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SERUM PFOS LEVELS ARE RELATED TO AN INCREASED BIOLOGICAL AGE AS CALCULATED BY DNA METHYLATION ANALYSIS

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Introduction

Perfluorinated compounds (PFASs), is a group of man-made chemicals manufactured since several decades. They are high volume chemicals used in a variety of consumer products like water-repellant cloths, cosmetics, frying pans, etc., but are also a major ingredient of fire-fighting foam. PFASs are very persistent in the biota and are found in almost all humans when measured in the blood. Following oral exposure, PFASs accumulate in the circulation, liver and kidney (1), but not to a major degree in adipose tissue (2). The estimated half-life is in the 4-6 year range for the most commonly found PFASs, PFOS and PFOA.

During the recent years, more and more evidence are accumulating that PFASs are endocrine disrupting compounds and have adverse health effects. Reproductive effects, as well as effects on the thyroid, glucose and lipid metabolism have been described.

It is a well-known clinical fact that humans age in an individualized manner in that the rate of ageing processes differs between individuals. The consequence is that the "biological age" well could differ from the chronological age. Recently, modern techniques to measure the degree of DNA methylation at multiple sites across the genome have shown that the degree of methylation change during ageing and that differential methylation at certain sites are linked to age. Based on information on the degree of methylation at such sites, "biological age" could be calculated (3,4) and an increased calculated biological age compared to the chronological age has recently been linked to a poor survival (5).

The present study was undertaken to test the hypothesis that high levels of PFASs are associated with a high biological age. To test this hypothesis, we used data from around 1,000 subjects in the Prospective Study of the Vasculature in Uppsala Seniors (PIVUS) study in which we have measured serum levels of the two most abundant PFASs, PFOS and PFOA, as well as calculated biological age from information of differential methylation (6).

Methods and methods

"Biological age" was calculated by use of methylation data in around 1,000 subjects in the Prospective Study of the Vasculature in Uppsala Seniors (PIVUS) study (50% women all aged 70 years at the examination). The difference between "biological" and chronological age was calculated (DiffAge). Methylation sites across the genome were assayed using the Illumina HumanMethylation450k Beadchip, which detects methylation based on genotyping of bisulfite-converted genomic DNA, covering 482,421 CpG-sites and 3,091 non-CpG sites. A quantile normalization of the signal intensities was performed per individual and undertaken separately for type-I and type-II probes of the chip. Beta-values were then calculated as the percentage methylation at a site, ranging from 0 to 1. A total of 20,522 methylation sites were excluded from the analysis since their probes mapped to multiple locations in the genome with at least two mismatches, in accordance with other investigators.

150 µL serum extracts were analyzed using an automated column-switching ultra-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method for determination of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS), The analytical procedure involves rapid protein precipitation using 96-well plates followed by instrumental analyses on a Acquity UPLC coupled to a Quattro Premier XE HPLC-MS/MS system (Waters Corporation, Milford, USA) with an atmospheric electrospray interface operating in negative ion mode system by injecting a 250 µL aliquot of the sample onto a C18 (2.1×20mm, 2.5µm) trap column connected to a C18 (2.1×100 mm, 1.7 µm) analytical column operated by a 6-port column switch valve. Quantitative analysis was performed

using the internal standards (internal standards, recovery standards and native calibration standards) being purchased from Wellington Laboratories (Guelph, Ontario, Canada). The method detection limits ranged between 0.01-0.17 ngmL-1 depending on the analyte (7).

Results and discussion
In analyses adjusted for sex only, PFOS, but not PFOA (p=0.19), levels were positively related to DiffAge (p=0.0061). Following adjustment for multiple life-style factors (education level, exercise habits, smoking, energy and alcohol consumption) and BMI, PFOS levels were significantly related to DiffAge (p=0.0074). Following this adjustment, also PFOA levels were related to DiffAge with borderline significance (p=0.056).
In conclusion, PFOS levels were related to DNA methylation-calculated "biological" age, giving further evidence for negative health effects in man of this major perfluorinated compound.

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