Early menarche and other endocrine disrupting effects of per- and polyfluoroalkyl substances (PFAS) in adolescents from Northern Norway. The Fit Futures study.

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Abstract

Background. Per- and polyfluoroalkyl substances (PFAS) comprise a large group of chemicals that are ubiquitous in the environment and include recognized persistent organic pollutants. The aim of this cross-sectional study was to investigate possible endocrine disrupting effects of different PFAS in adolescents.

Methods. Serum concentrations of PFAS, thyroid, parathyroid and steroid hormones were measured in 921 adolescents aged 15-19 years in the Fit Futures study, Northern Norway. The questionnaire included data on self-reported age at menarche and puberty development score (PDS). Multiple linear and logistic regression analyses and principle component analyses (PCA) were used to assess associations of PFAS with hormones concentrations and puberty indices.

Results. In girls, total PFAS (∑PFAS), perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), perfluorononanoate (PFNA), perfluorodecanoate (PFDA) were positively associated with dehydroepiandrosterone sulfate (DHEAS) and negatively associated with 11-deoxycorticosterone (11-DOC)/DHEAS ratio. In boys, the associations with 11-DOC/DHEAS ratio were positive for ∑PFAS, perfluoroheptanoate (PFHpA), perfluoroheptane sulfonate (PFHpS), PFOA, and PFOS. Perfluoroundecanoate (PFUnDA) was negatively associated with free thyroxine (fT4) and free triiodothyronine (fT3) in boys. PFNA and PFDA were also negatively associated with fT3 in boys. Serum parathyroid hormone concentration (PTH) was negatively associated with ∑PFAS and perfluorohexane sulfonate (PFHxS) in girls, and with PFOS in boys. PFDA and PFUnDA were positively associated with early menarche, while ∑PFAS and PFOA were positively associated with PDS in boys.

No associations of PFAS with serum testosterone, follicle-stimulating hormone, or luteinizing hormone were found in either sex. In girls, PFOA was positively associated with free testosterone index (FTI). In boys, PFOA was positively associated with androstendione and 17-OH-progesterone, while PFHpA was positively associated with estradiol.

Conclusions. Serum concentrations of several PFAS were associated with parathyroid and steroid hormones in both sexes, and with thyroid hormones in boys, as well as with early menarche in girls and higher PDS in boys.

Key words: PFAS, early menarche, thyroid hormones, steroid hormones, PTH, endocrine effects

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1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are industrially produced chemicals abundant in the environment worldwide. PFAS have unique hydrophobic and lipophobic properties and have therefore been widely industrially produced and applied for over 60 years. PFAS are still used in many consumer products: impregnated textiles and leather, waterproof clothes, food packages, cosmetics, fire-fighting foams, lubricants, ski waxes, coating agents, paints and stain repellent treated carpets (Paul et al., 2009). PFAS are omnipresent and can be found in food, drinking water, air, soil and within all other environmental compartments. However, dietary intake is the main route of exposure to these compounds in the general population (Domingo & Nadal, 2017; Jian et al., 2018), whereas other exposure pathways include inhalation and dermal contact. Most abundant PFAS, such as perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are defined in the Stockholm convention as persistent organic pollutants (POPs) (Schrenk D, 2020). Little is known about health effects of other PFAS that are not yet included in the Stockholm convention and are widely used to replace PFOS and PFOA in consumer products and other applications. There is increasing evidence that several PFAS may act as endocrine disrupting chemicals (EDC). PFAS may display EDC effects by affecting cellular functions via ligating intracellular receptors such as peroxisome proliferator-activated receptors (PPARs) that are regulators of lipid and carbohydrate metabolism, cell proliferation and inflammation (Lau et al., 2007; Rosen et al., 2017). Animal studies showed development of liver steatosis and obesity in the PFAS exposed mice (Attema et al., 2022; Das et al., 2017; Marques et al., 2020). However, different EDC effects of PFAS in humans are not fully understood. Exposure to PFAS was associated with dysregulation of thyroid function in several epidemiological studies (Aimuzi et al., 2020; Blake et al., 2018; Itoh et al., 2019; Lewis et al., 2015; Lin et al., 2013; Preston et al., 2020; Wang et al., 2014; Webster et al., 2016; Wen et

