Exposure to Perfluoroalkyl Substances and Glucose Homeostasis in Youth

Jesse A. Goodrich,1 Tanya L. Alderete,2 Britney O. Baumert,1 Kiros Berhane,3 Zanghua Chen,1 Frank D. Gilliland,1 Michael I. Goran,4,5 Xin Hu,6 Dean P. Jones,6 Katerina Margetaki,1 Sarah Rock,1 Nikos Stratakis,1 Damaskini Valvi,7 Douglas I. Walker,7 David V. Conti,1 and Leda Chatzi1

1Department of Population and Public Health Sciences, University of Southern California, Los Angeles, California, USA
2Department of Integrative Physiology, University of Colorado Boulder, Boulder, Colorado, USA
3Department of Biostatistics, Columbia University, New York, New York, USA
4Department of Pediatrics, Keck School of Medicine, Los Angeles, California, USA
5Department of Pediatrics, Icahn School of Medicine at the Mount Sinai, New York, New York, USA
6Department of Medicine, Emory University School of Medicine, Atlanta, Georgia, USA
7Department of Environmental Medicine and Public Health, Icahn School of Medicine at Mount Sinai, New York, New York, USA

BACKGROUND: Exposure to per- and polyfluoroalkyl substances (PFAS), a prevalent class of persistent pollutants, may increase the risk of type 2 diabetes.

OBJECTIVE: We examined associations between PFAS exposure and glucose metabolism in youth.

METHODS: Overweight/obese adolescents from the Study of Latino Adolescents at Risk of Type 2 Diabetes (SOLAR; n = 310) participated in annual visits for an average of 3.3 ± 2.9 y. Generalizability of findings were tested in young adults from the Southern California Children’s Health Study (CHS; n = 135) who participated in a clinical visit with a similar protocol. At each visit, oral glucose tolerance tests were performed to estimate glucose metabolism and β-cell function via the insulinogenic index. Four PFAS were measured at baseline using liquid chromatography–high-resolution mass spectrometry; high levels were defined as concentrations >66th percentile.

RESULTS: In females from the SOLAR, high perfluorohexane sulfonate (PFHxS) levels (≥2.0 ng/mL) were associated with the development of dysregulated glucose metabolism beginning in late puberty. The magnitude of these associations increased postpuberty and persisted through 18 years of age. For example, postpuberty, females with high PFHxS levels had 25-mg/dL higher 60-min glucose (95% CI: 12, 39 mg/dL; p < 0.0001), 15-mg/dL higher 2-h glucose (95% CI: 1.28 mg/dL; p = 0.04), and 25% lower β-cell function (p = 0.02) compared with females with low levels. Results were largely consistent in the CHS, where females with elevated PFHxS levels had 26-mg/dL higher 60-min glucose (95% CI: 6.0, 46 mg/dL; p = 0.01) and 19-mg/dL higher 2-h glucose, which did not meet statistical significance (95% CI: –1.39 mg/dL; p = 0.08). In males, no consistent associations between PFHxS and glucose metabolism were observed. No consistent associations were observed for other PFAS and glucose metabolism.

DISCUSSION: Youth exposure to PFHxS was associated with dysregulated glucose metabolism in females, which may be due to changes in β-cell function. These associations appeared during puberty and were most pronounced postpuberty. https://doi.org/10.1289/EHP9200

Introduction

Youth-onset type 2 diabetes is a growing epidemic (Nadeau et al. 2016). This disease displays a more aggressive phenotype than adult onset type 2 diabetes and results in the rapid progression of disease complications (Nadeau et al. 2016). According to the International Diabetes Federation, between 2013 and 2017 the prevalence of young-onset type 2 diabetes increased in every geographical region except South and Central America and sub-Saharan Africa (IDF 2013, 2017). In 2010, the estimated prevalence of youth-onset type 2 diabetes in the United States was 2.7 of 10,000 youth, and unless effective prevention strategies are implemented, this is projected to increase 4-fold by 2050 (Imperatore et al. 2012). In addition, youth of ethnic/racial minorities are at greater risk compared with non-Hispanic Whites. For example, in the United States, the annual increase in the incidence of youth-onset type 2 diabetes in Hispanic youth 10–19 years of age is nearly five times higher than in non-Hispanic Whites (Mayer-Davis et al. 2017). Thus, there is an urgent need for identifying modifiable risk factors that may help inform public health interventions to reduce the burden of youth-onset type 2 diabetes and specifically target health-disparate populations.

Experimental evidence has shown that endocrine-disrupting chemicals, including per- and polyfluoroalkyl substances (PFAS), may disrupt metabolic systems and increase the risk of type 2 diabetes (Heindel et al. 2017). PFAS have been used for decades as industrial surfactants and in textile coatings, firefighting foams, and many consumer products, including nonstick cookware, food containers, and waterproof clothing (De Silva et al. 2021). Because of their environmental stability and slow biodegradation and excretion rates, PFAS accumulate and persist in the environment (Sunderland et al. 2019). As such, humans are exposed to PFAS from a variety of sources, including drinking water and diet (Papadopoulou et al. 2019; De Silva et al. 2021). In the early 2000s, biomonitoring studies in the United States and around the world began to show nearly ubiquitous exposure to several PFAS, including PFSAs, perfluorooctane sulfonate (PFOS), and perfluorohexane sulfonic acid (PFHxS), as well as perfluoralkyl carboxylic acids (PFCAs), perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA) (Kannan et al. 2004; Kato et al. 2011). These PFSA and PFCA compounds are of particular concern because they are some of the most bioaccumulative PFAS compounds in human tissues, with estimated biological half-lives of 1.7–7.1 y for PFNA and PFDA, 5.8–18 y for PFOS, and 7.1–25 y for PFHxS (Zhang et al. 2013). Evidence from animal models suggest that PFAS can contribute to the pathogenesis of type 2 diabetes via effects on the pancreas, adipose tissue, and the immune system (DeWitt et al. 2012; Hines et al. 2009).
zebrafish exposed to PFOS exhibit altered pancreatic β-cell morphology and increased lipid accumulation compared with unexposed zebrafish (Sant et al. 2017, 2021). *In vitro* studies using a variety of different models have shown that PFAS can act on nuclear receptors, including peroxisome proliferator-activated receptor α (PPAR-α), PPAR-γ, and estrogen receptor-α (ERα), all of which play key roles in regulating glucose homeostasis (Cwinn et al. 2008; Jacobsen et al. 2018; Menger et al. 2020; Rosen et al. 2010, 2017; Sant et al. 2017, 2021; Takacs and Abbott 2007; Wolf et al. 2008).

