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Journal

International Journal of Hygiene and Environmental Health, 222(7)

ISSN

1438-4639

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Publication Date

2019-08-01

DOI

10.1016/j.ijheh.2019.07.002

Peer reviewed

HHS Public Access

Author manuscript

Int J Hyg Environ Health. Author manuscript; available in PMC 2020 August 01.

Published in final edited form as:

Int J Hyg Environ Health. 2019 August ; 222(7): 1038–1046. doi:10.1016/j.ijheh.2019.07.002.

Perfluorooctanoate and Changes in Anthropometric Parameters with Age in Young Girls in the Greater Cincinnati and San Francisco Bay Area

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Abstract

Background: Perfluorooctanoate (PFOA) is an endocrine disrupting chemical with ubiquitous exposure worldwide. Cross-sectional studies of the effect of PFOA on body mass index (BMI) have produced disparate findings, possibly related to age.

Hypothesis: Exposure to PFOA results in decreased BMI in young girls.

Methods: We conducted a study of per- and polyfluoroalkyl substance biomarkers, including PFOA, in girls from Greater Cincinnati (CIN, N=353) and the San Francisco Bay Area (SFBA, N=351). PFOA was measured in the baseline serum sample collected in 2004–2007 of 704 girls at age 6–8 years. Mixed effects models were used to derive the effect of PFOA on BMI, waist-to-height and waist-to-hip ratios over increasing age in this longitudinal cohort.

Results: Median PFOA serum concentrations were 7.3 (CIN) and 5.8 (SFBA) ng/mL, above the U.S. population median for children 12–19 years in 2005–2006 (3.8 ng/mL). Log-transformed serum PFOA had a strong inverse association with BMIz in the CIN girls (p=0.0002) and the combined two-site data (p=0.0008); the joint inverse effect of PFOA and Age*PFOA weakened at age at 10–11 years. However, in the SFBA group alone, the relationship was not significant (p=0.1641) with no evidence of changing effect with age. The effect of PFOA on waist:height ratio

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was similar to BMIz at both sites, but we did not find a significant effect of PFOA on waist:hip ratio in either the CIN or SFBA girls.

Conclusions: PFOA is associated with decreased BMI and waist:height ratio in young girls, but the strength of the relationship decreases with age. Site heterogeneity may be due to greater early life exposure in Cincinnati.

Background:

Per- and polyfluoroalkyl substances (PFAS), such as perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS), are surfactants that have wide consumer and industrial applications as well as known environmental persistence. PFAS are used in metal plating, semi-conductors, film processing and fire-fighting foams, resulting in ubiquitous exposure in the USA and throughout the world [1]. Accidental releases of these chemicals into drinking water sources are an additional source of exposure [2–4].

Although concentrations of PFOA and other PFAS in human serum have decreased as a result of cessation of production of PFOS in the USA in 2002 and curtailment of PFOA production shortly thereafter [5], these chemicals are still readily detectable in human serum [6]. In samples collected from National Health and Nutrition Examination Survey (NHANES) participants in 2009–2010, after changes in the manufacturing practices for some PFAS, sera concentrations of most PFAS had decreased (e.g., from a median PFOA of 5.60 ng/mL in 1999–2000 in children 12–19 years to 2.90 ng/mL in 2009–2010), but PFOA, PFOS, and other PFAS were still detected in over 98% of the samples [6].

The persistence of PFAS is related to the length of their carbon chain. Species variation in the biological half-life of PFAS is substantial, from hours in some rodent strains to several years in humans [7–9]. The perfluoroalkyl portions of these molecules (i.e., C_nF_{2n+1}–) have extreme resistance to environmental and metabolic degradation; the longer the carbon chain of the PFAS, the greater the persistence. PFOA and PFOS, with 7 and 8 carbons in the perfluoroalkyl chain, respectively, have greater environmental and biological persistence in humans (about 3–4 years) than those with shorter chains.

PFOA exposure during critical developmental periods has been reported to be associated with shifts in body weight. Prenatal PFOA exposure is associated with decreased birth weight in both mice and humans [10–15] although mice became heavier in young adulthood [16]. Cross-sectional studies in human populations generally have found that in adults, higher blood concentrations of PFOA have been associated with higher body mass index [9, 17, 18] while in children, both direct and inverse associations have been reported [13, 19–22]. Findings are inconsistent, which may relate to the cross-sectional study design, the age of the study participants, and the window and degree of exposure, and whether there were repeat measures of PFOA in the cohort study population.

The analysis reported here examines the longitudinal relationship between PFOA serum concentrations measured in young girls and the longitudinal changes in their body mass index (BMI), waist circumference to height ratio, and waist to hip circumference ratio with increasing age.

METHODS:

We used longitudinal data and PFAS serum biomarker measurements from the female puberty cohort of the Breast Cancer and the Environment Research Program to examine the relationship between internal exposure to PFOA and body mass index in girls age 6 to 14 years, and to study changes in the strength of that relationship as the girls aged. The puberty cohort study was designed to explore potential factors associated with earlier breast development and other pubertal milestones, with a primary focus on exposure to endocrine disrupting chemicals [23]. The study has been conducted at three sites in the USA: Mount Sinai School of Medicine (MSSM); Kaiser Permanente Northern California (girls living in the San Francisco Bay Area, SFBA); and Cincinnati Children's Hospital/ University of Cincinnati (CIN) although the MSSM site did not contribute to the current analysis. Girls were recruited at age 6–8 years in 2004–2007, and followed semi-annually or annually to observe onset and progression of pubertal maturation. Eligibility included age (6–8 years), female sex, and no underlying endocrine medical conditions. Descriptions of the puberty study and recruitment process are detailed elsewhere [24]. Measurements of PFAS in sera were made only in girls from the CIN and SFBA sites (N=704). The institutional review board (IRB) at each site approved the study; the Centers for Disease Control and Prevention (CDC) deferred to the IRB at each site as the IRB of record. A total of 1239 girls enrolled, and informed consent was obtained from parents or guardians and assent from the children. Baseline data were collected during 2004–2007.

