Folate concentrations and serum perfluoroalkyl and polyfluoroalkyl substance concentrations in adolescents and adults in the USA (National Health and Nutrition Examination Study 2003–16): an observational study

Yu Zhang, Vicente Mustieles, Yi-Xin Wang, Yang Sun, Juliana Agudelo, Zainab Bibi, Nicole Torres, Youssef Oulhote, Angela Slitt, Carmen Messerlian

Summary

Background Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are a family of highly fluorinated aliphatic compounds, which are widely used in commercial applications, including food packaging, textiles, and non-stick cookware. Folate might counteract the effects of environmental chemical exposures. We aimed to explore the relationship between blood folate biomarker concentrations and PFAS concentrations.

Methods This observational study pooled cross-sectional data from the National Health and Nutrition Examination Survey (NHANES) 2003 to 2016 cycles. NHANES is a population-based national survey that measures the health and nutritional status of the US general population every 2 years by means of questionnaires, physical examination, and biospecimen collection. Folate concentrations in red blood cells and in serum, and perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS) concentrations in serum were examined. We used multivariable regression models to assess the percentage change in serum PFAS concentrations in relation to changes in folate biomarker concentrations. We additionally used models with restricted cubic splines to investigate the shape of these associations.

Findings This study included 2802 adolescents and 9159 adults who had complete data on PFAS concentrations, folate biomarkers, and covariates, were not pregnant, and had never had a cancer diagnosis at the time of the survey. The mean age was 15·4 years (SD 2·3) for adolescents and 45·5 years (17·5) for adults. The proportion of male participants was slightly higher in adolescents (1508 [54%] of 2802 participants) than in adults (3940 [49%] of 9159 participants). We found negative associations between red blood cell folate concentrations and serum concentrations of PFOS (percentage change for a 2·7 fold-increase in folate level –24·36%, 95% CI –33·21 to –14·34) and PFNA (–13·00%, –21·87 to –3·12) in adolescents, and PFOA (–12·45%, –17·28 to –7·35), PFOS (–25·30%, –29·67 to –20·65), PFNA (–21·65%, –26·19 to –16·82), and PFHxS (–11·70%, –17·32 to 5·70) in adults. Associations for serum folate concentrations and PFAS were in line with those found for red blood cell folate levels, although the magnitude of the effects was lower. Restricted cubic spline models suggested linearity of the observed associations, particularly for associations in adults.

Interpretation In this large-scale, nationally representative study, we found consistent inverse associations for most examined serum PFAS compounds in relation to folate concentrations measured in either red blood cells or serum among both adolescents and adults. These findings are supported by mechanistic in-vitro studies that show the potential of PFAS to compete with folate for several transporters implicated in PFAS toxicokinetics. If confirmed in experimental settings, these findings could have important implications for interventions to reduce the accumulated PFAS body burden and mitigate the related adverse health effects.

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Department of Environmental Health (Y Zhang BA, Y Sun MD, Z Bibi BA, N Torres BA, C Messerlian PhD), **Department of Nutrition** (Y-X Wang PhD), **and Department of Epidemiology** (Y Sun, C Messerlian), **Harvard TH Chan School of Public Health, Boston, MA, USA; University of Granada, Center for Biomedical Research, Granada, Spain** (V Mustieles PhD); **Instituto de Investigación Biosanitaria Ibs Granada, Granada, Spain** (V Mustieles); **Consortium for Biomedical Research in Epidemiology and Public Health, Madrid, Spain** (V Mustieles); **University of Rhode Island, Kingston, RI, USA** (J Agudelo BS, A Slitt PhD); **Massachusetts General Hospital Fertility Center, Department of Obstetrics and Gynecology, Boston, MA, USA** (Z Bibi, N Torres, C Messerlian); **Department of Biostatistics and Epidemiology, School of Public Health and Health Sciences, University of Massachusetts Amherst, Amherst, MA, USA** (Y Oulhote PhD)

Correspondence to: Prof Carmen Messerlian, Department of Environmental Health, Harvard TH Chan School of Public Health, Boston, MA 02115, USA
cmesser@hsph.harvard.edu

Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are a family of highly fluorinated aliphatic compounds, which are widely used in commercial applications, including food packaging, textiles, and non-stick cookware.[1] Long-chain PFAS have very long half-lives (from 3 years to decades),[2,3] and are universally detected in populations worldwide.[4] PFAS have raised serious public health concern for their persistent nature and harmful health effects, including adverse pregnancy, birth, and developmental outcomes, altered immune function, liver and kidney diseases, lipid dysregulation, and cancer.[5,6]

Although direct exposure to PFAS from consumer products could be reduced through shifts in production, PFAS exposure driven by tap and groundwater contamination and accumulation in the food web persists over a long period.[7–9] Given the persistent nature of PFAS, it is crucial to find effective strategies to reduce PFAS burden

