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Per- and polyfluoroalkyl substances and calcifications of the coronary and aortic arteries in adults with prediabetes: Results from the diabetes prevention program outcomes study

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C.O.-Y. and M.S.-G. wrote the manuscript and performed statistical analyses. A.C., P.D.L., R.H., D.R.G., K.P.K., M-F.H., A.F.F., A.M.C., T.F. W., E.S.H., and E.O. provided critical input in the analyses and writing of the manuscript and were involved in planning the study.

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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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2021.106446>.

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Abstract

Background: Per- and polyfluoroalkyl substances (PFAS) are endocrine disrupting chemicals that have been associated with cardiovascular risk factors including elevated body weight and hypercholesterolemia. Therefore, PFAS may contribute to the development of atherosclerosis and cardiovascular disease (CVD). However, no previous study has evaluated associations between PFAS exposure and arterial calcification.

Methods and results: This study used data from 666 prediabetic adults enrolled in the Diabetes Prevention Program trial who had six PFAS quantified in plasma at baseline and two years after randomization, as well as measurements of coronary artery calcium (CAC) and ascending (AsAC) and descending (DAC) thoracic aortic calcification 13–14 years after baseline. We performed multinomial regression to test associations between PFAS and CAC categorized according to Agatston score [low (<10), moderate (11–400) and severe (>400)]. We used logistic regression to assess associations between PFAS and presence of AsAC and DAC. We adjusted models for baseline sex, age, BMI, race/ethnicity, cigarette smoking, education, treatment assignment (placebo or lifestyle intervention), and statin use. PFAS concentrations were similar to national means; 53.9% of participants had CAC > 11, 7.7% had AsAC, and 42.6% had DAC. Each doubling of the mean sum of plasma concentrations of linear and branched isomers of perfluorooctane sulfonic acid (PFOS) was associated with 1.49-fold greater odds (95% CI: 1.01, 2.21) of severe versus low CAC. This association was driven mainly by the linear (n-PFOS) isomer [1.54 (95% CI: 1.05, 2.25) greater odds of severe versus low CAC]. Each doubling of mean plasma N-ethyl-perfluorooctane sulfonamido acetic acid concentration was associated with greater odds of CAC in a dose-dependent manner [OR = 1.26 (95% CI: 1.08, 1.47) for moderate CAC and OR = 1.37 (95% CI: 1.07, 1.74) for severe CAC, compared to low CAC]. Mean plasma PFOS and n-PFOS were also associated with greater odds of AsAC [OR = 1.67 (95% CI: 1.10, 2.54) and OR = 1.70 (95% CI: 1.13, 2.56), respectively], but not DAC. Other PFAS were not associated with outcomes.

Conclusions: Prediabetic adults with higher plasma concentrations of select PFAS had higher risk of coronary and thoracic aorta calcification. PFAS exposure may be a risk factor for adverse cardiovascular health among high-risk populations.

Keywords

PFAS; Coronary artery calcium; Ascending thoracic aortic calcification; Descending thoracic aortic calcification; DPP; DPPOS

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are synthetic compounds commonly used in commercial applications and consumer products, such as stain-resistant and nonstick coatings, food packaging, and fire-fighting foams (Lindstrom et al., 2011). PFAS are durably persistent in the environment and some can also accumulate in wildlife and humans, earning them the nickname “forever chemicals.” For example, the half-life in humans has been estimated to range from 3.5 years for perfluorooctanoic acid (PFOA) and 7.3 years for perfluorohexane sulfonic acid (PFHxS) (Olsen et al., 2007; Stableski et al., 2017). The persistence and widespread use of some PFAS has resulted in ubiquitous human exposure exemplified by detectable PFAS serum concentrations in virtually all of the U.S. population (>97%) (Kato et al., 2011). The general population is exposed to PFAS primarily through diet (Lin et al., 2020), indoor environment (e.g., building materials, industrial fabrics, house dust) (de la Torre et al., 2019; Fraser et al., 2013), and contaminated drinking water (Hu et al., 2016; Hu et al., 2019).

Previous studies from our group have shown associations between PFAS exposure and atherosclerosis risk factors such as hyperlipidemia, adiposity, and diabetes (Lin et al., 2019; Cardenas et al., 2018; Cardenas et al., 2017). The underlying mechanism that can explain an association of PFAS exposure with atherosclerosis risk also include inflammation (Liao et al., 2012), reactive oxygen species generation (Qian et al., 2010), endothelial dysfunction (Lin et al., 2016) and PPAR-gamma inactivation (Wen et al., 2016). However, few studies have investigated the extent to which PFAS are related to the vascular process of atherosclerosis (Lin et al., 2016; Lin et al., 2013; Lind et al., 2018; Lind et al., 2017). Carotid intima media thickness (cIMT) and coronary artery calcification (CAC) (Zeb and Budoff, 2015) are noninvasive measures of atherosclerosis and are associated, independent of conventional risk factors, with future incidence of cardiovascular disease (CVD) such as coronary heart disease (CHD) and stroke (Zaid et al., 2017). The epidemiologic evidence, although scarce, suggests an association between PFAS exposure and greater cIMT. For instance, perfluorooctane sulfonic acid (PFOS) was significantly associated with cIMT in a cohort of young adults from Taiwan (Lin et al., 2013). Similarly, perfluoroundecanoic acid (PFUnDA) was associated with cIMT and carotid plaques in women, but not in men, from Uppsala Sweden (Lind et al., 2017).

CAC may be a better cardiovascular risk predictor than cIMT (Zaid et al., 2017) or Framingham Risk Score (Zeb and Budoff, 2015; Hecht and Narula, 2012). Calcium is deposited in the aortic wall as part of the development of atherosclerotic plaques by a process histologically similar to bone formation (Budoff et al., 2011). CAC is commonly quantified using Agastston scores, which summarize the degree and extent of calcium contained in the four major coronary arteries (Burge et al., 2016). The standard CAC scan also includes parts of the ascending and descending aorta, which can be analyzed for the presence of calcifications in the entire thoracic aorta (TAC), as well as ascending (AsAC) and descending aorta calcifications (DAC). Aortic calcifications contribute to arterial stiffness and cardiovascular risk (Budoff et al., 2011).

To our knowledge, no previous study has examined associations between PFAS exposure and major vascular calcifications. Therefore, we aimed to test the associations between plasma PFAS concentrations and coronary and aortic calcifications among individuals from the Diabetes Prevention Program (DPP) who had prediabetes at baseline and may be more susceptible to the cardiovascular effects associated with PFAS exposure. We hypothesized that plasma PFAS would be positively associated with risk of CAC and TAC.

