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Prenatal Exposure to Per- and Polyfluoroalkyl Substances (PFAS) and Cardiometabolic Risk in British Youth

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Prenatal Exposure to Per- and Polyfluoroalkyl Substances (PFAS) and Cardiometabolic Risk in British Youth

By

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Bachelor of Arts
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Abstract

Prenatal Exposure to Per- and Polyfluoroalkyl Substances (PFAS) and Cardiometabolic Risk in British Youth
By Natalie Hurlock

Per- and polyfluoroalkyl substances (PFAS), coined as “forever chemicals”, are man-made chemicals commonly used in household products like nonstick cookware and coatings to make surfaces water and stain resistant. Humans are exposed to low-levels of PFAS in their everyday lives, while some communities face heightened exposure from contaminated drinking water or proximity to manufacturing sites. We investigated associations between serum concentrations of perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorohexane sulfonic acid (PFHxS), and perfluorononanoic acid (PFNA) in pregnant women and eight cardiometabolic biomarkers in their children at age 9. The study population included 553 mother-child dyads from the Avon Longitudinal Study of Parents and Children (ALSPAC). Associations were modeled by multivariable linear regression and adjusted for maternal education, pre-pregnancy BMI, age, and smoking status. PFOS concentrations were the highest and the most consistently associated with cardiometabolic outcomes compared to the other PFAS examined. Among girls, 1-ng/mL higher prenatal PFOA and PFOS concentrations were associated with 3.32% (95% confidence interval (95% CI): 0.04, 6.70) and 0.77% (95% CI: 0.16, 1.39) higher triglycerides, respectively, and 0.5-ng/mL higher prenatal PFNA was associated with 13.77% (95% CI: 0.19, 29.19) higher triglycerides. Prenatal PFOS was associated with higher cholesterol among girls and boys (percent difference (%Δ) = 0.35% (95% CI: 0.14, 0.55) among girls and %Δ = 0.29% (95% CI: 0.02, 0.56) among boys). Lastly, prenatal PFAS were consistently associated with lower adiponectin levels among boys (%Δ associated with 1-ng/mL higher prenatal PFOA = -4.42% (95% CI: -7.63, -1.11) and %Δ associated with 1-ng/mL higher prenatal PFOS = -0.78% (95% CI: -1.53, -0.03), %Δ associated with 0.5-ng/mL higher prenatal PFNA = -20.43% (95% CI: -31.32, -7.82)). Overall, in this subsample of 9-year-old British boys and girls, findings suggest that PFAS are positively associated with triglycerides and cholesterol and inversely associated with adiponectin, varying by sex. There was little evidence of associations between PFAS and HDL, LDL, HbA1c, and IL-6 in the present subsample and all associations with CRP were null.

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Introduction

Childhood obesity is a pressing public health issue and has reached epidemic levels in both developing and developed countries.[1,2] The consequences are vast and burdensome as overweight and obese children are more likely to be overweight in adulthood and face an increased risk of developing chronic diseases at younger ages.[3] Childhood obesity is driven by social and environmental factors and is a primary determinant and predictor of cardiometabolic risk in adulthood.[4,5] Cardiometabolic risk factors are those commonly associated with increased risk of cardiovascular disease and type 2 diabetes and can be used in primary prevention efforts to target individuals at risk and reverse negative health outcomes.[4] Traditional cardiometabolic risk factors include age, race, sex, family history, hypertension, dysglycemia, dyslipidemia, and smoking, while emerging research establishes the importance of abdominal obesity, insulin resistance, inflammation, diet, physical activity, and psychosocial stress.[6] These risk factors are interrelated and interact through metabolic pathways in the body. Emerging evidence suggests environmental exposures such as man-made endocrine disrupting chemicals (EDCs) may predispose individuals to excess weight gain and adverse health effects by altering metabolic pathways and disrupting glucose and lipid homeostasis.[7,8]

Per- and polyfluoroalkyl substances (PFAS) are a class of EDCs used to make protective coatings on household products such as carpet, furniture, food packaging, and nonstick cookware.[9] Prevalent in our everyday environments, exposure to PFAS is widespread and pervasive. PFAS commonly detected in human serum samples include perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorohexane

sulfonic acid (PFHxS), and perfluorononanoic acid (PFNA). PFAS were detected in 97–100% of serum samples collected from Americans who participated in the 2011-2012 National Health and Nutrition Examination Survey (NHANES).[10] In addition to their widespread prevalence, PFAS do not readily break down and therefore, bioaccumulate in the environment and body. To depict the persistent nature of these synthetic chemicals, half-life estimates in human blood are 2.3 to 3.8 years for PFOA, 5.4 years for PFOS, 8.5 years for PFHxS, and 2.5 to 4.3 years for PFNA.[11-15] Alarmingly, PFAS have also been detected in umbilical cord blood and amniotic fluid of pregnant women.[16] This indicates prenatal exposure during critical periods of development and potential for negative long-term health effects.[17]

Existing epidemiologic studies have linked prenatal exposure to PFAS to adiposity measured by body mass index (BMI), waist circumference, and percent body fat, while few studies have investigated the relationship between prenatal PFAS exposure and blood lipid and glucose levels.[7,8,18,19] Evidence suggests positive associations between prenatal PFAS and cholesterol and low-density lipoprotein (LDL) concentrations, but overall trends are inconsistent due to study population characteristics such as age, location, health status, and PFAS exposure level.[9,17,20-23] Results also vary by each PFAS studied. To our knowledge, no studies to date have investigated the relationship between PFAS and inflammatory biomarkers like C-reactive protein (CRP) and interleukin-6 (IL-6) in adults or adolescents.

The current study uses data from the Avon Longitudinal Study of Parents and Children (ALSPAC) to explore the relationship between maternal serum concentrations of PFOA, PFOS, PFHxS, and PFNA during pregnancy and cardiometabolic risk in

children at age 9 measured by blood levels of triglycerides, cholesterol, high-density lipoprotein (HDL), LDL, adiponectin, hemoglobin A1c (HbA1c), CRP, and IL-6.