In humans, longitudinal studies examining the associations between exposure to PFAS and the risk of type 2 diabetes in adults have reported conflicting results. Although some studies have found that exposure to PFOS and PFOA increase the risk of type 2 diabetes (Cardenas et al. 2019; Sun et al. 2018), others have reported null or even weak inverse associations (Charles et al. 2020; Donat-Vargas et al. 2019). However, human and animal studies suggest that exposure during sensitive periods of development, including childhood, may predispose individuals to type 2 diabetes later in life because this is when cellular differentiation and development of important metabolic tissues occurs (Heindel 2019; Hines et al. 2009). During puberty, glucose homeostasis, insulin sensitivity, and β-cell function—which are highly predictive of developing type 2 diabetes (Nadeau et al. 2016)—undergo significant changes (Kelly et al. 2011). This suggests that puberty may be a window of increased susceptibility to the potential diabetogenic effects of PFAS.

Our previous work with a subset of 40 participants from the Study of Latino Adolescents at Risk (SOLAR) cohort suggested that childhood PFAS exposure is associated with longitudinal alterations of clinical markers for type 2 diabetes in youth (Alderete et al. 2019), something that could have far-reaching consequences for metabolic health over the life span. To our knowledge, no previous studies have examined the associations of PFAS exposure with longitudinal alterations of glucose metabolism, insulin resistance, or β-cell function during puberty, a critical time window for developing effective interventions that can stop or delay type 2 diabetes development (Perreault et al. 2012). Therefore, the primary aim of this study was to examine the associations of childhood PFAS exposure with longitudinal alterations in glucose metabolism, insulin sensitivity, and β-cell function in two independent cohorts of overweight/obese adolescents and young adults with a history of overweight or obesity during childhood.

**Methods**

**Study Populations**

**The SOLAR cohort.** Data were collected using samples from the SOLAR study, which is a prospective study of 328 children with obesity who were recruited in two waves between 2001 and 2012 (Alderete et al. 2017; Goran et al. 2004). Participants underwent annual clinical visits at the University of Southern California General Clinical Research Center or Clinical Trials Unit for a median of 2.2 y. Briefly, children were included if they met the following criteria: a) were between 8 and 13 years of age; b) had a body mass index (BMI) ≥85th percentile based on their age and sex; c) were free of type 1 or type 2 diabetes; d) were of Hispanic/Latino ethnicity (all four grandparents Hispanic/Latino by self-report); e) had a direct familial history of type 2 diabetes; and f) were not taking any medications known to influence insulin/glucose metabolism. This study included Hispanic and Latino individuals because studies examining the role of environmental exposures on the risk of type 2 diabetes among this minority population is lacking. Participants were included in the present analysis if there was sufficient fasting plasma sample volume from their first or second visit to measure PFAS concentrations, resulting in 312 participants. One participant had no available glucose concentrations from the oral glucose tolerance test (OGTT), and quality assurance/quality control analysis of the raw liquid chromatography–high-resolution mass spectrometry (LC-HRMS) results identified one outlier. Both participants were removed prior to data analysis, resulting in a total sample size of 310 participants. A population flowchart for the SOLAR cohort is presented in Figure S1. Only 41 visits with 29 participants were performed after the age of 20 y. Of these 29 participants, all had reached Tanner Stage 5 prior to 20 years of age, so all visits at >20 years of age were excluded from analysis. Ethics approval for this study was provided by the University of Southern California institutional review board. Prior to participation, written informed assent/consent was obtained from participants and their guardians.

**The Children’s Health Study cohort.** To test the generalizability of associations between childhood PFAS exposure and glucose homeostasis from the SOLAR cohort, samples were analyzed in a cohort of 137 young adults from the Metabolic and Asthma Incidence Research (Meta-AIR) study (Kim et al. 2019), a subset of participants from the Southern California Children’s Health Study (CHS) (McConnell et al. 2015). Meta-AIR participants completed a single clinical visit at the Clinical Trials Unit or the Diabetes and Obesity Research Institute at the University of Southern California between 2014 and 2018. At the time of the clinical visit, participants were between 18 and 23 years of age, were free from type 1 or type 2 diabetes, and were not taking any medications known to influence insulin/glucose metabolism. Meta-AIR participants were selected from the CHS cohort on the basis of a history of overweight or obesity in the 9th or 10th grade (ages 14–15 y), defined as an age- and sex-specific BMI (BMI ≥85th percentile). Initial characterization of LC-HRMS results identified one outlier sample, which was removed prior to analysis. One participant with a fasting glucose level of >200 mg/dL was removed from analysis, resulting in a total sample size of 135 participants. A population flowchart for the CHS cohort is presented in Figure S1. Ethics approval was provided by the University of Southern California institutional review board, and all participants gave written informed consent.

**Clinical Outcomes**

At each clinical visit, participants from both the SOLAR and the CHS cohorts underwent a 2-h OGTT. Following baseline blood sampling, participants consumed a glucose load of 1.75 g glucose per kg body mass, to a maximum of 75 g. In the SOLAR cohort, blood was sampled at 15, 30, 45, 60, and 120 min post glucose challenge; in the CHS cohort, samples were collected at 30, 60, 90, and 120 min post challenge. Blood samples for analysis of glucose concentrations were collected into tubes containing potassium oxalate and sodium fluoride, and samples for analysis of insulin concentrations were collected into tubes containing sodium heparin. These samples were centrifuged to obtain plasma, which was then used to measure glucose and insulin concentrations. Fasting whole blood samples were used to measure glycated hemoglobin (HbA1c). Additional blood samples from the fasting blood draw were collected into sodium heparin, sodium citrate (SOLAR cohort only), and ethylenediaminetetraacetic acid collection tubes. Following centrifugation, and the resulting plasma was frozen at −80°C for future analysis.

