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Association of per- and polyfluoroalkyl substances with thyroid homeostasis during pregnancy in the SELMA study

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ABSTRACT

Objectives: To investigate the association of exposure to per- and polyfluoroalkyl substances (PFAS) during early pregnancy with markers of the maternal thyroid system.

Methods: Serum concentrations of seven PFAS as well as thyroid stimulating hormone (TSH), free and total thyroxine (FT4 and TT4), free and total triiodothyronine (FT3 and TT3) were measured in pregnant women in early pregnancy in the Swedish Environmental Longitudinal, Mother and child, Asthma and allergy (SELMA) study. Outcomes were concentrations of TSH and thyroid hormones, FT4/FT3 or TT4/TT3 ratios, TSH/FT4 ratio as a marker of the negative feedback loop, TT4/FT4 or TT3/FT3 ratios as markers of the binding of thyroid hormones to binding proteins.

Results: The study population comprised 2,008 women with median (95% range) gestational age of 10 (6–14) weeks. There was no association between PFAS and TSH. Higher PFNA, PFDA, PFHpA and PFOA levels were associated with a higher FT4 (largest effect estimate for PFDA: β [95% CI]: 0.27 [0.10 to 0.45], $P = 0.002$). Higher PFUnDA levels, but no other PFAS, were associated with a lower FT3 (β [95% CI]: −0.05 [-0.09 to −0.01], $P = 0.005$). Higher PFUnDA levels were associated with lower TT4 (β [95% CI]: −1.58 [-3.07 to −0.09]) and there was an inverted U-shaped association of PFOS with TT4 ($P = 0.03$). Higher PFDA, PFUnDA, PFHpA levels were associated with a lower TT3. Overall, higher PFAS concentrations were associated with a higher FT4/FT3 ratio and a higher TT4/TT3 ratio. There was no association of PFAS with the TSH/FT4 ratio. Higher concentrations of several PFAS were associated with lower TT4/FT4 and TT3/FT3 ratios.

Conclusions: These findings translate results from experimental studies suggesting that exposure to PFAS may interfere with the thyroid system during pregnancy. Further experimental studies should take into account human evidence to better understand the potential underlying mechanisms of thyroid disruption by PFAS exposure.

1. Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are a group of persistent and bio-accumulating chemicals with a wide range of applications in consumer and industrial products, resulting in ubiquitous human exposure (Lau et al., 2007). Biomonitoring data also shows that virtually all people have measurable levels of PFAS in serum (Sunderland et al., 2019). Observational data have shown that exposure to PFAS has been associated with endocrine disruption, alteration of the immune system (e.g. suppression of antibody production in response to

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antigens), and a higher risk of developing malignancies of the breast and liver, metabolic diseases, reproductive disorders in humans (Kahn et al., 2020; Fenton et al., 2020; Coperchini et al., 2020) and adverse fetal growth and neurodevelopment (Wikström et al., 2020; Oh et al., 2021; Mughal et al., 2018). Experimental studies showed that PFAS can disrupt various components of the thyroid system in humans for example by binding to transthyretin (Weiss et al., 2009; Ren et al., 2016), by decreasing the activity of thyroid peroxidase enzyme (Song et al., 2012) and increasing intrathyroidal deiodinase enzyme gene expression while decreasing the hepatic expression of the same genes (Yu et al., 2009).

Thyroid hormone is a regulator of fetal growth and development (Korevaar et al., 2017). However, fetal thyroid hormone availability relies on the placental transfer of maternal thyroid hormones, especially during the first half of pregnancy, which makes pregnancy a sensitive period for any form of thyroid disruption (Demeneix, 2019). While already mild alterations in thyroid hormone homeostasis may affect fetal neurodevelopment (Korevaar et al., 2017; Demeneix, 2019), several studies have also shown that maternal exposure to PFAS during pregnancy is associated with a higher risk of adverse neurodevelopmental outcomes in the offspring, such as behavioral difficulties and autism spectrum disorder (Oh et al., 2021; Luo et al., 2020). If and to what extent maternal PFAS exposure affects thyroid homeostasis during pregnancy remains unknown. The currently available human studies remain inconclusive with varied results (Coperchini et al., 2020; Boesen et al., 2020), and most studies have only focused on concentrations of thyroid hormones or TSH as outcomes without utilizing their measurements to have a more in-depth look at functions of the thyroid system (such as thyroid hormone metabolism or balance of the pituitary-thyroid axis), and as a result key experimental findings have not yet been translated to human data. Therefore, we aim to investigate the association of serum PFAS with various components of the maternal thyroid system during pregnancy.

