Early Life Exposures to Perfluoroalkyl Substances in Relation to Adipokine Hormone Levels at Birth and During Childhood

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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 to 2000. Five PFASs were measured in maternal pregnancy serum and in 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 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 to 13 years were mostly null. The inverse associations with leptin and adiponectin were seen mainly in females, whereas the inverse PFAS associations with resistin levels were seen mainly in males. Estimates for significant associations (P value <0.05) suggested mean decreases in hormone levels (range) by 38% to 89% for leptin, 16% to 70% for adiponectin, and 33% to 62% for resistin for each twofold increase in serum PFAS concentration.

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. (***J Clin Endocrinol Metab* 104: 5338–5348, 2019**)

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Abbreviations: BMI, body mass index; EM, effect modification; GEE, generalized estimating equation; ln, natural logarithm; MIREC, Maternal-Infant Research on Environmental Chemicals; PFAS, perfluoroalkyl substance; PFDA, perfluorodecanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate.

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 more than 6 decades (7). These substances have long half-lives in human tissues [estimated at up to 7 years, depending on the PFAS (8, 9)], and recent human biomonitoring studies showed widespread exposure, with the most highly detected PFASs being perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS), perfluorodecanoic acid (PFDA), and perfluorononanoic acid (PFNA) (10). Nonoccupational 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 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 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), whereas the third study found nonsignificant positive associations between maternal serum PFOA and cord blood leptin and adiponectin concentrations (21). Only one study has evaluated childhood serum adipokines, and it reported no association between maternal serum PFAS concentrations and the child's serum concentration of leptin or adiponectin at age 8 years (22).

Because of the sparsity of data on the effect 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 prenatal exposures in particular, 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 Tórshavn 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 up 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 prepregnancy body mass index [BMI; *i.e.,* weight in kilograms/(height in meters)^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 preparturition examination (approximately 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 intake, 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 World Health Organization growth reference data (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—PFOS, PFOA, PFHxS, PFNA, and PFDA—were measured using high-pressure liquid chromatography with tandem mass spectrometry. All analyzed samples contained PFAS concentrations above the limit of detection (>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 with ELISA at the Department of Clinical Biochemistry at Rigshospitalet, Copenhagen

University Hospital. All samples were measured in duplicate using *in vitro* diagnostic–certified assays from BioVendor (Brno, Czech Republic). According to the manufacturer, interassay coefficients of variance for leptin, adiponectin, and resistin were 6.7% and 4.4% at concentrations of 15.39 and 29.34 ng/mL, 5.8% and 6.2% at concentrations of 9.41 and 17.74 μg/mL, and 7.0% and 8.1% at concentrations of 6.66 and 23.52 ng/mL, respectively.

Statistical analysis

Descriptive analyses included the comparison of exposure-outcome covariates between mother-child pairs who were included and excluded from analysis and between male and female offspring included in the analysis. To normalize the right-skewed distributions, we used $\log_2$-transformation for PFAS concentrations and the natural logarithm (ln) for leptin, adiponectin, and resistin concentrations. Generalized additive models (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 generalized additive model >0.10 for all PFAS-hormone pairs); 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 equation (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 13 years. To evaluate potential windows of effect susceptibility, we included in the GEE models an interaction term between PFAS exposure and age at hormone assessment and assessed whether associations differed according to the age at which the hormones were measured (age 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. Because of prior evidence suggesting that PFAS associations with metabolic outcomes may differ according to sex (2, 4, 5, 21), we examined effect modification (EM) 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 afterward in multivariable-adjusted models including confounders selected using directed acyclic graphs (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, prepregnancy BMI, gestational weight gain, and maternal fish intake during pregnancy. The multivariable-adjusted models of postnatal PFAS concentrations (at ages 5, 7, and 13 years) were adjusted for the same set of covariates, including further 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 model adjustment for gestational age, birth weight, child BMI, and pubertal stage to avoid overadjustment bias for an intermediate factor, as these covariates may also be predictors of adipokine hormone levels in cord blood and/or child serum and potential mediators in the association 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 adjustments in the maternal serum PFAS models for birth weight (grams) and in the child serum PFAS models for the BMI age- and sex-specific *z* scores at the same age as PFAS assessment. We also evaluated the influence of pubertal stage on the associations of interest by adjusting for the 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 (StataCorp LP) and R version 3.5.1 (www.r-project.org). 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

