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Exploratory assessment of perfluorinated compounds and human thyroid function

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ABSTRACT

Thyroid hormones play critical roles in human neurodevelopment and adult neurocognitive function. Persistent organohalogen pollutants, such as perfluorinated compounds (PFCs), may interfere with thyroid homeostasis and thus exposures to these compounds might represent risk factors for neurologic and cognitive abnormalities. In this study, serum specimens collected from thirty-one licensed anglers in New York State were analyzed for levels of thyroid stimulating hormone (TSH), free thyroxine (FT_4), perfluorodecanoic acid (PFDA), perfluorononanoic acid (PFNA), perfluoroheptanoic acid (PFHpA), perfluorohexanesulfonate (PFHxS), perfluorooctanoic acid (PFOA), perfluorooctanesulfonate (PFOS), perfluorooctanesulfonamide (PFOSA), and perfluoroundecanoic acid (PFUnDA). PFOS and PFOA occurred in the highest concentrations with geometric means of 19.6 ng/mL (95% CI 16.3–23.5) and 1.3 ng/mL (95% CI 1.2–1.5), respectively. In a cross-sectional analysis, no statistically significant associations were detected for PFCs, or their sum, with TSH or FT_4 at $\alpha = 0.05$. However, *post hoc* power analyses, though limited, suggested that moderate increases in sample size, to 86 and 129 subjects, might facilitate 80% power to detect statistically significant associations for FT_4 and PFDA ($\beta = 0.09$) and PFUnDA ($\beta = 0.08$), respectively. The consumption of sportfish may have contributed to PFDA ($r = 0.52$, $P = 0.003$) and PFUnDA ($r = 0.40$, $P = 0.025$) levels. This preliminary study does not indicate associations between non-occupational PFCs exposures and thyroid function. However, the possibility for weak associations for FT_4 with PFDA and PFUnDA, PFCs measured in low concentrations, is raised. Given the ubiquity of PFCs in the environment and the importance of thyroid function to neurodevelopmental and neurocognitive endpoints, a confirmatory study is warranted.

1. Introduction

Suitable circulating thyroid hormone levels are critical for proper human neurodevelopment (reviewed by [1]) and adult neurocognitive function (reviewed by [2]). Alterations of circulating thyroid hormone have been associated with adverse neurodevelopmental endpoints [3] and with behavioral and psychiatric pathologies (reviewed by [4,5]). Persistent organohalogen pollutants (POPs), such as polychlorinated biphenyls for example, may interfere with thyroid homeostasis (reviewed by [6]) and/or neurologic function in children [7] and adults [8].

Perfluorinated compounds (PFCs) comprise a distinct family of POPs which are distributed widely [9], biomagnify in aquatic food chains [10], are highly persistent [11], and may interfere with thyroid function (reviewed by [12]). These compounds are structurally homologous to free fatty acids [13], with which they compete for serum protein binding sites *in vivo* [14]. Albumin, a serum protein found in high relative concentrations in humans, serves as an important regulator of bio-availability, binding approximately 10% of circulating thyroid hormone [15]. Circulating serum albumin also tightly binds PFCs [14].

Several animal studies raise the possibility that PFCs, administered at high doses, may be thyroid toxicants [16–20]. However, criticisms regarding the use of analog methods for the assessment of free hormone in some of these latter studies have been recently raised [21]. A limited number of studies have considered 'background', or non-occupational, levels of human exposure to PFCs and thyroid function, and these have been null to date [22,23]. Several publications considering higher levels of human exposure to PFCs, those encountered in occupational settings, have reported statistically, though not clinically, significant associations with thyroid function [24–26].

The aim of the current study was to screen hypotheses regarding potential associations between serum concentrations for eight measured PFCs, and their sum, with levels of thyroid stimulating hormone (TSH) and free thyroxine (FT_4) measured in a subsample of New York State Angler Cohort Study (NYSACS) participants. New York

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State sportfish consumption is potentially an important source of exposure to PFCs [27] and thus anglers might be considered a suitable population for preliminary studies of non-occupational exposures and thyroid function. The goal of this study was to evaluate the necessity for, and to generate specific testable hypotheses and recommendations for a comprehensive study.

2. Methods

2.1. Sample selection

Described elsewhere in detail [28], the NYSACS was a prospective investigation of POPs and potential health effects among 18,082 licensed New York State sportfish anglers and their partners residing in 16 New York State counties contiguous to Lakes Erie and Ontario. The current study considers a subgroup of 31 of 38 NYSACS participants who had completed the 'Dioxin Exposure Substudy' (DES) component, which has also been described in detail in a prior publication [29]. DES participants were recruited to maximize the range of sportfish-consumption-related exposure to POPs contingent on self-reported sportfish consumption and sera concentrations of PCB IUPAC #153 [30]. Between 1995 and 1997, participants completed a detailed exposure questionnaire including queries regarding sportfish and game consumption, lifestyle and demographic factors, and medical history. In addition, a 450 mL sample of blood was donated for toxicologic analysis.

Age, gender [31,32], body mass index (BMI) [33], cigarette smoking (reviewed by [34]), history of physician-diagnosed goiter or thyroid condition [35], race/ethnicity [31], the self-reported use of thyroid medication or medications that might alter thyroid hormone concentrations, and the self-reported consumption of sportfish caught from New York State waters in the approximate year prior to the study (6/1/94 to 6/1/95), were considered covariates of potential importance. Informed consent was provided by each participant and this study was approved by the Human Subjects Institutional Review Boards of the State Universities of New York (SUNY) at Albany and Buffalo, and of the New York State Department of Health (NYSDOH).

