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Early life exposures to perfluoroalkyl substances in relation to adipokine hormone levels at birth and during childhood

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Conflicts of interest: All authors declare that they have no conflict of interest, financial or otherwise. PG recently served as a health expert for the State of Minnesota in a lawsuit against a PFAS-producing company.
**Precis:** Prenatal and early postnatal exposures to perfluoroalkyl substances were associated with decreases in adipokine hormone levels over the childhood period, in a sex-specific manner.

**Word count:** 4267
Abstract

Background: Birth cohort studies have linked exposure to perfluoroalkyl substances (PFASs) with child anthropometry. Metabolic hormone dysregulation needs to be considered as a potential adverse outcome pathway. We examined the associations between PFAS exposures and concentrations of adipokine hormones from birth to adolescence.

Methods: We studied 80 mother-child pairs from a Faroese cohort born in 1997-2000. Five PFASs were measured in maternal pregnancy serum and child serum at ages 5, 7 and 13 years. Leptin, adiponectin and resistin were analyzed in cord serum and child serum at the same ages. We fitted multivariable-adjusted generalized estimating equations to assess the associations of PFASs at each age with repeated adipokine concentrations at concurrent and subsequent ages.

Results: We observed tendencies of inverse associations between PFASs and adipokine hormones specific to particular ages and child sex. Significant associations with all adipokines were observed for maternal and child 5-year serum PFAS concentrations, whereas associations for PFASs measured at ages 7-13 years were mostly null. The inverse associations with leptin and adiponectin were mainly seen in females, whereas the inverse PFAS associations with resistin levels were mainly seen in males. Estimates for significant associations (at p-value<0.05) suggested mean decreases in hormone levels (range) by 38%-89% for leptin, 16%-70% for adiponectin, and 33%-62% for resistin for each 2-fold increase in serum-PFAS concentrations.

Conclusions: These findings suggest adipokine hormone dysregulation in early life as a potential pathway underlying PFAS-related health outcomes, and underscore the need to further account for susceptibility windows and sex-dimorphic effects in future investigations.
Introduction

Increasing evidence shows associations between early life exposure to perfluoroalkyl substances (PFASs) and the subsequent risk of obesity and diabetes [1-6]. However, potential mechanisms underlying the metabolic toxicity of PFASs remain largely understudied, especially in children. PFASs are persistent organic pollutants with water and oil repelling properties that have been used in the manufacturing of many household products such as cookware, clothing and carpets for over six decades [7]. These substances have long half-lives in human tissues (estimated up to 7 years, depending on PFASs [8, 9]), and recent human biomonitoring studies show widespread exposure with the most highly detected PFASs being perfluorooctanesulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS), perfluorodecanoic acid (PFDA), and perfluorononanoic acid (PFNA) [10]. Non-occupational exposure to PFASs occurs through the ingestion of contaminated water and food (e.g., fish) [8, 11], as well as transfer from the mother to the child through the placenta and breast-milk in early life [12-14].

Previous prospective studies in populations from the Faroe Islands and other regions have documented inverse associations between prenatal PFAS exposure and birth weight [5], and positive associations with overweight in childhood [1, 3, 4], or adulthood [2]. These associations may be mediated in part through alterations in adipokine hormone regulation or secretion, as suggested by rodent studies [15, 16]. Adipokine hormones, such as leptin, adiponectin and resistin, are secreted by adipocytes and play a key role in energy metabolism, inflammation and other pathways involved in the development of obesity and related comorbidities [17, 18]. Among three previous studies that have examined associations with adipokine hormone levels in children, one reported an inverse association between maternal serum PFOS concentrations and
leptin in cord blood, but no association with adiponectin [19]. Another study found a positive association between maternal serum PFOS and cord blood adiponectin but no association with leptin [20], while the third study found non-significant positive associations between maternal serum PFOA and cord blood leptin and adiponectin concentrations [21]. Only one study has evaluated childhood serum adipokines, and reported no association between maternal serum-PFAS concentrations and the child’s serum concentrations of leptin or adiponectin at age 8 years [22].

Because of sparse data available on the impact of PFAS exposures on adipokine hormone regulation, we evaluated the associations between age-specific serum-PFAS concentrations with adipokine levels in cord blood and child serum in a longitudinal birth cohort with extended follow-up through early adolescence. We hypothesized that especially prenatal exposures, as indicated by maternal serum-PFAS concentrations in pregnancy, alter metabolic programming and adipokine hormone levels over the childhood period, and further examined the associations of postnatal PFAS exposures for comparison purposes.