Compared with excluded mother-child pairs, mother-child pairs included in the analysis presented, on average, higher PFAS concentrations in maternal and child serum but did not differ regarding birth weight, child BMI, and maternal characteristics including important confounders (36). The analysis population included 39 males (49%) and 41 females (51%). Most children had at least one older sibling (80%) and had been exclusively breastfed for <6 months (71%), and their mothers had reported not smoking 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 concentration and PFDA the lowest in maternal and child serum (36). During the study period, PFOS and PFHxS concentrations tended to decrease, whereas no clear temporal change was seen for other PFASs. Correlations (Pearson *r*) of PFAS concentrations ($\log_2$-transformed) across ages were in the following ranges: [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 (36). Within-age PFAS correlations were in the range [0.08, 0.80] for maternal serum, [0.22, 0.78] for serum at age 5 years, [0.16, 0.65] for serum at age 7 years, and [0.33, 0.85] for serum at age 13 years.

All adipokine concentrations were higher in cord blood than in child serum at later examinations [Fig. 1; numeric data shown in (36). Over the childhood period (ages 5 to 13 years), serum leptin concentrations significantly increased, whereas serum adiponectin concentrations slightly decreased and resistin concentration

Table 1. Main Characteristics of the Faroese Mother-Child Pairs Included in the Analysis

	All Children (n = 80)		Males (n = 39)	Females (n = 41)	
Characteristic	**n**	**% or Mean ± SD**	**% or Mean ± SD**	**% or Mean ± SD**	***P*-Sex Difference[a]**
Maternal age, y	80	30.5 ± 5.2	30.1 ± 4.9	30.8 ± 5.5	0.58
Prepregnancy BMI, kg/m^2	80	24.3 ± 4.5	24.5 ± 5.1	24.2 ± 4.0	0.81
Social class					
Low	31	38.7%	38.5%	39.0%	0.92
Middle	28	35.0%	33.3%	36.6%	
High	21	26.3%	28.2%	24.4%	
Parity					
No older siblings	16	20.0%	20.5%	19.5%	0.91
≥1 older siblings	64	80.0%	79.5%	80.5%	
Smoking in pregnancy					
No	63	78.8%	76.9%	80.5%	0.70
Yes	17	21.2%	23.1%	19.5%	
Fish intake in pregnancy					
<2 dinners per wk	24	29.9%	27.2%	32.9%	0.37
≥2 dinners per wk	56	70.1%	63.8%	67.1%	
Gestational age, wk	80	39.7 ± 1.2	39.5 ± 1.4	39.9 ± 1.1	0.16
Gestational weight gain, kg	80	14.9 ± 6.3	16.2 ± 6.7	13.7 ± 5.7	0.08
Gestational diabetes					
No	74	92.0%	91.9%	92.1%	0.95
Yes	6	8.0%	8.1%	7.9%	
Birth weight, g	80	3779 ± 533	3801 ± 571	3758 ± 500	0.72
Exclusive breastfeeding					
<6 months	56	70.9%	71.8%	70%	0.86
≥6 months	23	29.1%	28.2%	30%	
Child age at 5-y examination	80	4.9 ± 0.05	4.9 ± 0.05	4.9 ± 0.06	0.64
Child fish intake at 5 y					
<2 dinners per wk	27	33.7%	28.2%	39.0%	0.31
≥2 dinners per wk	53	66.3%	71.8%	61.0%	
z BMI at age 5 y (SD)	80	0.40 ± 0.8	0.37 ± 0.6	0.42 ± 0.9	0.78
Overweight at age 5 y[b]					
No	64	80.0%	84.6%	75.6%	0.31
Yes	16	20.0%	15.4%	24.4%	
Child age at 7-y examinations	80	7.5 ± 0.09	7.5 ± 0.07	7.5 ± 0.10	0.10
z BMI at age 7 y (SD)	80	0.44 ± 0.8	0.47 ± 0.8	0.40 ± 0.9	0.69
Overweight at age 7 y[b]					
No	61	76.2%	79.5%	73.2%	0.51
Yes	19	23.8%	20.5%	26.8%	
Child age at 13-y examination	80	13.4 ± 0.3	13.3 ± 0.4	13.4 ± 0.3	0.17
z BMI at age 13 y (SD)	80	0.40 ± 0.9	0.38 ± 0.9	0.41 ± 0.9	0.88
Overweight at age 13 y[b]					
No	58	72.5%	66.7%	78.1%	0.25
Yes	22	27.5%	33.3%	21.9%	
Puberty status at 13 y					
Tanner stage ≤3	46	59.7%	69.5%	51.2%	0.10
Tanner stage >3	31	40.3%	30.5%	48.8%	

