Non-Targeted Metabolomic Profiling to Study Possible Metabolic Effects of PFASs in a Population-based Study

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Introduction

Perfluoroalkyl substance (PFAS) exposure affects human populations worldwide and an integral part of the exposure has been found to arise from ingestion of contaminated food and drinking water. Human exposure to PFASs has been implicated in the development of a number of diseases, including hypercholesterolemia and type 2 diabetes [1, 2]. The biochemical mechanism underlying PFASs-induced action has been suggested to include activation of the peroxisome proliferator-activated receptors (PPARs) alpha and gamma [3]. These nuclear receptors play a fundamental role in lipid and adipose tissue homeostasis. However, despite these findings, data to support the multiple potential biochemical mechanisms through which PFASs induce effects in humans are limited. Recently, advances in mass spectrometry and bioinformatics technologies have enabled global analysis of circulating metabolites in the context of large epidemiological studies. A number of metabolites in circulation (e.g., fatty acids, bile acids, glycerophospholipids) have been put forward as prognostic biomarkers in the development of type 2 diabetes and cardiovascular disease [4, 5]. However, whether PFASs affect the levels and profiles of metabolites in human circulation remains unclear. The aim of this study was to investigate the effect of PFASs levels on the metabolome by determining both circulating levels of PFASs and global metabolite profiles in 1,016 individuals from a population-based study.

Materials and methods

Study population and sample collection

The participants in the PIVUS study were randomly selected from the general population in the community of Uppsala, Sweden. Invitation letters were sent between April 2001 and June 2004 and within two months of each of the participants 70th birthday. The target sample population was 2, 025 participants out of which 1, 016 participated.

Serum and plasma were collected in the morning after overnight fast. After the samples were collected (1-2 mL vials), the vials were placed in freezers (-20 °C) until used for chemical analysis. The study was approved by the Ethics Committee of the University of Uppsala and the participants gave written informed consent.

PFAS analysis

Briefly, internal standards and 150 µL plasma or serum were added to a Ostro Sample Preparation 96-well plate 25mg (Waters Corporation, Milford, USA) followed by 450 µL 1% formic acid in acetonitrile. Samples were filtered and transferred to vials containing recovery standard and evaporated down to 250 µL using nitrogen. Finally, 750 µL 0.1 M formic acid in water was used to dilute the sample prior to the instrumental analysis. Mass analysis was performed on a UPLC-MS/MS (Waters Corporation, Milford, USA) system by injecting a 250 µL aliquot of the sample onto a C18 (2.1×20mm, 2.5µm) trap column connected to a C18 (2.1×100 mm, 1.7 µm) analytical column by a 6-port column switch valve. Analytes were analyzed on a MS/MS system run in electrospray ionization mode (ESI). Quantitative analysis of the PFASs was performed using the internal standard method. The method employed to all samples was successfully validated in terms of accuracy and precision, detailed information about the method validation can be found elsewhere [6].

Metabolomics profiling

Non-targeted metabolite profiling was performed using methods previously described in detail [7]. Briefly, serum samples were thawed and 100 µL of serum was transferred to a 96-well plate and 400 µL methanol was added to precipitate proteins. Separation and data acquisition was performed on Acquity UPLC coupled to a Xevo G2 Q-TOFMS (Waters Corporation, Milford, USA) with an atmospheric electrospray interface operating in positive ion mode. Non-consecutive duplicate sample aliquots of 1 µL were injected onto an Acquity UPLC BEH C8 (1.8 µM, 1.0 x 100 mm) analytical column held at 50°C using a gradient of water, methanol, and formic acid. Mass analysis was performed in the full scan MS mode (m/z 50-1200) at 6 V and fragmentation scans were collected in the MS^E mode using a collision ramp of 15-30V.

Data processing and metabolite annotation

Raw data were processed using XCMS software [8]. A detailed description of the data processing and annotation approach used for this study can be found elsewhere. Overall, this approach enabled the successful identification of ~200 metabolites in serum samples from participants in the PIVUS study. The metabolites detected come from diverse compound classes including amino acids and derivatives, peptides, steroids and steroid derivatives, alcohols and polyols, and various compounds related to the lipid metabolism such as fatty acids, glycerophospholipids, sphingolipids, and glycerolipids.

Statistical analysis

Multivariable linear regression models were used to assess the association between PFASs and metabolites following adjustment for sex and smoking, exercise habits, education, energy and alcohol intake. Only 6 PFASs with a

detection rate >75% were included in the final analyses Metabolites with a detection rate >30% and with a significant *p-value* ($p<4.11x10-5$) after Bonferroni multiple-testing adjustment are reported.