Research in context

Evidence before this study

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are manmade chemicals that are widely used in diverse commercial applications, leading to universal population exposure. Long-chain PFAS are extremely persistent and have long biological half-lives in humans. PFAS exposure is linked to numerous adverse health outcomes, including immune dysregulation, metabolic syndrome, and cancer. Identifying effective strategies to reduce the PFAS body burden is crucial to mitigate their harmful health effects. We searched PubMed for studies published in English from the earliest date in the database to Jan 1, 2022, using the search terms ("diet" OR "food" OR "nutrition" OR "nutrient" OR "folate" OR "folic acid") AND ("PFAS" OR "per- and polyfluoroalkyl substances" OR "perfluoroalkyl substances" OR "polyfluoroalkyl substances"). Previous studies have found that dietary consumption of some food groups was inversely associated with serum PFAS concentrations. However, few studies have explored which nutrients contributed to this reduction. Folate has been shown to reduce the body burden of environmental pollutants and counteract the detrimental health effects of environmental exposures. Evidence suggests that folate and PFAS could share similar transport carriers, potentially leading to an inverse association between them in humans.

Added value of this study

We investigated a large sample of 2802 adolescents and 9159 adults from a nationally representative cross-sectional study in the USA. We found an association between reductions in serum PFAS concentrations with increases in red blood cell folate concentrations. Specifically, we found strong and robust negative associations between folate concentrations in red blood cells—a marker of medium-term folate intake—and serum concentrations of perfluorooctane sulfonic acid (PFOS) and perfluorononanoic acid (PFNA) among adolescents, and perfluorooctanoic acid, PFOS, PFNA, and perfluorohexane sulfonic acid among adults. Similar negative associations were found between serum folate concentrations and PFAS concentrations, although these associations were generally attenuated and in line with the short-term nature of serum folate measurement.

Implications of all the available evidence

We found consistent negative associations for most examined serum PFAS compounds in relation to folate concentrations measured in either red blood cells or serum among both adolescents and adults in the USA. These findings are supported by mechanistic in-vitro studies that show the potential of PFAS to compete with folate for several transporters implicated in PFAS toxicokinetics. If validated in experimental studies, our findings could have important implications for public health interventions that aim to reduce the accumulated PFAS body burden and related adverse health effects.

in humans. Nutrition has long been proposed as a modulator to combat the toxicity of environmental pollutants.[10–13] Folate (vitamin B9) is of particular interest for its essential role in one-carbon metabolism, which is fundamental in maintaining cell function.[14] Studies show that folate is able to increase arsenic excretion in humans[15,16] and counteract the detrimental health effects of environmental chemical exposures.[17–20]

Previous studies showed that folate and PFAS share transport carriers, including folate receptor α and those in the ATP-binding cassette transporter family,[21–25] indicating potential competition between PFAS and folate. Thus, an opposing relationship between folate and PFAS could exist, implying a lower rate of PFAS absorption and higher rate of PFAS excretion due to competition with folate.[26–32] Observational studies found that higher dietary intake of vegetables, fruits, grains, and beans, which are major sources of folate, was negatively associated with PFAS concentrations in serum or in plasma.[33–37] We propose that these negative associations might in part be driven by the antagonist kinetic actions of folate and PFAS.

In this study, we aimed to examine whether there was an association between serum PFAS concentrations and folate concentrations in red blood cells and serum among adolescents and adults from the general US population by use of pooled cross-sectional data from the National Health and Nutrition Examination Survey (NHANES) 2003 to 2016 cycles.

Methods

Study design and population

This observational study pooled cross-sectional data from the NHANES 2003 to 2016 cycles. NHANES is a population-based national survey that measures the health and nutritional status of the US general population every 2 years by means of questionnaires, physical examination, and biospecimen collection. Detailed study procedures of NHANES have been described by the US Centers for Disease Control and Prevention (CDC).[38]

In this study, we included survey participants aged 12 years and older who had complete data on concentrations of PFAS in serum, folate biomarkers in red blood cells and in serum, and covariates (as stated in the covariates section). Because pregnant women (physiologically) and cancer patients (pathophysiologically) have different folate metabolism,[39–41] we excluded participants who had a positive pregnancy test at the time of survey or self-reported as ever having had a cancer diagnosis. A flowchart of study participants is presented in the figure. Ethical approval of NHANES was obtained from the National

Center for Health Statistics. All participants provided written consent to participate in NHANES.

Folate biomarker measurement

Peripheral blood samples were collected at mobile examination centers for all study participants.[42] Folate concentrations in red blood cells and in serum were measured using the Quantaphase II Folate radioassay kit (Bio-Rad Laboratories, Watford, UK) for the 2003–04 and 2005–06 NHANES cycles.[43,44] In the 2007–08 and 2009–10 cycles, serum folate and whole-blood folate concentrations were measured by microbiological assay,[45] from which folate concentrations in red blood cells were calculated. Starting at the 2011–12 cycle and onwards, five folate forms, including 5-methyl-tetrahydrofolate, pteroylglutamic acid, 5-formyl-tetrahydrofolate, tetrahydrofolate, and 5,10-methenyl-tetrahydrofolate, were measured in serum using isotope-dilution high performance liquid chromatography coupled to tandem mass spectrometry. The total folate concentration in serum was calculated by summing the five folate forms for these cycles. Red blood cell folate was calculated using the data of total folate concentrations in serum and whole-blood folate concentrations, which were measured by microbiological assay.[46] Detailed laboratory methods and quality control can be found in the laboratory procedure manuals of the NHANES study.[43–49]

