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Maternal Plasma per- and Polyfluoroalkyl Substance Concentrations in Early Pregnancy and Maternal and Neonatal Thyroid Function in a Prospective Birth Cohort: Project Viva (USA)

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Background: Prenatal exposure to some per- and polyfluoroalkyl substances (PFASs) may disrupt maternal and neonatal thyroid function, which is critical for normal growth and neurodevelopment.

Objectives: We examined associations of PFAS exposure during early pregnancy with maternal and neonatal thyroid hormone levels.

Methods: We studied 732 mothers and 480 neonates in Project Viva, a longitudinal prebirth cohort in Boston, Massachusetts. We quantified six PFASs, including perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), and maternal thyroid hormones [thyroxine (T_4), Free T_4 Index (FT_4I), thyroid stimulating hormone (TSH)] in plasma samples collected at a median 9.6 wk gestation and neonatal T_4 levels from postpartum heel sticks. We estimated associations of PFAS concentrations with thyroid hormone levels using covariate-adjusted linear regression models and explored effect measure modification by maternal thyroid peroxidase antibody (TPOAb) status and infant sex.

Results: PFAS concentrations were not associated with maternal T_4, but PFOA, perfluorohexane sulfonate (PFHxS), and 2-(N-methyl-perfluorooctane sulfonamido) acetate (MeFOSAA) were inversely associated with maternal FT_4I [e.g., −1.87% (95% confidence interval (CI): −3.40, −0.31) per interquartile (IQR) increase in PFOA]. PFAS concentrations [PFOA, PFOS, and perfluorononanoate (PFNA)] were inversely associated with TSH levels in TPOAb-positive women only. Prenatal PFOS, PFOA, and PFHxS concentrations were inversely associated with T_4 levels in male [e.g., PFHxS, quartile 4 vs.1: −2.51 μg/dL (95% CI: −3.99, −1.04)], but not female neonates [0.40 μg/dL (95% CI: −0.98, 1.79)].

Conclusions: In this study, prenatal exposure to some PFASs during early pregnancy was inversely associated with maternal FT_4I and neonatal T_4 in male infants. These results support the hypothesis that prenatal exposure to PFASs influences thyroid function in both mothers and infants. https://doi.org/10.1289/EHP2534

Introduction

Per- and polyfluoroalkyl substances (PFASs) are synthetic compounds commonly used in commercial and consumer products, such as stain-resistant and nonstick coatings, food packaging, and firefighting foams (Lindstrom et al. 2011). PFASs can persist in the environment and the human body for long periods (2–5 y) (Olsen et al. 2007). The persistence and widespread use of some PFASs has resulted in ubiquitous human exposure exemplified with detectable PFAS serum concentrations in the majority of the U.S. population (Calafat et al. 2007). Humans are exposed primarily through dietary intake of contaminated foods and the indoor environment, although exposure through contaminated drinking water is also a concern (Hu et al. 2016).

Both toxicological and epidemiologic evidence suggests that PFAS exposure may alter thyroid function (Ballesteros et al. 2017). Normal thyroid function is critical for proper fetal and neonatal growth and neurodevelopment. The fetus is completely dependent on maternal thyroid hormones during early pregnancy until 18–20 wk gestation, when fetal thyroid hormone production begins (Fisher 1997). Maternal thyroid dysfunction, especially during early pregnancy, has been associated with adverse pregnancy and developmental outcomes, such as impaired fetal growth, preterm delivery, and neurodevelopmental deficits, including lower IQ in children (de Escobar et al. 2004). Even subtle deficits in maternal thyroid function, such as hypothyroxinemia, can impair neurodevelopment in children (Henrichs et al. 2013; Min et al. 2016). Altered neonatal thyroid function has also been associated with neurodevelopmental and cognitive deficits (Lyall et al. 2016; Rose et al. 2006; Simic et al. 2009).

Toxicology studies have consistently demonstrated that PFASs can disrupt thyroid function in animals, often leading to hypothyroidism, characterized by decreased total or free thyroxine (T_4) and increased thyroid stimulating hormone (TSH), or hypothyroxinemia, defined by decreased free T_4 (fT_4) levels without a concomitant increase in TSH (Boas et al. 2012; Zoeller 2010). These animal studies have predominantly focused on PFOA and PFOS, but recent evidence indicates that effects of PFASs on thyroid function may vary across individual PFAS chemicals (Ren et al. 2015, 2016; Weiss et al. 2009). Studies of the effects of PFAS exposure on thyroid function in humans have not been as consistent. Previous studies conducted in pregnant women have found associations of higher PFAS serum or plasma concentrations, with higher

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TSH levels and/or lower T_4 or triiodothyronine (T_3) levels, suggesting a hypothyroid effect (Berg et al. 2015, 2016; Wang et al. 2013, 2014), whereas others have found no associations (Chan et al. 2011) or associations in the opposite direction (Yang et al. 2016). Although it is difficult to examine patterns of individual PFAS-thyroid associations across studies due to differences in PFASs analyzed, population exposure patterns, and reporting, the most consistently reported associations appear to be between PFHxS and PFOS exposure and increased TSH levels (Ballesteros et al. 2017). Most of the previous studies have had relatively small sample sizes and have varied in geographic location, sample timing (trimester of pregnancy), thyroid hormones measured, and concentration distributions of PFASs in the population. Prior studies have mainly been cross-sectional in nature, have generally not controlled for potential confounding by pregnancy-induced physiological factors (Loccisano et al. 2013; Zantour et al. 2013), and have not been able to examine potential mechanisms behind the associations of PFASs with thyroid function. A small study in Canadian pregnant women reported effect measure modification of these associations by thyroid peroxidase antibody (TPOAb) status, a marker of thyroid autoimmunity (i.e., Graves' disease or Hashimoto's thyroiditis) (Webster et al. 2014). The authors hypothesized that the autoimmune damage to the thyroid could make individuals more susceptible to thyroid-altering effects of PFASs, given an already decreased capacity to synthesize thyroid hormones.

Fewer studies have been conducted in neonates, and those studies' results have been even less consistent. Studies have reported both positive and negative associations of cord blood, heel stick, or prenatal PFAS concentrations with neonatal T_4, T_3, and/or TSH (Berg et al. 2016; de Cock et al. 2014; Kim et al. 2011; Shah-Kulkarni et al. 2016; Wang et al. 2014). Two studies reported sex-specific differences in associations of cord blood PFASs with neonatal thyroid hormones (de Cock et al. 2014; Shah-Kulkarni et al. 2016). To date, studies have varied in timing of PFAS measurement (cross-sectional at birth vs. prenatal), hormones measured, matrix analyzed for PFASs and hormones (cord blood vs. heel sticks), and geographical location, which can influence population demographics and PFAS exposure distributions.

