulin, HOMA-IR [calculated using the updated HOMA model (Levy, Matthews, & Hermans, 1998)], plasma IL-6, IL-8, and TNF-α, CRP, GSH, GSSG, CyS/CySS.

We assessed PFAS intensity as a continuous and dichotomous variable (where 0=0.0 intensity, 1=intensity greater than 0). Fasting blood glucose, fasting insulin, HOMA-IR, total cholesterol, HDL cholesterol, and LDL cholesterol were assessed as both continuous and categorical variables. Cut-off values were determined using World Health Organization standards for BMI, values from Gayoso-Diz et al. (2013) for HOMA-IR, and guidelines from the National Cholesterol Education Program Adult Treatment Panel III for fasting glucose, fasting

insulin, and total, HDL, and LDL cholesterol. $P < 0.05$ was considered statistically significant. All analyses were performed using SAS (version 9.4, SAS Institute, Cary, NC).

Results

Subject Characteristics

Baseline demographic and clinical characteristics are detailed in Table 1. We included a total of 179 participants (115 women, 64 men; 37 [20.67%] under the age of 40; 137 [76.6%] Caucasian) in our analysis. Mean age at the baseline CHDWB visit was 49.5 years (+/- 10.23). Over 95% of participants were nonsmokers. Education ranged from high school (8th grade) to six years of post-graduate school, but most participants reported four years of college (24%) or two years of graduate school (15%). The majority of our participants reported an average annual household income between $100,000-150,000.

For the metabolic outcomes, 41% of individuals in this cohort had a normal BMI (18-24.9 kg/m2), 89% had normal fasting glucose (<100 mg/dL), 78% had normal fasting insulin (<8 uIU/mL), 80% had normal HOMA-IR (<1.8 mass units) (Table 2). For cholesterol, 54% had normal total cholesterol (<200 mg/dL), 91% had high HDL cholesterol, and 58% had optimal LDL cholesterol (Table 2).

HRM Results

Over 10,800 features were detected in the CHDWB dataset. We detected three PFASs in the cohort including PFOS, PFNA, and PFHxS. Non-zero intensity values for PFOS, PFNA, and PFHxS were detected in 95%, 66%, and 80% of participants, respectively. PFAS intensities are shown in Figure 1A-C.

Results from the linear regression models showed that intensities of PFASs significantly differed by sex for PFOS ($p=0.05$) and PFHxS ($p<0.001$) (Table 3, Figure 2). Intensities were similar across age, race, BMI, household income, and number of years of education. Therefore, we only adjusted for sex in our linear regression analyses for the primary and secondary endpoints.

Primary Endpoint Results

Visceral fat mass was positively associated with PFOS ($p=0.02$) and PFHxS ($p=0.04$) (Table 4). However, after controlling for sex, these associations were no longer statistically significant. SAT mass was negatively associated with PFNA and PFHxS before adjusting for sex ($p=0.02$); however, the association was not significant after adjustment ($p=0.25$) (Table 4).

Secondary Endpoint Results

Intensities of PFASs were not significantly associated with fasting insulin, fasting glucose, HOMA-IR, total cholesterol, HDL cholesterol, or LDL cholesterol (Supplementary Table 1). PFASs were also not associated with higher body composition measurements (Supplementary Table 2). Likewise, there were no significant relationships between PFASs and the proinflammatory markers or the oxidative stress measurements (Supplementary Tables 3 and 4). Even when assessing PFAS as a dichotomous variable, no statistically significant associations were present (Supplementary Table 5).

Discussion

As other POPs have been shown to correlate with adiposity and metabolic derangements, we hypothesized that higher levels of PFASs would be associated with increased VAT and SAT mass. Additionally, we hypothesized that higher levels of PFASs would be associated with metabolic outcomes such as increased insulin resistance, hyperglycemia, higher plasma concentrations of pro-inflammatory cytokines, and greater oxidative stress. However, in this study, PFASs were not significantly, independently associated with adiposity or other biochemical measures of metabolic health.