PFAS measurement in serum

Serum PFAS concentrations were measured for a random subsample of about a third of the total NHANES population in each cycle. Serum PFAS concentrations were measured using online solid-phase extraction coupled to high-performance liquid chromatography–isotope dilution–tandem mass spectrometry. In cycles from 2003–12, four PFAS compounds were measured in serum, including perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorohexane sulfonic acid (PFHxS), and perfluorononanoic acid (PFNA). In cycles in 2013–14 and 2015–16, four isomers of PFOA and PFOS were measured using the same methods, including linear PFOA, sum of branched isomers of PFOA, linear PFOS, and the sum of monomethyl branched isomers of PFOS. Limits of detection for the examined PFAS compounds changed slightly over the cycles and are presented in the appendix (p 1). Concentrations below the limits of detection in each cycle were replaced by the limit of detection divided by the square root of 2 (imputed for <1·2% of participants; appendix p 3).[50] Detailed quality control information can be found in NHANES laboratory procedure manuals.[42,51]

Covariates

Demographic covariates were obtained by self-reported questionnaires, including age (continuous), sex (male or female), race or ethnicity (non-Hispanic White, non-Hispanic Black, Hispanic, or other), highest educational level for adults (less than middle school, middle school, high-school graduate, college degree, and college graduate or above), and income-to-poverty ratio (continuous). The income-to-poverty ratio was the ratio of family income to poverty guidelines. Trained health technicians measured the height and weight of participants. BMI at or above the 95th percentile for a child's sex and age based on US CDC growth charts was considered obese for adolescents and BMI at or above 30 kg/m² was considered obese for adults.[52,53] Dietary data were collected by two 24-h diet recalls, which spanned 3–10 days. We calculated the Alternative Healthy Eating Index 2010 (AHEI-2010) score using the mean dietary intake of the two diet recalls. AHEI-2010 is a measure of diet quality that contains 11 food and nutrient components, with higher scores reflecting better diet quality. Detailed description of the AHEI-2010 can be found elsewhere.[54] Dietary supplementation intake, including folic acid intake, was added to the 24-h dietary interviews in the 2007–08 cycle (available for 1567 adolescents and 6439 adults).

See Online for appendix

Statistical analysis

Descriptive statistics were calculated for the distribution of participant characteristics, folate biomarkers, and PFAS compounds. We further compared the characteristics for the original NHANES population who had data on folate

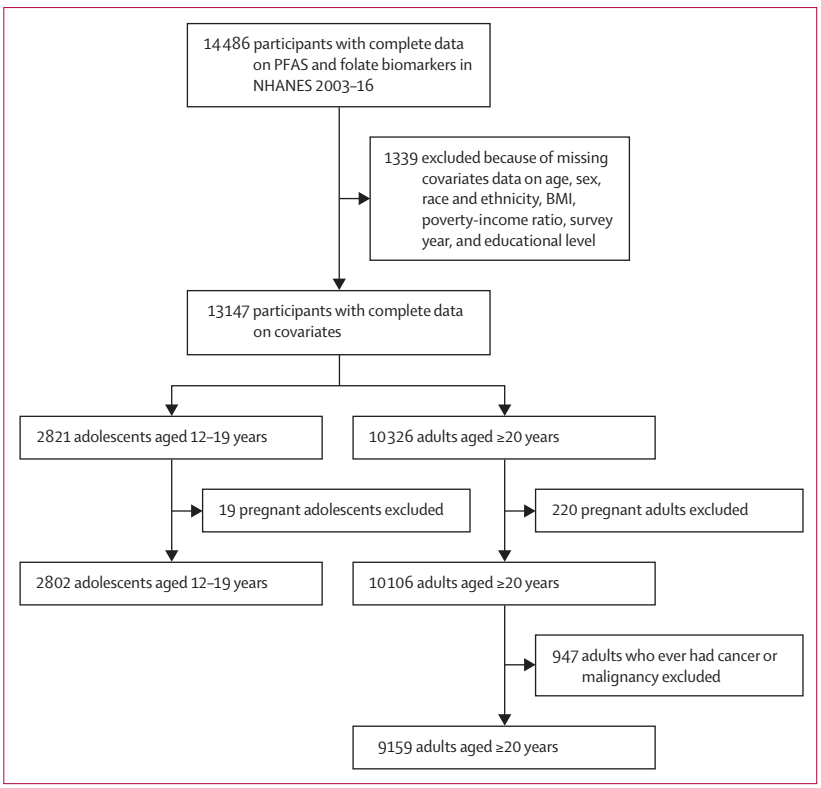

Figure: **Participant flowchart, NHANES 2003–16**
NHANES=National Health and Nutrition Examination Survey. PFAS=perfluoroalkyl and polyfluoroalkyl substances.

