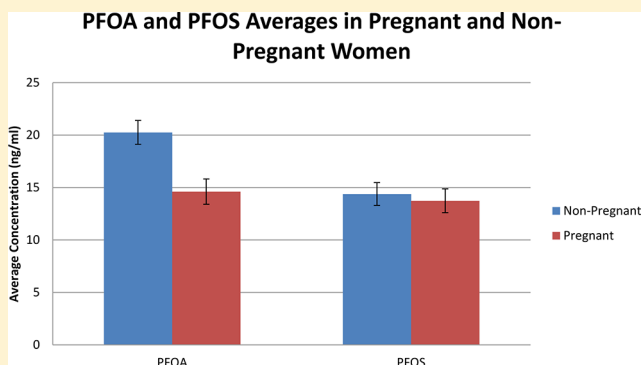


Circulating Maternal Perfluoroalkyl Substances during Pregnancy in the C8 Health Study

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ABSTRACT: Perfluoroalkyl substances are manmade chemicals used in many consumer products and have become ubiquitous in the environment. Animal studies and a limited number of human studies have demonstrated developmental effects in offspring exposed to perfluoroalkyl substances in utero, but the implications of timing of in utero exposure have not been systematically investigated. The present study investigated variation in perfluorocarbon levels of 9952 women of childbearing age who had been exposed to perfluorooctanoic acid (PFOA) in drinking water contaminated by industrial waste. An analysis of variance with contrast was performed to compare the levels of PFOA and perfluorooctanesulfonic acid (PFOS) in pregnant and nonpregnant women overall and during each trimester of pregnancy. We found that pregnant women had lower circulating PFOA and PFOS concentrations in peripheral blood than nonpregnant women and that PFOA levels were consistently lower throughout all trimesters for pregnancy, suggesting transfer to the fetus at an early stage of gestation. These results are discussed in the context of the endocrine-disrupting properties of perfluoroalkyl substances that have been characterized in animal and human studies. Our conclusion is that further, systematic study of the potential implications of intrauterine perfluorocarbon exposure during critical periods of fetal development is urgently needed.



INTRODUCTION

Perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are manmade perfluoroalkyl substances historically used in many consumer and industrial products. PFOA has been used primarily in the linings of cookware and food containers, and PFOS in stain-resistant clothing and textiles, cleaning agents (waxes and floor polishes), and paint and varnish.¹ Because of their widespread use, perfluoroalkyl substances have become ubiquitous in the environment and can be found in soil, water, wildlife, and in measurable quantities in most humans,² even those living in remote areas.³ Data from a representative random sample (National Health and Nutrition Examination Survey, NHANES) in the United States indicate that 98% of participants had measurable quantities of PFOA and PFOS.⁴

Several animal studies have addressed the issue of reproductive toxicity. Leubker et al.⁵ and Grasty et al.⁶ found that pup perfluoroalkyl substance levels were proportional to serum concentrations in the mothers, indicating that females transferred perfluoroalkyl substances to their offspring. It has also been shown that daily perfluoroalkyl substance exposure in pregnant rats, mice, and rabbits resulted in complications varying from altered feed consumption to hepatomegaly, decreased litter size, and increased litter reabsorption.^{6–10} Additional studies have found complications in offspring exposed to perfluoroalkyl substances in utero that included a decreased rate of survival,¹¹ decreased fetal weight,¹⁰ and

developmental and birth defects (e.g., delayed ossification of bones, enlarged right atrium, cleft palate, and inhibition of lung maturation).^{8–11}

Results from these studies have raised concerns about the health consequences of perfluoroalkyl substance exposure in pregnant women. Although human research is limited, PFOA was documented in all 100 umbilical cord blood samples of one study,¹² and an association between maternal and fetal exposure to perfluoroalkyl substances has been documented through umbilical cord blood concentrations at birth that were proportional to maternal serum concentrations.^{13–18} The Danish Cohort Study reported that maternal serum levels of PFOA and PFOS decreased in the second trimester compared to the first,¹⁶ and a small study of 105 babies detected both PFOS and PFOA in umbilical cord blood. That same study showed that midpregnancy maternal serum PFOS levels were higher than the maternal values at delivery.¹⁷ Together these data lend strong support for transfer of perfluoroalkyl substances to the fetus in the latter stages of pregnancy. Further human research has found an inverse association between gestational age and circulating perfluoroalkyl substances in pregnant mothers,^{16,17} and data from a small sample

