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Serum perfluoroalkyl acids concentrations and memory impairment in a large cross-sectional study

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Summary

Article focus

- Cross-sectional association between serum level of Perfluorooctanate (PFOA), perfuorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA) and perfluorohexane sulfonate (PFHxS) and self-reported memory impairment in a population exposed to high levels of PFOA
- Potential interaction between the association of perfluoroclorinated compound with memory impairment by diabetes status

Key Message

- Inverse associations between PFOS and PFOA and memory impairment were highly statistically significant suggesting a potential antinflammatory effect exerted through PPAR agonism. Comparable inverse associations with PFNA and PFHxS were of borderline statistical significance
- Inverse associations of PFAAs with memory impairment were weaker or non-existent in diabetics than overall in non-diabetics. Analysis further stratified by specific diabetes medication use showed no variation in odds ratios more than explicable by chance given the number of tests made.

Strengths and limitations

- Very large sample size including 21,024 adults with measured serum level of perfluorinated compounds with a given geographical distribution allowing some multilevel modelling
- The cross-sectional nature of the design does not allow any causal inference and makes results particularly prone to reverse causality
- Self-reported is not an optimal method for estimating the degree of memory impairment in a population

ABSTRACT

Objectives – To examine the cross-sectional association between serum PFOA, PFOS, PFNA, and PFHxS concentrations with self-reported memory impairment in adults and the interaction of these associations with diabetes status

Design - Cross-sectional study

Setting – Population-based in Mid-Ohio Valley, West Virginia following contamination by a chemical plant

Participants - The C8 Health Project collected data and measured serum level of prefluoroclorinated compounds of 21,024 adults

Primary and secondary outcome measures – Self-reported memory impairment as defined by the question "have experienced short term memory loss?"

Results - A total of 4,057 subjects self-reported short-term memory impairment. Inverse associations between PFOS and PFOA and memory impairment were highly statistically significant with fully adjusted OR=0.93 (95% C.I. 0.90-0.96) for doubling PFOS and OR=0.96 (95% C.I. 0.94-0.98) for doubling PFOA concentrations. Comparable inverse associations with PFNA and PFHxS were of borderline statistical significance. Inverse associations of PFAAs with memory impairment were weaker or non-existent in diabetics than overall in non-diabetics.

Conclusion - An inverse association between PFAA serum levels and self-reported memory impairment has been observed in this large population-based, cross-sectional study stronger and more statistically significant for PFOA and PFOS.

INTRODUCTION

Perfluoroalkyl acids (PFAAs) are man-made compounds used during the manufacture of fluoropolymers including non-stick cookware and breathable, yet waterproof, fabrics. They can also result from the metabolism of fluorinated telomers, compounds used for food package coatings, carpet treatments, and stain-resistant fabric treatment. Perfluorooctanate (PFOA), perfuorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA) and perfluorohexane sulfonate (PFHxS) persist in the environment and are found in the blood of humans and many animal species throughout the world [1 2]. Potential sources of exposure to PFAAs in humans include drinking water, dust, breast milk, food packaging, ambient air, and occupational exposure [3-6].

In animal models, perfluoroalkyl chemicals can activate peroxisome proliferator-activated receptor alpha (PPARα), a ligand-activated transcription factor that regulates gene expression, lipid modulation, glucose homeostasis, cell proliferation and inflammation [7 8]. The PPAR receptor has been involved in the ageing process: PPARα null mice showed a decreased longevity compared with the wild-type due to non-neoplastic spontaneous ageing lesions which occurred with a higher incidence and a short latency in the PPARα null mice [9]. Also PPARγ variants were reported to be associated with longevity in humans with low insulin resistance [10 11]. Activation of the PPARγ receptor *in vitro* and *in vivo* also prevents the expression of inflammatory cytokines and other inflammatory mediators in brains of Alzheimer disease animal models [12 13]. In addition, PPARγ agonists have been demonstrated to suppress the Aβ-mediated activation of microglia *in vitro* and prevent cortical or hippocampal neuronal cell death [14-16]. PPARγ is also deeply involved in diabetes, given its ability to orchestrate the expression of genes involved in lipid metabolism, adipogenesis, and inflammation. It is activated by endogenous ligands (such as fatty acids and prostaglandins) or drugs such as thiazolidinedione. It is most highly expressed in adipocytes where it acts as the master regulator of adipogenesis via induction of adipogenic genes [17]. Therefore, in line with what was recently observed by Power et al[18], we hypothesised that increased exposure to PFAA could be associated with a better cognitive function.

The positive association between diabetes and cognitive impairment is well established [19]; some studies investigating the association between PFOA exposure and diabetes suggested the presence of an inverse association: a negative trend in diabetes occurrence by increasing serum PFOA deciles was found in a case–control study nested in a previous study based on the population investigated here [20 21].

From 1950-2005, a chemical plant in the Mid-Ohio Valley, West Virginia was responsible for emitting PFOA into the surrounding environment. In 2001, a group of residents from the nearby West Virginia and Ohio communities filed a class action lawsuit alleging health damage from drinking water supplies drawing on PFOA-contaminated groundwater [22]. Part of the pre-trial settlement of the class action lawsuit included a baseline survey, the C8 Health Project, conducted in 2005-2006, that gathered data from over 69,000 people from six contaminated water districts surrounding the plant [22]. In this population, PFOA concentrations ranged from US background levels to very high; overall PFOA levels are much higher in this population (geometric mean 42.6.0 ng/mL, 95% C.I. 41.8-43.3) than in the corresponding US population surveys (NHANES in same year mean 3.95 ng/mL, 95% C.I. 3.65-4.27)) [1 22]. The mean PFOS (geometric mean 22.4, 95% C.I. 22.2-22.6), PFNA (1.37, 95% C.I. 1.36-1.38), and PFHXs (3.18, 95% C.I. 3.15-3.22) closely resembled values from a nationally representative US sample form a similar time frame (mean PFOS 20.7, 95% C.I. 19.2-22.3; mean PFNA 0.97, 95% C.I. 0.82-1.14; and PFHXs 1.93, 95% C.I. 1.73-2.16) [1].

The present study uses these data to examine the cross-sectional association between serum PFOA, PFOS, PFNA, and PFHxS concentrations with self-reported memory impairment in adults, and its potential interaction with diabetes status.

METHODS

The Study population

This study is one of the C8 Science Panel Studies and uses information from questionnaires and blood tests collected in the C8 Health Project, supplemented by further information on classification by water district developed in a companion C8 Science Panel Study.

The C8 Health Project enrolled eligible subjects between August 2005 and August 2006. Individuals were eligible to participate in the C8 Health Project if they had consumed water for at least one year between 1950 and December 3, 2004 while living, working, or going to school in one of the following six water districts: Little Hocking Water Association of Ohio; City of Belpre, Ohio; Tupper Plains–Chester District of Ohio; Village of Pomeroy, Ohio; Lubeck Public Service District of West Virginia; Mason County Public Service District of West Virginia; or private water sources within aforementioned districts and areas of documented PFOA contamination. Details of the study enrolment process, including consenting procedures, have been described elsewhere [22].

The C8 Health Project collected data on 69,030 people. While it is not possible to estimate the participation rate for the C8 Health Project as it is not possible to estimate the number of total possible participants over 50 years of environmental contamination, a participation rate, based on US census counts of residents in the affected water districts during Project enrolment, have been estimated at around 80% [22]. In this population, the strongest predictor of PFOA serum concentration was residence in one of the contaminated water districts [23]; serum levels of other PFAAs do not show such geographic variation. Of the population, 21,724 older adults (aged ≥50 years) were considered for this analysis, and a total of 21,024 (96.8%) were included in the final analysis after exclusion of subjects with missing data on ethnicity, education level, socio-economic status, cigarette smoking, or BMI measurements.

Memory impairment definition

During the survey, all participants were asked if they "had experienced short term memory loss", the possible answers being "frequently", "sometimes", "rarely", and "never". Memory impairment was defined as reporting short-term memory loss frequently or sometimes. Severe memory impairment was defined as reporting frequent episodes of short term memory loss.

Laboratory analysis

Blood samples were obtained and processed at individual data collection sites. Samples were drawn into four tubes per participant, with a maximum 35 mL. Tubes were spun, aliquoted, and refrigerated until shipping. Samples were shipped on dry ice daily from each data collection site to the laboratory daily [22]. Participants were not asked to fast before blood sample withdrawal, but fasting status was recorded.

Laboratory analysis of PFAAs used an automated solid-phase extraction combined with reverse-phase high-performance liquid chromatography [24]. Analyses were conducted by the Exygen Research Inc., State College, PA, USA; an intra-laboratory quality assurance program was carried out by analysis of duplicate samples at AXYS Analytical Service Ltd., Sidney, BC, Canada [22]. The intra-laboratory coefficient of variation for both PFOA and PFOS measurements was 0.1; the inter-laboratory comparison coefficient of variation was 0.2 for PFOA and 0.1 for PFOS [22]. The detection limit for PFOA and PFOS was 0.5 ng/mL and observations below this limit were assigned a value of 0.25 ng/mL (n=32 and n=230 for PFOA and PFOS, respectively, for this study population). Both PFOA and PFOS concentration distributions were skewed to the right. Methods and results are reported according to STROBE-ME recommendations [25].

Statistical analysis

Associations between exposure (serum concentration of PFOA, PFOS, PFNA, and PFHxS) and memory impairment were studied using logistic regression. Minimally adjusted models included age, in one year age-band, race (white, black, and others), gender, and educational level (high school diploma or general educational development (GED), some college, bachelor degree or higher) (Model 1). Further adjusted models additionally included average household income (≤$10,000, $10,001-20,000, $20,001-30,000, $30,001-40,000, $40,001-50,000, $50,001-60,000, $60,001-70,000, >$70,000), physical activity, alcohol consumption (none, <1 drink/month, <1 drink/week, few drinks/week, >1 drink/day) and cigarette smoking (never smoker, former smoker, current smoker <10 cigarettes/day, current smoker 10-19 cigarettes/day, current smoker ≥20 cigarettes/day) (Model 2). Fully adjusted models included also body mass index (BMI) (underweight/normal weight; overweight; and obese class I, II, and III), and diabetes (Model 3). PFAA concentrations were log-transformed to reduce skewness. For each model the association between PFAAs and self-reported memory impairment was calculated for a doubling in PFAA concentration in a model with PFAA entered as numerical covariate and for quintile groups of the PFAA distribution. To explore possible differential effect of PFAA in sub-groups, analyses were further stratified by diabetes status and, among diabetics, by type of medications.