Methods

Study Population

The Avon Longitudinal Study of Parents and Children (ALSPAC) recruited 14,541 pregnant women whose expected delivery dates were between April 1, 1991 and December 31, 1992 from the Avon region in Great Britain. Participating mothers and children completed surveys and participated in clinical assessments. Details on study recruitment and ALSPAC methodology are described elsewhere.[24,25]

The present study uses a subset of data from the ALSPAC cohort to examine the relationship between prenatal exposure to PFAS and cardiometabolic risk factors at age 9. Data used in the present study were originally obtained to study associations between prenatal exposure to EDCs and growth and development in children. A nested case-control identified 448 mother-daughter dyads to study the association between prenatal exposure to EDCs and age at menarche among daughters.[26] Cases (n=218) included girls who had early menarche, defined as menarche before 11.5 years of age, and controls (n=230) included girls who had menarche at 11.5 years of age or after. In addition, 457 mother-son dyads from the ALSPAC cohort were identified in such a way as to maximize data on puberty and dual energy X ray absorptiometry (DXA) scans to measure body composition. This subset included sons who completed two or more puberty questionnaires before age 13 and had two or more DXA scans. Among the total sample of 905 dyads available for the present study, 252 mother-daughter dyads and 301 mother-

son dyads had complete data for maternal serum concentrations of PFAS and cardiometabolic risk factors at age 9, and this is the final subset used for analyses (Supplementary Figure 1).

Please note that the study website contains details of all the data that is available through a fully searchable data dictionary and variable search tool (http://www.bristol.ac.uk/alspac/researchers/our-data/). Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. Consent for biological samples has been collected in accordance with the Human Tissue Act (2004). Lastly, informed consent for the use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee at the time.

Exposure assessment

Maternal serum concentrations of PFAS were analyzed to assess prenatal exposure to PFAS. The following PFAS were examined in this analysis: PFOA, PFOS, PFHxS, and PFNA. Maternal serum samples were collected at median 19 weeks gestation (IQR: 11-33 weeks gestation) and stored at the University of Bristol. Maternal serum samples were transferred to the CDC's National Center for Environmental Health (NCEH) in the United States for analysis. Samples were analyzed by isotope dilution high-performance liquid chromatography-tandem mass spectrometry.[27,28] Among mothers of daughter, limits of detection were 0.10 ng/mL for PFOA and PFHxS, 0.20 ng/mL for PFOS, and 0.082 ng/mL for PFNA. Among mothers of sons, limits of detection were 0.10 ng/mL for all four PFAS analyzed. The four PFAS of interest were detected in all samples analyzed with the exception of one PFHxS and one PFNA sample

from mothers of daughters that were below the limit of detection and excluded from analyses.

Outcome assessment

Non-fasting blood samples were collected from ALSPAC children who attended a clinical assessment at age 9 and were immediately centrifuged and stored at -80°C. Samples were analyzed after median 7.5 years in storage and had not been previously thawed. Lipid assays were analyzed using a modification of the standard Lipid Research Clinics Protocol with enzymatic reagents, and LDL levels were calculated using the Friedewald equation. HbA1c was analyzed using a Menarini HA 8140 auto-analyzer. Measures of HbA1c were expressed as a percentage of total hemoglobin and were only available for a smaller subset of participants. Adiponectin and IL-6 biomarkers were measured by ELISA (R&D Systems, Abingdon, UK), and CRP was measured by automated particle-enhanced immunoturbidimetric assay (Roche, UK). All assay coefficients of variation (CV) were less than 5%.[29-31]

Covariates

Potential confounders were identified a priori based on previous literature and biological plausibility. The following covariates were considered: maternal race (white/non-white), maternal education (categorized as < O-level, O-level, > O-level where O-level is the ordinary level of education required at age 16), pre-pregnancy BMI (kg/m^2), maternal age at delivery (years), smoking during pregnancy (any/none), alcohol use during the first 1-3 months of pregnancy (any/none), and parity (nulliparous/multiparous). Maternal age was recorded by clinical staff and all other covariates were self-reported by mothers during pregnancy or shortly after giving birth.

We avoided covariates thought to be on the causal pathway between PFAS and cardiometabolic outcomes, such as child's BMI and diet.

Statistical analyses

All exploratory and statistical analyses were performed in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Descriptive analyses were performed for PFAS and cardiometabolic outcome variables, and we examined levels of PFAS across each covariate. Triglycerides, CRP, and IL-6 were severely right-skewed and all other outcome variables were approximately normal. All outcome variables were natural log-transformed for continuity in interpretation and R-square statistics were slightly higher when outcomes were log-transformed. Multivariable linear regression models were used to examine the association between maternal serum concentrations of PFAS during pregnancy and cardiometabolic risk factors in children at age 9. Final models were adjusted for maternal education, pre-pregnancy BMI, age, and smoking during pregnancy. Pre-pregnancy BMI and maternal age were included in models as continuous variables, and maternal education and smoking were included as categorical variables. All exposure and outcome variables were modeled as continuous variables. Alcohol consumption did not confound associations according to the 10% change in estimate method and therefore, was not included in the final models.[32] Boys and girls were analyzed separately due to differences in sampling methods. The subset of mother-daughter dyads used in the present analysis was weighted to account for the nested case-control study design and adjust for under-representation of the true number of girls without early menarche. The weight for cases (girls with menarche < 11.5 years) was 1, and the weight for controls (girls with menarche ≥ 11.5 years) was 15.1.[33] Percentage

differences in cardiometabolic outcome variables associated with 1-ng/mL higher PFOA, PFOS, and PFHxS were calculated as $\%\Delta = (\exp(\beta)-1) \times 100$, and percentage differences in cardiometabolic outcome variables associated with 0.5-ng/mL higher PFNA were calculated as $\%\Delta = (\exp(\beta \times 0.5)-1) \times 100$. An alpha level of 0.05 presented with a 95% confidence interval (95% CI) was used to determine significance for all statistical tests.

Results

Characteristics of the study population

Mothers included in the study population (N=553) were predominantly white (95.8%) and received ordinary levels of education or higher (79.2%). The majority of mothers had a normal pre-pregnancy BMI (69.8%) and were at least 25 years old at delivery (84.6%). Few reported smoking during pregnancy (12.8%), meanwhile over half of participating mothers reported drinking alcohol during the first 1-3 months of pregnancy (55.5%). Lastly, about half of the mothers had given birth before (50.6%) (Table 1). Of the four PFAS analyzed, PFOS concentrations were the highest while PFNA concentrations were the lowest and very narrow in range (Table 1). PFAS and covariate distributions among the 553 dyads with complete data did not vary greatly from the original sample of 905 dyads (Supplementary Table 1). In this subset of ALSPAC children, median values of cardiometabolic biomarkers were slightly higher in girls compared to boys for all outcome variables except HDL (Table 2).

PFAS & Lipids

Associations between PFOA, PFOS, and PFNA and lipid outcomes varied. In contrast, all associations between PFHxS and lipid outcomes were null. PFOA, PFOS,

and PFNA were positively associated with triglycerides among girls but not boys. For girls, 1-ng/mL increases in maternal serum PFOA and PFOS were associated with 3.32% (95% CI: 0.04, 6.70) and 0.77% (95% CI: 0.16, 1.39) higher triglycerides, respectively. A 0.5-ng/mL increase in PFNA was associated with 13.77% (95% CI: 0.19, 29.19) higher triglycerides among girls.