Blood glucose and insulin responses from the OGTT were used to calculate glucose and insulin areas under the curve (AUCs) as markers of glucose homeostasis using the trapezoidal method (Le Floch et al. 1990). The homeostatic model
assessment (HOMA-IR) was calculated to estimate insulin resistance using the formula \( \text{Gl}u_{0x} \times \text{Ins}_{0x}/405 \) (Gutch et al. 2015), the Matsuda index (ISI_{Matsuda}) was calculated to estimate insulin sensitivity using the formula 10,000/\sqrt{\text{Gl}u_{0} \times \text{Ins}_{0} \times \text{Gl}u \times \text{Ins}} (Matsuda and DeFronzo 1999), and the insulinogenic index (IGI) was calculated to estimate \( \beta \)-cell function using the equation \( (\text{Ins}_{30} - \text{Ins}_{0})/(\text{Gl}u_{30} - \text{Gl}u_{0}) \) (Nathan et al. 2007), where Glu_{0} and Ins_{0} are fasting glucose and insulin concentrations, Glu_{30} and Ins_{30} are glucose and insulin concentrations at 30 min, and Glu is the weighted average glucose and insulin concentration over the entire OGTT. Impaired fasting glucose was defined as a fasting glucose concentration of >100 mg/dL, and impaired glucose tolerance was defined as a 2-h glucose concentration of >140 mg/dL (Nathan et al. 2007).

**Plasma PFAS Concentrations**

Plasma PFAS concentrations were determined using LC-HRMS with reference standardization (Jin et al. 2020; Liu et al. 2020a). Briefly, 65-\( \mu \)L plasma aliquots were treated with two volumes of ice-cold acetonitrile to precipitate the proteins. A mixture of 10 stable isotope internal standards was included for quality control prior to protein precipitation. Following 30 min incubation on ice, samples were centrifuged for 10 min at 16,100 \( \times g \) at 4\(^\circ\)C. The supernatants were analyzed using reverse phase chromatography (Higgins Analytical Targa C18; 50 \( \times 2.1 \) mm) with an acetonitrile/ammonium acetate gradient, and analyte peaks detected using a Thermo Scientific Orbitrap Tribrid Fusion HRMS operated at 120,000 resolution. MS data were collected in negative electrospray ionization mode from mass-to-charge ratio (m/z) 85 to 1,275. Raw MS data was extracted using aplCMS (Yu et al. 2009) and xMSanalyzer (Uppal et al. 2013) for m/z, retention time, and integrated peak intensity. Samples were corrected for intra- and interbatch variability using a quality control sample-based random forest signal correction algorithm (Luan et al. 2018).

Samples were excluded from analysis if the LC-MS data did not meet quality control standards. For all samples, the sum of the absolute intensities for all detected features from the raw LC-MS data was calculated (\( \Sigma \text{Intensity} \)). Samples were excluded if the \( \Sigma \text{Intensity} \) was greater than five times the interquartile range from the median. Based on this criterion, one sample from the SOLAR and one sample from the CHS were excluded. For both samples, PFAS were below the limit of detection (LOD) for at least three of the four measured PFAS.

PFAS were identified by matching the accurate m/z for the deprotonated ion \([M-H]^{-}\)-adduct and matching the retention time to authentic standards analyzed using the same platform. Quantification of PFAS was performed following reference standardization protocol using certified mass fraction values of PFAS in National Institute of Standards and Technology Standard Reference Material 1950 (Go et al. 2015), which has been previously validated to provide quantitative measures of plasma PFAS (Alderete et al. 2017; Chen et al. 2020; Jin et al. 2020; Kingsley et al. 2019). The LODs for PFOS, PFHxS, PFNA, and PFDA were 0.02, 0.03, 0.1, and 0.2 ng/mL, respectively. In the SOLAR, the percentage of samples below the LOD were PFOS, 0%; PFHxS, 6%; PFNA, 1%; and PFDA, 56%. The corresponding values for the CHS cohort were PFOS, 0%; PFHxS, 0%; PFNA, 0%; and PFDA, 60%. Concentrations below the LOD were imputed as the LOD divided by 2.

Thresholds for high and low levels of PFAS exposure were based upon cohort-specific tertiles of serum PFAS concentrations. High levels were defined as the third tertile of exposure, and low levels were defined as the first and second tertiles of exposure, in accordance with previous literature (ATSDR 2018).

To assess the generalizability of associations between PFHxS and glucose homeostasis in youth to young adults, high and low levels of PFHxS exposure in the CHS cohort also used the 66th percentile thresholds from the SOLAR to define high and low PFHxS levels in the CHS cohort. In the CHS cohort, high vs. low levels of PFOS and PFNA were determined using cohort-specific thresholds. In both cohorts, >50% of samples exhibited PFDA levels below the LOD, so PFDA was analyzed as a binary variable (detected vs. nondetected).

**Covariates**

In both cohorts, height (in meters) and weight (in kilograms) were measured at each visit and were used to calculate BMI as kilograms per meter squared. Participants and their guardians also completed several questionnaires detailing health history, familial health history, and sociodemographic characteristics. In the SOLAR cohort, a physician performed a physical examination for determination of the Tanner stage (Marshall and Tanner 1969, 1970). Tanner staging is an objective measure of the stage of development of secondary sex characteristics during puberty. Briefly, on the Tanner scale, Stage 1 is the period before the development of secondary sex characteristics, Stages 2–4 comprise the period during which secondary sex characteristics begin to develop and when most females undergo menarche, and Stage 5 is the period when pubic hair develops and secondary sex organs reach adult size (Marshall and Tanner 1969, 1970). Tanner stages were classified as prepuberty (Tanner 1), early puberty (Tanner 2), puberty (Tanner 3), late puberty (Tanner 4), and postpuberty (Tanner 5). Socioeconomic status (SES) was assessed in the SOLAR using a modified version of the Hollingshead Four Factor Index (Alderete et al. 2017; Hollingshead 2011) and in the CHS using parental education (Kim et al. 2019). In the SOLAR, SES data was not available for 35 (11%) of participants, so for these individuals SES was coded as missing. As assessed using chi-square tests and independent t-tests, these participants did not differ in important metabolic or physical attributes, including sex, age, BMI, fasting glucose concentration, or 2-h glucose concentration (all \( p < 0.05 \)). No other covariates included in statistical models contained missing data.