	Adolescents (n=2802)	Adults (n=9159)
Age, years	15·43 (2·27)	45·47 (17·51)
BMI, kg/m²	24·13 (6·25)	28·77 (6·82)
Sex		
Male	1508 (53%)	4517 (49%)
Female	1294 (46%)	4642 (51%)
Race and ethnicity		
Non-Hispanic White	780 (59%)	3940 (68%)
Non-Hispanic Black	792 (14%)	1986 (11%)
Hispanic	988 (20%)	2355 (14%)
Other, including multiracial	242 (7%)	878 (7%)
Education level		
Less than middle school	NA	1020 (6%)
Middle school	NA	1362 (11%)
High school graduate	NA	2094 (23%)
College degree	NA	2659 (32%)
College graduate or above	NA	2024 (28%)
Income-to-poverty ratio		
<1	916 (23%)	1931 (14%)
≥1 to <2	761 (22%)	2504 (21%)
≥2	1125 (5%)	4724 (64%)

Data are mean (SD) or n (%). Statistical estimates accounted for the study sampling scheme, data clustering, and sample weights in NHANES to be nationally representative. NHANES=National Health and Nutrition Examination Survey. NA=not applicable.

Table 1: **Participant characteristics of adolescents aged 12–19 years and adults aged ≥20 years, NHANES 2003–16**

biomarkers and serum PFAS measurements with the study population included in our final analytical sample.

Because of the potential effect of heterogeneity by age, we separated the analyses for adolescents (aged 12–19 years) and adults (aged ≥20 years). In cycles from 2013–16 when isomers of PFOA and PFOS were measured, serum PFOA and PFOS concentrations were calculated as the sum of their isomer concentrations.[55]

Folate concentrations in red blood cells and in serum, and PFAS concentrations in serum were natural log-transformed to minimise the effect of outliers and to improve the interpretation of associational results. We applied multivariable linear regression models to examine the percentage change in serum concentrations of individual PFAS compounds per 2·7-fold increase in (ie, per unit increase in natural log-transformed) folate concentrations (in red blood cells and serum) for both adolescents and adults, adjusting for covariates.[56] Covariates were selected a priori as potential confounders using a directed acyclic graph (appendix p 12), and included age, sex, race and ethnicity, income-to-poverty ratio, BMI, and survey cycle.[57] For models in adults, we additionally adjusted for their highest educational level (categorical). Because diet quality is related to both folate and PFAS exposure,[34,58] we additionally adjusted the analyses for AHEI-2010 score.

To examine the cumulative influence of folate levels on PFAS, we additionally calculated the sum of total serum PFAS compounds as the linear combination of serum concentrations of the four measured PFAS compounds and evaluated the associations of red blood cells and serum folate concentrations with this total sum in both adolescents and adults.

Because intake of folic acid supplementation could alter folate but not PFAS concentrations directly, associations of folate and PFAS concentrations could differ among participants with or without folic acid supplementation intake. We stratified the analyses for participants who reported folic acid supplementation intake in at least one of the two dietary interviews versus participants who did not. We additionally examined effect heterogeneity by sex and obesity for the associations of folate biomarkers and individual PFAS compounds in both adolescents and adults. Because parity could potentially affect both folate biomarkers and PFAS concentrations,[59,60] we conducted sensitivity analyses by excluding women who self-reported as previously having a livebirth (36 adolescents and 3306 adults).

We also used models with restricted cubic splines to explore associations between folate biomarkers and individual PFAS compound concentrations in adolescents and adults.

To supplement the associations between folate biomarkers and PFAS, we additionally explored daily dietary folate equivalent intake and doses of daily folic acid supplementation intake in relation to serum PFAS concentrations. Dietary folate equivalent included natural folate from diet and folic acid from fortifications and supplementation. Daily dietary folate equivalent was estimated by taking the mean of dietary folate equivalent intake reported from two 24-h diet recalls. We categorised the population by their dietary folate equivalent intake as at or above versus below daily recommendation levels (300 µg/day for participants aged 12–13 years and 400 µg/day for participants aged ≥14 years). We used multivariable linear regression models to examine the percentage difference in serum PFAS concentrations comparing participants whose daily dietary folate equivalent intake was at or above versus below recommended levels.[61] We additionally examined the associations between doses of folic acid supplementation intake (per 100 µg/day increase) and serum PFAS concentrations, restricting the analyses to participants who reported having taken a folic acid supplement in at least one of the 24-h diet recalls (164 adolescents and 1673 adults).

Both descriptive and associational analyses followed the NHANES protocol of statistical analyses to obtain nationally representative statistics.[62] We accounted for the study sampling scheme, data clustering, and subsample weights in the analyses. Stratified analyses were conducted using the domain or tables statement of the SAS survey procedure. Models with restricted cubic splines were conducted using survey and splines

packages in R version 4.0.3. All other analyses were done in SAS version 9.4.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

This study included 2802 adolescents and 9159 adults who had complete data on PFAS concentrations, folate biomarkers, and covariates, were not pregnant, and had never had a cancer diagnosis at the time of the survey (table 1). The mean age was 15·4 years (SD 2·3) for adolescents and 45·5 years (17·5) for adults. The proportion of male participants was slightly higher in adolescents (1508 [53%] of 2802 participants) than in adults (4517 [49%] of 9159 participants). The mean BMI was 24·1 kg/m² (SD 6·3) in adolescents and 28·8 kg/m² (6·8) in adults. More than half of the population was non-Hispanic White in the adolescent group (59%) and adult group (68%). The proportion of people who had household incomes lower than the poverty line (income-to-poverty ratio <1) was higher in adolescents (916 [23%] of 2802 participants) than in adults (1931 [14%] of 9159 participants). The demographics, folate biomarkers levels, and serum PFAS concentrations in our study population were similar to the original NHANES population that had data on folate biomarkers and serum PFAS measurements (appendix p 2).