Methods
Study Population and Data Collection
We studied 80 subjects from a birth cohort of 656 mother-child pairs recruited during the third trimester of pregnancy at the National Hospital in Torshavn in the Faroe Islands between 1997 and 2000 [23]. Only singleton, full-term (>37 gestational weeks) births were included in the cohort and newborns were followed at birth and at ages 5, 7, and 13 years. For the purposes of this pilot investigation, we randomly selected 80 children included in the present analysis from
the totality of children who had complete PFAS data and available blood samples for hormone assessment up to age 13 years. The institutional review board at Harvard T.H. Chan School of Public Health and at the Faroe Islands approved the study protocol. Written informed consent was obtained from all mothers.

Information on the sex of the child, maternal age at delivery, maternal pre-pregnancy body mass index (BMI, i.e., weight in kg/[height in m]²), gestational age, gestational diabetes mellitus, gestational weight gain and offspring’s anthropometry at birth were obtained from medical records. Interviews with the mothers at the pre-parturition examination (~week 34) provided information on parity, maternal education, smoking during pregnancy, and family history of diabetes. Breastfeeding information was reported by the mothers at postnatal examinations. Additional information collected via questionnaires included maternal and child fish intakes, which is a known source of PFAS exposure in this population [24]. Children’s examinations at ages 5 (mean age±sd: 4.9±0.05), 7 (7.5±0.09) and 13 (13.4±0.3) years were conducted by an experienced pediatrician and included anthropometry and pubertal stage assessments (Tanner Scale). We calculated sex- and age- specific z-scores for BMI, and classified children as having overweight (including obesity) if they were at or above the sex- and age- specific 85th percentile of the 2007 WHO Growth Reference [25]. Blood samples were obtained from the mother in pregnancy, from the umbilical cord at birth, and from the child postnatally.

PFAS exposure assessment

Blood samples were extracted at 34 weeks (sd: ±0.3) of gestation and stored at -80°C until PFAS analysis was carried out, as previously detailed [23]. Five major PFASs were measured,
including PFOS, PFOA, PFHxS, PFNA, and PFDA, using high-pressure liquid chromatography with tandem mass spectrometry. All analyzed samples contained PFAS concentrations above the limit of detection (LOD) (>0.03 ng/mL).

**Adipokine hormone assessments**

Leptin, adiponectin, and resistin concentrations were measured in cord blood and child serum collected at the ages of 5, 7, and 13 years. Analyses were performed at the Department of Clinical Biochemistry at Rigshospitalet, Copenhagen University Hospital by enzyme-linked immunooassay (ELISA). All samples were measured in duplicate using IVD certified assays from BioVendor (BioVendor, Brno, Czech Republic). According to the manufacturer, inter-assay coefficients of variance for leptin, adiponectin, and resistin were 6.7% and 4.4% at concentrations 15.39 and 29.34 ng/mL, 5.8% and 6.2% at concentrations 9.41 and 17.74 µg/mL, and 7.0% and 8.1% at concentrations 6.66 and 23.52 ng/mL, respectively.

**Statistical analysis**

Descriptive analysis included the comparison of exposure-outcome covariates between mother-child pairs included and excluded from analysis, and between male and female offspring included in analysis. To normalize the right-skewed distributions, we used log2-transformation for PFAS concentrations and the natural logarithm (ln) for leptin, adiponectin, and resistin concentrations. Generalized additive models (GAMs) [26] were used to assess the linearity of relationships between PFAS and hormone concentration pairs. We did not observe deviations from linearity (P-gain from the GAM model>0.10 for all PFAS-hormone pairs) and thus, we
present effect estimates expressed as ln-unit change in hormone concentrations per doubling (log$_2$) of PFAS concentrations.

To investigate the associations between PFAS and hormone measures, we fitted longitudinal generalized estimating equations (GEE) models of repeated outcome measures with an unstructured correlation matrix and Gaussian family specification. In the GEE models, we evaluated the association of either maternal or child serum PFAS concentrations with hormone measures at the same and subsequent ages up to age 13 years. To evaluate potential windows of effect susceptibility, we included in the GEE models an interaction term between PFAS exposure and age of hormone assessment, and assessed whether associations differed according to the age at which the hormones were measured (ages 0, 5, 7 or 13 years). We also evaluated the cross-sectional associations of PFAS and hormone concentrations measured at age 13 years in linear regression models. Due to prior evidence suggesting that PFAS associations with metabolic outcomes may differ according to sex [2, 4, 5, 21], we examined effect modification by inserting cross-product terms (PFAS*sex) in the GEE and linear regression models.

