ORIGINAL ARTICLE

# Perfluoroalkyl Substances and Abdominal Aortic Calcification

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AQ5 **Objective:** To evaluate if serum perfluoroalkylated substances (PFAS) were associated with abdominal aortic calcification (AAC). **Methods:** We used weighted logistic regression to investigate the gender-specific association between PFAS serum levels and AAC more than or equal to 6 from dual-energy X-ray absorptiometry (DXA) scans of the thoraco-lumbar spine from AQ6 NHANES 2013–2014 survey participants aged more than or equal to 40 years. **Results:** After adjusting for confounding, none of log-transformed perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), perfluorohexane sulfonic acid (PFHxS), or perfluorononanoic acid (PFNA) were significantly associated with AAC for either men or women (adjusted odds ratios [ORs] ranged from 0.80 to 1.33, $P > 0.05$ each). For PFOA and PFOS, the association was positive only in women (although the difference was not statistically significant in either case). **Conclusion:** These findings do not provide general support for a relationship of PFAS exposure to AAC, although the results show a need for gender-specific consideration in a larger dataset.

**Keywords:** aorta, aortic calcification, atherosclerosis, cardiovascular diseases, perfluoroalkyl substances

Per- and polyfluoroalkyl substances (PFAS) are a collective name for a wide range of anthropogenic fluorinated substances characterized by a hydrophobic alkyl chain of varying length, and a hydrophilic end group. They have been used extensively in various polymer and surfactant industries, and they are found in fire-fighting foams, consumer products including surface-coated clothing, food packaging and food contact materials, and personal cosmetics emulsions among many other applications.[1] Due to long, widespread use and strong persistence, a broad range of PFAS have been detected in the environment, wildlife, and humans. Multiple human exposure pathways have been described, including food, water, breast milk, and airborne dust.[2] "Long chain" PFAS such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have extended half-lives in humans, leading to bioconcentration with implications for long-term effects of chronic exposure.[3]

Multiple toxic effects of PFAS exposure have been observed both in vivo and in vitro. Cross-sectional and longitudinal evidence from studies of several populations shows that PFAS serum levels correlate with chronic risk factors for cardiovascular disease such as higher total and low density lipoprotein (LDL) cholesterol levels both in adults and children.[4–11] This detrimental lipid association extends to clinically defined high cholesterol.[12–14] Toxicologic evidence provides a number of explanations for the lipid associations, including the appearance of steatosis in animal species exposed to PFAS.[15]

Measurable levels of PFAS have also been found from many human tissues including blood, kidney, lungs, liver, brain, and bone, and we and others have earlier shown that PFAS accumulate in bone and interfere with calcium trafficking and bone homeostasis.[16–19] Interestingly, PFAS exposure has been linked to cardiovascular changes, such as enhanced carotid artery wall thickness due to increased calcification.[20,21] Abdominal aortic calcific deposits have been shown to be risk factors for incident cardiovascular disease and mortality, and provide direct, quantifiable insight into vascular status.[22,23] However, no studies on PFAS exposure and abdominal aortic calcification currently exist.

In this cross-sectional NHANES 2013–2014 study, we investigated the gender-specific associations between PFAS serum levels and abdominal aortic calcification (primary analysis) and if the magnitude of these associations differed between men and women (secondary analysis).

## METHODS

### Study Methods and Participants

NHANES 2013–2014 publicly available data were utilized for this study. Survey design details and methodology can be accessed on the NHANES website.[24] In summary, NHANES is an ongoing survey of the non-institutionalized US population that utilizes a stratified, multistage probability sampling protocol. After acquiring written informed consent, participants' physical assessment, examination, and laboratory measures were completed in a mobile examination center (MEC). In a random, one-third subsample of participants aged 12 years and above, PFAS serum analysis was completed at the National Center for Environmental Health. During 2013 to 2014, lateral dual-energy X-ray absorptiometry (DXA) scans of the thoraco-lumbar spine were administered in the MEC to eligible survey participants 40 years of age and older.

Abdominal aortic calcification scores (AAC) can be accurately assigned on lateral spine images obtained with dual-energy X-ray absorptiometry (DXA)[25] and provide a simple evaluation of subclinical vascular disease.[26] The lateral spine scans provide AAC measurement for vertebrae L1 to L4. Several studies have documented that lateral spine images obtained with DXA to detect vertebral fractures can capture AAC with good sensitivity and

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Conflicts of Interest: A.D. has been a paid consultant to three communities seeking class action relief for PFAS contamination (payers include Faraci Lange, Motley Rice, Langrock Sperry, and Cohen Milstein), and a volunteer consultant to community leaders concerned about PFAS contamination in multiple settings. In addition, A.D. has provided unpaid lectures or service concerning PFAS to the Michigan State Medical State Medical Society, the US Government Accounting Office, the Australian Land and Groundwater Association, a committee of the National Academies of Sciences, Engineering and Medicine, the Agency for Toxics Substances and Disease Control, and the Centers for Disease Control and Prevention.

Clinical Significance: Abdominal aortic calcification (AAC) is a risk factor for atherosclerosis in multiple vascular beds. PFAS exposure might contribute to the risk, which could be important knowledge for clinical decision-making and environmental health recommendations. This study provides at most equivocal support for an association between PFAS exposure and increased AAC.