**Statistical Methods**

Differences in baseline participant characteristics between cohorts were tested using independent t-tests for continuous variables (BMI, OGTT glucose concentrations) and with chi-square tests for categorical variables (e.g., SES). To test for differences in the geometric means of PFAS levels between cohorts, independent t-tests were performed on log-transformed PFAS concentrations. For PFDA, the proportion of samples below the LOD was too high to provide a valid estimate of the geometric mean, so differences in baseline levels of PFDA were tested using chi-square tests to examine differences in the percentage of samples >LOD between cohorts.

Linear mixed effects models were used to examine the associations between childhood PFAS levels and changes in glucose homeostasis across visits (Diggle et al. 2002). Estimates of glucose homeostasis, insulin sensitivity, and \( \beta \)-cell function—which included HOMA-IR, ISI_{Matsuda}, IGI, HbA1c, glucose AUC, and insulin AUC—were modeled as separate outcomes. Each outcome was modeled as a function of age, Tanner stage, and high vs. low PFAS concentrations, and each model allowed for separate intercepts for each individual. In these models, age and Tanner stage were used to account for multiple visits per participant across time. Specifically, for \( i = \{1, \ldots , N\} \) individuals and \( j = \{1, \ldots , J_i\} \) visits, we used the following model: \( Y_{ij} = \gamma_0 + \)}

Environmental Health Perspectives 097002-3 129(9) September 2021
γ1PFASk,Tannerij + γ2Tannerij + γ3PFASk,Agelj + 0, + εikj, where 0, ~ N(0,σ2), are the residuals. OGTT glucose concentrations, which included fasting and 15-, 30-, 45-, 60-, and 120-min glucose concentrations, were jointly examined in a single expanded model. Specifically, for k = {1,…,6} OGTT sampling time points (min 0, 15, 30, 45, 60, and 120), i = {1,…,N} individuals, and j = {1,…,J} visits, we used the following model:

\[ Y_{ijk} = \gamma_0 + \gamma_1PFASk + \gamma_2Tannerij + \gamma_3PFASk,Agelj + \gamma_4kSexij + \epsilon_{ijk} \]

where \( \gamma_0 \sim N(0,\sigma_0) \) is the OGTT time point-specific intercept. For sex-stratified analyses, we used the following simplified model:

\[ Y_{ijk} = \gamma_0 + \gamma_1PFASk + \gamma_2Tannerij + \gamma_3PFASk,Agelj + \gamma_4kSexij + \epsilon_{ijk} \]

In all models, PFAS exposures were modeled as a dichotomous variable to avoid the additional complexity of modeling nonlinear dose-response relationships.

Significance of associations between PFAS exposure and outcomes were assessed using likelihood-ratio tests to compare the full hierarchical model (i.e., the model with PFAS exposure) to the null model (i.e., the model with no PFAS exposure). To address the issue of multiple comparisons, we calculated false discovery rate (FDR)-adjusted p-values using the Benjamini-Hochberg method (Benjamini and Hochberg 1995). FDR corrections were calculated separately for the SOLAR and the CHS cohorts and are represented as a q-value. A q < 0.05 was considered statistically significant.

Because glucose homeostasis, insulin sensitivity, and β-cell function exhibit substantial changes throughout puberty (Kelly et al. 2011), we hypothesized that PFAS exposure may have differential associations with glucose homeostasis, depending on Tanner stage. Therefore, for each model, we tested the interaction between PFAS levels and Tanner stage. Given that exposure to PFOS, PFHxS, PFNA, and PFDA have been shown to have sex-specific associations with adiposity outcomes (Haldorsson et al. 2012; Liu et al. 2018; Mora et al. 2017) and with glucose or lipid metabolism outcomes (Fleisch et al. 2017; He et al. 2018; Valvi et al. 2021), interactions between sex and PFAS exposure were tested. Models included three sex × exposure interaction terms: a sex × PFAS term, a sex × PFAS × Tanner stage term, and a sex × PFAS × age term. All PFAS, with the exception of PFDA, exhibited at least one significant sex × exposure interaction term at p < 0.05 for one or more primary outcomes (Table S1), so all analyses were stratified by sex. Selection of additional covariates were based on a directed acyclic graph (DAG; Figure S2). All models included adjustments for time varying (i.e., age, BMI) and time invariant (i.e., SES, study wave) covariates. One of the outcomes, IGI, was log transformed to satisfy modeling assumptions.

Where significant associations between PFAS exposure and primary outcomes were observed, we calculated marginal means and 95% confidence intervals (CIs) for the outcome for high vs. low PFAS levels at each Tanner stage. These estimates were calculated at the median age for each Tanner stage, holding BMI at the median. Estimated means and effect sizes were also calculated at age 18 y and Tanner Stage 5.

In the CHS, which included only one visit, associations between PFAS levels and glucose homeostasis parameters were tested using linear regression. In the CHS, all models included adjustments for ethnicity. All analyses were performed in R (version 4.0.2; R Development Core Team). Alpha was set to 0.05.

### Sensitivity and Bias Analysis

To assess the robustness of our significant findings in the SOLAR, we performed several sensitivity analyses. These analyses also help to address potential uncertainties in the structure of the DAG.

First, because obesity and BMI may be on the causal pathway linking PFAS exposure and glucose homeostasis (Inoue et al. 2020; Valvi et al. 2021), we performed a sensitivity analysis excluding BMI as a covariate in the model. Second, to determine the impact of modeling PFHxS exposure as a dichotomous vs. continuous variable, we tested the associations between PFHxS levels and 2-h OGTT glucose concentrations, modeling PFHxS as a log2-transformed continuous variable. To test the assumption of linearity, we applied generalized additive models using cross-sectional data at Tanner Stage 5. Following this step, for associations that exhibited nonlinear relationships between exposure and outcome, we refit these models after excluding participants with PFHxS levels below the LOD, and again tested for nonlinearity. This step was performed to ensure that the method of imputing values below the LOD did not impact the assessment of linearity. Because no models exhibited nonlinearity, linear regression models were fit to estimate the association between PFAS levels and glucose homeostasis outcomes.

Third, we performed a sensitivity analysis in the SOLAR cohort to examine whether excluding visits above age 20 y influenced our findings. For this analysis, we re-ran the OGTT glucose concentration analysis in the SOLAR, including all visits above the age of 20 y.