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Table 1. Select Characteristics of the Study Population^a

	nonpregnant women (<i>n</i> = 9454) ^b			pregnant women (<i>n</i> = 498) ^b		
	median	mean	std dev	median	mean	std dev
age (years)	30.74	30.44	±7.00	26.26	26.93	±5.14
hematocrit (%)	40.90	40.79	±3.04	36.90	37.06	±3.30
hemoglobin (g/dL)	13.80	13.74	±1.05	12.50	12.54	±3.30
hemoglobin/hematocrit ratio	0.34	0.34	±0.01	0.34	0.34	±0.01
parity (no. of times pregnant)	1.00	1.57	±1.48	2.00	1.75	±1.44
	nonpregnant women (<i>n</i> = 9454) ^b			pregnant women (<i>n</i> = 498) ^b		
	% of nonpregnant women	PFOA (ng/mL)	PFOS (ng/mL)	% of pregnant women	PFOA (ng/mL)	PFOS (ng/mL)
Highest Level of Education Obtained						
less than high school diploma	8.4	32.29	15.00	11.3	22.01	11.08
high school diploma or GED	34.1	44.56	16.88	29.8	22.03	12.93
some college	41.5	44.18	17.60	41.0	22.11	16.41
bachelor's degree or higher	16.0	40.21	17.60	17.9	35.84	15.70
Health Behaviors						
currently smokes	31.6	42.73	15.11	22.4	19.54	12.62
currently drinks	53.2	45.73	17.20	32.2	25.62	14.93
Average Yearly Household Income						
<\$10 000	14.6	34.21	15.58	17.8	20.76	12.76
\$10 000–19 999	16.8	39.28	16.10	20.0	19.26	14.10
\$20 000–29 999	15.8	40.28	16.80	15.2	22.18	16.30
\$30 000–39 999	13.7	41.67	17.36	9.7	33.04	15.34
\$40 000–49 999	11.0	43.86	17.74	13.3	26.83	15.29
\$50 000–59 999	8.8	55.67	17.85	8.30	26.25	14.56
\$60 000–69 999	7.0	48.28	18.03	5.7	23.22	17.66
≥\$70 000	12.4	51.31	18.68	10.0	40.06	13.95
Residential History of Perfluoroalkyl Substance Exposure						
high	41.0	77.14	17.83	37.0	45.61	15.58
medium	30.8	27.56	16.52	34.5	15.87	13.91
low	24.2	11.18	16.80	28.5	8.54	14.57

^aPercentages are rounded to the nearest decimal. ^bThis number reflects the total number of participants for the respective group. However, it varies slightly across cells due to missing values.

of women in NHANES indicate that pregnant women may have lower perfluoroalkyl substances levels than nonpregnant women.¹⁹

Thus, accumulating human and animal research raises concerns about in utero exposure to perfluoroalkyl substances. The purpose of the present study was to investigate variation in perfluoroalkyl substances levels in pregnant women during gestational trimesters and compare them with those of nonpregnant women in a large geographic area where exposure to PFOA in drinking water had resulted from toxic waste disposal. On the basis of earlier animal and human data, we hypothesized that redistribution of perfluoroalkyl substances to the fetus during gestation would result in lower circulating serum perfluoroalkyl substance levels in the peripheral blood of mothers during all trimesters of pregnancy compared with levels found in nonpregnant women.

