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Higher free thyroxine associated with PFAS exposure in first trimester. The Odense Child Cohort.

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i OPEN, University of Southern Denmark, J. B. Winsløws Vej 9A, 5000, Odense C, Denmark

A R T I C L E   I N F O

Keywords:
Perfluorooalkyl substances
Early pregnancy
Thyroxine
Thyrotropin
Thyroid hormones
Developmental toxicity

A B S T R A C T

Background: Perfluoroalkyl substances (PFAS) are endocrine disrupting chemicals with elimination half-lives ranging from four to eight years. Experimental studies found PFAS able to interfere with thyroid hormone-binding proteins. During the first 20 weeks of gestation (GW), the fetus is reliant on placental transfer of maternal thyroid hormones, mainly free thyroxine (FT4). However, previous studies investigating associations between exposure to PFAS and thyroid hormone status mainly focused on blood samples from late pregnancy or umbilical cord with mixed findings.

Objectives: To investigate associations between serum-PFAS concentrations and thyroid hormone status in early pregnancy as reflected by FT4 and thyroid-stimulating hormone (TSH).

Methods: In the Odense Child Cohort, a single-center study, we measured maternal pregnancy serum concentrations of five PFAS: perfluorohexane sulfonic acid (PFHxS), perfluoroctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA); and FT4 and TSH in 1048 pregnant women at median GW 12 (25th, 75th percentile: 10, 15). Multivariate linear regression models were performed to estimate associations between PFAS exposure and thyroid hormone status.

Results: A doubling in PFOS, PFOA, and PFNA concentrations was associated with an increment in FT4 concentration by 1.85% (95% CI: 0.66%, 3.05%), 1.29% (95% CI: 0.21%, 2.39%), and 1.70% (95% CI: 0.48%, 2.94%), respectively, in adjusted analyses. A statistically significant dose-response relationship was observed across exposure quartiles for PFOS, PFOA, and PFNA in the association with FT4. No association was found between concentrations of PFAS and TSH in adjusted analyses.

Conclusion: Exposure to PFOS, PFOA, and PFNA was associated with higher FT4 concentrations in women during early pregnancy. The potential clinical implications of these findings remain to be clarified.

1. Introduction

Perfluoroalkyl substances (PFAS) are a group of persistent environmental chemicals with fat, oil, and water repellent properties used, e.g., in food packaging and textiles ([ATSDR] ATSDR, 2018). PFAS exposure routes are mainly through diet and potentially through drinking water and via inhalation of house dust from PFAS treated materials. Elimination half-lives for common PFAS range from four to eight years in the human body, and PFAS are measurable in the majority of human
blood samples across populations (ATSDR) AfTSaDR, 2018). The two legacy PFAS, perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), have been phased out by some major manufacturers, however, they remain in the environment (ATSDR AfTSaDR, 2018; Glynn et al., 2012). Yet less studied PFAS are in current use, such as perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA) (Stubleski et al., 2016).

PFAS are characterized as endocrine disrupting chemicals and have the capability to interfere with the endocrine homeostasis, including thyroid hormone status (Ballesteros et al., 2017). Pregnancy represents a vulnerable window for environmental influence on fetal development. During pregnancy, the woman and especially the fetus are sensitive to disruption of the maternal thyroid gland. The fetus is reliant on placental transfer of maternal thyroid hormones, especially free thyroxine (FT4), during the first 18–20 weeks of gestation (Korevaar et al., 2017). The fetal thyroid gland starts to produce low concentrations of thyroxine (T4) from around 12 weeks of gestation (Alexander et al., 2017; Jansen et al., 2019), while the fetus still relies on maternal supply of iodine-containing thyroid hormone. By gestational week 28, the fetal thyroid gland secretes concentrations equivalent to circulating maternal concentrations (de Escobar et al., 2008). Hence, maternal thyroid hormones play a pivotal role in the fundamental process of the early fetal neural maturation and development (Bernal, 2007; Dussault and Ruel, 1987) and regulation of metabolism (Boas et al., 2012; Skovsager Andersen et al., 2021). FT4 may be an unsurpassed way of assessing thyroid status in first trimester compared to thyroid-stimulating hormone (TSH) (de Escobar et al., 2008). TSH assesses thyroid status during pregnancy (Alexander et al., 2017), however, TSH levels may not be completely accurate, as concentrations of human chorion gonadotropin (HCG) in first trimester indirectly reduce TSH secretion from the pituitary gland (de Escobar et al., 2008). FT4 is dependent on production and binding capacity to thyroid hormone-binding proteins, including transthyretin (TTR), thyroxin-binding globulin, and albumin (Liz et al., 2010) (Fig. 1). Among these proteins, TTR is interfered by PFAS, as suggested by experimental data (Chang et al., 2008; Weiss et al., 2009).