2. Methods

2.1. Study population

This study was set in the Swedish Environmental Longitudinal, Mother and child, Asthma and allergy (SELMA) study, a prospective population-based pregnancy cohort. SELMA was designed to investigate the impacts of early life exposure to environmental factors, on maternal and offspring health (Bornehag et al., 2012). In total 6,658 pregnant women were invited out of which 2,582 agreed to participate. At the first antenatal visit, blood samples were taken from pregnant women in weeks 3–27 of pregnancy (median 10 weeks) in the county of Värmland (Sweden) between September 2007 and March 2010. Informed written consent was given by participating families for collection of biological samples and participation in the SELMA study. The SELMA study has been approved by the regional ethical committee, Uppsala, Sweden (Dnr: 2007/062, DNR:2015/177).

2.2. Laboratory measurements

Serum samples were frozen at −80° Celsius and stored in a biobank at the Central Hospital in Karlstad. Serum thyroid stimulating hormone (TSH), free thyroxine (FT4), total thyroxine (TT4), free triiodothyronine (FT3), total triiodothyronine (TT3), thyroid peroxidase antibodies (TPOAb) and thyroglobulin antibodies (TgAb) were measured by electrochemoluminescence assays (Cobas® e601; Roche Diagnostics, Mannheim, Germany) at the Department of Clinical Chemistry, Máxima Medical Center (Veldhoven, The Netherlands). Between-run coefficients of variation were 2.1%, 3.5%, 3.8%, 3.8%, and 7.7% for TSH, FT4, TT4, FT3 and TT3, respectively. TPOAb positivity and TgAb positivity were defined as TPOAb > 34 IU/ml or TgAb > 115 IU/l (manufacturer cut-offs), and the coefficients of variation were 12.4% and 7.1% for TPOAb at 33 or 100 IU/l, respectively, 10.9% and 8.6% for TgAb at 76 and 218 IU/l, respectively.

2.3. Chemical analysis

Serum concentrations of 8 PFAS (Lindh et al., 2012) and cotinine, a biomarker of tobacco exposure (Axelsson et al., 2018), were analyzed using liquid chromatography - tandem mass spectrometry (LC-MS/MS; QTRAP 5500, AB Sciex, Framingham, MA). Briefly, 100 µl aliquots serum were added with labelled internal standards for all compounds. Proteins were precipitated using acetonitrile and the samples were shaken for 30 min, followed by centrifugation. The LODs ranged from 0.01 to 0.06 ng/mL. The laboratory participates in the Erlangen Round Robin inter-laboratory control program for several PFAS and cotinine and has qualified as a European Human Biomonitoring Initiative (HBM4EU) laboratory for the analysis of PFAS. Any PFAS detected in<50% of the samples was excluded from the analyses which was the case only for PFDoDA.

2.4. Outcomes

We studied various aspects of the thyroid hormone system based on previous literature. First, absolute serum concentrations of TSH, FT4, TT4, FT3 or TT3. Second, thyroid autoimmunity, as reflected by TPOAb and/or TgAb positivity (Song et al., 2012). Third, the ratio of FT4/FT3 or TT4/TT3 as a marker of peripheral T4 conversion by deiodinase enzymes (Yu et al., 2009). Fourth, the TSH/FT4 ratio as a proxy of the negative feedback system at the level of the pituitary (Rothacker et al., 2016). Finally, the TT4/FT4 or TT3/FT3 ratios as a marker of the binding of thyroid hormones to thyroid hormone binding proteins, mainly thyroxine binding globulin and transthyretin (Ishihara et al., 2003).