[a] *P* value from Pearson χ^2 test for categorical variables and Student *t* test for continuous variables.

[b] Overweight (including obesity) defined as a BMI *z* score specific for age and sex greater than or equal to the 85th percentile of the 2007 World Health Organization growth reference.

remained unchanged on average. Significant differences according to sex were seen for leptin, with higher concentrations at all ages in females than in males (Fig. 1) (36). The same was seen for resistin in cord blood (median: 34.6 ng/mL in females vs 27.1 ng/mL in males), with no clear difference by sex observed at later ages (Fig. 1) (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 (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) (36).

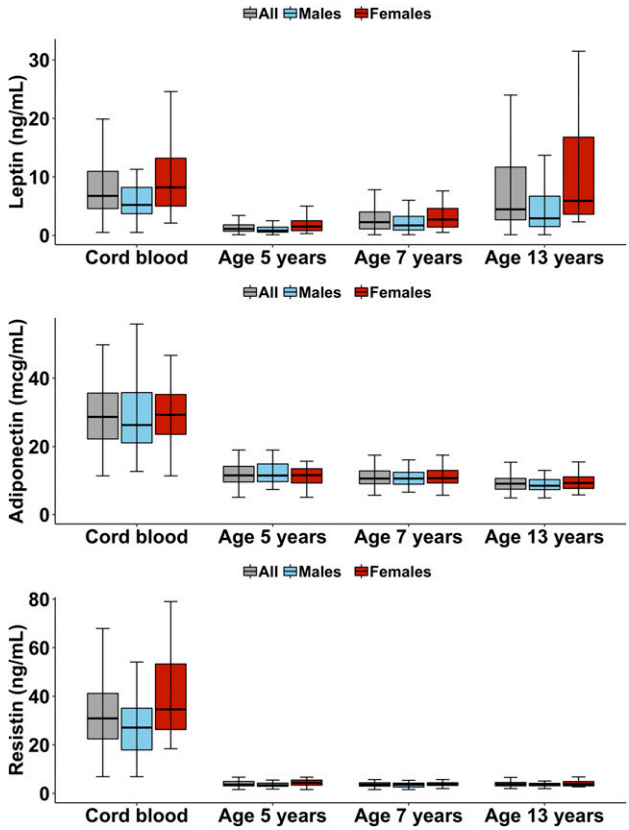

Figure 1. Distributions of adipokine hormone concentrations (median and interquartile range) at birth and over the childhood period overall and in males and females separately.

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

With regard to leptin [Fig. 2; numeric data shown in (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 were 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 years; −0.12 (−0.48, 0.23) for leptin at age 7 years; −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-year 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 years was significantly associated with lower leptin at ages 5, 7, and 13 years in females and with higher leptin concentrations at age 7 years 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 (Fig. 3) (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 were seen mostly for cord blood adiponectin, whereas they were null or nonsignificantly 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); 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)], which attenuated at a 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 analyses.

