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PFAS internal exposure levels across two generations and endocrine-metabolic biomarker associations in women from the French E3N-Generations cohort

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ABSTRACT

Background: Per- and polyfluoroalkyl substances (PFAS) are environmentally persistent chemicals suspected to act as endocrine-active compounds, disrupting lipid metabolism and hormonal homeostasis, while possible multigenerational effects are an emerging concern. Although legacy PFAS such as perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) have been restricted in France since 2009, the impact of these measures on long-term exposure remains unclear.

Objectives: We aimed to (1) compare serum PFAS concentrations across two generations of adult French women and identify determinants of exposure; (2) evaluate associations between PFAS (individually and as mixtures) and early hormonal and metabolic biomarkers; and (3) explore potential intergenerational associations between maternal PFAS exposure and adult daughters' biomarkers.

Methods: We analyzed serum PFAS concentrations among two generations of women from the E3N-Generations cohort (1995–1999 and 2024). Biomarkers included thyroid-stimulating hormone (TSH), testosterone, sex hormone–binding globulin (SHBG), triglycerides, and total, HDL, and LDL cholesterol. Multivariable models assessed determinants of exposure and PFAS–biomarker associations, with exploratory analyses of maternal–daughter pairs.

Results: Total PFAS concentrations decreased substantially over time, particularly for PFOS and PFOA, although these compounds remained the predominant PFAS detected in both generations. Sociodemographic, anthropometric, and reproductive factors were associated with PFAS serum concentrations, with generation-specific patterns. The results showed a positive cumulative association between PFAS and testosterone and a negative association with TSH and HDL cholesterol, while associations with other lipid biomarkers were heterogeneous. No consistent associations were observed between maternal PFAS concentrations and adult daughters' biomarkers.

Conclusions: Despite regulatory-driven declines, PFAS exposure remains widespread and associated with endocrine and metabolic biomarkers in adult women. These findings are consistent with the need for strengthened, class-based PFAS regulation and dedicated studies to rigorously evaluate potential multigenerational associations.

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1. Introduction

Per- and polyfluoroalkyl substances (PFAS) constitute a large family of synthetic chemicals that have been widely used since the 1940 s in various industrial applications and consumer products such as textiles, carpets, leather, footwear, food packaging, and cosmetics (Glüge et al., 2020). Their particular physicochemical properties, including resistance to heat, water, and oil, have made them commercially valuable but also environmentally problematic. The widespread use of PFAS has led to global environmental dissemination and human exposure (Glüge et al., 2020).

Among them, perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) have been the most extensively produced and studied. Their use has been restricted in France since 2009 under the European REACH regulation, and they were subsequently listed under the Stockholm Convention in 2009 and 2019, respectively. More recently, perfluorohexane sulfonic acid (PFHxS) was added to the Convention in 2022 (Plan d'action ministériel sur les PFAS [Internet], 2026). Despite these regulatory measures, PFAS remain highly persistent, bioaccumulative, and ubiquitous in the environment, leading to widespread contamination of the food chain, the primary source of exposure for the general population (Brunn et al., 2023). However, the effectiveness of the restrictions in the production and use of PFAS in reducing exposure levels in the population is still largely unknown. One of the major challenges is the lack of biomonitoring data from the 1990 s, as PFAS were not systematically included in national surveillance programs at that time. This gap hampers the ability to assess long-term trends in exposure and to determine whether regulatory actions have translated into measurable declines in body burdens. Consequently, generating robust comparative data between past and present exposure levels is essential to evaluate the real impact of regulatory interventions and to guide future risk assessment and management strategies.

Long-term exposure to PFAS is increasingly suspected to contribute to a wide range of adverse health effects, including endocrine disruption, metabolic alterations, immune dysfunction, and possibly cancer. Mounting epidemiological and experimental evidence supports the notion that PFAS interfere with metabolic and hormonal regulation. For example, PFAS exposure has been consistently associated with elevated serum total and LDL cholesterol concentrations, often considered early markers of cardiovascular risk. A systematic review concluded that compounds like PFOA and PFOS are associated with increased LDL, HDL, and total cholesterol, while results on associations with triglycerides are inconsistent (Ho et al., 2022). Indeed, mechanistic evidence suggests that PFAS may disrupt lipid homeostasis through activation of nuclear receptors (e.g., PPARα), interference with bile acid metabolism, and modulation of lipid transport and hormonal regulation (Beggs et al., 2016).

In addition to metabolic effects, PFAS act as endocrine disruptors impacting sex hormone regulation. Several studies and *meta*-analyses show negative associations between PFAS (notably PFOA and PFNA) and male testosterone levels (Li et al., 2024; Sang et al., 2024). In the general population, PFAS exposure has also been linked to alterations in testosterone, estradiol, and sex hormone-binding globulin (SHBG) in both men and women, with effects varying by age and compound (Xie et al., 2021). In fact, PFAS may interfere with steroidogenesis by affecting key enzymes or receptors involved in hormone synthesis, and they can alter liver production of SHBG, thereby modifying circulating hormones' levels (US EPA CFPH EA. US EPA [Reports and Assessments] [Internet]., 2022; Du et al., 2013). These hormonal alterations may represent early biological disturbances that, if sustained, could predispose to pathological conditions such as dyslipidemia, reproductive dysfunction, or metabolic disease.

However, the strength of current evidence is limited, largely due to a lack of comprehensive human data and difficulties in assessing low-dose, chronic exposures. Moreover, most previous studies investigated the exposure to one PFAS at a time, while it is essential to consider that in real life people are continuously exposed to multiple PFAS simultaneously which could have a joint action resulting in additive, synergistic or antagonistic toxicological effects. Not considering mixture effects can potentially lead to a biased estimation of the risk. Further, the possibility that adverse effects may be transmitted across generations is an emerging and critical concern that requires scientific evidence that is currently limited (Coperchini et al., 2024; Haimbaugh et al., 2022). Indeed, maternal PFAS exposure may contribute to offspring exposure both through placental transfer and breastfeeding, and may have long-term effects on the second generation (Cariou et al., 2015). Understanding intergenerational effects is particularly important for risk assessment and policy, as it implies that exposure may have consequences extending beyond directly exposed individuals. However, few studies have the appropriate design to investigate the effects of PFAS exposure across multiple generations in human populations. Mother–child cohorts, which recruit mothers during pregnancy and follow their children after birth, are well suited for such studies, yet follow-up often ends in early childhood, limiting conclusions about long-term effects in adulthood.

The present work aimed to address these knowledge gaps by pursuing three main objectives:

1. To measure and compare internal levels of PFAS exposure in two generations of adult women in France (1995–99 and 2024), and to identify the main determinants of exposure allowing the comparison of these determinants across generations.
2. To investigate associations between serum PFAS concentrations, individually and as mixtures, and early health biomarkers, focusing on established biological indicators of physiological responses in pathways likely to be affected by PFAS. These include thyroid-stimulating hormone (TSH), testosterone, sex hormone-binding globulin (SHBG), triglycerides, total cholesterol, HDL cholesterol, and LDL cholesterol.
3. To estimate the potential intergenerational correlations between maternal and daughters PFAS serum concentrations, and the associations between maternal PFAS levels and selected health biomarkers in daughters at adult age.