We examined associations of prenatal PFAS exposure with maternal and neonatal thyroid hormone levels in Project Viva, a prospective prebirth cohort. Due to our relatively large sample size and wealth of covariate information, we were able to assess potential effect modification by maternal TPOAb status and infant sex, as well as examine potential confounding by pregnancy-induced physiological factors. The present study is one of the largest to date and the first to quantify PFASs and thyroid hormones during early pregnancy.

Methods

Study Participants

Pregnant women were enrolled in Project Viva, a prospective prebirth cohort in the Boston, Massachusetts, area between 1999 and 2002 during their first prenatal visit (median 9.6 wk gestation) at Atrius Harvard Vanguard Medical Associates. Detailed subject recruitment and study protocols were previously described (Oken et al. 2015; Sagiv et al. 2015). Of the 2,128 mother–child pairs, 1,645 (77%) had maternal PFAS measurements from early pregnancy (median 9.6 wk gestation) plasma samples. We measured thyroid hormones in women with available blood samples from study visits before 14 wk gestation. In a previous study in Project Viva, eligible women with complete covariate information on first trimester diet and infant cognitive testing provided subsequent consent to us to obtain neonatal T_4 results from the New England Newborn Screening Program (NENSP). Of the 1,645 participants with PFAS measurements, 768 (47%) had maternal thyroid hormone measures, and 501 (30%) had available neonatal T_4 results. We excluded 36 women who reported prior or current diagnosis of thyroid disease and/or current thyroid medication use. The final analytic sample consisted of 732 pregnant women and 480 neonates.

Institutional review boards of all participating institutions approved all study protocols and all participating mothers provided written informed consent. The Centers for Disease Control and Prevention (CDC) laboratory's involvement did not constitute engagement in human-subjects research.

Plasma PFAS Concentrations

Maternal plasma samples were collected and stored as previously described (Sagiv et al. 2015). Samples were shipped to the Division of Laboratory Sciences at the CDC (Atlanta, GA), where staff quantified six PFASs [perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), perfluorononanoate (PFNA), perfluorohexane sulfonate (PFHxS), 2-(N-ethyl-perfluorooctane sulfonamido) acetate (EtFOSAA; also known as Et-PFOSA-AcOH), 2-(N-methyl-perfluorooctane sulfonamido) acetate (MeFOSAA; also known as Me-PFOSA-AcOH)] using previously published methods (Kato et al. 2011). The limits of detection (LOD) were 0.2 ng/mL for PFOS and 0.1 ng/mL for all other PFASs. PFASs were detected in 99–100% of samples; concentrations below the LOD were replaced by the LOD/$\sqrt{2}$ (Hornung and Reed 1990).

Maternal and Neonatal Thyroid Assays

TSH, total thyroxine (T_4), and triiodothyronine (T_3) resin uptake (T_3U) were measured in the same maternal plasma samples used for PFAS quantification using the Bayer Advia Centaur assay (Centaur; Bayer Diagnostics). T_3U is an indirect measure of thyroid hormone binding protein saturation by thyroxine (Dunlap 1990). The Free T_4 index (FT_4I) was calculated from total T_4 and T_3U (FT_4I = total $T_4 \times T_3$ Uptake). The FT_4I is an estimate of circulating free T_4 levels, which accounts for changes in T_4 levels due to changes in thyroid binding protein levels and saturation, such as increases in thyroxine binding globulin (TBG) during pregnancy (Lee et al. 2009). Maternal thyroid peroxidase antibody levels (TPOAb) were measured using the Nichols Advantage assay (Nichols Institute Diagnostics). The laboratory nonpregnancy reference ranges were: total T_4, 4.5–10.9 μg/dL; TSH, 0.35–5.50 mIU/L; FT_4I, 1.0-4.0; TPOAb 0–2.0 U/mL. We categorized women as TPOAb positive (>2.0 U/mL) or negative (≤2.0 U/mL), following the laboratory reference range. Eight participants had missing TSH data and were excluded from TSH models only.

We obtained neonatal total T_4 levels from NENSP, measured in whole blood on filter paper from heel sticks, as previously described (Oken et al. 2009).

Covariates

We used in-person interviews and questionnaires to collect data on maternal age, race/ethnicity, education, smoking habits, marital status, parity, dietary intake, vitamin/supplement use, and household income. Although we did not have a direct measure of maternal iodine status, such as urinary iodine concentration, we had information on maternal use of iodine-containing supplements and dietary intake of iodine-rich foods, including fish, dairy, and whole eggs from food frequency questionnaires administered at the time of maternal blood sampling. These measures have been used previously to adjust for iodine status in the Viva cohort (Oken et al. 2009). We abstracted date of birth from medical records, infant sex

from the postdelivery interview, and received data on age at heel stick (days) from NENSP. Gestational age was estimated from the date of the last menstrual period or second-trimester ultrasound if the two estimates differed by >10 days.

Both PFAS and thyroid hormone concentrations in plasma are affected by physiological changes during pregnancy (Loccisano et al. 2013; Zantour et al. 2013). To account for these changes, we assessed markers of pregnancy physiology and hemodynamics, including gestational age at blood draw, glomerular filtration rate (GFR), and plasma volume expansion (PVE). Higher GFR is associated with greater renal clearance of both PFAS and iodine during pregnancy (Loccisano et al. 2013; Sagiv et al. 2015; Zantour et al. 2013), which could potentially confound the potential associations of PFAS exposure with thyroid hormones during pregnancy. We calculated GFR from plasma creatinine levels using the Cockroft-Gault formula [GFR-CG = (140-age) × prepregnancy weight (kg) × 1.04/plasma creatinine (μmol/L)] (Morken et al. 2014). We used plasma albumin concentrations and gestational week at blood draw as proxy measures for PVE (Costantine 2014). Albumin is both a minor thyroid-binding protein as well as the major binding protein for PFASs in blood (Loccisano et al. 2013; Zantour et al. 2013). Plasma creatinine and albumin concentrations were quantified in the same samples used for maternal PFAS and thyroid hormone analysis at the Children's Hospital Boston Clinical and Epidemiologic Research Laboratory (Boston, MA).

