Early-life exposure to perfluorinated alkyl substances modulates lipid metabolism in progression to celiac disease

Lisanna Sinisalu, Partho Sen, Samira Salihović, Suvi Virtanen, Heikki Hyöty, Jorma Ilonen, Jorma Toppari, Riitta Veijola, Matej Orešič, Mikael Knip, Tuulia Hyötyläinen.

School of Science and Technology, Örebro University, Örebro, Sweden
Turku Bioscience Centre, University of Turku and Åbo Akademi University, Turku, Finland
School of Medical Sciences, Örebro University, Örebro, Sweden
National Institute for Health and Welfare, Nutrition Unit, Helsinki, Helsinki, Finland
Faculty of Medicine and Life Sciences, University of Tampere, Tampere, Finland
Fimlab Laboratories, Pirkanmaa Hospital District, Tampere, Finland
Immunogenetics Laboratory, Institute of Biomedicine, University of Turku, Turku, Finland
Clinical Microbiology, Turku University Hospital, Turku, Finland
Institute of Biomedicine, Centre for Integrative Physiology and Pharmacology, and Centre for Population Health Research, University of Turku, Turku, Finland
Department of Pediatrics, Turku University Hospital, Turku, Finland
Department of Paediatrics, PEDEGO Research Unit, Medical Research Centre, University of Oulu, Oulu, Finland
Department of Children and Adolescents, Oulu University Hospital, Oulu, Finland
Department of Women’s and Children’s Health, Karolinska Institutet, Stockholm, Sweden
Pediatric Research Center, Children's Hospital, University of Helsinki and Helsinki University Hospital, 00290 Helsinki, Finland; Research Program for Clinical and Molecular Metabolism, Faculty of Medicine, University of Helsinki, Helsinki, Finland
Center for Child Health Research, Tampere University Hospital, Tampere, Finland

*Shared senior authorship.

Correspondence:
Matej Orešič; Email: matej.oresic@utu.fi
Mikael Knip; Email: mikael.knip@helsinki.fi
Tuulia Hyötyläinen; MTM Research Centre, School of Science and Technology, Örebro University, 702 81 Örebro, Sweden. Email: tuulia.hyotylainen@oru.se; Phone: +46 19 303487
Abstract

OBJECTIVES

Celiac disease (CD) is a systemic immune-mediated disorder with increased frequency in the developed countries over the last decades implicating the potential causal role of various environmental triggers in addition to gluten. Herein, we apply determination of perfluorinated alkyl substances (PFAS) and combine the results with the determination of bile acids (BAs) and molecular lipids, with the aim to elucidate the impact of prenatal exposure on risk of progression to CD in a prospective series of children prior the first exposure to gluten (at birth and at three months of age).

METHODS

We analyzed PFAS, BAs and lipidomic profiles in 76 plasma samples at birth and at 3 months of age in the Type 1 Diabetes Prediction and Prevention (DIPP) study (n=17 progressors to CD, n=16 healthy controls, HCs).

RESULTS

Plasma PFAS levels showed a significant inverse association with the age of CD diagnosis in infants who later progressed to the disease. Associations between BAs and triacylglycerols (TGs) showed different patterns already at birth in CD progressors, indicative of different absorption of lipids in these infants.

DISCUSSION

PFAS exposure may modulate lipid and BA metabolism, and the impact is different in the infants who develop CD later in life, in comparison to HCs. The results indicate more efficient uptake of PFAS in these infants. Higher PFAS exposure during prenatal and early life may accelerate the progression to the disease in the genetically disposed children.
Study Highlights

WHAT IS KNOWN

The role of early life environmental triggers other than gluten in the development of CD has been indicated in several observational studies. This is supported by the findings showing dysregulation of lipids already prior the first introduction of gluten.

WHAT IS NEW HERE

We show that prenatal exposure to perfluorinated compounds is associated with changes in the lipid metabolism, most likely through the bile acids, and that a high exposure during prenatal and early life may accelerate the progression to clinical CD in the genetically disposed children.

TRANSLATIONAL IMPACT

Exposure to environmental chemicals may impact rate of progression to CD and should be considered as a potential risk factor of CD in larger clinical cohort settings.
Introduction

Celiac disease (CD) is a systemic immune-mediated disorder, which is triggered by gluten and other prolamins in genetically susceptible individuals. The frequency of CD has increased in the developed countries over the last decades, implicating the potential causal role of various environmental triggers in addition to gluten. Similarly to CD, the incidence of other autoimmune diseases such as type 1 diabetes (T1D) has also increased during the last decades. CD and T1D share common predisposing alleles in the class II HLA-region, and approximately 10% of patients with T1D also develop clinical CD, while subjects with CD are at-risk for developing T1D before 20 years of age. The possible triggers that have been indicated to affect the onset of CD include the composition of the intestinal microbiota, infant feeding, and the use of antibiotics.

The hygiene hypothesis, stating that a decrease of the infectious burden is associated with the rise of allergic and autoimmune diseases, has also been proposed in CD because several studies have shown that CD is more common in developed countries. Another possible explanation for varying incidence in different populations implicates the role of different infant feeding patterns (including the amount and timing of gluten introduction) in families with low socioeconomic status. Socioeconomic status (SOS) may also have a broader role in increasing the risk for CD, such as different exposures to environmental pollutants. Higher SOS has been in several studies linked with higher burdens of several persistent organic pollutants (POP), particularly for perfluorinated alkyl substances (PFAS). Exposure to POPs has, however, been poorly investigated as a risk factor for CD. In T1D, recent studies identified associations between the exposures to environmental chemicals and the increased risk of islet autoimmunity. We have shown that fetal exposure to PFAS increases the risk of T1D in genetically susceptible individuals, and further, that the metabolic profiles of the infants are affected by the prenatal exposure to PFAS. In particular, the PFAS exposure has a marked impact on bile acid (BA) profiles, which, in turn, were linked with the changes in specific
phospholipids, e.g., lysophosphatidylcholines (LPCs), phosphatidylcholines (PCs) and sphingomyelins (SMs). Similarly, we have recently identified systematic differences in plasma lipid profiles between children who later progressed to clinical CD during the follow-up, when compared to children who remained healthy. Importantly, these differences were observed prior to the first introduction of the gluten in the diet and before the first signs of CD-associated autoimmunity. Similar result were recently reported in an Italian cohort study.

In line with the observations of intestinal dysbiosis in CD, changes in lipid metabolism as well as the documented link of CD with liver disorders, it has been shown that circulating BAs are elevated in CD, including in children. BAs not only facilitate the digestion and absorption of dietary lipids in the small intestine, they are also important metabolic regulators involved in the maintenance of lipid and glucose homeostasis. BAs are produced in the liver, and their homeostasis is maintained through tightly controlled enterohepatic circulation. Moreover, there is a close interplay between BA and gut microbiota. Gut microbiota is involved in the biotransformation of secondary BAs, while BAs can modulate the microbial composition in the gut due to their antimicrobial activity as well as through regulation of the composition of the intestinal microbiota through farnesoid X receptor (FXR) and Takeda G-protein receptor 5 (TGR-5). PFAS, on the other hand, have been shown to undergo similar enterohepatic circulation as the BAs, and PFAS can also suppress the BA biosynthesis in the liver.

