3562 Per- and polyfluoroalkyl substances and epigenetic aging in U.S. airport firefighters

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Background and Purpose: Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals with water- and oil-resistant properties that have been in use since the mid-twentieth century. PFAS exposure has been associated with adverse health outcomes, including increased risk for cancers, reproductive consequences, obesity, and reductions in immune function. Firefighters are at increased risk of exposure to PFAS from occupational sources, including PFAS-containing aqueous firefighting foams (AFFF), mobilization from burning materials, and water- and oil- resistant turnout gear. Firefighters with aircraft rescue and firefighting (ARFF) duties, including those working at airports, may be at increased risk for PFAS exposure due to AFFF use. We previously identified an association between serum PFAS concentrations and accelerated epigenetic age in structural firefighters. Epigenetic age, approximated via various epigenetic clocks, is a biomarker of aging. Accelerated epigenetic age is associated with morbidity and mortality. In this cross-sectional study, we evaluate the association between serum PFAS and epigenetic age in ARFF firefighters. **Methods:** Serum concentrations of nine PFAS were quantified via on-line solid phase high-performance extraction liquid chromatography (HPLC) isotope dilution tandem mass spectrometry among a subset of 126 airport firefighters from the national Fire Fighter Cancer Cohort Study. Blood leukocyte DNA methylation was profiled via the Infinium EPIC array. Epigenetic age was approximated with the DNA methylation data via five widely used clocks (Horvath, Hannum, PhenoAge, Skin-Blood, and GrimAge). For eight detected PFAS, linear regression was used to test associations between PFAS and each clock, adjusting for confounders including chronological age, race, and sex. Seven frequently detected PFAS were modeled as continuous concentrations (natural log-transformed) while 2-(N-methyl-perfluorooctane sulfonamido) acetate (MeFOSAA) was dichotomized as detected versus not detected. **Results:** Perfluorohexane sulfonate, linear and branched perfluorooctane sulfonate isomers, linear perfluorooctanoate (n-PFOA), perfluorononanoate, perfluorodecanoate, and perfluoroundecanoate were significantly associated with accelerated epigenetic age according to the GrimAge clock with effect estimates ranging from 0.09 to 0.66 (p-values 0.003 to 0.01). n-PFOA was associated with decelerated epigenetic age (effect estimate -2.23, p=0.006) according to the Skin-Blood clock. Detectable MeFOSAA was inversely associated with GrimAge when compared to firefighters with no detected MeFOSAA (effect estimate -0.79, p=0.004). **Conclusions:** Serum concentrations of multiple PFAS were associated with accelerated epigenetic aging according to the GrimAge epigenetic clock. GrimAge was developed to be a better predictor of all-cause mortality and aging-related diseases compared to other epigenetic clocks. Thus, findings may have implications for PFAS-related morbidities. Decelerations in epigenetic age according to some clocks warrant further investigation. Given the exposure of firefighters to PFAS in their daily work and in their local environments and rapidly-evolving research on the health impacts of PFAS exposure, continued research, including longitudinal studies on PFAS and epigenetic aging, is needed. More data on the sources and impacts of specific PFAS and their impacts on human health are warranted to inform decision-making and best practices for municipalities with ARFF.

3563 Investigating the Effects of Developmental Perfluorooctanesulfonic Acid (PFOS) Exposures on Pancreatic Function and its Modulation by the Nrf2a Pathway in Zebrafish (*Danio rerio*)