Few studies (Aimuzi et al., 2020; Inoue et al., 2019; Preston et al., 2018) investigated associations between PFAS concentrations and thyroid status during the most vulnerable part of pregnancy, first trimester. In a recent large Chinese study, higher maternal concentrations of PFOA and PFHxS in early pregnancy were associated with increased concentrations of FT4, and higher concentrations of PFHxS were associated with lower concentrations of TSH (Aimuzi et al., 2020). No statistically significant associations were demonstrated between concentrations of PFAS and thyroid hormones in Danish pregnant women at median gestational week (GW) 8 (Inoue et al., 2019), but an American study found higher maternal concentrations of PFOA and PFHxS were associated with lower concentrations of calculated FT4 index at median GW 10 (Preston et al., 2018). Several studies have explored associations between maternal PFAS concentrations and thyroid hormones status during second and third trimester pregnancy with inconsistent findings (Ballesteros et al., 2017; Berg et al., 2015; Chan et al., 2011; Wang et al., 2013, 2014; Webster et al., 2014; Xiao et al., 2020), but generally PFAS tended to be negatively associated with T4 (Boesen et al., 2020; Kim et al., 2018).

**Fig. 1.** Plausible mechanistic sites of PFAS interaction with T4 during early pregnancy. TSH stimulates the thyroid gland to produce T4 and T3 (thick arrows), which circulate mainly bound to thyroid hormone binding proteins: T3, T4, TTR, or albumin (thin arrows). T4 may also undergo deiodination to T3 (hollow arrow). Dashed circles represent hypothetical potential PFAS target sites (lightning arrows) disrupting thyroid homeostasis. Colourbox.

Notes: PFAS, Perfluoroalkyl substances; T3, triiodothyronine; T4, Thyroxine; TSH, Thyroid-stimulating hormone; TTR, Transthyretin.

We hypothesized that exposure to PFAS in early pregnancy may disrupt thyroid hormone status, and we aimed to examine the association among pregnant women from a European single-center prospective study, the Odense Child Cohort (OCC), considering that previous studies primarily focused on blood samples from late pregnancy or umbilical cord reporting mixed findings.
2. Materials and methods

2.1. Study population

The present study is part of OCC (N = 2874), a longitudinal birth cohort conducted in Denmark (Kyhl et al., 2015). Women residing in the Municipality of Odense, Region of Southern Denmark were eligible for study participation and were recruited in early pregnancy (GW < 16) between 2010 and 2012. Following enrolment, the pregnant women were requested to donate a blood sample for PFAS and thyroid hormone status analysis and respond to a questionnaire on current health status and lifestyle (median GW (25th, 75th percentile): 12 (10, 15)).

In this study, we excluded multiple pregnancies (N = 56), miscarriages (N = 103), stillbirths (N = 10), and women without measured serum-PFAS concentrations and thyroid hormone status (N = 1657) (Fig. 2). Five women were pregnant more than once within the inclusion period, and only the first pregnancy was considered eligible for the present study. Finally, women taking prescribed thyroid medication (including Levothyroxine) or with unknown status regarding thyroid medication were excluded (N = 36) (Fig. 2).

2.2. PFAS assessment

Assessment of maternal serum-PFAS concentrations included the following compounds: PFHxS, PFOS, PFOA, PFNA, and PFDA. Maternal serum samples were pipetted into polypropylene cryotubes and stored at −80 °C before analysis (Dalsager et al., 2016). Blood samples collected at enrolment were analyzed for PFAS concentrations in two batches (Jensen et al., 2020). First, 649 blood samples were analyzed between September 2011 and September 2013, while the remaining 979 samples were analyzed in January 2019 (Jensen et al., 2020). All PFAS concentrations from both batches were estimated by using exactly the same automated on-line solid-phase extraction based analytical method on physically the same liquid chromatography and triple quadruple mass spectrometry (LC-MS/MS), and operated by the same laboratory assistant at the Department of Environmental Medicine, University of Southern Denmark (Jensen et al., 2015). Throughout the analyses, the within-batch coefficients of variation (CVs) were <3% and the between-batch CVs for both sets of data were <10.5% (Dalsager et al., 2021). A minor number of samples from the first data batch was reanalyzed with the second batch. The measured PFAS concentrations were in good agreement. The quality control process utilized for the batches in the two time periods included use of the certified reference material from National Institute of Standards and Technology, and samples from the German External Quality Assessment Scheme (G-EQUAS) in all series of samples analyzed, in order to ensure that the accuracy of the measurements of the reported PFAS were within the certified concentration ranges. The Department of Environmental Medicine participates in the G-EQUAS regarding analyses of biological materials organized by The German Society of Occupational Medicine. The Limit of

Fig. 2. Flowchart of participant exclusion and inclusion.
Quantification (LOQ) was 0.03 ng/mL for all compounds, and concentrations below the LOQ were reported as LOQ/2 (Supplemental Table 1).

2.3. Thyroid hormone analyses

Fasting blood samples from the time of inclusion were analyzed for FT4 (ACN code 10160), TSH (ACN code 10172), and thyroid peroxidase antibody (TPOab) (ACN code 10066) using electrochemiluminescence immunoassay on the E801 module at the Roche Cobas 8000 platform (Roche Diagnostics GmbH). The intra-assay coefficients of variation were 3.6 and 3.8% at concentrations of 16.2 and 35.1 pmol/L, respectively (FT4); 7.2 and 4.6% at concentrations of 0.08 and 11 mIU/L, respectively (TSH); and 15 and 12.5% at concentrations of 17.5 and 24 kIU/L, respectively (TPOab). Positive TPOab was defined as >34 kIU/L (Skovsager Andersen et al., 2021).