al., 2013). Some studies have also linked PFAS exposure to sex hormones disruption (Cui et al., 2020; Joensen et al., 2013; Lopez-Espinosa et al., 2016; Tsai et al., 2015; Wang et al., 2021) and showed a possible effect on onset of puberty in boys and girls (Carwile et al., 2021; Ernst et al., 2019). Exposure to PFAS was associated with disturbances in steroid synthesis both in animals and humans (Kang et al., 2016; Maisonet et al., 2015; Zhou et al., 2017). Few studies assessed EDC effects of PFAS exposure in children and teenagers (Lewis et al., 2015; Lin et al., 2013; Lopez-Espinosa et al., 2016; Tsai et al., 2015). Teenagers represent one of the most vulnerable groups for EDC effects due to the crucial hormonal changes occurring during growth and puberty.

The aim of this study was to investigate associations of various PFAS with thyroid, parathyroid, steroid and sex hormones, as well as with menstrual function and pubertal development in teenagers recruited from the general population of Northern Norway.

2. Methods

2.1 The study population

The first Fit Futures study (FF1) was conducted in 2010-2011 in the municipalities of Tromsø and Balsfjord in Northern Norway. All first-year high school students aged 15-19 years (n=1117) were invited to participate and 93% (n=1038) of the invited students attended the FF1 study. All participants visited the Clinical Research Unit at the University Hospital of North Norway (UNN), Tromsø, where trained research nurses did the clinical examinations, blood sampling, and interviews, and the participants filled in an electronic questionnaire. Time for blood sampling was registered and varied from 08:36 to 14:30. Body height and weight were measured to the nearest 0.1 cm and 0.1 kg on a Jenix DS 102 Stadiometer

(Dong Sahn Jenix, Seoul, Korea). Body mass index (BMI) was calculated as weight (kg)/height (m^2).

Non-fasting blood samples were obtained in special glass without background contamination with PFAS for 921 participants. Serum samples were stored at -35 ºC before the analyses of PFAS. Serum for hormones analyses was stored at -70 ºC. Altogether 435 girls and 486 boys provided blood samples for PFAS, steroid and thyroid hormone analyses. Of those, parathyroid hormone (PTH) and 25-OH-vitamin D were measured in 425 girls and 480 boys.

The interview of girls included questions about menstruation, pregnancy, and use of hormonal contraceptives. The following questions about menstruation were asked: Have you started menstruating? If you have started menstruating: how regular are your periods? (always regular, usually regular, irregular); If you have started menstruating and if you know the date of your last menstruation; what was the date of the first day of your last menstruation? If you have started menstruating, how old were you when you had your first menstrual period?

The participants filled in an electronic questionnaire with 143 questions concerning age at menarche and pubertal development, lifestyle, and health problems. All the boys were asked the following question about puberty: If you have got or started to get pubic hair, how old were you when you started to get pubic hair?". In a subgroup of boys (n=385), the Pubertal Development Score was calculated (PDS) (Petersen et al., 1988). It included the following questions: "Would you say that your growth in height has begun to spurt? Would you say that your body hair growth has begun to grow? Have you noticed a deepening of your voice? Have you begun to grow hair on your face?" Response options and points for each PDS question were: 1 point for "not yet started", 2 points for "barely started", 3 points for "definitely started", and 4 points for "seems complete". To assign a summary PDS for each boy, we calculated the sum of scores obtained from all questions and then divided it by four. Indices

coded on a 4-point PDS scale were: 1, no development; 2 , beginning development; 3, additional development; 4, development already passed (Petersen et al., 1988). PDS score was not used for girls in this study as the lowest age was 15 years and all the girls have achieved puberty.

We have compared the main characteristics of 117 participants without blood samples with 921 participants with blood samples that were included in the analyses (Table S1). Mean age, mean age at menarche in girls and mean PDS in boys were not significantly different between these groups. There was higher proportion of girls and slightly higher BMI in the group with missing blood samples.

2.2 Ethics

The Regional Committee for Medical and Health Research Ethics for Northern Norway (REC North 11694) has approved this study. Written, informed consent was obtained from all participants, for those younger than 16 years the written informed consent was provided by a parent/proxy.