2. Materials and methods

2.1. E3N-Generations cohort

The E3N-Generations cohort is a family-based study built upon the historical E3N cohort, which was initiated in 1990 with the enrollment of 98,995 women aged 40–65 years, all members of an insurance plan mainly covering people working in the national education system (Mutuelle Générale de l'Éducation Nationale). The cohort has been expanded to include family members of the E3N women, making it one of the few multigenerational prospective cohorts in Europe. Ultimately, the E3N-Generations cohort will encompass three generations: the original E3N women and the fathers of their children, constituting the first generation (E3N-G1); their children as the second generation (E3N-G2); and their grandchildren as the third generation (E3N-G3) (www.https://www.e3n-generations.fr/).

The detailed protocol of the E3N cohort (including only E3N-G1 women) has been previously described (Clavel-Chapelon et al., 1997; Clavel-Chapelon, 2015). Briefly, self-administered questionnaires are sent by post mail to participants every two to three years to collect information on lifestyle, reproductive and medical history, and overall health status. Between 1994 and 1999, participants were invited to donate blood, resulting in the collection of samples from approximately 25,000 women. Each sample was separated into 28 aliquots, including plasma, serum, buffy coat, leukocytes, and erythrocytes, and stored in plastic straws in liquid nitrogen containers (−196°C) for long-term storage.

The E3N-Generations cohort was launched in 2014 with the

beginning of the recruitment of the male partners of the first generation. Recruitment of men and women from the second generation began in 2018, while recruitment of participants from the third generation is planned for 2026. Participants of the second generation are invited regularly to answer questionnaires regarding their lifestyle, medical history and health status on a dedicated online platform.

2.2. Study population

The present study focused on mother–daughter pairs of the E3N-Generations cohort meeting a set of inclusion criteria. For mothers we included E3N-G1 women that 1) provided a blood sample between 1994 and 1999; 2) were free of cancer, diabetes, and cardiovascular diseases at the time of blood collection; 3) had at least three serum aliquots and one plasma aliquot still available in the biobank at the time of the study; and 4) were the biological mother of a daughter included in the second generation of the E3N-Generations cohort.

For daughters we included E3N-G2 women that 1) resided in the Île-de-France region at the moment of the present study; 2) had a mother selected according to the criteria described above; 3) were affiliated with or benefiting from a social security scheme; and 4) were not subject to legal protection measures (judicial safeguard, guardianship, or curatorship).

Initially, 759 women E3N-G2 were identified as meeting the inclusion criteria and were contacted by email to assess their interest in participating in the study. Of these, 321 agreed to take part in the study. However, 17 women were not residing in the Île-de-France region at the moment of the study, 1 was not enrolled in a social security scheme, and 2 were subject to legal protection measures. Consequently, 301 women were formally invited to participate. These participants were asked to provide written informed consent and to schedule a home appointment with study nurses for blood sampling. In total, 283 women arranged an appointment, and blood collection was successfully completed between March and July 2024 for 263 of them. For one E3N-G2 woman PFAS could not be measured in the blood sample and biomarkers could not be measured for another women. The flowchart summarizing the inclusion process of the E3N-G2 participants is presented in Supplementary Fig. 1.

Based on the E3N-G2 participants included, 250 women E3N-G1 were identified for the project. Among these, 9 mothers had two daughters enrolled in the study, and two mothers had three daughters enrolled. However, one E3N-G1 woman did not have sufficient biological material available in the biobank for the planned analyses, and for another E3N-G1 woman, the material was sufficient only for PFAS quantification but not for biomarker measurements.

2.3. Blood sample collection

For E3N-G2 participants, a blood sample of 35 mL was collected at home by a trained nurse, using eight tubes: three 5 mL SST tubes, four 4 mL EDTA tubes, and one 4 mL sodium fluoride tube. At the time of blood collection, participants were also asked to complete a short structured questionnaire administered by the nurse. The questionnaire collected the following information: fasting status since 8:00p.m. the previous evening (yes/no; if no, time since last food or drink other than water, expressed in hours), current height (cm) and weight (kg), and the presence of acute symptoms not usually experienced in the past seven days (fever, cough, breathing difficulties, or diarrhea; yes/no for each). Participants were also asked whether they had menstruated in the past seven days (yes/no) and whether they had taken any medication in the past 24 h (yes/no; if yes, the type of medication was recorded).

Blood samples were transferred by the nurse to the nearest collaborating laboratory of the Cerballiance network before 12:00 a.m. on the day of collection. Samples were kept refrigerated locally and centralized at the Cerballiance central laboratory within 24 h. At this laboratory, one SST tube (5 mL) and one sodium fluoride tube (4 mL) were used for the measurement of metabolic and hormonal biomarkers. The remaining two SST tubes and four EDTA tubes were centrifuged, aliquoted, and frozen at −80C. Aliquots were subsequently transported to the E3N-Generations biobank for storage located at the *Centre de Ressources Biologiques* of the Fondation Jean Dausset–CEPH.

2.4. PFAS measurement

E3N-G1 and E3N-G2 serum samples were transferred to the LABERCA's Human Biomonitoring (HBM) platform where the levels of 32 PFAS (PFBS, PFPeS, PFHxS, PFHpS, n-PFOS, Total-PFOS, PFNS, PFDS, PFUnDS, PFDoDS, PFTrDS, PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, PFOSA, N-MeFOSAA, N-EtFOSAA, GenX, DONA, F53B major, F53B minor, Capstone A, Capstone B, 6:2 FTS) were measured using fully validated (2002/657/CE decision) and accredited methods (ISO 17025, ISO9001:2015 standards), as previously described (Mancini et al., 2020; Fillol et al., 2021; Antignac et al., 2013). Briefly, 32 PFAS were determined conducting a preliminary alkaline digestion followed by a two-stage Solid Phase Extraction purification using polymeric Oasis HLB and graphitized carbon (ENVI-Carb) cartridges, before liquid chromatography coupled to tandem mass spectrometry (6410, Agilent Technologies, USA) measurement. Method performance was routinely monitored throughout the analytical sequence: the calibration curve (5-point) was verified every 100 samples, and calibration near the LOQ was checked every 20 samples. A procedural blank was analyzed every 10 samples to control for background contamination. Internal quality controls (IQC) were run at multiple concentration levels within each analytical batch to build control charts and verify compliance with Westgard rules. Intermediate precision and expanded measurement uncertainty ($k = 2$) were determined at three concentration levels (near-LOQ, medium, and high). Intermediate precision was further assessed by including 6 blind replicate pairs (two aliquots from the same subject analyzed under different sample IDs) within the analytical sequences; results were concordant across all PFAS analyzed, with (LOQs ranging from 0.05 to 0.5 ng/mL depending on the analyte). In addition, the laboratory both coordinates and participates in national and European interlaboratory comparison exercises and proficiency testing schemes (ISO 17043), including the QA/QC programs for PFAS established at EU level within the HBM4EU and PARC initiatives, thereby ensuring long-term method robustness and cross-batch, cross-study comparability of results.