2.5. Covariates

Maternal age, ethnicity, education level, parity, weight and height were ascertained using questionnaires. Data on weight and height were used to calculate BMI (kg/length^2) and was ascertained from the Swedish National Birth Register. Serum cotinine levels with the following cut-offs, below 0.2 ng/mL, 0.2–15 ng/mL or higher than 15 ng/mL, were used to categorize participants as non-smoker, passive smoker or active smoker, respectively.

2.6. Statistical analysis

TSH and PFAS concentrations were natural log-transformed to better deal with outlier effects and to normalize model residuals. We used Spearman's correlation coefficients to assess the correlations between PFAS concentrations. We utilized multivariable linear regression to study the association of PFAS with the thyroid system outcomes. All analyses were adjusted for maternal age, BMI, parity, smoking status (according to serum cotinine concentrations), education level, ethnicity, gestational age at the time of blood sampling, TPOAbs and TgAbs based on a directed acyclic graph (Textor et al., 2016) (Supplemental Fig. 1). We used restricted cubic splines with 3 knots to investigate potential non-linear associations between PFAS and thyroid system parameters.

Based on previous evidence that thyroid disruption by PFAS can be more prominent in TPOAb positive pregnant women (Coperchini et al., 2017), the product interaction term of TPOAb status was added to all TSH and FT4 analyses to investigate any effect modification by TPOAb status.

We utilized multiple imputation by chained equations to impute missing data of covariates, pooling 25 imputed datasets for analyses (Buuren et al., 2011). All statistical analyses were performed using R statistical software version 3.6.3 (packages *mice*; *rms* and *corrplot*; https://www.r-project.org/).

3. Results

After exclusions, the study population comprised 2,008 women (Fig. 1) with a mean (SD) age of 30.9 (4.9) years and median (95% range) gestational age of (6-14) weeks (Table 1). Serum concentration of PFAS are provided in Supplemental Table 1. PFNA, PFDA, PFHxS, PFOA and PFOS concentrations were above the LOD in 100% of cases, and 99.6% for PFUnDA, while PFHpA and PFDoDA concentrations were above the LOD in 73.4 % and 44.5%, respectively. The Spearman correlation coefficients between different PFAS ranged from 0.06 to 0.75 (Fig. 2).

3.1. PFAS and maternal TSH, thyroid hormones, TSH/FT4 ratio and thyroid autoimmunity

There was no association between PFAS and TSH (Table 2). Higher PFAS levels were associated with a higher FT4 (Table 2; largest effect estimate for PFDA: β [95% CI]: 0.27 [0.10 to 0.45], $P = 0.002$), but associations of PFUnDA, PFHxS and PFOS with FT4 did not reach statistical significance. The results of further analyses indicated that TPOAb positivity modifies the association of most PFAS with FT4 (P for interaction: 0.0008 to 0.025; Supplemental Table 2). The effect estimates of the association of PFNA, PFDA, PFUnDA, PFHxS, PFOA and PFOS with FT4 were up to 6-fold larger in the TPOAb positive women compared to TPOAb negative women (Supplemental Table 3). Moreover, there was no association of PFAS with the TSH/FT4 ratio (Table 2).

A higher PFUnDA concentration, but no other PFAS, was associated with a lower FT3 (Table 2; β [95% CI]: −0.05 [-0.09 to −0.01], $P = 0.005$). For total thyroid hormones, a higher PFUnDA concentration was associated with lower TT4 (Table 3, β [95% CI]: −1.58 [-3.07 to −0.09]) while there was an inverted U-shaped association of PFOS with TT4 ($P = 0.03$; Supplemental Fig. 2). Higher PFUnDA, PFHpA and PFOS concentrations were associated with a lower TT3 (Table 3), and this association was L-shaped for PFDA (Supplemental Figure 3). None of the PFAS were associated with thyroid autoimmunity (TPOAb and/or Tg antibody positivity combined) or isolated TPOAb positivity (Supplemental Table 4).