Four sensitivity analyses were carried out: firstly one analysis restricting the sample to 7,097 subjects aged 65 years and older. Secondly, an analysis conducted on the entire sample, but using as outcome definition only those reporting frequent episodes of memory loss. Third, an ordinal regression analysis with the outcome variable comprising the four original levels of self-reported frequency of episodes of memory loss. Fourth, we also considered the possibility that mobility (i.e. moving house measured as number of address during lifetime) might be associated with both memory loss and C8 and hence confound the association.

Finally, the geographical clustering of PFOA in water districts allowed use to decompose the overall estimate of association of PFOA with memory impairment into within and between water district components, by including as explanatory variables both water district mean logged PFOA serum concentration and potential deviations of individual's values from their district mean [26]. These two associations are subject to different biases, so help interpretation.

Role of funding

Funding for this work, the "C8 Science Panel Community Study at London School of Hygiene and Tropical Medicine - LSHTM", comes from the C8 Class Action Settlement Agreement (Circuit Court of Wood County, WV, USA) between DuPont and plaintiffs, which resulted from releases of perfluorooctanoate (PFOA, or C8) into drinking water. It is one of the C8 Science Panel Studies undertaken by the Court-approved C8 Science Panel established under the same Settlement Agreement. The task of the C8 Science Panel, of which Tony Fletcher is a member, is to undertake research in the Mid-Ohio Valley, and subsequently evaluate the results along with other available information to determine if there are any probable links between PFOA and disease. Funds were administered by the Garden City Group (Melville, NY) that reports to the Court. The authors of this manuscript declare that their ability to design, conduct, interpret, or publish research was unimpeded by and fully independent of the court and/or settling parties. In addition, they declare no competing financial interests. The LSHTM Ethics Committee approved this study.

RESULTS

A total of 4,462 subjects (21.2% of the entire population of 21,024 individuals aged 50 years or older) self-reported short-term memory impairment (**Error! Reference source not found.**): episodes of short-term memory loss were reported frequently by 1,115 subjects (5.3%); sometimes by 3,347 (15.9%); rarely by 4,283 (20.4%) and never by 12,279 (58.4%). Many personal characteristics were associated individually with memory loss, including higher age, lower socio-economic status, smoking, and diagnosis of diabetes (Table 1), though to what extent these reflected independent risk factors was not investigated.

Results from the logistic regression of association between PFAAs and memory impairment are shown in Table 2. Results for minimally, further and fully adjusted models were similar, so we show only further adjusted results in this table, but results for all models are in the on-line Table 1. Inverse associations between PFOS and PFOA and memory impairment were highly statistically significant with adjusted OR=0.93 (0.90-0.96) for PFOS and OR=0.96 (0.94-0.98) for PFOA for doubling PFAA concentrations. Inverse associations of similar magnitude with PFNA and PFHxS but of borderline statistical significance were found: OR=0.96 (0.92-1.02) for PFNA and OR=0.97 (0.94-1.00) for PFHxS. The analysis by PFAA quintile groups shows similar patterns.

Inverse associations of PFAAs with memory impairment were weaker or non-existent in diabetics than overall in non-diabetics (Table 3), though odds ratios were imprecise so this pattern was only significant for PFOA (p-value for interaction = 0.014). Analysis further stratified by specific diabetes medication use showed no variation in odds ratios more than explicable by chance given the number of tests made (on-line Table 2).

In the sensitivity analysis on subjects older than 65 years, the substantial reduction of the sample size resulted in loss of precision in odds ratios. However, there were associations of comparable magnitude in memory impairment with all PFAAs except for PFOA for which the association with memory impairment virtually disappears (OR= 0.99 (0.97-1.03)) (Table 4).

The analysis carried out on the entire sample, but restricting the definition of memory impairment to those who report frequent short-term memory loss episodes shows substantially unaltered associations for PFOA and PFNA, and somewhat reduced inverse associations for PFOS and PFHxS, but precision was reduced (Table 4). Analyses using ordinal regression in place of binary logistic regression gave similar results (Table 2, Table 3, and Table 4).

Mobility as indicated by count of addresses was not appreciably associated with C8, so results changed very little on inclusion this variable in our regression analysis and are not shown.

The analysis separating the PFOA-memory impairment association into within and between water district components found that within water districts individuals with high PFOA tended to have less memory impairment, as in the overall association (OR 0.94, 95%CI 0.91-0.98, scale and adjustments as before). However there was no tendency for water districts with high PFOA on average to a lower proportion of persons with memory loss (OR 1.00, 95%CI 0.97-1.03).

Extra data is available upon request by emailing Valentina Gallo (v.gallo@qmul.ac.uk).

DISCUSSION

An inverse association between PFAAs serum levels (including PFOS, PFOA, PNA, and PFHxS) and self-reported memory impairment has been observed in this large population-based, cross-sectional study. This association is stronger and more statistically significant for PFOA and PFOS.

It could be speculated that this effect might be mediated by the activation of the PPAR receptor by PFAAs. Activation of the PPAR receptors has been shown to decrease the secretion of proinflamatory cytokines and possibly increase phagocytosis of Aβ inclusions, probably thought activation of

microglia [27]. However there was suggestion that this effect of suppression of the activation of microglia was age-dependent or disease stage-dependent being not significant in patients with advanced AD [28 29]. PPAR agonist drugs, such as non-steroidal anti-inflammatory drugs (NSAID) and some anti-diabetics (i.e. thiazolodinedione or pioglitazone) have been proposed as preventive drugs for neurodegenerative conditions, including Alzheimer dementia [27 30].

In the present study, the inverse association between PFAAs and cognitive impairment was more evident in those without than with diabetes. This could be at least partially due to the fact that in diabetics PPAR receptors are more phosphorylated with a consequent reduced transcriptional activity [31 32], and the balance between PPARγ expression and activity levels is altered [31 33]. It is therefore reasonable to assume that the PPAR-agonist effect of PFAAs is different in subjects with and without PPAR-mediated metabolic changes such as diabetes. Also, it has been reported that PFAAs have a PPAR agonist effect, more prominently PPAR-α [34]; animal models suggest that PFOA has a stronger agonistic effect than PFOS [34]. Taken all together these results are compatible with an inverse association between PFAA and memory impairment among non-diabetics, and would be therefore compatible with a possible anti-inflammatory role exerted by PFAA on early symptoms of cognitive impairment.

That the association with PFOA was entirely within water districts, and not present at all between water districts despite large differences in (geometric) mean PFOA between districts (range 15.7 – 405.1) helps shed light on which biases the results are most vulnerable to. The between district estimate is not vulnerable to reverse causation and related biases at individual level, making this a more plausible explanation of the results. This association is, however, subject to bias by "ecologic" confounding by unmeasured factors differing across districts. This suggests that either the association documented at individual level could be confounded (e.g. by a common genetic variant related to both dementia risk and some excretion pathways); or that the association at the district level is biased towards the null (e.g. by confounding by socio-economic status). The notion that the association estimates found for PFOA are in the same direction of those found for PFNA and PFHXs, and in the majority of cases very consistent with those found for PFOS, however, tend to reinforce the notion of an inverse association between PFOA (and other PFAAs) and memory impairment at individual level. This suggests a common biological mechanism behind the findings.

Another alternative explanation of these findings is that the association between PFAAs and memory impairment is confounded by drinking water as inversely related to drinking artificially sweetened soft drinks. Fructose, currently the most used sweetener used in drinks as well as in a wide range of packaged food, has been associated with higher risk of dementia [35]. Assuming that the consumption

of water (contaminated by PFOA in this population case) is inversely related to the consumption of soft drinks, this would lead to an artificial association between PFOA and memory impairment. However, indirect evidences gathered mainly during intervention trials among adolescents suggest that soft drink consumption is independent from the amount of water consumed by individuals [36 37]. Also, if this was true one would expect that the pattern observed for PFOA to be substantially different from those observed for the other PFAAs, which is not in this case.

However, these findings should also be interpreted cautiously given the several limitations of the study. Firstly, given the cross-sectional nature of the study, reverse causality cannot be ruled out: subjects suffering from memory impairment could have drunk less water resulting in average lower levels of PFAA, although this is not a likely explanation given the consistency of the association across various PFAAs which have substantially different routes of exposure. Secondly, self-reported is not a very accurate method for ascertaining memory impairment, although errors in classification are likely to result in non-differential misclassification, biasing the estimate of association towards the null. Thirdly, the effects of PFAA have been mostly studied in relation to PPARα [7], while receptor mostly implicated in metabolic changes and diabetes and in dementia PPARγ [27]; however, these two belong to the same receptor family and some degree of cross-activation cannot be excluded, and the knowledge of their pleiotropic effects is currently advancing [38]. Lastly, the analysis of different anti-diabetic medications is particularly hampered by the fact that these were self-reported and not prompted by interviewers. This has likely led to low specificity and thus bias of the association (if any) towards the null.

On the other hand, strengths of this study include the fact that all showed estimates were adjusted for numerous potential confounders, including age in one-year age bands, making the effect of PFAA on memory impairment not likely to be confounded by lifestyle characteristics. Furthermore, these results are based on a very large population representative of the general population in West Virginia and Ohio [22], thus estimates are solid. Finally, the 21% prevalence of memory impairment is compatible and consistent with figures on prevalence of dementia reported for North America (Ferri et al, 2005).

In conclusion, these data show an inverse association between PFOA and PFOS exposure and self-reported memory-impairment, particularly in non diabetics. This can be potentially explained by preventive anti-inflammatory effect exerted by a PPAR agonist effect of these perfluoroclorinated compounds.