This study used untargeted metabolomic data to look at the relationship between exposure to PFASs, body composition, and related metabolic outcomes in an Atlanta cohort. We detected three out of the four prospective PFASs (PFOS, PFNA and PFHxS). While PFOA was expected to be present in this population, it was not detected. It is possible that this PFAS is present at levels below the limit of detection in this population.

VAT mass was positively associated with PFOS and PFHxS (Table 4). However, after controlling for sex, these associations were no longer statistically significant. There are sex differences in VAT and SAT storage in the body, with men generally having greater VAT than women (Demerath et al., 2007). There are a limited number of studies that have looked at the relationship between blood levels of PFASs and VAT mass. Recent literature suggests that prenatal exposure to PFASs could be associated with increases in adiposity among children (Braun et al., 2016; Mora et al., 2016). Another recently published study looked at gender and obesity-stratified models to explore the relationship between PFASs and lipids and found that obesity modifies the cross-sectional associations of PFASs with lipid concentrations (Jain &

Ducatman, 2019). Their findings suggested that higher levels of long chain PFASs were associated with greater susceptibility to elevated total cholesterol and LDL cholesterol in obese participants. Current evidence linking PFASs with higher fat mass is scarce and more research must be done to identify the true relationship between blood levels of PFASs and body composition.

There were no significant findings when we evaluated the relationship between PFAS intensity and metabolic outcomes. This cohort consisted of relatively healthy, actively working, primarily Caucasian individuals; thus, our results could be subject to selection bias. PFAS intensity in this study may not be representative of the entire Atlanta population, but could be for the Emory-Georgia Tech community. For the metabolic outcomes in Table 2, we found that 41% of individuals in this cohort had a normal weight BMI, 89% had normal fasting glucose, 78% had normal fasting insulin, and 80% had normal HOMA-IR. For cholesterol, 54% had normal total cholesterol, 91% had high HDL cholesterol, and 58% had optimal LDL cholesterol levels. Given that the cohort consisted of relatively healthy individuals, this could explain the lack of any significant findings.

Males had a higher PFAS intensities than females for all three chemicals. This is consistent with previous knowledge and studies (ATSDR, 2018; Calafat et al., 2007; Kato et al., 2011). Potential explanations could be due to gender differences in occupation, diet (fast food intake), and use of PFAS-containing products (waterproof textiles). However, this has not been studied to date and should be investigated further. It has also been suggested that women may have lower PFAS concentrations in blood due to PFAS loss through menstruation, childbirth, and breastfeeding (ATSDR, 2018).

Limitations of the study include using a cross-sectional study design, which prevents us from making any causal claims about our findings, and the relatively small sample size compared to studies using large national databases like NHANES.

In conclusion, this study did not find significant relationships between PFAS and adiposity or cardiometabolic risk factors, after accounting for sex differences. Inconsistencies exist in the current literature regarding the health effects of PFAS exposure which may be due to differences in study design, population demographics, or PFAS concentration distributions. More robust, longitudinal studies should be conducted to identify potential health effects of PFAS exposure.

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Figures and Tables

Table 1. Participant Demographics

Characteristic	Participants No. (%)
Overall	179
Sex	
Male	64 (35.8)
Female	115 (64.2)
Race/Ethnicity	
Caucasian	137 (76.6)
Other	42 (23.4)
Smoking Status	
Non-smokers	171 (95.5)
Smokers	8 (4.5)
Age (yrs)	
<40	37 (20.67)
40-44	19 (10.61)
45-50	27 (15.08)
50-54	33 (18.44)
55-60	34 (18.99)
60-64	19 (10.61)
>65	10 (5.59)
Education	
High School	6 (3.35)
College/University	69 (38.55)
Graduate School	69 (38.55)
Post-graduate School	35 (19.55)
Average Annual Household Income	
Not listed	10 (5.62)
$0 to $25,000	1 (0.56)
$25,000 to $50,000	15 (8.43)
$50,000 to $75,000	22 (12.36)
$75,000 to $100,000	22 (12.36)
$100,000 to $150,000	43 (24.16)
$150,000 to $200,000	19 (10.67)
$200,000 to $250,000	12 (6.74)
$250,000 to $300,000	5 (2.81)
Above $300,000	29 (16.29)