2.3 Laboratory analyses

The Environmental Pollutant Laboratory at the Department of Laboratory Medicine, UNN, performed all PFAS analyses. All the equipment for venous blood samples was tested for PFAS contribution and no substantial background PFAS contamination was detected.

The serum samples for PFAS analyses were stored at -35 ˚C and PFAS concentrations were measured after the completion of the survey. The sample preparation and analytical method for PFAS measurements has been described in detail previously (Huber & Brox, 2015). Briefly, ultra-high pressure liquid chromatography tandem-quadrupole mass-spectrometry (UHPLC-MS/MS, Waters Acquity UPLC system, Xevo TQ-S mass spectrometer, Waters, Milford, MA, USA) was applied for analysis of perfluorobutane sulfonate (PFBS),

perfluoropentane sulfonate (PFPS), PFHxS, perfluoroheptane sulfonate (PFHpS), PFOS, perfluorononane sulfonate (PFNS), perfluorodecane sulfonate (PFDS), perfluorododecane sulfonate (PFDoDS), perfluorooctane sulfonamide (PFOSA), perfluorohexanoate (PFHxA), perfluoroheptanoate (PFHpA), PFOA, perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorododecanoate (PFDoDA), perfluorotridecanoate (PFTrDA) and perfluorotetradecanoate (PFTeDA) concentrations in serum. Quantification was performed by the Masslynx and Targetlynx software (Version 4.1, Waters, Milford, MA, USA) using the internal-standard method with isotope-labelled PFAS. Four blank samples, four standard reference material (SRM) 1958 samples (NIST, Gaithersburg, MD, USA) and three bovine serum samples (Sigma Aldrich, Steinheim, Germany) were analyzed within each batch of 96 samples as quality controls. All the controls were within the acceptable limits. Analytical coefficients of variation (CVs) were below 10% for all PFAS except for PFUnDA with CV 12%.

Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), free thyroxine (fT4), free triiodothyronine (fT3), sex hormone binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS) and parathyroid hormone (PTH) analyses were performed at the Cobas 6000 instrument (Roche Diagnostics, F. Hoffmann-La Roche Ltd, Basel, Switzerland) at the Department of Laboratory Medicine, UNN, Tromsø. CVs for all measured hormones were <6%, except for PTH and DHEAS with CV<8%. The Department of Laboratory Medicine, UNN, participates successfully in several external quality control programmes for hormone analyses (Labquality, DEQAS) and in the international quality control programme for environmental pollutants (the Arctic Monitoring and Assessment (AMAP) Ring Test for Persistent Organic Pollutants in Human Serum, organized by the Laboratoire de Toxicologie, Institut National de Santé Publique du Quebec,

Canada). All the external quality controls during the time of the study were within the acceptable limits.

Steroid hormone analyses (testosterone, 17-hydroxyprogesterone, 11-deoxycortisol (11-DOC), androstenedione, estradiol, progesterone, 25-OH-vitamin D were performed in serum samples by LC-MS/MS methods at the Hormone laboratory, Haukeland University Hospital, Bergen. For testosterone and 17-hydroxyprogesterone, CV was <6%, for 11-DOC and androstendione, CV was <7%, for progesterone and 25-OH-vitamin D, CV was <10%. CV for estradiol was <13%. Both clinical laboratories in Tromsø and in Bergen are accredited by the ISO 15189 standard.

2.4 Definitions

Early menarche was defined as menstruation start at the age ≤11 years (Kim & Je, 2019; Lundblad & Jacobsen, 2018).

Late menarche in girls was defined as start of menstruation at the age >15 years (Kim & Je, 2019; Lundblad & Jacobsen, 2018).

Early puberty in boys was defined as having pubic hair at the age ≤9 years (Berberoglu, 2009; Oehme et al., 2020).

Late puberty in boys was defined as starting to develop pubic hair at the age >14 years (Bozzola et al., 2018; Haddad N.G, 2016).

Reference intervals for hormones in teenagers: TSH 0.5-4.4 IU/L, fT4 12-22 pmol/L, fT3 <8.5 pmol/L, PTH 1,1 - 6,8 pmol/L (NSMB, 2023).