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CONTRIBUTORSHIP: Dr Gallo had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: TF BA VG
Analysis and interpretation of data: VG TF BA
Drafting of the manuscript: VG
Data collection: TF
Critical revision of the manuscript for important intellectual content: CB GL

DATA SHARING: Extra data is available by emailing Valentina Gallo (v.gallo@qmul.ac.uk)

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Table 1: Participant Characteristics, Mid-Ohio Valley, 2005-2006 (N=21,024)

	All N=21,024*	Memory impaired N=4,462^
Males, n (%)	10,353 (49.2)	2,040 (19.7)
Females, n (%)	10,671 (50.8)	2,422 (22.7)
Age, median/mean (SD)	60.5/62.3 (9.0)	59.9/62.3 (9.4)
Age groups		
50-54 years	5,381 (25.6)	1,185 (22.0)
55-59 years	4,831 (23.0)	1,055 (21.8)
60-64 years	3,715 (17.7)	740 (19.9)
65-69 years	2,930 (13.9)	535 (18.3)
70-74 years	1,979 (9.4)	419 (21.2)
75-79 years	1,251 (6.0)	269 (21.5)
80+ years	937 (4.5)	259 (27.6)
Regular exercise, n (%)	6,774 (32.2)	1,306 (19.3)
BMI, n (%)		
Normal weight	5,100 (24.3)	1,051 (20.6)
Overweight	8,194 (39.0)	1,612 (19.7)
Obese class I	4,789 (22.8)	1,028 (21.5)
Obese class II	1,805 (8.6)	457 (25.3)
Obese class III	1,136 (5.4)	314 (27.6)
Household income, $/y n (%)		
≤10,000	1,486 (7.1)	448 (30.2)
10,001-20,000	3,059 (14.6)	757 (24.8)
20,001-30,000	3,281 (15.6)	751 (22.9)
30,001-40,000	2,936 (14.0)	572 (19.5)
40,001-50,000	2,135 (10.2)	422 (19.8)
50,001-60,000	1,815 (8.6)	359 (19.8)
60,001-70,000	1,367 (6.5)	268 (19.6)
>70,000	2,882 (13.7)	480 (16.7)
Undetermined	2,063 (9.8)	405 (19.6)
Education, n (%)		
< 12 years	3,310 (15.7)	845 (25.5)
HS diploma or GED	9,704 (46.2)	1,979 (20.4)
Some college	5,612 (26.7)	1,204 (21.5)
Bachelor degree or higher	2,398 (11.4)	434 (18.1)
Race, n (%)		
White	20,514 (97.6)	4,349 (21.2)
Black	213 (1.0)	38 (17.8)
Other	297 (1.4)	75 (25.3)
Alcohol consumption, n (%)		
None	13,276 (63.2)	2,848 (21.5)
< 1 drink/month	2,589 (12.3)	597 (23.1)
< 1 drink/week	1,530 (7.3)	309 (20.2)
Few drinks/week	2,087 (9.9)	397 (19.0)
1-3 drinks/day	805 (3.8)	142 (17.6)
>3 drinks/day	310 (1.5)	66 (21.3)
Undetermined	427 (2.0)	103 (24.1)
Smoking status, n (%)		
Never smoker	9,804 (46.6)	1,906 (19.4)
Former smoker	7,555 (35.8)	1,693 (22.5)
Current smoker < 10 cig/day	1,212 (5.8)	256 (21.1)
Current smoker 10-19 cig/day	1,260 (6.0)	310 (24.6)
Current smoker 20+ cig/day	1,213 (5.8)	297 (24.5)
Diabetes, n (%)	3,443 (16.4)	875 (25.4)
Thiazolidinedion use~	809 (23.5)	202 (25.0)
Other medications~	1,244 (36.1)	321 (25.8)
No medication~	1,390 (40.4)	352 (25.3)

*percentages refer to the proportion with respect to the entire population; ^percentages reflect the proportion of memory impaired in each category; percentages among diabetics only

Table 2: The association between PFAAs and self-report memory impairment in logistic regression for a doubling PFAA concentration, by quintiles of PFAAs, and in ordinal regression (n=21,024)

	Range (ng/mL)	Adjusted OR and 95% C.I.*
PFOS		0.93 (0.90-0.96)
1st quintile	0.25-14.4	Ref.
2nd quintile	14.5-20.4	0.96 (0.87-1.07)
3rd quintile	20.5-27.1	0.86 (0.78-0.96)
4th quintile	27.2-37.2	0.87 (0.78-0.96)
5th quintile	37.3-759.2	0.85 (0.76-0.94)
Trend		<0.001
Ordinal regression		0.95 (0.93-0.98)
PFOA		0.96 (0.94-0.98)
1st quintile	0.25-14.0	Ref.
2nd quintile	14.1-27.0	0.88 (0.79-0.97)
3rd quintile	27.1-53.8	0.83 (0.75-0.92)
4th quintile	53.9-118.1	0.79 (0.71-0.88)
5th quintile	118.3-22,412	0.79 (0.71-0.88)
Tend		<0.001
Ordinal regression		0.97 (0.96-0.98)
PFNA		0.96 (0.91-1.00)
1st quintile	0.25-0.90	Ref.
2nd quintile	1.0-1.2	0.86 (0.78-0.96)
3rd quintile	1.3-1.4	0.87 (0.77-0.98)
4th quintile	1.5-1.9	0.86 (0.77-0.95)
5th quintile	2.0-28.6	0.89 (0.80-0.99)
Trend		0.053
Ordinal regression		0.97 (0.94-1.01)
PFHxS		0.96 (0.93-0.99)
1st quintile	0.25-1.7	Ref.
2nd quintile	1.8-2.6	1.01 (0.91-1.12)
3rd quintile	2.7-3.6	1.02 (0.91-1.13)
4th quintile	3.7-5.6	0.93 (0.84-1.04)
5th quintile	5.7-232.6	0.89(0.79-0.99)
Trend		0.009
Ordinal regression		0.97 (0.94-0.99)

*Model adjusted for age (one-year age bands), ethnicity, gender, and school level (categorical), household income (categorical), physical activity, alcohol consumption (categorical, none/<1drink/month, < 1 drink/week, few drinks/week, 1-3 drinks/day, >3 drinks/day, undetermined), and cigarette smoking (categorical, never, former, < 10 cig/day, 12-20 cig/day, 20+ cig/day)

Table 3: The association between PFAAs and self-report memory impairment in logistic regression for a doubling PFAA concentration, by quintiles of distribution, and in ordinal regression by diabetes status (validated by clinical records)

	Range (ng/mL)	OR (95% CI)*	p for inter	N	OR (95% CI)*	p for inter
	PFOS N=17,832			**PFOA N=17,832**		
Non-diabetics		0.93 (0.90-0.96)†	-		0.95 (0.93-0.97) †	-
Ordinal regression		0.96 (0.93-0.99)			0.96 (0.95-0.98)	
1st quintile	0.25-14.5	Ref.		0.25-14.3	Ref.	
2nd quintile	14.6-20.5	0.96 (0.86-1.08)		14.4-27.2	0.85 (0.76-0.95)	
3rd quintile	20.6-27.0	0.90 (0.80-1.01)		27.3-54.3	0.82 (0.73-0.92)	
4th quintile	27.1-37.1	0.88 (0.78-0.99)		54.4-119.1	0.76 (0.68-0.86)	
5th quintile	37.2-759.2	0.85 (0.76-0.96)		119.2-8,416	0.75 (0.67-0.84)	
Trend		0.002			<0.001	
	N=3,192			**N=3,192**		
Diabetics		0.94 (0.88-1.02) †	0.698		1.02 (0.97-1.06) †	0.014
Ordinal regression		0.95 (0.90-1.01)			1.00 (0.97-1.04)	
1st quintile	0.25-13.6	Ref.		0.25-12.6	Ref.	
2nd quintile	13.7-20.0	1.06 (0.82-1.36)		12.7-25.4	1.04 (0.80-1.34)	
3rd quintile	20.1-27.3	0.82 (0.63-1.06)		25.5-48.0	0.88 (0.67-1.14)	
4th quintile	27.4-37.3	0.87 (0.67-1.13)		48.1-102.1	1.04 (0.80-1.35)	
5th quintile	37.4-272.0	0.90 (0.69-1.17)		102.4-22,412	1.09 (0.84-1.42)	
		0.162			0.543	
	PFNA			**PFHxS**		
	N=17,832			**N=17,832**		
Non-diabetics		0.95 (0.90-0.99) †	-		0.96 (0.93-0.99) †	-
Ordinal regression		0.97 (0.93-1.01)			0.97 0.94-0.99)	
1st quintile	0.25-0.9	Ref.		0.25-1.8	Ref.	
2nd quintile	1.0-1.2	0.86 (0.77-0.97)		1.9-2.6	0.98 (0.87-1.10)	
3rd quintile	1.3-1.5	0.85 (0.76-0.95)		2.7-3.7	0.99 (0.89-1.11)	
4th quintile	1.6-1.9	0.83 (0.73-0.93)		3.8-5.7	0.93 (0.82-1.05)	
5th quintile	2.0-28.6	0.88 (0.78-0.99)		5.8-232.6	0.88 (0.79-0.99)	
Trend		0.031			0.029	
	N=3,192			**N=3,192**		
Diabetics		1.01 (0.90-1.13) †	0.259	3,192	0.99 (0.92-1.06) †	0.683
Ordinal regression		0.99 (0.91-1.09)				
1st quintile	0.25-0.8	Ref.		0.25-1.6	Ref.	
2nd quintile	0.9-1.1	1.06 (0.80-1.40)		1.7-2.3	1.06 (0.81-1.38)	
3rd quintile	1.2-1.4	0.88 (0.66-1.17)		2.4-3.2	1.10 (0.85-1.42)	
4th quintile	1.5-1.8	1.03 (0.77-1.36)		3.3-5.0	1.02 (0.79-1.33)	
5th quintile	1.9-14.5	1.08(0.82-1.43)		5.1-99.7	1.00 (0.77-1.31)	
		0.620			0.942	

*using clinical record validated diagnosis of diabetes and self-reported use of medications, adjusted for age (one-year age bands), ethnicity, gender, school level (categorical), household income (categorical), physical activity, alcohol consumption (categorical, none/<1drink/month, < 1 drink/week, few drinks/week, 1-3 drinks/day, >3 drinks/day, undetermined), and cigarette smoking (categorical, never, former, < 10 cig/day, 12-20 cig/day, 20+ cig/day); † OR for doubling PFAA concentration

Table 4: Sensitivity analysis of the association between PFAAs and self-report memory impairment for a doubling PFAA concentration, by quintiles of PFAAs, and in ordinal regression on subjects aged 65 years and older (n=7,097), and using severe memory impairment as outcome measure (n=21,024).