2.5. Measurement of metabolic and hormone biomarkers

For both generations, seven biochemical biomarkers were quantified using standardized laboratory procedures. TSH and SHBG were measured by chemiluminescence immunoassays, while testosterone concentrations were determined using a radioimmunoassay. Triglycerides, total cholesterol, as well as HDL and LDL cholesterol, were all quantified using enzymatic colorimetric assays. All analyses were performed by the Cerballiance laboratory, accredited by COFRAC according to the NF EN ISO 15189 standard, and carried out under rigorous internal quality-control procedures to ensure measurement accuracy and reproducibility.

2.6. Statistical analyses

General characteristics of the two study populations (E3N-G1 and E3N-G2) were summarized using mean ± standard deviation for continuous variables and counts with percentages for categorical variables.

The PFAS included in the statistical analyses were those for which non-quantifiable values (< LLOQ, lower limit of quantification) were found in less than 25% of samples in both generations, namely Tot PFOS, n-PFOS, PFOA, PFUnDA, PFDA, PFHpS, PFHxS, and PFNA. For the included PFAS, non-quantifiable values were imputed as $LLOQ/\sqrt{2}$ (Estimation of Average Concentration in the Presence of Nondetectable

Values: Applied Occupational and Environmental Hygiene: Vol 5, No 1 [Internet], 2026). The number and proportion of non-quantified values, the minimum and maximum LLOQ for all PFAS, as well as the distribution of quantified values after imputation, are shown in Supplementary Table S1 and S2 separately for E3N-G1 and E3N-G2.

Spearman's rank correlation coefficients were used separately for each generation to assess correlations among the eight PFAS included in the analyses, visualized with heatmaps (R package corrplot).

PFAS's concentration distributions were compared between generations (E3N-G1 vs E3N-G2) using the Wilcoxon signed-rank test. Since some mothers had multiple daughters, the assumption of independent pairs was violated. To address this, mean concentrations across all daughters of a given mother were calculated, yielding a single value per mother. Changes in PFAS concentrations between mothers and daughters were assessed at the individual level. The absolute change (delta) was defined as the difference between the daughter's concentrations and the mother's concentration (delta=PFAS daughter – PFAS mother). The percentage decline in PFAS concentrations was calculated using the following formula: [(PFAS mother – PFAS daughter)]/PFAS mother] x 100. Both delta and percentage decline are presented as medians with interquartile ranges [IQR].

For all analyses described below, PFAS concentrations and biomarkers were log-transformed, and values exceeding six standard deviations above the mean were excluded from analyses.

Associations between anthropometric, sociodemographic, and lifestyle covariates and each PFAS were evaluated using multiple linear regression models with log10(PFAS) as the dependent variable. All covariates were included simultaneously to estimate their adjusted associations with the outcome. For all continuous variables, restricted cubic splines with a fixed number of three knots were used to model potential non-linear relationships. Covariates included in the model were: age at blood collection (years), annual change in BMI between inclusion and blood collection calculated as follows: (BMI at inclusion – BMI at blood collection) / N years between inclusion and blood collection (kg/m^2/year). Other covariates included in the model were: years since menopause (years), educational level (<12 years; 12–14 years; >14 years of studies), parity (nulliparous, 1 or 2 children; ≥3 children), breastfeeding history and duration (no breastfeeding; <6 months; ≥6 months). All covariates had less than 5% missing values which were imputed using the median for continuous variables and the modal category for categorical variables.

The associations between PFAS and health biomarkers (metabolic and hormonal biomarkers) were examined in the combined population (E3N-G1 + E3N-G2), using mixed-effects models to account for non-independence of mother–daughter pairs, with a random effect capturing intra-family variability. For the mixed models both PFAS and biomarkers of effect were log transformed better approximate a normal distribution. Models were first run unadjusted and then adjusted for age at blood collection, BMI at blood collection, and menopausal status at blood collection (pre-/post-menopause). For all continuous variables, restricted cubic splines with a fixed number of three knots were used to capture potential non-linear relationships. Additionally, quartile analyses were performed by modeling PFAS concentrations as categorical variables, with Q1 as the reference group. The overall p- value for quartile categories was obtained from a Type III Wald test. The exact number of individuals included in the analysis for each couple PFAS-biomarker is reported in Supplementary Table S3.

Bayesian Kernel Machine Regression (BKMR) was applied in the combined population (E3N-G1 + E3N-G2) to assess the association between PFAS mixture and seven biomarkers (Bayesian kernel machine regression for estimating the health effects of multi-pollutant mixtures | Biostatistics | Oxford Academic [Internet], 2026; Bobb et al., 2018). Due to the strong correlation between n-PFOS and total PFOS, only n-PFOS was retained.

For each biomarker, the BKMR model was specified as:

$$y_i = h(x_{i1}, \ldots, x_{im}) + \boldsymbol{\beta}^T \mathbf{c}_i + \varepsilon_i.$$

where y_i is the outcome variable (biomarker) for individual i (i = 1, …,n), $x_{i1},\ldots,x_{im}$ are the PFAS exposures, h(·) is a flexible exposure–response function defined by a nonparametric kernel, c_i is a vector of covariates (potential confounders), and β is the corresponding coefficient vector. Covariates included in the model were age, menopausal status and BMI all at blood sampling. The models were fitted using the kmbayes function from the bkmr R package (version 0.2.2). The Markov chain Monte Carlo sampler was run for a minimum of 100,000 iterations, with non-informative default priors as defined by the package.

For each outcome, the following metrics were reported: (i) posterior inclusion probabilities (PIPs) for each PFAS; (ii) univariate exposure–response functions with other PFAS fixed at the median; (iii) single-exposure risk summaries comparing the 75th vs. 25th percentile of each PFAS, with others fixed at the 25th, 50th, or 75th percentile; (iv) overall risk summaries comparing all PFAS set to the 20th–80th percentiles versus the 50th percentile; and (v) bivariate exposure–response functions to identify potential interactions, with non-parallel curves suggesting effect modification.

Correlations between maternal and daughter PFAS concentrations were evaluated using Spearman's rank correlation and illustrated with scatterplots. Because Spearman's test assumes independence of pairs, mother–daughter pairs from mothers with multiple daughters with PFAS measurements were excluded. Additionally, the one E3N-G1 woman without PFAS data and her daughter were excluded. The final mother-daughter correlation analysis of PFAS concentrations was therefore performed on 236 independent pairs.