Data collection methods were consistent at the two sites and included a yearly questionnaire and either annual (SFBA) or semi-annual (CIN) study clinical examinations. Girls in CIN were examined every six months (window of ± 4 weeks) between 2004 and 2010, and then yearly until 2014. At both sites, baseline and yearly questionnaires were administered to the girls' parents or guardians. Trained and certified female staff members performed standardized anthropometric measurements, using calibrated stadiometers and scales, as detailed previously [25, 26]. Weight, standing height, umbilical waist circumference and hip circumference were collected at baseline and at each follow-up visits thereafter using a standard protocol adapted from the National Growth and Health Study [27]. Children wore only light gowns or t-shirts and no shoes. All measurements were taken twice and the absolute difference was calculated. If the difference exceeded the specified tolerance level per National Growth and Health Study protocol, the measurement was repeated a third time, and the two closest measurements were averaged for statistical analyses. BMI was calculated as weight/(height)^2 in kilograms/meter^2, and then related to age- and sex-specific percentiles and z scores (BMIz) using the age-specific CDC growth charts [28]. BMIz, waist:height ratio and waist:hip ratio were used as the dependent variables in the mixed effects model analyses. The median number of measurements during the follow-up period was 7 (range, 3–13 measurements). Phlebotomies were performed at annual visits using pre-screened materials provided by CDC, and sera were aliquotted and frozen within hours of specimen collection.

Data regarding sociodemographic and other characteristics were collected from parents or guardian caretakers via self-completed (CIN) or interviewer-administered questionnaires (SFBA), conducted in English or Spanish. The questionnaires at the two sites contained the

exact same items, but for the initial examination cycles, the method of data collection was by in-person interview in SFBA and by mailed questionnaire in CIN. Socioeconomic status was represented by the highest attained education level of the primary caregiver at baseline, categorized as high school or less, college, and graduate/professional school. For the purpose of this analysis, race/ethnicity has been hierarchically categorized as Black, Hispanic, Asian and non-Hispanic White. Questionnaire data were used to determine the level of physical activity in the 12 months prior to the examination, characterized as hours of moderate or vigorous activity per week. Diet information was obtained by repeated 24-hour diet recall interviews at both sites, administered four times per year [29]

All materials used in collecting blood and processing the serum were provided by CDC. Serum was processed immediately and stored in polypropylene cryovials and placed in −80°C freezers (within <24 hours) until shipment to the CDC for analysis. Samples were refrigerated prior to freezing. Because of the strength of the molecular bonds in PFAS, and the very low likelihood of either biotransformation or biodegradation, the interval between collection and freezing is much less of a concern than for other environmental chemicals.

The participants at the CIN site were recruited through public and parochial schools in the greater Cincinnati metropolitan area (85% of participants) and through the Breast Cancer Registry of Greater Cincinnati (15% of participants). Recruitment through the Registry targeted those with a first or second degree family member with breast cancer. Eligible girls at the SFBA site were those who had been enrolled in the Kaiser Permanente system at San Francisco Bay Area clinics at birth and at the time of study recruitment.

Serum analyses: Concentrations of PFAS in all blood serums were measured at the CDC, using methods published previously [30, 31]. Briefly, after dilution with formic acid (and without protein precipitation), one aliquot of 100 μL serum was analyzed by online solid-phase extraction high performance liquid chromatography–tandem mass spectrometry to quantify trace concentrations of eight PFAS including PFOS and PFOA. Perfluorodecanoic acid (PFDA, also known before as PFDeA) was not measured for the early sample analyses. Most analyses incorporated isotopically-labeled internal standards for PFOS, PFOA and 2-(N-methyl-perfluorooctane sulfonamide) acetate (MeFOSAA, known before as Me-PFOSA-AcOH), 2-(N-ethyl-perfluorooctane sulfonamide) acetate (EtFOSAA, known before as Et-PFOSA-AcOH) and perfluorononanoate (PFNA). The CDC laboratory is certified according to the Clinical Laboratories Improvement Amendments, and its procedures incorporate quality control (QC) measures to ensure accuracy and precision of results, including annual proficiency testing compliance. A laboratory batch must meet QC criteria, including acceptable blanks, or the batch is entirely reanalyzed [32]. For our primary statistical analyses, we used PFAS measurements from the first serum sample collected from all girls. For CIN only, we had repeat measures of PFOA sera concentrations in samples one and three years after the baseline sample, and we used these in a site-specific analysis. There were no repeat measurements made for the SFBA girls.

Statistical analysis: Descriptive statistics were generated for demographic characteristics, PFOA biomarker and outcome variables. Most analyses used PFOA concentrations from the first serum serum obtained during the study. We used the CIN site-specific repeated

measures only for one sensitivity analysis. PFOA concentrations showed non-normal distribution characteristics. (skewness: 2.29–9.44) and therefore, were log-transformed (natural log base) to ensure normal distribution in the statistical analyses. In parametric analyses, we substituted the value LOD/√2 for concentrations below the LOD following the CDC practice [33]. For other analyses, baseline PFOA exposure was characterized by five categorical groups, defined by the quintiles of the distribution of the serum concentrations for both sites combined (Table 2). These common quintile group cut points also were used for the site-specific analyses, for comparison of the effect estimates across sites. Mixed effects models with imputation of missing data were then conducted to estimate the association of baseline PFOA serum concentration on the outcome variables of BMI z-score, waist:height ratio, and waist:hip ratio. Covariates in these models included other factors known to be associated with body mass in children such as race, highest level of parent education (of both parents), average daily Kcal intake over the last 12 months (from diet recalls) and physical activity (moderate + vigorous hours per week, over the last 12 months). Missing Kcal intake and physical activity were imputed by last observation carried forward, or if no prior observation were available, next observation carried backward.

Because others had found that the direction of the relationship between PFOA and BMI changed in adults, we also examined the change in the PFOA effect on BMI with change in age, using a PFOA*Age interaction term. For these analyses, girls were grouped by age at anthropometric measurements. We calculated the combined effect of PFOA and the PFOA by age interaction, which represents the combined effect of PFOA and the change in that effect with change in age, calculated as $(\beta_{PFOA} + \beta_{PFOA*AGE})*\mu_X$ where X equals the log transformed value of PFOA for the observations in the age strata, μX is the mean of X, βPFOA, βPFOA*AGE are the coefficient of the term, PFOA and coefficient of the interaction term between PFOA and age respectively. Additional mixed effects model analyses were conducted using repeat measurements of serum PFOA in CCIN girls only, obtained one and three years after the baseline measurements.