	Range (ng/mL)	OR (95% C.I.)[b] N=7,097 Restricted to those aged 65+*	Range (ng/mL)	OR (95% C.I.)[b] N=21,024 Severely memory impaired^
PFOS		0.95 (0.90-1.00)		0.96 (0.90-1.02)
Ordinal regression		0.98 (0.94-1.03)		
1st quintile	0.25-15.3	Ref.	0.25-14.4	Ref.
2nd quintile	15.4-22.0	0.99 (0.83-1.20)	14.5-20.4	1.13 (0.94-1.35)
3rd quintile	22.1-28.9	0.95 (0.79-1.14)	20.5-27.1	0.92 (0.76-1.11)
4th quintile	29.0-4.0	0.97 (0.81-1.16)	27.2-37.2	0.92 (0.75-1.12)
5th quintile	40.1-759.2	0.84 (0.70-1.01)	37.3-759.2	0.92 (0.75-1.12)
Trend		0.079		0.094
PFOA		0.99 (0.97-1.03)		0.95 (0.92-0.98)
Ordinal regression		1.00 (0.97-1.03)		
1st quintile	0.25-15.0	Ref.	0.25-14.0	Ref.
2nd quintile	15.1-29.6	0.91 (0.75-1.09)	14.1-27.0	0.84 (0.70-1.01)
3rd quintile	29.7-56.8	0.90 (0.75-1.08)	27.1-53.8	0.85(0.71-1.02)
4th quintile	56.9-123.0	0.84 (0.70-1.01)	53.9-118.1	0.79 (0.66-0.96)
5th quintile	123.1-5,994.8	0.99(0.83-1.19)	118.3-22,412	0.75 (0.61-0.91)
Tend		0.680		0.003
PFNA		0.95 (0.87-1.02)		0.92 (0.85-1.00)
Ordinal regression		0.99 (0.93-1.07)		
1st quintile	0.25-0.8	Ref.	0.25-0.90	Ref.
2nd quintile	0.9-1.1	0.88 (0.73-1.06)	1.0-1.2	0.89 (0.74-1.07)
3rd quintile	1.2-1.4	0.81 (0.67-0.98)	1.3-1.4	0.82 (0.66-1.01)
4th quintile	1.5-1.8	0.82 (0.68-0.99)	1.5-1.9	0.85 (0.71-1.02)
5th quintile	1.9-11.7	0.88 (0.73-1.07)	2.0-28.6	0.79 (0.65-0.97)
Trend		0.177		0.023
PFHxS		0.96 (0.91-1.01)		0.98 (0.93-1.04)
Ordinal regression		0.98 (0.93-1.02)		
1st quintile	0.25-1.9	Ref.	0.25-1.7	Ref.
2nd quintile	2.0-2.8	0.98 (0.82-1.18)	1.8-2.6	1.10(0.91-1.33)
3rd quintile	2.9-3.9	0.95 (0.79-1.15)	2.7-3.6	1.04 (0.86-1.27)
4th quintile	4.0-6.0	0.98 (0.82-1.17)	3.7-5.6	0.91 (0.75-1.12)
5th quintile	6.1-232.6	0.86 (0.71-1.03)	5.7-232.6	0.98 (0.80-1.19)
Trend		0.139		0.283

[b]Model 2 includes age (one-year age bands), ethnicity, gender, and school level (categorical), household income (categorical), physical activity, alcohol consumption (categorical, none/<1drink/month, < 1 drink/week, few drinks/week, 1-3 drinks/day, >3 drinks/day, undetermined), and cigarette smoking (categorical, never, former, < 10 cig/day, 12-20 cig/day, 20+ cig/day)

* Sensitivity analysis including subjects aged 65 years or older only (N=7,097)

^ Sensitivity analysis using a more restrictive definition of memory impairment (those reporting frequent episode of short-term memory loss only, cases = 1,115)

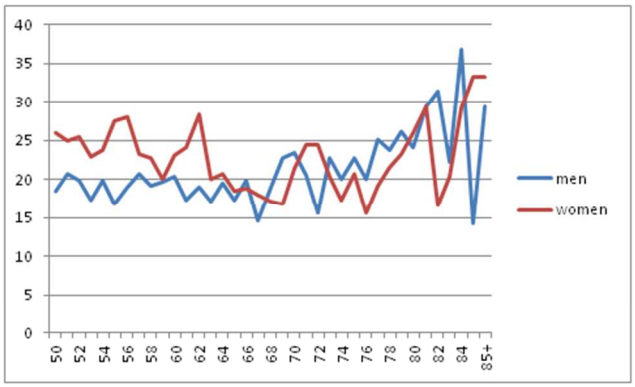

Figure 1: Prevalence of self-reported short-term memory impairment by age and sex in the study population
203x162mm (96 x 96 DPI)

Online Table 1: The association between PFAAs and self-reported memory impairment in logistic regression for a doubling PFAA concentration, by quintiles of PFAAs, and in ordinal regression (n=21,024).

	Range (ng/mL)	Model 1[a]	Model 2[b]	Model 3[c]
PFOS		0.92 (0.89-0.95)	0.93 (0.90-0.96)	0.93 (0.90-0.96)
Ordinal regression		0.95 (0.92-0.97)	0.95 (0.93-0.98)	0.96 (0.93-0.98)
1st quintile	0.25-14.4	Ref.	Ref.	Ref.
2nd quintile	14.5-20.4	0.95 (0.85-1.05)	0.96 (0.87-1.07)	0.97 (0.88-1.08)
3rd quintile	20.5-27.1	0.84 (0.76-0.93)	0.86 (0.78-0.96)	0.87 (0.79-0.97)
4th quintile	27.2-37.2	0.83 (0.75-0.93)	0.87 (0.78-0.96)	0.88 (0.79-0.97)
5th quintile	37.3-759.2	0.81 (0.73-0.91)	0.85 (0.76-0.94)	0.86 (0.77-0.96)
Trend		<0.001	<0.001	0.001
PFOA		0.95 (0.94-0.97)	0.96 (0.94-0.98)	0.96 (0.94-0.98)
Ordinal regression		0.97 (0.95-0.98)	0.97 (0.96-0.98)	0.97 (0.96-0.99)
1st quintile	0.25-14.0	Ref.	Ref.	Ref.
2nd quintile	14.1-27.0	0.86 (0.78-0.96)	0.88 (0.79-0.97)	0.88 (0.80-0.98)
3rd quintile	27.1-53.8	0.82 (0.74-0.91)	0.83 (0.75-0.92)	0.84 (0.76-0.93)
4th quintile	53.9-118.1	0.77 (0.70-0.86)	0.79 (0.71-0.88)	0.81 (0.73-0.90)
5th quintile	118.3-22,412	0.76 (0.69-0.85)	0.79 (0.71-0.88)	0.80 (0.72-0.90)
Tend		<0.001	<0.001	<0.001
PFNA		0.94 (0.90-0.98)	0.96 (0.91-1.00)	0.96 (0.92-1.01)
Ordinal regression		0.96 (0.93-0.99)	0.97 (0.94-1.01)	0.98 (0.94-1.01)
1st quintile	0.25-0.90	Ref.	Ref.	Ref.
2nd quintile	1.0-1.2	0.85 (0.77-0.94)	0.86 (0.78-0.96)	0.87 (0.78-0.96)
3rd quintile	1.3-1.4	0.85 (0.76-0.95)	0.87 (0.77-0.98)	0.88 (0.78-0.98)
4th quintile	1.5-1.9	0.83 (0.75-0.92)	0.86 (0.77-0.95)	0.86 (0.78-0.95)
5th quintile	2.0-28.6	0.85 (0.76-0.94)	0.89 (0.80-0.99)	0.90 (0.81-1.01)
Trend		0.004	0.053	0.079
PFHxS		0.95 (0.92-0.98)	0.96 (0.93-0.99)	0.97 (0.94-1.00)
Ordinal regression		0.96 (0.94-0.99)	0.97 (0.94-0.99)	0.97 (0.95-0.99)
1st quintile	0.25-1.7	Ref.	Ref.	Ref.
2nd quintile	1.8-2.6	1.00 (0.90-1.11)	1.01 (0.91-1.12)	1.02 (0.91-1.13)
3rd quintile	2.7-3.6	1.00 (0.90-1.11)	1.02 (0.91-1.13)	1.03 (0.93-1.15)
4th quintile	3.7-5.6	0.91 (0.82-1.02)	0.93 (0.84-1.04)	0.96 (0.86-1.06)
5th quintile	5.7-232.6	0.86 (0.77-0.96)	0.89(0.79-0.99)	0.92 (0.82-1.02)
Trend		0.001	0.009	0.053

Online Table 2: The association between PFAAs and self-report memory impairment for a doubling PFAA concentration and by tertiles of distribution by self-reported anti-diabetic treatment