Fourth, to assess potential bias resulting from an unknown or unmeasured confounder, we calculated the E-value (Ding and VanderWeele 2016; VanderWeele and Ding 2017). The E-value is a quantitative measure of the magnitude of an unknown or unmeasured confounder required to fully explain the association between exposure and outcome. Because our main findings were between PFHxS and OGTT glucose concentrations at Tanner Stages 4 and 5 in females, we first calculated the crude risk ratio (RR0n) for the association of PFHxS with impaired glucose tolerance at the first visit post-puberty in females. Using this value, we calculated the E-value (Ding and VanderWeele 2016; VanderWeele and Ding 2017).

### Results

Table 1 describes baseline participant characteristics, and Table 2 describes baseline PFAS levels in the SOLAR and the CHS cohorts. Geometric means of serum PFAS concentrations of PFOS and PFNA were 147% and 66% higher, respectively, in the SOLAR cohort vs. the CHS cohort (both p < 0.05). PFHxS concentrations were 39% higher in females from the SOLAR compared with females from the CHS cohort (p = 0.05), but 25% lower in males from the SOLAR cohort compared with males from the CHS cohort (p = 0.04; Table 2). Based upon tertiles of PFAS concentrations in the SOLAR, high PFAS levels were defined as serum concentrations of >20.8 ng/dL for PFOS, 2.0 ng/dL for PFHxS, and 0.7 ng/dL for PFNA. These values are similar to the median concentrations of PFAS in 12- to 19-y-old adolescents from the United States in the 2003–2004 National Health and Nutrition Examination Survey (NHANES), including PFOS [median (95% CI) from NHANES 2003–2004: 19.9 ng/dL (95% CI: 17.8, 22.0 ng/dL)], PFHxS [2.40 ng/dL (95% CI: 1.80, 3.20 ng/dL)] and PFNA [0.80 ng/dL (95% CI: 0.70, 1.00 ng/dL)] (CDC 2019).

### PFHxS Levels and OGTT Glucose Responses

Youth exposure to PFHxS was associated with impaired glucose homeostasis in females, including higher glucose levels during an OGTT. Associations between PFHxS and altered glucose concentrations during the OGTT were observed in females from the SOLAR cohort [likelihood-ratio \( \chi^2 = 79.6 \); degrees of freedom (df): 36; p < 0.001; q = 0.0022; Figure 1A; Table S2]. There were
also associations between PFHxS and altered glucose concentrations during the OGTT in males from the SOLAR cohort (χ² = 63.3; df: 36; p = 0.003; q = 0.062) and in females from the CHS cohort, (χ² = 11.2; df: 5; p = 0.048; q = 0.51; Figure 1B), although neither remained significant after corrections for multiple comparisons (Table S2).

In females, the association between PFHxS and elevated glucose concentrations during an OGTT became more pronounced with increasing pubertal development (Figure 1, Table 3; Table S3). In late puberty, high vs. low PFHxS levels were associated with a 21-mg/dL higher glucose concentration at 60 min (95% CI: 10, 31 mg/dL; p = 0.0001) and a 12-mg/dL higher 2-h glucose concentration (95% CI: 1, 22 mg/dL; p = 0.03). Postpuberty, PFHxS was associated with reduced glyceremic control following an oral glucose challenge. High vs. low PFHxS levels were associated with a 25-mg/dL higher glucose concentration at 60 min (95% CI: 12, 39 mg/dL; p < 0.0001), and a 15-mg/dL higher 2-h glucose level (95% CI: 1, 28 mg/dL; p = 0.04). Results were largely consistent among young adult females from the CHS where elevated PFHxS levels were associated with a 26-mg/dL higher glucose concentration at 60 min (95% CI: 6, 46 mg/dL; p = 0.02). Elevated PFHxS levels were also associated with a 19-mg/dL higher 2-h glucose concentration, although this association failed to meet statistical significance (95% CI: –1, 39 mg/dL; p = 0.09).

Table 1. Baseline participant characteristics in n = 310 overweight and obese adolescents recruited from Southern California between 2001 and 2012 (SOLAR cohort) and in n = 135 young adults recruited from Southern California between 2014 and 2018 (CHS cohort).

<table>
<thead>
<tr>
<th>Participant characteristics</th>
<th>SOLAR cohort</th>
<th>CHS cohort</th>
<th>SOLAR vs. CHS p-value&lt;sup&gt;d&lt;/sup&gt;</th>
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<tr>
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<td>Males</td>
<td>Females</td>
<td>Males</td>
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<tr>
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<td>11.3 ± 1.3</td>
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<td>BMI (kg/m²)</td>
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<td>28.5 ± 5.7</td>
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<td>Visits per subject (n)</td>
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<td>71 (40)</td>
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<tr>
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<td>6 (3)</td>
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<td>Wave 2 (2010–2012)</td>
<td>29 (22)</td>
<td>48 (27)</td>
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<td>SES</td>
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<td>Completed college</td>
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<td>High school graduate</td>
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<td>79 (44)</td>
<td>12 (20)</td>
</tr>
<tr>
<td>Partial college</td>
<td>30 (23)</td>
<td>59 (33)</td>
<td>12 (20)</td>
</tr>
<tr>
<td>Completed college</td>
<td>14 (11)</td>
<td>21 (12)</td>
<td>14 (24)</td>
</tr>
<tr>
<td>Graduate professional training</td>
<td>2 (2)</td>
<td>1 (1)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Missing</td>
<td>17 (13)</td>
<td>17 (10)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>2-h OGTT measures</td>
<td>Fasting glucose (mg/dL)</td>
<td>88.0 ± 6.5</td>
<td>132</td>
</tr>
<tr>
<td>2-h glucose (mg/dL)</td>
<td>126 ± 18</td>
<td>132</td>
<td>123 ± 18</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.6 ± 0.3</td>
<td>68</td>
<td>5.5 ± 0.3</td>
</tr>
</tbody>
</table>

Note: Data presented as geometric mean (95% confidence interval) or n (%); CHS, Children’s Health Study; HbA1c, glycated hemoglobin; OGTT, oral glucose tolerance test; SD, standard deviation; SES, socioeconomic status; SOLAR, Study of Latino Adolescents at Risk of Type 2 Diabetes.

<sup>a</sup>Pubertal status defined as prepuberty (Tanner Stage 1), puberty (Tanner Stages 2–4), and postpuberty (Tanner Stage 5).

<sup>b</sup>Hollingshead Four Factor Index uses information on parental education and occupation to calculate the SES of a single family. Scores range from 8 to 19 for unskilled laborers and up to 55 to 66 for higher-level executives.