Here we hypothesized that early life PFAS exposure affects the BA profiles of children and explains the association between the early changes in lipid profiles in children who later progress to clinical CD. We investigated the impact of early-life exposure to PFAS in children who progressed to clinical CD during the follow-up, and compared the exposures to those from children who remained healthy during the follow-up. In addition, we also analyzed BAs in the same samples, and performed integrative analysis of previously published lipidomic profiles, together with PFAS and BA levels.

Methods
Study design

The current study is a part of the Type 1 Diabetes Prediction and Prevention study in Finland (DIPP), which is an ongoing prospective birth cohort study initiated in 1994. In DIPP, parents of newborn children at the university hospitals of Turku, Tampere and Oulu in Finland are asked for permission to screening for T1D-conferring HLA risk alleles in the umbilical cord blood. Families of children identified as having an increased HLA-conferred risk for T1D are invited to join the study. This study included children born at Oulu and Tampere University Hospitals between 1994 and 2003. Our analysis included children born at Tampere University Hospital between August 1999 and September 2005. During that period, 23,839 children were screened at birth for increased risk of T1D, and 2,642 eligible children were enrolled in the follow-up and had at least two visits to the study clinic. These children carry the high-risk HLA DQB1*02/*03:02 genotype or the moderate-risk HLA-DQB1*03:02/x genotype (x ≠ DQB1*02, 03:01, or 06:02). More than 1,200 of these children took part in the DIPP-CD study. Because of the enrolment criteria 60% of the children were male. At each visit, the families were interviewed for dietary changes, infections, growth, important family related issues and the children gave a non-fasting venous blood sample. The children were followed for 4 CD-related antibodies: anti-transglutaminase 2 (anti-TG2), anti-endomysium (EMA), antigliadin (AgA-IgG and AgA-IgA) and anti-reticulin (ARA) antibodies, and for 4 T1D-associated autoantibodies: islet cell autoantibodies (ICA), autoantibodies against insulin (IAA), tyrosine phosphatase-like protein (IA-2A) and glutamate decarboxylase (GADA). IgA deficiency was excluded. All children participating in this study were of Caucasian origin. None of the mothers had CD. The maternal health care system in Finland gives recommendations as to the starting ages for different solid foods for infants and these are generally followed. For this reason, exclusive breast feeding and addition of new food ingredients to the infants’ diets were very uniform in this study. The children of the CD follow-up cohort were annually screened for anti-TG2 antibodies using a commercial kit (Celikey Pharmacia Diagnostics, Freiburg, Germany). If a child’s sample was found positive, all previous and forthcoming samples were analyzed for the
entire set of CD-related antibodies. A duodenal biopsy was recommended for all anti-TG2-positive children. If the biopsy was consistent with the ESPGHAN criteria of 1990, a gluten-free diet (GFD) was recommended. None of the children participating in this study had any T1D-associated autoantibodies in any samples during the follow-up. The first exposure to gluten in this study was at the median age of five months (Table 1).

We have randomly selected 17 children with biopsy proven CD (progressors) and a matched control for each progressor, with the similar risk HLA alleles, born within ±1 month of each other, having had each sample taken within ±1 month of each other and living in the same region of the country throughout the whole follow-up period. Samples at birth (cord blood) and at 3 months of age were analyzed. Altogether, 76 plasma samples from children developing CD and from their HCs were analyzed. The clinical and genetic data of the participants are found in Table 1.

The ethics committees of the participating university hospitals approved the study. Written informed consent was obtained from the parents for HLA-screening, autoantibody analysis and intestinal biopsies.

Analysis of PFAS and BAs

The BAs and PFAS were analyzed using the established method as described previously. In brief, the sample preparation was done with 25 mg Ostro Protein Precipitation and Phospholipid Removal 96-well plate (Waters Corporation, Milford, USA), using 30 μL of serum and a set of BA and PFAS internal standards. Matrix-matched calibration standards were made using newborn bovine serum. Analyses were performed on an Acquity UPLC system coupled to a triple quadrupole mass spectrometer (Waters Corporation, Milford, USA) with an atmospheric electrospray interface operating in negative ion mode. Aliquots of 10 μL of samples were injected into the Acquity UPLC BEH C18 2.1 mm × 100 mm, 1.7 μm column (Waters Corporation). A trap column (PFC Isolator column, Waters Corporation) was installed.
between the pump and injector and used to retain fluorinated compounds originating from the HPLC system and the mobile phase. The eluent system consisted of (A) 2 mM NH4Ac in 70% MiliQ: 30% methanol and (B) 2 mM NH4Ac in 100% methanol. The gradient was programmed as follows: 0–1 min, 1 % solvent B; 1–13 min, 100 % solvent B; 13-16 min, 100 % solvent B; 16-17 min, 1 % solvent B, flow rate 0.3 mL/min. The total run time for UPLC-MS/MS analysis was 17 minutes, while the total run time for each sample injection was 20 minutes, including the reconditioning of the analytical column. MS analysis was performed in multiple reaction monitoring (MRM) mode and experimental details of the MS/MS method are given in Supplementary Table S1.

**Lipidomic analyses**

The lipidomic analyses were performed as described previously. The plasma samples (10 μL) were extracted using a modified version of the previously published Folch procedure. The samples were analyzed using an ultra-high-performance liquid chromatography quadrupole time-of-flight mass spectrometry method (UHPLC-Q-TOF-MS from Agilent Technologies (Santa Clara, CA, USA). The analysis was done on an ACQUITY UPLC® BEH C18 column (2.1 mm × 100 mm, particle size 1.7 μm) by Waters (Milford, USA). Internal standard mixture was used for normalization and lipid-class specific calibration was used for quantitation as previously described. Quality control was performed throughout the dataset by including blanks, pure standard samples, extracted standard samples and control plasma samples. Relative standard deviations (%RSDs) for lipid standards representing each lipid class in the control plasma samples (n=8) and in the pooled serum samples (n = 20) were on average 11.7% (raw variation). The lipid concentrations in the pooled control samples was on average 8.4% and 11.4% in the standard samples. This shows that the method is reliable and repeatable throughout the sample set. MS data processing was performed using open source software MZmine 2.18.

**Statistical analyses**
Statistical analysis was performed using R (v3.6.0) statistical programming language. Previously published lipidomics datasets was obtained from MetaboLights with the study identifier MTBLS729. The data was log-transformed and missing values were imputed by half of the row’s minimum. The datasets were auto-scaled prior to multivariate analyses.