MATERIALS AND METHODS

Participants. Participants were 9952 women between the ages of 18 and 42 years enrolled in the C8 Health Project, which resulted from a class action suit. The “class” was defined as individuals in West Virginia or Ohio who had consumed contaminated drinking water at a residence, place of employment, or school within a specific area of West Virginia and Ohio for at least a year between 1950 and December 3, 2004. Details have been described elsewhere.²⁰ Because of the circumstances of the study, the average level of exposure in these participants

was higher than in NHANES.¹⁹ Eligibility requirements for inclusion of women in the study were that their residential water level exposure was classified as consistent with respect to geographic area prior to measurement (as described below), that they were of childbearing age (defined as 18–42 years), and that they had responded to the question on pregnancy status. There were 498 pregnant women and 9454 nonpregnant women who met the inclusion criteria. Eligible enrolled participants filled out surveys with demographic, medical, and other information and submitted a voluntary blood sample between August 1, 2005 and August 31, 2006. A detailed description of the consent, surveys, blood processing, and blood sample storage is available online at <http://www.hsc.wvu.edu/som/cmed/c8/>. Month of pregnancy was measured by self-report. The study was cross-sectional, meaning that perfluorocarbon measurements were done once in each woman.

Residential History of Exposure. Because this was an exposure study, a complete residential history was obtained from each participant to provide an indication of consistency and amount of exposures. Participants were eligible for the study if they had regularly consumed drinking water from one of the six water districts of PFOA exposure based on residence or having regularly worked or gone to school there. Interpreting the exposure of participants who had never lived in the C8 Health Study water districts but were included due to regular exposure in their workplaces or schools would have been

Table 2. PFOA and PFOS Levels in Pregnant and Nonpregnant Women

	PFOA (ng/mL)				PFOS (ng/mL)			
	mean	median	min	max	mean	median	min	max
all women (<i>n</i> = 9952)	42.26	17.50	0.25	8162.80	17.01	15.20	0.25	106.60
nonpregnant women (<i>n</i> = 9454)	43.19	17.80	0.25	8162.80	17.13	15.30	0.25	106.60
pregnant women (<i>n</i> = 498)	24.49	12.20	1.10	295.60	14.71	12.90	0.25	56.00
women in first trimester (<i>n</i> = 128)	25.42	12.40	1.50	227.30	16.32	15.00	1.80	56.00
women in second trimester (<i>n</i> = 193)	24.92	12.50	1.10	295.60	14.46	12.40	0.25	46.50
women in third trimester (<i>n</i> = 166)	23.69	12.00	1.40	194.50	13.63	12.10	1.30	41.50

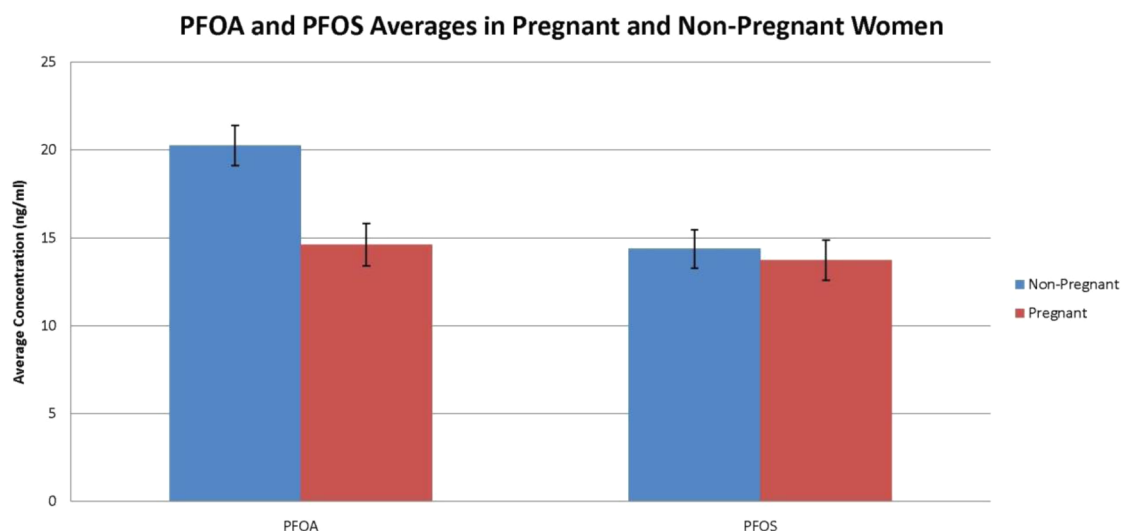


Figure 1. Average PFOS and PFOA concentrations in pregnant and nonpregnant women.

difficult. Also, including women who had moved between districts with high, medium, or low levels of exposure would also have made interpretation difficult. Therefore, nonresidents and inconsistent residents were excluded from the analyses. There were 1604 women excluded due to not having resided in any of the C8 Health Study water districts, and 1750 were excluded due to inconsistent residential history. This left 498 pregnant women and 9454 nonpregnant women whose data were analyzed.