2. Methods

2.1. Study population

2.1.1. Diabetes Prevention Program (DPP) and DPP outcomes study

(DPPOS)—The DPP study has been extensively described elsewhere (Cardenas et al., 2017; Program, 1999). Briefly, the DPP trial included individuals at high risk of developing type 2 diabetes from 27 clinical centers across the United States ($n = 3234$). Participants were recruited between July 1996 and May 1999 and were randomly assigned to 1 of 3 interventions: metformin 850 mg twice daily; a placebo medication twice daily; or a program of lifestyle modification with intensive training in diet, physical activity, and behavior modification. Inclusion criteria were age ≥ 25 years, body mass index (BMI) ≥ 24 kg/m² (≥ 22 kg/m² in Asian Americans), fasting plasma glucose levels between 95 and 125 mg/dL, and impaired glucose tolerance (2-hours after oral glucose load of 140–199 mg/dL) (Knowler et al., 2002). At the end of DPP, all surviving consented participants ($n = 3149$) of the 3 original DPP treatment arms were invited to participate in the Diabetes Prevention Program Outcome Study (DPPOS), regardless of diabetes status, and 2776 participants accepted (Diabetes Prevention Program Research, 2015; Goldberg et al., 2017).

For our studies of PFAS exposures with cardiovascular risk, we restricted eligibility to individuals in the lifestyle modification or the medication placebo control arms with available stored plasma samples collected at baseline and at two years after randomization. We did not include the metformin-treated group due to unknown interactions between PFAS and the pharmacological intervention on health outcomes and lack of experimental data (Cardenas et al., 2017). 957 participants had stored plasma samples available for quantification of PFAS. The present study included 666 participants who also had coronary artery calcification measurements 13 to 14 years after baseline randomization (Fig. 1).

The institutional review board at each clinical center approved the protocol, and all participants provided written informed consent for DPP/DPPOS. For the current analyses, the Institutional Review Board of Harvard Pilgrim Health Care reviewed and approved all study protocols. The involvement of the Centers for Disease Control and Prevention (CDC) laboratory did not constitute engagement in human subjects' research (Cardenas et al., 2017).

2.2. Coronary and aortic artery calcifications

At the 10th annual follow-up visit of the DPPOS study, DPPOS investigators measured CAC as well as descending and ascending aorta - calcification. Computed tomography was performed by certified technologists using prospectively ECG-triggered scan acquisition at

50% of the R-R interval with a multidetector system, acquiring a block of four 2.5-mm slices for each cardiac cycle in a sequential or axial scan mode. Participants were scanned twice, and the CAC measurement was calibrated against a phantom of known physical calcium concentration. A radiologist or cardiologist read all computed tomography scans at the central reading center (Los Angeles Biomedical Research Institute at Harbor-UCLA, Torrance, CA) in a manner blinded to patient characteristics and treatment assignment. Discrepancies were reviewed, and agreement was obtained through consensus. For each scan, a total phantom-adjusted averaged CAC Agatston score (Agatston et al., 1990) was calculated, defined as the sum of calcium measures from the left main, left anterior descending, circumflex, and right coronary arteries (Goldberg et al., 2017).

CAC Agatston score is interpreted as: 0 indicating the absence of calcified plaque, 1 to 10 minimal plaque, 11–100 mild plaque, 101–400 moderate plaque, and >400 severe plaque (Hecht, 2015). Because of the low number of participants with minimal plaque ($n = 57$), we categorized CAC status into three groups: a reference group (absence or minimal plaque, $n = 307$), a moderate group (mild and moderate plaque, $n = 255$), and a severe group (severe plaque, $n = 104$).

Ascending and descending thoracic aortic calcification (AsAC and DAC, respectively) were measured from the aortic annulus to the lower edge of the pulmonary artery (AsAC) and from the lower edge of the pulmonary artery to the cardiac apex (DAC) and were quantified by using the same lesion definition for coronary calcification. We categorized AsAC into two categories: participants with Agatston score of 0 ($n = 610$) and participants with detectable Agatston score ($n = 51$). For DAC, 372 individuals had Agatston score of 0 and 276 with detectable Agatston scores. The main analysis examined CAC Agatston score as the outcome; and evaluated TAC (including AsAC and DAC) as secondary analyses. We used the mean score of the two scans for all analyses.

2.3. Quantification of PFAS in plasma

Blood was collected at baseline and at the 2nd annual visit during DPP, and plasma was stored at the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) central repository. We quantified PFAS in the plasma using solid-phase extraction followed by high-performance liquid chromatography coupled to isotope dilution-tandem mass spectrometry at the CDC (Kato et al., 2011). We included PFAS with >80% detection in all analyses: linear PFOS (n-PFOS); sum of perfluoromethylheptane sulfonic acid isomers (Sm-PFOS); linear PFOA (n-PFOA); sum of perfluoromethylheptanoic and perfluorodimethylhexanoic acids (Sb-PFOA); PFHxS, N-ethyl-perfluorooctane sulfonamido acetic acid (EtFOSAA); N-methyl-perfluorooctane sulfonamido acetic acid (MeFOSAA); and perfluorononanoic acid (PFNA). The limit of detection (LOD) was 0.1 ng/mL for all PFAS. We imputed concentrations <LOD with LOD/ (2) (Hornung and Reed, 1990). We calculated total PFOS (PFOS = n-PFOS + Sm-PFOS) and total PFOA (PFOA = n-PFOA + Sb-PFOA) summing concentrations of branch and linear isomers, and we imputed concentrations <LOD before summation, as has been done previously (Cardenas et al., 2017; CDC, 2019).

2.4. Covariates

Baseline participants' characteristics such as age, sex, race/ethnicity, treatment assignment, education, income, marital status, alcohol intake, and cigarette smoking were assessed by research staff employing structured questionnaires. Also at baseline, we obtained total daily energy intake during the previous 12 months via a validated semiquantitative food frequency questionnaire as previously described (Lin et al., 2020). Systolic and diastolic BP was measured semiannually by study staff according to standard protocols (Diabetes Prevention Program Outcomes Study Research et al., 2013). Additional baseline characteristics considered included BMI, triglycerides and non-HDL cholesterol. Fasting triglycerides and total plasma cholesterol were determined enzymatically; HDL cholesterol was analyzed using dextran sulfate-magnesium precipitating procedure. Non-HDL cholesterol was calculated subtracting HDL cholesterol from total cholesterol levels (Lin et al., 2019).

2.5. Statistical methods

We calculated median and interquartile ranges for all PFAS and CAC. Because the distributions of all PFAS were right-skewed, we log (base 2)-transformed them to approximate normality. We examined correlations between PFAS concentrations using Spearman correlations coefficients.

We fitted multinomial logistic regression models to estimate associations between plasma PFAS concentrations and CAC categories. In addition to modeling CAC in three categories, we tested CAC as continuous (log CAC + 1) using Tobit regression models, a method previously reported to account for the value of zero (Ma et al., 2010). We also examined associations between PFAS and presence or absence of CAC (>0 or = 0, respectively). Few participants had AsAC greater than zero (n = 51), so we used a binary outcome variable and employed logistic regression models. To be consistent with AsAC, DAC was modeled as binary in logistic regression models. Each PFAS was evaluated as independent model.