We conducted the same analyses using waist:height and waist:hip ratio as dependent variables, and with age at the anthropometric measurements in all models. Unlike BMI percentile, these ratios were not standardized for age of the girl. All analyses were conducted using SAS version 9.2 (Cary, NC).

RESULTS

Of the 704 girls in the cohort from these two sites, mean age at enrollment was 7.3 years and at date of sample collection was 7.8 years (Table 1); serum samples of the SFBA girls were collected at a slightly older mean age than CIN girls [6.75 (±0.46) years vs 6.70 (±0.61)] years. Girls from the CIN site had parents that were somewhat more educated than the SFBA girls' parents. Cohort members were distributed across four racial groups, although most of the Asian and Hispanic girls were from the SFBA site (Table 1). Most girls (70.6%) had a BMI below the 85th percentile for their age (CDC, 2000) at enrollment, with a BMI percentile median value of 49.6. For girls with a BMI percentile ≥ 85th percentile, the median BMI percentile value was 94.9 (data not shown). The proportion of girls whose BMI percentile was beneath the CDC 85th percentile for age was the same at the two sites (70.8%

of CIN girls versus 70.4% of SFBA girls). Both the mean waist:height and waist:hip ratios were almost identical for the two sites (Table 1).

The median serum PFOA concentration differed for the two sites ($p<0.001$) (Table 2). Baseline examination PFOA serum concentrations in the greater Cincinnati (CIN) area girls were higher than general population values for children [34], especially those living in an area of northern Kentucky, and also higher than in girls in the SFBA [4]. Median concentration of PFOA significantly differed by study site (7.3 ng/mL in CIN vs. 5.8 ng/mL in SFBA girls) and the range of values also differed (<LOD to 55.9 ng/mL in CIN girls vs. 2.4 to 18.2 ng/mL in SFBA girls) (Table 2). PFOA sera concentrations exceeded the NHANES 2005–2006 95^{th} percentile value for children 12–19 years (8.4 ng/mL) in 38.6% of the CIN site girls (136/352) and in 14% of the SFBA girls (50/351). The geometric mean concentrations (adjusted for race/ethnicity, study site, BMI percentile at enrollment and age at sample collection) for PFOA were significantly lower in Black, Asian and Hispanic girls than in Whites ($p<0.01$) (not shown). Girls at the CIN site had significantly higher adjusted geometric mean concentrations of PFOA than SFBA girls ($p<0.001$). When girls were placed into quintiles of PFOA serum concentration, the SFBA group, as expected, had a larger number in the lowest quintile (Ns=102 vs. 47), and the CIN group had the largest number in the highest quintile (Ns=35 vs. 105) (Table 2).

In the mixed effects analyses, log-transformed baseline PFOA was strongly inversely associated with BMIz in the CIN girls ($p=0.001$) and less so in the SFBA girls ($p=0.22$) (Table 3), indicating that girls with higher serum PFOA concentrations had lower BMIs. In analyses combining the girls from the two sites, in fully adjusted models, significant inverse relationships were noted for log-transformed PFOA ($p=0.001$), Asian race, and higher parental education but not other covariates. In analyses where PFOA concentrations were modeled as categorical variables, again, higher PFOA serum concentrations were associated with lower BMIz in the combined sample (top two exposure categories, $p=0.001$, $p=0.044$) and CIN girls (top exposure category, $p=0.001$, and borderline for second exposure category, $p=0.061$) (Table S1, Figure 1),. When PFOA*Age at exam interaction terms were included in the models with log-transformed PFOA, the combined effect of PFOA and the PFOA*age interaction first decreased and then increased in strength with increasing age in CIN girls (Figure 2, Table S2), indicating first a decrease and then an increase in BMI with age. The effect estimates appeared to remain fairly consistent across age for the SFBA girls.

Observations in each of the age group strata did not include identical sets of girls. In order to test whether this difference in the number of girls in various age strata may have created the finding of a change in the relationship between PFOA and BMIz with age in the CIN girls, we conducted a sensitivity analysis using just the CIN girls with BMIz observations in the age 12–13 age strata. In these 281 girls, with almost the same number of observations in each of the age groups, we found the same effects as we had in the entire group of CIN girls (Figure 2, dashed line; also Table S3).

We then used the repeat measurements of PFOA, available in the CIN girls only, in statistical analyses. For 306 girls we had a repeat measurement one year later, and for 42 we had a repeat measurement three years from the baseline. In the CIN cohort, the annualized

decrease in serum concentration between baseline and one year later was 1.29 ng/mL and the annualized decrease between the first repeat and the second (two years later) was 1.00 ng/mL per year. For the 306 girls for whom we had two measurements, the Spearman rank order correlation between the baseline measures and the repeat measures one year later was 0.84 ($p<0.0001$). When the repeat measurements were used in the mixed effects model analyses with all of the girls (N=333), the effect of PFOA on BMIz was no longer significant ($p=0.249$) and was much decreased in magnitude, with a decrease in BMIz of only −0.028 per log-transformed unit of PFOA, compared to the analysis without repeat measures where the decrease in BMIz was −0.333. The changes in the strength of these effect estimates is likely attributable to the decrease in serum PFOA with time, through excretion including possibly menstruation during the latter time periods. Restricting the analysis to only the girls with at least one repeat measure (N=295) resulted in a decrease in BMIz of only −0.033. When we examined the combined effect of PFOA and the PFOA*age interaction, we noted a similar pattern of a decreasing and then increasing BMI with increasing age (Table 4, Figure 3), regardless of whether all girls were included in the analysis or just those with repeat measures.

Waist:height ratio also was inversely associated with PFOA in the CIN girls, as expected since BMI and waist:height ratio are highly correlated (Table S4) [24]. The decrease in waist:height ratio with increasing serum PFOA concentration increased with age in the CIN girls (Figure S1) but the effect of PFOA serum concentration on waist:height ratio in the SFBA did not change with age, and then became unstable. We also noted this age-related inverse PFOA serum concentration effect with waist:hip ratio in the Cincinnati girls, but in the SFBA girls was again relatively unchanged and then unstable (Figure S2). In analyses without the PFOA*age interaction, waist:hip ratio was not associated with PFOA serum concentration at either site (Table S5).