	Range (ng/ML)	N	PFOS OR (95% CI)*	Range (ng/ML)	PFOA OR (95% CI)*	Range (ng/ML)	PFNA OR (95% CI)*	Range (ng/ML)	PFHxS OR (95% CI)*
Thiazolidinedione use		809	1.00 (0.86-1.16)		0.97 (0.88-1.07)		0.94 (0.74-1.19)		1.02 (0.87-1.20)
Ordinal regression			1.06 (0.93-1.20)		1.03 (0.95-1.11)		1.02 (0.84-1.25)		1.05 (0.92-1.20)
1st tertile	0.25-17.9		Ref.	1.1-17.5	Ref.	0.25-1.0	Ref.	0.25-1.9	Ref.
2nd tertile	18.0-29.9		0.76 (0.50-1.16)	17.6-49.7	0.72 (0.47-1.10)	1.1-1.5	0.83 (0.54-1.26)	2.0-3.5	1.56 (1.02-2.38)
3rd tertile	30.1-104.9		0.93 (0.61-1.42)	19.9-8,068	0.81 (0.53-1.24)	1.6-14.7	0.79 (0.51-1.23)	3.6-84.0	1.13 (0.72-1.77)
p-value for trend			0.737		0.333		0.309		0.628
Other medications		1,244	0.90 (0.80-1.01)		1.00 (0.93-1.07)		0.95 (0.79-1.15)		0.91 (0.81-1.03)
Ordinal regression			0.92 (0.83-1.01)		1.00 (0.93-1.07)		0.94 (0.81-1.10)		0.94 (0.86-1.04)
1st tertile	0.25-17.9		Ref.	0.25-20.5	Ref.	0.25-1.1	Ref.	0.25-2.1	Ref.
2nd tertile	18.0-29.8		0.75 (0.54-1.04)	20.6-63.2	0.99 (0.71-1.39)	1.2-1.6	0.72 (0.52-1.01)	2.2-3.6	0.99 (0.71-1.38)
3rd tertile	29.9-218.0		0.68 (0.48-0.95)	63.4-2,316.2	0.92 (0.66-1.29)	1.7-6.0	0.85 (0.61-1.20)	3.7-99.7	0.82 (0.58-1.16)
p-value for trend			0.023		0.644		0.341		0.259
No medication		1,390	0.95 (0.85-1.07)		1.00 (0.94-1.08)		1.03 (0.87-1.23)		1.01 (0.90-1.13)
Ordinal regression			0.94 (0.86-1.03)		1.00 (0.95-1.06)		0.98 (0.85-1.13)		0.99 (0.90-1.08)
1st tertile	0.25-18.3		Ref.	0.7-20.2	Ref.	0.25-1.0	Ref.	0.25-2.1	Ref.
2nd tertile	18.4-29.3		1.11 (0.81-1.52)	20.3-63.4	1.05 (0.77-1.44)	1.1-1.5	1.01 (0.72-1.40)	2.2-3.7	0.93 (0.68-1.28)
3rd tertile	29.4-272.0		1.02 (0.74-1.40)	63.5-22,412	0.99 (0.72-1.37)	1.6-14.5	1.12 (0.81-1.54)	3.8-43.3	0.99 (0.72-1.37)
p-value for trend			0.897		0.984		0.473		0.957

*using clinical record validated diagnosis of diabetes and self-reported use of medications, adjusted for age (one-year age bands), ethnicity, gender, school level (categorical), household income (categorical), physical activity, alcohol consumption (categorical, none/<1drink/month, < 1 drink/week, few drinks/week, 1-3 drinks/day, >3 drinks/day, undetermined), and cigarette smoking (categorical, never, former, < 10 cig/day, 12-20 cig/day, 20+ cig/day); † OR for doubling PFAA concentration

Table S1
(Gallo et al. [2011] PLoS Med; doi:10.1371/journal.pmed.1001117)

The Strengthening the Reporting Observational studies in Epidemiology – Molecular Epidemiology (STROBE-ME) Reporting Recommendations: Extended from STROBE statement

Item	Item number	STROBE Guidelines	Extension for Molecular Epidemiology Studies (STROBE-ME)
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	**ME-1** State the use of specific biomarker(s) in the title and/or in the abstract if they contribute substantially to the findings
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	
Introduction			
Background rationale	2	Explain the scientific background and rationale for the investigation being reported	**ME-2** Explain in the scientific background of the study how/why the specific biomarker(s) have been chosen, potentially among many others (e.g., others are studied but reported elsewhere, or not studied at all)
Objectives	3	State specific objectives, including any pre-specified hypotheses	**ME-3** *A priori* hypothesis: if one or more biomarkers are used as proxy measures, state the *a priori* hypothesis on the expected values of the biomarker(s)
Methods			
Study design	4	Present key elements of study design early in the paper	**ME-4** Describe the special study designs for molecular epidemiology (in particular nested case/control and case/cohort) and how they were implemented
Biological sample collection			**ME-4.1** Report on the setting of the biological sample collection; amount of sample; nature of collecting procedures; participant conditions; time between sample collection and relevant clinical or physiological endpoints.
Biological sample storage			**ME-4.2** Describe sample processing (centrifugation, timing, additives, etc).
Biological sample processing			**ME-4.3** Describe sample storage until biomarker analysis (storage, thawing, manipulation, etc).
Biomarker biochemical characteristics			**ME-4.4** Report the half-life of the biomarker, and chemical and physical characteristics (e.g., solubility).
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	
Participants	6	(a) Cohort study—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up Case-control study—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants	**ME-6** Report any habit, clinical conditions, physiological factor, or working or living condition that might affect the characteristics or concentrations of the biomarker
		(b) Cohort study—For matched studies, give matching criteria and number of exposed and unexposed Case-control study—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	
Data source/measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	**ME-8** Laboratory methods: report type of assay used, detection limit, quantity of biological sample used, outliers, timing in the assay procedures (when applicable) and calibration procedures or any standard used
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	**ME-12** Describe how biomarkers were introduced into statistical models
		(b) Describe any methods used to examine subgroups and interactions	
		(c) Explain how missing data were addressed	
		(d) Cohort study—If applicable, explain how loss to follow-up was addressed Case-control study—If applicable, explain how matching of cases and controls was addressed Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(e) Describe any sensitivity analyses	

Validity/reliability of measurement and internal/external validation			**ME-12.1** Report on the validity and reliability of measurement of the biomarker(s) coming from the literature and any internal or external validation used in the study.
Results			
Participants	13	(a) Report the numbers of individuals at each stage of the study—e.g., numbers potentially eligible, examined for eligibility, con-firmed eligible, included in the study, completing follow-up, and analysed	**ME-13** Give reason for loss of biological samples at each stage
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14	(a) Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential con-founders	
		(b) Indicate the number of participants with missing data for each variable of interest	
		(c) Cohort study—Summarise follow-up time (e.g., average and total amount)	
Distribution of biomarker measurement			**ME-14.1** Give the distribution of the biomarker measurement (including mean, median, range, and variance)
Outcome data	15	Cohort study—Report numbers of outcome events or summary measures over time Case-control study—Report numbers in each exposure category, or summary measures of exposure Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included	
		(b) Report category boundaries when continuous variables were categorized	
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	**ME-19** Describe main limitations in laboratory procedures
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	**ME-20** Give an interpretation of results in terms of *a-priori* biological plausibility
Generalisability	21	Discuss the generalisability (external validity) of the study results	
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	
Ethics			**ME-22.1** Describe informed consent and approval from ethical committee(s). Specify whether samples were anonymous, anonymised or identifiable

Serum perfluoroalkyl acids concentrations and memory impairment in a large cross-sectional study

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Serum perfluoroalkyl acids concentrations and memory impairment in a large cross-sectional study

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Summary

Article focus

- Cross-sectional association between serum level of Perfluorooctanate (PFOA), perfuorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA) and perfluorohexane sulfonate (PFHxS) and self-reported memory impairment in a population exposed to high levels of PFOA
- Potential interaction between the association of perfluoroalkyl acids (PFAAs) with memory impairment by diabetes status

Key Message

- Inverse associations between PFOS and PFOA and memory impairment were statistically significant perhaps due to a potential anti-inflammatory effect exerted through PPAR agonism. Comparable inverse associations with PFNA and PFHxS were of borderline statistical significance
- Inverse associations of PFAAs with memory impairment were weaker or non-existent in diabetics than overall in non-diabetics. Analysis further stratified by specific diabetes medication use showed no variation in odds ratios more than explicable by chance given the number of tests made.

Strengths and limitations

- Very large sample size including 21,024 adults with measured serum level of Perfluoroalkyl acids (PFAAs) with a given geographical distribution allowing some multilevel modelling
- The cross-sectional nature of the design does not allow any causal inference and makes results particularly prone to reverse causality
- Self-reported is not an optimal method for estimating the degree of memory impairment in a population

ABSTRACT

Objectives – To examine the cross-sectional association between serum PFOA, PFOS, PFNA, and PFHxS concentrations with self-reported memory impairment in adults and the interaction of these associations with diabetes status

Design - Cross-sectional study

Setting – Population-based in Mid-Ohio Valley, West Virginia following contamination by a chemical plant

Participants - The C8 Health Project collected data and measured serum level of PFAAs of 21,024 adults aged 50+ years

Primary outcome measure – Self-reported memory impairment as defined by the question "have experienced short term memory loss?"

Results - A total of 4,057 subjects self-reported short-term memory impairment. Inverse associations between PFOS and PFOA and memory impairment were highly statistically significant with fully adjusted OR=0.93 (95% C.I. 0.90-0.96) for doubling PFOS and OR=0.96 (95% C.I. 0.94-0.98) for doubling PFOA concentrations. Comparable inverse associations with PFNA and PFHxS were of borderline statistical significance. Inverse associations of PFAAs with memory impairment were weaker or non-existent in diabetics than overall in non-diabetics.

Conclusion - An inverse association between PFAA serum levels and self-reported memory impairment has been observed in this large population-based, cross-sectional study stronger and more statistically significant for PFOA and PFOS. The associations can be potentially explained by preventive anti-inflammatory effect exerted by a PPAR agonist effect of these PFAAs, but confounding or even reverse causation cannot be excluded as an alternative explanation.