<sup>c</sup>Household education takes into account the average parental education level in a single household and is used as the education variable for the Hollingshead Four Factor Index of SES.

<sup>d</sup>p-Values calculated with independent t-tests for continuous variables (BMI, OGTT glucose concentrations) and with chi-square tests for categorical variables (SES). Comparisons between cohorts were performed without stratification (overall) as well as stratified by sex (females and males).

Table 2. Baseline levels of perfluoroalkyl substances in n = 310 overweight and obese adolescents recruited from Southern California between 2001 and 2012 (SOLAR cohort) and in n = 135 young adults recruited from Southern California between 2014 and 2018 (CHS cohort).

<table>
<thead>
<tr>
<th>Perfluoroalkyl substance</th>
<th>SOLAR cohort</th>
<th>CHS cohort</th>
<th>SOLAR vs. CHS p-value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td>PFOS (ng/mL)</td>
<td>11.3 (9.3, 13.6)</td>
<td>10.9 (9.1, 13.6)</td>
<td>3.5 (3.1, 4.0)</td>
</tr>
<tr>
<td>PFHxS (ng/mL)</td>
<td>1.1 (0.8, 1.4)</td>
<td>1.1 (0.8, 1.3)</td>
<td>0.8 (0.6, 1.0)</td>
</tr>
<tr>
<td>PFNA (ng/mL)</td>
<td>0.58 (0.53, 0.64)</td>
<td>0.64 (0.60, 0.69)</td>
<td>0.35 (0.33, 0.38)</td>
</tr>
<tr>
<td>PFDA (% above LOD)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55 (42)</td>
<td>81 (46)</td>
<td>22 (37)</td>
</tr>
</tbody>
</table>

Note: Data presented as geometric mean (95% confidence interval) or n (%); CHS, Children’s Health Study; LOD, limit of detection; PFDA, perfluorodecanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOS, perfluorooctane sulfonate; SOLAR, Study of Latino Adolescents at Risk of Type 2 Diabetes.

<sup>a</sup>Proportion of samples below the LOD was too high to provide a valid estimate of the geometric mean. LOD for PFOS, PFHxS, PFNA, and PFDA were 0.02, 0.03, 0.1, and 0.2ng/mL, respectively.

<sup>b</sup>p-Values to test for differences in the geometric means between cohorts were calculated using independent t-tests on log-transformed PFAS concentrations. For PFDA, differences in the proportion of samples above the LOD between cohorts were tested using chi-square tests.
In adolescent males from the SOLAR, the association between PFHxS levels and glucose responses during the OGTT were less pronounced. In contrast to females, there was no evidence of a clear association between PFHxS exposure and altered glycemic control at any developmental stage. Where there were significant associations with glucose concentrations during the OGTT, PFHxS was associated with lower glucose levels (Table 3). In addition, there was no association between PFHxS levels and altered glucose concentrations during the OGTT in males from the CHS ($\chi^2 = 1.6$; df: 5; $p = 0.90$; Table 3).

**PFHxS Levels and Markers of Glucose Homeostasis**

Consistent with the positive associations observed between childhood PFHxS exposure and glucose levels during the OGTT, we also found that PFHxS exposure was associated with increases in glucose AUC, increases in HbA1c, and declines in $\beta$-cell function (Figure 2; Tables S2 and S4). In females from the SOLAR, PFHxS levels were positively associated with a glucose AUC ($\chi^2 = 23$; df: 6; $p = 0.0008$; $q = 0.023$; Figure 2A; Table S4). In females from the SOLAR, PFHxS was also associated with a higher HbA1c ($\chi^2 = 13.9$; df: 6; $p = 0.03$; $q = 0.17$; Figure 2C; Table S4), and a lower $\beta$-cell function as measured using the IGI ($\chi^2 = 14.0$; df: 6; $p = 0.03$; $q = 0.17$; Figure 2D; Table S4) although these associations were not significant after adjusting for multiple comparisons (Table S2). The relationships between PFHxS levels and these markers of glucose homeostasis and $\beta$-cell function were dependent on pubertal status, with significant associations observed during and postpuberty. In late puberty, elevated childhood PFHxS levels were associated with an 8.9% higher glucose AUC ($p = 0.003$). Postpuberty, elevated PFHxS levels were associated with a 10.6% higher glucose AUC ($p = 0.0004$) as well as a 25% lower IGI ($p = 0.02$). In young adult females from the CHS, there were no significant associations between PFHxS levels and markers of glucose homeostasis (Table S2). In males from both the SOLAR and the CHS, PFHxS levels were associated with a lower IGI ($\chi^2 = 23$; df: 6; $p = 0.0008$; $q = 0.023$; Figure 2A; Table S4). In young adult males from the CHS, PFHxS was associated with a 20.4% lower IGI ($p = 0.0017$; Figure 2B; Table S4) although these associations were not significant after adjusting for multiple comparisons (Table S2).

### Table 3. Differences in glucose concentrations in individuals with high PFHxS levels (≥2.0 ng/dL) vs. individuals with low PFHxS levels during a 2-h oral glucose tolerance test in $n = 310$ overweight and obese adolescents recruited from Southern California between 2001 and 2012 (SOLAR cohort) and in $n = 135$ young adults recruited from Southern California between 2014 and 2018 (CHS cohort).

<table>
<thead>
<tr>
<th>Sex</th>
<th>Glucose (mg/dL at each time point)</th>
<th>SOLAR</th>
<th>CHS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prepuberty (Tanner 1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>Fasting</td>
<td>36/143</td>
<td>79/139</td>
</tr>
<tr>
<td></td>
<td>30-min</td>
<td>13 (0, 27)</td>
<td>4 (−5, 13)</td>
</tr>
<tr>
<td></td>
<td>60-min</td>
<td>−4 (−18, 10)</td>
<td>4 (−3, 15)</td>
</tr>
<tr>
<td></td>
<td>2-h</td>
<td>−2 (−15, 12)</td>
<td>4 (−5, 13)</td>
</tr>
<tr>
<td>Male</td>
<td>Fasting</td>
<td>−7 (−15, 0)</td>
<td>−2 (−12, 7)</td>
</tr>
<tr>
<td></td>
<td>30-min</td>
<td>−4 (−12, 4)</td>
<td>−10 (−20, 0)</td>
</tr>
<tr>
<td></td>
<td>60-min</td>
<td>−3 (−11, 5)</td>
<td>−1 (−11, 8)</td>
</tr>
<tr>
<td></td>
<td>2-h</td>
<td>−7 (−15, 0)</td>
<td>−6 (−15, 4)</td>
</tr>
</tbody>
</table>

Note: Results are displayed as the difference and 95% confidence interval between individuals with high (≥2.0 ng/dL) and low PFHxS levels at baseline. Estimates were calculated at the median age and BMI for each Tanner stage using linear mixed effects models. Estimates were corrected for socioeconomic status, and for the SOLAR study, recruitment wave. BMI, body mass index; CHS, Children’s Health Study; PFHxS, perfluorohexane sulfonic acid; SOLAR, Study of Latino Adolescents at Risk of Type 2 Diabetes.