2.4. Covariates

Maternal age at delivery and parity were derived from hospital records using maternal civil registration numbers. Pre-pregnancy BMI, smoking status (yes/no) during pregnancy, and educational level (high school or less; high school +1–4 years; high school +5 or more years) were extracted from questionnaires. If educational information was missing in questionnaires, they were retrieved from hospital records. Maternal national origin (European; non-European) was based on information about the country of birth acquired from Odense Municipality. A questionnaire was distributed to the pregnant women at GW 28, where the women were asked to declare, if they took any medications during pregnancy. Additionally, they were requested to specify use of over-the-counter and prescription medication, including name of the drug, dosage, duration, and period of gestational use (for the following gestational categories: GW 4–9, 10–14, 15–19, 20–24, and 25–29).

2.5. Ethical approval

The study was performed in accordance with the Helsinki Declaration II and approved by the Regional Scientific Ethical Review Committee for Southern Denmark (Project ID S-20090130) and the Danish Data Protection Agency (J.no. 18/15692). All participants received written and oral information and provided their written consent for participation in the study.

2.6. Statistical analyses

Concentrations of PFAS, FT4, and TSH during pregnancy were normally distributed and reported as median and 90% range (5%, 95% percentiles) in subgroups according to maternal and child characteristics. Differences in concentrations of PFAS and thyroid hormones between subgroups of maternal and offspring descriptive characteristics were analyzed using Kruskal-Wallis or Wilcoxon’s rank sum test (non-normally distributed variables). Characteristics of included mother-child pairs were compared to the rest of the women in OCC using t-test (normally distributed variables) or Wilcoxon’s rank sum test between groups, and Chi square test for categorical variables. Correlations between PFAS were tested by pairwise Spearman correlations.

Associations between concentrations of PFAS and FT4 and TSH during pregnancy were investigated using linear regression models. As concentrations of PFAS, FT4, and TSH were ln-transformed to obtain normally distributed residuals and variance homogeneity, the β-estimates were transformed to express percent change in thyroid hormone concentration for a two-fold increase in the PFAS concentration. Serum PFAS concentrations were divided into quartiles, and a test for linear trend across exposure groups were performed to assess dose-response-association. In the quartile analyses, the β-estimates express percent change in thyroid hormone concentration in the respective exposure quartile compared to first quartile (reference).

Confounders and intermediate factors were identified using directed acyclic graphs (DAGs) based on a priori review of published evidence (Supplemental Fig. 1). Maternal serum-PFAS concentrations have been found to change across categories of parity (Brantsaeter et al., 2013), age, and education (Bjerrregaard-Olesen et al., 2016). Age and socio-economic status may be strong predictors for the maternal thyroid hormone status during pregnancy (Jasim and Gharib, 2018; Knudsen et al., 2003; Mehran et al., 2019). Hence, we adjusted for age (continuous), parity status (nulliparous; parous), and educational level (high school or less; high school +1–4 years; high school +5 or more years).

Previous studies demonstrated the effects of PFAS on thyroid hormone status to be affected by TPOab (Aimuzu et al., 2020; Inoue et al., 2019; Preston et al., 2018). Hence, effect modification by TPO status on the association between concentrations of PFAS and thyroid hormones was evaluated by including an interaction term between PFAS concentrations and TPO status in the linear regression models. The interaction models were used to obtain TPOab status specific (TPOab positive and negative) results.

In sensitivity analyses, the adjusted linear regression models were additionally adjusted for maternal pre-pregnancy BMI and smoking status, as these factors might mediate or confound the association between PFAS exposure and concentrations of thyroid hormones (Mehran et al., 2019) (Fig. S1).

Model assumptions of all models were validated through comprehensive residual analyses. Augmented component-plus-residual plot was conducted to identify potential non-linearities in the data, and no indication of non-linearity was observed in any of the models. Normality and homoscedasticity of residuals in models were inspected graphically, and multicollinearity was assessed formally using variance inflation factors. A two-sided significance level of 5% was used. Data were analyzed using STATA/IC version 17.0 (StataCorp, College Station, TX, USA).

3. Results

A total of 1007 pregnant women with both serum-PFAS concentrations and thyroid hormone concentrations were eligible for inclusion in the final analyses. The included pregnant women had a mean age of 30.2 (±4.5 SD) years at the time of parturition, were predominantly nulliparous (58.7%) and of European origin (97.2%), 18.9% were in the highest educational group (completed high school +5 or more years), and 3.4% smoked during pregnancy (Table 1). The women gave birth (47% girls and 52% boys) after a median gestation of 40.1 (5th, 95th percentile: 37.4, 41.9) weeks. A total of 8.4% (n = 85) women were TPOab positive (Table 2). The included pregnant women were similar to the rest of the participants in OCC in regard to maternal age, pregnancy BMI, educational level, gestational age, and sex of the child, but they smoked less and were more often nulliparous and of European origin (Supplemental Table 2).