Blood Sample Processing and Laboratory Methods. Participants voluntarily submitted up to 26 mL of blood for analysis in the C8 Health Study, and the resulting serum was banked during the collection period (2005–2006). Perfluoroalkyl substance concentrations were quantified at Exygen Research Inc., State College, PA. The analytical protocol was a modification of a previously described procedure combining protein precipitation extraction and reverse-phase high-performance liquid chromatography–tandem mass spectrometry. Spectrometric detection was performed on a triple-quadrupole mass spectrometer in selected reaction monitoring mode, monitoring for the individual *m/z* transitions for each of the 10 perfluoroalkyl substances and the ^{13}C -PFOA internal standard. A description of the perfluoroalkyl acid analytic techniques and quality assurance protocols for the C8 Health Project has been published elsewhere.²⁰

Statistical Analyses. Statistical analyses were performed by use of JMP/PRO 10 visualization software (SAS Institute Inc., Cary NC). Because month of gestation was self-reported and calculating exactly when the pregnancy started was not exact, months 1 and 2 were combined in the analyses. An analysis of variance and covariance with a contrast statement comparing

pregnant and nonpregnant women was calculated to investigate the differences in PFOA and PFOS concentrations between pregnant and nonpregnant women. Separate analyses were also calculated to compare nonpregnant women with those in each trimester of pregnancy (defined as months 1–3, 4–6, and 7–9). The natural logarithm for PFOA and PFOS was used in all analyses to mitigate the effects of outliers. All analyses were controlled for level of education and income (proxies for socioeconomic status that could influence simultaneous exposure to other toxicants based on living area within the water district); age (older women may have longer exposure times); parity (increased number of pregnancies may decrease perfluorocarbon levels),¹⁶ and smoking and alcohol consumption (which can impair blood flow to the placenta),^{21,22} as well as a surrogate for plasma volume, which changes during pregnancy²³ and could potentially affect interpretation of the perfluoroalkyl substance measurements. Direct measures of plasma volume such as Evan's blue dye²⁴ were not available in the data set, so we performed plasma volume adjustments with two different surrogate measures: hematocrit and the hemoglobin/hematocrit ratio. Hematocrit is a crude estimate of overall plasma volume, that is, the percentage of blood volume consisting of packed red blood cells, and the hemoglobin/hematocrit ratio adds additional information concerning whether red blood morphology might be contributing to a hemoglobin value. If the hemoglobin/hematocrit ratio decreases and there is no other reason for reduced red blood cell mass, the reduction is attributed to increased plasma volume. Equations were calculated with both versions with similar results. The proxy for plasma volume described in the Results section is hemoglobin/hematocrit

Cross-Sectional PFOA and PFOS Averages in each Trimester

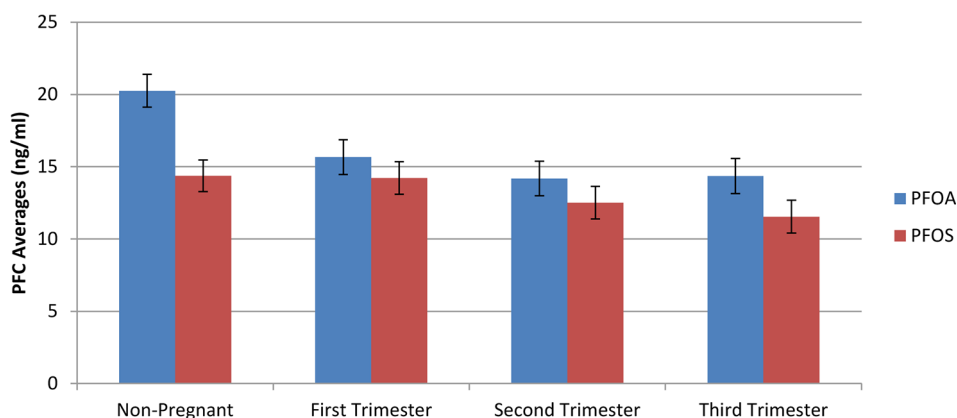


Figure 2. Cross-sectional maternal perfluorocarbon concentrations by trimester.

ratio. Covariates were allowed to enter the equation in order of importance.