*Number of observations per developmental stage for females and males (n female observations/m male observations).
CHS, PFHxS levels were not associated with markers of glucose homeostasis, insulin sensitivity, or \( \beta \)-cell function (Table S2). In both the SOLAR and the CHS cohorts, PFOS, PFNA, and PFDA were not associated with alterations in glucose homeostasis in males or females after adjusting for multiple comparisons (Tables S5–S9).

**Sensitivity and Bias Analysis**

In the sensitivity analysis to examine the associations between PFHxS and OGTT glucose concentrations after excluding BMI from the statistical models, results remained largely unchanged (Table S10). In the sensitivity analysis to examine the associations between PFHxS and OGTT glucose concentrations after including all visits above the age of 20 y, results again remained largely unchanged (Table S11). Similarly, in the sensitivity analysis to examine the impact of modeling PFHxS as a continuous vs. categorical variable, the main findings remained qualitatively unchanged (Table S12).

To perform a quantitative bias analysis, we first performed a cross-sectional analysis at Tanner Stage 5 in females from the SOLAR cohort and found that high PFHxS levels at baseline were associated with a 2.56-fold (95% CI: 1.29, 5.1) higher risk of developing impaired glucose tolerance after puberty. Based on this relative risk, the \( E \)-value was 4.6, indicating that an unknown confounder would have to be associated with both PFHxS levels and impaired glucose tolerance by a relative risk of 4.6 to explain the observed associations between PFHxS and glucose homeostasis.

**Discussion**

To our knowledge, this is the first study to examine associations of PFAS with longitudinal alterations in glucose metabolism among adolescents and young adults. In females from the SOLAR, we found that childhood exposure to PFHxS was associated with several risk factors for type 2 diabetes, especially after puberty, including higher 2-h glucose concentrations and a lower \( \beta \)-cell function using the IGI. These associations began to appear in late puberty but were most pronounced postpuberty and persisted through 18 years of age. These findings were replicated in an independent cohort of young adults from the CHS, where elevated PFHxS levels were associated with higher glucose concentrations 60 min and 2-h post glucose challenge in females. In males, there was a trend for negative associations between PFHxS levels and glucose concentrations during the OGTT at Tanner Stage 5, but these associations did not meet the threshold for statistical significance after corrections for multiple comparisons. These results suggest that exposure to PFHxS impairs glucose tolerance in adolescents and young adults and that these associations may be explained by changes in \( \beta \)-cell function. In addition, we observed that these associations were stronger in females compared with males.

This study addresses a key gap in the literature examining associations between PFAS with longitudinal alterations in glucose metabolism among adolescents and young adults. In adults, several studies have addressed this question, but results have been discordant. For example, elevated plasma concentrations of PFOA and PFOS have been linked to an increased risk of developing type 2 diabetes in females from the Nurses’ Health Study II (Sun et al. 2018) and in males and females from the Diabetes Prevention Program.
trial and Diabetes Prevention Program Outcomes Study (Cardenas et al. 2019). In contrast, in females from the Norwegian Women and Cancer Study, there were no associations between PFAS and the risk of type 2 diabetes (Charles et al. 2020), and in males and females from the Swedish-based Västerbotten Intervention Program cohort, PFOA was found to have a slightly negative association with the risk of type 2 diabetes (Donat-Vargas et al. 2019).

In children, limited research exists examining the associations between PFAS exposure and the risk of type 2 diabetes, but several studies have examined the associations between PFAS exposure and anthropometric markers of adiposity. For example, prenatal exposure to PFHxS and other PFAS has been linked to increases in markers of adiposity at 7.7 years of age in children from Project Viva, but only in females (Mora et al. 2017). In a study of Danish women and offspring, prenatal exposure to PFOA was associated with increased markers of adiposity at 20 years of age, but again only in females (Halldorsson et al. 2012). In children from the European Youth Heart Study, childhood exposure to PFOS and PFOA have been linked to alterations in an estimate of β-cell function at 15 and 21 years of age, but this study was limited by the fact that only fasting blood samples were used to estimate markers of β-cell function and insulin sensitivity (Domazet et al. 2016). To our knowledge, the present study is the first research to incorporate longitudinal measures of glucose homeostasis using 2-h OGTTs, which permit accurate estimates of insulin sensitivity and β-cell function.

The potential mechanisms linking PFHxS exposure and the development of impaired glucose tolerance and β-cell dysfunction remain unclear. In vitro studies in pancreatic human cells show that PFHxS interacts with a variety of transcription factors and nuclear receptors that play important roles in β-cell function. Specifically, PFHxS and other PFAS have been shown to decrease the activity of PPAR-α and PPAR-γ (DeWitt et al. 2012; Rosen et al. 2017) and has been shown to down-regulate several transcription factors, including pancreatic and duodenal homeobox 1 (PDX1) and forkhead box A1 (FOXA1) (Liu et al. 2020b). PPAR-γ, PDX1, and FOXA1 are all important for pancreatic development and the risk of type 2 diabetes because of their roles in regulating β-cell differentiation, proliferation, and survival (Fujimoto and Polonsky 2009). In our study, we found that PFHxS was associated with reduced β-cell function in females, but these associations did not appear until after puberty.