We selected potential confounders *a priori* based on published literature and by drawing a directed acyclic graph (DAG, Supplemental Fig. 1). The final list of confounders included in all models were sex, age group (categorical), BMI (continuous), race/ethnicity (categorical), education (categorical), cigarette smoking (categorical), treatment assignment and statin use (Lind et al., 2017; Erbel et al., 2014; Kalsch et al., 2017; Kronmal et al., 2007; Kiramijyan et al., 2013). PFAS were not included as covariates in the models.

We considered other potential baseline confounders including total energy intake and alcohol intake. However, they did not significantly change the parameter estimates, thus we removed them for model parsimony. We did not include biochemical markers of cholesterol or inflammation as covariates because they may act as mediators in the causal pathway between PFAS and CAC. Finally, we did not adjust for multiple testing because PFAS concentrations were related and not independent.

We performed several sensitivity analyses, 1) multinomial, logistic and Tobit regression models were further adjusted for diet, C-reactive protein, alcohol intake and lipid levels; 2) we tested effect modification by sex and treatment arm, by adding a multiplicative interaction term of PFAS and sex or treatment in the models; we considered evidence for

effect modification at $P_{\text{interaction}} < 0.15$; 3) we ran multinomial models without adjustment for BMI or statin use to assess whether or not we observed a change in our estimates ($>10\%$); 4) finally, we performed quantile-based g-computation (Keil et al., 2020) to estimate the associations of the average plasma concentrations of baseline and year 2 measures of the 6 PFAS as a mixture on the odds of CAC using the gqcomp R package. As the package had not been adopted for performing multinomial logistic regression, we modeled CAC outcome as different sets of binary variables: moderate /high vs low, moderate vs low, and high vs low. We presented the joint association for each of the outcomes with overall model confidence bounds estimated by 10,000 bootstraps. The adjusted models controlled for sex, age group, BMI, race/ethnicity, education, cigarette smoking, treatment assignment and statin use.

We present 95% Confidence Intervals (95% CI) and estimates for all tests to evaluate our hypotheses. All analyses were performed using R version 3.3.0 (Vienna, Austria) and SAS Studio 3.6 (SAS Institute, Cary NC).

3. Results

3.1. Characteristics of the study population

The characteristics of the included DPP study participants at baseline and CAC measurements at the 10th annual follow-up of DPPOS ($N = 666$) are shown in Table 1. Most participants were female (65.6%), Caucasian (53.7%), 40 years or older (89.5%) and 66.5% had obesity. Almost half of the participants reported college as the highest level of education (49.9%). 5% of the participants currently smoked and 12.3% reported an annual household income lower than USD\$20,000. CAC Agatston score ranged from 0 to 3519 and median (IQR) for CAC Agatston score was 18.30 (185). We found variation in the distribution of CAC categories across participants' characteristics in this population. Namely, the percentage of severe CAC was higher in men than women (35.8% vs. 5%, respectively) and higher in Caucasians (19.0%) compared to African American (9.2%) or Hispanic of any race (9.8%). CAC increased with increasing age ($r_{\text{spearman}} = 0.43$; $P\text{-Value} < 0.001$) and was inversely correlated with BMI ($r_{\text{spearman}} = -0.23$; $P\text{-Value} < 0.0001$) (data not shown). Among those with severe CAC, 51% ($n = 53$) had obesity, while only 3.5% ($n = 4$) had normal weight. Finally, the percentage of severe CAC was higher in current smokers than never smokers (Table 1).

3.2. PFAS concentrations

Our group has previously reported that plasma PFOS and PFOA concentrations at baseline differed by sex, race and education, but not according to BMI, cigarette smoking and treatment assignment (Cardenas et al., 2017). We evaluated plasma PFAS concentrations at baseline and at the 2nd annual visit of DPP. Because of the relatively long half-lives and relative stability of plasma concentrations across two years, we employed mean plasma PFAS concentration at baseline and two years as our main exposure variable (Table 2). Mean plasma PFAS concentrations were positively correlated (Fig. 2).

3.3. Associations between PFAS and coronary artery Calcification

In unadjusted analyses, several PFAS (PFOS, n-PFOS, Sm-PFOS, PFOA, n-PFOA, PFHxS and PFNA) were associated with higher odds of severe CAC, compared to low CAC (Supplemental Table 1). However, after adjustment for potential confounders, only PFOS and n-PFOS remained associated (Table 2). Each doubling of mean plasma PFOS was associated with higher odds (OR = 1.49; 95% CI: 1.01–2.21) of severe CAC compared to low CAC (Table 2). The association between PFOS and severe CAC was driven mainly by the n-PFOS isomer. Namely, each doubling of mean n-PFOS was associated with higher odds (OR = 1.54; 95% CI: 1.05, 2.25) of severe CAC compared to low CAC (Table 2).

Unadjusted analyses showed no associations between EtFOSAA and CAC (Supplemental Table 1). However, after adjustment for potential confounders, mean plasma EtFOSAA concentration was associated with higher CAC risk in a dose–response manner. Each doubling of EtFOSAA was associated with greater odds (OR = 1.26; 95% CI: 1.08, 1.47) of moderate CAC and greater odds (OR = 1.37; 95% CI: 1.07, 1.74) of severe CAC, compared to low CAC (Table 2).

Among all PFAS, only EtFOSAA was associated with CAC when employing logistic and Tobit regression models. This finding is consistent with the dose–response association between EtFOSAA and CAC in multinomial models. After adjusting for potential confounders, each doubling of mean plasma EtFOSAA was associated with higher odds of CAC [(CAC > 0 vs. CAC = 0; OR = 1.16; 95% CI: 1.0, 1.36). Results from Tobit regression models showed that EtFOSAA at baseline (beta: 0.22 and 95% CI: 0.050–0.40), two-years (beta: 0.16 and 95% CI: 0.01, 0.31) and mean (beta: 0.27 and 95% CI: 0.07–0.46) were associated with greater CAC measurements (Supplemental Fig. 2).

3.4. Associations between PFAS and thoracic aortic Calcification

Agatston scores for AsAC ranged from 0 to 11483, and for DAC ranged from 0 to 509. In unadjusted models we found significant associations between PFOS, n-PFOS, Sm-PFOS and PFNA and higher odds of AsAC (Supplemental Table 2). However, after adjustment for potential confounders, only PFOS and n-PFOS remained associated (Fig. 3A). Each doubling of mean plasma PFOS was associated with higher odds of AsAC (OR = 1.67; 95% CI: 1.10–2.54), and this was driven mainly by n-PFOS (AsAC OR = 1.70; 95% CI: 1.13–2.56 per doubling of n-PFOS) (Fig. 3A). In unadjusted models, PFHxS and PFNA were associated with higher odds of DAC (vs no DAC), but the association was no longer present after adjustment for potential confounders (Supplemental Table 2, Fig. 3B). We found no significant associations of EtFOSAA with AsAC or DAC. We found no effect modification by sex or study arm for AsAC or DAC.