Finally, the association between maternal serum PFAS concentrations and hormonal and metabolic biomarkers in daughters was assessed using linear regression models. Models were adjusted for daughters' PFAS levels as well as for age, menopausal status, and the BMI of the daughter at blood sampling. Given the moderate correlations between maternal and daughter PFAS concentrations (r = 0.20–0.30), including both in the same model did not raise collinearity concerns. A random intercept for sisters' pairs was not included, as the intra-family variance could not be estimated due to the small number of mothers with multiple daughters.

The exact number of individuals included in each statistical analyses performed is summarized in Supplementary figure 2.

All tests were two-sided, and the threshold for statistical significance was set at $p < 0.05$. Data management was performed using SAS version 9.4 (SAS Institute, Inc., Cary, North Carolina) and statistical analyses were performed in R version 4.4.1.

3. Results

3.1. Study population and PFAS concentrations among the two generations

A total of 250 women from E3N-G1 were included. At blood collection, the mean age was 55.5 years, 74% were postmenopausal, and the mean BMI was 23.3 kg/m^2. Most participants (61%) had > 14 years of education; 49% had one or two children, and 51% had three or more (Table 1). Twelve PFAS (37.5%) had < 25% of samples below the LLOQ, namely Tot PFOS, n-PFOS, PFOA, PFUnDA, PFDA, PFHpS, PFHxS, PFNA, N-EtFOSAA, N-MeFOSAA, PFOSA, and PFHpA. The highest mean (SD) concentrations were observed for Tot PFOS (29.5 ± 14.5 ng/mL) and n-PFOS (15.5 ± 7.6 ng/mL), while the lowest was for PFUnDA (0.15 ± 0.09 ng/mL). Moreover, 18 PFAS (54.5%) had more than 80% of samples below the LLOQ, while 3 PFAS (9.1%) had between 25% and 80% of samples below the LLOQ Table 2.

Among the 263 E3N-G2 participants, the mean age and BMI at blood collection were 53.5 years and 23.8 kg/m^2, respectively. Overall, 54% were postmenopausal, 87% had > 14 years of education, 19% were nulliparous, 57% had one or two children, and 24% had three or more (Table 1). In this generation, 8 PFAS (25%) had < 25% of samples below the LLOQ, including Tot PFOS, n-PFOS, PFOA, PFUnDA, PFDA, PFHpS,

Table 1
Main characteristics of the E3N-G1 and E3N-G2 women included in the present study.

	E3N-G1 (N = 250)	E3N- G2 (N = 263)
Age at inclusion (years)	48.31 (6.01)	49.45 (8.07)
Age at blood collection (years)	55.49 (5.99)2	53.54
Missing	(0.8%)	(7.98)0 (0.0%)
Body mass index at inclusion in the cohort (kg/m^2)	22.02 (2.87) 3 (1.2%)	23.37 (4.85)8
Missing		(3%)
Body mass index at blood collection (kg/m^2)	23.34 (3.46) 3 (1.2%)	23.82 (5.14)8
Missing		(3%)
Variation of BMI (kg/m^2 per year)	0.19 (0.21)3	0.15 (0.98)
Missing	(1.2%)	8 (3%)
School educational level (years)		
<12	13 (5.20)	2 (0.76)
12–14	81 (32.40)	31 (11.79)
>14	152 (60.80)	228 (86.69)
Missing	4 (1.60)	2 (0.76)
Parity		
Nulliparous	0 (0.00)	50 (19.01)
One or two children	122 (48.80)	149 (56.65)
More than two children	128 (51.20)	64 (24.33)
Menopausal status at blood collection		
Postmenopausal	186 (74.40)	143 (54.37)
Premenopausal	62 (24.80)	120 (45.63)
Missing	2 (0.80)	0 (0.00)
Age at menopause in post- menopaused women (years)	50.70 (3.51)8 (3.2%)	50.37 (3.45)5
Missing		(1.9%)
Time since menopause at blood collection (years)	4.77 (5.83)10 (4%)	4.72 (6.07) 5
Missing		(1.9%)
History of breastfeeding		
No breastfeeding	49 (19.60)	79 (30.04)
Cumulative duration of breastfeeding < 6 months	107 (42.80)	80 (30.42)
Cumulative duration of breastfeeding ≥ 6 months	84 (33.60)	96 (36.50)
Missing	10 (4.00)	8 (3.04)
	(N = 248)	**(N = 262)**
Testosterone (nmol/L)	2.12 (0.72)	1.93 (0.72)
TSH (mUI/L)	1.80 (1.67)	2.27 (1.17)
SHBG (nmol/L)	71.35 (28.85)	73.14 (32.05)
Triglycerides (mmol/L)	1.07 (0.45)	1.13 (1.32)
Cholesterol (mmol/L)	5.48 (0.81)	5.64 (1.15)
HDL (mmol/L)	1.71 (0.38)	1.85 (0.43)
LDL (mmol/L)	3.49 (0.77)	3.37 (0.94)

N (%); Mean (SD).

PFHxS, and PFNA. Tot PFOS (3.75 ± 2.4 ng/mL) and n-PFOS (2.04 ± 1.55 ng/mL) showed the highest mean concentrations, while PFHpS (0.08 ± 0.05 ng/mL) had the lowest. Twenty-one PFAS (63.6%) had over 80% of samples below the LLOQ, and 3 PFAS (9.1%) had between 25 and 80% of samples below the LLOQ.

It is noteworthy that four PFAS shifted from having fewer than 25% non-quantified values in E3N-G1 to more than 25% non-quantified values in E3N-G2. Specifically, N-EtFOSAA decreased dramatically from being quantified in 100% of E3N-G1 samples to less than 1% in E3N-G2. Similarly, the detection frequency of N-MeFOSAA and PFOSA dropped from 100% in E3N-G1 to 12% and 5% in E3N-G2, respectively. Finally, PFHpA declined from 90% detection in E3N-G1 to 45% quantified values in E3N-G2.

The distributions of the eight PFAS with < 25% of samples below the LLOQ in both generations, as well as N-EtFOSAA, N-MeFOSAA, PFOSA and PFHpA in E3N-G1, were visualized using histograms (Supplementary Fig. 3).

Mean (SD) levels of the metabolic and hormonal biomarkers measured separately in the two generations are reported in table 1.

Median concentrations of all PFAS included in the analyses were found significantly lower in E3N-G2 compared with E3N-G1, as summarized in table 2. A marked decline between mothers and daughters was observed for PFOS, PFOA, and PFHpS, with median decreases ranging from approximately 76% to 88%, whereas more moderate reduction were found for the other PFAS, with declines ranging from about 25% to 33%.