We evaluated associations first in models adjusted only for child sex and age, and afterwards in multivariable-adjusted models including confounders selected using directed acyclic graphs (DAGs) [27, 28] based on prior evidence [1-6, 11-14, 19-22, 24, 29-34]. The models of maternal serum PFAS concentrations included sex, child age at examination, parity, maternal age, pre-pregnancy BMI, gestational weight gain, and maternal fish intake during pregnancy. The multivariable-adjusted models of postnatal PFAS concentrations (at ages 5, 7 and 13) were adjusted for the same set of covariates, while further including adjustment for breastfeeding and
the child’s fish intake [13, 24]. Social class, maternal smoking and gestational diabetes were not associated with PFAS exposure in this population [5] and were omitted from the statistical models to optimize study precision [35]. We also omitted from the main analysis models adjustment for gestational age, birth weight, child BMI and pubertal stage to avoid over-adjustment bias for an intermediate factor, because these covariates may be also predictors of adipokine hormone levels in cord blood and/or child serum, and potential mediators in the associations of prior PFAS exposure with adipokine hormone levels at later ages [5, 30].

In sensitivity analyses, we evaluated whether child weight status at baseline mediates the associations with hormone levels at later ages, by adjusting in the maternal serum PFAS models for birth weight (g), and in the child serum PFAS models for the BMI age-and-sex specific z-scores at same age as PFAS assessment. We also evaluated the influence of pubertal stage in the associations of interest, by adjusting for child’s pubertal stage in the statistical models of the cross-sectional analysis at age 13 years. Finally, we evaluated whether adjustment for maternal serum PFAS concentrations confounds the associations seen between child serum PFAS concentrations and hormone levels.

All statistical analyses were carried out with Stata 14.1 and R version 3.5.1. To interpret findings, we emphasized the consistency of association patterns and the magnitude and precision of effect estimates rather than relying only on p-values. For effect modification (by age or sex) we used a p-value<0.10 as the level of statistical significance.
Results

Mother-child pairs included in the analysis, compared to those excluded, presented on average higher PFAS concentrations in maternal and child serum, but did not differ in regard to birth weight, child BMI and maternal characteristics including important confounders (Supplementary Table 1, [36]). The analysis population included 39 (49%) males and 41 (51%) females. Most children had at least one older sibling (80%), had exclusively breastfed for less than 6 months (71%), and their mothers had reported not to have smoked during pregnancy (79%) (Table 1).

The prevalence of overweight (including obesity) in children increased from 20% to 28% from ages 5 to 13 years (Table 1). Maternal characteristics and breastfeeding duration did not significantly differ between male and female offspring (Table 1). PFOS showed the highest, and PFDA the lowest, concentrations in maternal and child serum (Supplementary Table 2, [36]).

During the study period, PFOS and PFHxS concentrations tended to decrease, while no clear temporal change was seen for other PFASs. Correlations (Pearson r) of PFAS concentrations (log2-transformed) across ages were in the ranges of [0.13, 0.80] for PFOS, [0.13, 0.44] for PFOA, [-0.11, 0.87] for PFHxS, [0.21, 0.52] for PFDA and [0.21, 0.47] for PFNA (Supplementary Figure 1,[36]). Within-age PFAS correlations were in the range [0.08-0.80] for maternal serum, [0.22, 0.78] for age-5 child serum, [0.16, 0.65] for age-7 child serum and [0.33, 0.85] for age-13 child serum.

All adipokines were detected at the highest concentrations in cord blood compared to child serum at later examinations (Figure 1; numeric data are shown in Supplementary Table 3, [36]). Over the childhood period (ages 5 to 13 years), serum-leptin concentrations significantly increased, while serum-adiponectin slightly decreased, and resistin remained unchanged on
average. Significant differences according to sex were seen for leptin, with higher concentrations at all ages in females compared to males (Figure 1 and Supplementary Table 3, [36]). The same was seen for resistin in cord blood (medians, 34.6 ng/mL in females versus 27.1 ng/mL in males) with no clear difference by sex observed at later ages (Figure 1 and Supplementary Table 3, [36]). Correlations (Pearson r) of hormone concentrations across ages were weak to moderate, with ranges of [0.22, 0.73] for leptin, [0.22, 0.62] for adiponectin, and [0.03, 0.41] for resistin (Supplementary Figure 2, [36]). Cord blood leptin was positively correlated with birth weight (r=0.42) and with child BMI z-score at age 13 years (r=0.16), whereas cord blood adiponectin and resistin levels were inversely correlated with birth weight and postnatal BMI z-scores (-0.18<r<-0.10 for adiponectin, and -0.22<r<-0.16 for resistin) (Supplementary Figure 1, [36]).