Hypothyreoidism was defined as TSH >4.4 IU/L and low/normal fT4 (≤ 22 pmol/L) or use of Levothyroxine (T4) (ATC-code H03AA01).

Hyperthyreoidism was defined as TSH < 0.5 IU/L and normal/high fT4 (≥12 pmol/L)

Low PTH was defined as PTH < 1.1 pmol/L

High PTH was defined as PTH > 6.8 pmol/L

Free testosterone index (FTI) was calculated as testosterone (nmol/l) x 10 / SHBG (nmol/l).

Statistical analyses

Statistical analyses were performed by the SPSS program (IBM Corp. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). Sex differences in PFAS concentrations were evaluated by the non-parametric Mann-Whitney U test because PFAS concentrations were not normally distributed.

Associations between PFAS and serum hormone concentrations were assessed by multiple linear regression analyses with hormone concentrations as dependent variables. Because of the skewed distribution, all PFAS concentrations were log10-transformed before linear regression analyses. Hormone concentrations as covariates for the regression models were not log-transformed as they were nearly normally distributed.

The Targetlynx-software was used to calculate the limit of detections (LOD) for each individual sample (LOD*i*). Values below the LOD*i* were replaced by LOD*i* divided by 2 to reduce possible bias of left censored data analyses.

Only PFAS with detection rate over 70% were included in multiple linear regression analyses: PFHxS (mean LOD 0.02 ng/mL), PFHpA (mean LOD 0.07 ng/mL), PFHpS (mean LOD 0.01 ng/mL), PFOA (mean LOD 0.30 ng/mL), PFOS (mean LOD 0.04 ng/mL), PFNA (mean LOD 0.03 ng/mL), PFDA (mean LOD 0.03 ng/mL), PFUnDA (mean LOD 0.03 ng/mL). We have

performed both individual PFAS and principle component analyses (PCA) for the mixture of PFAS. In our previous publication (Averina et al., 2019) about possible health effects of PFAS we have seen that sometimes the sum of all measured PFAS (∑PFAS) had stronger association with health outcomes than individual PFAS. Therefore, we have used ∑PFAS in this article in addition to the individual PFAS analyses. Age, BMI for both sexes and the use of hormonal contraceptives for girls were included in the regression models as covariates. Several potential confounders were considered such as educational level of parents, ethnicity of the adolescents (Norwegian/ indigenous Sami people/Other), smoking. These variables had no association with PFAS and therefore were not included in the regression models. Analyses for steroid hormones were performed separately for girls who did not use hormonal contraceptives. Multiple linear regression analyses were performed with PDS as a dependent variable for boys and age at menarche for girls with log10-tranformed PFAS concentrations, age and BMI as covariates. Additionally, principle component analysis was performed for PFAS serum concentrations.

Logistic regression analyses were used to examine the associations between PFAS and early menarche, irregular menstruation, early or late puberty, hypothyreoidism, hyperthyreoidism, low PTH, high PTH, serum testosterone and FTI below 25^{th} percentiles. All analyses were performed stratified by sex as previous studies showed sex differences for PFAS effects (Wen et al., 2013). An association with p-value < 0.05 was considered statistically significant.

Human endocrine system has multiple hormones; therefore, we have performed several statistical tests that reflect the complexity of the endocrine system. Each hormone/health outcome was tested for associations with different PFAS individually and then in the principle component analysis. We did not use Bonferroni correction as it is a very conservative approach and was considered not to be necessary here, because we had clear biologically plausible hypotheses about the possible endocrine disrupting effects of PFAS.

3. Results

General characteristics of the study population and PFAS concentrations are presented in Table 1. Detection rates for PFHxS, PFOA, PFOS, PFNA, PFDA were 100%, for PFHpS 98%, PFUnDA 95%, and for PFHpA 75%. Almost all girls (99.4%) had started menstruating and 14.5% of those had irregular periods. Altogether 33.3 % of girls used hormonal contraception. Mean age at menarche was 12.7 years (SD 1.16) with range 9-17 years.

For boys, mean 4-point PDS scale in boys was 3.31 (SD 0.44) with range 2-4 points. Altogether 9.6% of boys had PDS of 4 points, which indicates completed puberty, while 56.6% had PDS between 3 and 4 points.