All PFAS related exposure markers were found positively correlated together within both generations (Supplementary Fig. 4). Overall, stronger inter-PFAS congener correlations were observed in E3N-G2, likely reflecting the more homogeneous distribution of PFAS concentrations, particularly due to the lower levels of PFOS and PFOA, which were more closely aligned with those of the other congeners.

3.2. PFAS exposure determinants among the women of the two generations

Among E3N-G1 women, adjusted linear regression models examining anthropometric, sociodemographic, and lifestyle factors in relation to log(PFAS) concentrations revealed several noteworthy patterns. Age at blood collection was non-linearly associated with PFDA, PFNA, and PFUnDA. Annual variation in BMI was inversely associated with PFUnDA blood levels. Time since menopause showed overall positive trend with all PFAS, with the exception of PFDA and PFUnDA; this relationship was linear only for PFHxS and PFOA. No clear trend was highlighted in relationship with women's education levels, while having more than 2 children was associated with lower blood levels for all PFAS but this association did not reach statistical significance. Finally, breast feeding for more than 6 months was inversely associated with all PFAS, although this association was statistically significant only for PFOA (Supplementary Fig. 5).

In E3N-G2 women, positive linear associations were observed between age at blood sample and PFAS blood levels, although this association was statistically significant only for PFHpS, PFHxS, n-PFOS, and total PFOS. Conversely, annual variation in BMI was negatively and linearly associated with PFDA, PFHpS, PFNA, n-PFOS, and total PFOS. Time since menopause showed a decreasing non-linear association only with PFUnDA. Educational attainment was also related to PFAS concentrations and overall displayed a positive trend, in particular, women with > 14 years of education had significantly higher levels of PFDA, PFUnDA, and n-PFOS. No clear trend could be observed in relation to the number of children. Statistically significantly lower concentrations of PFDA, PFHpS, PFNA, PFOA, n-PFOS, and total PFOS were observed in women with 1 or 2 children, compared with both nulliparous women and those with three or more children. No association with breastfeeding could be highlighted with regard to PFAS blood levels in E3N-G2 women (Supplementary Fig. 6).

3.3. Associations between PFAS levels and the metabolic and hormonal biomarkers: Mono-pollutant approach

Results from the adjusted mixed-effects models to assess the relationship between PFAS levels and the metabolic and hormonal biomarkers in the combined population (E3N-G1 + E3N-G2), highlighted positive linear associations between most PFAS (with the exception of PFHxS) and testosterone levels. In contrast, PFDA, PFHxS, PFUnDA, and total PFOS were inversely and linearly associated with TSH levels. For PFHpS, PFNA, PFOA, and n-PFOS, the associations with TSH were statistically significant but non-linear, displaying biphasic trends (Fig. 1, Supplementary Table S4 a and b).

Regarding lipid biomarkers, overall, an inverse trend was observed between PFAS levels and triglycerides, nevertheless, PFOA was the only PFAS statistically significantly associated with triglyceride

Table 2
Median concentrations and Q1-Q3 of PFAS in the overall study population, and in E3N-G1 and E3N-G2, compared using the Wilcoxon signed-rank test, and changes between mother and daughters pairs (delta and % decline).

	All (N = 498)	G1 (N = 249)	G2 (N = 249*)	Global p-value	Delta median [IQR]	% decline median [IQR]
Perfluorodecanoic acid (PFDA)	0.16 [0.11–0.22]	0.18 [0.14–0.24]	0.13 [0.09–0.19]	<0.0001	−0.05 [-0.11–0.01]	31.58 [-5.56–52.94]
Perfluoroheptanesulfonic acid (PFHpS)	0.16 [0.07–0.32]	0.32 [0.24–0.38]	0.07 [0.05–0.11]	<0.0001	−0.22 [-0.31–-0.16]	75.76 [63.64–84.78]
Perfluorohexanesulfonic acid (PFHxS)	1.23 [0.81–1.69]	1.41 [1.04–1.85]	0.98 [0.66–1.50]	<0.0001	−0.28 [-0.84–0.11]	25.17 [-8.82–54.12]
Perfluorononanoic acid (PFNA)	0.42 [0.29–0.61]	0.52 [0.38–0.68]	0.33 [0.23–0.47]	<0.0001	−0.15 [-0.36–-0.02]	32.43 [5.13–56.36]
Perfluorooctanoic acid (PFOA)	2.18 [0.89–5.78]	5.79 [3.92–7.97]	0.89 [0.61–1.27]	<0.0001	−4.92 [-6.84–-2.98]	84.13 [75.9–89.91]
Perfluoroundecanoic acid (PFUnDA)	0.11 [0.08–0.17]	0.13 [0.10–0.18]	0.09 [0.06–0.14]	<0.0001	−0.04 [-0.09–0.01]	33.33 [-10–61.11]
Linear isomer of perfluorooctanesulfonic acid (n-PFOS)	6.13 [1.58–14.08]	14.10 [11.18–18.08]	1.58 [1.09–2.45]	<0.0001	−12.18 [-15.95–-9.21]	88.47 [83.12–92.09]
Total PFOS	10.95 [3.05–27.29]	27.29 [21.41–34.26]	3.05 [2.14–4.58]	<0.0001	−23.22 [-30.67–-17.78]	88.45 [82.65–91.67]

*For mothers with more than one daughter included in the study, only one value, corresponding to the mean of the PFAS's concentration in the daughters, was used.