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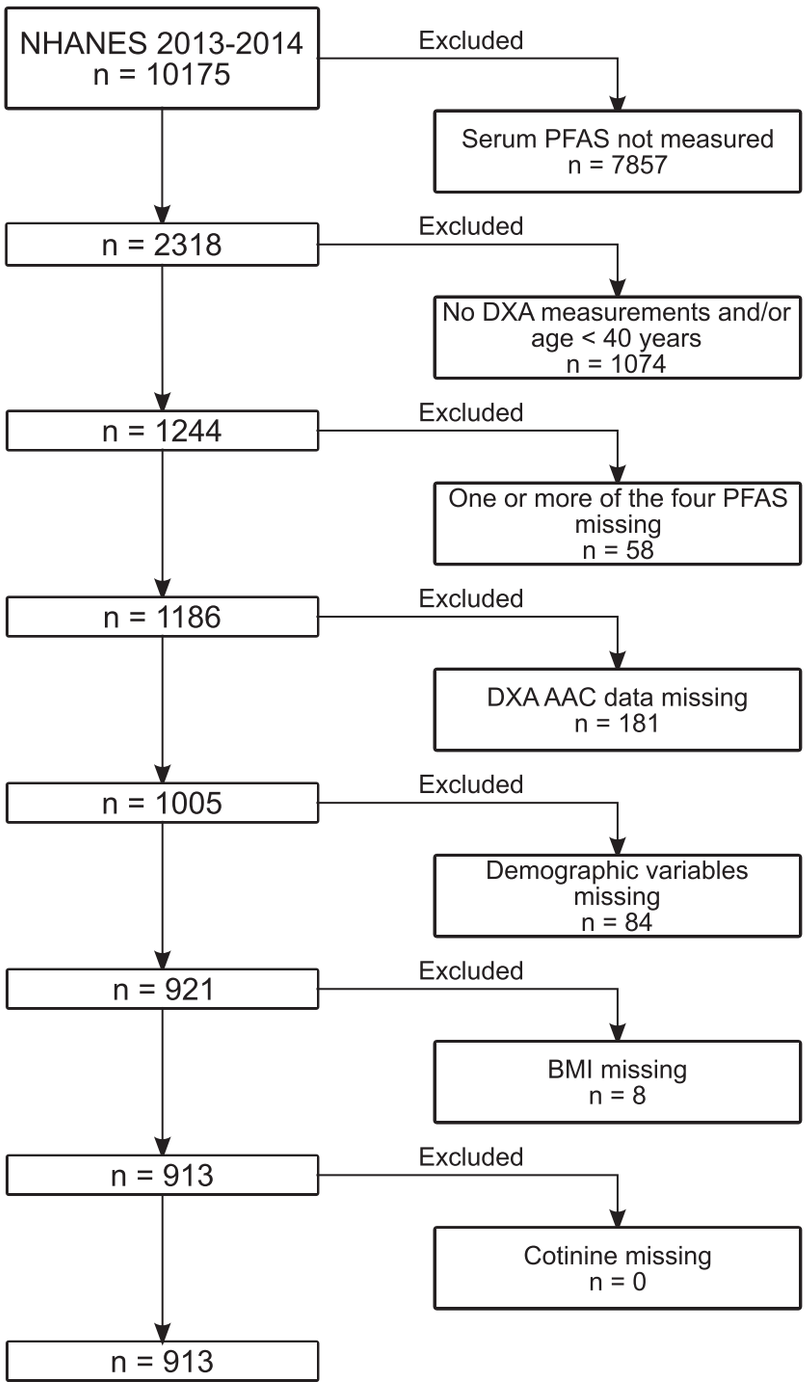


**FIGURE 1.** A flow chart depicting selection of the study population.

specificity.[27,28] The quality of image obtained with lateral spine scans using DXA is comparable to the image resolution of a radiograph, with much lower radiation dose with DXA scan compared with a standard radiograph exposure.[27] The present study comprised of 913 participants (424 men, 489 women) aged 40 to 80 years who had AAC data, serum concentrations of four PFAS (PFOA, PFOS, perfluorohexane sulfonic acid or PFHxS, and perfluorononanoic acid or PFNA) and complete information on covariates. Pregnant women (positive urine pregnancy test and/or self-report) were ineligible for the DXA examination. Participants were excluded (for reasons other than pregnancy) from the DXA scan if they had self-reported weight more than 450 pounds (DXA table limitation) or self-reported history of radiographic contrast material (barium) use in past 7 days, or were excluded from all spine scans if they reported a Harrington Rod in the spine for scoliosis. A study population selection flow chart is shown in Fig. 1.

## Dual X-Ray Absorptiometry (DXA) and Abdominal Aortic Calcification Data

The lateral thoraco-lumbar spine scans were obtained on Hologic Discovery model A densitometers (Hologic, Inc., Marlborough, MA), that used software version Apex 3.2. The DXA examinations were conducted by trained and certified radiology technologists. Further details of the DXA examination protocol are documented in the Body Composition Procedures Manual on Quality Assurance & Quality Control.[24] Rigorous quality control included daily phantom scanning schedule. Detailed information about the AAC assessment by DXA is available online https://wwwn.cdc.gov/Nchs/Nhanes/2013-2014/DXXAAC_H.htm.
Images were read by a single reader who was trained by Dr John Schousboe, at NHANES quality control center at the University of California, San Francisco (UCSF), Department of Radiology using standard radiologic techniques and NHANES protocols.

Semi-quantitative degrees of calcification scoring for AAC-24 were used.[26,27] The most common scoring system in use today is the 24-point semi-quantitative scoring method developed by Kauppila in the Framingham study.[26] Using this method, the anterior and posterior aortic walls are divided into four segments corresponding to the arterial areas in front of lumbar vertebrae L1 to L4. Within each of these eight segments, vascular calcification is visually scored as a diffuse white stippling of the aorta extending out to the aortic wall or as white diffuse linear calcification of the anterior and/or posterior vascular walls. Aortic calcification is scored as 0 if there is no calcification, and as 1 if one-third or less of the vascular wall in that segment is calcified, as 2 if more than one-third but less than or equal to two-thirds of the aortic wall is calcified, or as 3 if more than two-thirds of the aortic wall is calcified. Therefore, scores can range from 0 to 6 for each vertebral level, and the total score ranges from 0 to 24.

There is high reliability (inter-rater and intra-rater) of the AAC-24-point scale score on standard lateral spine radiographs, with reported intraclass correlation coefficients (ICC) of 0.90 or higher.[26] For the current study, participants' aortic calcification score was dichotomized as less than 6 (''low''; referent), and more than or equal to 6 (''high''). Cutoffs of 6 and 5 have been used interchangeably in earlier studies.[29]

## PFAS Assay

Detailed information about the analytical procedure used in NHANES for PFAS is available online. Serum measures of four PFAS (PFOA, PFOS, PFHxS, and PFNA), were included in this analysis. Briefly, online-solid phase extraction coupled to high performance liquid chromatography-turbo ion spray ionization-tandem mass spectrometry is used for the quantitative detection of PFAS including PFHxS, PFNA.[30] Following dilution with formic acid, one aliquot of 100 μL of serum is injected into a commercial column switching system allowing for concentration and chromatographic separation of the analytes.

During the NHANES 2013–2014 cycle, lab methods were modified to include both linear and branched isomers of PFOS and PFOA.[31] Therefore, concentrations of linear PFOA (n-PFOA), sum of branched isomers of PFOA (Sb-PFOA, branched PFOA isomers), linear PFOS (n-PFOS), and sum of perfluoromethylheptane sulfonate isomers (Sm-PFOS, monomethyl branched PFOS isomers) were measured in serum. n-PFOA, Sb-PFOA, n-PFOS, and Sm-PFOS were quantified with on-line solid phase extraction coupled to isotope dilution-high performance liquid chromatography tandem mass spectrometry.