PFOS, PFOA, PFNA, and PFDA were detectable in all samples in this study, while 0.8% (8/1007) of the included women had a PFHxS concentration below the LOQ (Supplemental Table 1). Nulliparous women had statistically significantly higher concentrations of all five PFAS compared to parous women (Table 1). PFOS and PFOA concentrations decreased with increasing maternal age (Table 1); the estimates were similar and in the same direction when stratifying according to parity, though no longer statistically significant. Weak to strong correlations were found between the five PFAS concentrations, as the Spearman correlation coefficients ranged from 0.33 (PFHxS and PFDA) to 0.64 (PFOA and PFNA) (Supplemental Table 3).

Concentrations of FT4 decreased with higher BMI, and TSH concentrations were higher in nulliparous and non-smoking women (Table 2). FT4 concentrations were lower in pregnant women with male child compared to parous women (Table 1). PFOS and PFOA positive women had statistically significantly higher concentrations of TSH compared to TPOab negative women, whereas there was no significant difference in PFAS exposure
concentrations or main characteristics between the two groups (Supplemental Table 4).

In crude analyses, PFOS, PFOA, and PFNA were associated with higher concentrations of FT4 (Table 3). After adjusting for confounders, a two-fold increase in concentrations of PFOS, PFOA, and PFNA was associated with an increment in concentrations of TSH by 6.85% (95% CI: 0.13%, 4.77%) (PFNA). A statistically significant dose-response association was found for FT4 in the fourth quartile compared to the first quartile statistically significantly increased FT4 concentrations: 2.82% (95% CI: 0.53%, 5.16%) (PFOS); 2.74% (95% CI: 0.26%, 5.28%) (PFOA); and 2.42% (95% CI: 0.13%, 4.77%) (PFNA). A statistically significant dose-response relationship was observed across exposure quartiles for PFOS, PFOA, and PFNA (p-value for linear trend <0.01) in the association with FT4 (Table 3).

In crude models, a two-fold increase in PFHxS concentrations was associated with an increment in concentrations of TSH by 6.85% (95% CI: 1.18%, 12.84%) (Table 3). After adjusting for confounders, no statistically significant continuous or dose-response associations were found between any of the PFAS concentrations and TSH (Table 5).

Table 1
Maternal pregnancy serum-PFAS concentrations (ng/mL) according to maternal and offspring characteristics.

<table>
<thead>
<tr>
<th>Variables</th>
<th>PFHxS</th>
<th>PFOS</th>
<th>PFOA</th>
<th>PFNA</th>
<th>PFDA</th>
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<tr>
<td>N (%)</td>
<td></td>
<td></td>
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<td></td>
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<td>&lt;25</td>
<td>106</td>
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<td>0.14</td>
<td>0.81</td>
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<td>(10.5)</td>
<td></td>
<td>8.09</td>
<td>(3.52, 16.75)</td>
<td>1.91</td>
<td>(0.75, 4.19)</td>
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<td>25-29</td>
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<td>0.73</td>
<td>0.34</td>
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<td></td>
<td>8.09</td>
<td>(3.54, 16.53)</td>
<td>1.78</td>
<td>(0.74, 4.01)</td>
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<td>0.11</td>
<td>0.72</td>
<td>0.33</td>
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<td>(0.63, 3.81)</td>
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<td>0.32</td>
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<td>7.72</td>
<td>(3.58, 15.30)</td>
<td>1.68</td>
<td>(0.68, 3.92)</td>
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<tr>
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<td>28</td>
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<td>0.85</td>
<td>0.12</td>
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<tr>
<td>(2.8)</td>
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<td>4.63</td>
<td>(1.14, 11.65)</td>
<td>1.00</td>
<td>(0.31, 4.03)</td>
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<tr>
<td>Child Characteristics</td>
<td></td>
<td></td>
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<tr>
<td>Boys</td>
<td>522</td>
<td>0.35</td>
<td>0.13</td>
<td>0.76</td>
<td>0.35</td>
</tr>
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<td>(51.8)</td>
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<td>7.93</td>
<td>(3.56, 15.31)</td>
<td>1.69</td>
<td>(0.66, 4.03)</td>
</tr>
<tr>
<td>Girls</td>
<td>472</td>
<td>0.32</td>
<td>0.09</td>
<td>0.75</td>
<td>0.32</td>
</tr>
<tr>
<td>(46.9)</td>
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<td>7.42</td>
<td>(3.39, 15.02)</td>
<td>1.61</td>
<td>(0.63, 3.81)</td>
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<tr>
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<td>0.32</td>
<td>0.09</td>
<td>0.75</td>
<td>0.32</td>
</tr>
</tbody>
</table>

Note: Data are presented as median (5th, 95th percentile) and numbers (%). Abbreviations: BMI, Body mass index; PFDA, Perfluorodocanoic acid; PFHxS, Perfluorohexanesulfonic acid; PFOS, Perfluorooctanoic acid; PFOA, Perfluorooctanoic acid; PFNA, Perfluorononanoic acid. P-value, when comparing PFAS concentrations between subgroups according to maternal and child characteristics using Kruskal-Wallis or Wilcoxon rank sum tests.
In pregnant women with TPOab negative status, higher concentra-
tions of FT4 in crude and adjusted models (Supplemental Table 5).
Corresponding estimates for TPOab positive women were
closer to the null, but they were not statistically significant different
from the estimates in TPOab negative women (p < 0.01) (Sup-
plemental Table 5).