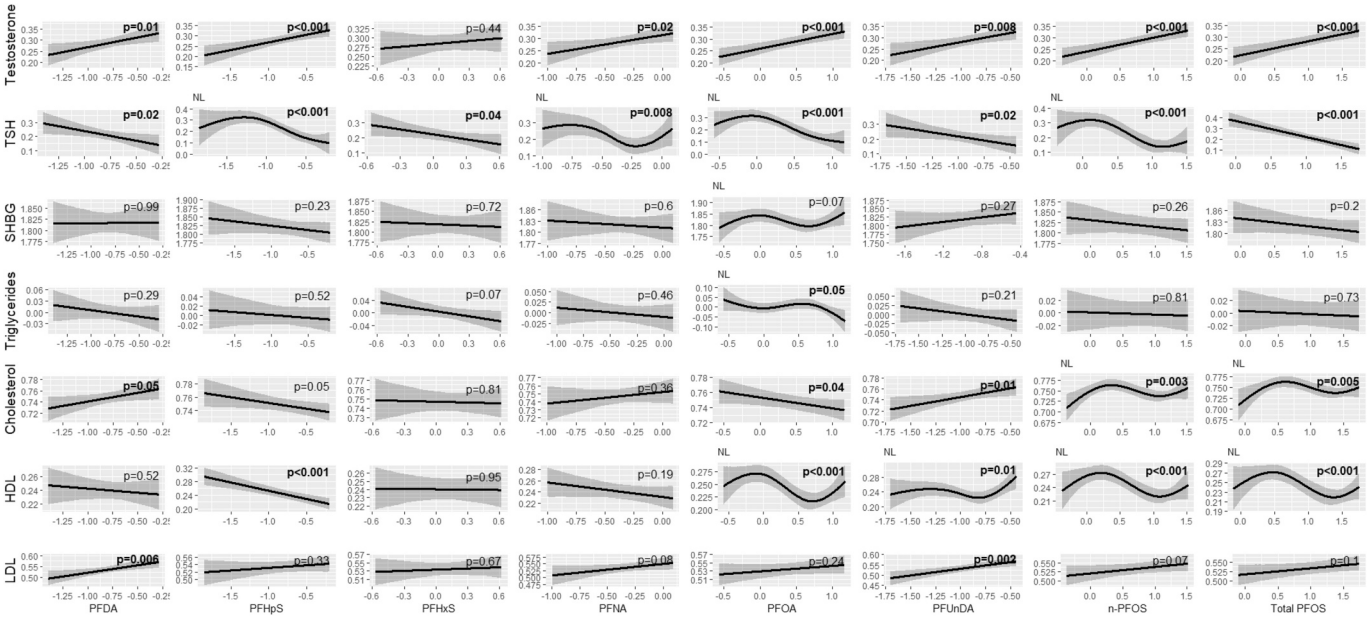

Fig. 1. Shape of the associations between PFAS levels and metabolic and hormonal biomarkers estimated using mixed-effects models adjusted on age (years), BMI (kg/m^2), and menopausal status (pre-/post-menopause) at blood collection in the combined population (E3N-G1 + E3N-G2). The reported P value refers to the overall statistical significance of the model. Details on regressions coefficients are presented on Supplementary Tables 4 a and 4b.

concentrations, showing a non-linear inverse relationship. Divergent associations were observed for total cholesterol: PFDA and PFUnDA were positively and linearly associated, PFOA was negatively and linearly associated, and n-PFOS and total PFOS showed significant non-linear associations. Complex biphasic or triphasic non-linear relationships were also detected for PFOA, PFUnDA, n-PFOS, and total PFOS in relation to HDL cholesterol, while PFHpS showed an inverse linear relationship. For LDL cholesterol, overall, the associations between PFAS levels marked a positive trend, which was statistically significant for PFDA and PFUnDA. Finally, no clear association was observed between PFAS concentrations and SHBG levels (Fig. 1, Supplementary Table S4 a and b).

3.4. Associations between PFAS levels and the metabolic and hormonal biomarkers: Multi-pollutant approach

Overall, in the combined population (E3N-G1 + E3N-G2), BKMR models indicated that higher cumulative PFAS exposure was associated with higher testosterone levels (Fig. 2). In contrast, higher cumulative exposure was associated with lower TSH and HDL concentrations (Fig. 2). Beyond these overall mixture-related associations, the results did not suggest the presence of two-way interactions between PFAS based on graphical inspection of bivariate exposure–response cross-sections. All results from the BKMR models are summarized in Supplementary Fig. 7.

Intergenerational correlations between maternal and daughters PFAS serum concentrations, and associations between maternal PFAS levels and selected health biomarkers in daughters Overall, intergenerational correlations between maternal and daughter PFAS blood levels were found modest, ranging from $\rho = 0.15$ (PFOA, p-value = 0.02) to $\rho = 0.35$ (total PFOS, p-value < 0.001) (Supplementary Fig. 8).

When investigating the relationship between maternal serum PFAS concentrations and hormonal and metabolic biomarkers in daughters, no association was observed (Supplementary Table S5).

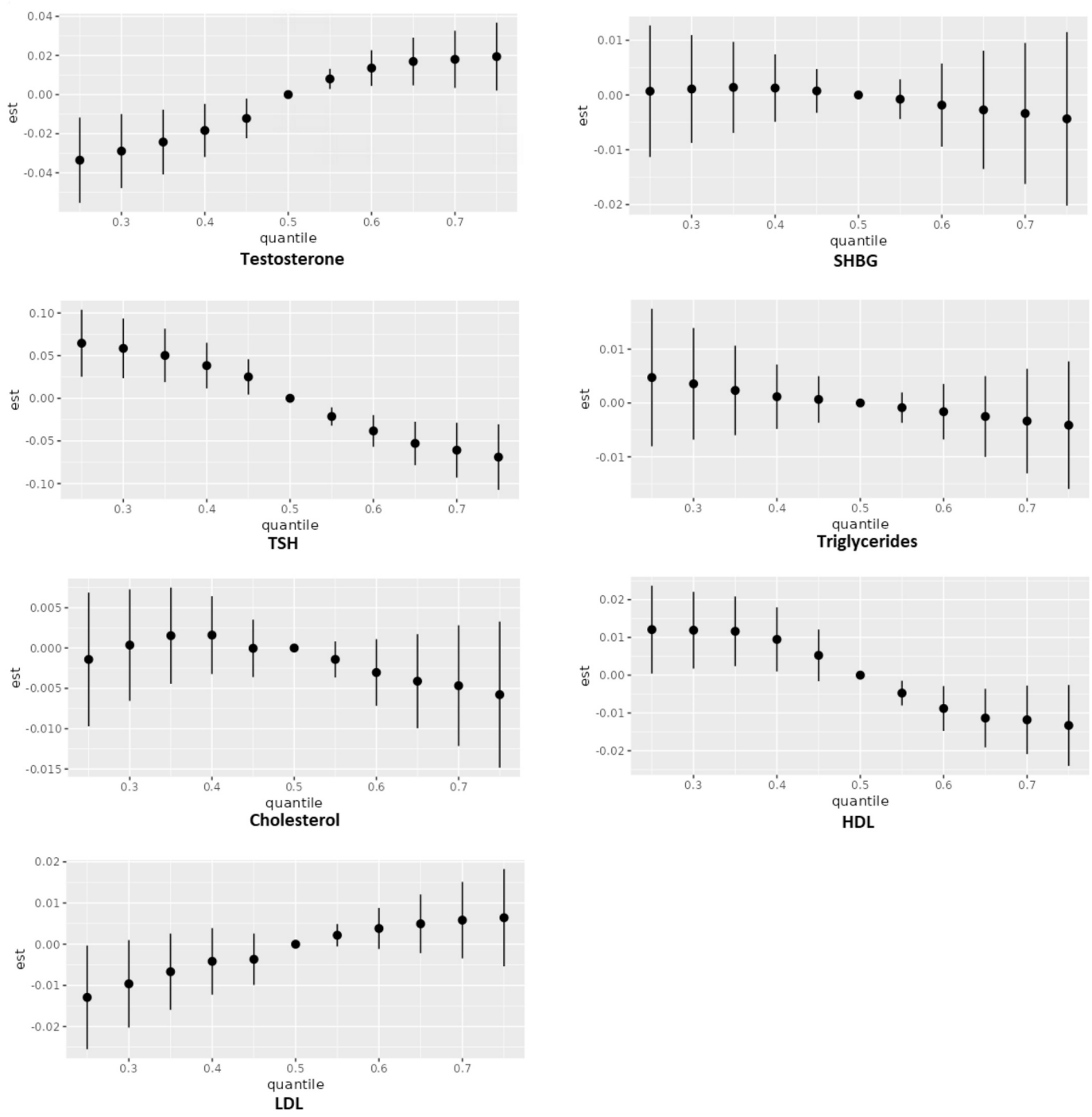

Fig. 2. Results of the BKMR model estimating the association between cumulative exposure to 7 PFAS and testosterone, SHBG, TSH, Triglycerides, Cholesterol, HDL, and LDL levels, adjusted age, menopausal status and BMI all at blood sampling in the combined population (E3N-G1 + E3N-G2). The y-axis (est) corresponds to the difference in the estimated biomarker value when all PFAS are fixed at the percentile indicated on the x-axis, relative to when all PFAS are fixed at their median values. Ninety-five percent credible intervals are presented.