To estimate the total (sum of the isomers measured) concentrations of PFOS and PFOA for each participant, the concentrations of n-PFOA and Sb-PFOA were summed to obtain total PFOA, n-PFOS and Sm-PFOS were summed for PFOS. The lower limit of detection (LLOD) for the four PFAS was 0.10 ng/mL. PFAS results below the LLOD were imputed with a value 0.07 derived by dividing lower limit of detection (0.10) by square root of 2 (1.41) (LLOD/sqrt (2); https://wwwn.cdc.gov/Nchs/Nhanes/2013-2014/PFAS_H.htm). Samples below LLOD for each PFAS compound are listed in Table S1, http://links.lww.com/JOM/B50.

## Covariates

Covariates selected a priori included age, race/ethnicity, gender, income, and smoking (serum cotinine) (Fig. 2).[21,32] Body mass index (BMI) was also considered, however due to the possible association of PFAS with lipid metabolism (in the causal pathway between PFAS and AAC), we did not adjust for BMI, a potential mediator. We did, however, carry out a sensitivity analysis in which BMI was included in the model, with results shown in the Supplemental material (the results were qualitatively similar: Table S2, http://links.lww.com/JOM/B51).

Selection of covariates was based on previous studies linking PFAS and cardiovascular diseases.[6,33] As compared with men, an increased intima-media thickness has been seen in women,[34] although this association has not been observed in all studies.[35,36] Racial and ethnic differences in aortic calcification have been documented in populations,[37] and socio-economic deprivation

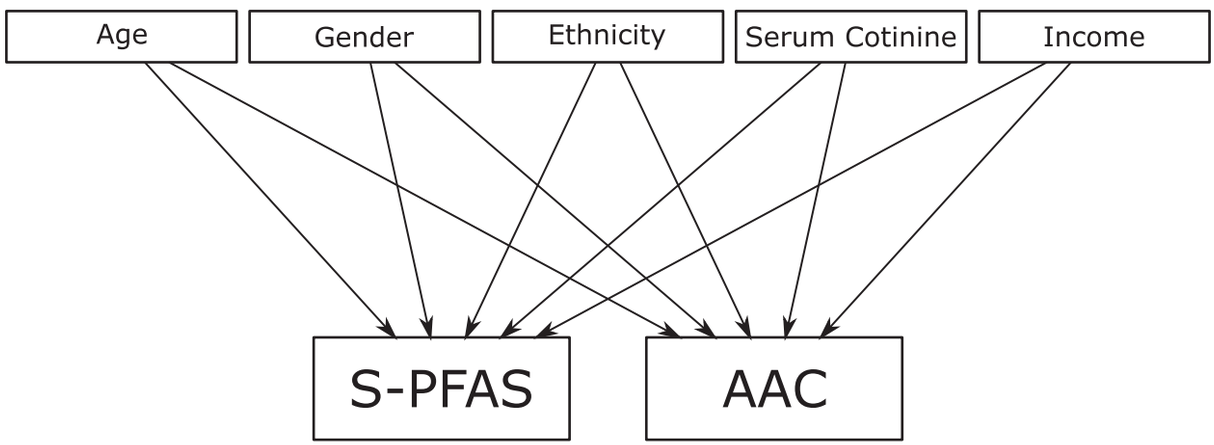


**FIGURE 2.** Previously observed relationships between study covariates, serum PFAS, and abdominal aortic calcification. AAC, abdominal aortic calcification; S-PFAS, serum PFAS.

 

has been associated with higher arterial calcification risk.[38] Sociodemographic information such as age, gender, race/ethnicity, and income, were self-reported and recorded using interviewer-administered questionnaires. Age was included as a continuous variable. Race/ethnicity was grouped as non-Hispanic White, non-Hispanic Black, Mexican American or Other Hispanic, and other race—including multi-racial. Annual household income was grouped as less than $25,000, $25,000 to less than $55,000, and $55,000+.

Body weight was measured to the nearest 0.01 kg using an electronic load cell scale and standing height was measured with a fixed stadiometer. BMI was calculated as body weight (kilograms) divided by height (meters squared) and categorized as underweight (less than 18.5 $kg/m^2$), normal (18.5 to less than 25 $kg/m^2$), overweight (25 to less than 30 $kg/m^2$), and obese (more than or equal to 30 $kg/m^2$). Serum cotinine levels less than 1.0 ng/mL were categorized as non-smoker, 1.0 to 9.9 ng/mL as environmental tobacco smoke (ETS) exposure, and more than or equal to 10.0 ng/mL as current smoker.[39,40] As there were only 21 individuals in the ETS group, during final analysis ETS and current smokers were analyzed as one group.

## Statistical Analysis

Population characteristics, outcomes, and exposures were summarized as survey weighted mean ± standard error (SE) or unweighted frequency and survey weighted proportion (%). Differences in characteristics between genders were tested using Student 2-tailed *t* test or Rao Scott Chi-square test as recommended by the National Center for Health Statistics (NCHS).[41] As decided a priori, analyses were conducted to examine relationships between PFAS and abdominal aortic calcification overall and stratified by gender.

Weighted unadjusted and adjusted binary logistic regression was used to assess the association between PFAS and the binary outcome AAC24 more than or equal to 6, including odds ratios (OR) and 95% confidence intervals. The exposure variables for our primary (confirmatory) analysis were the four PFAS congeners individually as continuous log transformed variables, separately for men and women. We used a Bonferroni correction to adjust for multiple testing over these eight tests. Secondary (exploratory) analyses included a summed PFAS total created to assess the combined effect of the four PFAS congeners as a continuous variable, the summed PFAS and each of the four congeners as categorical variables derived using gender-specific quartile (Q1: referent, lowest; Q4 highest) cut points, and comparisons of the magnitudes of associations between genders. Gender-specific analyses and the comparison between genders were done using a model that included gender interacted with all other variables.

Due to having non-normal distributions, continuous PFAS variables were natural log-transformed (Ln-PFAS) (each of the four individually, as well as the sum). Analyses were adjusted for age, gender, ethnicity, serum cotinine, and income.

We considered using ordinal logistic regression with cutoffs of 3 and 6 for AAC24. However, when comparing to separate binary logistic regressions using each cutoff, the adjusted ORs differed enough in magnitude to indicate a failure of the proportional odds assumption, although statistical significance was almost identical using either cutoff. Thus, our conclusions would be the same had we used 3 as the cutoff rather than 6. We also checked to see if our conclusions would change had we used a cutoff of 5 instead of 6 and they did not.