Statistical Analyses

We examined associations of prenatal plasma PFAS concentrations with maternal T_4, FT_4I, TSH, and neonatal T_4 levels using separate multivariable linear regression models for each PFAS and hormone pair. Thyroid hormone levels were modeled as continuous outcomes. T_3 Uptake measures are used to create the FT_4I and not as an independent marker of thyroid function. However, for a secondary analysis, we modeled associations of PFAS concentrations with T_3U levels, which represent the saturation of thyroid hormone binding proteins in the serum and could provide insight into potential binding mechanisms that may explain associations with thyroid hormones. Maternal TSH levels and FT_4I were skewed, so we natural-log transformed them to meet model assumptions. We report model regression coefficients as percent difference for ease of interpretation [% difference = (exp (beta) − 1) × 100%]. We utilized generalized additive models (GAMs) with continuous PFAS plasma concentrations to assess linearity of our models. To reduce the influence of potential outliers and examine possible dose–response relationships, we first modeled PFAS concentrations as categorical variables using quartiles, and as associations appeared roughly linear across quartiles, we then modeled them as continuous variables. Continuous model associations are reported per interquartile range (IQR) increase in PFAS concentrations.

We controlled for covariates in our models that were potentially associated with plasma PFAS concentrations or circulating thyroid hormone levels. Base model covariates were selected *a priori* based on previous literature (Berg et al. 2015; Oken et al. 2009; Pearce et al. 2008; Sagiv et al. 2015; Wang et al. 2014) and directed acyclic graphs. Maternal thyroid models included: maternal age (years), smoking habits [during pregnancy, former (not during pregnancy), never], race/ethnicity (black, white, other), parity (0, 1, ≥2), and gestational week at blood draw. Neonatal thyroid models included: maternal model covariates and infant sex, gestational age at birth (weeks), route of delivery, and age at heel stick (days)]. Next, we assessed potential confounding by hemodynamic factors [eGFR (mL/min per 1.73 m^2), albumin (g/dL)]. We assessed an additional set of potential confounding variables associated with both PFAS concentrations and thyroid hormones in bivariate analyses by adding them individually to the base adjusted models [maternal education (<college, college, >college), first-trimester dairy intake (servings per week), and first-trimester fish intake (servings per week)]. Covariates were included in final models if they changed beta estimates appreciably. Final maternal thyroid-hormone models were adjusted for maternal age, race/ethnicity, smoking habits, parity, gestational week at blood draw, and fish intake. Neonatal T_4 models were adjusted for maternal age, race/ethnicity, smoking habits, parity, gestational week at blood draw, infant sex, age at heel stick, gestational age, and route of delivery. Gestational age and route of delivery are often strong predictors of neonatal thyroid levels (Aktas et al. 2017; Herbstman et al. 2008a; Oken et al. 2009) and were therefore considered as potential confounders. However, we recognize that these variables could lie on the causal pathway between PFAS exposure and altered neonatal thyroid function. Therefore, we performed a sensitivity analysis excluding these covariates from our neonatal models to assess changes in model results. As >99% of participants had complete covariate data, all models were run using complete case analysis, excluding those with missing data (maternal $n = 6$, neonatal $n = 15$).

We assessed effect measure modification by TPOAb status in our maternal models by stratification and tested for heterogeneity of the stratum-specific model estimates using two-sample z-tests (Buckley et al. 2016). In our neonatal models, we assessed effect measure modification by infant sex by stratification and heterogeneity testing using two sample z-tests.

Because maternal thyroid function can affect neonatal thyroid function, maternal thyroid hormone levels may be on the causal pathway between prenatal PFAS concentrations and neonatal T_4 levels (Kuppens et al. 2011). Therefore, we chose not to control for maternal thyroid-hormone levels in our primary analyses and instead performed secondary analyses, adding each maternal thyroid hormone to our neonatal T_4 models to assess changes in beta estimates.

We performed additional sensitivity analyses to assess the robustness of the results. To assess the potential influence of incomplete neonatal thyroid development, we excluded subjects with gestational ages <37 weeks from the neonatal T_4 models. To attempt to account for significant variation in neonatal T_4 levels due to the postnatal TSH surge, we stratified analyses by age at heel stick (<2, ≥2 days).

We utilized R version 3.2.4 for GAMs (R Foundation for Statistical Computing); all other analyses were conducted in SAS version 9.3 (SAS Institute Inc.).

Results

Participant characteristics of the analytic sample and the excluded sample are displayed in Table 1. Maternal participants averaged 32.5 years of age, were primarily white, college graduates, and evenly split between parous and nulliparous. Mean gestational week at time of maternal plasma sample collection was 10.0, mean gestational age at birth was 39.5 wk, and mean age at heel stick was 2.0 d (range 0.5–10.1). Maternal characteristics and PFAS concentrations of the subjects in the neonatal T_4 analysis were similar to those of the full analytic sample (data not shown). Participants in the analytic sample were more likely to be white and of higher socioeconomic status (SES) (higher maternal education, partner education, household income) in comparison with excluded participants (Table 1). When stratified by TPOAb status, TPOAb-positive women were more likely to be white and parous in comparison with TPOAb-negative women (data not shown).

Table 2 summarizes the distributions of maternal and neonatal thyroid-hormone markers and maternal plasma PFAS concentrations. As expected, neonatal T_4 levels were significantly higher

Table 1. Maternal and neonatal characteristics in the analytic study population and excluded population.