Overall, we observed some significant associations with adipokine hormone levels for prenatal and postnatal PFAS exposures (i.e. at ages 5 and/or 7) that in most occasions significantly differed according to the age of hormone assessment (Figures 2-4 and numeric data shown in Supplementary Tables 4-6, [36]). The cross-sectional analyses at age 13 years, showed no significant association between PFASs and adipokines (shown in Supplementary Tables 4-6, [36]).

With regard to leptin (Figure 2; numeric data shown in Supplementary Table 4, [36]), maternal PFOA concentrations were associated with lower leptin levels in cord blood (adjusted β [95%CI] per PFOA doubling= -0.91 [-1.74, -0.09]), but not in child serum at later ages (p-age EM interaction=0.03). Maternal PFHxS concentrations were not associated with cord blood leptin, and inversely associated with child serum leptin levels (adjusted β [95%CI] per PFHxS
doubling=−0.41 [−0.77, −0.06] for leptin at age 5; −0.12 [−0.48, 0.23] for leptin at age 7; −0.35 [−0.73, 0.02] for leptin at age 13 years) (p-age EM interaction=0.12). For maternal PFDA associations also differed across ages (p-age EM interaction=0.03), with a positive association seen only with age-13 leptin levels in males (adjusted β [95%CI] per PFDA doubling = 1.21 [0.11, 2.31]) but not females (0.04 [−0.85, 0.92]). We found no significant associations for leptin and maternal serum PFOS and PFNA. For postnatal PFAS exposures, an overall pattern of inverse associations was seen in females only; child serum-PFOS was significantly associated with lower concurrent/subsequent leptin levels in females (p-sex EM=0.02 for 5-year serum PFOS and 0.04 for 7-year serum PFOS) and child serum-PFDA at age 5 was significantly associated with lower leptin at ages 5, 7 and 13 years in females, and with higher leptin at age 7 in males (p-sex EM=0.01). No significant associations were found for child serum PFOA, PFHxS and PFNA and leptin at any age and sex.

For adiponectin (Figure 3 and Supplementary Table 5, [36]), we found significant associations only for maternal serum PFASs and no association for child serum PFASs. Associations for maternal PFOS, PFDA and PFNA were inverse in females and mostly seen for cord blood adiponectin, while null or non-significant positive in boys (p-sex EM=0.10 for PFOS, 0.09 for PFDA and 0.04 for PFNA). For maternal serum PFHxS, associations did not differ by sex (p-sex EM = 0.99), and overall, inverse associations were seen with adiponectin in cord blood (adjusted β [95%CI] per PFHxS doubling = −0.26 [−0.45, −0.26]) and in 5-year child serum (−0.18 [−0.35, −0.02]), that attenuated at later age (p-age EM = 0.04). No association was seen for maternal PFOA, and for postnatal PFASs with adiponectin, overall, or in sex-stratified analysis.
For resistin, associations with maternal and child serum PFASs mostly differed by sex (Figure 4 and Supplementary Table 6, [36]). In males, maternal serum PFOS, PFDA and PFNA were significantly associated with lower resistin in cord blood (adjusted β [95%CI] per PFOS doubling = -0.98 [-1.55, -0.41]), and also at age 5, but not at later ages (P-age EM=0.15 for PFOS, 0.11 for PFDA, and 0.12 for PFNA). In females, PFOS, PFDA and PFNA associations with resistin were non-significant at all ages (P-sex EM = 0.06 for PFOS, 0.03 for PFDA and 0.09 for PFNA). Inverse association was otherwise seen only for maternal PFOA and child serum resistin at age 5 overall (adjusted β [95%CI] per PFOA doubling -0.46 [-0.86, -0.05], p-sex EM=0.41), while a positive association was seen for maternal PFHxS and cord blood resistin in females only (adjusted β [95%CI] per PFHxS doubling = 0.30 [0.08, 0.52] vs 0.01[-0.27, 0.28] in males). Child serum PFOS at ages 5 and 7 was associated with lower resistin at ages 7-13 in either sex, though associations were of somewhat stronger magnitude in females. Other child serum PFASs were not significantly associated with resistin.