SAS PROC SURVEYLOGISTIC was used to account for the complex NHANES survey design. Sampling weights (subsample weights found in the NHANES PFAS dataset), strata, and primary sampling units were adjusted for in all analyses as recommended by SAS survey procedures (SAS Institute, Inc., version 9.4), applying the Taylor series linearization method for the calculation of SEs. All tests were two-tailed. The primary analyses, eight tests of gender-specific associations between each of four log-transformed individual congeners and AAC24, was carried out at the familywise 5% significance level. Although *P*-values are also reported for the secondary analyses, these should be considered as exploratory.

# RESULTS

## Characteristics of Study Population

Table 1 displays the weighted descriptive statistics overall and by gender. Mean age of this group was 57.4 (SE: 0.6) years; with no significant difference in mean age among male and female participants. Smoking status was comparable across genders, with about three-fourths of the population being non-smokers. The study population predominantly comprised of non-Hispanic White participants. Overall BMI distribution was mostly overweight and obese, but when compared by gender, women, and men showed different proportions of overweight and obesity. Although the majority of study participants overall had an annual household income of at least $55,000, the income distribution varied significantly across gender ($P < 0.001$).

## Aortic Calcification Scores and Serum PFAS Concentrations

Table 2 displays the weighted descriptive statistics by aortic calcification score (AAC24 less than 6 vs more than or equal to 6). High aortic calcification score (AAC24 more than or equal to 6) was detected in 9.8% of the population overall with comparable proportions across gender. Overall, the sample number and weighted proportion for each individual AAC-score were 0 = 631 (71.6%); 1 = 33 (3.6%); 2 = 53 (5.8%); 3 = 38 (3.7%); 4 = 41 (3.8%); 5 = 17 (1.6%); 6 = 17 (1.7%); and more than 6 = 83 (8.1%). Mean age was significantly higher in individuals with high aortic calcification score ($P < 0.001$). No significant difference across AAC categories was noted for gender, BMI, or smoking. The race distribution was significantly different across AAC categories, with NH White making up a larger proportion of the high AAC group ($P = 0.010$). Individuals in the high AAC group had a lower proportion of annual household income in the $55,000+ range. Individual and summed serum PFAS concentrations were significantly higher in the high AAC group ($P < 0.05$) for all but PFOA.

## Binary Logistic Regression Results

Unadjusted results are presented in supplementary material (Table S3, http://links.lww.com/JOM/B52) and adjusted results are shown in Table 3 and Fig. 3. After adjusting for confounding, none of log-transformed PFOA, PFOS, PFHxS, or PFNA were significantly associated with AAC24 more than or equal to 6 for either men or women (adjusted ORs ranged from 0.80 to 1.33, $P > 0.05$ each even before the Bonferroni correction). Similarly, no significant association was noted for the continuous Ln-summed PFAS variable, or any quartile variable, overall or by gender (secondary analyses).

In the secondary analysis comparing genders, the association between continuous PFAS and AAC was positive in women and negative in men for PFOA (adjusted OR = 1.15 vs 0.80), PFOS (adjusted OR = 1.33 vs 0.81), and summed PFAS (adjusted OR = 1.31 vs 0.86), but none of these differences were statistically significant (Fig. 1). When considering the quartile variables, PFOA ($P = 0.033$) and summed PFAS ($P = 0.001$) demonstrated a significant difference between genders. In both cases, for men, those in the 2nd quartile had the greatest odds of AAC24 more than or equal to 6, while for women those in the 4th quartile had the greatest odds.

# DISCUSSION

Long chain PFAS were not significantly associated with abdominal aortic calcifications after adjustment for age, gender,

 

AQ8 **TABLE 1.** Characteristics of 2013–2014 NHANES Participants, Distribution of Serum Perfluoroalkyl Substances, Overall, and by Sex (Mean, SE, and % Are Survey Weighted)

| | | Overall ($n = 913$) | | Male ($n = 424$, 48.0%) | | Female ($n = 489$, 52.0%) | | |
|---|---|---|---|---|---|---|---|---|
| **Characteristic** | **Level** | **No.** | **Mean ± SE or %** | **No.** | **Mean ± SE or %** | **No.** | **Mean ± SE or %** | ***P*-Value*** |
| Age, yr | | | 57.4 ± 0.6 | | 57.1 ± 0.8 | | 57.7 ± 0.7 | 0.471 |
| BMI, kg/m$^2$ | Underweight | 17 | 1.6 | 5 | 0.8 | 12 | 2.4 | <0.001 |
| | Normal | 239 | 24.4 | 99 | 19.0 | 140 | 29.3 | |
| | Overweight | 342 | 38.4 | 196 | 47.9 | 146 | 29.6 | |
| | Obese | 315 | 35.6 | 124 | 32.3 | 191 | 38.7 | |
| Smoking status† | ETS/Current smoker | 232 | 25.9 | 119 | 26.5 | 113 | 25.4 | 0.752 |
| | Non-smokers | 681 | 74.1 | 305 | 73.6 | 376 | 74.6 | |
| Race/ethnicity | NH White | 408 | 72.1 | 181 | 72.3 | 227 | 71.9 | 0.635 |
| | NH Black | 174 | 9.5 | 87 | 8.6 | 87 | 10.2 | |
| | Mex-Am-Hispanic | 208 | 11.2 | 103 | 12.1 | 105 | 10.5 | |
| | Other races | 123 | 7.2 | 53 | 6.9 | 70 | 7.4 | |
| Annual income | <$25,000 | 268 | 21.1 | 108 | 15.4 | 160 | 26.4 | <0.001 |
| | $25,000 to <$55,000 | 253 | 26.0 | 115 | 26.7 | 138 | 25.2 | |
| | $55,000+ | 392 | 52.9 | 201 | 57.9 | 191 | 48.4 | |
| AAC24 | Low score (<6) | 813 | 90.2 | 380 | 90.3 | 433 | 90.1 | 0.964 |
| | High score (≥6) | 100 | 9.8 | 44 | 9.7 | 56 | 9.9 | |
| PFOA, ng/mL‡ | | | 2.30 ± 0.11 | | 2.53 ± 0.08 | | 2.11 ± 0.15 | 0.015 |
| PFOS, ng/mL‡ | | | 6.25 ± 0.34 | | 7.90 ± 0.49 | | 5.04 ± 0.29 | <0.001 |
| PFHxS, ng/mL‡ | | | 1.61 ± 0.09 | | 2.06 ± 0.10 | | 1.29 ± 0.09 | <0.001 |
| PFNA, ng/mL‡ | | | 0.80 ± 0.04 | | 0.87 ± 0.05 | | 0.74 ± 0.03 | 0.006 |
| Sum_PFAS, ng/mL‡ | | | 11.9 ± 0.46 | | 14.2 ± 0.60 | | 10.1 ± 0.53 | <0.001 |

AQ9 *P-values for differences between males and females: continuous variables: weighted *t* test; categorical variables: Rao-Scott chi-square.
†Smoking categories based on serum cotinine concentration, ETS: environmental tobacco smoke.
‡Geometric mean and SE; *t* test performed on log transformed variables.