INTRODUCTION

Perfluoroalkyl acids (PFAAs) are man-made compounds used during the manufacture of fluoropolymers including non-stick cookware and breathable, yet waterproof, fabrics. They can also result from the metabolism of fluorinated telomers, compounds used for food package coatings, carpet treatments, and stain-resistant fabric treatment. Perfluorooctanate (PFOA), perfuorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA) and perfluorohexane sulfonate (PFHxS) persist in the environment and are found in the blood of humans and many animal species throughout the world [1 2]. Potential sources of exposure to PFAAs in humans include drinking water, dust, breast milk, fish and other foods, food packaging, ambient air, and occupational exposure [3-6].

In animal models, perfluoroalkyl chemicals can activate peroxisome proliferator-activated receptor alpha (PPARα), a ligand-activated transcription factor that regulates gene expression, lipid modulation, glucose homeostasis, cell proliferation and inflammation [7 8]. The PPAR receptor has been involved in the ageing process: PPARα null mice showed a decreased longevity compared with the wild-type due to non-neoplastic spontaneous ageing lesions which occurred with a higher incidence and a short latency in the PPARα null mice [9]. Also PPARγ variants were reported to be associated with longevity in humans with low insulin resistance [10 11]. Activation of the PPARγ receptor *in vitro* and *in vivo* also prevents the expression of inflammatory cytokines and other inflammatory mediators in brains of Alzheimer disease animal models [12 13]. In addition, PPARγ agonists have been demonstrated to suppress the Aβ-mediated activation of microglia *in vitro* and prevent cortical or hippocampal neuronal cell death [14-16]. PPARγ is also deeply involved in diabetes, given its ability to orchestrate the expression of genes involved in lipid metabolism, adipogenesis, and inflammation. It is activated by endogenous ligands (such as fatty acids and prostaglandins) or drugs such as thiazolidinedione. It is most highly expressed in adipocytes where it acts as the master regulator of adipogenesis via induction of adipogenic genes [17]. However, a study *in vitro* showed that PFOA and PFOS activate differentially PPARα and PPARγ receptors, but it is not possible to directly extrapolate these results to toxicity studies *in vivo* [18]. Therefore, in line with what was recently observed by Power et al[19], we hypothesised that increased exposure to PFAA could be associated with a better cognitive function.

The positive association between diabetes and cognitive impairment is well established [20]; some studies investigating the association between PFOA exposure and diabetes suggested the presence of an inverse association: a negative trend in diabetes occurrence by increasing serum PFOA deciles

was found in a case–control study nested in a previous study based on the population investigated here [21 22]; but not in others [23 24].

From 1950-2005, a chemical plant in the Mid-Ohio Valley, West Virginia was responsible for emitting PFOA into the surrounding environment. In 2001, a group of residents from the nearby West Virginia and Ohio communities filed a class action lawsuit alleging health damage from drinking water supplies drawing on PFOA-contaminated groundwater [25]. Part of the pre-trial settlement of the class action lawsuit included a baseline survey, the C8 Health Project, conducted in 2005-2006, that gathered data from over 69,000 people from six contaminated water districts surrounding the plant [25]. In this population, PFOA concentrations ranged from US background levels to very high; overall PFOA levels are much higher in this population (geometric mean 42.6.0 ng/mL, 95% C.I. 41.8-43.3) than in the corresponding US population surveys (NHANES in same year mean 3.95 ng/mL, 95% C.I. 3.65-4.27) [1 25]. The mean PFOS (geometric mean 22.4, 95% C.I. 22.2-22.6), PFNA (1.37, 95% C.I. 1.36-1.38), and PFHXs (3.18, 95% C.I. 3.15-3.22) closely resembled values from a nationally representative US sample form a similar time frame (mean PFOS 20.7, 95% C.I. 19.2-22.3; mean PFNA 0.97, 95% C.I. 0.82-1.14; and PFHXs 1.93, 95% C.I. 1.73-2.16) [1].

The present study uses these data to examine the cross-sectional association between serum PFOA, PFOS, PFNA, and PFHxS concentrations with self-reported memory impairment in adults, and its potential interaction with diabetes status.

METHODS

The Study population

This study is one of the C8 Science Panel Studies and uses information from questionnaires and blood tests collected in the C8 Health Project, supplemented by further information on classification by water district developed in a companion C8 Science Panel Study.

The C8 Health Project enrolled eligible subjects between August 2005 and August 2006. Individuals were eligible to participate in the C8 Health Project if they had consumed water for at least one year between 1950 and December 3, 2004 while living, working, or going to school in one of the following six water districts: Little Hocking Water Association of Ohio; City of Belpre, Ohio; Tupper Plains–

Chester District of Ohio; Village of Pomeroy, Ohio; Lubeck Public Service District of West Virginia; Mason County Public Service District of West Virginia; or private water sources within aforementioned districts and areas of documented PFOA contamination. Details of the study enrolment process, including consenting procedures, have been described elsewhere [25].

The C8 Health Project collected data on 69,030 people. The participation rate for the C8 Health Project based on US census counts of residents in the affected water districts during Project enrolment, have been estimated at around 80% [25]. In this population, the strongest predictor of PFOA serum concentration was residence in one of the contaminated water districts [26]; serum levels of other PFAAs do not show such geographic variation. Of the population, 21,724 older adults (aged ≥50 years) were considered for this analysis, and a total of 21,024 (96.8%) were included in the final analysis after exclusion of subjects with missing data on ethnicity, education level, socio-economic status, cigarette smoking, or BMI measurements.

Memory impairment definition

During the survey (2005-2006), all participants were asked if they "had experienced short term memory loss", the possible answers being "frequently", "sometimes", "rarely", and "never". The principle analyses assessed memory impairment defined as reporting short-term memory loss frequently or sometimes, compared to rarely and never. Memory impairment ever was also considered, defined as reporting any memory loss and compared to the never category.

Laboratory analysis

Blood samples were obtained and processed at individual data collection sites. Samples were drawn into four tubes per participant, with a maximum 35 mL. Tubes were spun, aliquoted, and refrigerated until shipping. Samples were shipped on dry ice daily from each data collection site to the laboratory daily [25]. Participants were not asked to fast before blood sample withdrawal, but fasting status was recorded.

Laboratory analysis of PFAAs used an automated solid-phase extraction combined with reverse-phase high-performance liquid chromatography [27]. Analyses were conducted by the Exygen Research Inc., State College, PA, USA; an intra-laboratory quality assurance program was carried out by analysis of duplicate samples at AXYS Analytical Service Ltd., Sidney, BC, Canada [25]. The intra-laboratory coefficient of variation for all PFAAs measurements was 0.1; the inter-laboratory comparison coefficient of variation was 0.2 for PFOA and PFNA, 0.1 for PFOS, and not applicable for PFHxA as all in the second lab measurement values were below level of detection[25]. The detection

limit for all PFAAS was 0.5 ng/mL and observations below this limit were assigned a value of 0.25 ng/mL (*n*=16, *n*=101, n=532, and n=387for PFOA, PFOS, PFNA, and PFHxS, respectively, for this study population). All PFAAs concentration distributions were skewed to the right. Methods and results are reported according to STROBE-ME recommendations [28].

Statistical analysis

Associations between exposure (serum concentration of PFOA, PFOS, PFNA, and PFHxS) and memory impairment were studied using logistic regression. Minimally adjusted models included age, in one year age-band, race (white, black, and others), gender, and educational level (high school diploma or general educational development (GED), some college, bachelor degree or higher) (Model 1). Further adjusted models additionally included average household income (≤$10,000, $10,001-20,000, $20,001-30,000, $30,001-40,000, $40,001-50,000, $50,001-60,000, $60,001-70,000, >$70,000), physical activity, alcohol consumption (none, <1 drink/month, <1 drink/week, few drinks/week, >1 drink/day) and cigarette smoking (never smoker, former smoker, current smoker <10 cigarettes/day, current smoker 10-19 cigarettes/day, current smoker ≥20 cigarettes/day) (Model 2). Fully adjusted models included also body mass index (BMI) (underweight/normal weight; overweight; and obese class I, II, and III), and diabetes (Model 3). PFAA concentrations were log-transformed to reduce skewness. For each model the association between PFAAs and self-reported memory impairment was calculated for a doubling in PFAA concentration in a model with PFAA entered as continuous covariate, for quintile groups of the PFAA distribution, and by ordinal regression analysis with the outcome variable comprising the four original levels of slef-reported frequency of episodes of memory loss, again in relation to a doubling of PFAAs. To explore possible differential effect of PFAA in sub-groups, analyses were further stratified by diabetes status and, among diabetics, by type of medications.

The following four sensitivity analyses were carried out: firstly one analysis restricting the sample to 7,097 subjects aged 65 years and older. Secondly, an analysis conducted on the entire sample, but using as outcome definition those reporting any memory loss (frequently, sometimes, and rarely). Our final sensitivity analysis utilises the geographical clustering of PFOA exposure by water districts which allowed use to decompose the overall estimate of association of PFOA with memory impairment into within and between water district components, by including as explanatory variables both water district mean logged PFOA serum concentration and the deviations of individual's values from their district mean [29]. These two associations are subject to different potential biases, so help interpretation.

Role of funding

Funding for this work, the "C8 Science Panel Community Study at London School of Hygiene and Tropical Medicine - LSHTM", comes from the C8 Class Action Settlement Agreement (Circuit Court of Wood County, WV, USA) between DuPont and plaintiffs, which resulted from releases of PFOA (or C8) into drinking water. It is one of the C8 Science Panel Studies undertaken by the Court-approved C8 Science Panel established under the same Settlement Agreement. The task of the C8 Science Panel, of which Tony Fletcher is a member, is to undertake research in the Mid-Ohio Valley, and subsequently evaluate the results along with other available information to determine if there are any probable links between PFOA and disease. Funds were administered by the Garden City Group (Melville, NY) that reports to the Court. The authors of this manuscript declare that their ability to design, conduct, interpret, or publish research was unimpeded by and fully independent of the court and/or settling parties. In addition, they declare no competing financial interests. The LSHTM Ethics Committee approved this study.