For resistin, associations with maternal and child serum PFASs differed mostly by sex (Fig. 4) (36). In males, maternal serum PFOS, PFDA, and PFNA concentrations were significantly associated with lower resistin levels in cord blood [adjusted β (95% CI) per PFOS doubling = −0.98 (−1.55, −0.41)] and also at age 5 years, 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 nonsignificant at all ages (*P*-sex EM = 0.06 for PFOS; 0.03 for PFDA; and 0.09 for PFNA). An inverse association was otherwise seen only for maternal PFOA and child serum resistin at age 5 years overall [adjusted β (95% CI) per PFOA doubling = −0.46 (−0.86, −0.05); *P*-sex EM = 0.41], whereas 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 years was associated with lower resistin at ages 7 to 13 years in either sex, though associations were somewhat stronger in females. Other child serum PFASs were not significantly associated with resistin.

In sensitivity analyses (36), the inclusion of birth weight (grams) in the multivariable adjusted models tended to strengthen the associations between maternal

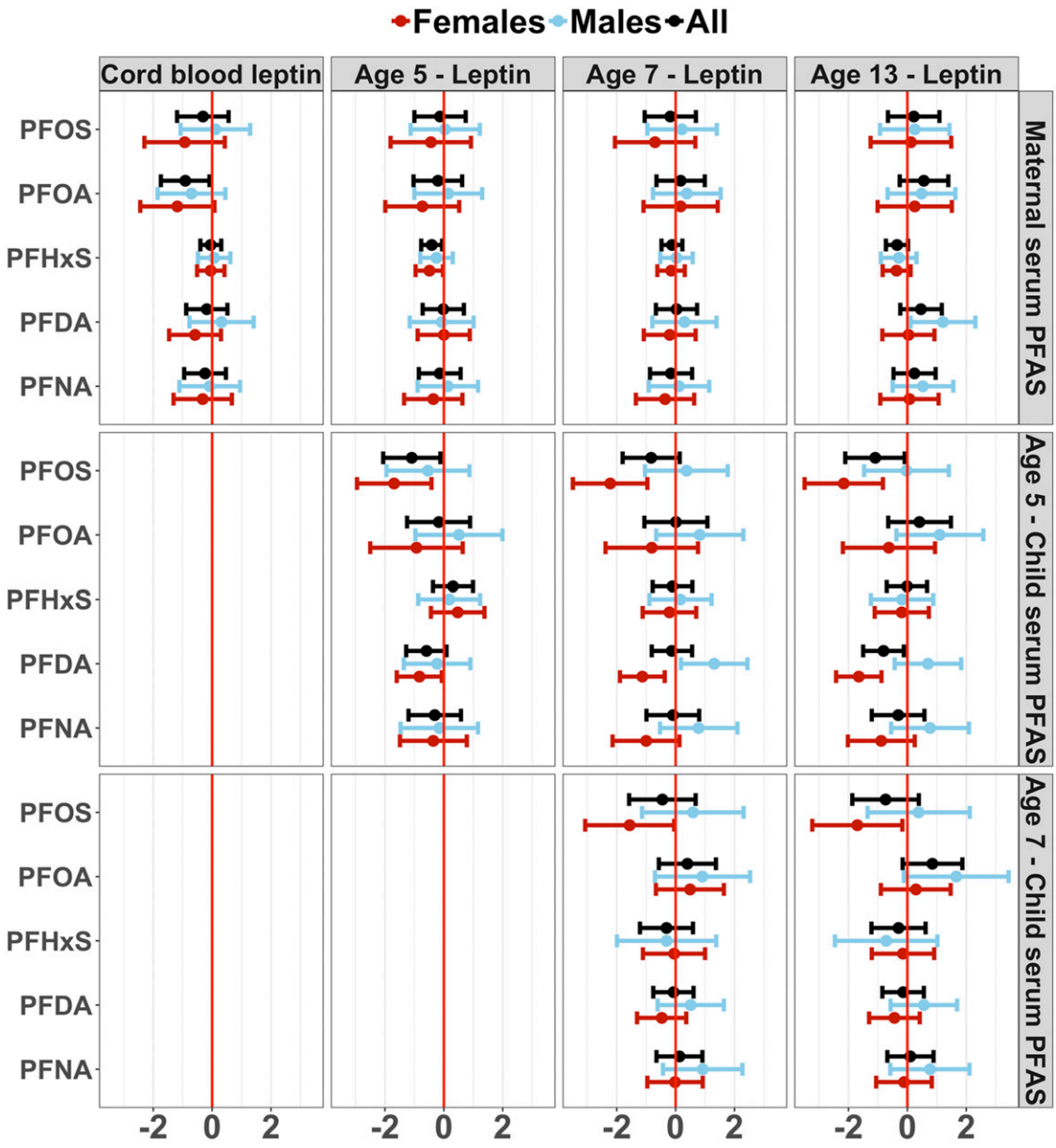

Figure 2. Adjusted GEE effect estimates [β (95% CI)] for the association of PFAS concentrations (log_2) in maternal or child serum with repeated (concurrent and subsequent) leptin concentrations (ln). Effect estimates for maternal serum PFASs were adjusted for child sex, exact age at examinations, maternal 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 were additionally adjusted for breastfeeding and child fish intake.

serum PFASs and leptin in cord blood and child serum [relative change in effect estimates after birth weight adjustment (range) = (14%, 80%)] (36). Birth weight adjustment did not substantially change the effect estimates (*i.e.*, relative change <5%) for adiponectin (36) and resistin (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%)] (36) and slightly attenuated the significant associations between child serum PFASs and resistin [change in effect estimates (range) = (−3%, 35%)] (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 (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-year child serum PFASs and concurrent/subsequent levels of leptin and adiponectin, whereas it slightly attenuated the associations between age 5-year child serum PFASs and resistin levels (36). Adjustment for maternal serum PFASs did not confound the associations of child serum PFASs at 7 or 13 years with any