RESULTS

Descriptive characteristics of pregnant and nonpregnant women are listed in Table 1. Their PFOS and PFOA concentrations are listed in Table 2. Both PFOS and PFOA are lower in pregnant women than in nonpregnant women, and this is reflected also in the covariates in Table 1.

Comparison of PFOA and PFOS in Pregnant versus Nonpregnant Women. Assumptions for normality in this population were met. After all covariates were controlled for (age, alcohol consumption, smoking, educational level, hemoglobin/hematocrit ratio, parity, residential exposure, and income), the results of the analysis of variance (ANOVA) using month as a predictor (months 1 and 2 were combined) showed significant differences between pregnant and nonpregnant women in PFOA ($F = 17.33$; $p < 0.001$) and PFOS ($F = 9.78$; $p = 0.0018$). Pregnant women in the study population had lower circulating levels of PFOA¹⁵ than nonpregnant women. Mean values for pregnant and nonpregnant women are shown in Figure 1. In the figures, natural log values have been converted to analog values to make interpretation easier.

Perfluorocarbon Concentrations by Trimester of Pregnancy versus Nonpregnancy. To examine perfluoroalkyl substance concentrations during different stages of fetal development, we also analyzed the data by trimester. After the same covariates were controlled for, comparison of nonpregnant women to women in each trimester of pregnancy showed significantly lower levels of PFOA in pregnant women in the second and third trimesters of pregnancy (second trimester $T = 2.81$, $p = 0.005$; third trimester $T = 2.67$, $p = 0.008$) than in nonpregnant women and a strong trend toward lower concentrations in the first trimester ($T = 1.85$, $p = 0.06$). However, PFOS concentrations in pregnant women were significantly lower than those in nonpregnant women only during the third trimester $T = 3.05$, $p = 0.002$, although the tendency in the second semester was strong ($T = 1.79$, $p = 0.07$). The perfluoroalkyl substance concentrations by trimester of pregnancy compared with nonpregnant women are shown in Figure 2.

DISCUSSION

These data from a population of women exposed to PFOA in drinking water substantiate the earlier indications from NHANES that circulating levels do indeed drop in pregnant women.¹⁹ Pregnant women in our study had consistently lower levels of circulating PFOA in all trimesters of pregnancy (strong tendency in the first trimester and significant tendency in the second and third trimesters), as well as lower concentrations of PFOS that reached significance during the third trimester of pregnancy. Since pregnant women do not menstruate, they might have been expected, on the basis of earlier reported hysterectomy data,²⁵ to exhibit higher levels of circulating perfluoroalkyl substances than nonpregnant women. Not only were their PFOA levels not higher, they were lower. One plausible explanation for the lower peripheral blood levels in pregnant women is redistribution to the fetus. This explanation is consistent with existing animal data, which have demonstrated transfer of PFOS to the fetus,^{5,6} and with human data showing the presence of both PFOS and PFOA in the cord blood of neonates from exposed mothers.^{14–17} Furthermore, a recent cross-sectional study in humans reported steadily increasing PFOS in amniotic fluid by gestational week.²⁶ The results of these studies further support our hypothesis that maternal perfluoroalkyl substances are offloaded into the fetus throughout the course of pregnancy.