DISCUSSION

The Ohio River is a drinking water source for many persons living in the Mid-Ohio Valley, including the greater Cincinnati area. In 2009, water concentrations of PFOA in the Ohio River ranged from 2.5 ng/L in Pittsburgh, Pennsylvania (upstream of a plant processing PFAS), to 35.2 ng/L in Ravenwood, West Virginia, and 13.1 ng/L (13,100 ppt) in Cincinnati, Ohio at two locations downriver [35]. The current EPA drinking water advisory for the combined concentrations of PFOS and PFOA is 70 ppt (or ng/mL) [36]. In sera of adult residents of the greater Cincinnati area collected in 1991–1993, the adjusted geometric mean concentrations for persons in northern Kentucky was 13.5 ng/mL and for Cincinnati was 16.4 ng/mL [34]. Many production facilities of the semi-conductor industry are located in the San Francisco Bay area; PFOA is used in semi-conductor production. To our knowledge, the only publicly-available information about PFOA water concentrations in the SFBA is from the public water testing mandated by EPA [37], and no PFOA was detected in drinking water in 2013–2016 [38].

Findings of previous studies by us [4, 39] suggest that the primary exposure route for greater Cincinnati girls was drinking water and that the source was an industrial facility upriver from Cincinnati, in West Virginia. Since production at this facility has been curtailed since

2002 and ceased in 2011, it is likely that the serum concentrations of the CIN girls were higher around the time of their birth, supported by serum concentrations measured in the Cincinnati area adult cohort in the 1990s (adjusted geometric mean 16.4 ng/mL) and PBPK modeling that we have performed [39]. In the current study, the strength of the inverse association between serum PFOA concentrations and BMI weakened with age, an effect modification that may explain the differences in the findings of other studies of children versus those of adults. The direction of the relationship changed when going from the 10–11 year age group to the 12–13 year age group, about the time of menarche. Waist:height ratio also was inversely associated with PFOA in the CIN girls, as expected because these two measures are highly correlated. Waist:hip ratio was not associated with PFOA serum concentration at either site. The mechanism by which PFOA alters body composition is not known. Altered hepatic function with PFOA exposure has been demonstrated in multiple rodent studies [40], which may consequently alter cholesterol and steroid metabolism with downstream effects on body composition in humans [41–43].

There is ample evidence that children are exposed to PFAS [44], both *in utero* and during development. PFAS have been detected in cord blood, breast milk, infant formula and food [45–48] and cord blood and breast milk concentrations are correlated with maternal serum concentrations [46, 49–51] [52]. The binding characteristics of PFOA to serum albumin allow it to readily cross the placenta with greater efficiency than other PFAS [14] [53], exposing the developing fetus to chronic low-dose levels of PFOA throughout pregnancy. Having been breast fed is associated with higher PFOA serum concentrations in children [29] and decrease in the body burden in mothers [54].

There is a large body of scientific evidence that PFOA can have disruptive biologic effects on the metabolism and growth of animal species and humans, but the findings of studies of the effects on birthweight and body weight of children are equivocal. Studies in animals have shown that low-dose intrauterine exposure to PFOA disrupts normal fetal growth and development resulting in lower birth weight of pups with exposure to PFOA [10] [11]. Many studies in humans have reported an inverse association between maternal or cord blood PFOA serum concentration and birth outcomes such as lower birth weight, smaller head circumference and ponderal index, and lower gestational age [12–14, 51, 55, 56]. Several studies in girls have noted lower birthweight with maternal exposure [14],[13]. However, two recent analyses of PFOA in maternal serum did not reveal any relationship with birthweight adjusted for gestational age [57] [58].

In humans, the direction of the change (increase or decrease in body weight or BMI) seems to be dependent on the age at exposure to PFOA (window of susceptibility) [23], the degree of exposure, and the age at BMI or adiposity measurements. Some studies have reported higher serum PFOA concentrations appear to be associated with lower BMI in girls, such as those from the greater Cincinnati area, while others report a direct relationship. Many studies report that the effect is either limited to, or stronger in, females. Among studies of prenatal exposure, higher prenatal concentrations of PFOA in Project Viva mothers (median, 5.6 ng/mL) were not associated with adiposity in girls at both early (median 3.2 years of age) and mid-childhood (median 7.7 years) [19]. An analysis of a Danish birth cohort also found no association between BMI at age seven and maternal serum concentrations of

PFOA, but the maternal serum concentrations were relatively low (median 2.0 ng/mL) [59]. A prospective cohort study of Norwegian and Swedish mothers found a direct relationship between maternal serum concentrations of PFOA and PFOS and their children's BMIz at 5 years of age, although the concentrations found in maternal serum collected in 1986–1988, PFOA (1.64 and 2.33) and PFOS (9.62 and 16.3 ng/mL), were much lower than those reported for adult women in the USA [20]. In the Avon longitudinal cohort, maternal serum concentrations of PFOA were associated with body fat percent at age 9 years, but only in offspring of mothers who had obtained a certificate of secondary education, and not among those with a university education [60]. HØyer et al. [21] examined multiple anthropometric measures in children from Greenland and the Ukraine, and found that waist:height ratio was slightly greater with higher maternal prenatal PFOS (median 10.8 ng/mL) and PFOA (median 1.3 ng/mL) concentrations [21]. Braun et al. [22], in an analysis using a Cincinnati area birth cohort of both girls and boys (with no overlap with our study participants), reported greater adiposity in both sexes among children age 8 years whose mothers had higher serum PFOA concentrations, although there are differences in the cohorts. Pregnant women in the HOME cohort were recruited in March 2003 to January 2006, and only from Ohio [22], whereas the girls in our puberty cohort were enrolled in 2004–2006 from both Ohio and Kentucky, at ages 6–8 years, and their mothers were pregnant 1997–2000 when serum concentrations in the greater Cincinnati probably were higher. PFOA median serum concentration of mothers was 5.3 ng/mL. Of note, the median serum concentrations of the HOME study children were 5.4 ng/mL at 3 years of age and 2.4 ng/mL at 8 years of age [61], lower than in our CIN girls, whose median PFOA serum concentration was 7.3 ng/mL. Maisonet et al. in a study of British girls, reported that offspring of mothers with higher prenatal serum PFOS concentrations had lower weight at birth but greater weight at 20 months, after adjustment for birth weight [13]. This study also reported that exposure to PFOA exemplified a "critical window" effect, with high concentrations of PFOA during the post-weaning period showing lower body weight gain and PFOA concentrations during adulthood having no effect on body weight gain.