Characteristic	Study participants		Excluded participants	
	n	Mean ± SD or *n* (%)	*n*	Mean ± SD or *n* (%)
Maternal/family characteristics	732		1,396	
Age at enrollment (y)	732	32.5 ± 4.7	1,396	31.5 ± 5.4
First trimester dietary intake				
fish (daily servings)	728	0.2 ± 0.2	1,049	0.2 ± 0.3
whole eggs (weekly servings)	728	0.3 ± 0.3	1,049	0.3 ± 0.3
total dairy (weekly servings)	728	2.7 ± 1.5	1,049	2.6 ± 1.5
Gestational week at blood draw	732	10.0 ± 2.2	936	10.2 ± 2.4
eGFR (mL/min/1.73 m^2)	729	115.3 ± 61.8	901	105.4 ± 36.1
Albumin (g/dL)	684	5.8 ± 1.3	907	6.2 ± 1.1
Race/ethnicity	732		1,372	
black		74 (10)		274 (20)
white		561 (77)		838 (61)
other		97 (13)		260 (19)
Parity	732		1,396	
0		368 (50)		649 (47)
1		253 (35)		508 (36)
≥2		111 (15)		239 (17)
Smoking history	730		1,377	
never		510 (70)		933 (68)
former		145 (20)		253 (18)
during pregnancy		75 (10)		191 (14)
Education	732		1,372	
<college		188 (26)		556 (41)
college		284 (39)		459 (33)
>college		260 (35)		357 (26)
Iodine-containing supplements	728		1,348	
no		678 (93)		1,306 (97)
yes		50 (7)		42 (3)
TPOAb status	732		45	
negative (≤2 U/mL)		632 (86)		15 (33)
positive (>2 U/mL)		100 (14)		30 (67)
Partner education	689		1,215	
<college		193 (28)		482 (40)
College		263 (38)		408 (34)
>college		233 (34)		325 (27)
Household income	695		1,179	
<$40K		71 (10)		222 (19)
$40K–70K		174 (25)		261 (22)
>$70K		450 (65)		696 (59)
Child characteristics	480		1,648	
Age at heel stick (days)	467	2.0 ± 0.7	30	1.9 ± 0.6
Gestational age (weeks)	480	39.5 ± 1.9	1,648	39.4 ± 2.0
Sex	480		1,648	
male		244 (51)		852 (52)
female		236 (49)		796 (48)
Cesarean	479		1,619	
no		371 (77)		1,229 (76)
yes		108 (23)		390 (24)

Note: eGFR, estimated glomerular filtration rate; SD, standard deviation; TPOAb, thyroid peroxidase antibodies.

in females in comparison with males [Mean ± standard deviation (mean ± SD): females, 18.0 ± 4.0 μg/dL; males, 16.9 ± 4.2 μg/dL, $p = 0.004$]. Maternal FT_4I was positively correlated with T_4 and negatively correlated with TSH, but maternal T_4 and TSH were weakly but not significantly correlated with each other. As expected based on previous work in Project Viva (Oken et al. 2009), maternal thyroid hormones were not correlated with neonatal T_4 levels (see Table S1).

Maternal plasma PFAS distributions are summarized in Table 2. PFOS was the dominant PFAS (median 24.0 ng/ml), followed by PFOA (5.6 ng/ml). PFAS distributions were similar in excluded participants in comparison with those in both the maternal and neonatal analytic data sets (see Table S2). In general, PFASs were moderately to strongly correlated with each other (Spearman r_s range: 0.19–0.74) (see Table S1).

As previously described in this population, T_4 and FT_4I levels were significantly lower, and TSH levels were higher, in women who were older, white, and TPOAb positive (Pearce et al. 2008). FT_4I was also lower in women who reported smoking during pregnancy. Maternal use of iodine-containing supplements was not associated with maternal or neonatal thyroid hormones (Oken et al. 2009; Pearce et al. 2008). Gestational week at blood draw was weakly correlated with maternal T_4 ($r_s = 0.23$, $p < 0.0001$), and FT_4I ($r_s = -0.19$, $p < 0.0001$), but not TSH ($r_s = 0.06$, $p = 0.10$). Plasma albumin levels and eGFR were not significantly correlated with thyroid hormone levels. As previously reported, plasma PFAS concentrations were lower in women who were older, parous, nonsmokers, had higher education attainment, higher eGFR, and lower plasma albumin levels (Sagiv et al. 2015).

Prenatal PFAS Plasma Concentrations and Maternal Thyroid Hormones

Adjusted cross-sectional associations of quartiles of first-trimester maternal PFASs and thyroid hormones are presented in Figure 1.

Table 2. Prenatal plasma PFAS and maternal and neonatal hormone distributions in the analytic study population.

Analyte	Maternal analysis ($n = 732$)		Neonatal analysis ($n = 480$)	
	Median (25th–75th percentile)	Range	Median (25th–75th percentile)	Range
Prenatal plasma PFAS (ng/mL)				
PFOS	24.0 (17.6–32.6)	2.8–115	23.5 (17.3–31.1)	4.6–115
PFOA	5.6 (3.9–7.7)	0.3–36.7	5.5 (4.0–7.6)	0.9–20.1
PFHxS	2.4 (1.6–3.8)	<LOD–43.2	2.3 (1.6–3.8)	<LOD–43.2
PFNA	0.6 (0.5–0.8)	<LOD–6.0	0.6 (0.5–0.9)	<LOD–6.0
EtFOSAA	1.1 (0.7–1.7)	<LOD–33.6	1.0 (0.7–1.7)	<LOD–33.6
MeFOSAA	1.8 (1.2–2.9)	0.1–29.7	1.8 (1.2–2.8)	0.1–29.7
Thyroid hormones				
Total T_4 (μg/dL)	9.9 (8.7–11.2)	3.9–24.4	17.3 (14.7–20.1)	3.7–35.7
Free T_4 Index	2.1 (1.9–2.3)	1.3–6.0	NA	NA
TSH (mIU/mL)[a]	1.2 (0.7–1.9)	<0.01–21.4	NA	NA

Note: The limits of detection (LOD) were 0.2 ng/mL for PFOS and 0.1 ng/mL for all other PFASs. EtFOSAA, 2-(N-ethyl-perfluorooctane sulfonamide) acetate; MeFOSAA, 2-(N-methyl-perfluorooctane sulfonamide) acetate; PFAS, per- and polyfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate; T_4, thyroxine; TSH, thyroid stimulating hormone.
[a]$n = 724$, 8 participants missing TSH values.

We found no meaningful associations of maternal PFAS concentrations with T_4 or TSH. Concentrations of PFOA (Q2 & Q4 vs. Q1), PFHxS (Q4 vs. Q1), and MeFOSAA (Q4 vs. Q1) were significantly inversely associated with FT_4I levels (effect estimates, see Table S3). The associations of other PFASs with FT_4I were consistent in direction of association but effect estimates were weaker.

Our GAMs showed no evidence of significant departure from linearity overall in the models. Results from adjusted linear PFAS models were similar to the quartile models. An IQR increase in PFOA concentrations was associated with a 1.9% (−3.4, −0.3) decrease in FT_4I. There were no meaningful associations of PFAS concentrations with T_4 or TSH (Table 3).