3.5. Sensitivity analyses

Additional adjustment for diet, C-reactive protein, alcohol intake and lipid levels in multinomial and logistic models resulted in no appreciable change in estimates (<10% changes in regression coefficients). Moreover, we found no effect modification by sex or study arm for the association between PFAS and CAC or PFAS and TAC. Multinomial models for PFAS and CAC without adjustment for BMI or statin use yielded similar

results than those models with BMI and statin use adjustment. Regarding quantile g-based computation, each quantile increases in the plasma concentrations of the 6 PFAS as a mixture was associated with 31% (95% CI: 5%, 63%) higher odds of having moderate or high CAC compared to low CAC. However, after adjusting for covariates, the effect diminished to 6% and the 95% CI cross the null suggesting no statistically significant effect. We observed similar finding comparing high vs low CAC; the model comparing moderate CAC vs low CAC did not showed statistically significant finding before and after adjustment for covariates (Table 3).

4. Discussion

In this study of adults at high risk of type 2 diabetes, we observed that greater plasma concentrations of PFOS and EtFOSAA were associated with higher odds of severe CAC up to 14 years later. Additionally, we found positive associations with PFOS and n-PFOS and AsAC, but not with DAC. These findings suggest that PFOS and EtFOSAA may be risk factors for atherosclerosis in adults.

So far as we are aware, our study is the first to assess associations between plasma PFAS concentrations and CAC and TAC scores. Compared with previous studies on other atherosclerosis outcomes, we showed consistent positive relationship between certain PFAS and atherosclerosis risk markers. For example, Lin and colleagues (Lin et al., 2013) reported in a cross-sectional study that adolescents and young adults with higher plasma PFOS had higher cIMT (0.434 mm, 0.446 mm, 0.458 mm, 0.451 mm across PFOS quartiles; P for trend <0.001). A study among elderly participants showed positive associations between PFUnDA and carotid plaque in women (OR 1.59, 95% CI: 1.03–2.43), but not in men (OR 0.93, 95% CI: 0.62–1.42) (Lind et al., 2017). Both studies reported sex-specific effects, stronger in women (Lin et al., 2013; Lind et al., 2017), suggesting PFAS may act through mechanisms that disrupt sex hormones (Lin et al., 2013; Lind et al., 2017; Zhou et al., 2017). Even though we observed higher prevalence of severe CAC in men than women in our study population, our results showed no effect modification by sex for associations with CAC. These differences between our findings and previous studies might be due to CAC vs. cIMT measurements, atherosclerosis stage (adolescents, elderly and adults), genetic background, diet, among others.

The mechanisms by which PFAS may increase atherosclerosis risk are not well understood. Previous experimental studies showed that PFOS exposure could induce inflammatory responses (Liao et al., 2012), trigger reactive oxygen species generation (Qian et al., 2010; Hu and Hu, 2009; Sun et al., 2018), increase endothelial cells' permeability (Qian et al., 2010; Wang et al., 2011), and enhance the ability of endothelial cell to express adhesion molecules such as ICAM-1 (intercellular adhesion molecule-1) (Liao et al., 2012). Activated endothelial cells that express adhesion molecules (sICAM-1, VCAM-1, E-selectine) are able to attract circulating monocytes toward the atherosclerotic lesion and worsen atherosclerosis (Marchio et al., 2019). Epidemiologic studies have also shown that PFOS can lead to endothelial dysfunction (Lin et al., 2013), which predispose to atherosclerotic plaque formation (Mahdi et al., 2019). In fact, a recent study demonstrated that the association between PFOS and cIMT increase was accompanied with an increase in endothelial

dysfunction biomarkers in young adults from Taiwan (Lin et al., 2013). Another molecular target of PFOS relevant to atherosclerosis is the peroxisome proliferator activated receptor gamma (PPAR-gamma) (Ivanova et al., 2017; Xu et al., 2016). PPAR-gamma activation is a pharmacological target to reduce blood pressure and atherosclerosis risk (Ivanova et al., 2017). The activation of PPAR-gamma was observed in one cell line study (Xu et al., 2016) but not in others (Wen et al., 2016; Takacs and Abbott, 2007). In their study, Xu and colleagues (Xu et al., 2016) demonstrated that PFOS administration in mice increased adipogenesis thorough Nrf2 (Nuclear factor [erythroid-derived 2]-like 2) and PPAR gamma activation. More studies are needed to clarify the role of PPAR-gamma in the association between PFOS and atherosclerosis risk.

PFAS may also increase atherosclerosis risk by modifying the sensitivity to risk factors for atherosclerosis such as hyperlipidemia, elevated blood pressure, diabetes and adiposity. Previous studies have demonstrated associations between PFOS concentrations and blood pressure increase in adolescents (Ma et al., 2019) and Chinese adults (Bao et al., 2017). Moreover, PFOS concentrations have been associated with higher lipid levels (Frisbee et al., 2010; Steenland et al., 2009), uric acid increase (Steenland et al., 2010) and insulin resistance (Cardenas et al., 2017; Lin et al., 2009). All these factors are implicated in atherosclerosis pathophysiology (Marchio et al., 2019). We previously reported that participants in DPP/DPPOS with higher plasma PFOS had greater development of adiposity over time (Cardenas et al., 2018) and higher risks of incident hypercholesterolemia and hypertriglyceridemia (Lin et al., 2019) in the placebo group but not in the lifestyle intervention group. However, we did not detect associations between plasma PFOS and hypertension risk in a previous analysis using the same study population (Lin et al., 2020). Therefore, it is plausible that higher PFOS concentrations increased atherosclerosis risk by increasing blood lipid concentrations and adiposity. However, additional research is needed to confirm our hypothesis.

The associations between EtFOSAA and atherosclerosis risk factors are scarce. Our group previously showed in this population that each doubling in EtFOSAA was associated with 17% greater odds of prevalent microvascular disease (Cardenas et al., 2019).