The geometric mean of red blood cell folate concentrations was lower in adolescents than in adults (353·4 ng/mL *vs* 408·1 ng/mL), whereas the geometric means for serum folate concentrations were similar among the two age groups (15·4 ng/mL in adolescents *vs* 14·9 ng/mL in adults; appendix p 3). All four PFAS compounds had around or above 99% detection rates for both adolescents and adults (appendix p 3). The serum PFAS concentrations were higher in adults than in adolescents except for PFHxS, for which the serum concentration was slightly higher in adolescents (appendix p 3).

Folate biomarker concentrations were similar in male and female adolescents, and were higher in female adults than in male adults (appendix p 4). However, male participants generally had higher serum PFAS concentrations than female participants in both adolescents and adults (appendix p 4). Participants with obesity had lower serum PFAS and serum folate concentrations but higher red blood cell folate concentrations compared with participants who were not obese in both adolescents and adults (appendix p 4).

In adolescents, covariate-adjusted models showed negative associations between red blood cell folate and serum PFOS and PFNA concentrations, and serum folate and PFOS concentrations (table 2). Specifically, a 2·7-fold increase in red blood cell folate concentrations was associated with a 24·36% (95% CI –33·21 to –14·34) reduction in serum concentration of PFOS and a 13·00% (–21·87 to –3·12) reduction in serum concentration of PFNA. A 2·7-fold increase in serum folate concentrations was associated with an 11·57% (95% CI –17·87 to –4·79) decrease in serum PFOS concentration. Additional adjustment for diet quality did not change these findings (table 2). No associations were found for red blood cell or serum folate concentration with other PFAS compounds among adolescents.

In adults, the associations between red blood cell folate and serum PFAS concentrations were more consistent than findings in adolescents. We found that folate concentrations in red blood cells were negatively associated with serum concentrations of all four PFAS compounds in crude analyses, covariate-adjusted analyses, and analyses additionally adjusted for diet quality in adults (table 3). Strong associations were found for red blood cell folate concentrations and serum PFOS and PFNA concentrations in adults. Specifically, we found that a 2·7-fold increase in red blood cell folate concentrations was associated with a 25·30% (95% CI –29·67 to –20·65) reduction in serum concentration of PFOS and a 21·65% (–26·19 to –16·82) reduction in serum concentration of PFNA, after adjusting for covariates. Red blood cell folate concentrations were also negatively associated with serum PFOA (percentage change –12·45%, 95% CI –17·28 to –7·35) and PFHxS (–11·70%, –17·32 to 5·70) concentrations. Associations of PFAS concentrations and serum folate concentrations were generally weaker compared with those for red blood cell folate concentrations. Serum folate concentrations were negatively associated with serum concentrations of PFOA (percentage change –5·01%, 95% CI –8·35 to –1·54), PFOS (–12·81%, –16·15 to –9·35), and

	Unadjusted percentage change (95% CI)	Adjusted* percentage change (95% CI)	Adjusted† percentage change (95% CI)
Folate in red blood cells‡			
PFOA	–36·43% (–41·41 to –31·04)	–7·34% (–16·57 to 2·91)	–7·92% (–17·76 to 3·11)
PFOS	–65·00% (–68·00 to –61·00)	–24·36% (–33·21 to –14·34)	–25·50% (–34·75 to –14·93)
PFNA	–18·74% (–28·83 to –7·23)	–13·00% (–21·87 to –3·12)	–15·03% (–24·12 to –4·87)
PFHxS	–27·18% (–37·67 to –14·92)	–12·29% (–26·12 to 4·12)	–12·82% (–27·21 to 4·43)
Folate in serum‡			
PFOA	–16·19% (–21·50 to –10·51)	3·82% (–2·32 to 10·34)	4·16% (–2·31 to 11·05)
PFOS	–43·70% (–48·67 to –38·25)	–11·57% (–17·87 to –4·79)	–11·75% (–17·96 to –5·07)
PFNA	–5·62% (–13·71 to 3·21)	–1·25% (–7·14 to 5·01)	–1·84% (–7·87 to 4·59)
PFHxS	–13·12% (–24·03 to –0·64)	–3·94% (–15·51 to 9·20)	–3·16% (–15·17 to 10·55)

Data are percentage change (95% CI). PFAS=perfluoroalkyl and polyfluoroalkyl substances. PFHxS=perfluorohexane sulfonic acid. PFNA=perfluorononanoic acid. PFOA=perfluorooctanoic acid. PFOS=perfluorooctane sulfonic acid. *n=2802; models were adjusted for age (continuous), sex (binary), race (categorical), BMI (continuous), poverty-to-income ratio (continuous), and survey cycle (categorical). †n=2700; models were adjusted for age (continuous), sex (binary), race and ethnicity (categorical), BMI (continuous), poverty-to-income ratio (continuous), survey cycle (categorical), and Alternative Healthy Eating Index 2010 score (continuous). ‡Folate biomarkers were the independent variables and the individual serum PFAS concentrations were the dependent variables.