Table 2. Participant Metabolic Results

BMI Classification (kg/m^2)	
Normal (18.5-24.9)	73 (40.78)
Overweight (25.0-29.9)	64 (35.75)
Obese (≥30.0)	42 (23.46)
Fasting Blood Glucose (mg/dL)	
Normal (<100)	160 (89.39)
Above normal (≥100)	19 (10.61)
Fasting Insulin (uIU/mL)	
Normal (<8)	139 (77.65)
Above normal (≥8)	40 (22.35)
HOMA-IR (mass units)	
Normal (<1.8)	141 (78.77)
Above normal (≥1.8)	38 (21.23)
Total Cholesterol (mg/dL)	
Normal (<200)	98 (54.75)
Above normal (≥200)	81 (42.25)
HDL Cholesterol (mg/dL)	
Low (<40)	10 (9.26)
High (≥60)	98 (90.74)
LDL Cholesterol (mg/dL)	
Optimal (<100)	75 (58.33)
Above optimal (≥100)	105 (41.67)

Cut-off values were determined using World Health Organization standards for BMI, values from Gayoso-Diz et al. (2013) for HOMA-IR, and guidelines from the National Cholesterol Education Program Adult Treatment Panel III for fasting glucose, fasting insulin, and total, HDL, and LDL cholesterol.

Table 3. Linear Regression Results for Sex

PFAS	Parameter Estimate	Standard Error	P-value
PFOS	-0.02	0.008	0.04*
PFNA	-0.01	0.006	0.06
PFHxS	-0.03	0.006	<0.0001*

*Indicates a statistically significant result

Table 4. Linear Regression Results for Visceral and Subcutaneous Adipose Tissue

PFAS	Parameter Estimate	Standard Error	P-value	Adjusted Parameter Estimate	Standard Error	Adjusted P-value
VISCERAL ADIPOSE TISSUE MASS						
PFOS	0.083	0.032	0.02*	0.043	0.029	0.14
PFNA	0.041	0.023	0.14	0.014	0.021	0.49
PFHxS	0.064	0.026	0.04*	-0.014	0.026	0.60
LOG SUBCUTANEOUS ADIPOSE TISSUE MASS						
PFOS	0.125	0.006	0.51	0.009	0.006	0.19
PFNA	-0.004	0.004	0.98	0.001	0.004	0.78
PFHxS	-0.373	0.005	0.02*	-0.006	0.005	0.25