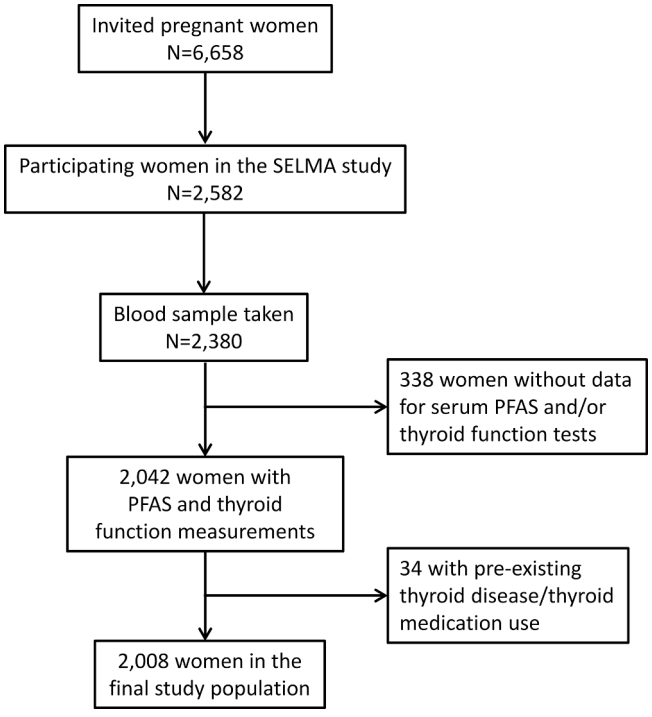

Fig. 1. Flowchart of the study population.

Table 1
Characteristics of the study population.

Characteristics	N = 2,008
Thyroid-stimulating hormone (mU/L)	1.30 (0.11–4.13)
Free thyroxine (pmol/L)	15.0 (11.4–19.5)
Total thyroxine (nmol/L)	118 (81–166)
Free triiodothyronine (pmol/L)	4.67 (3.72–5.96)
Total triiodothyronine (nmol/L)	1.93 (1.27–2.90)
Thyroid peroxidase antibodies positivity	206 (10.3)
Thyroglobulin antibodies positivity	159 (7.9)
Gestational age (weeks)	10 (6–14)
Age (years)	30.9 (4.9)
BMI (kg/m^2)	24.8 (4.5)
Parity, n (%)	
0	894 (45)
1	713 (36)
≥2	389 (19)
Ethnicity, n (%)	
Western	1,935 (97)
Non-western	61 (3)
Serum cotinine levels, n (%)	
Non-smoker: <0.2 ng/mL	1,699 (85.1)
Passive smoker: 0.2–15 ng/mL	117 (5.9)
Active smoker: >15 ng/mL	180 (9)
Education level, n (%)	
Low	83 (4)
Medium	724 (36)
High	1,189 (60)

Data are median (95% range), mean (SD) or number (percentage) as appropriate.

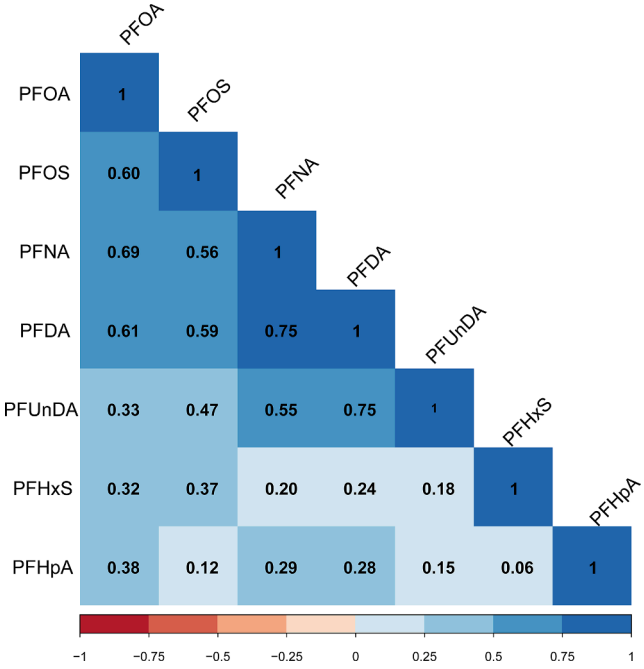

Fig. 2. Correlations of maternal serum PFAS.