**TABLE 2.** Characteristics of 2013–2014 NHANES Participants, Distribution of Serum Perfluoroalkyl Substances, by Aortic Calcification Status AAC24 (Mean, SE, and % Are Survey Weighted)

| | | Aortic Calcification Score (AAC24), $n = 913$ | | | | |
|---|---|---|---|---|---|---|
| | | Low Score (<6) ($n = 813$, 90.2%) | | High Score (≥6) ($n = 100$, 9.8%) | | |
| **Characteristic** | **Level** | **No.** | **Mean ± SE or %** | **No.** | **Mean ± SE or %** | ***P*-Value*** |
| Gender | Male | 380 | 48.0 | 44 | 47.7 | 0.964 |
| | Female | 433 | 52.0 | 56 | 52.3 | |
| Age, yr | | | 55.8 ± 0.6 | | 71.9 ± 0.8 | <0.001 |
| BMI, kg/m$^2$ | Underweight | 14 | 1.5 | 3 | 2.4 | 0.334 |
| | Normal | 212 | 24.6 | 27 | 22.3 | |
| | Overweight | 299 | 37.4 | 43 | 47.1 | |
| | Obese | 288 | 36.4 | 27 | 28.2 | |
| Smoking status† | ETS/Current smoker | 208 | 26.3 | 24 | 22.3 | 0.552 |
| | Non-smokers | 605 | 73.7 | 76 | 77.7 | |
| Race/ethnicity | NH White | 342 | 70.9 | 66 | 82.7 | 0.010 |
| | NH Black | 158 | 9.6 | 16 | 7.9 | |
| | Mex-Am-Hispanic | 196 | 11.9 | 12 | 5.6 | |
| | Other races | 117 | 7.6 | 6 | 3.8 | |
| Income | <$25,000 | 232 | 20.4 | 36 | 28.0 | 0.063 |
| | $25,000 to <$55,000 | 221 | 25.4 | 32 | 31.5 | |
| | $55,000+ | 360 | 54.3 | 32 | 40.5 | |
| PFOA, ng/mL‡ | | | 2.27 ± 0.10 | | 2.59 ± 0.20 | 0.070 |
| PFOS, ng/mL‡ | | | 6.08 ± 0.32 | | 8.16 ± 0.96 | 0.011 |
| PFHxS, ng/mL‡ | | | 1.56 ± 0.10 | | 2.16 ± 0.22 | 0.018 |
| PFNA, ng/mL‡ | | | 0.78 ± 0.03 | | 0.96 ± 0.10 | 0.018 |
| Sum PFAS, ng/mL‡ | | | 11.6 ± 0.45 | | 15.1 ± 1.20 | 0.004 |

*P-values for differences between AAC24 binary categories (less than 6, more than or equal to 6): continuous variables: weighted *t* test; categorical variables: Rao-Scott chi-square.
†Smoking categories based on serum cotinine concentration, ETS: environmental tobacco smoke.
‡Geometric mean and SE; *t* test performed on log transformed variables.

**TABLE 3.** Adjusted Binary Logistic Regression Analysis of Perfluoroalkyl Substances and Aortic Calcification in NHANES 2013–2014 Participants (Survey Weighted) (OR: Odds Ratio) (PFAS = PFOA + PFOS + PFHxS + PFNA) (Shading = Primary Analysis)

| | | Overall ($N = 913$) | | Male ($N = 424$) | | Female ($N = 489$) | | M vs F |
|---|---|---|---|---|---|---|---|---|
| **Exposure** | **Level** | **Adjusted* OR (95% CI)** | ***P*‡** | **Adjusted† OR (95% CI)** | ***P*‡** | **Adjusted† OR (95% CI)** | ***P*‡** | ***P*** |
| Ln-PFOA | | 1.00 (0.71, 1.40) | 0.990 | 0.80 (0.48, 1.33) | 0.386 | 1.15 (0.62, 2.15) | 0.656 | 0.422 |
| PFOA quartiles | Q1 (ref) | | 0.902 | | 0.496 | | 0.203 | 0.033 |
| | Q2 | 1.21 (0.37, 3.92) | 0.750 | 2.66 (0.27, 26.4) | 0.403 | 0.47 (0.16, 1.34) | 0.156 | |
| | Q3 | 1.05 (0.52, 2.14) | 0.893 | 1.65 (0.34, 8.17) | 0.537 | 0.60 (0.20, 1.78) | 0.359 | |
| | Q4 | 1.27 (0.64, 2.49) | 0.495 | 1.05 (0.21, 5.15) | 0.956 | 1.31 (0.53, 3.25) | 0.563 | |
| Ln-PFOS | | 1.03 (0.81, 1.30) | 0.837 | 0.81 (0.49, 1.34) | 0.420 | 1.33 (0.78, 2.26) | 0.297 | 0.268 |
| PFOS quartiles | Q1 (ref) | | 0.544 | | 0.149 | | 0.654 | 0.546 |
| | Q2 | 1.31 (0.62, 2.80) | 0.483 | 1.18 (0.41, 3.41) | 0.761 | 1.35 (0.33, 5.46) | 0.677 | |
| | Q3 | 0.84 (0.43, 1.66) | 0.624 | 0.40 (0.14, 1.20) | 0.103 | 1.58 (0.45, 5.52) | 0.475 | |
| | Q4 | 1.35 (0.56, 3.28) | 0.504 | 0.75 (0.20, 2.85) | 0.675 | 2.43 (0.60, 9.92) | 0.215 | |
| Ln-PFHxS | | 1.12 (0.74, 1.69) | 0.597 | 1.18 (0.65, 2.13) | 0.593 | 1.06 (0.65, 1.73) | 0.832 | 0.745 |
| PFHxS Quartiles | Q1 (ref) | | 0.097 | | 0.557 | | 0.291 | 0.836 |
| | Q2 | 0.63 (0.22, 1.80) | 0.383 | 0.72 (0.25, 2.01) | 0.526 | 0.44 (0.10, 1.89) | 0.269 | |
| | Q3 | 0.98 (0.30, 3.23) | 0.972 | 1.21 (0.22, 6.79) | 0.825 | 0.61 (0.19, 1.93) | 0.400 | |
| | Q4 | 1.33 (0.43, 4.13) | 0.624 | 1.53 (0.38, 6.18) | 0.552 | 0.93 (0.23, 3.73) | 0.914 | |
| Ln-PFNA | | 1.32 (0.87, 2.00) | 0.190 | 1.31 (0.47, 3.69) | 0.606 | 1.31 (0.71, 2.44) | 0.391 | 0.999 |
| PFNA quartiles | Q1 (ref) | | 0.743 | | 0.642 | | 0.104 | 0.151 |
| | Q2 | 1.04 (0.32, 3.39) | 0.944 | 0.70 (0.13, 3.82) | 0.675 | 1.54 (0.34, 7.02) | 0.578 | |
| | Q3 | 1.20 (0.50, 2.87) | 0.681 | 0.43 (0.10, 1.92) | 0.271 | 2.67 (0.84, 8.48) | 0.097 | |
| | Q4 | 1.47 (0.52, 4.14) | 0.469 | 0.85 (0.19, 3.84) | 0.828 | 2.49 (0.51, 12.2) | 0.260 | |
| Ln-PFAS | | 1.04 (0.78, 1.39) | 0.772 | 0.86 (0.49, 1.50) | 0.585 | 1.31 (0.69, 2.49) | 0.410 | 0.418 |
| PFAS quartiles | Q1 (ref) | | 0.067 | | 0.058 | | 0.315 | 0.001 |
| | Q2 | 1.42 (0.61, 3.31) | 0.412 | 2.54 (0.95, 6.77) | 0.062 | 0.48 (0.12, 1.90) | 0.295 | |
| | Q3 | 0.89 (0.35, 2.27) | 0.811 | 0.61 (0.22, 1.71) | 0.346 | 1.16 (0.30, 4.42) | 0.829 | |
| | Q4 | 1.77 (0.86, 3.62) | 0.120 | 1.52 (0.45, 5.09) | 0.499 | 2.03 (0.51, 8.09) | 0.315 | |