*Indicates a statistically significant result

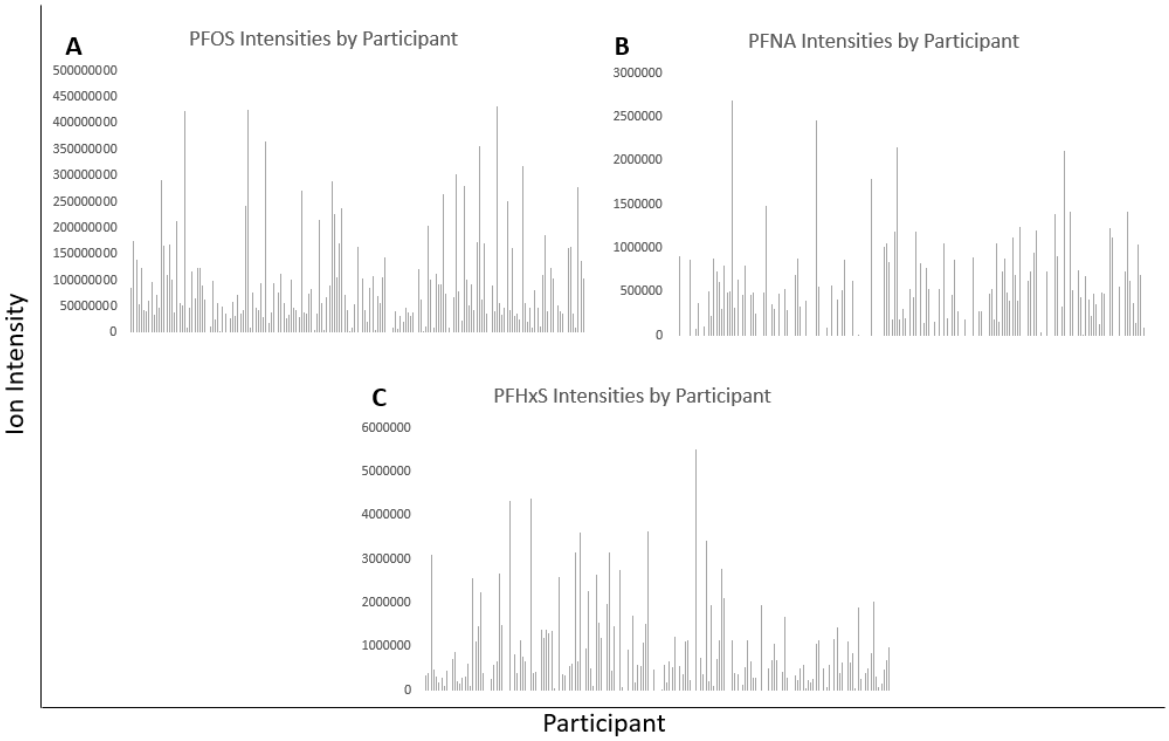

Figure 1A-C. Individual ion intensities for PFOS (A), PFNA (B), and PFHxS (C).