3.2. PFAS and thyroid hormone metabolism

Higher PFAS levels were associated with a higher FT4/FT3 ratio and a higher TT4/TT3 ratio indicative of reduced conversion of the prohormone T4 to T3 by deiodination (Table 3), although the associations of PFNA, PFHxS and PFOA with FT4/FT3 ratio and PFOA with TT4/TT3 ratio were not statistically significant. The effect estimates for FT4/FT3 ratio ranged from a β per one log unit change of PFAS (95% CI) of 0.03 (0.0004 to 0.07) for PFOS to 0.06 (0.02 to 0.09) for PFHpA (Table 3) and the effect estimates for TT4/TT3 ratio ranged from 0.87 (0.14 to 1.60) for PFHxS to 1.99 (1.18 to 2.79) for PFOS (Table 3).

Table 2
Association of serum concentrations of per- and polyfluoroalkyl substances (PFAS) with TSH, FT4 and FT3 concentrations.

PFAS	TSH		FT4		FT3		TSH/FT4 ratio	
	β (95% CI)	P value	β (95% CI)	P value	β (95% CI)	P value	β (95% CI)	P value
PFNA	0.02 (-0.04 to 0.09)	0.47	0.21 (0.05 to 0.38)	0.009	0.01 (-0.03 to 0.06)	0.52	0.0003 (-0.004 to 0.004)	0.89
PFDA	0.009 (-0.06 to 0.08)	0.81	0.27 (0.10 to 0.45)	0.002	0.007 (-0.04 to 0.05)	0.78	−0.0005 (-0.005 to 0.004)	0.80
PFUnDA	0.05 (-0.01 to 0.11)	0.10	0.08 (-0.06 to 0.21)	0.26	−0.05 (-0.09 to −0.01)	0.005	0.001 (-0.001 to 0.005)	0.30
PFHxS	0.01 (-0.05 to 0.08)	0.72	0.13 (-0.01 to 0.28)	0.07	0.01 (-0.03 to 0.05)	0.63	0.001 (-0.002 to 0.005)	0.61
PFHpA	0.003 (-0.06 to 0.07)	0.92	0.23 (0.07 to 0.40)	0.004	−0.01 (-0.06 to 0.03)	0.50	0.00008 (-0.004 to 0.004)	0.96
PFOA	0.06 (-0.01 to 0.13)	0.12	0.20 (0.03 to 0.36)	0.02	0.04 (-0.001 to 0.09)	0.05	0.002 (-0.001 to 0.007)	0.21
PFOS	0.03 (-0.04 to 0.10)	0.43	0.16 (-0.001 to 0.33)	0.05	0.007 (-0.04 to 0.05)	0.73	0.001 (-0.003 to 0.005)	0.62

Betas (95% CI) are calculated with a multi-variable linear regression model for each PFAS separately (natural log-transformed), adjusted for gestational age at the time of sampling, maternal age, thyroid peroxidase antibodies, thyroglobulin antibodies, smoking status (according to serum cotinine), body mass index, education, ethnicity and parity.

Table 3
Association of serum concentrations of per- and polyfluoroalkyl substances (PFAS) with maternal total T4 and total T3 concentrations as well as FT4/FT3 and TT4/TT3 ratios.