Studies examining BMI in adults, in both animals and humans, have reported an association with exposure to PFOA, particularly *in utero* or prenatal exposure. Hines et al. (2009) reported that low-dose levels of in-utero exposure to PFOA in mice resulted in increased body and organ weight gain and rate of weight gain in mid-life adult mice [62]. Halldorsson et al. [17] reported that the young adult female (but not male) offspring of mothers who had the highest PFOA serum concentrations had both significantly higher BMI ($p = 0.001$) and greater waist circumference ($p = 0.006$) at mean age 20 years. In a study of mid-Ohio Valley residents, neither male nor female adults who were exposed to PFOA during the first three years of life were at increased risk of being overweight or obese as adults [18]. For about half of the study participants, the estimate of their early life PFOA exposure was above the NHANES geometric mean for adults in 2005–2006 [63]. However, in a recent randomized clinical trial of weight loss in adults, persons with higher PFOA had greater decline in resting metabolic rate during the weight loss period, and less gain in resting metabolic rate and more increase in the visceral fat mass during the weight regain period and also gained more weight [9]. These equivocal findings, in both children and adults, are likely related to

different exposures at different windows of susceptibility [23], but support an exposure effect mechanism that alters body composition.

When compared with previous studies of PFOA and anthropometry in children, our study has numerous strengths. Our sample of girls is large and racially diverse; socioeconomic position was similar in girls from the two sites [64]. Body measurements were made at dedicated study clinical visits by trained study personnel, and because of the prospective design of the study, we have repeat measurements of anthropometry across time. PFAS measurements were made using state-of-the art methods by a CLIA laboratory with top quality assurance standards. Information on covariates was collected using standardized data collection methods. Using mixed effects models in our statistical analysis, where each clinical examination was an observation, allowed us to use all of the data, both the semi-annual examinations at the CIN site and the annual examinations at the SFBA site. Because of the semi-annual clinical visits, there were a greater number of observations from the Cincinnati site in these analysis records, which improved the precision of the Cincinnati site effect estimates. Limitations of the study include drawing participants from only two urban/suburban areas of the USA, and self-selection by parents and girls who were willing to participate in a multi-year study with multiple research clinical visits. In these analyses, we used serum measurements of the PFAS, obtained during childhood, whereas the exposure in utero or the peri-natal period may represent the most important window of susceptibility. However, because of the long half-life of PFOA and several of these PFAS, it is likely that the relative ranking would remain consistent if earlier measurements were available. The decrease in the strength of the PFOA effect with age observed by us was based on an analysis of baseline PFOA measurements only, so would not be a result of dilution of PFOA in serum with increasing blood volume with growth [65], or due to loss of PFOA due to menstration but also may indicate that the earlier in life internal exposure (prenatal) may be more strongly related to the effect on body composition. The median PFOA serum concentration in a large study California women in 2011–2015 was 2.47 ng/mL [66], which were the earliest measurements that we were able to locate. These levels were higher than women in the U.S. general population in 2013–2014 (1.67 ng/ml) (CDC) [6], and similar to adults in the greater Cincinnati population in 2011–2013 [39]. However, in serum obtained in 1997–2000 from persons living in the northern Kentucky counties (part of greater Cincinnati and included in the recruitment area for the CIN girls), which was around the time that the girls in this study were born, serum concentrations were considerably higher (15.2 ng/mL) [39]. Ongoing birth cohort studies with long-term follow-up and multiple PFAS measures will be able to address the questions of differential effect of exposure at various windows of susceptibility.

Our study shows that BMI and waist:height ratio are lower in girls with higher peripubertal serum PFOA concentrations, but as the girls age, the direction of this relationship reverses around the age of 11–12 years, a time at which menarche would be expected. For each log-transformed ng/mL increase in serum concentration above the U.S. population median value in girls 6–8 years, we found that BMIz was decreased by 0.333 units in the CIN girls, who probably had higher serum concentrations *in utero* and earlier in life. The timing of the greatest exposure relative to windows of susceptibility may explain the heterogeneity in the PFOA effect on body weight detected at two sites of our study population. While the

mechanism behind these PFOA-related changes in body weight and composition in girls not entirely clear, it is likely that the inverse relationship to body weight is related to an older age at initial breast development (thelarche) and menarche as others have noted [67] and we intend to study in our cohort. We hope to follow these girls into adulthood, to assess the effect of PFOA exposure on adult body weight and potential risk of adverse breast health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding: U01ES012770, U01ES012801, U01ES019435, U01ES019453, U01ES019457, U01ES026119, P30ES006096, T32-ES10957, UL1RR024131, CSTA-UL1RR026314