CAC burden has been strongly and independently associated with increased incidence of CHD and CVD events beyond traditional risk factors (Detrano et al., 2008; Gibson et al., 2014), and it is part of American Heart Association and American College of Cardiology clinical cardiovascular risk guidelines (Dzaye et al., 2019). However, there is increasing interest in imaging techniques targeting other vascular beds such as the thoracic aorta. While coronary calcification occurs within the intimal layer of the coronary arteries and is almost exclusively associated with atherosclerosis, calcification of the aortic wall can occur both in the intima and medial layers and it is associated with increased arterial stiffness (Kim et al., 2017). We are aware of only one report showing associations between PFAS serum concentrations and arterial stiffness. Koshy et al. 2017 observed associations of PFOS (unit change and 95% CI: 0.30 [−0.01, 0.62]; P-Value = 0.06) and PFOA (unit change and 95% CI: 0.45 [0.04, 0.87]; P-Value = 0.03) with brachial artery distensibility (BrachD), a rapid method of accurately assessing the relative stiffness of a peripheral artery (Koshy et al., 2017). Since 2002 there is an effort to reduce production and emission of PFAS in the US,

moreover other PFAS manufacturers committed to reduce production and emission of PFOA and its precursors by 2015 (Cardenas et al., 2017). Although, PFAS exposure has decreased along the time, PFAS also have long elimination half-lives in humans in the range of 3.5 to 8.5 y (Olsen et al., 2007). It is possible that the associations observed in this study would be a reflect of the constant and chronic exposure. Another possibility, it is that PFAS could have a long-term effect on coronary and artery calcification, however the study design cannot allow us to identify susceptibility windows. Future studies should be conducted to assess the associations of PFAS exposure with vascular calcification or arterial stiffness.

Our results should be interpreted in light of our study's strengths and limitations. The strengths are the prospective study design that allowed us to confirm the chronological sequence of PFAS concentrations and outcome relationships prospectively across many years. PFAS are correlated, therefore we did not include all of the PFAS in the same model so the issue of correlated exposure (confounding by co-exposure) is discarded (Weisskopf et al., 2018). Moreover, we evaluated the association of 6 PFAS as a mixture on the odds of CAC and we did not find significant results. Our study population was diverse with over 40% of the study population from racial/ethnic minorities. We were able to control for important confounding factors such as diet, medication use, alcohol intake and cigarette smoking; however, we cannot exclude residual confounding (e.g., familial hypercholesterolemia) due to unmeasured variables. The present study included 666 participants with available data on plasma PFAS and coronary artery calcification. It would be reasonable to assume that the missingness of data information was independent of PFAS levels. Therefore, selection bias attributable to informative missingness is unlikely. Another limitation of our study is the lack of repeated CAC measurements to assess plaque progression, so future research would benefit from studies on development of plaque progression and atherosclerosis-related disorders, such as myocardial infarction and stroke. Also, our results might not be representative of the general population because DPP participants were adults with prediabetes recruited for a diabetes prevention trial. Another limitation is the lack of measurement of other endocrine disrupting chemicals and we did not adjust our results for multiple comparisons. However, we based our conclusions on estimation of associations and our findings were in the direction expected.

5. Conclusion

In summary, our findings suggest that PFOS, n-PFOS and Et-FOSAA are associated with coronary and aortic calcifications over 10–14 years follow-up among individuals at high risk for diabetes. Exposures to PFAS are ubiquitous and may be an environmental risk factor for CVD events among high-risk populations. Overall, we did not observe a statistically significant joint association between the PFAS mixture with CAC after adjusting for covariates. Further studies are warranted to confirm this association, identify the underlying mechanisms and test the generalizability of findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

| | |
|----------------|--|
| PFAS | Per- and polyfluoroalkyl substances |
| CAC | coronary artery calcium |
| AsAC | ascending thoracic aortic calcification |
| DAC | descending thoracic aortic calcification |
| PFOS | perfluorooctane sulfonic acid (sum of linear and branched isomers) |
| n-PFOS | linear perfluorooctane sulfonic acid |
| Sm-PFOS | perfluoromethylheptane sulfonic acids |
| PFOA | perfluorooctanoic acid (sum of linear and branched isomers) |
| n-PFOA | linear perfluorooctanoic acid |
| Sb-PFOA | branched perfluorooctanoic acids |
| PFHxS | perfluorohexane sulfonic acid |
| EtFOSAA | N-ethyl-perfluorooctane sulfonamido acetic acid |
| MeFOSAA | N-methyl-perfluorooctane sulfonamido acetic acid |
| PFNA | perfluorononanoic acid |
| DPPOS | Diabetes Prevention Program Outcomes Study |
| BMI | body mass index |