PFAS	TT4		TT3		FT4/FT3 ratio		TT4/TT3 ratio	
	β (95% CI)	P value	β (95% CI)	P value	β (95% CI)	P value	β (95% CI)	P value
PFNA	0.12 (-1.65 to 1.90)	0.89	−0.03 (-0.06 to 0.001)	0.06	0.03 (-0.0001 to 0.07)	0.05	1.13 (0.33 to 1.93)	0.005
PFDA	0.42 (-1.47 to 2.33)	0.66	Non-linear†	0.001	0.05 (0.01 to 0.08)	0.008	1.46 (0.60 to 2.31)	0.0009
PFUnDA	−1.58 (-3.07 to −0.09)	0.03	−0.06 (-0.09 to −0.03)	<0.0001	0.05 (0.02 to 0.08)	0.0006	0.95 (0.28 to 1.63)	0.005
PFHxS	1.59 (-0.03 to 3.22)	0.05	−0.001 (-0.03 to 0.03)	0.90	0.02 (-0.008 to 0.05)	0.15	0.87 (0.14 to 1.60)	0.018
PFHpA	−0.59 (-2.36 to 1.17)	0.50	−0.06 (-0.09 to −0.02)	0.0007	0.06 (0.02 to 0.09)	0.0006	1.50 (0.71 to 2.30)	0.0002
PFOA	1.61 (-0.20 to 3.43)	0.08	0.001 (-0.03 to 0.03)	0.93	0.01 (-0.02 to 0.05)	0.40	0.70 (-0.12 to 1.53)	0.09
PFOS	Non-linear*	0.03	−0.06 (-0.10 to −0.03)	0.0003	0.03 (0.0004 to 0.07)	0.04	1.99 (1.18 to 2.79)	<0.0001

Betas (95% CI) are calculated with a multivariable linear regression model for each PFAS separately (natural log-transformed), adjusted for gestational age at the time of sampling, maternal age, smoking status (according to serum cotinine), body mass index, education, ethnicity, parity, thyroid peroxidase antibodies and thyroglobulin antibodies.
* Supplemental Figure 2.
† Supplemental Figure 3.

3.3. PFAS and possible displacement of thyroid hormones from distributor proteins (Ratios of TT4/FT4 and TT3/FT3)

Higher PFDA, PFUnDA or PFHpA levels were associated with a lower TT4/FT4 ratio (Table 4, Supplemental Figure 4). Moreover, except for PFHxS and PFOA, higher PFAS concentrations were linked with a lower TT3/FT3 ratio (Table 4), and the association of PFDA was L-shaped (Supplemental Figure 4).

Table 4
Association of serum concentrations of per- and polyfluoroalkyl substances (PFAS) with maternal TT4/FT4 and TT3/FT3 ratios.

PFAS	TT4/FT4 ratio		TT3/FT3 ratio	
	β (95% CI)	*P* value	β (95% CI)	P value
PFNA	−0.09 (-0.19 to 0.006)	0.06	−0.008 (-0.01 to −0.002)	0.006
PFDA	Non-linear*	0.001	Non-linear*	0.0005
PFUnDA	−0.14 (-0.22 to −0.05)	0.0008	−0.006 (-0.01 to −0.001)	0.006
PFHxS	0.03 (-0.05 to 0.12)	0.48	−0.001 (-0.006 to 0.004)	0.68
PFHpA	−0.15 (-0.25 to −0.05)	0.001	−0.01 (-0.01 to −0.004)	0.0003
PFOA	−0.006 (-0.10 to 0.09)	0.89	−0.003 (-0.008 to 0.003)	0.32
PFOS	−0.09 (-0.19 to 0.001)	0.05	−0.01 (-0.02 to −0.007)	<0.0001

Betas (95% CI) are calculated with a multivariable linear regression model for each PFAS separately (natural log-transformed), adjusted for gestational age at the time of sampling, maternal age, smoking status (according to serum cotinine), body mass index, education, ethnicity, parity, thyroid peroxidase antibodies and thyroglobulin antibodies.
* Supplemental Figure 4.