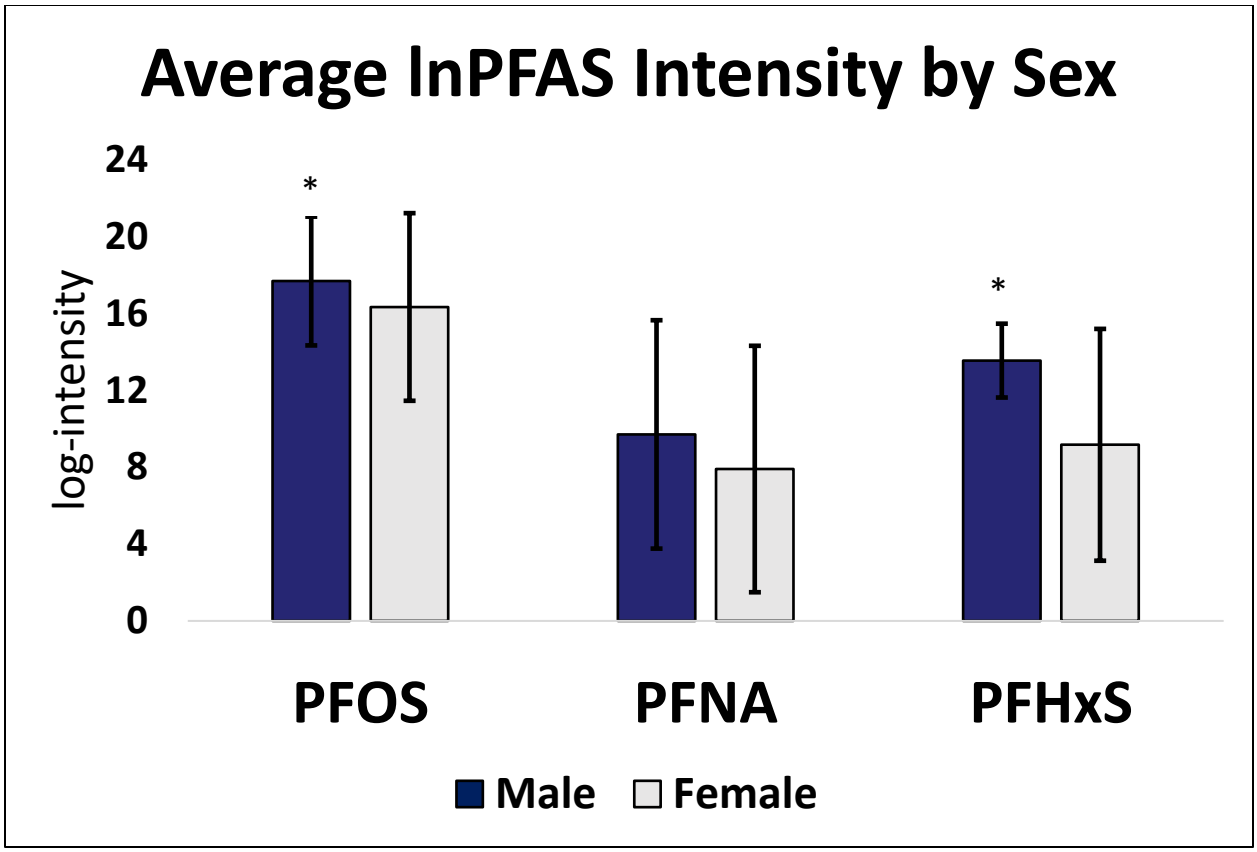

Figure 2. Average lnPFAS intensity by sex. Average lnPFAS intensity for males is depicted in blue while average lnPFAS intensity for females is depicted in grey. Error bars are representative of standard deviation. *Represents a statistically significant result.

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Supplementary Information

Supplementary Table 1. Linear Regression Results for PFASs vs. Measures of Insulin Resistance

PFAS	Parameter Estimate	P-Value	Adjusted Parameter Estimate	Adjusted P-Value
LOG FASTING GLUCOSE				
PFOS	0.004	0.11	0.003	0.20
PFNA	-0.003	0.15	-0.003	0.07
PFHxS	0.0001	0.95	-0.002	0.34
LOG FASTING INSULIN				
PFOS	0.010	0.44	0.008	0.52
PFNA	0.009	0.29	0.008	0.35
PFHxS	0.005	0.64	0.001	0.91
LOG HOMA-IR				
PFOS	0.013	0.30	0.001	0.40
PFNA	0.006	0.49	0.005	0.61
PFHxS	0.005	0.65	-0.0009	0.94
LOG TOTAL CHOLESTEROL				
PFOS	0.316	0.64	0.374	0.59
PFNA	-0.764	0.11	-0.745	0.13
PFHxS	-0.497	0.37	-0.459	0.45
LOG TRIGLYCERIDES				
PFOS	0.0120	0.13	0.009	0.26
PFNA	0.0007	0.90	-0.001	0.80
PFHxS	0.005	0.48	-0.003	0.69
HDL				
PFOS	-0.164	0.60	0.111	0.71
PFNA	-0.281	0.18	-0.152	0.45
PFHxS	-0.265	0.28	0.221	0.38
LDL				
PFOS	0.154	0.79	0.064	0.91
PFNA	-0.491	0.23	-0.561	0.17
PFHxS	-0.307	0.52	-0.598	0.25

*Indicates a statistically significant result

Supplementary Table 2. Linear Regression Results for PFASs vs. Body Composition

PFAS	Parameter Estimate	P-Value	Adjusted Parameter Estimate	Adjusted P-Value
LOG TOTAL BODY FAT (KG)				
PFOS	0.125	0.51	0.009	0.19
PFNA	-0.004	0.98	0.001	0.78
PFHxS	-0.373	0.02*	-0.007	0.25
PERCENT REGION FAT				
PFOS	0.023	0.87	0.175	0.15
PFNA	-0.073	0.46	0.024	0.78
PFHxS	-0.400	0.0005*	-0.090	0.40
ANDROID REGION FAT				
PFOS	0.193	0.33	0.267	0.18
PFNA	0.066	0.64	0.112	0.42
PFHxS	-0.223	0.17	-0.088	0.62
ANDROID COMPOSITION FAT				
PFOS	0.013	0.09	0.011	0.16
PFNA	0.003	0.52	0.002	0.70
PFHxS	-0.0007	0.91	-0.006	0.36
GYNOID REGION FAT				
PFOS	-0.146	0.40	0.131	0.20
PFNA	-0.176	0.15	0.005	0.95
PFHxS	-0.598	<0.0001*	0.002	0.98
GYNOID COMPOSITION FAT				
PFOS	0.00004	0.10	0.005	0.29
PFNA	-0.003	0.42	0.0002	0.96
PFHxS	-0.013	0.004*	-0.002	0.58
LEAN FAT				
PFOS	0.568	0.14	0.019	0.95
PFNA	0.345	0.21	-0.017	0.93
PFHxS	1.065	0.0008*	-0.163	0.51
LOG COMPOSITION FAT				
PFOS	0.00604	0.34	0.008	0.22
PFNA	-0.0002	0.97	0.0009	0.84
PFHxS	-0.008	0.13	-0.005	0.36