3.1 Steroid hormones

Linear regression analyses showed that ∑PFAS, PFOA, PFOS, PFNA, PFDA were positively associated with DHEAS in all girls after adjustment for age, BMI, and hormonal contraceptive use and also in girls who did not use hormonal contraceptives (Table 2). In boys, there was an inverse statistically significant association of PFHpS and PFHpA with DHEAS (Table 2). Other PFAS had no statistically significant association with DHEAS.

∑PFAS, PFHpS, PFOA, PFOS were significantly positively associated with 11-DOC in boys, whereas no statistically significant association was seen in girls, except for a borderline inverse association (p=0.04) for PFNA in girls who did not use hormonal contraceptives (Table 3). PFHxS, PFHpA and PFDA were not significantly associated with 11-DOC in both sexes (data not shown).

In girls, 11-DOC/DHEAS ratio was negatively associated with ∑PFAS, PFOA, PFOS, PFNA, PFDA, while in boys the ratio was positively associated with ∑PFAS, PFHpA, PFHpS, PFOA, PFOS (Table 4).

Principle component analysis (PCA) of PFAS serum concentrations was performed. After the Scree plot evaluation and elimination of all components with eigenvalues <1.0, three principle components (PC) were retained for boys (PC1: PFNA; PFDA, PFUnDA; PC2: PFHxS, PFHpS, PFOS; PC3: PFHpA, PFOA) and two PCs for girls (PC1: PFOA, PFNA, PFDA, PFUnDA; PC2: PFHxS, PFHpS, PFOS). PC scores were generated from the PCA and included as covariates in the regression analyses instead of the ΣPFAS variable and the individual PFAS variables.

PC2 score in girls and PC3 score in boys were significantly associated with DHEAS in the multiple linear regression analysis. Other PC scores were not statistically significantly associated with DHEAS when included in the regression models together with PC2 score in girls and PC3 score in boys. The results of the PCA compared with the regression analysis for single PFAS indicate that these associations were mostly due to PFOS effect in girls and PFHpA effect in boys, as other single PFAS that defined these PCs were not significantly associated with DHEAS.

PC1 score in girls and PC2 and PC3 scores in boys were significantly associated with the 11-DOC/DHEAS ratio in the multiple linear regression analysis. Other PC scores were not significantly associated with the 11-DOC/DHEAS ratio. The results of the PCA together with regression analyses for single PFAS showed that PFNA in girls and PFHpS, PFOA in boys contributed most to the significant associations of these PCs with 11-DOC/DHEAS ratio.

In girls, log10-transformed PFOA was positively associated with FTI (β=0.078 (95% CI 0.016 to 0.140), p=0.013) after adjustment for age, time for blood sampling, BMI and hormonal contraceptive use.

In boys, log10-tranformed PFOA was positively associated with androstendione (β=0.71 (95% CI 0.07 to 1.36), p=0.029) and 17-OH-progesterone (β=0.86 (95% CI 0.03 to 1.70),

p=0.043) after adjustment for age, BMI and time for blood sampling. ∑PFAS, PFHpA, PFHpS, PFHxS, PFOS, PFNA, PFDA, PFUnDA were not associated with serum testosterone, FTI, androstendione or serum 17-OH-progesterone concentrations after adjustment for age, time for blood sampling and BMI in boys and girls and additional adjustment for hormonal contraceptive use in girls (results not shown). Log10-tranformed PFHpA was positively associated with estradiol in boys (β=0.002 (95% CI 0.001 to 0.003), p=0.029) after adjustment for age, BMI and time for blood sampling.

Analyses in girls who did not use hormonal contraceptives (n=290) showed no associations between PFAS and serum testosterone, estradiol, FTI, androstendione and 17-OH-progesterone concentrations adjusted for age, BMI, time since last menstruation and time for blood sampling (results not shown). Additionally, for boys, we performed logistic regression analysis with serum testosterone below 25th percentile (<12 nmol/L in this population) and FTI below 25th percentile (<4.5 for boys in this study population) as dependent variables. No significant associations were found with PFAS concentration quartiles after adjustments for age and BMI.