To integrate across different data types, we applied sparse generalized correlation discriminant analysis via the DIABLO framework, part of mixOmics package (v6.10.8). The method constructs components across different data blocks, by maximizing their covariance with each other and a given response (Y) variable. Heterogenous data such as PFAS, BAs and lipid levels were partitioned into three different blocks and regressed to a binary response variable, i.e., CD progressors (CD) or HCs. Regularized sparse partial least squares discriminant (sPLS-DA) models were fitted. The optimal number of components that achieve the best performance based on the overall error rate or Balanced Error Rate (BER) were determined. Block sPLS-DA models were developed at two time-points, i.e., at birth (cord blood) and at 3 months of age. Moreover, these models were cross-validated by 5-fold cross-validation (CV) with (N =100 repeats). The final model performances were assessed by area under the curve (AUC), overall misclassification error rate, and BER generated using 'perf', 'auroc', 'block.splsda' functions.

The key predictors/contributors that are jointly associated with the response variable of interest across all the input data matrices were identified by their Variable Importance (VI) score, i.e (absolute weighted loadings). The direction (up or down) of contribution of a particular predictor was determined by estimating the mean relative log fold change in the CD progressors vs. HCs at a particular time-point.

In order to understand which variables influence the outcomes, multivariate correlations were performed by a method described in. In addition, bivariate correlation was performed using Pearson correlation. Debiased Sparse Partial Correlation algorithm (DSPC) was used for
estimating partial correlation networks, visualized by the *MetaboAnalyst 4* version with cutoff values on correlations between +/- 0.22 to 0.75. Univariate analysis (unpaired two sample t-test and paired t-test) using the 't.test' function, was deployed to identify mean differences in the concentration of individual PFAS and BAs between CD progressors vs. HCs at birth and at 3 months of age. Libraries/packages such as 'Heatmap.2', 'mixOmics', 'boxplot', 'beanplot', 'gplot' and 'ggplot2' were used for data visualization.

**Results**

**Levels of PFAS and BAs in the infants**

Seven PFAS compounds were detected in the samples, namely PFHpA, PFHxS, PFOA, PFNA, PFOS, PFDA and PFUnDA, both at birth and at three months of age (*Supplementary Table S2*). PFOS and PFOA had highest concentrations and they were detected in the majority of samples, whilst PFDA and PFNA were detected in less than 10% of the samples and were excluded from further analyses. The levels of PFAS were lower in the cord blood (CB) than at the age of three months (*Figure 1A-C*). No significant differences were observed between cases and controls either at birth or at 3 months of age (*Figure 1A-C*). Interestingly, the levels of the total PFAS were elevated from birth to 3 months of age was significant only for the CD group (fold change (FC) 3 months vs. at birth = 1.72, \( p = 0.003 \) *Supplementary Table S3*) although also controls had an increasing trend (FC = 1.02, \( p = 0.35 \)). Among the individual PFAS, PFOA showed nearly 2-fold increase, being significant in both groups (*Figure 1A-C* (*Supplementary Table S3*).

In addition to the two primary BAs, we measured five primary/conjugated and secondary BAs (*Supplementary Table S2*). The levels of secondary BAs were mostly below the detection limits, particularly in the cord blood samples, where the total BA pool consisted mainly of GCA and GCDCA. Similarly, as for PFAS, no significant differences were observed between cases
and controls either at birth or at 3 months of age (Figure 1D-F). The BA profiles at birth and at 3 months of age were markedly different in both groups (Figure 1D-F). The total BA pool showed significant, almost 7-fold upregulation (p = 0.0001). Particularly, GHCA and CDCA, which were close the limits of detection at birth, have increased substantially with age.

**Associations between PFAS, BA and lipid levels**

Partial correlation network analysis between PFAS, BAs and lipids (classes) measured in the cord blood showed different pattern of association in HCs (Figure 2A) and CD progressors (Figure 2B). In the HCs, the level of deoxycholic acid (DCA) was inversely linked with the sex of the infant, however, such association was not observed in CD progressors. Interestingly, DCA concentration was inversely linked to the level of PFOA. The CD group showed strong association between the age of diagnosis and PFOA exposure, at both time points (Figure 2B, Supplementary Figure 1). In general, PFAS had a direct association with several lipid classes. The age of diagnosis was also negatively associated with total TGs at birth. The association showed a clearly different pattern in the controls, both at birth and at the later time point (Figure 2).

To understand how the individual PFAS and BAs are associated with the molecular lipid species, we performed multivariate and bivariate correlation analysis.

Bivariate correlation analysis identified different in correlation patterns in HCs and in CD progressor group (Figure 3). In the control group at birth, there was significant correlations mainly between the levels of the lipid classes and only total concentration of TGs showed an association with the levels of BAs (Figure 3A). In CD progressor group, we observed significant correlation between the levels of BAs (GUDCA, GHCA, CDCA) and with multiple lipid classes at cord blood (PE, TG, SM) (Figure 3B). At three months of age, the patterns were different from the samples at birth. In the control group, specific BAs (CDCA, GUDCA, TCDCA and GCHA) showed significant association with lipid concentrations, mainly with cholesterol esters (CEs) and TGs, with different patterns depending on the fatty acyl saturation
level (Figure 3C). Only weak correlation between the levels of PFAS and LPCs was observed while overall the PFAS levels were not associated with either lipids or BAs. In CD progressors, the patterns were different from the controls also at three months of age (Figure 3D). In this group, PFAS (PFOS and PFUnDA) levels and concentration of TGs showed significant correlations, particularly with TGs containing saturated FAs. In addition, concentrations of BAs were also associated with those of TGs (DCA, GCA, CDCA and TCDA, total BAs), the directions being dependent on the bile acid species.

The main difference between the groups was the absence of correlation between the LPCs and the other phospholipid classes (PC, SM, PC) in CD progressors, while there was a strong and significant co-regulation of these lipid classes in the control group. At birth, there were no significantly different individual lipids between the groupss, however, the LPCs showed overall increased levels and most of the PCs decreased levels. LPC/PC ratio, with selected PC species containing PUFAs, showed significant upregulation in the CD group (FC=1.3, p=0.01).

**Molecular Predictors of PFAS, BA and Lipids in CD progression**

Multivariate correlation analysis showed strong associations (r > 0.8) among PFAS, BAs and molecular lipids such as cholesterol esters (CEs), lysophosphatidylcholines (LPCs), phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), sphingomyelin (SMs) and triacylglycerols (TGs) (Figure 4A). These associations become more complex at three months of age (Figure 4D).