4. Discussion

In our study, PFAS internal exposure profiles differed markedly between the two E3N generations. Detection frequencies and concentrations were consistently higher in E3N-G1 than in E3N-G2, with median levels of PFAS detected in both generations significantly lower in E3N-G2. Total PFOS, n-PFOS, and PFOA were the most abundant compounds in both groups, yet their concentrations dropped substantially over time. Notably, four PFAS, N-EtFOSAA, N-MeFOSAA, PFOSA, and PFHpA, shifted from being almost universally quantified in E3N-G1 to largely non-detectable in E3N-G2. Although women in the two generations are not directly comparable in all respects, it is plausible that the differences in PFAS levels reflect regulatory changes that occurred between the two collection periods. Legacy compounds such as PFOS, PFOA, and their precursors (e.g., N-EtFOSAA, N-MeFOSAA, PFOSA) have been progressively phased out in Europe since the early 2000 s, which is consistent with the marked reduction in their detection frequencies and concentrations in E3N-G2. At the same time, the persistence of measurable levels of long-chain PFAS (e.g., PFOA, PFOS, PFUnDA, PFDA) suggests ongoing exposure through food or

environmental reservoirs. The other detected PFAS are also classified as long-chain compounds; however, most have not yet been subject to direct regulatory restrictions, with the notable exception of PFHxS, which has been restricted in Europe since 2023. The more limited decline observed for these compounds between the two generations could reflect their continued use and environmental persistence, despite their gradual substitution by industry.

In both E3N generations, sociodemographic, anthropometric, and reproductive factors were associated with PFAS blood concentrations, though patterns varied. In E3N-G1, age showed non-linear associations with several PFAS, while in E3N-G2 positive linear associations were observed, reaching significance for PFHpS, PFHxS, n-PFOS, and total PFOS. BMI change was inversely associated with several PFAS, especially among E3N-G2 women, suggesting that changes in body weight and adiposity may influence circulating PFAS concentrations and estimated body burdens (Cardenas et al., 2018; Lind et al., 2022). However, the relationship between body fat dynamics and PFAS blood levels is not yet fully understood. Time since menopause was more consistently associated with higher PFAS levels in E3N-G1 than in E3N-G2, supporting the idea that reduced menstrual elimination contributes to accumulation (Park et al., 2019). Parity and breastfeeding showed non-significant inverse trends with E3N-G1 concentrations, consistent with PFAS transfer during pregnancy and lactation (Mamsen et al., 2019; Lehmann et al., 2018), whereas no clear trend was observed in E3N-G2. Interestingly, higher education was associated with elevated levels of certain PFAS only in E3N-G2. The results are in favor of the hypothesis that differences in lifestyle (in particular food habits) and/or exposure pathways associated with the socio-economic status may play an important role in the different exposure profiles observed in the two generation, although further studies are needed. Taken together, these findings highlight the influence of age, reproductive history, and body composition on PFAS body burdens, while also suggesting that sociodemographic factors may shape exposure patterns differently across generations. It is also plausible that the different levels of exposure between generations modulated the strength of these associations: at higher concentrations, physiological processes such as menstrual period and lactation may account for a larger proportion of elimination, whereas at lower background levels, their relative impact becomes smaller and harder to detect. Conversely, when exposures decline, other determinants such as education, diet, or consumer product use may emerge more clearly as sources of variability.

In the analyses combining both E3N generations, PFAS exposure was broadly associated with hormonal and lipid biomarkers, though patterns varied by congener and outcome. Most PFAS (except PFHxS) showed positive linear associations with testosterone, while PFDA, PFHxS, PFUnDA, and total PFOS were inversely associated with TSH. Other congeners (PFHpS, PFNA, PFOA, n-PFOS) displayed significant biphasic associations with TSH, underscoring the potential for non-linear endocrine responses. Regarding lipids, an overall null association was observed with triglycerides, except for PFOA that shown a non-linear inverse association, while associations with cholesterol fractions were divergent: PFDA and PFUnDA were positively related to total and LDL cholesterol, while PFOA was negatively associated with total cholesterol, and several congeners displayed complex non-linear relationships with HDL. No clear association emerged for SHBG. The results of the BKMR for the cumulative risk strengthen the results observed in the single pollutant approach, highlighting a positive association between PFAS cumulative exposure and testosterone's levels and a negative association with TSH levels. Interestingly the association between PFAS and HDL levels appeared stronger when considering PFAS cumulatively, compared to what was highlighted for the congeners individually.

These findings align with a growing body of literature suggesting that PFAS act as endocrine and metabolic disruptors, though their effects are heterogeneous and often congener-specific. The positive association with testosterone supports prior evidence that PFAS can interfere with steroidogenesis, whereas inverse or non-linear associations with TSH are consistent with studies describing thyroid disruption through binding to transport proteins and feedback regulation (Coperchini et al., 2021; PFAS Regulates Steroidogenesis in the Male Gonad [Internet], 2025). Divergent associations with lipid fractions likely reflect both the hepatic activity of PFAS (e.g., PPAR signaling) and the complexity of lipid metabolism, where effects may differ by lipoprotein class (Hari et al., 2024). The frequent detection of non-linear (biphasic or triphasic) relationships suggest that the underlying mechanisms may be based on non-monotonic dose–response patterns, previously observed in the presence of endocrine disruption (Vandenberg et al., 2012).