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Background and Purpose: Perfluorooctanesulfonic acid (PFOS) is a legacy, ubiquitous contaminant that was historically used as a surfactant in several consumer and industrial products. Exposure to this compound has been associated with increased risk for metabolic dysfunctions, including diabetes. The pancreatic beta-cells are the only insulin-producing cells in the body. Therefore, proper development of this organ is crucial for maintaining glucose homeostasis. In zebrafish (*Danio rerio*), embryonic exposure to PFOS has been shown to impact pancreatic organogenesis and disrupt the redox environment (i.e., inducing oxidative stress). However, we do not fully understand the effects of exposure on pancreatic function or the role of adaptive antioxidant defenses, such as the transcription factor nuclear factor erythroid-2 (Nrf2), in modulating these effects. This study aims to investigate insulin release upon glucose stimulation in zebrafish larvae exposed to PFOS. **Methods:** Wildtype and mutant zebrafish embryos, carrying a loss-of-function mutation affecting the binding domain of the ortholog Nrf2a, were exposed daily to 0, 8 or 16 µM PFOS beginning at 3 hours post fertilization (hpf). Embryos were dechorionated at 24 hpf and solutions were refreshed daily. At 72 hpf, half of the embryos from each group were challenged with 20 mM glucose for 24 hours. At 96 hpf, larvae were rinsed, fixed, permeabilized, stained with an anti-insulin antibody, and imaged with a Nikon A1 confocal microscope. Whole-mount immunofluorescence was used to detect changes in the islet (retained insulin) and in the whole-body (proxy for released insulin). **Results:** Exposure to 16 µM PFOS alone significantly decreased (52%) islet insulin fluorescence while increased (13%) whole-body fluorescence in Nrf2a wildtype larvae. Interestingly, when challenged with 20 mM glucose, no changes in islet insulin fluorescence were observed in pancreatic beta-cells. However, there was a significant dose-dependent increase in released insulin (whole-body) intensity in larvae exposed to 8 and 16 µM PFOS (19 and 34%, respectively). While PFOS alone did not alter insulin levels in Nrf2a mutants, embryos exposed to 16 µM PFOS and challenged with glucose had a significant increase (63%) in islet fluorescence intensity. No changes were observed in whole-body fluorescence upon glucose challenge in Nrf2a mutant larvae. Regardless of treatment, Nrf2a mutant larvae had lower insulin intensity compared to Nrf2a wildtype. **Conclusions:** Overall, PFOS exposure altered the response to a 20 mM glucose challenge in distinct ways in Nrf2a wildtype and mutant embryos, which suggests that activation of adaptive antioxidant defenses by Nrf2a does not fully protect larvae from PFOS-induced toxicity. Ultimately, this work demonstrates that PFOS disrupts beta-cell function in zebrafish, potentially impacting glucose homeostasis. This work was supported by R01ES025748.

3564 Identifying chemical and biological determinants of PFAS with long half-life using physiologically-based toxicokinetic modeling

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Background and Purpose: Humans are exposed to the environmental contaminants per- and polyfluoroalkyl substances (PFAS) through consumer products, contaminated food and water sources. Highly persistent, they have unusually long half-lives in the body and their bioaccumulation is associated with adverse health effects including cancer and thyroid hormone disruption. Long-chain PFAS such as perfluorohexanesulfonic acid (PFHxS, 6 carbons), perfluorooctanesulfonic acid (PFOS, 8 carbons) and perfluorooctanoic acid (PFOA, 8 carbons) have half-life estimates of 7, 3.3-5.4 and 1.3-3.9 years respectively, while short-chain PFAS such as perfluorobutanoic acid (PFBA, 4 carbons) and perfluorobutanesulfonic acid (PFBS, 4 carbons) have estimated half-lives of 3 and 28 days respectively. To date, it remains unclear what features can clearly distinguish PFAS exhibiting long half-lives from those with short half-lives. Carbon chain length is often cited as a structural discriminant, but this simple relationship does not always hold true. With over 14,000 PFAS to be assessed, a reliable and quick approach to identify PFAS with long half-lives is needed for risk prioritisation and identification of PFAS that require in-depth risk assessments. **Methods:** We performed a comparative analysis of the 5 aforementioned PFAS using bottom-up, physiologically-based toxicokinetic (PBTK) models. We generated an extensive panel of *in vitro* transporter kinetic data for hepatic transporters OATP1B1, OATP1B3, OATP2B1 and NTCP; renal transporters OAT1, OAT3, OAT4 and URAT1; lactate transporter MCT1 and plasma protein binding measurements. The kinetic data were scaled to *in vivo* clearances using quantitative proteomics-based *in vitro*-to-*in vivo* extrapolation (IVIVE), and then used to parameterize our *in silico* PBTK models without relying on animal data. **Results:** Our simulations successfully recapitulated the trend of half-lives for PFAS where PFBA<PFBS<PFOA<PFOS<PFHxS, as similarly observed in biomonitoring studies. Kinetic analyses showed that short half-life PFAS were substrates of fewer transporters than long half-life PFAS, and exhibit lower intrinsic clearances for these transporters. Sensitivity analyses indicated renal (and not biliary) clearance as the major elimination pathway of PFAS. For long half-life PFAS, their *in vivo* persistence is largely driven by extensive renal reabsorption, which was less prominent for short-chain PFAS. In addition, short half-life PFAS exhibit a higher unbound fraction in plasma, resulting in a greater contribution of renal filtration to their elimination. We identified 4 features that clearly distinguish short versus long half-life PFAS, namely susceptibility to transport by URAT1, OAT4 and MCT1, as well as the degree of plasma protein binding. **Conclusions:** These learnings have been developed into a systematic screening methodology to identify PFAS with high risk of exhibiting long half-lives as part of a risk prioritisation strategy. We are expanding our screenings to more PFAS to demonstrate the robustness of this approach in identifying long half-life PFAS.

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