Next, we selected those lipids that showed significant differences between CD and HCs in infancy in our previously reported studies. Together these lipids with the BAs and PFAS were subjected to multiblock (MB) analysis. MB analysis identified PFAS, BA and molecular lipid species as predictors (discriminative features), that help to classify CD progression vs. HCs. The association between top predictors (high variable importance scores) are shown in (Figure 4A, D).
In cord blood, the levels of PFOA and PFOS were identified as the top linear predictors of CD progression (Figure 4B). On the other hand, the levels of primary BAs such as GCA and GHCA are the key predictors of CD progression (Figure 3C). Moreover, elevated concentration of PFOA in the CD progressors is positively associated with the levels of GCA and GHCA. It suggests that prenatal exposure to PFOA and/or PFOS might accelerate the risk of CD progression. Presumably, it alters the BA and lipid metabolism. Interestingly, lipids such as CE(18:1) (intermediate of BA synthesis) were identified as predictors of CD progression; the levels of PFOA and PFOS were negatively correlated with those of CEs (Figures 4A and 5A). In addition, many ether-linked PCs such as PC(O-34:3), PC(O-36:3,4) and PC(O-38:4,5,6) were identified as key contributors, that were downregulated in the CD progressors as compared to the HCs (Figures 4A and 5A).

At three months of age, concentration of PFHxS was marked as a top predictor associated with CD progression, along with levels of PFOA and PFOS. Moreover, the level of PFHxS was elevated in the CD progressors as compared to HCs (Figures 1A, 4E and 5B). At this age, elevated levels of secondary BAs such as DCA and CDCA were marked as the key predictors for CD progression (Figure 4F). As stated before, the level of PFOA was negatively correlated with that of DCA (Figure 2B), presumably, there is an interaction between the PFAS and BAs in the CD progressors. Moreover, PFOA is inversely linked to age of diagnosis of CD (Figure 2B and Figure 6). Besides, the MB model identified several PCs, SM(d36:2), PC(O-38:4,5) as key predictors that discriminate the lipid profiles of CD progressors from the HCs (Figure 5B).

Discussion

We observed dysregulation between intralipid class regulation at birth in those infants that progressed to CD, particularly in LPC/PC ratios. PFOS and PFHxS levels were also positively associated with LPCs in the CD progressors, but not in HCs. However, at three months, the ratio was similar in both groups. Increased LPCs have been indicated to be characteristic for CD progression at later time points, both in our earlier study as well as in another recent study.
which showed increased LPCs at those 4 month old infants that progressed later to CD. Furthermore, our results showed that the associations between BAs and complex lipids showed different patterns already at birth in those infants that later developed CD as compared with healthy controls. Particularly, significant correlations were observed between the TGs and specific BAs already at birth in the CD progressors. At three months of age, both groups showed significant correlation between the lipids and BAs, however, the patterns were different. Particularly, at this later time point, CD group showed significant positive correlations between the different classes of TGs while the healthy controls showed the opposite correlations. Thus, the data agree well with our earlier study, in which we observed distinct lipid changes in children that later developed CD, even prior to the first exposure to dietary gluten, particularly in the TG class. The data suggested that the specific TGs, found elevated in CD progressors, may be due to a host response to compromised intake of essential lipids in the small intestine, requiring de novo lipogenesis. The current study suggests that the changes in TG metabolism are also related to alteration in BA metabolism, and that early-life exposure to PFAS may contribute to these changes. Currently, there is very limited amount of data on BA and lipid metabolism in infants. The BA pool in the fetus and in newborns is unique. Over 90% of fetal BAs are conjugated forms of the primary BAs, cholic acid (CA) and chenodeoxycholic acid (CDCA), because the intestinal bacteria necessary to transform primary BAs into secondary BAs are thought to be absent in utero, or based on recent findings, very different than later in life. The enterohepatic circulation of BAs in utero is also minimal. Instead, there is a transplacental gradient for BAs in the fetal-to-mother direction, except for secondary and tertiary BAs, which are more abundant in maternal serum.

PFAS exposure has been shown to impact BA metabolism through several mechanisms. BAs and PFAS have similar enterohepatic circulation, and it has been indicated that 7-alpha-hydroxylase (CYP7A1), which catalyzes the first and rate-limiting step in the classical pathway of formation of BAs from cholesterol, may be down-regulated by PFAS. This may lead to increased re-uptake of BAs, which would generate negative feedback loops via the farnesyl-
X-receptor and subsequently reduce their *de novo* synthesis\textsuperscript{25,42}. It has also been shown that PFOA inhibits the function of the hepatocyte nuclear factor 4α\textsuperscript{43}, which plays a central role in the regulation of BA metabolism in the liver, and is linked both with the synthesis and conjugation of primary BAs. However, in neonates, the alternative (acidic) pathway is the major pathway for bile acid synthesis and this pathway is governed by mitochondrial sterol 27-hydroxylase (CYP27A1), which can initiate a process independent of CYP7A1. Only after weaning, CYP7A1 is expressed and the classic pathway becomes the major pathway for bile acid synthesis in adult liver\textsuperscript{25}. There is currently no data on the impact of PFAS exposure on the regulation of the CYP27A1. On the other hand, the impact of PFAS on the TG metabolism may be modulated through the bile acids, as the bile acid receptor FXR has also a regulatory role in triglyceride metabolism\textsuperscript{44-46}. In humans, the recent data suggests that FXR is not activated directly by PFAS\textsuperscript{44}. In the liver, FXR activation, by, e.g., bile acids, would result in downregulation of CYP7A1, which in addition to inhibition of the classical BA synthetic pathway also reduces the expression of several genes mediating free fatty acid synthesis, thereby attenuating *de novo* lipogenesis\textsuperscript{25}. Thus, activation of FXR modulates free FA oxidation and TG clearance to the circulation.

The acylalkylPCs type of lipids that showed significant association with the CD progression were positively associated with the levels of total BAs and PFHxS, indicating potentially disturbed hepatic synthesis of these lipids, particularly as the intra-lipid regulation between these lipids and particularly TGs was disturbed in CD progressors. These ether lipids, in addition to their structural roles in cell membranes, are thought to function as endogenous antioxidants, and emerging studies suggest that they are involved in cell differentiation and signaling pathways\textsuperscript{47}. Interestingly, these lipids have shown to be endogenous antigens to activate invariant natural killer T cells (iNKT)\textsuperscript{48}, which are subset of innate immune cells. Recently, therapeutic potential of iNKT cell antigens against autoimmunity have been suggested\textsuperscript{49}. Thus, the reduced levels of the alkyl ether lipids could suggest compromised response to oxidative stress. Interestingly, these lipids have previously been found to be
associated with disease development of pediatric CD\textsuperscript{5,21} and type 1 diabetes\textsuperscript{50}, thus indicating that they may play an important role in the development of an autoimmune disorders. Due to their role in cell membranes, the decrease in ether-linked PCs particularly, PC(O-38:4,5) might also attribute to compromised intestinal permeability in the CD progressors.