4. Discussion

We investigated the cross-sectional association of maternal serum PFAS concentrations with various markers of the maternal thyroid system in early pregnancy, identifying that higher concentrations of PFAS are associated with higher FT4 concentrations especially in TPOAb positive women. Our findings correspond well with a Chinese study of 1885 pregnant women, where higher PFNA was associated with a higher FT4 in TPOAb positive women and in general the effect estimates were larger in the TPOAb positive group compared to the negative (Aimuzi et al., 2020). However, in a Canadian study of 494 pregnant women higher PFAS levels were associated with lower FT4 only among TPOAb negative women (Reardon et al., 2019). Furthermore, in two other small studies, the association of PFAS with TSH or FT4 did not differ according to thyroid antibodies status (Itoh et al., 2019; Lebeaux et al., 2020). While the smaller sample sizes of these studies can be a reason for their contradictory results (not being able to detect differences), various potential mechanisms could underlie the associations with FT4 and effect modification by TPOAb positivity. First, PFAS could directly affect the susceptibility to thyroid autoimmunity, but we could not identify any association of PFAS exposure with thyroid antibodies (as a marker for thyroid autoimmunity). Second, there could be indirect effects causing thyroid autoimmunity, but the limited experimental data on potential cytotoxic effects of PFAS on thyroid cells show no cytotoxic effects of short-chain PFAS (Croce et al., 2019), or only with very high doses (Coperchini et al., 2015). Several studies have shown that PFAS can suppress the immune system (Fenton et al., 2021; Peden-Adams et al., 2008; Grandjean et al., 2012). In view of all the currently available evidence, it is hard to define mechanisms that might explain our observation of associations of PFAS with FT4 in TPOAb positive subjects. However, it appears that the effect is not due to any influence of PFAS on thyroid autoimmunity but rather might arise from the susceptibility of

TPOAb positive women to thyroidal stress. There are currently too few experimental data to explain the potential underlying mechanisms of the effects of PFAS on TSH or FT4 in TPOAb positive women. Nonetheless, our results can provide new orientations for experimental studies to explore the potential underlying mechanisms that have not yet been investigated in experimental settings.

We did not find any association between PFAS exposure with TSH, while higher concentrations of several PFAS were associated with higher FT4 levels. The results of previous epidemiological studies on the association of PFAS with TSH in pregnant women are heterogeneous, either showing that higher serum concentrations of PFAS were associated with higher TSH (Reardon et al., 2019; Xiao et al., 2020; Berg et al., 2015; Webster et al., 2014; Wang et al., 2014), lower TSH (Kato et al., 2016; Yang et al., 2016) or a lack of association with TSH (Itoh et al., 2019; Lebeaux et al., 2020; Preston et al., 2020; Inoue et al., 2019). Experimental studies have shown that exposure to PFAS does not affect mRNA expression of the TSH receptor or TSH concentrations in rats (Yu et al., 2009; Ramhøj et al., 2020), did not affect TRβ mRNA expression in silver female eels (Couderc et al., 2016) or TSH-stimulated cAMP production in cultured human thyroid cells (Croce et al., 2019); all of which can indicate that exposure to PFAS might not affect the TSH balance. In line with these results, we did not find any association of PFAS with the TSH/FT4 ratio, indicating that the HPT-axis was not be affected by PFAS.

In the current study, there was no association of PFAS with FT3 except for the association of a higher PFUnDA with lower FT3, similar to the findings of a small Norwegian study including 375 pregnant women for PFUnDA (Berg et al., 2015). On the other hand, in a Chinese study (n = 1,111), higher PFNA and PFHxS were associated with a higher FT3 (Aimuzi et al., 2020) while in a Japanese cohort (N = 701), Canadian cohort (N = 494) and a cohort from the US (N = 468) there was no association of PFAS with FT3 (Reardon et al., 2019; Itoh et al., 2019; Lebeaux et al., 2020). Based on these results it could be concluded that in general there is no association between PFAS and maternal FT3 concentrations.