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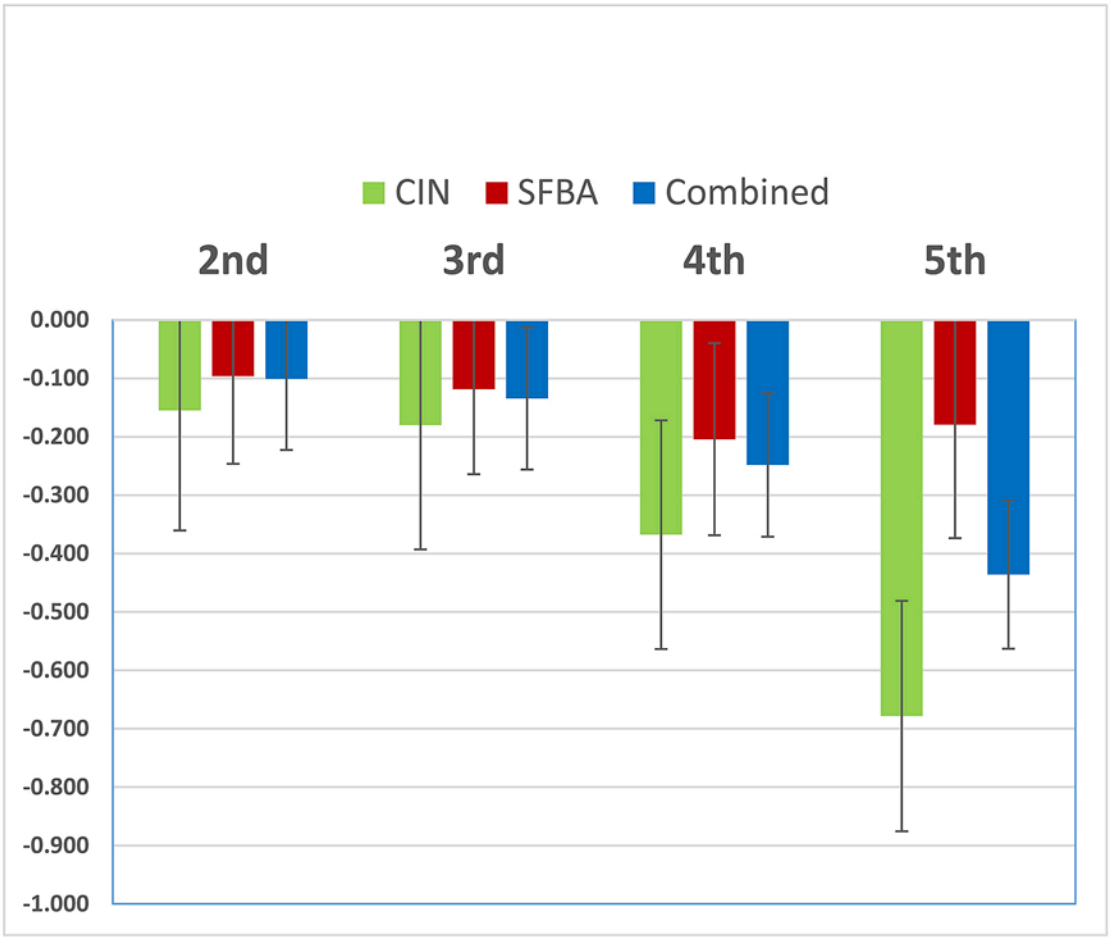

Figure 1: Effect of PFOA expoexposure on BMI[1] (quintile β estimates)[2]

[1]The effects of PFOA and other covariates on BMIz were derived from a model which contained race/ethnicity, parent education, PFOA, age at examination, average kilocalories in prior 12 months, hours of moderate or vigorous physical activity in last 12 months

[2] PFOA exposure was modeled as quintile groups of the values of the PFOA biomarker

CIN=Cincinnati; SFBA-San Francisco Bay Area

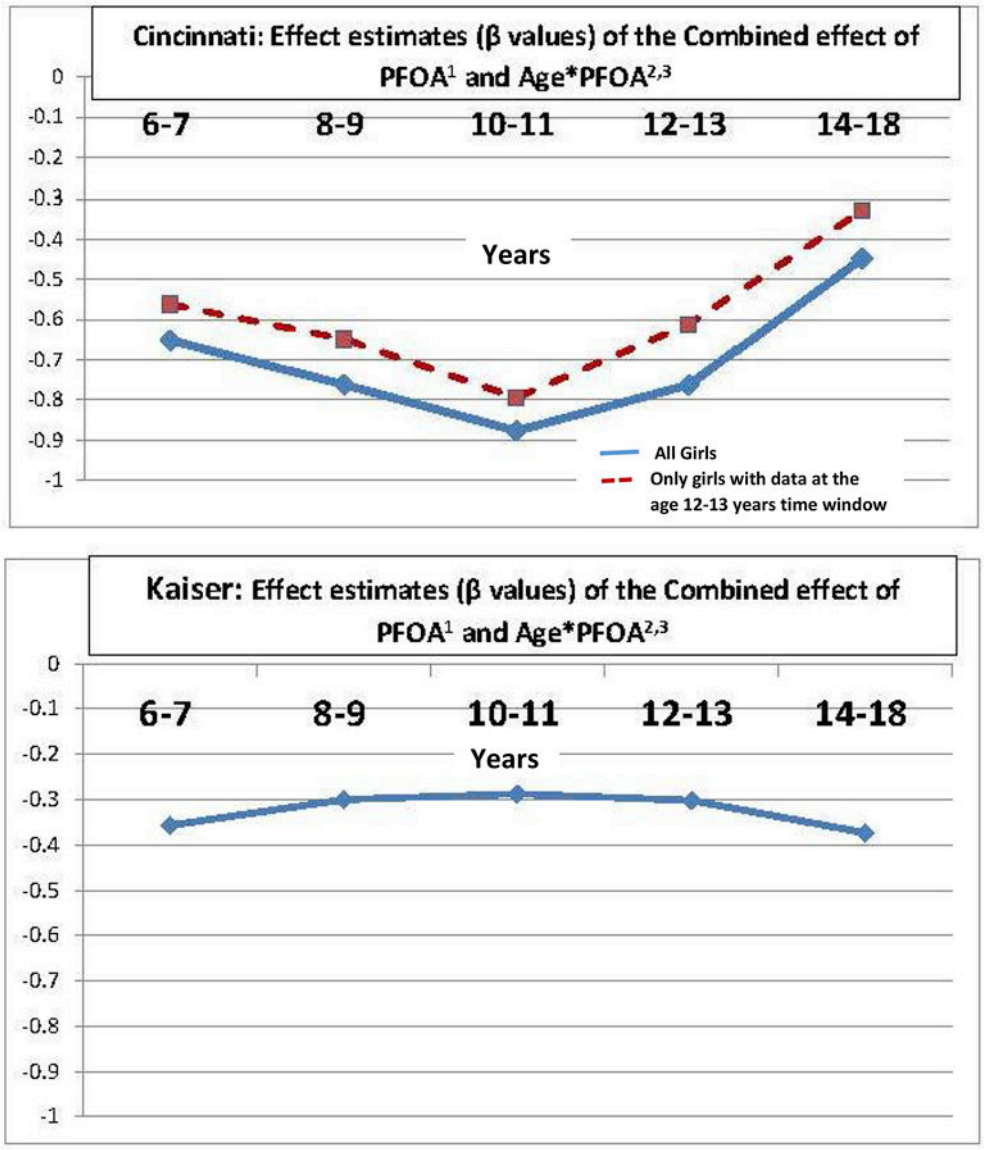

Figure 2: Effect estimates (β values) of the combined effect of PFOA and Age on BMIz