*Adjusted for age, gender, ethnicity, serum cotinine, and income.
†Adjusted for age, ethnicity, serum cotinine, and income.
‡*P*-values in the referent row are 3 df *P*-values testing the overall association for each quartile variable.

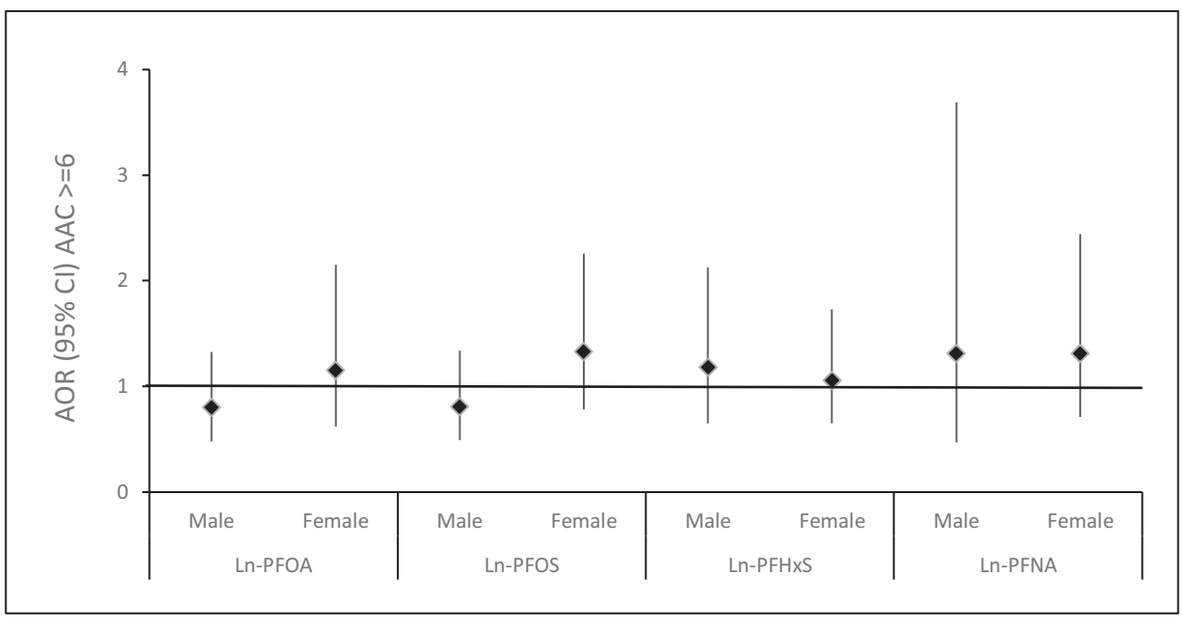


**FIGURE 3.** Adjusted odds ratios (AOR) and 95% confidence intervals (95% CI) of aortic calcification score more than or equal to 6 and serum perfluoroalkyl substances in NHANES 2013–2014 participants.

race/ethnicity, smoking status, and SES. For PFOA, PFOS, and summed PFAS, associations were only positive among women, although these differences between genders were not statistically significant. The observed associations do not endorse a relationship between PFAS exposure and AAC. The positive trend in women could be due to chance or unmeasured confounding and, in an initial paper on this topic, they are more useful for hypothesis generation than hypothesis testing. Alternatively, the absence of clearer statistical associations for the quartile comparisons may be due to the limited population size with calcification scores more than or equal to 6, only 100 participants with scores more than or equal to 6, compared with 813 participants with scores less than 6 (Table 1).

Serum biomarkers or models of PFAS exposure have been associated with disruption of lipid metabolism in multiple cross-sectional, and longitudinal studies of human populations.[4–14,42] Independent of PFAS exposure, it is understood that an elevation of blood cholesterol levels is associated with increased risk of abdominal aortic calcifications.[43] Fewer studies address PFAS and hypertension, which is a stronger risk factor for atherosclerotic disease. Recent cross-sectional work in NHANES-enrolled adolescents, and in a separate young adult prediabetics with substantial PFAS exposure, suggest a possible relationship of PFAS to this atherosclerotic risk factor as well.[12,44,45] Our results do not support a current addition of AAC to the list of associated risk factors. The presence of a weak, non-significant association in a planned gender comparison opens the question of whether an association would be seen in a larger sample size. In contrast, serum PFOS concentration has been associated with direct ultrasound measures of carotid artery intima thickness in young adults.[21] Associations of PFAS exposure to carotid intima thickness have also been detected in longitudinal studies.[34] Endothelial cell damage has been suggested as a precipitating physiology for the association of carotid intima thickness to PFAS exposure.[46] Concerning the specific topic of PFAS and aortic calcification, our findings are mostly nil.