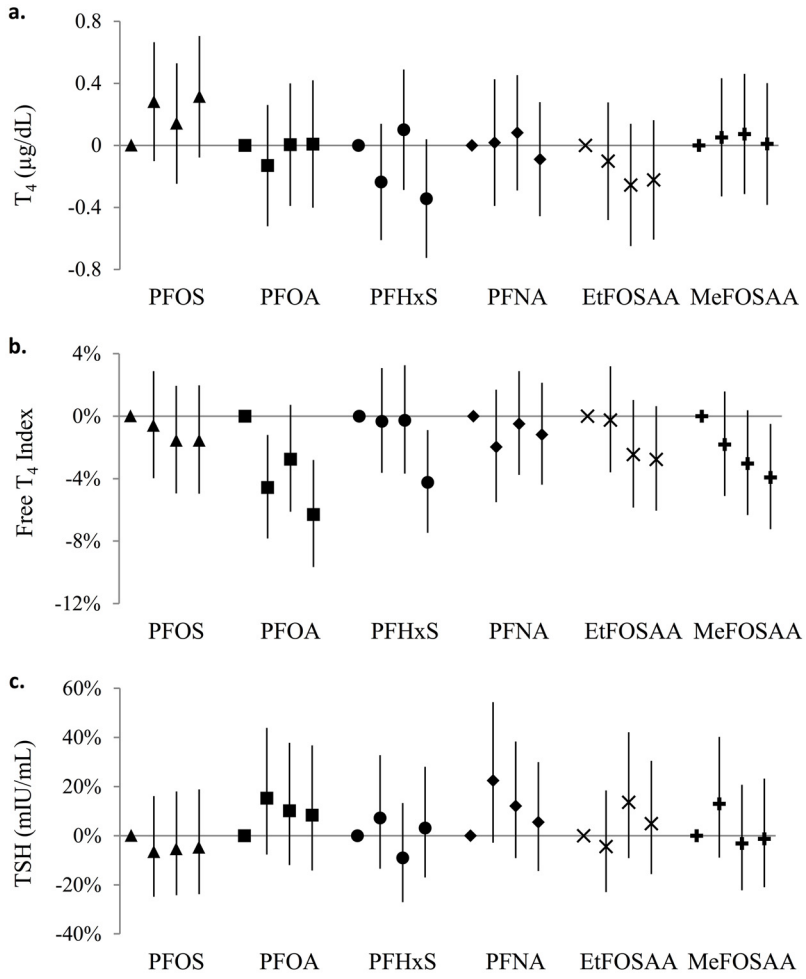

Figure 1. Effect estimates and 95% confidence intervals (CI) for associations of quartiles of plasma PFASs with (a) T_4 ($n = 726$), (b) Free T_4 Index ($n = 726$), and (c) TSH ($n = 718$) in early pregnancy maternal plasma samples. Effect estimates represent difference in (a) T_4 (μg/dL) or percent difference in (b) Free T_4 Index and (c) TSH for PFAS Quartiles 2–4 versus Quartile 1. Models were adjusted for maternal age, race/ethnicity, smoking status, fish intake, parity, and gestational week at blood draw. The horizontal line represents a null association.

Table 3. Adjusted difference in maternal thyroid hormone levels per IQR increase in plasma PFAS concentrations ($n = 726$).

	Total T_4 (μg/dL)	Free T_4 index	TSH[a] (mIU/mL)
PFAS	Beta (95% CI)	% difference (95% CI)	% difference (95% CI)
PFOS	0.01 (−0.14, 0.16)	−1.04 (−2.36, 0.29)	0.90 (−7.27, 9.80)
PFOA	0.09 (−0.08, 0.27)	−1.87 (−3.40, −0.31)	0.28 (−9.26, 10.8)
PFHxS	−0.05 (−0.14, 0.04)	−0.60 (−1.39, 0.19)	2.89 (−2.12, 8.17)
PFNA	−0.05 (−0.16, 0.05)	−0.57 (−1.52, 0.40)	−0.27 (−6.19, 6.03)
EtFOSAA	−0.01 (−0.09, 0.06)	0.07 (−0.61, 0.77)	−0.06 (−4.31, 4.38)
MeFOSAA	0.03 (−0.10, 0.15)	−0.82 (−1.93, 0.29)	0.57 (−6.27, 7.92)

Note: Adjusted for maternal age, race/ethnicity, smoking status, fish intake, parity, and gestational week at blood draw; $n = 6$ participants excluded due to missing covariate data.
[a]$n = 718$, 8 participants missing TSH values.

Maternal T_3U was inversely associated with plasma PFAS concentrations. IQR increases in PFOA, PFOS, and MeFOSAA were associated with −2.8% (−4.2, −1.4), −1.1% (−2.3, 0.1), and −2.1% (−2.2, −0.2) decreases in mean T_3U levels, respectively.

Effect Modification by TPOAb Status

In quartile models, cross-sectional associations of maternal PFAS concentrations with TSH differed according to TPOAb status (Table 4). Associations in TPOAb-negative women were null. In TPOAb-positive women, we saw large but imprecise inverse associations of IQR increases in PFOA, PFOS, and PFNA with TSH (z-test EMM p-values: PFOA, 0.08; PFOS, 0.05; PFNA, 0.03). Patterns of associations were similar in quartile models, but estimates were less precise. TPOAb status did not modify associations of PFAS concentrations with T_4 or FT_4I.

Prenatal Plasma PFAS Concentrations and Neonatal Thyroid Hormones

Figure 2 shows the effect estimates and 95% CIs from adjusted quartile models in the full sample (a) and stratified by sex (b, c). In all neonates, we found a consistent inverse association of several PFASs with neonatal T_4 levels: PFHxS Q3 vs. Q1: −1.0 μg/dL (−2.0, 0.03), Q4 vs. Q1: −1.1 μg/dL (−2.1, −0.1); PFOS Q4 vs. Q1: −1.1 (−2.1, −0.1); and PFOA Q4 vs. Q1: −1.1 μg/dL (−2.2, −0.1). Results for PFNA, EtFOSAA, and MeFOSAA were null. In continuous PFAS models (Table 5), we saw a suggestive inverse association between an IQR increase in prenatal PFOA concentrations and neonatal T_4 [PFOA: −0.4 μg/dL (−0.9, 0.1)]. All other PFASs except PFNA were weakly inversely associated with neonatal T_4.

When quartile models were stratified by infant sex, we found inverse associations of PFHxS, PFOA, and PFOS with T_4 among males, but no associations among females (Figure 2). In males, we found a monotonic decrease in T_4 levels with each quartile increase in concentrations of PFHxS (Q2–Q4 vs. Q1), PFOS (Q2–Q4 vs. Q1), and PFOA (Q3 & Q4 vs. Q1) (effect estimates, see Table S4). Results from continuous models are displayed in Table 5. In males, IQR increases in PFOA and PFHxS were associated with −0.7 μg/dL (−1.4, 0.1) and −0.5 μg/dL (−0.8, −0.1) decreases in T_4, respectively. Stratified model estimates were significantly different for PFHxS (z-test p-value = 0.02).