In linear regression analyses LH and FSH serum concentrations were not associated with ∑PFAS, PFHpA, PFHpS, PFHxS, PFOA, PFOS, PFNA, PFDA, PFUnDA after adjustment for age, BMI and FTI in boys, and for age, BMI, time since last menstruation, estradiol concentrations and hormonal contraceptive use in girls (results not shown). Separate analyses in girls who did not use hormonal contraceptives showed similar results (results not shown).

3.2 Age at menarche

Altogether 15.6% of girls reported early menarche. Increased serum concentrations of PFDA and PFUnDA over 4th quartiles were significantly positively associated with early menarche after adjustment for age and BMI (Table 5). Multiple linear regression analyses with

adjustment for age and BMI showed that age at menarche was negatively associated with both log10-transformed PFDA (β=-0.43 (95% CI -0.85 to -0.01), p=0.044) and log10-transformed PFUnDA (β= -0.52 (95% CI -0.91 to -0.12), p=0.011). BMI was considered to be a confounder and not a mediator for the association between early menarche and PFDA, PFUnDA, as these PFAS were slightly stronger associated with early menarche in the logistic regression models with BMI than in the models without BMI (data not shown).

Other PFAS were not associated with age at menarche in this study. Very few girls (0.2%) reported a late menarche and no statistical analysis for late menarche was performed.

3.2 Irregular menstruation

Logistic regression analyses showed that serum concentrations of ∑PFAS, PFHpA, PFHpS, PFHxS, PFOA, PFOS, PFNA, PFDA, PFUnDA had no association with self-reported irregular menstruation in girls who did not report use of hormonal contraception (n=290) after adjustment for age and BMI (results not shown).

3.4 Puberty in boys

The 4-point PDS scale in boys was positively associated with log10-transformed ∑PFAS (β=0.28 (95% CI 0.01 to 0.55), p=0.043) and log10-transformed PFOA (β=0.42 (95% CI 0.09 to 0.75), p=0.013) concentrations after adjustment for age and BMI.

Altogether 2% of boys had early puberty, and 3% of boys had late puberty. There were no significant associations between early or late puberty in boys and serum PFAS concentrations.

3.5 PTH

Altogether 6.6% of boys and 3.5% of girls had high PTH. There were no cases of PTH below the reference interval in this population. Linear regression analysis showed that PTH serum

concentrations were significantly inversely associated with log10-transformed ∑PFAS concentrations in both sexes after adjustment for age, BMI and 25-OH-vitamin D concentrations (β=-0.81 (95% CI -1.48 to -0.15), p=0.017 in girls and β=-0.95 (95% CI -1.82 to -0.07), p= 0.034 in boys). However, this association became not significant in boys after additional adjustment for serum calcium concentrations (Table 6). The results of regression analyses for single PFAS showed that PFHxS in girls and PFOS in boys had the inverse associations with PTH concentrations after adjustment for age, BMI, 25-OH-vitamin D and serum calcium (Table 6). Logistic regression analyses showed no significant association of high PTH with PFAS (results not shown).

3.6 Thyroid hormones

Linear regression analyses stratified by sex showed that TSH serum concentrations were not associated with serum concentrations of ∑PFAS, PFHpA, PFHpS, PFHxS, PFOA, PFOS, PFNA, PFDA, PFUnDA in boys or in girls.

In boys, after adjustment for age and BMI, serum fT4 concentrations were negatively associated with log10-transformed PFUnDA concentration (β= -1.36 (95% CI -2.20 to -0.51), p=0.002), while fT3 serum concentrations were negatively associated with log10-transformed PFNA (β= -0.89 (95% CI -1.52 to -0.26), p=0.006), PFDA (β= -0.63 (95% CI -1.20 to -0.06), p=0.029) and PFUnDA concentrations (β= -0.43 (95% CI -0.78 to -0.09), p=0.014).

PC1 score was significantly positively associated with serum fT3 in boys in the multiple linear regression analysis. As PFNA, PFDA and PFUnDA, which defined the PC1, were strongly positively correlated, their effects on fT3 are difficult to separate from each other.