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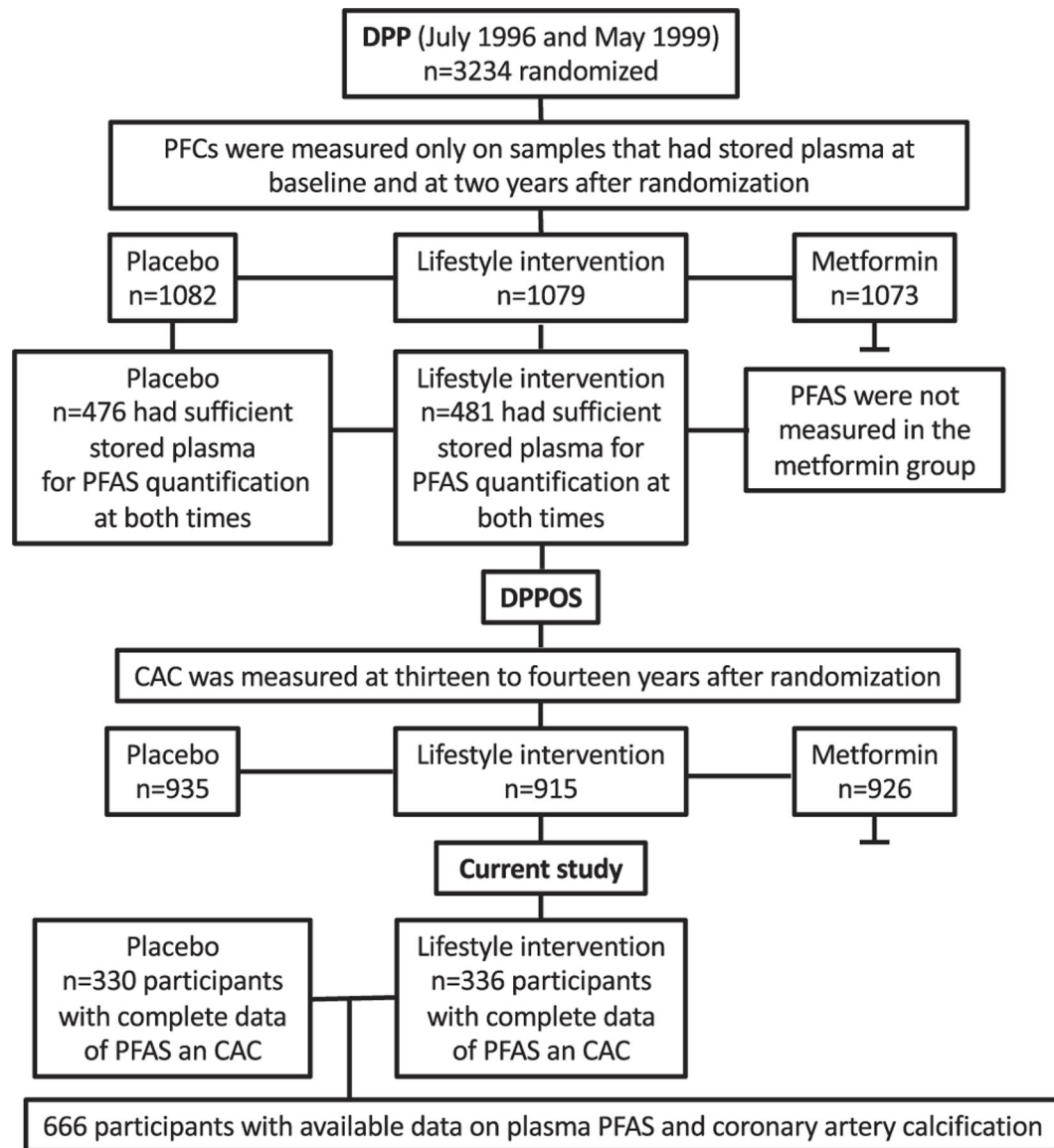
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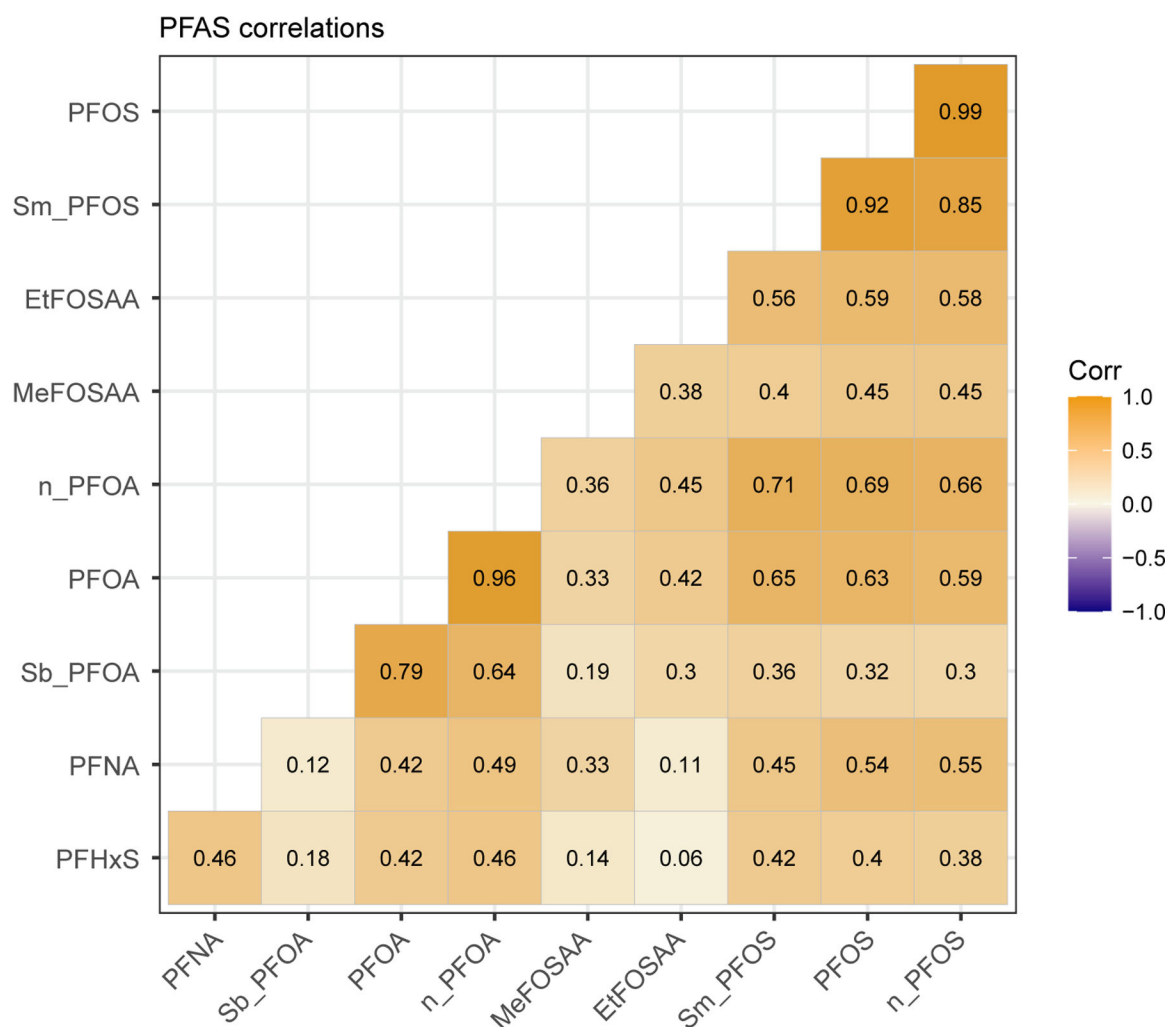
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**Fig. 1.**

Study flowchart of participants with available data for PFAS and CAC. Per- and polyfluoroalkyl substances (PFAS); coronary artery calcium (CAC); Diabetes Prevention Program (DPP); Diabetes Prevention Program Outcome Study (DPPOS).

**Fig. 2.**

Spearman correlation coefficients for plasma PFAS mean concentrations; PFOS: perfluorooctane sulfonic acid (sum of linear and branched isomers); n-PFOS: linear perfluorooctane sulfonic acid; Sm-PFOS: perfluoromethylheptane sulfonic acids; PFOA: perfluorooctanoic acid (sum of linear and branched isomers); n-PFOA: linear perfluorooctanoic acid; Sb-PFOA: branched perfluorooctanoic acids; PFHxS: perfluorohexane sulfonic acid; EtFOSAA: N-ethyl-perfluorooctane sulfonamido acetic acid; MeFOSAA: N-methyl-perfluorooctane sulfonamido acetic acid; PFNA: perfluorononanoic acid.