Aortic calcification is a risk factor for atherosclerotic disease. High level of AAC is a risk factor for incident cardiovascular disease and mortality.[22,23,25,47] PFAS exposure induces oxidative stress.[48,49] Circulating biomarkers of thrombosis are elevated in plasma of mice exposed to PFAS mixtures.[50] In humans from the high exposure areas around Veneto Italy, biomarkers of PFAS exposure were associated with altered platelet membrane fluidity and increased platelet aggregation.[51] However, these indications of the biologic plausibility of increased risk of atherosclerosis following PFAS exposure does not mean it is consistently identified in human studies.

PFAS exposure has been associated with cardiovascular disease as previously been reported from NHANES data[6,33] but findings are not consistent. The actual incidence of coronary artery disease is not necessarily increased in longitudinal studies of representative populations based on death or hospital admissions records.[35]

An inherent and important limitation of this work is that NHANES data are cross-sectional. Based on the long (more than 2 years) half-lives[52,53] of long chain PFAS measured in this study, the contemporaneous measure of serum PFAS and aortic calcification scores implies information that is influenced by years of exposure, but that is still a short time period compared with our current understanding of the decades long development of vascular disease in humans. An additional potential limitation is the use of a binary outcome variable. We chose to use a categorical version of AAC24 due to the large number of zero values (69% of the sample). Based on these limitations and long exposure times for PFAS, similar research in a high exposure population is an important need.

## REFERENCES

1. EFSA Panel on Contaminants in the Food Chain (EFSA CONTAM Panel), Schrenk D, Bignami M, et al. Risk to human health related to the presence of perfluoroalkyl substances in food. *Efsa J*. 2020;18:e06223.
2. Buck RC, Franklin J, Berger U, et al. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. *Integr Environ Assess Manag*. 2011;7:513–541.
3. Chang ET, Adami H, Boffetta P, Wedner HJ, Mandel JS. A critical review of perfluorooctanoate and perfluorooctanesulfonate exposure and immunological health conditions in humans. *Crit Rev Toxicol*. 2016;46:279–331.
4. Lind PM, Lind L. Are persistent organic pollutants linked to lipid abnormalities, atherosclerosis and cardiovascular disease? A review. *J Lipid Atheroscler*. 2020;9:334–348.
5. Jensen RC, Andersen MS, Larsen PV, et al. Prenatal exposures to perfluoroalkyl acids and associations with markers of adiposity and plasma lipids in infancy: an odense child cohort study. *Environ Health Perspect*. 2020;128:77001.
6. Shankar A, Xiao J, Ducatman A. Perfluorooctanoic acid and cardiovascular disease in US adults. *Arch Intern Med*. 2012;172:1397–1403.
7. Frisbee SJ, Shankar A, Knox SS, et al. Perfluorooctanoic acid, perfluorooctanesulfonate, and serum lipids in children and adolescents: results from the C8 health project. *Arch Pediatr Adolesc Med*. 2010;164:860–869.
8. Fu Y, Wang T, Fu Q, Wang P, Lu Y. Associations between serum concentrations of perfluoroalkyl acids and serum lipid levels in a chinese population. *Ecotoxicol Environ Saf*. 2014;106:246–252.
9. Eriksen KT, Raaschou-Nielsen O, McLaughlin JK, et al. Association between plasma PFOA and PFOS levels and total cholesterol in a middle-aged danish population. *PLoS ONE*. 2013;8:e56969.
10. Seo S, Son M, Choi S, Lee D, Chang Y. Influence of exposure to perfluoroalkyl substances (PFASs) on the korean general population: 10-year trend and health effects. *Environ Int*. 2018;113:149–161.
11. Blomberg AJ, Shih Y, Messerlian C, Jørgensen LH, Weihe P, Grandjean P. Early-life associations between per- and polyfluoroalkyl substances and serum lipids in a longitudinal birth cohort. *Environ Res*. 2021;200:111400.
12. Lin PD, Cardenas A, Hauser R, et al. Per- and polyfluoroalkyl substances and blood lipid levels in pre-diabetic adults-longitudinal analysis of the diabetes prevention program outcomes study. *Environ Int*. 2019;129:343–353.
13. Steenland K, Tinker S, Frisbee S, Ducatman A, Vaccarino V. Association of perfluorooctanoic acid and perfluorooctane sulfonate with serum lipids among adults living near a chemical plant. *Am J Epidemiol*. 2009;170:1268–1278.
14. Winquist A, Steenland K. Modeled PFOA exposure and coronary artery disease, hypertension, and high cholesterol in community and worker cohorts. *Environ Health Perspect*. 2014;122:1299–1305.
15. Blake BE, Fenton SE. Early life exposure to per- and polyfluoroalkyl substances (PFAS) and latent health outcomes: a review including the placenta as a target tissue and possible driver of peri- and postnatal effects. *Toxicology*. 2020;443:152565.
16. Koskela A, Koponen J, Lehenkari P, Viluksela M, Korkalainen M, Tuukkanen J. Perfluoroalkyl substances in human bone: concentrations in bones and effects on bone cell differentiation. *Sci Rep*. 2017;7:6841.
17. Khalil N, Ebert JR, Honda M, et al. Perfluoroalkyl substances, bone density, and cardio-metabolic risk factors in obese 8–12 year old children: a pilot study. *Environ Res*. 2018;160:314–321.
18. Di Nisio A, Rocca MS, De Toni L, et al. Endocrine disruption of vitamin D activity by perfluoro-octanoic acid (PFOA). *Sci Rep*. 2020;10:16789.
19. Perez F, Nadal M, Navarro-Ortega A, et al. Accumulation of perfluoroalkyl substances in human tissues. *Environ Int*. 2013;59:354–362.
20. Lind PM, Salihovic S, van Bavel B, Lind L. Circulating levels of perfluoroalkyl substances (PFASs) and carotid artery atherosclerosis. *Environ Res*. 2017;152:157–164.
21. Lin CY, Lin LY, Wen TW, et al. Association between levels of serum perfluorooctane sulfate and carotid artery intima-media thickness in adolescents and young adults. *Int J Cardiol*. 2013;168:3309–3316.
22. Criqui MH, Denenberg JO, McClelland RL, et al. Abdominal aortic calcium, coronary artery calcium, and cardiovascular morbidity and mortality in the multi-ethnic study of atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2014;34:1574–1579.
23. Wilson PW, Kauppila LI, O'Donnell CJ, et al. Abdominal aortic calcific deposits are an important predictor of vascular morbidity and mortality. *Circulation*. 2001;103:1529–1534.
24. CDC. National Center for Health Statistics. National Health and Nutrition Examination Survey: Questionnaires, Datasets, and Related Documentation;

 