In girls, there were no significant associations between all measured serum PFAS and fT3 and fT4. There were 34 cases (3.7%) of hypothyreoidism and 14 cases (1.5%) of hyperthyreoidism in this study population. There were no associations between all measured

PFAS and hypothyreoidism or hyperthyreoidism in this population assessed by logistic regression models adjusted for age, sex and BMI.

4. Discussion

In the present study we found that serum concentrations of several PFAS were associated with thyroid, parathyroid and steroid hormone serum concentrations. We observed sex-dependent associations of PFAS with steroid hormones. In girls, there was a positive association between several PFAS and DHEAS and negative association of several PFAS with 11-DOC/DHEAS ratio. In boys, the associations between 11-DOC/DHEAS ratio and several PFAS were positive. The 11-DOC/DHEAS ratio reflects glucocorticoid/androgen balance. Our study observed that PFAS were associated with balance shift towards androgens in girls, and balance shift towards glucocorticoids in boys. A previously published study investigating prenatal exposure reported a negative association between glucocorticoid/androgenic hormone ratio and PFOS in cord blood samples (Goudarzi et al., 2017). These samples were from neonates of both sexes, but it is known that both steroid hormones and PFOS concentrations in cord blood are influenced by concentrations of those in the mother's blood (Manzano-Salgado et al., 2015; Miranda & Sousa, 2018).

The physical changes of early pubertal maturation are preceded by biochemical changes that consist of increased production of DHEA and DHEAS that have androgen effects (Oberfield et al., 2011). Other studies showed that DHEAS concentrations were associated with earlier pubertal maturation in girls (thelarche, pubarche, and menarche) (Merino et al., 2019; Pereira et al., 2017). Our study found a positive association of some PFAS (i.e., PFDA and PFUnDA) with early menarche. Our results are consistent with results of a Danish study that found that PFOS, PFNA, PFDA were associated with earlier onset of puberty in girls (Ernst et al., 2019).

Serum concentrations of PFOS were four times higher in the Danish population, while PFNA and PFDA concentrations were similar to our study and PFUnDA was not reported previously. A study from US found later onset of puberty in girls exposed to PFOS, PFOA and PFDA (Carwile et al., 2021). However, this US study measured only prenatal PFAS exposure which is not necessarily reflecting exposure later in life. The prenatal serum concentrations of PFOS, PFOA and PFDA were twice as high compared to our study. Previously published studies on early menarche and PFAS reported exposure to different PFAS mixtures and different PFAS concentrations. Besides, the populations may differ genetically, phenotypically and have different lifestyles as well as dietary habits, which also may influence susceptibility to PFAS effects, and which might explain the inconsistencies in findings from different studies regarding PFAS exposure related to age at menarche. Several population studies have shown previously that earlier menarche is associated with higher BMI, more mental problems, substance use and psychological disorders in adolescence and higher all-cause and cardiovascular mortality and risk of chronic diseases (e.g., breast cancer, diabetes, obesity, and metabolic syndrome) in adulthood (Jacobsen et al., 2007; Kim & Je, 2019; Lee et al., 2022). Early menarche is therefore not an advantage and the association between higher concentrations of PFAS and early menarche should be a matter of concern.

In boys, there was a positive association of ∑PFAS and PFOA with the PDS, which reflects possible earlier pubertal development associated with PFOA exposure. Interestingly, PFOA was also weakly positively associated with androstendione in boys. ∑PFAS, PFHpS, PFOA and PFOS were also positively associated with 11-DOC, a steroid hormone with mineralocorticoid activity that acts as a precursor to aldosterone. That could be a possible mechanism explaining the positive association between ∑PFAS, PFOA, PFOS and hypertension found in the same population (Averina et al., 2021).

Several previous studies reported negative associations between PFAS and FSH, LH, testosterone and FTI (Cui et al., 2020; Joensen et al., 2013; Lopez-Espinosa et al., 2016; Luo et al., 2021; Nian et al., 2020; Tsai et al., 2015). Our study found no significant associations of PFAS with these hormones and FTI in boys, but a positive association of PFOA with FTI in girls. However, PFAS concentrations in general were much lower in our study compared with the previously published literature, which might explain the difference in results. Thus, a study in young children that reported a negative association of PFOS and PFOA with testosterone had respectively 3.5 and 17 times higher PFOS and PFOA serum concentrations than our study population (Lopez-Espinosa et al., 2016). Age of participants and PFAS exposure window may also differ between the studies, which may influence the results.