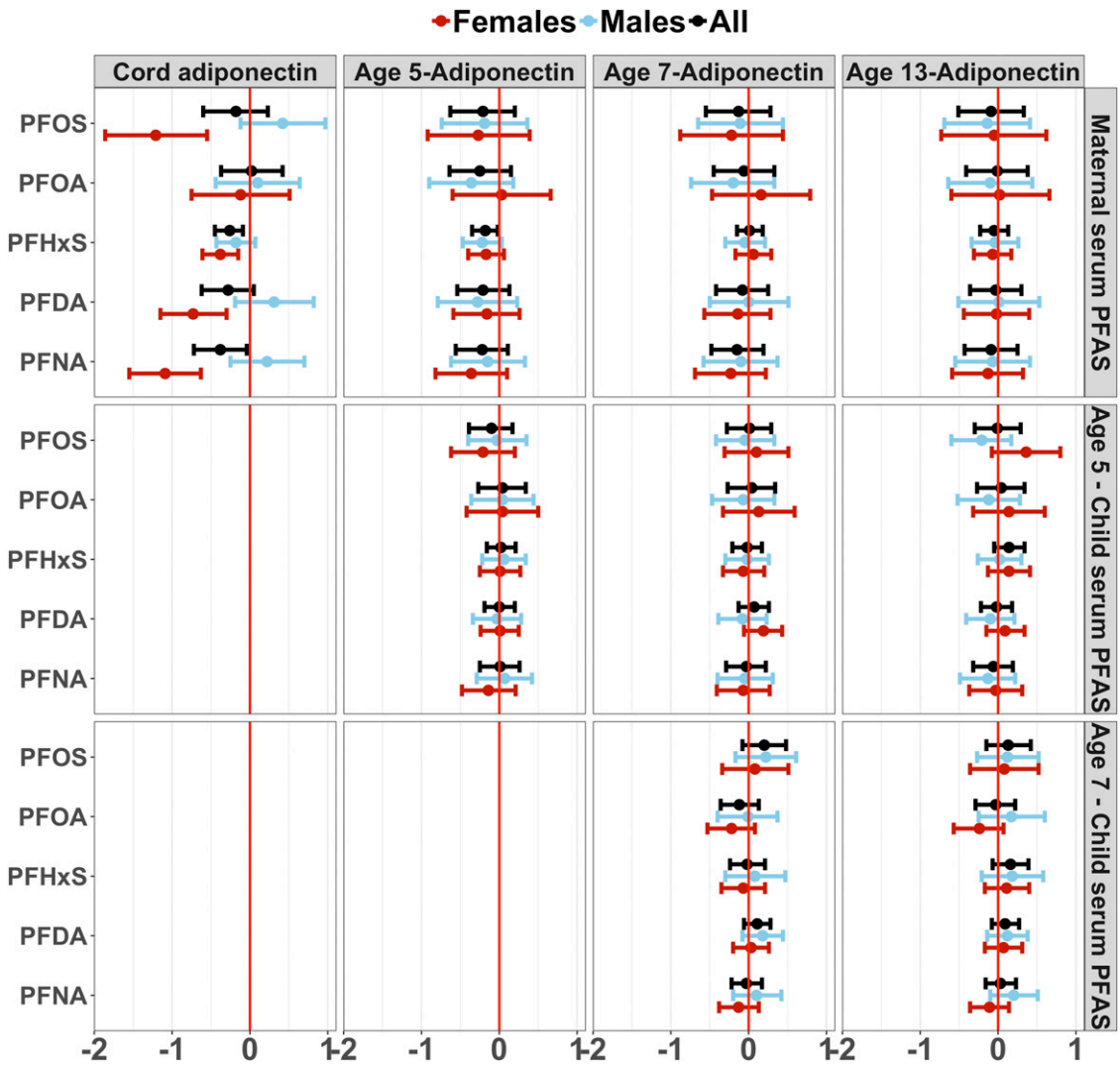

Figure 3. Adjusted GEE effect estimates [β (95% CI)] for the association of PFAS concentrations (log_2) in maternal or child serum with repeated (concurrent and subsequent) adiponectin concentrations (ln). Effect estimates for maternal serum PFASs were adjusted for child sex, exact age at examinations, 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 were additionally adjusted for breastfeeding and child fish intake.

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 exposure to major PFASs and serum concentrations of adipokine hormones at relevant susceptibility windows, often specific to child sex. Significant associations were seen almost exclusively for PFAS concentrations measured in maternal serum and in child serum at age 5 years rather than PFASs measured at ages 7 to 13 years, 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 may 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 to 2.22 for leptin, 0.18 to 1.21 for adiponectin, and 0.41 to 0.98 for resistin, which translates to percentage mean decreases in hormone levels ranging from 38% to 89% for leptin, 16% to 70% for adiponectin, and 33% to 62% for resistin for each doubling of PFAS exposure. These changes in hormone concentrations are relatively