There are a number of potential consequences of fetal exposure to perfluoroalkyl substances that could have major consequences for subsequent child development. Our data show that the first trimester is the beginning of a decline in maternal circulating PFOA levels, indicating that redistribution of this chemical occurs during a critical period of gestational development. This is a period during which structures of the nervous, cardiovascular, digestive, respiratory, and endocrine systems and the kidneys are being formed,²⁷ and the potential for epigenetic influences is particularly critical. Extensive data indicate that epigenetic influences during critical periods of fetal development can cause permanent changes in metabolism and chronic disease susceptibility,²⁸ and an increasing body of research shows that a complex combination of adult health-related disorders can originate from developmental events that occur in utero even without direct effects on pregnancy or birth weight.²⁹ A great many epigenetic changes that occur in utero

do not manifest until later in childhood or even adulthood and may therefore not be immediately identifiable as birth outcomes. Because animal research has shown that perfluorocarbon exposure to the fetus is, in fact, associated with epigenetic changes, this issue is important. It has been demonstrated in rat L02 liver cells³⁰ that there is a dose-related increase in methylation (a process that turns genes off) of the glutathione-S-transferase promoter (GSTP), a gene that encodes an enzyme involved with detoxification metabolism and susceptibility to cancer.³¹ DNA methylation in human cord serum has also been demonstrated to be inversely associated with serum PFOA.³² The implications of these methylation changes have not been investigated, but given the increasing evidence of maternal–fetal transfer of these chemicals, the need for such research is urgently needed. Animal research on other toxicants (e.g., vinclozolin) has demonstrated that embryonic exposure was associated with tissue abnormalities including prostate, kidney, immune system, and testis, as well as tumor development in the adult F1 generation as well as in subsequent generations (F2–F4).³³

The relevance of vinclozolin is that it is an endocrine disruptor, and endocrine disruption has been an important focus of research related to perfluoroalkyl substances.^{34,35} Although endocrine-disrupting consequences of fetal exposure to perfluoroalkyl substances, have to our knowledge not yet been investigated, the fact that exposure to other endocrine-disrupting chemicals in utero has been associated with the above-mentioned abnormalities, as well as with human urogenital malformations and cancer,^{36–38} impaired reproductive function and infertility,³⁸ increased risk of breast cancer,³⁹ and intellectual impairment and neurodevelopmental changes manifesting in children,⁴⁰ raises cause for concern. Whether or not these effects also generalize to PFOA and PFOS remains to be seen; however, accumulating data suggest that this is an important area for future investigation.

The C8 Health Study has reported that, in adults, PFOS and PFOA are associated with endocrine disruption related to thyroid dysfunction.⁴¹ In analyses stratified by age and gender, both PFOA and PFOS were shown to be associated with significant elevations in serum thyroxine and a significant reduction of T3 uptake in all participants. These effects were significantly stronger in women. The pattern found in those data were interpreted as being consistent with what occurs with the use of exogenous estrogens in patients, namely, an increase in thyroid binding globulin (TBG) but not thyroid-stimulating hormone (TSH), an increase in total thyroxine, and a decrease in T3 uptake. The limitation of that study was that the only binding protein actually measured was albumin, which binds a much smaller amount of thyroxine than TBG. However, albumin showed the same positive association with serum PFOA and PFOS that would have been expected from TBG.

The fact that PFOS and PFOA are associated with thyroid dysfunction in adults has implications for pregnant mothers. It has been demonstrated in clinical studies that thyroid hormone reaches the fetus and affects gene expression in the fetal brain.⁴² In fact, thyroid hormones control neuronal and glial proliferation in certain brain regions and contribute to the regulation of neuronal migration and differentiation.⁴³ Thus, factors that disrupt thyroid function in the mother have the potential to affect brain development in the fetus.⁴² In fetal fluids, a major proportion of T4 is not protein-bound (i.e., it is “free”) and is correlated to that in maternal serum.⁴⁴ The primary research focus with respect to maternal thyroid

function and subsequent fetal and child development has been on the damaging effects of hypothyroidism to the central nervous system and cognitive development.^{45–49} Hypothyroidism has not been observed in the C8 data set. Rather the effect of perfluoroalkyl substances on thyroid function was an increase in T4 and circulating T3 (based on a reduction in T3 uptake). However, increases in maternal thyroid hormones are also associated with biochemical disturbances in the fetus, including effects on the neurotransmitters acetylcholine, dopamine, and serotonin.⁵⁰ Again, the accumulating data indicate the need for more systematic research on fetal exposure to PFOS and PFOA.