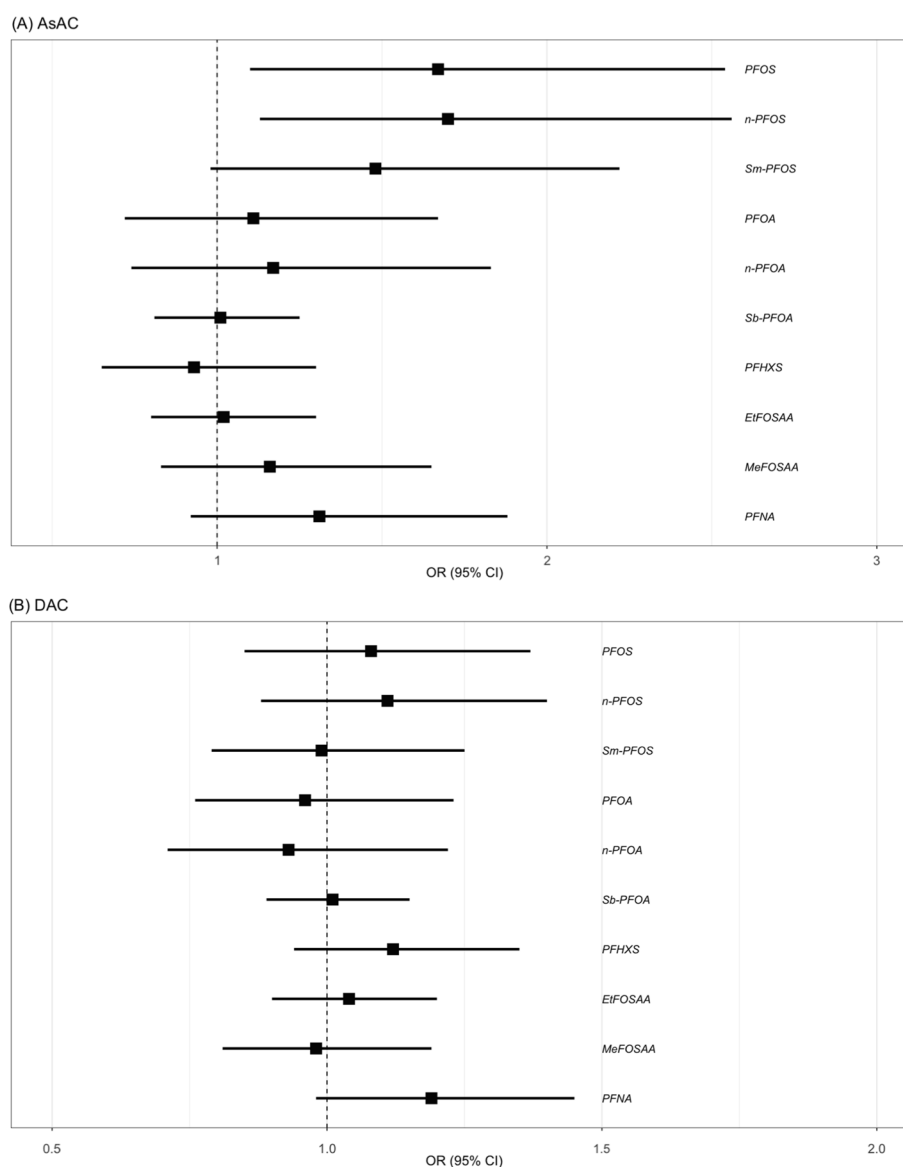


Fig. 3. Forest plot for logistic regression models to assess mean plasma PFAS and risk of AsAC or DAC. All PFAS were log2 transformed; AsAC: ascending aortic calcification; DAC: descending aortic calcification; For (A) AsAC = 0 was used as reference (N = 610); For (B) DAC = 0 was used as reference (N = 372); values represent Odds Ratio (OR) and 95% Confidence Interval (95% CI). All models were adjusted for sex, age, body mass index, race/ethnicity, cigarette smoking, education, treatment assignment and statin use. For abbreviations of PFAS, see Table 2.

Table 1

Baseline characteristics of participants with PFAS and coronary artery calcification measurements from the Diabetes Prevention Program and Outcome Study (DPP/DPPOS) (N = 666).

| Characteristics (N = 666) | All participants | Low CAC (0–10) | Medium CAC (11–400) | Severe CAC (>400) | P-value |
|--|------------------|----------------|---------------------|-------------------|---------|
| Overall | 666 (100) | 307 (46.1) | 255 (38.3) | 104 (15.6) | |
| Participant sex | | | | | |
| Male | 229 (34.4%) | 55 (24.0%) | 92 (40.2%) | 82 (35.8%) | <0.0001 |
| Female | 437 (65.6%) | 252 (57.7%) | 163 (37.3%) | 22 (5.0%) | |
| Treatment arm | | | | | |
| Lifestyle intervention | 336 (50.5%) | 167 (49.7%) | 120 (35.7%) | 49 (14.6%) | 0.17 |
| Placebo | 330 (49.5%) | 140 (42.4%) | 135 (40.9%) | 55 (16.7%) | |
| Race/ethnicity | | | | | |
| Caucasian | 358 (53.7%) | 152 (42.5%) | 138 (38.5%) | 68 (19.0%) | 0.006 |
| African American | 142 (21.3%) | 75 (52.8%) | 54 (38.0%) | 13 (9.2%) | |
| Hispanic of any race | 133 (20.0%) | 66 (49.6%) | 54 (40.6%) | 13 (9.8%) | |
| All other | 33 (5.0%) | 14 (42.4%) | 9 (27.3%) | 10 (30.3%) | |
| Age (years) | | | | | |
| <40 | 70 (10.5%) | 54 (77.1%) | 15 (21.4%) | 1 (1.4%) | <0.0001 |
| 40–44 | 79 (11.9%) | 52 (65.8%) | 23 (29.1%) | 4 (5.1%) | |
| 45–49 | 158 (23.7%) | 93 (58.9%) | 59 (37.3%) | 6 (3.8%) | |
| 50–54 | 125 (18.8%) | 49 (39.2%) | 58 (46.4%) | 18 (14.4%) | |
| 55–59 | 100 (15%) | 35 (35.0%) | 42 (42.0%) | 23 (23.0%) | |
| 60–64 | 71 (10.7%) | 16 (22.5%) | 41 (57.7%) | 14 (19.7%) | |
| 65 | 63 (9.4%) | 8 (12.7%) | 17 (27.0%) | 38 (60.3%) | |
| BMI classification (kg/m²) | | | | | |
| Normal (18.5 – 24.9) | 19 (2.9%) | 7 (36.8%) | 8 (42.1%) | 4 (21.1%) | 0.007 |
| Overweight (25.0 – 29.9) | 204 (30.6%) | 83 (40.7%) | 74 (36.3%) | 47 (23.0%) | |
| Obesity (>30.0) | 443 (66.5%) | 217 (49.0%) | 173 (39.0%) | 53 (12.0%) | |
| Education | | | | | |
| <High school | 31 (4.6%) | 14 (45.2%) | 14 (45.2%) | 3 (9.6%) | 0.08 |
| High school/GED | 125 (18.8%) | 63 (50.4%) | 42 (33.6%) | 20 (16.0%) | |