Note: Data are presented as median (5th, 95th percentile) and numbers (%).
Abbreviations: BMI, Body mass index; FT4, Free thyroxine; TPOab, Thyroid peroxidase antibody; TSH, thyroid-stimulating hormone.

P-value, when comparing thyroid hormones concentrations and TPO status between subgroups according to maternal and child characteristics using Kruskal-Wallis,

Table 2
Concentrations of thyroid hormones and antibodies according to maternal and offspring characteristics.

<table>
<thead>
<tr>
<th>Variables</th>
<th>N (%)</th>
<th>FT4 (pmol/L) Median (5th, 95th percentile)</th>
<th>TSH (mIU/L) Median (5th, 95th percentile)</th>
<th>TPOab positive N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>1007 (100)</td>
<td>13.8 (11.4, 17.1)</td>
<td>1.4 (0.3, 3.4)</td>
<td>85 (8.4%)</td>
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<tr>
<td>Maternal characteristics</td>
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<tr>
<td>Maternal age (years)</td>
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</tr>
<tr>
<td>&lt;25</td>
<td>106 (10.5)</td>
<td>14.2 (11.3, 18.8)</td>
<td>1.3 (0.2, 3.2)</td>
<td>7 (6.6%)</td>
</tr>
<tr>
<td>25-29</td>
<td>353 (35.1)</td>
<td>13.8 (11.4, 17.0)</td>
<td>1.4 (0.5, 3.7)</td>
<td>31 (8.8%)</td>
</tr>
<tr>
<td>30-34</td>
<td>368 (36.5)</td>
<td>13.8 (11.3, 17.0)</td>
<td>1.5 (0.3, 3.5)</td>
<td>30 (8.2%)</td>
</tr>
<tr>
<td>&gt;35</td>
<td>180 (17.9)</td>
<td>13.7 (11.4, 17.2)</td>
<td>1.3 (0.3, 3.3)</td>
<td>17 (9.4%)</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>0.09</td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>Missing</td>
<td>12 (1.2)</td>
<td>0.01</td>
<td></td>
<td>0.12</td>
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<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nulliparous</td>
<td>591 (58.7)</td>
<td>13.8 (11.3, 17.2)</td>
<td>1.5 (0.4, 3.8)</td>
<td>48 (8.1%)</td>
</tr>
<tr>
<td>Parous</td>
<td>416 (41.3)</td>
<td>13.8 (11.4, 17.1)</td>
<td>1.2 (0.3, 3.2)</td>
<td>37 (8.9%)</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>0.78</td>
<td></td>
<td>&lt;0.01</td>
</tr>
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<td>Smoking</td>
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<tr>
<td>Yes</td>
<td>34 (3.4)</td>
<td>13.3 (11.7, 18.1)</td>
<td>1.0 (0.2, 2.0)</td>
<td>1 (2.9%)</td>
</tr>
<tr>
<td>No</td>
<td>960 (95.5)</td>
<td>13.8 (11.3, 17.1)</td>
<td>1.4 (0.3, 3.4)</td>
<td>84 (8.8%)</td>
</tr>
<tr>
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<tr>
<td>Educational level</td>
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<tr>
<td>High school or less</td>
<td>293 (29.1)</td>
<td>13.8 (11.4, 18.1)</td>
<td>1.3 (0.3, 3.2)</td>
<td>26 (8.9%)</td>
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<tr>
<td>High school 1–4 years</td>
<td>512 (50.8)</td>
<td>13.7 (11.3, 16.9)</td>
<td>1.5 (0.3, 3.7)</td>
<td>41 (8.0%)</td>
</tr>
<tr>
<td>High school &gt;5 or more years</td>
<td>190 (18.9)</td>
<td>13.9 (11.4, 16.6)</td>
<td>1.5 (0.3, 3.5)</td>
<td>17 (9.0%)</td>
</tr>
<tr>
<td>Missing</td>
<td>12 (1.2)</td>
<td>0.47</td>
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<td>0.25</td>
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<tr>
<td>National origin</td>
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<tr>
<td>European</td>
<td>979 (97.2)</td>
<td>13.8 (11.4, 17.1)</td>
<td>1.4 (0.3, 3.5)</td>
<td>84 (8.6%)</td>
</tr>
<tr>
<td>Non-European</td>
<td>28 (2.8)</td>
<td>13.6 (11.3, 18.5)</td>
<td>1.2 (0.5, 2.9)</td>
<td>1 (3.6%)</td>
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<tr>
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</tr>
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<td>Child sex</td>
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</tr>
<tr>
<td>Boys</td>
<td>522 (51.8)</td>
<td>13.7 (11.6, 16.9)</td>
<td>1.4 (0.3, 3.3)</td>
<td>44 (8.4%)</td>
</tr>
<tr>
<td>Girls</td>
<td>472 (46.9)</td>
<td>13.9 (11.3, 17.2)</td>
<td>1.4 (0.3, 3.5)</td>
<td>41 (8.7%)</td>
</tr>
<tr>
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<tr>
<td>p-value</td>
<td></td>
<td>0.02</td>
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</tbody>
</table>

Note: Data are presented as median (5th, 95th percentile) and numbers (%).