β-cell activity increases during puberty in order to compensate for a decrease in peripheral insulin sensitivity (Kelly et al. 2011; Kelsey et al. 2020), and in healthy individuals, this pancreatic β-cell compensation occurs without developing β-cell fatigue postpuberty (Kelly et al. 2011; Kelsey et al. 2020). However, some individuals fail to maintain β-cell compensation during this period and begin to develop β-cell fatigue (Kelsey et al. 2020), which is a significant risk factor for developing type 2 diabetes later in life (Fujimoto and Polonsky 2009). During puberty, when β-cell activity increases in order to maintain glucose homeostasis, the effects of exposure to PFHxS on PPAR-γ, PDX1, and FOXA1 may be amplified, which could lead to β-cell dysfunction and death. Future work is necessary to fully elucidate the mechanisms underlying pubertal-specific associations of PFAS exposure with insulin sensitivity and β-cell function.

In the present study, we found that PFHxS had a strong relationship with glucose homeostasis, whereas associations of other PFAS with glucose homeostasis were null. Currently, the research examining mechanisms linking exposure to PFAS with metabolic disorders remain unclear. PFHxS has an estimated biological half-life of between 7 and 25 y, which is the longest half-life of any of the PFAS measured in the present study (Olsen et al. 2007; Zhang et al. 2013). Therefore, one explanation for our findings is that PFHxS is simply more bioaccumulative than other PFAS. However, further research is needed to examine the potential mechanisms explaining the differential associations of PFHxS in contrast to other PFAS on glucose homeostasis.

One of our key findings was that childhood PFHxS levels were associated with alterations in glucose homeostasis in females and not males. Although direct comparisons to previous literature are difficult, at least four previous studies have reported similar sex-specific associations between PFHxS exposure and adiposity or glucose homeostasis outcomes. For example, Mora et al. (2017) found that prenatal PFHxS levels were positively associated with subscapular to triceps skinfold thickness ratio at mid childhood in females but not males (sex × exposure interaction <0.01). Similarly, in a cohort of overweight/obese adults undergoing a 2-y diet intervention, Liu et al. (2018) reported that baseline PFHxS levels were positively associated with weight regain after the first 6 months in females but not in males (sex × exposure interaction =0.01). PFHxS levels have also been reported to have sex-specific associations with measures of insulin resistance and sensitivity. In a prospective cohort of 665 mother–child pairs, prenatal PFHxS levels were found to increase insulin sensitivity (as evident by a negative association with HOMA-IR) at mid childhood in males, but this association was not present in females (exposure × sex interaction p =0.04) (Fleisch et al. 2017). Finally, Valvi et al. (2021) reported that PFHxS exposure was associated with decreased insulin sensitivity in young female adults (as evident by a positive association with HOMA-IR and a negative association with the Matsuda index using a 2-h OGTT), but these associations were null in males.

Despite previous epidemiological evidence showing that PFAS have sex-specific associations with glucose metabolism, the mechanisms underlying these findings remain unclear. One possible mechanism is that PFAS have been shown to modulate ER-α activity (Rosen et al. 2017), which could lead to sex-specific effects, especially during and after puberty. Estrogen receptors play an important role in β-cell function and survival, which results in the sexual dimorphism of the pancreas beginning in puberty (Gannon et al. 2018). As a result, females have a greater β-cell mass compared with males after puberty (Gannon et al. 2018). PFHxS-associated alterations in ER-α activity during puberty could explain our finding that PFHxS was associated with worse β-cell function after puberty in females but not males. Future work is required to better understand why associations between PFAS and the risk of type 2 diabetes appear stronger in females vs. males.

The present study has several strengths. First, we used two independent cohorts to replicate our findings, which improves the generalizability of our results. In addition, both cohorts performed 2-h OGTTs, the clinical gold standard to screen for and diagnose type 2 diabetes (Jesudason et al. 2003). In the SOLAR, Tanner stage was determined by a physician to determine sexual maturity, which allowed us to examine associations of PFAS with risk factors for type 2 diabetes at different stages of development. We also were able to account for several known confounders related to glucose homeostasis, including SES and BMI. We also performed multiple sensitivity analysis and found that the associations between PFAS and glucose homeostasis persisted regardless of modeling assumptions. For example, association between PFHxS and glucose homeostasis outcomes were present when the exposure was analyzed as continuous or categorical.

Despite these study strengths, there are some limitations worth noting. First, participants from the CHS were measured at only a single time point, limiting the ability to draw conclusions about the associations between PFAS exposure and longitudinal alterations in glucose homeostasis in young adults. Second, the
LOD for PFDA (0.2 ng/dL) was relatively high compared with other PFAS in our study, which helps to explain the low detection rate for PFDA. Third, 6% of participants in the SOLAR cohort had PFHxS levels below the LOD, and analytic assumptions about values below the LOD can impact effect estimates. We imputed values below the LOD as the LOD divided by 2, which is a common imputation approach for values below the LOD (Schisterman et al. 2006). When analyzing environmental exposures as continuous variables, this deterministic imputation method may bias results toward the null. However, we observed nonlinear relationships between PFHxS and glucose homeostasis outcomes, and therefore we performed our main analyses using categorical exposure variables. Analyzing PFHxS as categorical removes the possibility of bias due to the imputation method because all imputation methods would place values below the LOD in the lowest exposure category. Fourth, the difference in ethnic composition of the cohorts makes it difficult to directly compare results between studies. However, the use of individual participant data from two different cohorts with varied background characteristics and the absence of heterogeneity between individual cohort effect estimates suggests the findings are not restricted to one cohort and improves the generalizability of our findings. Finally, owing to the observational design of this study, it is possible that residual confounding due to an unmeasured or unknown variable could have influenced our results. However, we found that an unknown confounder would have to jointly increase the relative risk of both higher PFHxS levels and of pre-diabetes by a factor of 4.6 to fully explain away the observed associations. To put this in context, in children, none of the traditional risk factors for type 2 diabetes—such as genetic predisposition, family history of disease, diet, or sedentary activity—have this magnitude of effect estimates (Harrison et al. 2003; Rodd et al. 2018; Scott et al. 2017). Therefore, it seems unlikely that residual confounding due to an unmeasured or unknown confounder could fully explain the associations between PFHxS exposure at baseline and glucose homeostasis.

In conclusion, this study supports the hypothesis that PFAS exposure impairs glucose tolerance in adolescents and young adults. We observed that the associations between PFAS exposure and impaired glucose tolerance were stronger in females compared with males, and our results provide evidence that these associations may be driven by changes in β-cell function. These findings suggest that childhood exposure to PFHxS could predispose females to type 2 diabetes later in life.

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