2.2. Laboratory analysis

Blood specimens were collected using a standard protocol and allowed to clot, yielding serum that was used in a previously described series of toxicologic analyses [29]. Remaining specimens were divided into aliquots, labeled, and archived at −70 °C at the Center for Preventive Medicine at the University at Buffalo.

2.2.1. Thyroid marker analysis

In April 2003, following a median 6.5 years (range 5.3–7.6) of storage at −70 °C, serum aliquots were transported on dry ice to a commercial laboratory for thyroid function marker analyses. TSH was measured using a 3rd generation immunometric chemiluminescent sandwich assay and FT_4 by a competitive chemiluminescent immunoassay. An Immulite® 2000 analyzer (Diagnostics Products Corporation, Los Angeles, CA) immunoassay system, as well as proprietary testing reagents, was employed in determinations. Three quality control samples, at three different hormone concentrations, were included in each sample run. No inter-assay CV exceeded 12.5%. Due to concerns regarding the quality of other measured thyroid hormone markers, as previously described [29], only TSH and FT_4 concentrations were available for this study. Stability of these thyroid function markers has been previously reported following more than 10 years of storage at −25 °C [36].

2.2.2. Chemical analysis

In January 2006, following a median 9.1 years (range 8.1–10.3) of storage at −70 °C, remaining serum aliquots were transported on dry ice to the Wadsworth Center, NYSDOH, for the analysis of PFCs. These included five perfluoroalkyl carboxylates, perfluorodecanoic acid (PFDA), perfluoroheptanoic acid (PFHpA), perfluorononanoic acid (PFNA), perfluorooctanoic acid (PFOA), and perfluoroundecanoic acid (PFUnDA); and three perfluoroalkyl sulfonates, perfluorohexanesulfonate (PFHxS), perfluorooctanesulfonate (PFOS), and perfluorooctanesulfonamide (PFOSA). An ion-pairing extraction procedure was employed using aliquots of approximately 0.5 mL from 31 subjects with sufficient sample volume available. Determinations were conducted using a high-performance liquid chromatograph (HPLC) with electrospray tandem mass spectrometry (ES-MS/MS), as previously described [9,37]. Intra-assay CVs for perfluorobutanesulfonate (PFBS) recovery standards ranged from 5–10% and the mean internal standard recovery was 99.9% (SD 11.9%). Method blanks contained only trace levels of PFHpA and PFOA. Method blank values were subtracted prior to reporting.

Limits of detection (LODs) for PFNA and PFOA were calculated as three times the concentrations detected in method blanks (PFNA = 0.50 ng/mL and PFOA = 0.80). LODs for other analytes were determined as the lowest concentrations on calibration curves (PFDA = 0.20 ng/mL, PFHpA = 0.15, PFHxS = 0.15, PFOS = 2.00, PFOSA = 0.20, and PFUnDA = 0.02). For PFHpA no values exceeded the LOD, and only 9.7% of values did so for PFOSA. These analytes were thus not further considered. Values below LODs were censored as $LOD/\sqrt{2}$ prior to data analysis, assuming non-detectable values were normally distributed [38].

2.3. Statistical analysis

2.3.1. Univariate and bivariate analysis

Statistical analysis was conducted using SAS v. 9.1.3 (SAS Institute Inc. Cary, NC). Descriptive statistics are presented for individual PFCs, and their sum (ΣPFCs), as well as TSH and FT_4. Normality assessment for continuously distributed variables using the Shapiro–Wilk test indicated the necessity for log transformation of PFCs and TSH prior to parametric analysis. Pearson and Spearman correlation analysis, as appropriate, and analysis of variance (ANOVA) were employed to evaluate bivariate associations between age, BMI, gender, cigarette smoking, TSH, FT_4, and PFCs as appropriate. In addition, we employed the self-reported average number of sportfish meals caught from New York State waters, in the approximate year prior to the study (6/1/94–6/1/95), as a broad marker of sportfish exposure. This variable was presumed to be the most valid among those sportfish data collected during this study, given the short duration between the exposure interval and study completion. Statistical significance was defined as $P<0.05$ for a two-tailed test.

2.3.2. Multivariable analysis

PFC variables were simultaneously entered as potential predictors, using a forward stepwise selection procedure, for TSH and FT_4 as dependent variates, to generate multivariable linear regression models. Separate regression models were also fitted for TSH and FT_4 as dependent variates on each PFC variable as a predictor. Covariates of potential importance were included in regression models where bivariate associations were of, at minimum, 'borderline' statistical significance ($P<0.10$) for associations with a PFC variable, and with FT_4 or TSH as appropriate. Influential observations, defined as those with 'dfbeta' scores exceeding $2/\sqrt{n} = 0.36$ for the PFC coefficient, were excluded and regression analysis repeated [39]. The tenability of statistical assumptions necessary for general linear modeling was evaluated graphically [40].

2.3.3. Power analysis

To facilitate interpretation of study results, *post hoc* power analysis was conducted for non-statistically significant individual linear regression models using the program PS Power and Sample Size Calculations [41]. Sample sizes required to generate statistically