In sensitivity analyses, estimates from the multiple linear regression models did not change materially after additionally adjusting for pre-pregnancy BMI and smoking status (Supplemental tables 6a-b).

4. Discussion

Our main study finding was that higher maternal serum concentra-
tions of PFOS, PFOA, and PFNA in early pregnancy were associated with increased concentrations of FT4 during early pregnancy in this European single-center cross-sectional study. This is supported by experimental studies demonstrating that PFAS have affinity for the thyroid hormone-binding protein, TTR (Chang et al., 2008; Weiss et al., 2009). In accor-
dance with our results, a recent large Chinese study also demonstrated that higher maternal PFOA concentrations were associated with increased concentrations of FT4 among pregnant women around GW 13 (Aimuzu et al., 2020). While a study based on the Danish National Birth Cohort (DNBC) reported no association between continuous and catego-
rical PFAS exposures and FT4 at GW 8 (Inoue et al., 2019). As opposed
to our single-center study, the DNBC study relied on pooled national data across several sub-cohort samples (Inoue et al., 2019). In our study, concentrations of PFAS and thyroid hormones were measured at the same laboratory. By contrast, PFAS concentrations from the DNBC were measured at two different laboratories, and slightly higher absolute concentrations of PFOS and PFOA were reported at one laboratory compared to the other (Inoue et al., 2019). Our maternal concentrations of serum FFNA and PFDA from early pregnancy (GW 12) were somewhat higher compared to the DNBC at GW 8 (1996–2002) (Inoue et al., 2019), whereas serum PFHxS, PFOS, and PFOA in our study were lower compared to DNBC (Inoue et al., 2019). The difference in study sampling (single-center vs. national), temporal and/or spatial changes in PFAS exposures, and selection of laboratories may explain the discrepancy in findings between our study and the DNBC (Inoue et al., 2019), despite both studies were performed in Denmark. In contrast, one Boston study reported that higher concentrations of PFOA and PFHxS from around GW 10 were associated with lower concentrations of the maternal FT4 index, which is an older method for estimating circulating FT4 based on a calculation from total T4 and triiodothyronine resin uptake (Preston et al., 2018). As opposed to the calculated FT4 index applied in the Boston study (Preston et al., 2018), our study and others (Aimuzu et al., 2020).
sulfonic acid; PFNA, Perfluorononanoic acid; TSH, thyroid-stimulating hormone. 

Abbreviations: FT4, Free thyroxine; PFDA, Perfluorodecanoic acid; PFHxS, Perfluorohexane sulfonic acid; PFOA, Perfluorooctanoic acid; PFOS, Perfluorooctane sulfonic acid; PFOA, Perfluorooctanoic acid; PFOS, Perfluorooctane sulfonic acid; PFNA, Perfluorononanoic acid; TSH, thyroid-stimulating hormone. 

that this difference between studies in methodological calculation when 

et al., 1992), which in most clinical situations have supplanted the 

assessing FT4 concentrations may have an impact on findings and 

for the quartiles expresses the percentage change in thyroid hormones in the 2nd, 3rd, and 4th quartile compared to the 1st quartile. 

Note: Percentage change (β) in marker of maternal thyroid hormone status with a two-fold increase in pregnancy serum-PFAS concentration (ng/mL). The β-coefficient for the quartiles expresses the percentage change in thyroid hormones in the 2nd, 3rd, and 4th quartile compared to the 1st quartile. 

Abbreviations: FT4, Free thyroxine; PFDA, Perfluorodecanoic acid; PFHxS, Perfluorohexane sulfonic acid; PFOA, Perfluorooctanoic acid; PFOS, Perfluorooctane sulfonic acid; PFNA, Perfluorononanoic acid; TSH, thyroid-stimulating hormone. 

* Adjusted for age, parity, and educational level. 

2020; Inoue et al., 2019) relied on assessed FT4 measurements (Wong et al., 1992), which in most clinical situations have supplanted the calculated FT4 index (Midgley, 2001). Hence, it must be acknowledged that this difference between studies in methodological calculation when assessing FT4 concentrations may have an impact on findings and challenge comparability. 

We observed no association between maternal concentrations of PFAS and TSH after adjusting for confounders. In line with our findings, the Boston (Preston et al., 2018) and Danish (Inoue et al., 2019) studies likewise observed no association between maternal concentrations of PFAS and TSH at GW 10 and 8, respectively. In contrast, the Chinese study reported that higher PFHxS concentration (though not PFOS and PFOA) were associated with lower concentrations of TSH at GW 13 (Aimuzi et al., 2020). In comparison to our study, the median PFHxS concentration was twice as high in the Chinese study. However, TSH may be less reliable during first trimester, as the pronounced rise in concentrations of HCG stimulates T4 secretion and inhibits TSH from the pituitary gland (de Escobar et al., 2008). 