PFOS was positively associated with cholesterol among girls and boys. A 1-ng/mL increase in PFOS was associated with 0.35% (95% CI: 0.14, 0.55) higher cholesterol among girls and 0.29% (95% CI: 0.02, 0.56) higher cholesterol among boys. In addition, a 1-ng/mL increase in PFOA was associated with 1.25% (95% CI: 0.02, 2.48) higher cholesterol among boys. A 0.5-ng/mL increase in PFNA was associated with 5.43% (95% CI: 0.90, 10.16) higher cholesterol among girls.

Weak associations between PFOS and HDL and LDL were observed among girls only. A 1-ng/mL increase in PFOS was associated with 0.33% (95% CI: 0.01, 0.65) higher HDL and 0.37% (95% CI: 0.01, 0.74) higher LDL among girls.

PFAS & Glucose Regulation

Evidence of an inverse association between PFAS and adiponectin is consistent among boys but not girls. For boys, a 1-ng/mL increases in PFOA and PFOS were associated with 4.42% (95% CI: -7.63, -1.11) and 0.78% (95% CI: -1.53, -0.03) lower adiponectin. Additionally, a 0.5-ng/mL increase in PFNA was associated with 20.43% (95% CI: -31.32, -7.82) lower adiponectin among boys. Contradictory to this trend, a 1-ng/mL increase in PFOS was weakly associated with 0.57% (95% CI: 0.03, 1.11) higher adiponectin among girls.

In this analysis, evidence of a relationship between PFAS and HbA1c was weak. A 1-ng/mL increase in PFHxS was associated with 0.27% (95% CI: 0.02, 0.51) higher HbA1c among girls, while all other associations were null.

PFAS & Inflammation

Associations between PFAS and CRP were null among girls and boys. Associations between PFAS and interleukin-6 were mostly null but indicate an inverse relationship. A 0.5-ng/mL increase in PFNA was associated with 21.58% (95% CI: -38.43, -0.10) lower IL-6 among girls, and a 1-ng/mL increase in PFHxS was associated with 1.89% (95% CI: -3.71, -0.03) lower IL-6 among boys.

Discussion

We hypothesized that maternal serum concentrations of PFAS during pregnancy would be associated with higher cardiometabolic risk in children at age 9 indicated by higher triglyceride, cholesterol, LDL, HbA1c, CRP, and IL-6 and lower HDL and adiponectin concentrations. After adjusting for confounders in multivariable regression analyses, results from the present study support positive associations with triglycerides and cholesterol and an inverse association with adiponectin, varying by sex. Among the PFAS evaluated, PFOS was most consistently associated with cardiometabolic outcomes, while PFHxS was the least.

Evidence of the relationship between prenatal PFAS and cholesterol is consistent with the literature, while triglyceride and LDL results diverge from the existing literature. A nationally representative cross-sectional study of 815 US adolescent participants in the National Health and Nutrition Examination Survey (NHANES) from 1999-2008 analyzed

boys and girls together and observed positive associations between serum concentrations of PFOA and PFOS and total cholesterol and LDL and null associations with HDL and triglyceride levels.[21] These results using NHANES data are consistent with those from a study that used a small subset of 111 ALSPAC mother-daughter dyads to examine prenatal exposure to PFOA and PFOS and blood lipids at age 7.[17] In the present study, associations between prenatal exposure to PFAS and LDL were largely null. In addition, prenatal PFAS was positively associated with triglycerides among girls in the present subsample. Several cohort studies have determined that cardiometabolic risk factors in childhood are best measured at or after age 9, which could explain the difference in results from the previous ALSPAC study.[34]

Trends in associations between early PFAS exposure and glucose regulation are inconsistent in the literature and have been shown to vary among adolescents and adults.[9,19,22,35] The inverse relationship observed between prenatal PFAS and adiponectin among ALSPAC boys in the present study adds to the literature and agrees with our hypothesis.

To our knowledge, the relationship between prenatal PFAS exposure and inflammatory biomarkers has not been previously studied in adult or adolescent populations. IL-6 is synthesized at the initial stage of inflammation and triggers release of CRP into the bloodstream.[36] Therefore, inverse associations between prenatal PFAS and IL-6 observed in the present study could indicate a disruption in immune response and could in turn, explain null associations with CRP.

While the difference in sampling methods between girls and boys could affect results, variation by sex is also consistent with previous literature. A large US birth

cohort of about 1,000 pregnant women and offspring with follow-up at median age 7.7 observed positive associations between prenatal PFOA, PFOS, PFHxS, and PFNA and BMI, waist circumference, and total body fat among girls only.[8] Similarly, a prospective Danish cohort of 665 pregnant women and offspring with follow-up at age 20 observed positive associations between maternal serum concentrations of PFOA during pregnancy and BMI and waist circumference among female but not male offspring.[19]

Experimental studies have evaluated several potential mechanisms through which PFAS may affect weight gain and cardiometabolic risk. First, PFAS are hypothesized to alter gene expression of peroxisome proliferator-activated receptors, regulators of lipid and lipoprotein metabolism. Through this mechanism, PFAS could elevate blood lipids and prompt predisposition to excess weight gain and hypertension. [8,9,20] In addition, PFAS have endocrine disrupting properties which can explain their influence on glucose regulation, immune response, and the difference in effect between males and females since the endocrine system regulates a network of glands and organs through hormones released in the bloodstream. [37,38]

Definitions for metabolic syndrome and cardiometabolic risk factors have been adapted for children, but there is less consensus because complications such as pubertal insulin resistance and lipid variation by age and race make it difficult to define what abnormal and normal levels are.[4,39,40] The World Health Organization (WHO), the European Group for the Study of Insulin Resistance (EGIR), the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III), and the International Diabetes Foundation (IDF) offer definitions of metabolic syndrome that include the following key components: abdominal obesity measured by waist circumference,

hyperglycemia measured by fasting glucose or insulin resistance, dyslipidemia measured by abnormal triglyceride and HDL levels only, and hypertension.[41] Therefore, there is less consensus but growing interest in LDL, CRP, IL-6, adiponectin, and HbA1c because these biomarkers have been tracked from childhood into adulthood and are known predictors of cardiometabolic outcomes.[5,42]