[1] The effects of PFOA and other covariates on BMIz were derived in a model which contained race/ethnicity, parent education, PFOA, age at examination, average kilocalories in prior 12 months, hours of moderate or vigorous physical activity in last 12 months

[2]Age was modeled in four categories (6–7, 8–9, 10–11, 12–13 and 14 or older) with age 8–9 serving as the comparison group. The combined effect of PFOA and age for each age group was calculated as βPFOA + βPFOA*AGE)*μX where X is the log transformed values of PFOA for the observations in the age strata, μX is the mean of X, βPFOA, βPFOA*AGE are the coefficient of the term, PFOA and coefficient of the interaction term between PFOA and age respectively.

[3] Dashed line represents the mixed effects sensitivity analysis, with a set of observations restricted to those of girls with a observations in the age 12–13 years strata

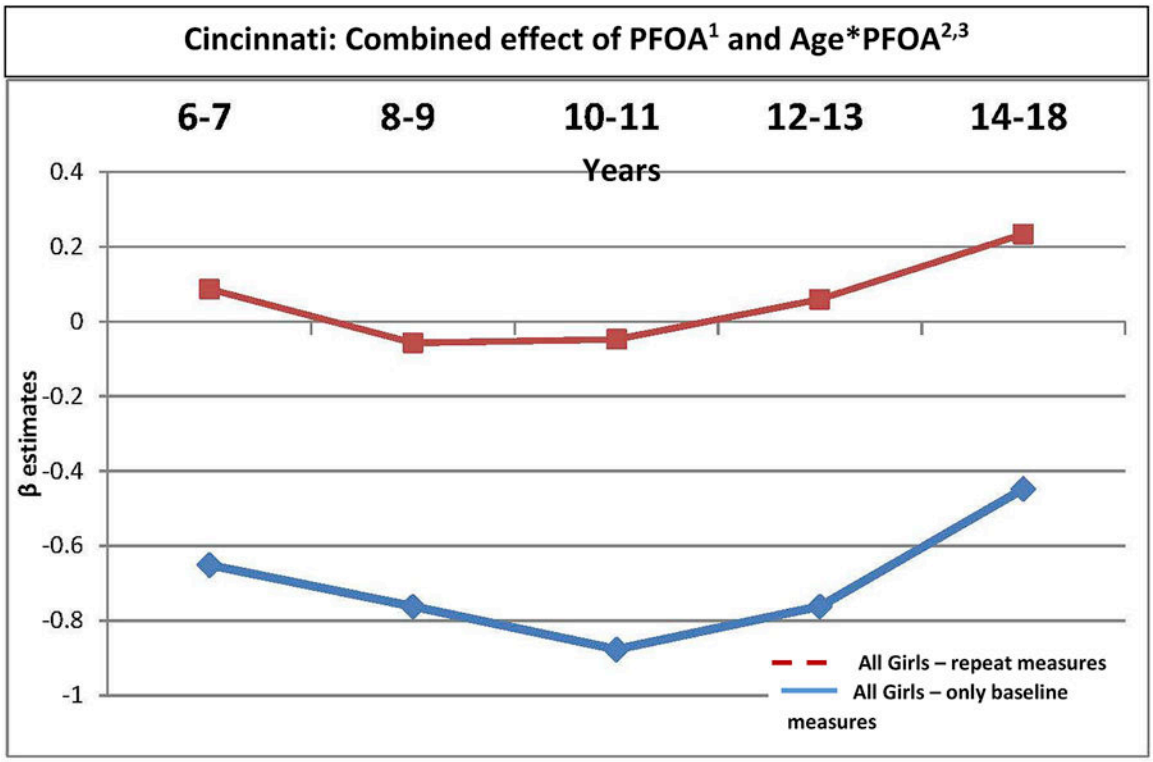

Figure 3: Combined effect of PFOA and Age on BMIz in analyses with repeat measurements of serum PFOA, Cincinnati site only

[1]The effects of PFOA and other covariates on BMIz were derived from a model which contained race/ethnicity, parent education, PFOA, age at examination, average kilocalories in prior 12 months, hours of moderate or vigorous physical activity in last 12 months

[2]Age was modeled in four categories (6–7, 8–9, 10–11, 12– 13 and 14 or older) with age 8–9 serving as the comparison group. The combined effect of PFOA and age for each age group was calculated as βPFOA + βPFOA*AGE)*μX where X is the log transformed values of PFOA for the observations in the age strata, μX is the mean of X, βPFOA, βPFOA*AGE are the coefficient of the term, PFOA and coefficient of the interaction term between PFOA and age respectively.

[3] The **dashed line** represents the β effect estimates that resulted from the mixed effects models analysis which include all of the girls and also the repeat measures of serum PFOA. The **solid line** represents the β effect estimates when only the baseline serum PFOA measurements were included in the model (which is the same as the blue line in Figure 2).

Table 1:

Study Population Demographic Characteristics

Characteristic	Greater Cincinnati		San Francisco Bay Area	
	N	%	N	%
Participants with PFC measures	353	50.1%	351	49.9%
Age at sample collection (years)				
6.0–6.9	59	16.7%	62	17.7%
7.0–7.9	137	38.8%	176	50.1%
≥8.0	157	44.5%	113	32.2%
Race/Ethnicity				
Black	119	33.7%	77	21.9%
Hispanic	14	4.0%	84	23.9%
Asian	5	1.4%	41	11.7%
White	215	60.9%	149	42.5%
Parental Education				
≤ HS Diploma	33	9.3%	65	18.5%
Technical or Associate Degree	116	32.9%	110	31.3%
Bachelor's Degree	103	29.2%	101	28.8%
Master's Degree or Higher	83	23.5%	44	12.5%
BMI For Age at Sample				
Below the 85th Percentile	250	70.8%	247	70.4%
At or Above the 85th Percentile	103	29.2%	104	29.6%
Kcal consumed/day				
Mean (sd) - all records	1.656	(0.378)	1.584	(0.356)
Waist Height Ratio				
Mean (sd) - all records	0.450	(0.040)	0.460	(0.045)
Waist Hip Ratio				
Mean (sd) - all records	0.840	(0.039)	0.854	(0.040)