Taken together, our results show that PFAS exposure may modulate lipid and BA metabolism, and the impact is different in the infants who develop CD later in life, in comparison with healthy controls. Although we did not observe any significant differences in the levels of PFAS exposure in those children that later developed CD, which may be due to the small sample size, we did observe a significant increase in their levels in progressors to CD from the time of birth to three months of age, suggesting more efficient uptake of PFAS. Furthermore, the age of diagnosis was strongly associated with the PFAS exposure. Our study thus suggests that further investigations of the impacts of exposures to environmental chemicals are merited.

**Conflicts of interest**

**Guarantor of the article:** Tuulia Hyötyläinen, Ph.D.

**Specific author contributions:** T.H., M.K., M.O.: conception and design of the study; L.S., S.S., S.V., H.H., J.I., J.T., R.V., M.O., M.K., T.H.: generation, collection, assembly, analysis, and/or interpretation of data; T.H. and M.O.: drafting or revision of the manuscript; all authors: approval of the final version of the manuscript.

**Financial support:** This study was supported by funding from Vetenskapsrådet (to T.H.; grant no. 2016-05176) and Formas (to T.H. and M.O.; grant no. 2019-00869).

**Potential competing interests:** None to report.

**Acknowledgments**
We thank the families participating in the DIPP study for making this study possible. We also thank the expert staff of the DIPP study for their excellent work with the participating research families and sample collection.

References

1. Caio G, Volta U, Sapone A, et al. Celiac disease: a comprehensive current review. *BMC Medicine*. 2019;17(1):142.

2. Holmes GKT, Muirhead A. Epidemiology of coeliac disease in a single centre in Southern Derbyshire 1958–2014. 2017;4(1):e000137.

3. Assa A, Frenkel-Nir Y, Leibovici-Weissman Ya, et al. Anthropometric measures and prevalence trends in adolescents with coeliac disease: a population based study. 2017;102(2):139-144.

4. Ilonen J, Kiviniemi M, Lempainen J, et al. Genetic susceptibility to type 1 diabetes in childhood – estimation of HLA class II associated disease risk and class II effect in various phases of islet autoimmunity. 2016;17(S22):8-16.

5. Sen P, Carlsson C, Virtanen SM, et al. Persistent Alterations in Plasma Lipid Profiles Before Introduction of Gluten in the Diet Associated With Progression to Celiac Disease. *Clin Transl Gastroenterol*. 2019;10(5):1-10.

6. Rewers M, Liu E, Simmons J, Redondo MJ, Hoffenberg EJ. Celiac disease associated with type 1 diabetes mellitus. *Endocrinol Metab Clin North Am*. 2004;33(1):197-214, xi.

7. Scaramuzza AE, Mantegazza C, Bosetti A, Zuccotti GV. Type 1 diabetes and celiac disease: The effects of gluten free diet on metabolic control. *World J Diabetes*. 2013;4(4):130-134.

8. Ludvigsson JF, Ludvigsson J, Ekbom A, Montgomery SM. Celiac disease and risk of subsequent type 1 diabetes: a general population cohort study of children and adolescents. *Diabetes Care*. 2006;29(11):2483-2488.

9. Kirchberg FF, Werkstetter KJ, Uhl O, et al. Investigating the early metabolic fingerprint of celiac disease – a prospective approach. *Journal of Autoimmunity*. 2016;72:95-101.

10. Lionetti E, Catassi C. The Role of Environmental Factors in the Development of Celiac Disease: What Is New? *Diseases*. 2015;3(4):282-293.

11. Whyte L, Kotecha S, Watkins W, Jenkins H. Coeliac disease is more common in children with high socio-economic status. 2014;103(3):289-294.
12. Zingone F, West J, Crooks CJ, et al. Socioeconomic variation in the incidence of childhood coeliac disease in the UK. 2015;100(5):466-473.
13. Olén O, Bihagen E, Rasmussen F, Ludvigsson JF. Socioeconomic position and education in patients with coeliac disease. Digestive and Liver Disease. 2012;44(6):471-476.
14. Tyrrell J, Melzer D, Henley W, Galloway TS, Osborne NJ. Associations between socioeconomic status and environmental toxicant concentrations in adults in the USA: NHANES 2001–2010. Environment International. 2013;59:328-335.
15. Buekers J, Colles A, Cornelis C, Morrens B, Govarts E, Schoeters G. Socio-Economic Status and Health: Evaluation of Human Biomonitored Chemical Exposure to Per- and Polyfluorinated Substances across Status. 2018;15(12):2818.
16. Conway B, Innes KE, Long D. Perfluoroalkyl substances and beta cell deficient diabetes. Journal of Diabetes and its Complications. 2016;30(6):993-998.
17. Nisticò L, Iafusco D, Galderisi A, et al. Emerging Effects of Early Environmental Factors over Genetic Background for Type 1 Diabetes Susceptibility: Evidence from a Nationwide Italian Twin Study. The Journal of Clinical Endocrinology & Metabolism. 2012;97(8):E1483-E1491.
18. Predieri B, Iughetti L, Guerranti C, Bruzzi P, Perra G, Focardi SE. High Levels of Perfluorooctane Sulfonate in Children at the Onset of Diabetes. Int J Endocrinol. 2015;2015:234358.
19. Stene LC, Gale EAM. The prenatal environment and type 1 diabetes. Diabetologia. 2013;56(9):1888-1897.
20. McGlinchey A, Sinioja T, Lamichhane S, et al. Prenatal Exposure To Environmental Chemicals Modulates Serum Phospholipids In Newborn Infants, Increasing Later Risk Of Type 1 Diabetes. 2019:588350.
21. Auricchio R, Galatola M, Cielo D, et al. A Phospholipid Profile at 4 Months Predicts the Onset of Celiac Disease in at-Risk Infants. Scientific Reports. 2019;9(1):14303.
22. Hoffmanová I, Sánchez D, Tučková L, Tlaskalová-Hogenová H. Celiac Disease and Liver Disorders: From Putative Pathogenesis to Clinical Implications. Nutrients. 2018;10(7):892.
23. Vuoristo M, Miettinen TA. The Role of Fat and Bile Acid Malabsorption in Diarrhoea of Coeliac Disease. Scandinavian Journal of Gastroenterology. 1987;22(3):289-294.
24. Ejderhamn J, Samuelson K, Strandvik B. Serum Primary Bile Acids in the Course of Celiac Disease in Children. 1992;14(4):443-449.
25. Chiang JY. Recent advances in understanding bile acid homeostasis. F1000Research. 2017;6:2029-2029.
26. Barrasa JI, Olmo N, Lizarbe MA, Turnay J. Bile acids in the colon, from healthy to cytotoxic molecules. Toxicology in Vitro. 2013;27(2):964-977.
27. Ramírez-Pérez O, Cruz-Ramón V, Chinchilla-López P, Méndez-Sánchez N. The Role of the Gut Microbiota in Bile Acid Metabolism. Annals of Hepatology. 2017;16:S21-S26.
28. Zhao W, Zitzow JD, Ehresman DJ, et al. Na+/Taurocholate Cotransporting Polypeptide and Apical Sodium-Dependent Bile Acid Transporter Are Involved in the Disposition of Perfluoroalkyl Sulfonates in Humans and Rats. Toxicological sciences: an official journal of the Society of Toxicology. 2015;146(2):363-373.
29. Salihović S, Dickens AM, Schoultz I, et al. Simultaneous determination of perfluoroalkyl substances and bile acids in human serum using ultra-high-performance liquid chromatography–tandem mass spectrometry. Analytical and Bioanalytical Chemistry. 2019.
30. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem. 1957;226(1):497-509.
31. Pluskal T, Castillo S, Villar-Briones A, Oresic M. MZmine 2: modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. BMC Bioinformatics. 2010;11:395.
32. R Development Core Team. R: A language and environment for statistical computing. In: Vienna: R Foundation for Statistical Computing; 2018: http://www.R-project.org.
33. Rohart F, Gautier B, Singh A, Lê Cao K-A. mixOmics: An R package for ‘omics feature selection and multiple data integration. PLoS computational biology. 2017;13(11):e1005752.
34. Le Cao KA, Boitard S, Besse P. Sparse PLS discriminant analysis: biologically relevant feature selection and graphical displays for multiclass problems. BMC Bioinformatics. 2011;12:253.
35. Westerhuis JA, Hoefsloot HC, Smit S, et al. Assessment of PLSDA cross validation. Metabolomics. 2008;4(1):81-89.
36. Chong J, Wishart DS, Xia J. Using MetaboAnalyst 4.0 for Comprehensive and Integrative Metabolomics Data Analysis. 2019;68(1):e86.
37. Basu S, Duren W, Evans CR, Burant CF, Michailidis G, Karnovsky A. Sparse network modeling and metscape-based visualization methods for the analysis of large-scale metabolomics data. Bioinformatics (Oxford, England). 2017;33(10):1545-1553.
38. Jebbink J, Veenboer G, Boussata S, et al. Total bile acids in the maternal and fetal compartment in relation to placental ABCG2 expression in preeclamptic pregnancies complicated by HELLP syndrome. Biochimica et biophysica acta. 2015;1852(1):131-136.
39. Seki Y, Matsushita M, Kimura A, et al. Maternal and fetal circulation of unusual bile acids: A pilot study. 2011;53(6):1028-1033.