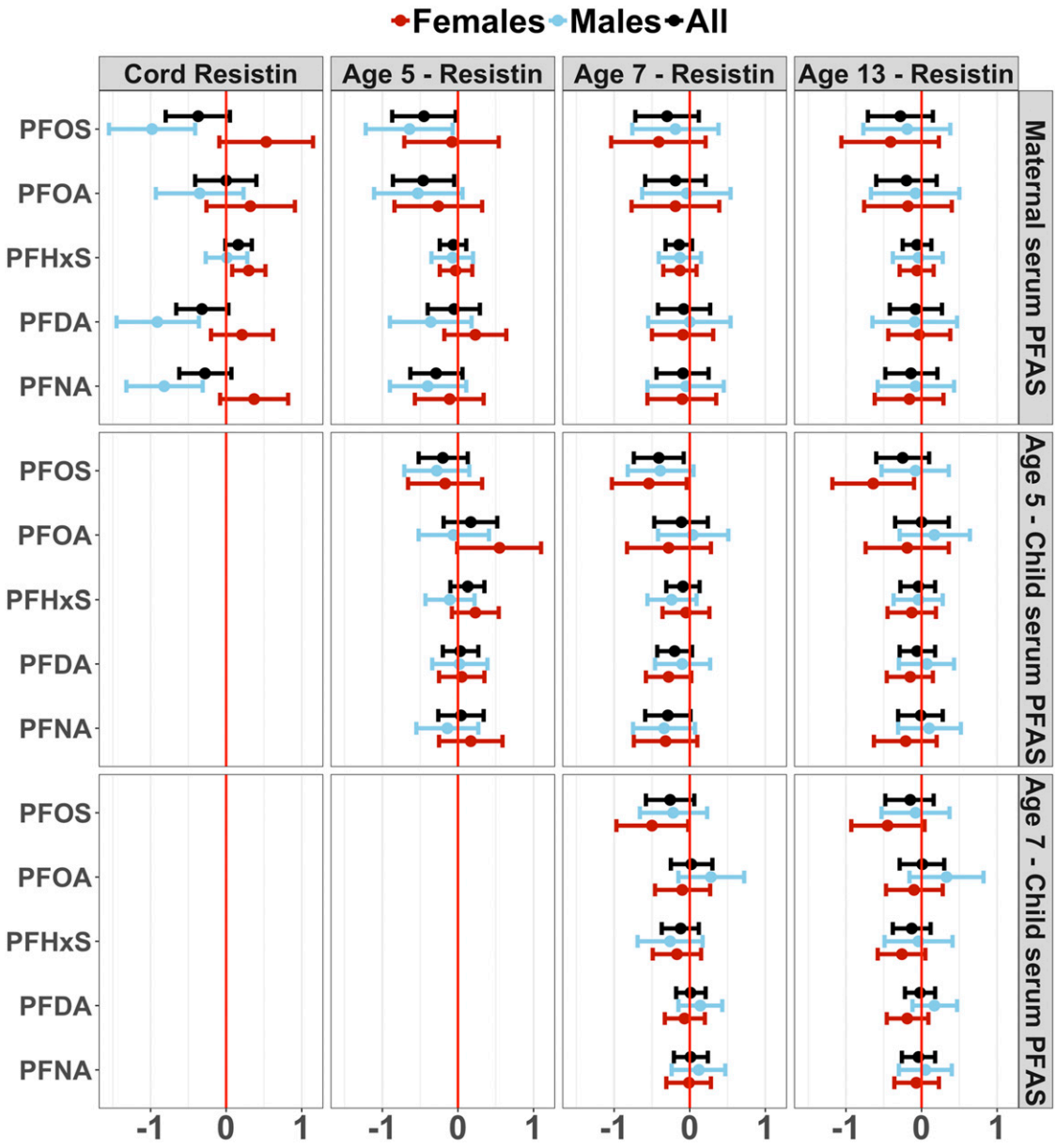

Figure 4. Adjusted GEE effect estimates [β (95% CI)] for the association of PFAS concentrations (log_2) in maternal or child serum with repeated (concurrent and subsequent) resistin concentrations (ln). Effect estimates for maternal serum PFASs were adjusted for child sex, exact age at examinations, 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 were additionally adjusted for breastfeeding and child fish intake.