2020. Available at: https://Wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2013. Accessed December 17, 2020.

25. Schousboe JT, Lewis JR, Kiel DP. Abdominal aortic calcification on dual-energy X-ray absorptiometry: methods of assessment and clinical significance. *Bone*. 2017;104:91–100.
26. Kauppila LI, Polak JF, Cupples LA, Hannan MT, Kiel DP, Wilson PWF. New indices to classify location, severity and progression of calcific lesions in the abdominal aorta: a 25-year follow-up study. *Atherosclerosis*. 1997;132:245–250.
27. Schousboe JT, Wilson KE, Kiel DP. Detection of abdominal aortic calcification with lateral spine imaging using DXA. *J Clin Densitom*. 2006;9:302–308.
28. Bazzocchi A, Ciccarese F, Diano D, et al. Dual-energy X-ray absorptiometry in the evaluation of abdominal aortic calcifications. *J Clin Densitom*. 2012;15:198–204.
29. Leow K, Szulc P, Schousboe JT, et al. Prognostic value of abdominal aortic calcification: a systematic review and meta-analysis of observational studies. *J Am Heart Assoc*. 2021;10:e017205.
30. Kuklenyik Z, Needham LL, Calafat AM. Measurement of 18 perfluorinated organic acids and amides in human serum using on-line solid-phase extraction. *Anal Chem*. 2005;77:6085–6091.
31. Benskin JP, De Silva AO, Martin JW. Isomer profiling of perfluorinated substances as a tool for source tracking: a review of early findings and future applications. *Rev Environ Contam Toxicol*. 2010;208:111–160.
32. Khalil N, Chen A, Lee M, et al. Association of perfluoroalkyl substances, bone mineral density, and osteoporosis in the U.S. population in NHANES 2009–2010. *Environ Health Perspect*. 2016;124:81–87.
33. Huang M, Jiao J, Zhuang P, Chen X, Wang J, Zhang Y. Serum polyfluoroalkyl chemicals are associated with risk of cardiovascular diseases in national US population. *Environ Int*. 2018;119:37–46.
34. Lind PM, Salihovic S, Stubleski J, Kärrman A, Lind L. Changes in plasma levels of perfluoroalkyl substances (PFASs) are related to increase in carotid intima-media thickness over 10 years - a longitudinal study. *Environ Health*. 2018;17:59.
35. Mattsson K, Rignell-Hydbom A, Holmberg S, et al. Levels of perfluoroalkyl substances and risk of coronary heart disease: findings from a population-based longitudinal study. *Environ Res*. 2015;142:148–154.
36. Hutcheson R, Innes K, Conway B. Perfluoroalkyl substances and likelihood of stroke in persons with and without diabetes. *Diab Vasc Dis Res*. 2020;17:1479164119892223.
37. Allison MA, Budoff MJ, Nasir K, et al. Ethnic-specific risks for atherosclerotic calcification of the thoracic and abdominal aorta (from the multi-ethnic study of atherosclerosis). *Am J Cardiol*. 2009;104:812–817.
38. Kim D, Diez Roux AV, Kiefe CI, Kawachi I, Liu K. Do neighborhood socioeconomic deprivation and low social cohesion predict coronary calcification?: the CARDIA study. *Am J Epidemiol*. 2010;172:288–298.
39. Hukkanen J, Jacob P, Benowitz NL. Metabolism and disposition kinetics of nicotine. *Pharmacol Rev*. 2005;57:79–115.
40. CDC (Centers for Disease Control and Prevention). Fourth Report on Human Exposure to Environmental Chemicals, 2009. Atlanta, GA: U.S. Department Of Health And Human Services, Centers For Disease Control And Prevention; 2019. Available at: https://Www.cdc.gov/exposurereport/. Accessed December 23, 2020.
41. NCHS (National Center for Health Statistics) NHANES tutorials; 2020. Available at: Https://Wwwn.cdc.gov/nchs/nhanes/tutorials/default.aspx. Accessed December 23, 2020.
42. Fenton SE, Ducatman A, Boobis A, et al. Per- and polyfluoroalkyl substance toxicity and human health review: current state of knowledge and strategies for informing future research. *Environ Toxicol Chem*. 2021;40:606–630.
43. Barascuk N, Ganz M, Nielsen M, et al. Abdominal aortic calcification quantified by the morphological atherosclerotic calcification distribution (MACD) index is associated with features of the metabolic syndrome. *BMC Cardiovasc Disord*. 2011;11:75.
44. Ma S, Xu C, Ma J, et al. Association between perfluoroalkyl substance concentrations and blood pressure in adolescents. *Environ Pollut*. 2019;254:112971.
45. Pitter G, Zare Jeddi M, Barbieri G, et al. Perfluoroalkyl substances are associated with elevated blood pressure and hypertension in highly exposed young adults. *Environ Health*. 2020;19:102.
46. Lin CY, Chen PC, Lo SC, Torng PL, Sung FC, Su TC. The association of carotid intima-media thickness with serum level of perfluorinated chemicals and endothelium-platelet microparticles in adolescents and young adults. *Environ Int*. 2016;94:292–299.
47. Bolland MJ, Wang TKM, van Pelt NC, et al. Abdominal aortic calcification on vertebral morphometry images predicts incident myocardial infarction. *J Bone Miner Res*. 2010;25:505–512.
48. Omoike OE, Pack RP, Mamudu HM, et al. Association between per and polyfluoroalkyl substances and markers of inflammation and oxidative stress. *Environ Res*. 2021;196:110361.
49. Qian Y, Ducatman A, Ward R, et al. Perfluorooctane sulfonate (PFOS) induces reactive oxygen species (ROS) production in human microvascular endothelial cells: role in endothelial permeability. *J Toxicol Environ Health A*. 2010;73:819–836.
50. Deng P, Wang C, Wahlang B, Sexton T, Morris AJ, Hennig B. Co-exposure to PCB126 and PFOS increases biomarkers associated with cardiovascular disease risk and liver injury in mice. *Toxicol Appl Pharmacol*. 2020;409:115301.
51. De Toni L, Radu CM, Sabovic I, et al. Increased cardiovascular risk associated with chemical sensitivity to perfluoro-octanoic acid: role of impaired platelet aggregation. *Int J Mol Sci*. 2020;21:0108.
52. Li Y, Fletcher T, Mucs D, et al. Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water. *Occup Environ Med*. 2018;75:46–51.
53. Xu Y, Fletcher T, Pineda D, et al. Serum half-lives for short- and long-chain perfluoroalkyl acids after ceasing exposure from drinking water contaminated by firefighting foam. *Environ Health Perspect*. 2020;128:77004.

 

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