According to the American Heart Association, abnormal lipid levels in mg/dL for children 10-19 years of age are characterized as ≥130 triglycerides (≥100 for 0-9 years of age), ≥200 total cholesterol, <40 HDL, and ≥130 LDL.[43] Using receiver operator characteristic curves (ROC curves), low adiponectin levels have been classified as below 5.5 and 5.75 μg/mL in predicting metabolic syndrome in at-risk adults.[44,45] A European study aimed to establish reference values for adiponectin in normal weight children ages 3-9 years old from the Identification and prevention of Dietary- and lifestyle-induced health EFfects in Children and infantS (IDEFICS) cohort, and 3rd, 50th, and 97th percentiles for adiponectin at age 8-8.9 years old were 3.64, 10.11, and 17.43 μg/mL among girls and 3.53, 8.60, 17.15 μg/mL among boys. Adiponectin levels in overweight and obese children were slightly lower.[46] The American Diabetes Association establishes an HbA1c reading of less than 5.7% as normal, 5.7% to 6.4% as prediabetic, and 6.5% or higher as diabetic in youth and adults.[47] Lastly, cutoffs for abnormal CRP and IL-6 levels in children are not well documented in the literature, but elevated CRP and IL-6 levels associated with cardiovascular events in adults have been classified as ≥2 mg/L and ≥5 pg/mL, respectively.[48,49]

Given this context, generally all median cardiometabolic outcomes in the present cohort meet normal levels by standards discussed in the literature and summarized above

(Table 2). Median triglyceride levels could be considered borderline, meanwhile the percentages of participants with abnormal cholesterol, HDL, and LDL levels were less than 15%. In addition, overall median adiponectin levels were 11.77 (IQR: 8.64-15.58) μg/mL, and median HbA1c measured as a percentage was 4.90 (IQR: 4.80-5.10). A small percentage of the present cohort (5.8%) had adiponectin levels below 5.75 μg/mL, and only 3 participants (<1%) had abnormal HbA1c levels. Lastly, overall median CRP and IL-6 levels were very low as we would expect to see in children. Only 6.7% and 2.2% of the present cohort had elevated CRP and IL-6 levels, respectively (Supplementary Table 2). Therefore, we expected to detect very small associations due to the generally healthy cardiometabolic profile and age of this cohort. While it may be difficult to discern the biological significance of a less than 5% difference in blood lipids or glucose-regulating proteins, it is plausible that small changes in cardiometabolic predictors over time may lead to clinical health effects, and a small change at the individual level may have implications at the population level, especially when the exposure is prevalent.[50,51] In addition, a less than 5% difference in biomarkers that reflect long-term levels rather than tightly controlled and fluctuating biomarkers may be clinically significant even while modest. For example, clinicians interpret a 0.5% difference in HbA1c, a marker of one's average blood glucose levels over the last 2-3 months, as a significant change in glycemic control.[52]

Strengths of this study include prospective study design and reliable biological measures of four PFAS and eight cardiometabolic biomarkers, but there are some potential limitations as well. First, generalizability of results is limited due to the demographics and geography of the overall ALSPAC cohort, being mainly white and

from the UK. In addition, the subsample of women included in our analyses were more likely to be highly educated and non-smokers compared to the overall ALSPAC cohort.[53] Inclusion criteria for this subsample also required children to complete at least 2 puberty questionnaires and boys, additionally, completed at least two DXA scans. This shows a level of engagement and motivation that may differ from the general cohort as loss to follow-up is generally more common among individuals who are of lower socioeconomic status and less healthy.[54] In addition, boys may have more complete data than girls because they were already in clinic for their DXA scan and were able to conveniently give a blood sample at age 9 when cardiometabolic outcomes were being analyzed.

There is also potential for bias due to self-reported and missing covariate data. While women may be more honest about alcohol consumption in the first 1-3 months of pregnancy because they may not have known they were pregnant yet, smoking and alcohol use during pregnancy are commonly under-reported behaviors. In addition, educational status was used as a proxy for socioeconomic status because many women did not report their income. Socioeconomic status is an important indicator of environmental factors related to increased exposure to PFAS such as housing, contaminated water, and occupation and increased risk of adverse childhood experiences that could raise cardiometabolic risk.[55] Thus, residual confounding may exist by socioeconomic status, but the effect size is likely to be small.[56]

Blood samples from children at age 9 were taken non-fasting, but recent practice with support from large prospective studies has shifted towards taking non-fasting samples because lipids and lipoproteins change minimally during a normal day, and both non-fasting and fasting samples are effective predictors of cardiovascular outcomes.[43,57]

In addition, we hypothesize that fasting status would not influence prenatal exposure to PFAS and thus, not confound the relationship between prenatal PFAS and blood lipid levels at age 9.

Conclusions

This study adds to the literature on prenatal PFAS and cardiometabolic risk factors, especially CRP, IL-6, and HbA1c that had not been studied before. Specifically, associations observed among this British cohort provide evidence for the risk of low-level everyday exposure to PFAS on cardiometabolic profiles among healthy children whereas cardiometabolic risk factors have been commonly studied among at-risk populations or cohorts of children who are overweight or obese. Results are modest among this subset from the ALSPAC cohort but are consistent with results from previous studies that also observed variation by sex and PFAS specific results. In conclusion, we observed positive associations between prenatal PFAS and cholesterol among girls and boys and triglycerides among girls only, and an inverse association with adiponectin among boys only.

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Tables

Table 1. Characteristics of subsample of mothers (N=553) from the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort and distribution of maternal serum concentrations of perfluoroalkyl substances (ng/mL) across covariates.