Endocrine disruption is not the only threat from exposure to PFOS and PFOA. These chemicals have also been shown to be associated with increased total and low-density lipoprotein (LDL) cholesterol in children and adults.^{51,52} The significance of this is that maternal hypercholesterolemia is associated with increased formation of fatty streaks in human fetal arteries⁵³ as well as accelerated atherosclerosis progression in childhood.⁵⁴

Overall, studies investigating the effects of intrauterine perfluoroalkyl substance exposure on child development in humans are limited, and most have not investigated outcomes associated with endocrine disruption. Studies of infant allergies and infectious diseases from mothers exposed to perfluoroalkyl substances during pregnancy have found an inverse association between maternal PFOA levels during pregnancy and IgE levels in cord blood of infant girls,⁵⁵ as well as negative associations between PFOS and PFOA and anti-diphtheria prebooster antibody concentrations.⁵⁶ Consistent with the developmental origins of disease hypothesis, PFOA concentrations in pregnant mothers have also been found to be positively associated with body mass index (BMI) and waist circumference among their 20-year-old daughters⁵⁷ and with the daughters’ biomarkers of adiposity (e.g., insulin, leptin, and leptin/adiponectin ratio) while being inversely associated with their adiponectin.⁵⁷

The comparison of maternal PFOS and PFOA concentrations during pregnancy with those of nonpregnant women is complex because of the significant changes in plasma volume that occur. One weakness of our study is that we were forced to use a proxy for plasma volume in our analyses because the data set did not contain a direct measure. Interestingly, previously published data from this same cohort reported that levels of perfluoroalkyl substances were higher in women who had had hysterectomies (i.e., were not menstruating);²⁵ the implication is that our comparison may actually be conservative. If nonmenstruating women accumulate more perfluoroalkyl substances, then pregnant women who also do not menstruate may actually be transferring higher concentrations to the fetus than would be expected from comparing concentrations between pregnant and nonpregnant women as a whole.

A second limitation to our study is that the month of pregnancy is measured by self-report. Because women use more than one way to measure pregnancy onset (e.g., date of last menstrual period, date of conception), our designation of trimesters is not exact. Despite this fact, there is no reason to assume any systematic bias with respect to this variable. Another study limitation is that we were not able to adjust for thyroid binding globulin, which varies greatly during pregnancy, because it was not part of the data set. Problems of this type with covariates are often a trade-off in large population databases. These databases provide a lot of power for investigating population effects but do not always contain the covariates one would desire. We nevertheless believe these

analyses are informative. The fact that the results were stronger for PFOA than for PFOS may reflect the results of a Chinese study that reported a higher partition ratio of PFOA through placental barrier and lactation than for PFOS.⁵⁸ There is an additional covariate we would have liked to analyze but could not, namely, history of lactation. Breastfeeding could potentially influence the level of maternal perfluoroalkyl substances by off-loading some of them into the baby. This is a limitation of the study. Fortunately, there is no reason to believe that history of lactation would vary by water district and thus introduce systematic bias. The major strength of this study is that the population was large enough to compare perfluorocarbon levels at different trimesters of pregnancy with those of nonpregnant women.

Although there has been some human research with regard to perfluoroalkyl substances and developmental outcomes, it has not been systematic. Study designs have tended to measure exposure at more advanced stages of gestation, and reports of clinical outcomes have not, for the most part, been theory-driven. Because of the tremendous strides that have been made in the field of epigenetics, a whole new avenue for investigating fetal origins of adult disease has emerged. Given the accumulating data on perfluoroalkyl substances and endocrine disruption and the indications that PFOA is entering the fetus in the first trimester of development, more systematic research needs to be applied to the epigenetic effects of these chemicals on the developing fetus.

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Author Contributions

All authors have given approval to the final version of the manuscript. Contributions of individual authors area as follows: B.J. contributed to the statistical analyses and wrote the first draft of the manuscript; G.H. was the statistician; A.M.D. contributed to editing the document and interpretation of results and was the technical consultant on PFOS and PFOA; C.P. contributed to discussions concerning implications of the results for fetal development; and D.T. contributed specific information on surrogates for plasma volume. S.S.K. designed the study, contributed to the analyses, interpreted the result, and did a lot of the writing and editing.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

PFOS perfluorooctanesulfonic acid

PFOA perfluorooctanoic acid

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