Circulating levels of perfluoroalkyl substances (PFASs) and carotid artery intima-media thickness - a longitudinal study over 10 years.

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Introduction

Per- and polyfluoroalkyl substances (PFASs) make up a vast class of compounds that are used as processing aids during manufacturing, and are applied to a wide variety of industrial and household products due to their unique surfactant-like properties. The production of PFASs was initiated in the late 1940s and since then PFASs have become ubiquitous in the environment and general populations.

A number of adverse health effects have been linked to elevated levels of PFASs exposure. However, there is no uniform depiction on the cardiovascular effects in response to PFAS exposure, and most recent studies have been based on occupational or accidental occurrences with a gender bias in favor of men. At present, there is limited information on the relationship between exposure to PFASs and atherosclerosis in a gender-balanced sample of the general population. One cross-sectional study found a relationship between PFOS levels and the carotid artery intima-media thickness (IMT), especially in women [2] and we have previously published a link between levels of PFUnDA and overt atherosclerotic plaques in women only [3].

The studies which have evaluated the association between carotid atherosclerosis and PFASs so far have been cross-sectional, and since such studies are subjected to a high risk of reverse causation, we have now analyzed plasma PFAS levels and carotid artery intima-media thickness at three occasions during a 10-year period (2001-2014) in the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study [4]. In the present study, we evaluated if the change in plasma concentration of PFASs was related to the change in IMT over this 10-year period.

Materials and Methods

The carotid artery was assessed by external B-mode ultrasound imaging (Acuson XP128 with a 10 MHz linear transducer, Acuson Mountain View, California, USA) in 1,016 subjects aged 70 years in the population-based PIVUS study (50% women). The measurements were repeated at ages 75 and 80 years.

The images were digitized and imported into the AMS (Artery Measurement Software) automated software for dedicated analysis of intima-media thickness. The IMT was evaluated in the far wall in the common carotid artery

(CCA) 1-2 cm proximal to the bulb. A maximal 10 mm segment with good image quality was chosen for intima-media thickness -analysis from the carotid artery.

The analytical method used to determine plasma concentrations of PFASs in all samples at 70, 75 and 80 years was successfully validated in terms of recovery, accuracy, and precision [5, 6]. Briefly, the sample preparation included rapid protein precipitation of 150 µL of plasma samples with 1% formic acid in acetonitrile and filtration using an Ostro 96-well plate (Waters Corp.). The extracts were analyzed for 14 PFASs using automated column-switching with an Acquity ultra-performance liquid chromatograph coupled to a Quattro Premier XE tandem mass spectrometer (UPLC-MS/MS) system (Waters Corporation, Milford, USA) operating in negative electrospray ionization mode. 250 µL aliquot of the final extract was injected onto a C18 (2.1×20mm, 2.5µm) trap column connected to a C18 (2.1×100 mm, 1.7 µm) analytical column by a 6-port column switch valve. Quantitative analysis of the PFASs was performed using a matrix-matched calibration curve and isotope dilution; all standards (i.e., ^{13}C labelled internal standards, ^{13}C labelled recovery standards and native calibration standards) were purchased from Wellington Laboratories (Guelph, Ontario, Canada). The current study evaluated the 8 PFASs for which >75% of the population showed measurable levels above the limit of detection; perfluoroheptanoic acid (PFHpA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorohexane sulfonic acid (PFHxS), the linear isomer of perfluorooctane sulfonic acid (L-PFOS), perfluorooctanoic acid (PFOA) and perfluorooctane sulfonamide (PFOSA).

The relationships between the changes in the PFASs' concentrations vs the change in IMT over the 10 years were evaluated by mixed random effects models with adjustment for sex, blood pressure, fasting glucose, LDL- and HDL-cholesterol, BMI, smoking, and statin use.

Results and discussion

IMT increased 0.058 mm during the 10-year period ($p<0.0001$). Following adjustment for baseline values of PFASs (age 70) and sex, the changes in plasma levels of 6 of the 8 evaluated PFASs were significantly related to the change in IMT over the 10-year follow-up period ($p<0.0062$ using Bonferroni correction for 8 tests). The relationship between the change in IMT and the change in PFUnDA levels is graphically shown in figure 1 as an example. Further adjustment for traditional CV risk factors (HDL and LDL-cholesterol, smoking, systolic blood pressure, statin use, fasting glucose, and serum triglycerides) only marginally affected these relationships.

No interactions between the changes in PFASs and sex regarding the change in IMT was found.

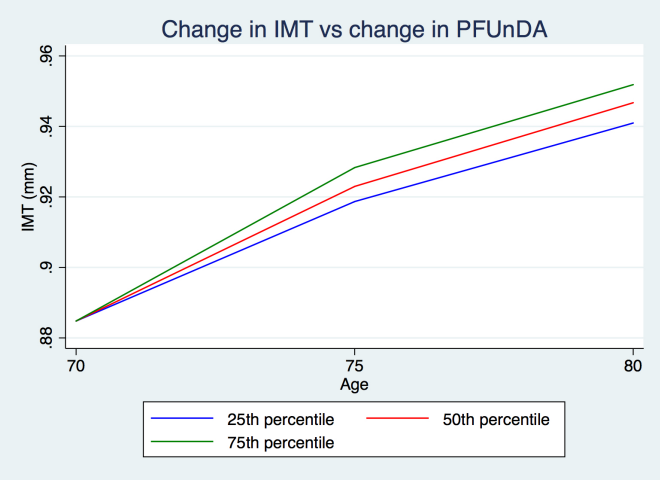

Figure 1. The graph shows the changes over time in intima-media thickness (IMT) for the 25th, 50th and 75th percentile change in PFUnDA. The value of PFUnDA at age 70 is set at the median for all three groups of change in PFUnDA to improve the interpretation of the graph.

The present study strengthens previous cross-sectional findings of a possible role of PFASs in the development of atherosclerosis [2, 3]. The longitudinal design of the present study reduces the risk of reverse causation seen in cross-sectional studies.

It has previously been shown that PFASs levels in humans are related to cholesterol metabolism [7]. However, in the present study the inclusion of statins, HDL- and LDL-cholesterol as confounders did not alter the associations, suggesting that PFASs are linked to IMT by other mechanisms.

In conclusion, the changes in plasma levels of the majority of the measured PFASs were significantly related to the change in IMT over the 10-year follow-up period, suggesting that PFASs might be involved in atherosclerosis development.

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