Table 2: **Percentage change in serum PFAS concentrations per 2·7-fold increase in folate biomarker concentrations among adolescents aged 12–19 years, National Health and Nutrition Examination Survey 2003–16**

	Unadjusted percentage change (95% CI)	Adjusted* percentage change (95% CI)	Adjusted† percentage change (95% CI)
Folate in red blood cells‡			
PFOA	−25·49% (−30·06 to −20·63)	−12·45% (−17·28 to −7·35)	−12·03% (−17·16 to −6·59)
PFOS	−47·00% (−50·00 to −43·00)	−25·30% (−29·67 to −20·65)	−24·30% (−28·81 to −19·50)
PFNA	−21·86% (−27·26 to −16·06)	−21·65% (−26·19 to −16·82)	−21·24% (−26·06 to −16·11)
PFHxS	−12·07% (−18·44 to −5·19)	−11·70% (−17·32 to −5·70)	−10·66% (−16·57 to −4·33)
Folate in serum‡			
PFOA	−12·39% (−15·98 to −8·65)	−5·01% (−8·35 to −1·54)	−4·76% (−8·21 to −1·19)
PFOS	−25·86% (−29·60 to −21·93)	−12·81% (−16·15 to −9·35)	−11·82% (−15·17 to −8·35)
PFNA	−12·90% (−16·29 to −9·36)	−11·85% (−14·72 to −8·88)	−11·36% (−14·39 to −8·23)
PFHxS	−4·49% (−9·55 to 0·85)	−1·72% (−6·65 to 3·46)	−0·82% (−5·88 to 4·51)

Data are percentage change (95% CI). PFAS=perfluoroalkyl and polyfluoroalkyl substances. PFHxS=perfluorohexane sulfonic acid. PFNA=perfluorononanoic acid. PFOA=perfluorooctanoic acid. PFOS=perfluorooctane sulfonic acid. *n=9159; models were adjusted for age (continuous), sex (binary), race and ethnicity (categorical), BMI (continuous), poverty-to-income ratio (continuous), educational level (categorical), and survey cycle (categorical). †n=8625; models were adjusted for age (continuous), sex and ethnicity (binary), race and ethnicity (categorical), BMI (continuous), poverty-to-income ratio (continuous), educational level (categorical), survey cycle (categorical), and Alternative Healthy Eating Index 2010 score (continuous). ‡Folate biomarkers were the independent variables and the individual serum PFAS concentrations were the dependent variables.

Table 3: **Percentage change in serum PFAS concentrations per 2·7-fold increase in folate biomarker concentrations among adults aged ≥20 years, National Health and Nutrition Examination Survey 2003–16**

PFNA (–11·85%, –14·72 to –8·88), but not PFHxS (–1·72%, –6·65 to 3·46). Additionally adjusting for diet quality did not change the findings (table 3).

Higher red blood cell and serum folate levels were significantly associated with decreases in the total sum of PFAS concentrations in both adolescents and adults (appendix p 5). After stratifying analyses by folic acid supplementation intake, the associations in the main analyses generally appeared stronger among participants who reported folic acid supplementation intake in at least one of the two 24-h diet recalls among adults. The patterns were less consistent in adolescents, which might be because of the smaller sample size of adolescents who reported folic acid supplementation (164 participants; appendix p 6).

After stratifying analyses by sex, no consistent sex heterogeneity was found for associations between red blood cell folate and PFOS and PFNA, and serum folate and PFOS among adolescents (appendix p 7). The negative associations of red blood cell and serum folate with PFOS concentration were stronger in magnitude in adolescents with obesity, whereas the association between red blood cell folate and PFNA was similar among adolescents with obesity and adolescents who were not obese (appendix p 8).

By contrast, in adults, associations between folate biomarkers and PFAS concentrations appeared slightly stronger among females and adults without obesity (appendix pp 7–8). The associations between all four PFAS compounds and red blood cell folate, and between PFOA and serum folate were stronger in female participants, whereas the associations for PFOS and PFNA in relation to serum folate were similar in male and female participants (appendix p 7). The associations in the main analyses were also slightly stronger in magnitude among adults without obesity compared with adults with obesity (appendix p 8). Excluding women who self-reported a previous livebirth did not materially change the findings (appendix p 9).

Models of restricted cubic splines showed slightly decreasing linear relationships between red blood cell and serum folate and PFOS concentration among adolescents (appendix p 13). Other dose–response relationships between folate biomarkers and PFAS concentrations appeared flat among adolescents. In adults, clear linear negative relationships were seen between red blood cell folate concentrations and all four serum PFAS serum concentrations, while inverse U-shapes were found for serum folate and PFOS and PFNA concentrations in adults (appendix p 14).