Table 2:

PFOA Serum Concentrations by Site

	Cincinnati		San Francisco		Combined	
	N		**N**		**N**	
	353		351		704	
N < LOD (%)	1 (0.3%)		0 (0.0%)		1 (0.0%)	
Median (ng/mL)	7.3		5.8		6.4	
Mean (SD)(ng/mL)	9.50(7.41)		6.13(2.31)		7.82(5.74)	
Range (ng/mL)	0.07 – 55.9		2.40-18.20		0.07–55.9	
Quintile group median	ng/mL	N	ng/mL	N	ng/mL	N
PFOA≤4.6 ng/mL	4.0	47	4.0	102	4.0	149
4.6<PFOA≤5.7 ng/mL	5.2	64	5.2	72	5.2	136
5.7<PFOA≤7.1 ng/mL	6.4	53	6.4	86	6.4	139
7.1<PFOA≤9.1 ng/mL	8.2	84	7.9	56	8.0	140
PFOA>9.1 ng/mL	13.4	105	10.4	35	12.1	140

Table 3:

Effect of Serum PFOA Concentration on BMIz, for Each Site and the Two Sites Combined

	Cincinnati				San Francisco				Combined			
	N=333 girls; 3823 records				N=334 girls; 2574 records				N= 667 girls; 6397 records			
	95% CI β				95% CI β				95% CI β			
Variable	**β est**	**Lower**	**Upper**	**p-value**	**β est**	**Lower**	**Upper**	**p-value**	**β est**	**Lower**	**Upper**	**p-value**
Log transformed Serum PFOA	−0.333	−0.529	−0.137	0.001	−0.185	−0.481	0.111	0.220	−0.264	−0.416	−0.112	0.001
Race[1]												
Black	0.238	−0.038	0.515	0.091	0.262	−0.023	0.546	0.071	0.250	0.053	0.447	0.013
Hispanic					0.256	−0.042	0.555	0.093	0.263	−0.006	0.531	0.056
Asian					−0.465	−0.801	−0.129	0.007	−0.520	−0.850	−0.191	0.002
White (ref)	0.000	***	***	***	0.000	***	***	***	0.000	***	***	***
Parental Education[2]												
≤ HS Diploma or some college/vocat	−0.148	−0.557	0.261	0.479	−0.083	−0.406	0.241	0.617	−0.099	−0.354	0.157	0.450
MS or higher	−0.179	−0.446	0.088	0.188	−0.380	−0.626	−0.135	0.002	−0.284	−0.464	−0.103	0.002
Assoc or Bachelor's Degree (ref)	0.000	***	***	***	0.000	***	***	***	0.000	***	***	***
Average Kcal (in prior 12 months)	0.138	0.099	0.178	<.0001	0.016	−0.042	0.073	0.594	0.103	0.071	0.135	<.0001
Physical Activity (Hour/wk, moderate + vigorous, in prior 12 months)	−0.001	−0.004	0.001	0.340	−0.006	−0.011	−0.001	0.024	−0.002	−0.005	0.000	0.057

[1] For Race, White was the referent group

[2] For Parental Education, Assoc or Bachelor's Degree was the referent group

Table 4.

Effect of Serum PFOA Concentration and PFOA*Age Interaction on BMIz using PFOA Repeat Measurements, for All Cincinnati Girls and for Just Cincinnati Girls with Repeat Measurements. All girls Just those with repeat measurements

	All girls				Just those with repeat measurements			
	N=333 girls; 3823 records				N=281 girls; 3571 records			
		95% CI β				95% CI β		
Variable	**β est**	**Lower**	**Upper**	**p-value**	**β est**	**Lower**	**Upper**	**p-value**
Log transformed Serum PFOA	−0.028440	0.079	0.020	0.249	−0.332	0.083	0.016	0.188
PFOA * 6–7 years	0.070400	0.001	0.128	0.016	0.065	0.006	0.125	0.032
PFOA * 8–9 years	***	***	***	***	***	***	***	***
PFOA * 10–11 years	0.001385	0.054	0.057	0.961	−0.005	0.062	0.052	0.853
PFOA * 12–13 years	0.065970	0.003	0.129	0.041	0.052	0.013	0.117	0.115
PFOA * 14–18 years	0.176400	0.116	0.237	<.0001	0.170	0.108	0.231	<.0001
Age at exam, 6–7 years	−0.200400	0.324	−0.077	0.002	−0.178	0.307	−0.048	0.007
Age at exam, 8–9 years	***	***	***	***	***	***	***	***
Age at exam, 10–11 years	0.007758	0.101	0.117	0.889	0.012	0.100	0.124	0.834
Age at exam, 12–13 years	−0.018100	0.133	0.097	0.758	−0.008	0.126	0.110	0.897
Age at exam, 14–18 years	−0.151200	2.261	−0.041	0.007	−0.145	0.258	−0.033	0.012
Race[1]								
Black	0.395300	0.128	0.662	0.004	0.364700	0.088	0.641	0.010
White	***	***	***	***	0.000	***	***	***
Education[2]								
≤ HS Diploma or some college/vocat	−0.059370	−0.473	0.354	0.778	0.074	−0.349	0.498	0.731

	All girls				Just those with repeat measurements			
	N=333 girls; 3823 records				N=281 girls; 3571 records			
	95% CI β				95% CI β			
Variable	**β est**	**Lower**	**Upper**	**p-value**	**β est**	**Lower**	**Upper**	**p-value**
		-				-		
MS or higher	−0.230900	0.501	0.039	0.094	−0.213	0.494	0.069	0.382
Assoc or Bachelor's Degree	***	***	***	***	0.000	***	***	***
Average Kcal (in prior 12 months)	0.082730	0.042	0.123	<.0001	0.089	0.048	0.130	<.0001
		-				-		
Physical Activity (Hour/wk, moderate + vigorous, in prior 12 months)	0.001	0.002	0.003	0.688	0.001	0.002	0.003	0.688

[1] For Race, White was the referent group

[2] For Parental Education, Assoc or Bachelor's Degree was the referent group