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, leptin levels have also been reported to decrease on average from birth to early childhood and afterward rise in mid-childhood, as body fat mass increases prepuberty, whereas adiponectin levels decreased over the infancy and early childhood periods and remained at lower ranges in later childhood (37, 38). In Faroese children, we also found higher leptin concentrations in females than in males, which agrees with previous studies that reported sex-specific hormone levels in cord blood and/or child serum (38, 39). Resistin trajectories are currently underexplored in children; thus, it is yet unclear whether the observed pattern in Faroese children, which suggests 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 Maternal-Infant Research on Environmental Chemicals (MIREC) study for maternal serum PFOS concentrations and leptin in cord blood (19) and with an inverse cross-sectional association reported between child serum PFOA and leptin levels in 665 children at age 8 years from the US Project Viva study

(22). However, the MIREC study, which is the largest conducted so far (n = 1175), reported null associations for maternal serum PFOA and cord blood leptin and no association with cord blood adiponectin levels, whereas in the current 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 with the exposure ranges reported in the MIREC and Hokkaido studies [*e.g.,* the interquartile range for maternal serum PFOS was (23.3, 35.5 ng/mL) in the Faroese study vs (3.2, 6.8 ng/mL) in the MIREC study and (3.7, 6.7 ng/mL) in the Hokkaido study]. Sex was considered a modifying factor in both studies, but sex-stratified estimates were not reported, preventing direct comparison of sex-specific results. However, sex-stratified analysis of maternal serum PFASs and cord blood leptin levels in the recent US 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 nonsignificant (21). This agrees with the potentially higher susceptibility to leptin decreases suggested in females in this study. Of note, there is large inconsistency in the associations of each specific PFAS across studies, likely because of different exposure ranges and moderate correlations among PFASs, which do not allow ascribing an effect to a specific compound in population studies. One more consideration is that no previous study examined resistin, a promising marker of insulin resistance and adipose tissue inflammation (40) that 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 in the secretion of several hormones, including adipokines. Developmental low-dose PFOA exposure in mice induced latent effects with increases in serum leptin level and body weight when the mice reached midlife (21 to 33 weeks), 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 to 7 weeks had elevated serum concentrations of leptin and adiponectin, as well as impaired glucose tolerance (15). Plausible molecular mechanisms underlying these effects may include the known actions of PFASs as agonists of the peroxisome proliferator-activated receptor-gamma (42), which is considered a key regulator of adipocyte differentiation and metabolic functions in rodents and humans (43, 44). However, mechanisms could substantially differ between rodents and humans; therefore, further investigations in *in vitro* human tissue models are needed. This can be particularly important for resistin, for which secretion and functional activity has differed remarkably between rodents and humans (40).

Findings from this study complement evidence about the adverse metabolic potential of PFASs provided by our previous investigations in the Faroese population, in which we showed associations of PFAS 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 weight gain in childhood (45, 46). Further, lower adiponectin levels 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 levels detected in adults with obesity and type 2 diabetes mellitus (48). Thus, altered adipokine hormone secretion in early life may serve as early predictors of PFAS-associated metabolic outcomes at later life stages.

In addition, because adiposity correlates with adipokine hormone secretion, we prospectively examined the influence of weight status as an indirect measure of adiposity. Birth weight did not 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 can be confounded and/or mediated to some extent by child adiposity. This is an important consideration in the interpretation of findings from cross-sectional studies focusing on postnatal exposure periods.

Study limitations include the sample size and lack of detailed dietary information that did not allow us to rule out unmeasured confounding. However, we were able to adjust for maternal and child fish intake, which is an important PFAS exposure source in the Faroese population (24). Glomerular filtration rate during pregnancy may also be a relevant confounder not accounted for in our study, as suggested by a 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 in this analysis had somewhat higher PFAS

concentrations than those excluded from it; 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. Furthermore, the Faroese are a relatively homogenous population with respect to socioeconomic status, which may reduce confounding.

Conclusion

Findings from this pilot study suggest an overall 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 than for exposures at later ages and further differed by sex. Although results should be interpreted with caution because of the small sample size, our findings support adipokine hormone dysregulation as a potential mechanistic pathway for adverse health outcomes of PFASs and underscore the need to account for susceptibility windows and sex-dimorphic effects in future investigations.

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Additional Information

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Disclosure Summary: P.G. recently served as a health expert for the State of Minnesota in a lawsuit against a PFAS-producing company. All authors have nothing to declare, financial or otherwise.

Data Availability: Restrictions apply to the availability of data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

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