In the supplemental analyses of dietary folate intake and serum PFAS concentrations, no obvious associations were seen for dietary folate equivalent above versus below recommended levels or for folic acid supplementation intake and serum PFAS concentrations among adolescents (appendix pp 10–11). However, adults with daily dietary folate equivalent intake at or above the recommended level had lower serum PFOS (percentage difference –7·29%, 95% CI –12·14 to –2·17) and PFNA (–8·40%, –12·56 to –4·05) concentrations, compared with adults whose daily dietary folate equivalent intakes were below the recommended level (appendix p 10). Among adults who reported having taken folic acid supplementation in diet recalls, each 100-µg increase in daily folic acid supplementation intake was associated with decreases of 1·24% (95% CI –2·24 to –0·23) in serum PFOS concentration and 1·38% (–2·34 to –0·41) in serum PFNA concentration (appendix p 11).

Discussion

In this large-scale, representative, cross-sectional study, we found strong negative associations between red blood cell folate concentrations and serum PFOS and PFNA concentrations among adolescents, and between red blood cell folate concentrations and serum PFOA, PFOS, PFNA, and PFHxS concentrations among adults. Negative associations were also found between serum folate and PFOS concentrations among adolescents, and between serum folate and PFOA, PFOS, and PFNA concentrations among adults. The findings were more pronounced among participants who took folic acid supplementations. Models with restricted cubic splines supported the linearity of the associations observed, particularly among adults. Consistently, our supplemental analyses of dietary folate intake supported that optimal folate intake and a higher dose of folic acid supplementation were both associated with lower serum PFOS and PFNA concentrations in adults.

Findings reported by previous dietary studies were in line with our study, and generally showed negative

associations for PFAS concentrations in plasma and serum in relation to dietary intake of soy, beans, grain, vegetables, and fruits, which are foods containing high amounts of folate.[33–37,63–67] For example, a NHANES study using data from the 2003–08 cycles reported that PFOA was inversely correlated with the intake of beans in the general US population.[63] Another cross-sectional study in the USA found that vegetable and fruit consumption during the past year was negatively associated with plasma PFAS concentrations, particularly PFOS.[33] A study in China found that the intake frequency of soy was negatively associated with plasma concentrations of PFOS and PFNA among women of reproductive age.[36] A study in Singapore observed that plasma PFOA and PFOS concentrations were inversely correlated with grain intake, and plasma PFOS concentration was inversely correlated with intake of soy products.[64]

To our knowledge, this is the first study to examine the associations between folate biomarkers and PFAS concentrations in blood. Previous studies examined dietary food intake, rather than biomarkers, in relation to PFAS concentrations in serum and plasma. Compared with diet recall, the folate biomarker data in our study provided a more objective and accurate measurement of this specific nutrient, which has substantial implications for mechanistic experiments and intervention studies. Further adjustment for AHEI-2010 score in our analyses limited the possibility of confounding by diet and supported the negative associations between folate and serum PFAS concentrations.

Two studies have reported similar negative associations between serum PFAS concentrations and blood folate biomarker levels among adolescents and adults in the NHANES, but with smaller sample sizes.[68,69] However, these two studies hypothesised that higher PFAS exposure disrupts folate metabolism and thus results in lower folate concentrations, although no clear mechanism exists to support this hypothesis. By contrast, our hypothesis that folate might lower serum PFAS concentrations is novel and works in the opposite direction on the mode of action between folate and PFAS. Our analyses and findings for folate biomarkers and dietary folate intake in relation to reduced serum PFAS concentrations were robust and provide strong support for our hypothesis. Importantly, because folic acid supplementation could only alter folate biomarker concentrations and not PFAS directly, the stronger associations between folate and PFAS concentrations that we found among participants who took folic acid supplementation support the hypothesis that folate reduces PFAS absorption or increases its excretion. Furthermore, supplemental analyses using dietary folate intake as the exposure group provided more direct evidence that folate might reduce PFAS concentrations in serum. Additionally, another study by our group showed that red blood cell folate levels could modify the associations between serum PFAS concentrations and rubella and mumps antibody levels among adolescents in the USA, where the negative association between PFAS and antibody levels was only found in adolescents with low red blood cell folate status and not among those with higher folate status.[70] The cumulative evidence indicates that folate might reduce PFAS levels and mitigate PFAS-related health outcomes.

The associations with serum PFAS concentrations were generally stronger for folate concentrations measured in red blood cells compared with folate in serum. Folate in red blood cells (mean lifespan around 3–4 months) reflects the relatively long-to-medium-term intake of folate from food, folic acid supplementation, or both, whereas serum folate reflects recent folate intake.[71] We found that recent daily dietary folate equivalent intake and folic acid supplementation intake were negatively associated with PFOS and PFNA concentrations among adults, which was consistent with our findings on serum folate. Our findings on the strong negative associations between red blood cell folate and PFAS concentrations indicated that long-term folate intake might be more relevant to reduced serum PFAS concentrations. Given the long half-lives of PFAS compounds, the fact that in this study stronger associations were found with red blood cell folate adds biological plausibility to our findings.