In sensitivity analyses (Supplementary Figures 2-5, [36]), the inclusion of birth weight (g) in the multivariable adjusted models tended to strengthen the associations between maternal serum PFASs and leptin in cord blood and child serum (relative change in effect estimates after birth weight adjustment [range]=[14%, 80%]) (Supplementary Figure 2, [36]). Birth weight adjustment did not substantially change the effect estimates (i.e., relative change < |5| %) for adiponectin (Supplementary Figure 3, [36]) and resistin (Supplementary Figure 4, [36]). Further, adjustment for child BMI z-scores substantially attenuated the significant associations of child serum PFAS concentrations and leptin (change in effect estimates after BMI z-score adjustment [range]= [-9%, -103%]) (Supplementary Figure 2, [36]), and slightly attenuated the significant
associations between child serum PFASs and resistin (change in effect estimates [range]= -3%, 35%) (Supplementary Figure 4, [36]). Adjustment for child BMI z-scores at baseline did not change the associations between child serum PFASs and adiponectin at any age, which remained null (Supplementary Figure 3, [36]). Moreover, adjustment for pubertal stage did not change the null cross-sectional associations seen between PFASs and adipokines at age 13 years (data not shown). Finally, adjustment for maternal serum PFAS concentrations in the models strengthened the associations between age-5 child serum PFASs and concurrent/subsequent levels of leptin and adiponectin, whereas it slightly attenuated the associations between age-5 child serum PFASs and resistin levels (Supplementary Figure 5, [36]); adjustment for maternal serum PFASs was not shown to confound the associations of child serum PFASs at 7 or 13 years with any adipokine hormones under study (i.e., relative change in effect estimates < |5| %), data not shown).

Discussion

This is the first longitudinal study evaluating the associations of maternal and child serum PFAS concentrations with adipokine levels over the course of childhood. Our findings suggest an overall pattern of mostly inverse associations between exposures to major PFASs and serum concentrations of adipokine hormones at relevant susceptibility windows, often specific to child sex. Significant associations were almost exclusively seen for PFAS concentrations measured in maternal serum and in child serum at age 5, rather than PFASs measured at ages 7-13 years, thus suggesting that any potential causal effect on adipokine secretion is initiated prenatally or early postnatally. Further, we observed a more consistent pattern of inverse associations for early-life PFAS exposures and leptin and adiponectin levels in females, and for prenatal PFAS exposures
and resistin levels in males, suggesting that metabolic hormone disruption by PFASs could
substantially differ by sex. These findings underscore the need to further account for exposure-
effect susceptibility windows and sexually dimorphic effects in future study designs.

The observed significant inverse associations indicated mean decreases in ln-unit hormone levels
in the range of 0.49-2.22 for leptin, 0.18-1.21 for adiponectin and 0.41-0.98 for resistin, which
translates to % mean decreases in hormone levels ranging from 38% to 89% for leptin, 16%-70%
for adiponectin, and 33%-62% for resistin, for each doubling of PFAS exposure. These changes
in hormone concentrations are relatively large and can be of clinical importance. The hormone
patterns observed in Faroese children are comparable to those reported in other populations. In
European and Mexican American children on average, leptin levels have also been reported to
decrease from birth to early childhood, and afterwards rise in mid-childhood, as body fat mass
increases pre-puberty, while adiponectin levels decreased over the infancy and early childhood
periods and remained at lower ranges in later childhood [37, 38]. In the Faroese children we also
found higher leptin concentrations in females compared to males, which is in agreement with
previous studies that have reported sex-specific hormone levels in cord blood and/or child serum
[38, 39]. Resistin trajectories are currently underexplored in children, and thus, it is yet unclear
whether the observed pattern in Faroese children, suggesting higher concentrations in cord blood
and in female infants, replicates in other populations.

The pattern of inverse associations we observed for leptin is in partial agreement with an inverse
association reported in the Canadian MIREC study for maternal serum PFOS concentrations and
leptin in cord blood [19], and an inverse cross-sectional association reported between child
serum PFOA and leptin in 665 children from the US Project Viva study at age 8 years [22].