Table 4. Adjusted difference in maternal TSH (mIU/mL) per IQR increase in plasma PFAS concentrations stratified by TPOAb status.

	TPOAb Negative ($n = 620$)[a]	TPOAb Positive ($n = 98$)[a]
PFAS	% difference (95% CI)	% difference (95% CI)
PFOS	2.84 (−6.02, 12.5)	−16.4 (−29.8, −0.38)
PFOA	0.88 (−9.22, 12.1)	−19.0 (−35.1, 1.15)
PFHxS	3.04 (−2.51, 8.91)	−4.77 (−12.7, 3.88)
PFNA	0.78 (−5.46, 7.44)	−16.1 (−27.7, −2.56)
EtFOSAA	0.24 (−4.16, 4.83)	−4.87 (−15.9, 7.56)
MeFOSAA	0.30 (−6.92, 8.09)	−5.82 (−19.0, 9.58)

Note: Adjusted for maternal age, race/ethnicity, smoking status, fish intake, parity, and gestational week at blood draw; EMM z-test p-values: PFOS, 0.05; PFOA, 0.08; PFHxS, 0.13; PFNA, 0.03; EtFOSAA, 0.29; MeFOSAA, 0.30. TPOAb, thyroid peroxidase antibody.
[a]TPOAb negative, ≤2 IU/mL; TPOAb positive, >2 IU/mL).

Sensitivity Analyses

Excluding gestational age and route of delivery from our neonatal models did not appreciably change our results (see Table S5); therefore, we included these covariates in our final adjusted models. When we restricted neonatal models to subjects with heel sticks ≥2 days ($n = 175$) to account for the postdelivery TSH surge, adjusted effect estimates did not meaningfully differ from results in all neonates (see Table S5). Excluding neonates born <37 weeks gestation ($n = 28$) did not appreciably change the results (see Table S5). Additionally, controlling for maternal thyroid-hormone levels in our neonatal models did not meaningfully change the effect estimates (see Table S5). Maternal TPOAb status was not associated with plasma PFAS concentrations (see Table S6).

Discussion

We found inverse associations of PFOA, PFHxS, and MeFOSAA concentrations with FT_4I levels in maternal plasma collected cross-sectionally during early pregnancy, but did not find meaningful associations of PFAS concentrations with maternal T_4 levels. PFOA, PFOS, and PFNA concentrations were inversely associated with TSH in TPOAb-positive women only; associations in TPOAb-negative women and the full population were null. In neonates, higher prenatal PFOA, PFOS, and PFHxS concentrations from early pregnancy were associated with lower postpartum T_4 levels in males, but not females.

Our main results are generally consistent with reports from animal studies, which tend to report decreases in total T_4 levels following exposure to PFASs, usually PFOA or PFOS (Boas et al. 2012; Zoeller 2010). There are multiple potential mechanisms for the thyroid-disrupting effects of PFASs, including competitive binding to thyroid-hormone binding proteins (Weiss et al. 2009), increased hepatic clearance of T_4, increased conversion of T_4 to T_3 by type 1 deiodinase (Yu et al. 2009), and reduced responsiveness of the hypothalamic-pituitary-thyroid axis (Long et al. 2013).

Most PFASs have a high binding affinity for albumin (D'eon et al. 2010) and transthyretin (Chang et al. 2008; Weiss et al. 2009), but have low to no binding affinity for thyroxine-binding globulin (Ren et al. 2016). Although transthyretin is the major thyroid-binding protein in rats, thyroxine-binding globulin is the major thyroid-binding protein in humans (Zantour et al. 2013). Our study does not support competitive binding of PFAS to thyroid-binding proteins as the main mechanism behind the observed inverse associations with FT_4I. We found that higher concentrations

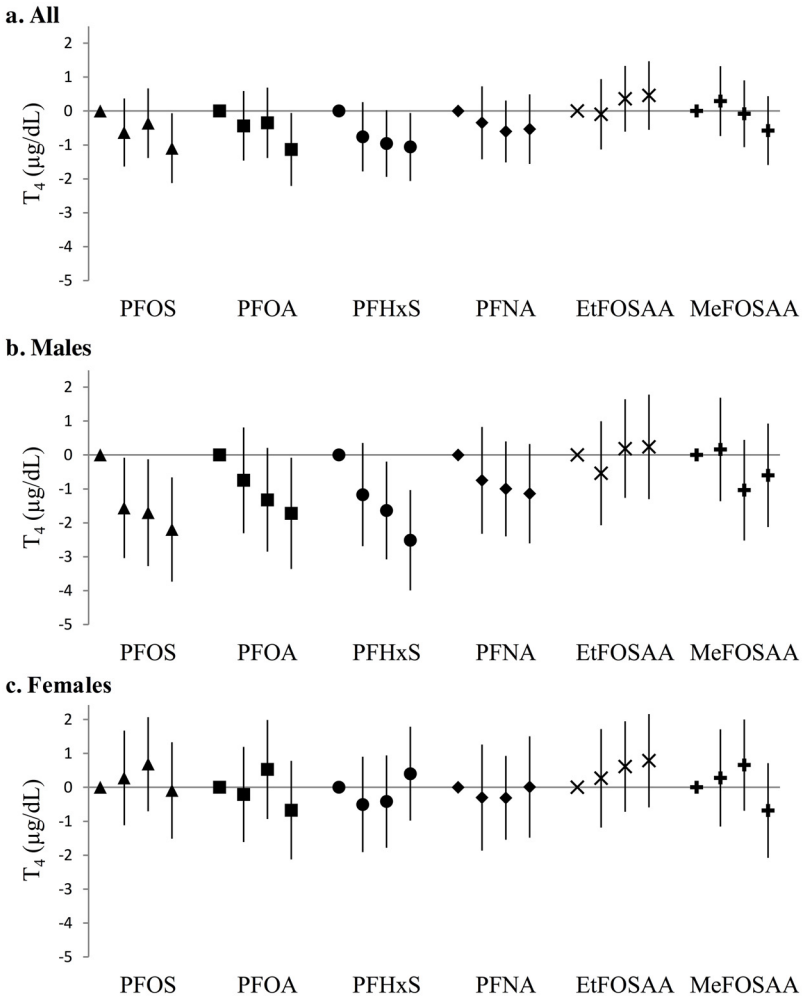

Figure 2. Effect estimates and 95% confidence intervals (CI) for associations of quartiles of prenatal plasma PFASs with neonatal T_4 in (a) all neonates ($n = 465$), (b) males ($n = 236$), and (c) females ($n = 229$). Models were adjusted for maternal age, race/ethnicity, smoking status, parity, gestational week at maternal blood draw, cesarean delivery, gestational age, and age at heel stick. (a) Models additionally adjusted for infant sex. The horizontal line represents a null association. EMM z-test *p*-values (Q2, Q3, Q4): PFOS, 0.31, 0.13, 0.29; PFOA, 0.40, 0.20, 0.32; PFHxS, 0.16, 0.16, 0.01; PFNA, 0.10, 0.39, 0.11; EtFOSAA, 0.36, 0.23, 0.39; MeFOSAA, 0.22, 0.15, 0.40.