TPO is the primary enzyme involved in thyroid hormone production, and the presence of TPO antibodies (TPOab) may inhibit TPO and induce autoimmune thyroiditis (Czarnocka et al., 1985; Taurog et al., 1996). In the present study, 8.4% of the women were TPOab positive, which is comparable to another Danish study during early pregnancy (Knudsgaard et al., 2020), but it was somewhat lower compared to other studies, where the prevalence ranged from 12.1% in a Chinese setting (Aimuzi et al., 2020) to 14% in an American setting (Preston et al., 2018). In our study, higher concentrations of PFOS, PFOA, and PFNA were associated with lower concentrations of TSH in the TPOab positive women (Preston et al., 2018). In the Chinese study did not demonstrate any effect modification by TPOab status between PFAS and TPOab status in relation to outcomes FT4 and TSH. This likely, we observed no statistically significant interaction terms between maternal concentrations of PFAS and TSH at GW 10 and 8, respectively. In contrast, the Chinese study reported that higher PFHxS concentration (though not PFOS and PFOA) were associated with lower concentrations of TSH at GW 13 (Aimuzi et al., 2020). In comparison to our study, the median PFHxS concentration was twice as high in the Chinese study. However, TSH may be less reliable during first trimester, as the pronounced rise in concentrations of HCG stimulates T4 secretion and inhibits TSH from the pituitary gland (de Escobar et al., 2008). 

Table 3 

<table>
<thead>
<tr>
<th>Compound</th>
<th>FT4</th>
<th></th>
<th>TSH</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (95% CI)</td>
<td>p</td>
<td>β (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td>PFHxS</td>
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<tr>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Adjusted * (N = 995)</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>1st (0.15 &gt; 0.23) (N = 247)</td>
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</tr>
<tr>
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<tr>
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<tr>
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</tr>
<tr>
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<td>Adjusted * (N = 995)</td>
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<tr>
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<tr>
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<tr>
<td>p-trend</td>
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</tbody>
</table>

2020; Inoue et al., 2019) relied on assessed FT4 measurements (Wong et al., 1992), which in most clinical situations have supplanted the calculated FT4 index (Midgley, 2001). Hence, it must be acknowledged that this difference between studies in methodological calculation when assessing FT4 concentrations may have an impact on findings and challenge comparability. 

We observed no association between maternal concentrations of PFAS and TSH after adjusting for confounders. In line with our findings, the Boston (Preston et al., 2018) and Danish (Inoue et al., 2019) studies likewise observed no association between maternal concentrations of PFAS and TSH at GW 10 and 8, respectively. In contrast, the Chinese study reported that higher PFHxS concentration (though not PFOS and PFOA) were associated with lower concentrations of TSH at GW 13 (Aimuzi et al., 2020). In comparison to our study, the median PFHxS concentration was twice as high in the Chinese study. However, TSH may be less reliable during first trimester, as the pronounced rise in concentrations of HCG stimulates T4 secretion and inhibits TSH from the pituitary gland (de Escobar et al., 2008). 

TPO is the primary enzyme involved in thyroid hormone production, and the presence of TPO antibodies (TPOab) may inhibit TPO and induce autoimmune thyroiditis (Czarnocka et al., 1985; Taurog et al., 1996). In the present study, 8.4% of the women were TPOab positive, which is comparable to another Danish study during early pregnancy (Knudsgaard et al., 2020), but it was somewhat lower compared to other studies, where the prevalence ranged from 12.1% in a Chinese setting (Aimuzi et al., 2020) to 14% in an American setting (Preston et al., 2018). In our study, higher concentrations of PFOS, PFOA, and PFNA were associated with higher concentrations of FT4 in pregnant women with TPOab negative status, but not among pregnant women with TPOab positive status. Hypothetically, the presence of TPOab may competitively bind to the same potential interaction sites as PFAS, hence the non-significant findings among TPOab positive women. Interest- ingly, we observed no statistically significant interaction terms between the PFAS and TPOab status in relation to outcomes FT4 and TSH. This indicated no statistically significant difference in slopes for TPOab positive and TPOab negative pregnant women. Accordingly, the Boston study did not demonstrate any effect modification by TPOab status between PFAS concentrations and FT4 index among pregnant women during early pregnancy, but PFOS and PFNA were associated with lower TSH in the TPOab positive women (Preston et al., 2018). In the Chinese study, no associations were found between concentrations of PFAS, and FT4 and TSH among TPOab negative pregnant women during early pregnancy, but PFNA concentrations were associated with higher FT4 among TPOab positive pregnant women (Aimuzi et al., 2020).
Comparing study findings in relation to TPOab status may be challenged by differential cut-off levels for TPOab positivity, thus variability in sample sizes of TPOab positive women. Differences in PFAS exposure and descriptive characteristics between TPOab status may also play a role.