Characteristic	Frequency[a] n (%)	PFOA Median (IQR)	PFOS Median (IQR)	PFHxS Median (IQR)	PFNA Median (IQR)
Mothers of daughters (N = 252)					
Overall	252 (100)	3.7 (2.8-4.7)	19.4 (15.2-24.7)	1.6 (1.2-2.1)	0.5 (0.4-0.7)
Maternal race	n = 244				
White	238 (97.5)	3.8 (2.9-4.7)	19.8 (15.3-24.8)	1.6 (1.2-2.1)	0.5 (0.4-0.7)
Non-white	6 (<5)	2.6 (2.2-2.9)	16.1 (14.5-19.2)	1.6 (1.3-1.7)	0.5 (0.3-0.7)
Maternal education[b]	n = 242				
< O-level	41 (16.9)	3.6 (2.8-4.5)	18.0 (14.9-22.8)	1.6 (1.3-2.2)	0.5 (0.3-0.6)
O-level	79 (32.6)	3.6 (2.8-5.0)	19.7 (15.3-25.8)	1.5 (1.1-2.1)	0.5 (0.4-0.7)
> O-level	122 (50.4)	3.9 (2.8-4.7)	20.3 (15.2-25.0)	1.6 (1.2-2.1)	0.5 (0.4-0.7)
Pre-pregnancy BMI, kg/m2	n = 235				
< 18.5 (underweight)	10 (4.3)	3.5 (2.8-4.8)	15.5 (12.4-26.8)	1.5 (0.9-2.0)	0.5 (0.3-0.6)
18.5 - 24.99 (normal weight)	167 (71.1)	3.8 (2.8-4.7)	19.8 (15.3-25.1)	1.6 (1.2-2.1)	0.5 (0.4-0.7)
25-29.99 (overweight)	39 (16.6)	3.5 (2.9-4.7)	19.8 (17.1-26.2)	1.7 (1.4-2.2)	0.6 (0.4-0.7)
>= 30 (obese)	19 (8.1)	3.1 (2.6-4.3)	17.4 (13.1-21.1)	1.3 (1.2-1.7)	0.4 (0.3-0.6)
Maternal age at delivery, years	n = 250				
< 25	48 (19.2)	3.9 (3.1-4.8)	19.7 (13.9-23.5)	1.5 (1.2-2.1)	0.5 (0.4-0.6)
25 - 29	90 (36.0)	3.6 (2.9-4.7)	19.1 (15.0-24.8)	1.6 (1.1-2.0)	0.5 (0.4-0.7)
>= 30	112 (44.8)	3.7 (2.7-4.6)	19.6 (15.7-25.6)	1.7 (1.3-2.4)	0.5 (0.4-0.7)
Smoking during pregnancy	n = 238				
No	194 (81.5)	3.8 (2.8-4.7)	20.0 (15.4-25.1)	1.6 (1.2-2.1)	0.5 (0.4-0.7)
Yes	44 (18.5)	3.4 (2.7-4.6)	17.3 (13.6-22.0)	1.7 (1.3-2.5)	0.4 (0.3-0.6)
Alcohol during pregnancy	n = 240				
No	110 (45.8)	3.7 (2.8-4.7)	19.3 (14.9-24.6)	1.6 (1.2-2.1)	0.5 (0.4-0.6)
Yes	130 (54.2)	3.8 (2.9-4.6)	19.8 (15.4-24.8)	1.6 (1.2-2.2)	0.6 (0.4-0.7)
Parity	n = 236				
Nulliparous	113 (47.9)	4.5 (3.6-5.2)	21.4 (17.5-25.6)	1.6 (1.4-2.1)	0.6 (0.5-0.7)
Multiparous	123 (52.1)	3.1 (2.4-3.8)	18.0 (14.2-23.7)	1.5 (1.1-2.1)	0.5 (0.3-0.6)
Mothers of sons (N = 301)					
Overall	301 (100)	3.0 (2.3-3.8)	13.8 (10.9-17.7)	1.8 (1.3-2.4)	0.4 (0.3-0.4)

Maternal race	n = 294				
White	292 (99.3)	3.0 (2.3-3.8)	13.9 (10.9-17.9)	1.9 (1.4-2.4)	0.3 (0.3-0.4)
Non-white	2 (<5)	4.2 (2.1-6.3)	14.2 (12.7-15.6)	2.7 (1.3-4.1)	0.3 (0.2-0.3)
Maternal education[b]	n = 296				
< O-level	59 (19.9)	2.8 (2.4-3.6)	14.1 (11.0-17.7)	1.8 (1.4-2.3)	0.4 (0.3-0.5)
O-level	98 (33.1)	3.1 (2.1-3.8)	15.0 (12.0-19.1)	1.8 (1.3-2.2)	0.4 (0.3-0.4)
> O-level	139 (47.0)	3.0 (2.3-3.9)	13.6 (10.6-17.1)	1.9 (1.4-2.5)	0.3 (0.2-0.4)
Pre-pregnancy BMI, kg/m2	n = 281				
< 18.5 (underweight)	11 (3.9)	3.0 (2.2-4.1)	13.2 (10.1-19.2)	1.6 (1.2-2.4)	0.3 (0.2-0.4)
18.5 - 24.99 (normal weight)	219 (77.9)	3.0 (2.3-3.8)	14.0 (11.0-18.0)	1.9 (1.4-2.5)	0.4 (0.3-0.5)
25-29.99 (overweight)	40 (14.2)	2.8 (2.3-4.0)	13.6 (11.1-17.1)	2.0 (1.3-2.5)	0.3 (0.3-0.4)
>= 30 (obese)	11 (3.9)	3.2 (2.8-3.9)	14.7 (10.2-18.0)	1.6 (1.1-2.1)	0.3 (0.3-0.4)
Maternal age at delivery, years	n = 300				
< 25	34 (11.3)	3.0 (2.2-3.9)	12.8 (10.6-18.0)	1.7 (1.1-2.0)	0.4 (0.2-0.5)
25 - 29	121 (40.3)	3.2 (2.4-3.8)	14.2 (12.0-18.4)	1.8 (1.3-2.2)	0.4 (0.3-0.5)
>= 30	145 (48.3)	2.9 (2.2-3.7)	13.7 (10.7-17.1)	1.9 (1.5-2.5)	0.3 (0.2-0.4)
Smoking during pregnancy	n = 291				
No	264 (90.7)	3.0 (2.3-3.8)	14.0 (10.9-17.7)	1.8 (1.3-2.3)	0.3 (0.3-0.4)
Yes	27 (9.3)	3.1 (2.5-3.7)	13.2 (11.0-18.5)	2.2 (1.7-2.8)	0.4 (0.3-0.5)
Alcohol during pregnancy	n = 297				
No	120 (40.4)	3.0 (2.2-3.8)	13.9 (10.6-17.7)	1.8 (1.3-2.2)	0.4 (0.3-0.5)
Yes	177 (59.6)	3.0 (2.4-3.7)	13.7 (11.5-18.0)	1.9 (1.4-2.5)	0.4 (0.3-0.4)
Parity	n = 293				
Nulliparous	136 (46.4)	3.5 (2.7-4.2)	14.3 (11.6-18.0)	2.0 (1.4-2.6)	0.4 (0.3-0.5)
Multiparous	157 (53.6)	2.6 (2.1-3.3)	13.6 (10.8-16.9)	1.8 (1.3-2.2)	0.3 (0.2-0.4)

Abbreviations: PFOA perfluorooctanoic acid; PFOS perfluorooctane sulfonic acid; PFHxS perfluorohexane sulfonic acid; PFNA perfluorononanoic acid; IQR interquartile range.

[a] Covariate information was missing among mothers of daughters and sons, including race (n=15, 2.7%), education (n=15, 2.7%), pre-pregnancy BMI (n=37, 6.7%), age at delivery (n=3, 0.5%), smoking status during pregnancy (n=24, 4.3%), alcohol consumption during pregnancy (n=22, 4.0%), and parity (n=24, 4.3%).