Results and discussion

As shown in Figure 1, levels of five PFASs were found to be related to 17 metabolites belonging to the class of amino acids (1), cinnamic acids (1), purines (1), fatty acids (4), and glycerophospholipids (10) following adjustment for sex and life-style factors (smoking, exercise habits, education, energy and alcohol intake). Moreover, the metabolic fingerprint was shown to be significantly different ($p<0.0001$) among the 6 PFASs evaluated. Mainly lipid pathways were related with PFASs exposure, where the strongest associations were observed for the long-chain analogues PFNA and PFUnDA thus suggesting potentially different biochemical pathways of PFNA and PFUnDA action in humans when compared to PFHxS, PFOS, PFHpA, and PFOA. Our results show the usefulness of metabolomics as a tool to study potential metabolic effects associated with exposure to environmental chemicals.

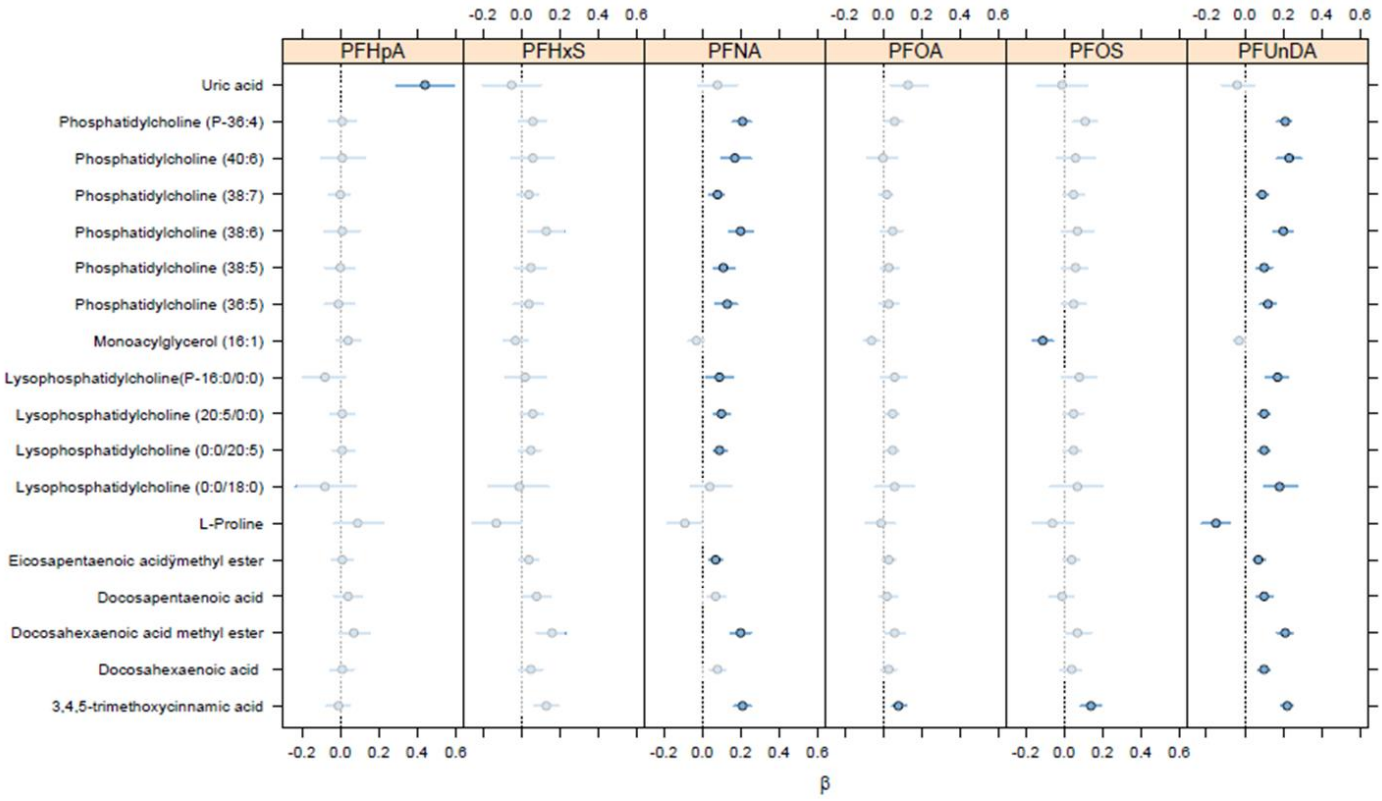

Figure 1. Identification of circulating metabolites associated with plasma concentrations of PFASs ($ngmL^{-1}$). Only significant associations are highlighted as bold.

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