These observations are broadly consistent with international evidence. Analyses from NHANES 2015–2016 reported positive associations between several PFAS (PFDeA, PFOA, PFOS) and free testosterone in women aged 20–49 years (Xie et al., 2021), whereas the SWAN cohort did not observe strong associations during the menopausal transition, highlighting potential age- and reproductive-stage differences (Harlow et al., 2021). Regarding thyroid outcomes, several studies investigated the impact of PFAS on thyroid function and TSH levels in particular, with some studies reporting non-linear or biphasic patterns (Chung et al., 2025; Li et al., 2021; Du et al., 2024). For lipids, the positive associations we observed with LDL and total cholesterol are consistent with most studies documenting higher cholesterol levels with PFAS exposure (Haug et al., 2023; Hussey et al., 2025; Ji et al., 2025; Batzella et al., 2024), whereas the overall lack of a clear association with triglycerides is aligned with previous studies reporting null associations (Haug et al., 2023; Liu et al., 2020), although other authors had highlighted positive associations between PFAS and triglycerides (Hussey et al., 2025). Moreover, the divergent association of PFAS on HDL (negative) and LDL (positive) is consistent with what has been observed also for other environmental endocrine disruptors contaminants, such as PCB (Suarez-Lopez et al., 2019; Patel et al., 2012), although divergent results have been observed for PFAS (Haug et al., 2023; Batzella et al., 2024).

BKMR analyses also showed no clear interaction between PFAS for any metabolic or hormonal biomarker. The absence of detectable interactions between PFAS suggests that their associations with metabolic and hormonal biomarkers are largely independent and additive, supporting the use of a cumulative exposure approach for risk assessment.

Finally, in this exploratory analysis, the relationship between maternal PFAS concentrations and hormonal and metabolic biomarkers in daughters at adult age was examined. The results suggest the lack of association between maternal PFAS levels and daughters' levels of hormonal and metabolic biomarkers. Nevertheless, these findings should be interpreted cautiously, as the analysis was exploratory and intended to generate hypotheses rather than establish causal relationships.

Several limitations should be considered when interpreting the findings of this study. Due to logistical constraints, only E3N-G2 women residing in the Île-de-France region were eligible for inclusion, which may limit the generalizability of our results. It is worth noting, however, that the full E3N-Generations cohort, like most cohort studies, is not intended to be representative of the general French population. The relatively small number of first- and second-generation participants reduced our ability to precisely characterize the influence of individual characteristics and behaviors on internal PFAS exposure, and limited direct comparisons between generations. For the same reason, the statistical power of both the linear regression models and the BKMR analyses was restricted, preventing us from conducting stratified analyses. Moreover, PFAS concentrations were measured only once, preventing the assessment of temporal changes in internal exposure levels. Finally, biomarkers of exposure and hormonal and metabolic biomarkers were measured simultaneously, which limits the ability to draw causal inferences from the observed associations.

Despite these limitations, we were able to identify several noteworthy patterns in PFAS determinants that differed between the two generations, as well as consistent associations with multiple hormonal

and metabolic biomarkers. Another important limitation relates to the assessment of potential multigenerational associations. Ideally, such an investigation would require prospectively collected data on maternal exposure during pregnancy, together with long-term follow-up of daughters into adulthood. Implementing such a design would require more than 50 years of follow-up and is therefore extremely challenging in practice. In light of this constraint, we conducted exploratory analyses using maternal PFAS levels measured years after pregnancy, under the assumption that women with high exposure in the late 1990 s were likely to be highly exposed 10–20 years earlier. This assumption should be kept in mind when interpreting the findings, which should be viewed with caution. As emphasized, our aim in examining the associations between maternal PFAS levels and daughters' hormonal and metabolic biomarkers was not to establish causality but to generate hypotheses for future research.

This study also presents several strengths. First, to the best of our knowledge, it is the first investigation to compare PFAS concentrations measured in blood samples collected in the 1990 s with those measured today in similarly defined populations. This provides a rare opportunity to evaluate temporal trends in internal exposure. By assessing 32 PFAS compounds, this study was able to characterize complex exposure profiles and describe their evolution over time among French adult women. Furthermore, the availability of data on multiple PFAS enabled the use of mixture approach (BKMR), offering a more realistic representation of real-life exposure conditions. Because PFAS concentrations were substantially higher in E3N-G1 than in E3N-G2, including both generations in the models created a wider exposure gradient, allowing better identification of exposure–response relationships. Finally, measuring simultaneously a broad panel of hormonal and metabolic biomarkers allowed for a more holistic evaluation of the potential effects of PFAS on women's health.

In conclusion, overall PFAS concentrations declined over time, with the decrease being more pronounced for compounds that have been formally restricted, such as PFOA and PFOS. Nevertheless, these substances remain the most abundant PFAS in both generations. This pattern likely reflects the regulatory measures implemented in France over the past decades and highlights the ongoing need to implement and reinforce restrictions for all PFAS to further reduce exposure. Across both generations of French women, sociodemographic, anthropometric, and reproductive characteristics were associated with PFAS blood concentrations, although the patterns were not entirely consistent, suggesting that these factors may influence exposure differently across generations. Regarding biological responses, PFAS were generally positively associated with testosterone, inversely associated with TSH, and showed divergent relationships with lipid biomarkers, negative for HDL and positive for LDL. Finally, taken together, these findings highlight substantial temporal shifts in PFAS exposure and reveal meaningful associations with key hormonal and metabolic biomarkers in adult women. Additional research in other population subgroups is warranted, and there is a pressing need for dedicated studies designed to rigorously investigate the potential multigenerational effects of PFAS exposure.

Author agreement Statement

I, the undersigned Corresponding Author, on behalf of all authors of this manuscript, declare that this manuscript is original, has not been published previously, and is not currently under consideration for publication elsewhere.

I confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfy the criteria for authorship but are not listed. I further confirm that the order of authors listed in the manuscript has been approved by all authors.

I understand that, as Corresponding Author, I am the sole contact for the editorial process and am responsible for communicating with the other authors regarding the progress of the submission, the submission of revisions, and the final approval of proofs.

On behalf of all authors, I confirm our agreement to the submission of this manuscript to Environment International.

Ethics approval

The E3N Generations study was approved by the French National Commission for Data Protection and Privacy (ClinicalTrials.gov identifier: NCT03285230). The E3N Generations blood collection study was approved by the Bicêtre ethics committee (CPP-IDF-VII, IRB # IORG0001140). All participants included in the E3N Generations study gave written informed consent, and informed consent was obtained for novel biomarkers testing.

CRediT authorship contribution statement

Francesca Romana Mancini: Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Claire Perrin:** Writing – review & editing, Methodology, Formal analysis. **Régine Billmann:** Writing – review & editing, Project administration. **Hélène Blanché:** Resources, Data curation. **Pauline Frénoy:** Writing – review & editing, Methodology. **Xuan Ren:** Writing – review & editing, Methodology. **Grégoire Petitjean:** Resources. **Ronan Pommereuil:** Resources. **German Cano-Sancho:** Writing – review & editing, Validation, Resources, Formal analysis, Conceptualization. **Bruno Le Bizec:** Resources. **Jean-Philippe Antignac:** Writing – review & editing, Resources, Conceptualization. **Gianluca Severi:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization.

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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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2026.110462.

Data availability

The authors do not have permission to share data.

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