[b] Defined by highest achieved qualification, where O-levels (ordinary levels) are required and completed at the age of 16.

Table 2. Distribution of cardiometabolic outcomes in subsample of 9-year-old British boys and girls from the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort (N=553).

Cardiometabolic Outcome	Median (IQR) *Overall (n=553)*	Median (IQR) *Girls (n=252)*	Median (IQR) *Boys (n=301)*
Triglycerides (mg/dL)	87.68 (65.54-125.77)	93.88 (69.97-129.31)	84.14 (63.77-124.00)
Cholesterol (mg/dL)	161.64 (145.79-178.27)	164.73 (145.40-182.52)	158.93 (146.17-174.79)
HDL (mg/dL)	52.20 (45.24-59.94)	50.27 (42.15-58.01)	53.36 (46.79-61.87)
LDL (mg/L)	87.01 (75.37-103.80)	92.57 (77.20-107.46)	84.53 (74.73-99.38)
Adiponectin ($\boldsymbol{\mu}$g/mL)	11.77 (8.64-15.58)	11.97 (8.42-15.31)	11.48 (8.78-15.74)
Hemoglobin A1c (%)	4.90 (4.80-5.10)	5.0 (4.80-5.10)	4.90 (4.70-5.10)
C-reactive protein (mg/L)	0.21 (0.11-0.52)	0.30 (0.16, 0.69)	0.16 (0.10-0.37)
Interleukin-6 (pg/mL)	0.83 (0.50-1.52)	0.96 (0.59, 1.72)	0.73 (0.44-1.28)

Table 3. Percent differences (95% CI) in cardiometabolic outcomes associated with 1-ng/mL increase in PFAS adjusted for maternal education, pre-pregnancy BMI, age, and smoking status.

		PFOA		PFOS		PFHxS		PFNA[a]	
Cardiometabolic outcomes	*n*	% Difference (95% CI)	*p*-value	% Difference (95% CI)	*p*-value	% Difference (95% CI)	*p*-value	% Difference (95% CI)	*p*-value
Triglycerides (mg/dL)									
Girls	226	3.32 (0.04, 6.70)	0.048*	0.77 (0.16, 1.39)	0.01*	0.29 (-0.87, 1.47)	0.31	13.77 (0.19, 29.19)	0.047*
Boys	273	2.32 (-1.41, 6.20)	0.23	0.53 (-0.29, 1.36)	0.21	0.45 (-0.42, 1.32)	0.62	15.26 (-1.87, 35.39)	0.08
Cholesterol (mg/dL)									
Girls	226	0.51 (-0.61, 1.65)	0.37	0.35 (0.14, 0.55)	0.001*	0.29 (-0.11, 0.70)	0.16	5.43 (0.90, 10.16)	0.02*
Boys	273	1.25 (0.02, 2.48)	0.046*	0.29 (0.02, 0.56)	0.03*	0.20 (-0.09, 0.48)	0.18	-0.14 (-5.31, 5.31)	0.96
HDL (mg/dL)									
Girls	226	-0.49 (-2.17, 1.21)	0.57	0.33 (0.01, 0.65)	0.045*	0.37 (-0.23, 0.98)	0.23	0.47 (-6.04, 7.42)	0.89
Boys	273	1.57 (-0.45, 3.63)	0.13	0.18 (-0.27, 0.62)	0.44	-0.07 (-0.54, 0.40)	0.75	-0.73 (-9.04, 8.33)	0.87
LDL (mg/dL)									
Girls	226	1.07 (-0.86, 3.04)	0.28	0.37 (0.01, 0.74)	0.046*	0.36 (-0.34, 1.06)	0.31	7.54 (-0.31, 16.00)	0.06
Boys	273	0.86 (-1.17, 2.94)	0.41	0.30 (-0.16, 0.75)	0.20	0.22 (-0.26, 0.70)	0.37	-3.74 (-11.90, 5.18)	0.40
Adiponectin (ng/ml)									
Girls	226	-0.06 (-2.87, 2.83)	0.97	0.57 (0.03, 1.11)	0.04*	0.54 (-0.48, 1.58)	0.30	-1.79 (-12.23, 9.88)	0.75
Boys	272	-4.42 (-7.63, -1.11)	0.01*	-0.78 (-1.53, -0.03)	0.04*	-0.51 (-1.30, 0.29)	0.21	-20.43 (-31.32, -7.82)	0.003*
Hemoglobin A1c (%)									
Girls	82	0.16 (-0.52, 0.85)	0.64	0.06 (-0.03, 0.16)	0.20	0.27 (0.02, 0.51)	0.03*	1.70 (-1.12, 4.61)	0.24
Boys	138	-0.10 (-0.81, 0.61)	0.78	0.15 (-0.02, 0.32)	0.08	0.10 (-0.02, 0.23)	0.11	2.77 (-0.70, 6.37)	0.12
C-reactive protein (mg/l)									

Girls	226	0.43 (-7.97, 9.59)	0.92	0.38 (-1.26, 2.05)	0.65	2.39 (-0.77, 5.66)	0.14	19.14 (-15.48, 67.95)	0.32
Boys	273	-1.62 (-11.14, 8.93)	0.75	-0.16 (-2.39, 2.11)	0.89	1.59 (-0.78, 4.03)	0.19	-6.52 (-39.93, 45.47)	0.76
Interleukin 6 (pg/ml)									
Girls	226	0.36 (-5.67, 6.77)	0.91	-0.92 (-2.06, 0.24)	0.12	-0.99 (-3.18, 1.24)	0.38	-21.58 (-38.43, -0.10)	0.049*
Boys	271	2.38 (-5.61, 11.04)	0.57	-0.95 (-2.71, 0.83)	0.29	-1.89 (-3.71, -0.03)	0.046*	15.73 (-18.60, 64.54)	0.41