40. Macias RIR, Marin JJG, Serrano MA. Excretion of biliary compounds during intrauterine life. World journal of gastroenterology. 2009;15(7):817-828.

41. Beggs KM, McGreal SR, McCarthy A, et al. The role of hepatocyte nuclear factor 4-alpha in perfluorooctanoic acid- and perfluorooctanesulfonic acid-induced hepatocellular dysfunction. Toxicology and applied pharmacology. 2016;304:18-29.

42. Pandak WM, Kakiyama G. The acidic pathway of bile acid synthesis: Not just an alternative pathway. Liver Research. 2019;3(2):88-98.

43. Buhrke T, Kruger E, Pevny S, Rossler M, Bitter K, Lampen A. Perfluorooctanoic acid (PFOA) affects distinct molecular signalling pathways in human primary hepatocytes. Toxicology. 2015;333:53-62.

44. Behr A-C, Plinsch C, Braeuning A, Buhrke T. Activation of human nuclear receptors by perfluoroalkylated substances (PFAS). Toxicology in Vitro. 2020;62:104700.

45. Bjork JA, Butenhoff JL, Wallace KB. Multiplicity of nuclear receptor activation by PFOA and PFOS in primary human and rodent hepatocytes. Toxicology. 2011;288(1):8-17.

46. Zhang L, Nichols RG, Correll J, et al. Persistent Organic Pollutants Modify Gut Microbiota-Host Metabolic Homeostasis in Mice Through Aryl Hydrocarbon Receptor Activation. Environmental health perspectives. 2015;123(7):679-688.

47. Dean JM, Lodhi IJ. Structural and functional roles of ether lipids. Protein & Cell. 2018;9(2):196-206.

48. Facciotti F, Ramanjaneyulu GS, Lepore M, et al. Peroxisome-derived lipids are self antigens that stimulate invariant natural killer T cells in the thymus. Nature Immunology. 2012;13(5):474-480.

49. Van Kaer L, Wu L. Therapeutic Potential of Invariant Natural Killer T Cells in Autoimmunity. Front Immunol. 2018;9:519-519.

50. Oresic M, Gopalacharyulu P, Mykkanen J, et al. Cord serum lipidome in prediction of islet autoimmunity and type 1 diabetes. Diabetes. 2013;62(9):3268-3274.
Table 1. Demographic and clinical characteristics of the study subjects.

|                             | Celiac Disease | Healthy Controls |
|-----------------------------|----------------|------------------|
| Number of subjects         | 17             | 16               |
| Gender                     |                |                  |
| Male (median)              | 10             | 8                |
| Female (median)            | 7              | 8                |
| Age of first gluten intake (median) | 5.25 months | 5.3 months |
| Age of first tTGA (median)(seroconversion) | 58 months | -- |
| First tTGA level in plasma (median EIU) | 116.8      | --               |
| Endoscopy Age (median)     | 58.5 months   | --               |
| tTGA level after GFD (median EIU) | 7.1         | --               |
| Total follow-up age from birth (median) | 6.08 years | 6.2 years |
Figure 1. Bean plots showing distribution of selected PFAS and BAs in CD progressors and HCs. A-C) Levels (log₂ intensities) of selected PFAS in the cord blood (0 Mo) and at 3 months (3 Mo) of age in the CD progressors (CD) and HCs. D-F) Levels (log₂ intensities) of selected BAs in the cord blood and at 3 months of age. The red dotted line denotes the mean of the population. The black solid lines in the bean plots represent the group mean. The group mean difference between case-control at a particular time-point was tested by unpaired two-sample t test. Mean difference between cord blood and 3 months samples obtain from the same infants were tested by paired t test. CD, HCs and BA denotes CD progressors, healthy controls and bile acids respectively.
Figure 2. Molecular network of PFAS (yellow color), BAs (violet color), lipid classes (grey color), and clinical variables (orange color) measured in the cord blood: A) Healthy controls. B) CD progressors. Red and blue lines denote positive and negative correlations respectively. The width of the lines represent the strength of correlation.
Figure 3. Correlation plots of PFAS, BA and lipid classes in (A) Controls at birth, (B) CD at birth, (C) controls at 3 months and (D) CD at three months. Red and blue colors denote positive and negative correlations respectively. Significant Pearson correlations are marked with *.
Figure 4. Integrative multiblock component analysis of PFAS, BAs and molecular lipids in the CD progressors vs. HCs. A, D) Circos plots (2 principal components) showing overall association (full-design matrix) between covariates of PFAS, BAs and molecular lipids in the cord blood(CB), and at 3 months of age. Red and blue line denotes positive and negative correlations respectively. Sparse generalized correlation\textsuperscript{33} (coefficient, \( r > 0.5 \)) are shown. B-C) Horizontal barplots showing top selected predictors/contributors/discriminators from each PFAS and BA data blocks, where the length of bar corresponds to the absolute loading weights (variable importance) of the feature, that tends to discriminate CD progressors vs. HCs at birth. The direction of regulation of the predictors are shown with light brown and blue colors that corresponds to the CD progressors or HCs respectively; wherever the mean intensity of the predictor is maximum. E-F) Key predictors of PFAS and BAs, that helped to classify CD progressors vs. HCs at 3 months (3 Mo) of age. The model performance on each block of data are given by cumulative (principal components) area under the curves (AUCs).
Figure 5. Key lipid contributors/predictors, that jointly (together with PFAS and BAs), aided in the separation of CD progressors vs. HCs, at birth and at 3 months of age. The direction of regulation of the predictors are shown with light brown and blue colors that corresponds to the CD progressors or HCs respectively; wherever the mean intensity of the predictor is maximum.
Figure 6. Correlation of PFOA concentration at birth and the age of diagnosis.
Supplementary Figure 1. (A) correlation plots of lipid-BA and lipid-PFAS (3 months), Pearson correlations, significant correlations marked with * (p<0.0001 ****, p<0.001 ***, p<0.05 **, p<0.1 *) and (B) Partial correlation network of the previously identified panel of CD-related lipids, BAs and PFAS (at birth and at three months of age).