| Characteristics (N = 666) | All participants | Low CAC (0–10) | Medium CAC (11–400) | Severe CAC (>400) | P-value |
|---------------------------------------|------------------|----------------|---------------------|-------------------|---------|
| College | 332 (49.9%) | 163 (49.1%) | 126 (38.0%) | 43 (12.9%) | |
| Graduate school | 178 (26.7%) | 67 (37.6%) | 73 (41.0%) | 38 (21.3%) | |
| Cigarette Smoking [†] | | | | | |
| Never smoker | 392 (59.0%) | 211 (53.8%) | 140 (35.7%) | 41 (10.5%) | <0.0001 |
| Former smoker | 240 (36.0%) | 88 (36.7%) | 97 (40.4%) | 55 (22.9%) | |
| Current smoker | 34 (5.0%) | 8 (23.5%) | 18 (52.9%) | 8 (23.5%) | |
| Alcohol consumption | | | | | |
| Nondrinker | 347 (52.8%) | 180 (51.9%) | 126 (36.3%) | 41 (11.8%) | <0.0001 |
| <1 drink/week | 109 (16.6%) | 52 (47.7%) | 47 (43.1%) | 10 (9.2%) | |
| 1 drink/week | 201 (30.6%) | 67 (33.3%) | 81 (40.3%) | 53 (26.4%) | |
| Annual Household Income | | | | | |
| <\$20,000 | 82 (12.3%) | 35 (42.7%) | 34 (41.5%) | 13 (15.8%) | 0.18 |
| \$20,000 to <\$35,000 | 106 (15.9%) | 59 (55.7%) | 35 (33.0%) | 12 (11.3%) | |
| \$35,000 to <\$50,000 | 132 (19.8%) | 62 (47.0%) | 41 (31.0%) | 29 (22.0%) | |
| \$50,000 to <\$75,000 | 135 (20.3%) | 61 (45.2%) | 53 (39.3%) | 21 (15.5%) | |
| \$>75,000 | 161 (24.2%) | 65 (40.4%) | 73 (45.3%) | 23 (14.3%) | |
| Refused to answer | 50 (7.5%) | 25 (50.0%) | 19 (38.0%) | 6 (12.0%) | |

Data are expressed as n (%) CAC categories according to Agatston score; BMI. Body mass Index; GED. P-Values were obtained from Chi-Square test.

[†]Never smoker: an adult who has never smoked cigarettes or who smoked less than 100 cigarettes; Former smoker: an adult who smoked at least 100 cigarettes in their lifetime but who had quit smoking cigarettes at the time of interview; Current smoker, an adult who currently smokes cigarettes and had smoked more than 100 cigarettes in their lifetime.

Associations between mean log₂-transformed plasma PFAS concentrations and Coronary Artery Calcification (CAC) classification in the Diabetes Prevention Program.

Table 2

| PFAS | Median and IQR, (ng/mL) | Log 2 Values | CAC < 11 N = 307 | CAC (11–400) n = 255 | CAC (>400) n = 104 |
|--------------------------|-------------------------|--------------|--------------------|--------------------------|--------------------------|
| | | | OR (95% CI) | | |
| PFOS ^a | 27.55 (19.3) | 4.78 (1.01) | 1.0 (ref) | 1.20 (0.94, 1.53) | 1.49 (1.01, 2.21) |
| n-PFOA | 19.80 (14.5) | 4.31 (1.05) | 1.0 (ref) | 1.20 (0.94, 1.53) | 1.54 (1.05, 2.25) |
| Sm-PFOS | 7.52 (5.7) | 2.91 (1.10) | 1.0 (ref) | 1.15 (0.91, 1.46) | 1.32 (0.89, 1.94) |
| PFOA ^b | 5.35 (3.6) | 2.42 (0.98) | 1.0 (ref) | 1.17 (0.91, 1.50) | 1.05 (0.71, 1.57) |
| n-PFOA | 4.60 (2.9) | 2.20 (0.90) | 1.0 (ref) | 1.21 (0.92, 1.59) | 1.07 (0.70, 1.64) |
| Sb-PFOA | 0.55 (0.7) | −0.86 (1.51) | 1.0 (ref) | 1.06 (0.93, 1.21) | 1.08 (0.87, 1.34) |
| PFHxS | 2.30 (2.2) | 1.20 (1.30) | 1.0 (ref) | 1.10 (0.92, 1.33) | 1.0 (0.74, 1.36) |
| EtFOSAA | 1.10 (1.4) | 0.14 (1.66) | 1.0 (ref) | 1.26 (1.08, 1.47) | 1.37 (1.07, 1.74) |
| MeFOSAA | 1.10 (1.0) | 0.14 (1.28) | 1.0 (ref) | 1.07 (0.88, 1.29) | 1.35 (0.98, 1.87) |
| PFNA | 0.55 (0.5) | −0.86 (1.14) | 1.0 (ref) | 0.94 (0.77, 1.14) | 1.13 (0.81, 1.56) |

PFAS: per- and polyfluoroalkyl substances; PFOS: perfluorooctane sulfonic acid (sum of linear and branched isomers); n-PFOA: linear perfluorooctanoic acid; Sm-PFOS: perfluoromethylheptane sulfonic acids; PFOA: perfluorooctanoic acid (sum of linear and branched isomers); n-PFOA: linear perfluorooctanoic acid; Sb-PFOA: branched perfluorooctanoic acids; PFHxS: perfluorohexane sulfonic acid; EtFOSAA: N-ethyl-perfluorooctane sulfonamido acetic acid; MeFOSAA: N-methyl-perfluorooctane sulfonamido acetic acid; PFNA: perfluorononanoic acid.

PFAS were log₂ transformed from the mean of baseline and year 2 measurements; CAC: coronary artery calcification; values represent Odds Ratio (OR) and 95% Confidence Interval (95% CI); All models were adjusted for sex, age, body mass index, race/ethnicity, cigarette smoking, education, treatment assignment and statin use.

Significant results (P-Value <0.05) are highlighted in bold.

^aSummary measure calculated by adding up concentrations of linear and branched isomers (n-PFOS and Sm-PFOS).

^bSummary measure calculated by adding up concentrations of linear and branched isomers (n-PFOA and Sb-PFOA), with values <LOD imputed with LOD/2 before summation.

Table 3

Associations between the average plasma concentrations of baseline and year 2 measures of the 6 PFAS as a mixture and Coronary Artery Calcification (CAC).

| | CAC Outcomes | | |
|-----------------------|--|------------------------------------|-----------------------------------|
| | Moderate/high vs low OR (95% CI) | Moderate vs low Ψ (95% CI) | High vs low OR (95% CI) |
| Crude model | 1.31 (1.05, 1.63) | 1.25 (0.99, 1.58) | 1.41 (1.02, 1.95) |
| Adjusted model | 1.06 (0.97, 1.15) | 1.07 (0.95, 1.20) | 1.08 (0.91, 1.29) |

Note: OR interpreted as the odds ratio of CAC outcome per quantile increase in the plasma concentration of the 6 PFAS as a mixture adjusting for sex, age, race/ethnicity, baseline BMI, educational attainment, smoking status, baseline use of cholesterol medication and treatment assignment.

Significant results (P-Value < 0.05) are highlighted in bold.