One of the main results of our study is that higher exposures to PFAS were associated with a larger FT4/FT3 ratio which was due to elevated FT4 not accompanied by lower FT3 concentrations (except for exposure to PFUnDA). On the other hand, in general there were no associations of PFAS with TT4 but higher concentrations of most PFAS were with lower TT3 which resulted in a higher TT4/TT3 ratio. We utilized the ratios of free and total T4/T3 as markers of T4 deiodination, thus our results may imply that exposure to PFAS decreases the deiodination of T4 metabolism in the liver, placenta or target cells. In experimental studies, exposure to PFAS has been associated with lower FT4 or TT4 in rats (Yu et al., 2009; Chang et al., 2007; Martin et al., 2007), a decrease in hepatic mRNA expression of deiodinase type I but an increase in expression of type III deiodinase mRNA (Yu et al., 2009; Martin et al., 2007). Likewise, studies in chickens show that exposure to PFAS has been associated with increased hepatic and/or neuronal mRNA expression of deiodinase types II and III (Mattsson et al., 2019; Cassone et al., 2012; Vongphachan et al., 2011). While deiodinase type I is responsible for conversion of T4 to T3, type III is responsible for inactivation of T4 and T3 to reverse T3; therefore, if the same pattern of interference with gene expression of these two enzymes as explained above happens in humans, it might result in higher T4 and lower T3 concentrations which then can explain our findings.

In our study, higher exposures to most PFAS were associated with lower ratios of TT4/FT4 and TT3/FT3, markers of displacement of thyroid hormones from transport proteins. We speculate that higher exposure to PFAS in our study results in displacement of free thyroid hormones from binding proteins resulting in lower ratios of total to free thyroid hormones. This interpretation is supported by the findings of two experimental studies that show that PFAS can bind to human transthyretin with binding affinities comparable to T4 (Weiss et al., 2009; Ren et al., 2016). Another potential underlying mechanism by which higher exposure to PFAS could be associated with lower ratios of total to free thyroid hormones (binding proteins) is their reported hepatotoxic effects (higher liver enzymes or higher risk of nonalcoholic fatty liver disease) which can in turn reduce the production of proteins by liver (Fenton et al., 2021).

In this study we were able to utilize data from a large population-based cohort of pregnant women to translate findings of experimental studies on the effects of exposure to PFAS on thyroid system into human data. Our findings should be interpreted as an entirety, considering the links between all the components of the thyroid system that can be affected by PFAS (from pituitary to the target cells of thyroid hormones and from deiodinase enzymes to binding proteins) and various forms of evidence from human and experimental data. Although we did not have repeated measurements of serum PFAS during pregnancy, the long half-life of PFAS as well as their potential for bioaccumulation in human body, including in thyroid cells (Coperchini et al., 2017; Conti et al., 2020) mean that our measurements can be reflective of the exposures to PFAS that stretch back longer times. Another limitation of our study is that we did not have measurements of serum transthyretin, thyroid binding globulin or albumin which would have been beneficial to further investigate the underlying mechanisms of changes of total to free thyroid hormone ratios.

In conclusion, exposure to PFAS during early pregnancy is associated with the homeostasis of the maternal thyroid system on different levels. Further human and experimental studies are needed to replicate these results and large international collaborations between pregnancy cohorts in form of individual participant data *meta*-analyses can help to conduct stronger epidemiological investigations in the field.

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CRediT authorship contribution statement

Arash Derakhshan: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Andreas Kortenkamp:** Project administration, Conceptualization, Methodology, Writing – review & editing, Funding acquisition. **Huan Shu:** Methodology, Resources, Data curation, Writing – review & editing. **Maarten A.C. Broeren:** Methodology, Resources, Data curation, Writing – review & editing. **Christian H. Lindh:** Methodology, Resources, Data curation, Writing – review & editing. **Robin P. Peeters:** Project administration, Supervision, Conceptualization, Methodology, Resources, Writing – review & editing, Funding acquisition. **Carl-Gustaf Bornehag:** Project administration, Conceptualization, Methodology, Resources, Writing – review & editing, Funding acquisition. **Barbara Demeneix:** Project administration, Conceptualization, Methodology, Writing – review & editing, Funding acquisition. **Tim I.M. Korevaar:** Supervision, Conceptualization, Methodology, Investigation, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary material

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