Significant correlation was observed in the CD group between BAs and the lipids. Particularly, GCDCA, CDCA and DCA showed significant positive correlation with TGs of low carbon number and double bond count, while TCDCA and GUDCA showed negative association with PCs at three months of age. At the same time point, PFOA, PFOS and PFUnDA, and total PFAS showed positive correlation between the TGs containing low carbon number and double bond count and negative association with PCs. In the partial correlation network, PFOA and PFHxS showed negative association with the age of diagnosis and PFHxS further positive associations with CE(18:2) and PC(34:3) (Figure 4B). PFHpA showed negative association with one TG and one PC species and positive association with GHCA. BAs showed mainly negative associations with several lipid species.
## Supplementary Table S1. Acquisition parameters including the list or targets compounds ordered by retention time.

| Target analytes                | Abbreviation | Retention time | Precursor ion (m/z) | Product ion (m/z) | Cone (V) | Collision (eV) |
|-------------------------------|--------------|----------------|---------------------|-------------------|----------|----------------|
| Glycodehydrocholic acid       | GHCA         | 6.88           | 123.               | 9                 | 52.0     | 32.0           |
|                              |              |                | 74.0               |                   | 28.0     | 28.0           |
| Taurodehydrocholic acid       | TDCA         | 3.09           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 514.2              |                   | 50.0     | 42.0           |
| Perfluoropentanoic acid       | PFPeA        | 4.45           | 41.0               |                   | 74.0     | 32.0           |
|                              |              |                | 401.1              |                   | 24.0     | 24.0           |
| Perfluorobutane sulfonate     | PFBS         | 4.83           | 123.               | 9                 | 52.0     | 32.0           |
|                              |              |                | 298.9              |                   | 26.0     | 26.0           |
| Taur-o-mega-muricholic acid   | TMCA         | 5.38           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 514.2              |                   | 50.0     | 42.0           |
| Perfluorohexanoic acid        | PFHaA        | 5.82           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 312.8              |                   | 50.0     | 45.0           |
| Tauro-alpha-muricholic acid   | TaMCA        | 5.81           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 514.2              |                   | 50.0     | 42.0           |
| Tauro-beta-muricholic acid    | TbMCA        | 5.87           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 514.2              |                   | 50.0     | 42.0           |
| Glycochenodeoxycholic acid    | GCDCA        | 9.31           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 448.2              |                   | 50.0     | 50.0           |
| Glycocholic acid              | GCA          | 7.84           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 446.2              |                   | 50.0     | 50.0           |
| Glycohydeoxycholic acid       | GUDCA        | 7.10           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 448.2              |                   | 50.0     | 50.0           |
| Tauroursoxycholic acid        | TUDA         | 7.11           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 748.2              |                   | 50.0     | 50.0           |
| Glycoxydeoxycholic acid       | GHDA         | 7.39           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 448.2              |                   | 50.0     | 50.0           |
| Glycoxycholic acid            | GCA          | 7.84           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 446.2              |                   | 50.0     | 50.0           |
| 7-oxo-deoxycholic acid        | 7-oxo-DCA    | 7.61           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 405.2              |                   | 50.0     | 50.0           |
| 7-oxo-Muricholic acid         | 7-oxo-MCA    | 7.88           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 405.2              |                   | 50.0     | 50.0           |
| Pfluroxane sulfonate          | PFxS         | 7.95           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 398.9              |                   | 50.0     | 50.0           |
| Omega/Alpha-Muricholic acid   | w/a-MCA      | 7.97           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 407.2              |                   | 50.0     | 50.0           |
| Beta-Muricholic acid          | b-MCA        | 8.12           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 407.2              |                   | 50.0     | 50.0           |
| Perfluorooctanoic acid        | PFOA         | 8.89           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 413.0              |                   | 50.0     | 50.0           |
| Helicholic acid               | HCA          | 9.11           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 407.1              |                   | 50.0     | 50.0           |
| Ursodeoxycholic acid          | UDCA         | 9.25           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 391.1              |                   | 50.0     | 50.0           |
| Taurchenodeoxycholic acid     | TCDCA        | 9.26           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 498.2              |                   | 50.0     | 50.0           |
| Glycochenodeoxycholic acid    | GCDCA        | 9.31           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 448.2              |                   | 50.0     | 50.0           |
| Taurodeoxycholic acid         | TDCA         | 9.63           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 498.2              |                   | 50.0     | 50.0           |
| Glycoxydeoxycholic acid       | GDCA         | 9.70           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 488.2              |                   | 50.0     | 50.0           |
| Hyodeoxycholic acid           | HDCA         | 9.72           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 391.1              |                   | 50.0     | 50.0           |
| Cholic acid                   | CA           | 9.74           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 407.2              |                   | 50.0     | 50.0           |
| 12-oxo-Ilcholic acid          | 12-oxo-LCA   | 9.84           | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 399.1              |                   | 50.0     | 50.0           |
| Perfluorocotic acid           | PFNA         | 10.04          | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 463.0              |                   | 50.0     | 50.0           |
| Linear-perfluorooctane        | L-PFOS       | 10.27          | 123.               | 9                 | 70.0     | 50.0           |
|                              |              |                | 499.0              |                   | 50.0     | 50.0           |
|   | Name                                 | Abbreviation | m/z 11.92 | m/z 8.89 | m/z 7.95 | m/z 3.68 | m/z 12.70 | m/z 12.05 | m/z 11.78 | m/z 11.54 | m/z 11.92 | m/z 13.99 | m/z 13.28 | m/z 13.39 | m/z 12.70 | m/z 12.05 | m/z 11.78 | m/z 11.54 | m/z 11.92 | m/z 13.28 | m/z 13.39 | m/z 12.05 | m/z 11.78 | m/z 11.54 | m/z 11.92 | m/z 13.28 | m/z 13.39 | m/z 12.05 | m/z 11.78 | m/z 11.54 | m/z 11.92 | m/z 13.28 | m/z 13.39 | m/z 12.05 | m/z 11.78 | m/z 11.54 | m/z 11.92 | m/z 13.28 | m/z 13.39 | m/z 12.05 | m/z 11.78 | m/z 11.54 | m/z 11.92 | m/z 13.28 | m/z 13.39 | m/z 12.05 | m/z 11.78 | m/z 11.54 | m/z 11.92 | m/z 13.28 | m/z 13.39 | m/z 12.05 | m/z 11.78 | m/z 11.54 | m/z 11.92 | m/z 13.28 | m/z 13.39 | m/z 12.05 | m/z 11.78 | m/z 11.54 | m/z 11.92 | m/z 13.28 | m/z 13.39 | m/z 12.05 | m/z 11.78 | m/z 11.54 | m/z 11.92 | m/z 13.28 | m/z 13.39 | m/z 12.05 | m/z 11.78 | m/z 11.54 | m/z 11.92 | m/z 13.28 | m/z 13.39 | m/z 12.05 | m/z 11.78 | m/z 11.54 | m/z 11.92 | m/z 13.28 | m/z 13.39 | m/z 12.05 | m/z 11.78 | m/z 11.54 | m/z 11.92 | m/z 13.28 | m/z 13.39 | m/z 12.05 | m/z 11.78 | m/z 11.54 | m/z 11.92 | m/z 13.28 | m/z 13.39 | m/z 12.05 | m/z 11.78 | m/z 11.54 | m/z 11.92 | m/z 13.28 | m/z 13.39 | m/z 12.05 | m/z 11.78 | m/z 11.54 | m/z 11.92 | m/z 13.28 | m/z 13.39 | m/z 12.05 | m/z 11.78 | m/z 11.54 | m/z 11.92 | m/z 13.28 | m/z 13.39 | m/z 12.05 | m/z 11.78 | m/z 11.54 | m/z 11.92 | m/z 13.28 | m|
Supplementary Table S2. PFAS concentrations.