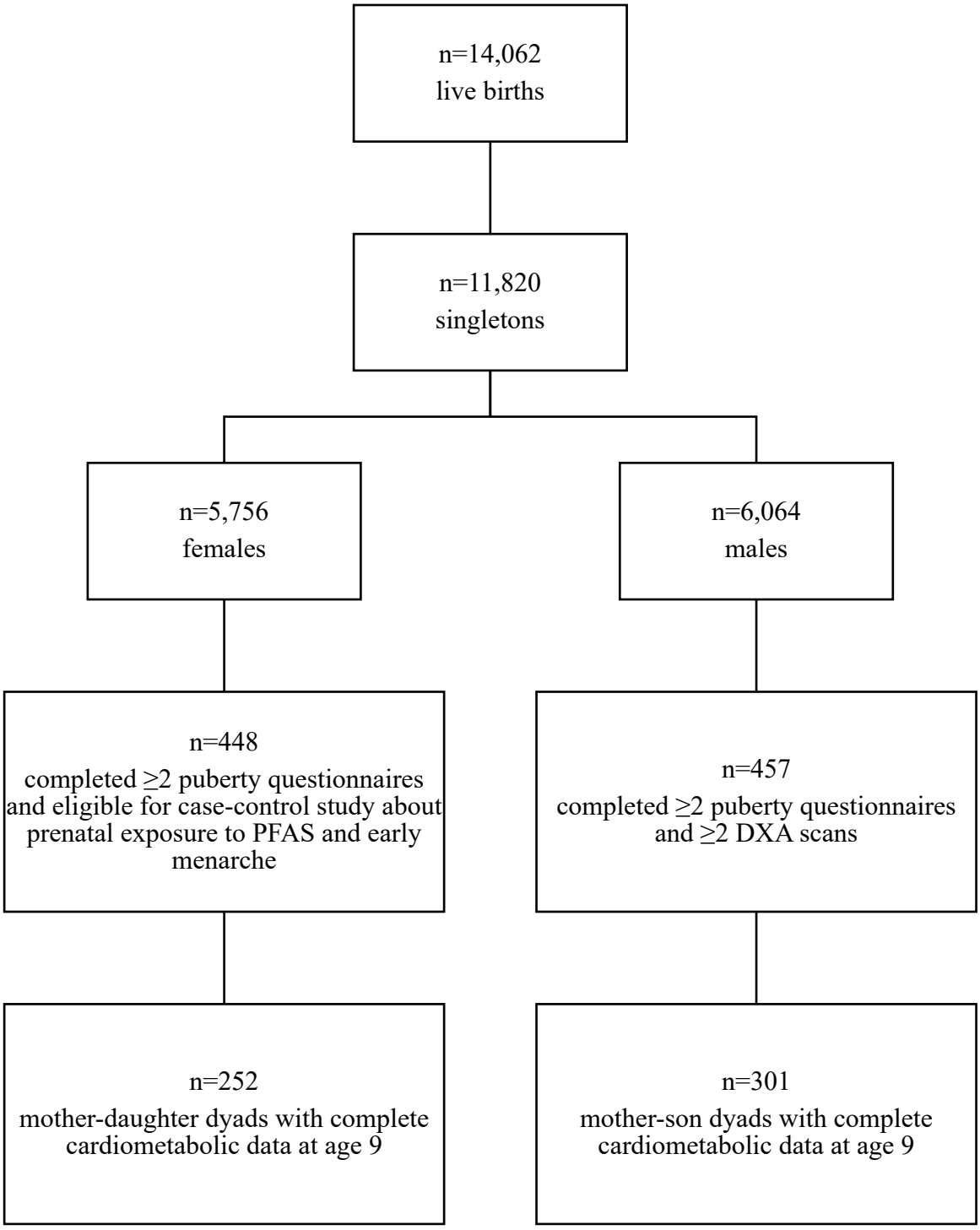
Abbreviations: PFOA perfluorooctanoic acid; PFOS perfluorooctane sulfonic acid; PFHxS perfluorohexane sulfonic acid; PFNA perfluorononanoic acid; % difference percent difference; 95% CI 95% confidence interval

[a] Percent difference (95% CI) in cardiometabolic outcomes associated with 0.5-ng/mL increase in PFNA

* Indicates p-value < 0.05

Figures

Figure 1. Flowchart of eligibility and exclusions.



Appendix

Supplementary Table 1. Characteristics of full subsample of mothers (N=905) from the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort and distribution of maternal serum concentrations of perfluoroalkyl substances (ng/mL) across covariates.

Characteristic	Frequency[a] n (%)	PFOA Median (IQR)	PFOS Median (IQR)	PFHxS Median (IQR)	PFNA Median (IQR)
Mothers of daughters (N = 448)					
Overall	448 (100)	3.7 (2.8-4.8)	19.8 (15.1-24.9)	1.6 (1.2-2.2)	0.5 (0.4-0.7)
Maternal race	n = 431				
White	423 (98.1)	3.8 (2.9-4.8)	19.9 (15.2-25.3)	1.6 (1.2-2.2)	0.5 (0.4-0.7)
Non-white	8 (<5)	2.3 (1.6-2.9)	14.6 (8.1-18.4)	1.4 (0.9-1.7)	0.5 (0.2-0.7)
Maternal education[b]	n = 429				
< O-level	89 (20.8)	3.6 (2.8-4.4)	18.2 (14.9-23.3)	1.6 (1.3-2.2)	0.5 (0.4-0.7)
O-level	140 (32.6)	3.7 (2.9-5.0)	19.6 (15.1-26.0)	1.6 (1.2-2.3)	0.6 (0.4-0.7)
> O-level	200 (46.6)	3.9 (2.8-4.8)	20.4 (15.2-25.3)	1.7 (1.2-2.2)	0.5 (0.4-0.7)
Pre-pregnancy BMI, kg/m2	n = 402				
< 18.5 (underweight)	18 (4.5)	3.5 (2.8-4.7)	16.9 (14.0-22.6)	1.5 (1.2-2.3)	0.5 (0.3-0.6)
18.5 - 24.99 (normal weight)	291 (72.4)	3.8 (2.8-4.8)	20.1 (15.0-25.5)	1.6 (1.2-2.2)	0.5 (0.4-0.7)
25-29.99 (overweight)	62 (15.4)	3.7 (3.2-4.9)	20.9 (17.5-25.6)	1.9 (1.5-2.5)	0.6 (0.4-0.7)
>= 30 (obese)	31 (7.7)	3.6 (2.7-5.0)	19.2 (13.8-23.4)	1.4 (1.2-2.3)	0.6 (0.3-0.7)
Maternal age at delivery, years	n = 445				
< 25	92 (20.7)	3.9 (3.0-4.8)	18.5 (14.1-23.1)	1.6 (1.2-2.1)	0.5 (0.4-0.6)
25 - 29	164 (36.9)	3.8 (3.0-4.9)	20.7 (15.4-25.4)	1.6 (1.2-2.1)	0.6 (0.4-0.7)
>= 30	189 (42.5)	3.6 (2.5-4.6)	19.7 (15.1-25.5)	1.7 (1.2-2.4)	0.5 (0.4-0.7)
Smoking during pregnancy	n = 427				
No	348 (81.5)	3.8 (2.8-4.9)	20.5 (15.4-25.6)	1.6 (1.2-2.2)	0.6 (0.4-0.7)
Yes	79 (18.5)	3.4 (2.9-4.4)	17.2 (13.4-21.4)	1.7 (1.3-2.4)	0.5 (0.3-0.7)
Alcohol during pregnancy	n = 428				
No	194 (45.3)	3.8 (2.8-4.9)	20.2 (15.3-25.3)	1.6 (1.2-2.2)	0.5 (0.4-0.7)
Yes	234 (54.7)	3.7 (2.9-4.7)	19.5 (15.2-24.8)	1.6 (1.2-2.2)	0.6 (0.4-0.7)
Parity	n = 419				
Nulliparous	208 (49.6)	4.4 (3.4-5.4)	21.5 (17.0-26.4)	1.8 (1.4-2.4)	0.6 (0.4-0.7)
Multiparous	211 (50.4)	3.1 (2.4-4.0)	18.2 (14.2-23.7)	1.5 (1.1-2.2)	0.5 (0.3-0.7)
Mothers of sons (N = 457)					