of PFASs were associated with decreased saturation of plasma-binding proteins. In our study, PFAS concentrations were inversely associated with T_3U. If PFASs were competitively binding to thyroid-binding proteins, we would expect that greater PFAS concentrations would be associated with higher T_3U due to increased saturation of plasma-binding proteins. It is more likely that the inverse associations between PFASs and FT_4I in our study resulted from increased metabolism of T_4 and/or conversion of T_4 to T_3 by type 1 deiodinase. Yu et al. (2009) found that thyroidal type 1 deiodinase and hepatic uridine diphosphoglucuronosyl transferase, both involved in conversion of T_4 to T_3, were significantly increased following PFOS exposure in rats (Yu et al. 2009). Increased conversion of T_4 to T_3 could result in lower free T_4 levels, but normal or elevated T_3 levels, which would result in stable or decreased TSH levels. Unfortunately, we did not measure T_3 levels in our study.

It is also important to note that many toxicology studies have looked only at effects of one or two PFASs, usually PFOS or

Table 5. Adjusted difference in neonatal T_4 (µg/dL) per IQR increase in prenatal plasma PFAS concentrations in all neonates and stratified by sex.

	All ($n = 465$)[a,b]	Male ($n = 236$)	Female ($n = 229$)
PFAS	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)
PFOS	−0.10 (−0.46, 0.25)	−0.24 (−0.76, 0.29)	−0.02 (−0.48, 0.52)
PFOA	−0.41 (−0.88, 0.06)	−0.69 (−1.44, 0.07)	−0.25 (−0.85, 0.36)
PFHxS	−0.15 (−0.38, 0.08)	−0.46 (−0.83, −0.10)	0.07 (−0.23, 0.37)
PFNA	0.05 (−0.29, 0.39)	−0.27 (−0.84, 0.30)	0.23 (−0.20, 0.66)
EtFOSAA	−0.08 (−0.27, 0.11)	0.20 (−0.30, 0.71)	−0.12 (−0.32, 0.08)
MeFOSAA	−0.12 (−0.43, 0.18)	−0.08 (−0.45, 0.29)	−0.28 (−0.86, 0.30)

Note: Adjusted for maternal age, race/ethnicity, smoking status, parity, gestational week at maternal blood draw, cesarean delivery, gestational age, and age at heel stick. EMM z-test *p*-values: EtFOSAA, 0.23; MeFOSAA, 0.31; PFHxS, 0.02; PFNA, 0.10; PFOA, 0.23; PFOS, 0.30.
[a]Additionally adjusted for infant sex.
[b]$n = 15$ participants excluded due to missing covariate data.

PFOA, and although PFASs share similar structural features, they may differ in mechanisms of action and potencies. Additionally, most animal studies use doses that are significantly higher than those experienced by general human populations, and interspecies differences may make it difficult to compare effects of PFASs on thyroid function in animals to effects in humans.

Inverse associations of maternal PFASs with FT_4I seen in our cohort were also reported in a Taiwanese cohort (free T_4) (Wang et al. 2014) and in TPOAb-positive women in a Canadian cohort (Webster et al. 2014). We found no associations between TSH and PFASs in the full Viva cohort, but in TPOAb-positive women, higher PFAS concentrations were associated with lower TSH. Although the majority of previous studies reported positive associations between PFASs and TSH (Berg et al. 2015; Wang et al. 2013, 2014; Webster et al. 2014), Yang et al. (2016) found an inverse association between PFAS concentrations and TSH in a Chinese cohort. Lack of consistency across studies could be due to the considerable variation in study design, including differences in timing of sample collection (trimester), hormones measured, population demographics, and distributions of PFAS concentrations.

Only one previous study assessed effect modification by TPOAb status in pregnant women (Webster et al. 2014). Contrary to our findings, they found that IQR increases in PFNA, PFOA, and PFOS concentrations were associated with increased TSH levels in TPOAb-positive women. However, that study was substantially smaller than ours ($n = 14$ TPOAb-positive women vs. $n = 98$ in our study), and results from both studies could be affected by limited sample sizes. Webster et al. collected blood samples later in pregnancy (15 and 18 wk gestation vs. median 9.6 wk in our study), and median PFAS concentrations were lower in Webster et al. (2014) in comparison with Project Viva (e.g., PFOS: 4.8 vs. 24.0 ng/mL in our study), which could affect comparability between studies. In a separate study, Webster et al. (2016) assessed effect modification by TPOAb status in the U.S. general population using NHANES data. The study examined the potential modifying effects of TPOAb status in combination with iodine status, another potential thyroid stressor. The study reported interquartile increases in serum PFAS concentrations were associated with increases in fT_3, fT_3/fT_4 ratios, TSH, total T_3 levels, and decreases in fT_4 levels exclusively in TPOAb-positive individuals who also had low urinary iodine levels. For those individuals with normal urinary iodine levels, associations were null regardless of TPOAb status. These results suggest that differences between our study findings and those of Webster et al. (2014) could potentially be due to differences in population iodine status. Unfortunately, we are unable to further explore these differences as we did not have a direct measure of maternal iodine status in our study. Additionally, in Project Viva, TPOAb-positive women were more likely to be white and parous in comparison with TPOAb-negative women. As parity is associated with both PFAS concentrations and potential differences in thyroid function, it is possible that confounding by parity related factors was not properly controlled for in models, leading to residual confounding of the PFAS-TSH association in our analysis. Ultimately, it remains unclear exactly how TPOAb status may modify the association between PFAS exposure and thyroid hormone levels. Further research is needed to understand these relationships.