|          | CTRL CB |          |          | CTRL CB |          |          | CTRL 3 months |          |          | CD 3 months |          |          |
|----------|---------|----------|----------|---------|----------|----------|---------------|----------|----------|-------------|----------|----------|
|          | median  | min      | max      | median  | min      | max      | median        | min      | max      | median      | min      | max      |
| PFHpA    | <LLQ    | <LLQ     | 378.39   | <LLQ    | <LLQ     | 0.50     | <LLQ          | <LLQ     | 0.69     | <LLQ        | <LLQ     | 1.09     |
| PFHxS    | 0.49    | 0.31     | 0.99     | 0.55    | 0.31     | 1.03     | 0.59          | 0.31     | 0.93     | 0.70        | 0.31     | 1.10     |
| PFOA     | 2.43    | 1.23     | 4.46     | 2.32    | 1.31     | 4.80     | 4.05          | 0.98     | 6.25     | 4.34        | 1.23     | 9.30     |
| PFOS     | 2.25    | 0.27     | 5.32     | 2.21    | 0.27     | 8.17     | 3.40          | 0.71     | 6.70     | 2.93        | 0.27     | 7.46     |
| PFUnDA   | <LLQ    | <LLQ     | 0.44     | <LLQ    | <LLQ     | 0.48     | <LLQ          | <LLQ     | 0.63     | <LLQ        | <LLQ     | 0.63     |
| PFAS     | 6.04    | 3.19     | 11.69    | 6.87    | 3.42     | 14.17    | 9.74          | 3.82     | 12.77    | 9.15        | 3.17     | 12.77    |
| TCDDCA   | 32.415  | <LLQ     | 32.415   | 17.295  | <LLQ     | 17.295   | 44.0125       | <LLQ     | 44.0125  | 72.2175     | <LLQ     | 72.2175  |
| GHCA     | <LLQ    | <LLQ     | <LLQ     | <LLQ    | <LLQ     | <LLQ     | 30.7825       | <LLQ     | 30.7825  | 28.8775     | <LLQ     | 28.8775  |
| GCA      | 171.015 | <LLQ     | 171.015  | 150.215 | <LLQ     | 150.215  | 88.62         | <LLQ     | 88.62    | 252.4375    | <LLQ     | 252.4375 |
| GUDCA    | <LLQ    | <LLQ     | <LLQ     | <LLQ    | <LLQ     | <LLQ     | 0.65          | <LLQ     | 0.65     | 1.575       | <LLQ     | 1.575    |
| GCDDA    | 205.3   | <LLQ     | 205.3    | 135.63  | 16.865   | 135.63   | 862.43        | 138.38   | 862.43   | 1391.333    | 176.295  | 1391.333 |
| CDCA     | <LLQ    | <LLQ     | <LLQ     | <LLQ    | <LLQ     | <LLQ     | 108.375       | <LLQ     | 108.375  | 126.3225    | <LLQ     | 126.3225 |
| DCA      | <LLQ    | <LLQ     | <LLQ     | <LLQ    | <LLQ     | <LLQ     | <LLQ          | <LLQ     | <LLQ     | 3.5775      | <LLQ     | 3.5775   |
| SUM BA   | 465.275 | <LLQ     | 465.275  | 392.87  | 16.865   | 392.87   | 1210.23       | 313.405  | 1210.23  | 2267.025    | 403.525  | 2267.025 |
Supplementary Table S3. Change of PFAS levels from cord blood to 3 months.

|       | PFHpA  | PFHxS | PFOA  | PFNA  | PFOS  | PFUnDA | SUM  |
|-------|--------|--------|-------|-------|-------|--------|------|
| Ctrl  | p      | 0.335  | 0.969 | 0.008 | 0.173 | 0.309  | 0.090 | 0.362 |
|       | Fold   | 1.00   | 0.95  | 1.43  | 1.00  | 0.90   | 1.00  | 1.02  |
| CD    | p      | 0.135  | 0.039 | 0.001 | 0.189 | 0.051  | 0.603 | 0.003 |
|       | Fold   | 1.00   | 1.13  | 1.87  | 1.00  | 1.66   | 1.00  | 1.70  |