Our data showed that PTH serum concentrations were inversely associated with ∑PFAS in both sexes before the adjustment for calcium. Further analyses showed that it was mostly due to the association of PTH with PFHxS in girls and PFOS in boys. Very few studies examined the association between PTH and PFAS. One study has reported reduced expression of PTH receptor gene in women after exposure to PFOS and PFOA (Galloway et al., 2015). PTH is important for control of the calcium serum concentration. PTH concentrations are influenced by vitamin D, which exerts negative feedback on PTH secretion. However, the negative association between PFHxS (in girls) and PFOS (in boys) and PTH remained significant after adjustment for vitamin D and calcium serum concentrations.

There were sex differences in associations of PFAS with thyroid hormones. PFNA and PFDA were associated with lower fT3, and PFUnDA was associated with lower fT4 and fT3 serum concentrations in boys, but not in girls. The US-NHANES study also reported sex differences in associations between PFAS and thyroid hormones: PFHxS exposure was associated with lower fT4 in men, but not in women (Wen et al., 2013). A study of prenatal PFAS exposure found that higher concentrations of ∑PFAS was associated with lower fT4 primarily in male

neonates (Preston et al., 2020). Previous population studies have also reported that several PFAS (i.e., PFHxS, PFOA, PFOS, PFNA, PFUnDA) were negatively associated with fT4 (Wang et al., 2014; Webster et al., 2016). These findings are supported by the results of animal studies in rats that showed reduced concentrations of fT3 and fT4 after exposure to PFOS (Davidsen et al., 2022; Yu et al., 2009). However, some human studies reported only a positive association with TSH concentrations and no association between PFAS and fT4, fT3 (Blake et al., 2018; Jain, 2013). The associations of PFAS with thyroid hormones may vary depending on thyroid antibodies and iodine status (Itoh et al., 2019; Webster et al., 2016). The NHANES study reported that PFHxS, PFOA, PFOS and PFNA were positively associated with fT3, fT3/fT4 and TSH in the group with joint exposure to high thyroid peroxidase antibodies and low iodine (Webster et al., 2016). Levels of exposure to different PFAS mixtures, and other factors such as iodine status and thyroid antibodies may vary in different study populations. The outcome of direction and extent of associations of PFAS with thyroid hormones may therefore show variation in different populations, and for different age and sex groups.

Study strengths and limitations

The main advantages of the present study are standardized measurements of hormones and PFAS and the high attendance rate. The analyses were performed by two laboratories accredited by ISO 15189 standard. Further, the collection of the samples and physical investigations were conducted by trained health care professionals, which guarantees a correct and reproducible performance through the entire study cohort. The attendance rate was high (93%), and the results are therefore considered highly representative for this population of young people. The limitation of this study is its cross-sectional design that does not permit to

make conclusions about causality of the found associations. Self-reported data on menstruation, puberty and age at menarche are also limitations of this study. However, a previous study of the reproducibility of self-reported age at menarche in adult women in the same community found very high reproducibility (Lundblad & Jacobsen, 2017).

5. Conclusions

Serum concentrations of several PFAS were associated with serum thyroid, parathyroid and steroid hormones. PFDA and PFUnDA were positively associated with early menarche, while ∑PFAS and PFOA were positively associated with the pubertal development score in boys. Further prospective studies are needed to investigate causality and to assess clinical importance of the EDC effects of PFAS.

CredIT author statement

Maria Averina: conceptualization, methodology, data curation, formal data analysis, writing-original draft preparation; Sandra Huber: PFAS analyses, data curation and validation, writing - review & editing; Bjørg Almås: resources, steroid hormone analysis, writing - review & editing; Jan Brox: resources, conceptualization, supervision, writing - review & editing; Bjarne K. Jacobsen: conceptualization, writing - review & editing; Anne-Sofie Furberg: resources, funding acquisition for investigation, project administration, writing - review & editing; Guri Grimnes: conceptualization, project administration, writing - review & editing.

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