However, the MIREC study, which is the largest conducted so far (n=1,175), reported null associations for maternal serum PFOA and cord blood leptin, and no association with cord blood adiponectin levels, whereas in the present study, we found inverse associations between maternal PFOA and cord blood leptin in either sex, and between maternal serum PFASs (PFOS, PFHxS, PFDA, and PFNA) and cord blood adiponectin in females. The Hokkaido study in Japan (n=168) reported null associations of maternal serum PFOS and PFOA with cord blood leptin, but a positive association between maternal serum PFOS and adiponectin [20]. One explanation for inconsistent findings across studies may be that PFAS concentrations in the Faroese population were substantially higher for all PFASs examined compared to the exposure ranges reported in MIREC and in Hokkaido study (e.g., IQR for maternal serum PFOS equal to [23.3, 35.5] ng/mL in the Faroese study vs [3.2, 6.8] ng/mL in MIREC and [3.7, 6.7] ng/mL in Hokkaido). Sex was considered as a modifying factor in both studies, but sex-stratified estimates were not reported, preventing the direct comparison of sex-specific results. However, sex-stratified analysis of maternal serum PFASs and cord blood leptin levels in the recent U.S. HOME study of 107 male and 123 female newborns suggested a pattern of inverse associations in females, and positive associations in males, even though most estimates were statistically non-significant [21]. This is in agreement with the potentially higher susceptibility to leptin decreases in females suggested by this study. Notably, there is large inconsistency in associations of each specific PFAS across studies, likely due to different exposure ranges, and the moderate correlations among PFASs that do not allow ascribing an effect to a specific compound in population studies. One more consideration is that no previous study has examined resistin, a promising marker of insulin
resistance and adipose tissue inflammation [40], which is far less studied than leptin and adiponectin [41].

Although mechanistic pathways are poorly understood, existing data from experimental rodent studies support the interference of PFASs with the secretion of several hormones, including adipokines. Developmental low-dose PFOA exposure of mice induced latent effects with increases in serum leptin and body weight when the mice reached mid-life (21-33 weeks), thus suggesting a potential leptin-resistance mechanism of sustained PFOA action [16]. However, higher PFOA exposure doses in utero led to opposite effects with long-term decreases in body weight in mice [16]. Further, one study found that PFOA-treated male mice at age 6-7 weeks presented elevated serum concentrations of leptin and adiponectin, as well as impaired glucose tolerance [15]. Plausible molecular mechanisms that underlie these effects may include the known actions of PFASs as agonists of the peroxisome proliferator-activated receptor- gamma (PPARg) [42], that is considered to be a key regulator of adipocyte differentiation and metabolic functions in rodents and humans [43, 44]. However, mechanisms could substantially differ between rodents and humans and therefore further investigations in in vitro human tissue models are needed. This can be particularly important for resistin, in which the secretion and functional activity has been shown to remarkably differ between rodents and humans [40].

Findings from this study compliment the evidence about the adverse metabolic potential of PFASs provided by our previous investigations in the Faroese population, where we showed PFAS associations with lower birth weight and higher BMI in childhood [3, 5]. Lower cord blood concentrations of leptin have been associated with lower birth weight and accelerated
Further, lower adiponectin in cord blood or child serum have been linked to higher adiposity and insulin resistance markers later in childhood \[39, 45, 47\], similar to lower adiponectin detected in adults with obesity and type 2 diabetes \[48\]. Thus, altered adipokine hormone secretion in early life might serve as early predictors of PFAS-associated metabolic outcomes at later life stages. Additionally, as adiposity correlates with adipokine hormone secretion, we examined prospectively the influence of weight status (as an indirect measure of adiposity). Birth weight was not seen to attenuate the associations of maternal serum PFAS concentrations, which strengthens the plausibility of a direct link of PFAS exposure with adipokine hormone regulation. However, adjustment for child BMI status at baseline attenuated a large proportion of estimated effects between postnatal PFAS exposures and leptin levels, suggesting that associations over childhood examinations could be confounded and/or mediated to some extent by child adiposity. This is an important consideration for the interpretation of findings from cross-sectional studies focusing on postnatal exposure periods.

Study limitations include the sample size and the lack of detailed dietary information that does not allow us to rule out unmeasured confounding. However, we were able to adjust for maternal and child fish intakes which is an important PFAS exposure source in the Faroese population \[24\]. Glomerular filtration rate during pregnancy might also be a relevant confounder not accounted in our study, as suggested by few recent studies on PFASs and child anthropometry at birth \[33, 34, 49\]. Further, extension of analyses to the whole cohort is needed to fully capture dose-response relationships, as children included versus those excluded from this analysis had somewhat higher PFAS concentrations and therefore we cannot rule out selection bias. The evaluation of associations in larger populations will further enhance precision for detecting sex-
specific effects. Study strengths include the prospective design with repeated PFASs and hormone measures, and the long follow-up period of this cohort. Further, the Faroese consist of a relatively homogenous population in respect to socioeconomic status, which may reduce confounding.