Published evidence from experimental studies suggest plausible biological explanations on how PFAS may interfere with the complex pathways of thyroid hormone status (Chang et al., 2008; Weiss et al., 2009). Interestingly, these studies demonstrated that PFAS competitively bind to one of three thyroid hormone-binding proteins, namely TTR (Chang et al., 2008; Weiss et al., 2009). TTR is a plasma protein that binds and transports mainly T4, and it also prevents variations in the accessibility of thyroid hormones by acting as a physiological buffer (Liz et al., 2010; Mimoto and Reteloff, 2020; Wieczorek and Ozyhar, 2021). Moreover, TTR plays a critical role in transplacental transport of maternal thyroid hormones to the fetus during early pregnancy (Landers et al., 2009). An experimental study demonstrated that major PFAS, including PFOA, PFOS, and PFHxS, showed increased affinity for TTR (Weiss et al., 2009) (Fig. 1). Correspondingly, Chang et al. demonstrated that a single dose of PFOS resulted in increased concentration of FT4 in adult rats, while TSH concentrations were unaffected (Chang et al., 2009). The competitive binding of PFAS on TTR may conceivably have implications on the amount of free thyroid hormones by displacing T4 from the TTR, hence resulting in increased concentrations of free/unbound thyroid hormones (FT4). Alternatively, the increased concentrations of FT4 could be explained by a potential PFAS-induced inhibition of deiodinase (Fig. 1), but an experimental study on male rats suggested an increase in thyroidal deiodinase (type 1) in response to elevated PFOS exposure (Yu et al., 2009). Our in vivo data may support the experimental study findings (Chang et al., 2008; Weiss et al., 2009) suggesting that PFAS can affect thyroid hormone status by competitively binding to the thyroid hormone transport proteins. A displacement of TTR bound T4 during pregnancy may potentially have implications on the transplacental transport of maternal thyroid hormones to the fetus during the sensitive windows of development.

The major strength of the present study is the large sample size of pregnant women with PFAS and thyroid status assessed in early pregnancy. In our study, maternal concentrations of legacy PFAS were measured in early pregnancy around the most sensitive stage of fetal thyroid development. In contrast, other studies (Berg et al., 2015; Chan et al., 2011; Wang et al., 2013, 2014; Webster et al., 2014; Xiao et al., 2020) determined PFAS concentrations in late pregnancy, where their serum concentrations are lower. Our single-time point measurement of exposure largely reflects the PFAS concentrations throughout early pregnancy due to the stable and long half-lives of PFAS in humans (ATSDDR) A1SdDR, 2018). Misclassification is most likely minimal, and any misclassification would be non-differential, as the trained health care professionals and pregnant women were blinded to PFAS concentrations and thyroid status.

Some potential limitations should be noted. We appreciate that the cross-sectional design of the study limits the ability to establish the true cause and effect relationship. Nonetheless, the long half-lives of PFAS would reflect exposure concentrations for an extended period. Unfortunately, data on thyroid hormone-binding proteins, triiodothyronine (T3), FT3, total T4, and iodine status were not available in the study. These data would have contributed to the complex interpretation and understanding of the potential PFAS disrupting effects on thyroid metabolism. We acknowledge the risk of chance findings due to multiple comparison bias, as associations were not adjusted for multiple testing. However, the study was based on an a priori hypothesis. The associations explored may have been affected by residual confounding from other unknown or unmeasured covariates related to maternal PFAS exposure and thyroid metabolism, such as exposure to correlated endocrine disrupting chemicals, diet, medication, or genetic disposition, although the covariates identified affected the findings only marginally. Finally, the pregnant women included in the study smoked less, had a higher pregnancy BMI, were more often nulliparous and of European origin compared to the rest of the women in OCC. Potential confounding by parity, smoking, and pre-pregnancy BMI was addressed by adjustment in the analyses and sensitivity analyses, which materially did not change the estimates. This potential lack of complete representability is unlikely to have affected the association between maternal PFAS concentrations and thyroid hormone status during pregnancy.

Our observed associations between exposure to PFAS and FT4 concentrations were small in magnitude, nonetheless, the effects may be greater in populations with higher concentrations of PFAS exposure. The clinical significance of these findings remains to be elucidated. At population level, the demonstrated potential disruption of maternal thyroid hormone status in response to PFAS exposure during early pregnancy may affect offspring neurodevelopment. Hence, the findings are of general public interest, which supports the necessity of a follow-up of offspring in the OCC to assess putative long-term implications on neurodevelopment.

5. Conclusion

Increased serum concentrations of PFOS, PFOA, and PFNA during early pregnancy were associated with increased concentrations of FT4, both as continuous variables and in dose-response relationships based on quartiles.

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Ethics

The study was performed in accordance with the Helsinki Declaration II and approved by the Regional Scientific Ethical Review Committee for Southern Denmark (Project ID S-20090130) and the Danish Data Protection Agency (j.no. 18/15692). All participants received written and oral information and provided their written consent for participation in the study.

Declaration of competing interest

The authors declare the following financial interests/personal relationships, which may be considered as potential competing interests: Philippe Grandjean has provided paid expert assistance in legal cases involving PFAS exposed populations. The other authors declare they have no actual or potential competing financial interests.

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Appendix A: Supplementary data

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