RESULTS

A total of 4,462 subjects (21.2% of the entire population of 21,024 individuals aged 50 years or older) self-reported short-term memory impairment (**Error! Reference source not found.**): episodes f short-term memory loss were reported frequently by 1,115 subjects (5.3%); sometimes by 3,347 (15.9%); rarely by 4,283 (20.4%) and never by 12,279 (58.4%). Many personal characteristics were associated individually with memory loss, including higher age, lower socio-economic status, smoking, and diagnosis of diabetes (Table 1), though to what extent these reflected independent risk factors was not investigated.

Results from the logistic regression of association between PFAAs and memory impairment are shown in Table 2. Results for minimally, further and fully adjusted models were similar, so we show only further adjusted results in this table, but results for all models are in the on-line Table 1. Inverse associations between PFOS and PFOA and memory impairment were highly statistically significant with adjusted OR=0.93 (0.90-0.96) for PFOS and OR=0.96 (0.94-0.98) for PFOA for doubling PFAA concentrations. Inverse associations of similar magnitude with PFNA and PFHxS but of borderline statistical significance were found: OR=0.96 (0.92-1.02) for PFNA and OR=0.97 (0.94-1.00) for PFHxS. The analysis by PFAA quintile groups shows similar patterns.

Inverse associations of PFAAs with memory impairment were weaker or non-existent in diabetics than for non-diabetics (Table 3), though odds ratios were imprecise, and the difference by diabetes status was only significant for PFOA (p-value for interaction = 0.014). Analysis further stratified by specific diabetes medication use showed no variation in odds ratios more than explicable by chance given the number of tests made (on-line Table 2).

In the sensitivity analysis on subjects older than 65 years, the substantial reduction of the sample size resulted in loss of precision in odds ratios. However, the points estimates of associations with memory impairment were of comparable magnitude for all PFAAs except PFOA for which the association with memory impairment was close to null (OR= 0.99 (0.97-1.03)) (Table 4).

The analysis carried out on the entire sample, comparing those with any memory impairment against those with no memory problems shows slightly weaker associations for each PFAAs but precision was reduced (Table 4). Analyses using ordinal regression in place of binary logistic regression yielded similar results to the logistic regressions (Table 2, Table 3, and Table 4).

The analysis separating the PFOA-memory impairment association into within and between water district components found that within water districts there was an inverse association between PFOA and memory impairment, as in the overall association (OR 0.94, 95%CI 0.91-0.98, scale and adjustments as before). However there was no association between geometric mean concentration by and memory impairment (OR 1.00, 95%CI 0.97-1.03, per doubling in geometric mean PFOA by district).

Extra data is available upon request by emailing Tony Fletcher (tony.fletcher@lshtm.ac.uk).

DISCUSSION

An inverse association between PFAAs serum levels (including PFOS, PFOA, PNA, and PFHxS) and self-reported memory impairment has been observed in this large population-based, cross-sectional study. This association is more clearly monotonic with increasing exposure, and more statistically significant for PFOA and PFOS. However, the consistent decrement for all PFAAs suggests a common mechanism.

It is plausible that PFAAs could have an effect on cognitive function via PPAR agonism. Although it is not clear to what extent PFAAs act differentially on PPAR receptors α and γ[18], it could be speculated

that this association might be mediated by the activation of the PPAR receptor by PFAAs. Activation of the PPARγ receptors has been shown to decrease the secretion of proinflamatory cytokines and possibly increase phagocytosis of Aβ inclusions, probably through activation of microglia[30]. However there was suggestion that this effect of suppression of the activation of microglia was age-dependent or disease stage-dependent being not significant in patients with advanced Alzheimer's disease (AD)[31 32]. PPAR agonist drugs, such as non-steroidal anti-inflammatory drugs (NSAID) and some anti-diabetics (i.e. thiazolodinedione or pioglitazone) have been proposed as preventive drugs for neurodegenerative conditions, including Alzheimer's dementia[30 33].

In a previous published study an inverse association between PFAAs and memory impairment was observed specifically among non-medicated diabetics[19]. In the present study, this pattern was not replicated, with the inverse association between PFAAs and cognitive impairment being more evident in those without diabetes; among diabetics, the association was not present, irrespective of treatment status. This finding could be explained by the fact that in diabetics PPAR receptors are more phosphorylated with a consequent reduced transcriptional activity [34 35], and the balance between PPARγ expression and activity levels is altered [34 36]. It is therefore possible – based on the present data – that the PPAR-agonist effect of PFAAs is different in subjects with and without PPAR-mediated metabolic changes such as diabetes. Also, it has been reported that PFAAs have a PPAR agonist effect, more prominently PPAR-α [37]; animal models suggest that PFOA has a stronger agonistic effect than PFOS [37]. Our findings of an inverse association between PFAA and memory impairment among non-diabetics, would therefore be compatible with a possible anti-inflammatory role exerted by PFAA on early symptoms of cognitive impairment.

There is some evidence of detrimental effects of PFAAs in neurodevelopment of mice affecting the cholinergic system and cognitive function[38-40], thus timing of exposure may also be relevant in order for the PFAAs to exert this hypothesised anti-dementing role. However, these findings should be interpreted cautiously given the limitations of the study. Firstly, given the cross-sectional nature of the study, reverse causality must be considered: subjects suffering from memory impairment could have consumed less of these compounds via water and food sources, though this is not a likely explanation given the consistency of the association across various PFAAs which have substantially different routes of exposure. Host characteristics such as genotype could be correlated with both some mechanism predisposing these symptoms and variation in PFAA excretion rates, thus leading to a confounded association with serum levels. Further, self-report is not a very accurate method for ascertaining memory impairment, although errors in classification would be expected to be non-differential misclassification, biasing the estimate of association towards the null. The effects of

PFAA have been mostly studied in relation to PPARα [7], while the receptor mostly implicated in metabolic changes and diabetes and in dementia PPARγ [30]; however, these two belong to the same receptor family and some degree of cross-activation cannot be excluded, and the knowledge of their pleiotropic effects is currently advancing [41]. Lastly, the classification into different anti-diabetic medications is uncertain as these were self-reported and not prompted by interviewers. However, we consider it very unlikely that any misreporting would be confounded with serum PFAAs. This would tend to low specificity and thus bias of the association (if any) towards the null.

On the other hand, strengths of this study include the fact that all showed estimates were adjusted for numerous potential confounders, including age in one-year age bands, making the effect of PFAA on memory impairment not likely to be confounded by lifestyle characteristics. Furthermore, these results are based on a very large population representative of the general population in West Virginia and Ohio [25], thus estimates are solid; and the 21% prevalence of memory impairment is compatible and consistent with figures on prevalence of dementia reported for North America (Ferri et al, 2005).

Individual serum levels reflect the contributions of both intake and retention/excretion rates. While we have no direct data on either of these components, the large differences in drinking water contamination and associated average population serum levels for PFOA in the 6 water districts, allow an estimate of the effect of exposure. That the association with PFOA was entirely within water districts, and not present at all between water districts despite large differences in (geometric) mean PFOA between districts (range 15.7 – 405.1) is suggestive of a bias operating at one or both of these levels. The between district estimate is not vulnerable to reverse causation or confounding at individual level, though some ecological confounding may operate if it happens to correlate with exposure level. Conversely the within district estimate but not between district estimate could reflect such individual confounding if present. Thus either the association documented at individual level could be confounded (e.g. by a common genetic variant related to both dementia risk and some excretion pathways); or that the association at the district level is biased towards the null (e.g. by confounding by socio-economic status). This sensitivity analysis cannot prove the presence of confounding at either level, but if the association had been consistent at both individual and district level that would have been more convincing of the association being due to PFAAs.

The size of the associations observed has both strong and weak aspects. The strong statistical significance suggests chance is an unlikely explanation. However, the odds ratios are only modestly different from one, 0.75 at the most extreme, so that biases are a more plausible explanation than they would be with more extreme ratios. In conclusion, these data show an inverse association

between PFOA and PFOS exposure and self-reported memory-impairment, particularly in non diabetics. This can be potentially explained by preventive anti-inflammatory effect exerted by a PPAR agonist effect of these PFAAs, but confounding or even reverse causation cannot be excluded as an alternative explanation.

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Table 1: Participant Characteristics, Mid-Ohio Valley, 2005-2006 (N=21,024)

	All N=21,024*	Memory impaired N=4,462^
Males, n (%)	10,353 (49.2)	2,040 (19.7)
Females, n (%)	10,671 (50.8)	2,422 (22.7)
Age, median/mean (SD)	60.5/62.3 (9.0)	59.9/62.3 (9.4)
Age groups		
50-54 years	5,381 (25.6)	1,185 (22.0)
55-59 years	4,831 (23.0)	1,055 (21.8)
60-64 years	3,715 (17.7)	740 (19.9)
65-69 years	2,930 (13.9)	535 (18.3)
70-74 years	1,979 (9.4)	419 (21.2)
75-79 years	1,251 (6.0)	269 (21.5)
80+ years	937 (4.5)	259 (27.6)
Regular exercise, n (%)	6,774 (32.2)	1,306 (19.3)
BMI, n (%)		
Normal weight	5,100 (24.3)	1,051 (20.6)
Overweight	8,194 (39.0)	1,612 (19.7)
Obese class I	4,789 (22.8)	1,028 (21.5)
Obese class II	1,805 (8.6)	457 (25.3)
Obese class III	1,136 (5.4)	314 (27.6)
Household income, $/y n (%)		
≤10,000	1,486 (7.1)	448 (30.2)
10,001-20,000	3,059 (14.6)	757 (24.8)
20,001-30,000	3,281 (15.6)	751 (22.9)
30,001-40,000	2,936 (14.0)	572 (19.5)
40,001-50,000	2,135 (10.2)	422 (19.8)
50,001-60,000	1,815 (8.6)	359 (19.8)
60,001-70,000	1,367 (6.5)	268 (19.6)
>70,000	2,882 (13.7)	480 (16.7)
Undetermined	2,063 (9.8)	405 (19.6)
Education, n (%)		
< 12 years	3,310 (15.7)	845 (25.5)
HS diploma or GED	9,704 (46.2)	1,979 (20.4)
Some college	5,612 (26.7)	1,204 (21.5)
Bachelor degree or higher	2,398 (11.4)	434 (18.1)
Race, n (%)		
White	20,514 (97.6)	4,349 (21.2)
Black	213 (1.0)	38 (17.8)
Other	297 (1.4)	75 (25.3)
Alcohol consumption, n (%)		
None	13,276 (63.2)	2,848 (21.5)
< 1 drink/month	2,589 (12.3)	597 (23.1)
< 1 drink/week	1,530 (7.3)	309 (20.2)
Few drinks/week	2,087 (9.9)	397 (19.0)
1-3 drinks/day	805 (3.8)	142 (17.6)
>3 drinks/day	310 (1.5)	66 (21.3)
Undetermined	427 (2.0)	103 (24.1)
Smoking status, n (%)		
Never smoker	9,804 (46.6)	1,906 (19.4)
Former smoker	7,555 (35.8)	1,693 (22.5)
Current smoker < 10 cig/day	1,212 (5.8)	256 (21.1)
Current smoker 10-19 cig/day	1,260 (6.0)	310 (24.6)
Current smoker 20+ cig/day	1,213 (5.8)	297 (24.5)
Diabetes, n (%)	3,443 (16.4)	875 (25.4)
Thiazolidinedion use~	809 (23.5)	202 (25.0)
Other medications~	1,244 (36.1)	321 (25.8)
No medication~	1,390 (40.4)	352 (25.3)