Conclusion

Findings from this pilot study suggest overall a pattern of inverse associations between exposures to major PFASs and adipokine hormone levels in early life. Associations were of larger magnitude for PFAS exposures in the prenatal and early childhood periods compared to exposures at later ages, and further differed by sex. Even though results should be interpreted with caution due to the small sample size, findings support adipokine hormone dysregulation as a potential mechanistic pathway for the adverse health outcomes of PFASs, and underscore the need to account for susceptibility windows and sex-dimorphic effects in future investigations.
References


    https://www.cdc.gov/exposurereport/index.html [accessed on 04/01/2019].


https://figshare.com/articles/Shelly_et_al_SUPPLEMENTAL_MATERIAL/8220569


Table 1. Main characteristics of the Faroese mother-child pairs included in analysis.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All children (n=80)</th>
<th>Males (n=39)</th>
<th>Females (n=41)</th>
<th>P-sex differencea</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>% or mean ± sd</td>
<td>% or mean ± sd</td>
<td>% or mean ± sd</td>
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<tr>
<td>Maternal age (years)</td>
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<td>30.5 ± 5.2</td>
<td>30.1 ± 4.9</td>
<td>30.8 ± 5.5</td>
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<td>Pre-pregnancy BMI (kg/m²)</td>
<td>80</td>
<td>24.3 ± 4.5</td>
<td>24.5 ± 5.1</td>
<td>24.2 ± 4.0</td>
</tr>
<tr>
<td>Social class</td>
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<tr>
<td>Low</td>
<td>31</td>
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<td>38.5%</td>
<td>39.0%</td>
</tr>
<tr>
<td>Middle</td>
<td>28</td>
<td>35.0%</td>
<td>33.3%</td>
<td>36.6%</td>
</tr>
<tr>
<td>High</td>
<td>21</td>
<td>26.3%</td>
<td>28.2%</td>
<td>24.4%</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>No older siblings</td>
<td>16</td>
<td>20.0%</td>
<td>20.5%</td>
<td>19.5%</td>
</tr>
<tr>
<td>≥1 older sibling</td>
<td>64</td>
<td>80.0%</td>
<td>79.5%</td>
<td>80.5%</td>
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<tr>
<td>Smoking in pregnancy</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>63</td>
<td>78.8%</td>
<td>76.9%</td>
<td>80.5%</td>
</tr>
<tr>
<td>Yes</td>
<td>17</td>
<td>21.2%</td>
<td>23.1%</td>
<td>19.5%</td>
</tr>
<tr>
<td>Fish intake in pregnancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2 dinners per week</td>
<td>24</td>
<td>29.9%</td>
<td>27.2%</td>
<td>32.9%</td>
</tr>
<tr>
<td>≥2 dinners per week</td>
<td>56</td>
<td>70.1%</td>
<td>63.8%</td>
<td>67.1%</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>80</td>
<td>39.7 ± 1.2</td>
<td>39.5 ± 1.4</td>
<td>39.9 ± 1.1</td>
</tr>
<tr>
<td>Gestational weight gain (kg)</td>
<td>80</td>
<td>14.9 ± 6.3</td>
<td>16.2 ± 6.7</td>
<td>13.7 ± 5.7</td>
</tr>
<tr>
<td>Gestational diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>74</td>
<td>92.0%</td>
<td>91.9%</td>
<td>92.1%</td>
</tr>
<tr>
<td>Yes</td>
<td>6</td>
<td>8.0%</td>
<td>8.1%</td>
<td>7.9%</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>80</td>
<td>3779 ± 533</td>
<td>3801 ± 571</td>
<td>3758 ± 500</td>
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<tr>
<td>Exclusive breastfeeding</td>
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<tr>
<td>&lt;6 months</td>
<td>56</td>
<td>70.9%</td>
<td>71.8%</td>
<td>70%</td>
</tr>
<tr>
<td>≥6 months</td>
<td>23</td>
<td>29.1%</td>
<td>28.2%</td>
<td>30%</td>
</tr>
<tr>
<td>Child age at 5-year exams</td>
<td>80</td>
<td>4.9 ± 0.05</td>
<td>4.9 ± 0.05</td>
<td>4.9 ± 0.06</td>
</tr>
<tr>
<td>Child fish intake at 5 years</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2 dinners per week</td>
<td>27</td>
<td>33.7%</td>
<td>28.2%</td>
<td>39.0%</td>
</tr>
<tr>
<td>≥2 dinners per week</td>
<td>53</td>
<td>66.3%</td>
<td>71.8%</td>
<td>61.0%</td>
</tr>
<tr>
<td>zBMI at age 5 years (sd)</td>
<td>80</td>
<td>0.40 ± 0.8</td>
<td>0.37 ± 0.6</td>
<td>0.42 ± 0.9</td>
</tr>
<tr>
<td>Overweight at age 5 yearsb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>64</td>
<td>80.0%</td>
<td>84.6%</td>
<td>75.6%</td>
</tr>
<tr>
<td>Yes</td>
<td>16</td>
<td>20.0%</td>
<td>15.4%</td>
<td>24.4%</td>
</tr>
<tr>
<td>Child age at 7-year exams</td>
<td>80</td>
<td>7.5 ± 0.09</td>
<td>7.5 ± 0.07</td>
<td>7.5 ± 0.10</td>
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<tr>
<td>zBMI at age 7 years (sd)</td>
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<td>0.44 ± 0.8</td>
<td>0.47 ± 0.8</td>
<td>0.40 ± 0.9</td>
</tr>
<tr>
<td>Overweight at age 7 yearsb</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>No</td>
<td>61</td>
<td>76.2%</td>
<td>79.5%</td>
<td>73.2%</td>
</tr>
<tr>
<td>Yes</td>
<td>19</td>
<td>23.8%</td>
<td>20.5%</td>
<td>26.8%</td>
</tr>
<tr>
<td>Child age at 13-year exams</td>
<td>80</td>
<td>13.4 ± 0.3</td>
<td>13.3 ± 0.4</td>
<td>13.4 ± 0.3</td>
</tr>
<tr>
<td>zBMI at age 13 years (sd)</td>
<td>80</td>
<td>0.40 ± 0.9</td>
<td>0.38 ± 0.9</td>
<td>0.41 ± 0.9</td>
</tr>
<tr>
<td>Overweight at age 13 years&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>58</td>
<td>72.5%</td>
<td>66.7%</td>
<td>78.1%</td>
</tr>
<tr>
<td>Yes</td>
<td>22</td>
<td>27.5%</td>
<td>33.3%</td>
<td>21.9%</td>
</tr>
<tr>
<td>Puberty status at 13 years</td>
<td></td>
<td></td>
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<tr>
<td>Tanner stage ≤ 3</td>
<td>46</td>
<td>59.7%</td>
<td>69.5%</td>
<td>51.2%</td>
</tr>
<tr>
<td>Tanner stage &gt;3</td>
<td>31</td>
<td>40.3%</td>
<td>30.5%</td>
<td>48.8%</td>
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</table>