Few studies have examined associations of PFAS exposure with neonatal thyroid hormones. In our study, we found an inverse association of prenatal PFAS concentrations with neonatal T_4 levels, consistent with results from a Taiwanese study of 116 neonates (Wang et al. 2014) and a South Korean study of 43 neonates (Kim et al. 2011). Two studies reported sex specific effects that were inconsistent with our results of inverse associations of PFASs with T_4 in male but not female neonates; neonatal T_4 levels were positively associated with PFASs in female but not male neonates in Dutch (PFOA) (de Cock et al. 2014) and South Korean infants (PFHxS and PFNA) (Shah-Kulkarni et al. 2016). The reason for this sex difference is unclear. In our cohort, male neonates had significantly lower T_4 levels in comparison with females, which may make males more susceptible to alterations in thyroid function (Chan et al. 2011; Herbstman et al. 2008b; Kuppens et al. 2011). However, T_4 levels were also slightly lower in males in comparison with females in both de Cock et al. (2014) and Shah-Kulkarni et al. (2016). The discrepancies in results among studies may be due to differences in study design, population demographics, or PFAS concentrations distributions. Although we measured PFASs prenatally, de Cock et al. (2014) and Shah-Kulkarni et al. (2016) measured PFASs at birth. Shah-Kulkarni et al. (2016) measured neonatal hormones in cord blood, whereas blood from neonatal heel sticks a few days after birth was used to measure neonatal hormones in de Cock et al. (2014) and in our study.

Reductions in FT_4I levels alone could potentially affect fetal growth and neurodevelopment. We measured maternal hormones during early pregnancy, when the fetus is completely dependent on the mother for thyroid hormones. Maternal hypothyroxinemia has been consistently associated with cognitive and psychomotor deficits in the offspring (de Escobar 2001, 2004; Henrichs et al. 2013). Offspring are particularly susceptible to these effects when hypothyroxinemia occurs during early pregnancy (de Escobar et al. 2004; Henrichs et al. 2013). The effects of low neonatal T_4 levels are less well understood, but altered neonatal thyroid function has been associated with reductions in neurocognitive test, IQ, and attention scores (Lyall et al. 2016; Rose et al. 2006; Simic et al. 2009).

The present study has several strengths and limitations. To our knowledge, this is the second largest study of PFAS concentrations and thyroid hormones in pregnant women, the largest study in neonates, and the first conducted in a U.S. cohort. Because of our large sample size, we were able to investigate effect measure modification by maternal TPOAb status in a much larger sample of women than previously reported and by sex in neonatal models. In our maternal analysis, PFASs and thyroid hormones were measured cross-sectionally, limiting our ability to establish temporality. However, this limitation is potentially offset, as several PFASs, including PFOA, PFOS, and PFHxS, have relatively long half-lives in plasma, measured in years; concentrations in a single blood sample are likely representative of an individual's exposure over relatively long time periods. Unlike previous studies, we collected maternal blood in the first trimester of pregnancy, when physiological changes such as plasma volume expansion and increased GFR are less likely to affect both PFAS and thyroid-hormone measurements in comparison with changes later in pregnancy. Unlike most previous studies, we were able to assess potential confounding effects by pregnancy-induced physiological factors, such as plasma volume expansion and increased GFR, by controlling for plasma albumin levels and eGFR in addition to controlling for gestational week at blood draw. However, in our study, albumin and eGFR did not confound the observed associations.

We estimated fT_4 using the FT_4I, not by equilibrium dialysis, considered the gold standard for fT_4 measurement during pregnancy. However, unlike fT_4 immunoassay methods, which are sensitive to alterations in binding proteins and prone to bias during pregnancy, the FT_4I accounts for alterations in binding proteins and has been shown to accurately represent fT_4 levels during pregnancy (Lee et al. 2009). Additionally, we did not measure T_3, which may have allowed us to better understand the relationship between PFASs and FT_4I and TSH. Unfortunately, we did not have a direct measure of maternal iodine status, an important determinant of maternal and neonatal thyroid function.

However, we did have information on use of iodine containing supplements and dietary intake of iodine-rich foods.

We measured neonatal T_4 in whole blood from heel sticks rather than cord blood; heel stick T_4 levels may be less influenced by stress during birth (Tehrani et al. 2003). However, our neonatal T_4 measurements came from the New England Newborn Screening Program with a median time of heel stick of 44 h post birth, and it is possible that the postbirth TSH surge could have caused substantial measurement error in our data. When we restricted our analyses to neonates with heel sticks performed $\geq$2 days, results were not meaningfully different than results in all neonates, suggesting that outcome misclassification from the postbirth TSH surge did not affect our results.

The Project Viva population is less racially diverse and of higher SES than the general population, limiting the generalizability of our results. However, the homogeneity of the cohort may have helped to reduce residual confounding, and concentrations of PFASs in the Viva population were comparable to those in the U.S. general population during the corresponding time period (Sagiv et al. 2015). We cannot rule out potential residual confounding from uncontrolled factors such as coexposures to other endocrine disrupting chemicals; however, previous studies have found low correlations between concentrations of PFASs and other chemical classes (Robinson et al. 2015; Rosofsky et al. 2017). It is possible that our results reflect confounding among the PFASs themselves. Due to the moderate to strong correlations among the PFASs, identifying such confounding and thoroughly teasing apart the independent associations would require additional analyses beyond the scope of the present study. Recent toxicological studies suggest that PFAS chemicals may differ in terms of magnitude and mechanism of effect on thyroid function (Long et al. 2013; Ren et al. 2016). In addition to possible confounding among the PFASs, there may be combined or joint effects of exposure to multiple PFASs on thyroid function. Although we modeled individual associations among single PFAS–thyroid hormone pairs, subsequent studies should examine both confounding from and joint effects of exposure to multiple PFASs using appropriate statistical methods (Braun et al. 2016; Taylor et al. 2016).

Conclusions

Women with higher plasma concentrations of PFOA, MeFOSAA, and PFHxS had significantly lower levels of FT_4I during early pregnancy in comparison with women with the lowest concentrations. In TPOAb-positive women, increases in PFOA, PFOS, and PFNA concentrations were associated with lower TSH levels. We saw no associations of PFAS concentrations with T_4 levels in women. In neonates, higher prenatal concentrations of PFHxS, PFOS, and PFOA were associated with lower T_4 levels among males. Differences in maternal FT_4I associated with PFASs were relatively small and may not be clinically relevant to individual pregnant women or their offspring. However, even small deficits in maternal free T_4, especially during early pregnancy, can have significant adverse effects on fetal growth and neurodevelopment (Henrichs et al. 2013; Min et al. 2016).

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