*Indicates a statistically significant result

Supplementary Table 3. Linear Regression Results for PFASs vs. Proinflammatory Cytokines

PFAS	Parameter Estimate	P-Value	Adjusted Parameter Estimate	Adjusted P-Value
LOG CRP				
PFOS	0.014	0.25	0.019	0.12
PFNA	0.009	0.27	0.013	0.14
PFHxS	-0.018	0.07	-0.009	0.39
LOF TNF-A				
PFOS	-0.010	0.55	-0.011	0.51
PFNA	-0.015	0.19	-0.016	0.17
PFHxS	-0.011	0.40	-0.016	0.27
LOG IL-8				
PFOS	0.010	0.24	0.011	0.23
PFNA	0.000002	0.10	0.00008	0.99
PFHxS	-0.005	0.43	-0.006	0.43
LOG IL-6				
PFOS	-0.005	0.52	-0.005	0.57
PFNA	0.001	0.83	0.001	0.80
PFHxS	-0.004	0.58	0.003	0.68

*Indicates a statistically significant result

Supplementary Table 4. Linear Regression Results for PFASs vs. Oxidative Stress

PFAS	Parameter Estimate	P-Value	Adjusted Parameter Estimate	Adjusted P-Value
CYS				
PFOS	0.037	0.31	0.055	0.13
PFNA	-0.027	0.27	-0.021	0.38
PFHxS	0.005	0.86	0.038	0.21
CYSS				
PFOS	-0.162	0.62	-0.133	0.69
PFNA	-0.392	0.07	-0.390	0.08
PFHxS	0.220	0.39	0.321	0.25
GSH				
PFOS	-0.005	0.64	-0.003	0.78
PFNA	-0.011	0.10	-0.011	0.12
PFHxS	-0.010	0.21	-0.008	0.37
GSSG				
PFOS	-0.001	0.02*	-0.001	0.02
PFNA	-0.0001	0.79	-0.0001	0.75
PFHxS	-0.0005	0.30	-0.0007	0.17

*Indicates a statistically significant result

Supplementary Table 5. Linear Regression Results for PFAS as a Dichotomous Variable (where 0=0.0 intensity, 1=intensity greater than 0)

PFAS	Parameter Estimate	P-Value	Adjusted Parameter Estimate	Adjusted P-Value
VAT MASS				
PFOS	0.938	0.11	0.610	0.25
PFNA	0.365	0.22	0.140	0.60
PFHxS	0.497	0.17	-0.264	0.44
LOG SAT MASS				
PFOS	0.080	0.486	0.117	0.29
PFNA	-0.016	0.786	0.0087	0.88
PFHxS	-0.171	0.013*	-0.101	0.14
LOG FASTING GLUCOSE				
PFOS	0.066	0.17	0.055	0.24
PFNA	-0.038	0.12	-0.045	0.06
PFHxS	-0.008	0.78	-0.035	0.24
LOG FASTING INSULIN				
PFOS	0.155	0.49	0.136	0.55
PFNA	0.109	0.34	0.097	0.40
PFHxS	0.045	0.74	0.002	0.99
LOG HOMA-IR				
PFOS	0.220	0.37	0.191	0.43
PFNA	0.072	0.56	0.052	0.68
PFHxS	0.037	0.80	-0.033	0.83
LOG TOTAL BODY FAT				
PFOS	0.119	0.35	0.133	0.26
PFNA	-0.00066	0.10	0.0136	0.82
PFHxS	-0.136	0.06	-0.104	0.18

*Indicates a statistically significant result