<sup>a</sup> p-value from Pearson chi-squared test for categorical variables and Student’s t-test for continuous variables.

<sup>b</sup> Overweight (including obesity) defined as a BMI z-score specific-for-age-and-sex≥ the 85<sup>th</sup> percentile of the 2007 WHO Growth reference.
Figure Legends

Figure 1. Distributions of adipokine hormone concentrations (median and interquartile range [IQR]) at birth and over the childhood period, overall and in males and females separately.
Figure 2. Adjusted GEE effect estimates\(^a\) (\(\beta [95\% \ CI]\)) for the association of PFAS concentrations (log\(_2\)) in maternal or child serum with repeated (concurrent and subsequent) LEPTIN concentrations (ln).

\(^a\) Effect estimates for maternal-serum PFASs are adjusted for child sex, exact age at examinations, maternal age, parity, prepregnancy BMI, gestational weight gain, maternal fish intake and an interaction term between PFAS*age at hormone assessments. Effect estimates for child-serum PFASs are additionally adjusted for breastfeeding, and child fish intake.
Figure 3. Adjusted GEE effect estimates\textsuperscript{a} ($\beta$ [95\% CI]) for the association of PFAS concentrations (log\textsubscript{2}) in maternal or child serum with repeated (concurrent and subsequent) ADIPONECTIN concentrations (ln).

\textsuperscript{a} Effect estimates for maternal-serum PFASs are adjusted for child sex, exact age at examinations, maternal age, parity, prepregnancy BMI, gestational weight gain, maternal fish intake and an interaction term between PFAS*age at hormone assessments. Effect estimates for child-serum PFASs are additionally adjusted for breastfeeding, and child fish intake.
Figure 4. Adjusted GEE effect estimates\textsuperscript{a} ($\beta$ [95\% CI]) for the association of PFAS concentrations (log\textsubscript{2}) in maternal or child serum with repeated (concurrent and subsequent) RESISTIN concentrations (ln).

\textsuperscript{a} Effect estimates for maternal-serum PFASs are adjusted for child sex, exact age at examinations, maternal age, parity, prepregnancy BMI, gestational weight gain, maternal fish intake and an interaction term between PFAS*age at hormone assessments. Effect estimates for child-serum PFASs are additionally adjusted for breastfeeding, and child fish intake.