Overall	457 (100)	3.0 (2.3-3.8)	13.8 (11.0-17.7)	1.9 (1.4-2.5)	0.4 (0.3-0.5)
Maternal race	n = 444				
White	441 (99.32)	3.0 (2.3-3.8)	13.9 (11.0-17.9)	1.9 (1.4-2.5)	0.4 (0.3-0.5)
Non-white	3 (<5)	2.1 (2.0-6.3)	12.7 (9.1-15.6)	1.3 (1.0-4.1)	0.2 (0.2-0.3)
Maternal education[b]	n = 446				
< O-level	96 (21.5)	2.8 (2.4-3.6)	13.9 (11.0-17.3)	1.9 (1.5-2.3)	0.4 (0.3-0.5)
O-level	154 (34.5)	3.1 (2.3-3.8)	14.8 (11.9-18.9)	1.8 (1.3-2.3)	0.4 (0.3-0.4)
> O-level	196 (44.0)	3.0 (2.3-3.9)	13.6 (10.7-17.2)	1.9 (1.4-2.5)	0.3 (0.3-0.4)
Pre-pregnancy BMI, kg/m2	n = 430				
< 18.5 (underweight)	15 (3.5)	3.0 (2.2-4.1)	13.2 (10.1-19.2)	1.6 (1.2-2.3)	0.3 (0.2-0.4)
18.5 - 24.99 (normal weight)	324 (75.4)	3.1 (2.3-3.8)	14.1 (11.2-18.0)	1.9 (1.4-2.5)	0.4 (0.3-0.5)
25-29.99 (overweight)	71 (16.5)	2.8 (2.4-3.8)	13.6 (10.9-17.4)	1.8 (1.4-2.5)	0.4 (0.3-0.5)
>= 30 (obese)	20 (4.7)	3.0 (2.5-3.5)	12.2 (10.7-15.2)	1.7 (1.3-2.0)	0.3 (0.3-0.4)
Maternal age at delivery, years	n = 453				
< 25	54 (11.9)	3.0 (2.3-3.7)	12.6 (10.6-16.9)	1.6 (1.1-1.9)	0.4 (0.3-0.4)
25 - 29	188 (41.5)	3.2 (2.5-4.0)	14.1 (11.9-18.8)	1.8 (1.4-2.5)	0.4 (0.3-0.5)
>= 30	211 (46.6)	2.9 (2.2-3.7)	13.9 (10.8-17.3)	1.9 (1.4-2.5)	0.4 (0.3-0.5)
Smoking during pregnancy	n = 441				
No	397 (90.0)	3.0 (2.3-3.8)	14.0 (11.1-17.9)	1.9 (1.4-2.4)	0.4 (0.3-0.5)
Yes	44 (10.0)	3.0 (2.4-3.6)	13.2 (11.0-17.2)	2.0 (1.7-2.6)	0.4 (0.3-0.5)
Alcohol during pregnancy	n = 412				
No	197 (44.0)	3.0 (2.3-3.8)	13.9 (10.6-17.7)	1.8 (1.4-2.4)	0.3 (0.3-0.5)
Yes	215 (56.0)	3.0 (2.3-3.7)	13.8 (11.5-17.4)	1.9 (1.4-2.5)	0.4 (0.3-0.4)
Parity	n = 442				
Nulliparous	213 (48.2)	3.4 (2.7-4.2)	14.3 (11.8-18.0)	2.0 (1.5-2.6)	0.4 (0.3-0.5)
Multiparous	229 (51.8)	2.6 (2.2-3.3)	13.6 (10.6-17.0)	1.8 (1.3-2.3)	0.3 (0.2-0.4)

Abbreviations: PFOA perfluorooctanoic acid; PFOS perfluorooctane sulfonic acid; PFHxS perfluorohexane sulfonic acid; PFNA perfluorononanoic acid; IQR interquartile range.

[a] Covariate information was missing among mothers of daughters and sons, including race (n=30, 3.3%), education (n=30, 3.3%), pre-pregnancy BMI (n=73, 8.1%), age at delivery (n=7, 0.8%), smoking status during pregnancy (n=37, 4.1%), alcohol consumption during pregnancy (n=65, 7.2%), and parity (n=44, 4.9%).

[b] Defined by highest achieved qualification, where O-levels (ordinary levels) are required and completed at the age of 16.

Supplementary Table 2. Percentage of subsample of 9-year-old British boys and girls from the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort with abnormal levels of cardiometabolic outcomes (N=553).

Abnormal level of cardiometabolic outcome	Frequency n (%)
[a]Triglycerides ≥100 mg/dL	236 (42.7)
[b]Cholesterol ≥200 mg/dL	44 (8.0)
[b]HDL <40 mg/dL	70 (12.7)
[b]LDL ≥130 mg/dL	18 (3.3)
[c]Adiponectin <5.75 μg/mL	32 (5.8)
[d]Adiponectin among girls <10.11 μg/mL	93 (36.9)*
[d]Adiponectin among boys <8.60 μg/mL	68 (22.6)**
[e]Hemoglobin A1c ≥5.7%	3 (<1)
[f]C-reactive protein ≥2 mg/L	37 (6.7)
[g]Interleukin-6 ≥5 pg/mL	12 (2.2)

[a] Guideline from American Heart Association for children 0-9 years old[43]

[b] Guideline from American Heart Association for children and adolescents 10-19 years old[43]

[c] Cutoff for low adiponectin levels determined among obese adults[45]

[d] 50th percentile values for adiponectin among girls and boys from a European cohort of healthy children at age 9[46]

[e] Guideline from American Diabetes Association for youth and adults[47]

[f] Guideline from American College of Cardiology and American Heart Association for adults[48]

[g] Cutoff for elevated IL-6 levels determined among adult coronary artery disease (CAD) patients[49]

* Percentage among girls only (n=252)

** Percentage among boys only (n=301)