A 2022 experimental study found that spinach and soybean, which contain high levels of folate, reduced the relative bioavailability of absorption for PFOA and its short-chain alternative, hexafluoropropylene oxide trimer acid, in mice,[72] providing preliminary experimental support for our findings. Several mechanisms might explain these results. Folate and PFAS are both substrates for several carriers, including folate receptor α,[73] breast cancer resistance protein,[21,23,74] P-glycoprotein,[23,74] multidrug resistance-associated proteins,[74] organic anion transporters, and organic anion-transporting polypeptides,[24,25,28–32,75] which are widely distributed in the human body, including in the renal tubules and gastrointestinal tract. Folate might compete with PFAS for carrier transport, decreasing the absorption of PFAS in the intestine or reducing the reabsorption of PFAS in the kidney, resulting in decreased PFAS concentrations in the blood. A higher renal clearance coupled with lower renal reabsorption of PFAS could synergistically contribute to a reduced PFAS body burden.

That our observed associations were less consistent in adolescents than adults might suggest heterogenous mechanisms of folate and PFAS at different developmental stages. Adolescents undergo physiological changes to reach maturity, whereas development is relatively stable or degenerating in adulthood.[76] Although lifestyle habits tend to be more stable in adults, adolescence also represents a dynamic period for behavioural and lifestyle habits. Furthermore, adults have a longer time for PFAS accumulation and generally present with higher PFAS concentrations than do adolescents. Another difference that should be noted when interpreting the associations in adolescents is that the sample size of adolescents was about three times smaller compared with adults. The

effect heterogeneity by sex among adults could have implications for the mechanisms of the associations between folate and PFAS concentrations. Compared with male participants, female participants have similar folate biomarker concentrations but lower serum PFAS concentrations, which could be due to loss through menstruation.[77,78] In a study using NHANES 2005–16 data, which examined dietary fibre intake and serum PFAS concentrations among adults, the negative associations between fibre intake and PFAS were also stronger among female participants, although the magnitudes of effect were generally small.[37] Our finding that associations were more apparent among adolescents with obesity and adults who did not have obesity should be interpreted with caution, given the small sample size of the population with obesity and the small magnitude of difference in associations. This finding might suggest that the antagonistic effect of folate on PFAS concentrations might differ by lipid or albumin levels. Nevertheless, there might be modest differences by sex and obesity, and any differences might also be due to unmeasured confounding due to other lifestyle factors.

A major strength of this study is the large sample size and the nationally representative nature of the NHANES design. The strict quality control of the NHANES guaranteed the accuracy of our exposure and outcome measurements. Our findings were robust and consistent across multiple sensitivity analyses, including the supplemental analyses on dietary folate intake. Admittedly, this study also has several limitations. First, the cross-sectional study design prevents us from establishing the temporality of exposure (folate biomarkers in blood) and outcome (PFAS compounds in serum), and causality cannot be established. A limitation of using biomarker data is that folate is regulated within the human body. From our findings, we cannot infer whether higher folate intake decreases the absorption of PFAS or accelerates the excretion of PFAS, or whether higher folate intake decreases PFAS concentration in serum while increasing PFAS in other body tissues, such as the liver and kidney. Future experimental studies are needed to investigate these mechanisms. Although we have adjusted for as many covariates as confounders in the analyses, unmeasured confounding cannot be ruled out. Residual bias from diet might exist, since 24-h diet recall is an imperfect measure of diet history. Nevertheless, using mean intake from two dietary recalls reflects a relatively stable diet pattern. Furthermore, the observed covariate-adjusted effect sizes were large. It is unlikely that the associations solely resulted from unmeasured confounding, which would require the uncontrolled confounding to be substantial.[79] Finally, we were unable to examine short-chain PFAS since the NHANES had few data on this, necessitating further research.

In conclusion, in this large-scale nationally representative study, we found negative associations for selected serum PFAS concentrations in relation to folate concentrations measured in both red blood cells and serum among adolescents and adults. The associations were stronger for folate measured in red blood cells and among adults. Dietary total folate intake was also inversely associated with PFAS concentrations. These results are supported by mechanistic in-vitro studies that show the potential of PFAS to compete with folate for several transporters implicated in PFAS toxicokinetics. Given the ubiquitous exposure, long half-lives, and health risks of PFAS, these findings might have important implications for interventions that aim to reduce the PFAS body burden. Further prospective and experimental studies are warranted to confirm or rule out a causal relationship between folate intake and PFAS concentrations, and to investigate related mechanisms.

Contributors

YZ contributed to the study conceptualisation, data curation, formal analysis, methodology, software, visualisation, writing the original draft, and reviewing and editing the manuscript. VM contributed to study conceptualisation, methodology, and reviewing and editing the manuscript. Y-XW contributed to methodology and reviewing and editing the manuscript. YS contributed to validating the data and reviewing and editing the manuscript. JA, ZB, NT, and YO contributed to reviewing and editing the manuscript. AS contributed to funding acquisition and reviewing and editing the manuscript. CM contributed to study conceptualisation, data curation, formal analysis, software, visualisation, funding acquisition, methodology, supervision, writing the original draft of the manuscript, and reviewing and editing the manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication. YZ and YS directly accessed and verified the underlying data reported in the manuscript.

Declaration of interests

We declare no competing interests.

Data sharing

Data and analytical code can be made available to others on request after publication. All requests should be made via email to the corresponding author.

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