*percentages refer to the proportion with respect to the entire population; ^percentages reflect the proportion of memory impaired in each category; percentages among diabetics only

Table 2: The association between PFAAs and self-report memory impairment in logistic regression for a doubling PFAA concentration, by quintiles of PFAAs, and in ordinal regression (n=21,024)

	Range (ng/mL)	Adjusted OR and 95% C.I.*
PFOS		0.93 (0.90-0.96)
1st quintile	0.25-14.4	Ref.
2nd quintile	14.5-20.4	0.96 (0.87-1.07)
3rd quintile	20.5-27.1	0.86 (0.78-0.96)
4th quintile	27.2-37.2	0.87 (0.78-0.96)
5th quintile	37.3-759.2	0.85 (0.76-0.94)
Trend		<0.001
Ordinal regression		0.95 (0.93-0.98)
PFOA		0.96 (0.94-0.98)
1st quintile	0.25-14.0	Ref.
2nd quintile	14.1-27.0	0.88 (0.79-0.97)
3rd quintile	27.1-53.8	0.83 (0.75-0.92)
4th quintile	53.9-118.1	0.79 (0.71-0.88)
5th quintile	118.3-22,412	0.79 (0.71-0.88)
Tend		<0.001
Ordinal regression		0.97 (0.96-0.98)
PFNA		0.96 (0.91-1.00)
1st quintile	0.25-0.90	Ref.
2nd quintile	1.0-1.2	0.86 (0.78-0.96)
3rd quintile	1.3-1.4	0.87 (0.77-0.98)
4th quintile	1.5-1.9	0.86 (0.77-0.95)
5th quintile	2.0-28.6	0.89 (0.80-0.99)
Trend		0.053
Ordinal regression		0.97 (0.94-1.01)
PFHxS		0.96 (0.93-0.99)
1st quintile	0.25-1.7	Ref.
2nd quintile	1.8-2.6	1.01 (0.91-1.12)
3rd quintile	2.7-3.6	1.02 (0.91-1.13)
4th quintile	3.7-5.6	0.93 (0.84-1.04)
5th quintile	5.7-232.6	0.89(0.79-0.99)
Trend		0.009
Ordinal regression		0.97 (0.94-0.99)

*Model adjusted for age (one-year age bands), ethnicity, gender, and school level (categorical), household income (categorical), physical activity, alcohol consumption (categorical, none/<1drink/month, < 1 drink/week, few drinks/week, 1-3 drinks/day, >3 drinks/day, undetermined), and cigarette smoking (categorical, never, former, < 10 cig/day, 12-20 cig/day, 20+ cig/day)

Table 3: The association between PFAAs and self-report memory impairment in logistic regression for a doubling PFAA concentration, by quintiles of distribution, and in ordinal regression by diabetes status (validated by clinical records)

	Range (ng/mL)	OR (95% CI)*	p for inter	N	OR (95% CI)*	p for inter
	PFOS N=17,832			**PFOA N=17,832**		
Non-diabetics		0.93 (0.90-0.96)†	-		0.95 (0.93-0.97) †	-
Ordinal regression		0.96 (0.93-0.99)			0.96 (0.95-0.98)	
1st quintile	0.25-14.5	Ref.		0.25-14.3	Ref.	
2nd quintile	14.6-20.5	0.96 (0.86-1.08)		14.4-27.2	0.85 (0.76-0.95)	
3rd quintile	20.6-27.0	0.90 (0.80-1.01)		27.3-54.3	0.82 (0.73-0.92)	
4th quintile	27.1-37.1	0.88 (0.78-0.99)		54.4-119.1	0.76 (0.68-0.86)	
5th quintile	37.2-759.2	0.85 (0.76-0.96)		119.2-8,416	0.75 (0.67-0.84)	
Trend		0.002			<0.001	
	N=3,192			**N=3,192**		
Diabetics		0.94 (0.88-1.02) †	0.698		1.02 (0.97-1.06) †	0.014
Ordinal regression		0.95 (0.90-1.01)			1.00 (0.97-1.04)	
1st quintile	0.25-13.6	Ref.		0.25-12.6	Ref.	
2nd quintile	13.7-20.0	1.06 (0.82-1.36)		12.7-25.4	1.04 (0.80-1.34)	
3rd quintile	20.1-27.3	0.82 (0.63-1.06)		25.5-48.0	0.88 (0.67-1.14)	
4th quintile	27.4-37.3	0.87 (0.67-1.13)		48.1-102.1	1.04 (0.80-1.35)	
5th quintile	37.4-272.0	0.90 (0.69-1.17)		102.4-22,412	1.09 (0.84-1.42)	
		0.162			0.543	
	PFNA			**PFHxS**		
	N=17,832			**N=17,832**		
Non-diabetics		0.95 (0.90-0.99) †	-		0.96 (0.93-0.99) †	-
Ordinal regression		0.97 (0.93-1.01)			0.97 0.94-0.99)	
1st quintile	0.25-0.9	Ref.		0.25-1.8	Ref.	
2nd quintile	1.0-1.2	0.86 (0.77-0.97)		1.9-2.6	0.98 (0.87-1.10)	
3rd quintile	1.3-1.5	0.85 (0.76-0.95)		2.7-3.7	0.99 (0.89-1.11)	
4th quintile	1.6-1.9	0.83 (0.73-0.93)		3.8-5.7	0.93 (0.82-1.05)	
5th quintile	2.0-28.6	0.88 (0.78-0.99)		5.8-232.6	0.88 (0.79-0.99)	
Trend		0.031			0.029	
	N=3,192			**N=3,192**		
Diabetics		1.01 (0.90-1.13) †	0.259	3,192	0.99 (0.92-1.06) †	0.683
Ordinal regression		0.99 (0.91-1.09)				
1st quintile	0.25-0.8	Ref.		0.25-1.6	Ref.	
2nd quintile	0.9-1.1	1.06 (0.80-1.40)		1.7-2.3	1.06 (0.81-1.38)	
3rd quintile	1.2-1.4	0.88 (0.66-1.17)		2.4-3.2	1.10 (0.85-1.42)	
4th quintile	1.5-1.8	1.03 (0.77-1.36)		3.3-5.0	1.02 (0.79-1.33)	
5th quintile	1.9-14.5	1.08(0.82-1.43)		5.1-99.7	1.00 (0.77-1.31)	
		0.620			0.942	

*using clinical record validated diagnosis of diabetes and self-reported use of medications, adjusted for age (one-year age bands), ethnicity, gender, school level (categorical), household income (categorical), physical activity, alcohol consumption (categorical, none/<1drink/month, < 1 drink/week, few drinks/week, 1-3 drinks/day, >3 drinks/day, undetermined), and cigarette smoking (categorical, never, former, < 10 cig/day, 12-20 cig/day, 20+ cig/day); † OR for doubling PFAA concentration

Table 4: Sensitivity analysis of the association between PFAAs and self-report memory impairment for a doubling PFAA concentration, by quintiles of PFAAs, and in ordinal regression on subjects aged 65 years and older (n=7,097), and using any memory impairment as outcome measure (n=21,024).

	Range (ng/mL)	OR (95% C.I.)[b] N=7,097 Restricted to those aged 65+*	Range (ng/mL)	OR (95% C.I.)[b] N=21,024 Any memory impairment^
PFOS		0.95 (0.90-1.00)		0.96 (0.94-0.99)
Ordinal regression		0.98 (0.94-1.03)		
1st quintile	0.25-15.3	Ref.	0.25-14.4	Ref.
2nd quintile	15.4-22.0	0.99 (0.83-1.20)	14.5-20.4	0.96 (0.88-1.05)
3rd quintile	22.1-28.9	0.95 (0.79-1.14)	20.5-27.1	0.90 (0.82-0.98)
4th quintile	29.0-4.0	0.97 (0.81-1.16)	27.2-37.2	0.94 (0.86-1.03)
5th quintile	40.1-759.2	0.84 (0.70-1.01)	37.3-759.2	0.93 (0.85-1.02)
Trend		0.079		0.121
PFOA		0.99 (0.97-1.03)		0.97 (0.96-0.99)
Ordinal regression		1.00 (0.97-1.03)		
1st quintile	0.25-15.0	Ref.	0.25-14.0	Ref.
2nd quintile	15.1-29.6	0.91 (0.75-1.09)	14.1-27.0	0.90 (0.82-0.98)
3rd quintile	29.7-56.8	0.90 (0.75-1.08)	27.1-53.8	0.86 (0.79-0.94)
4th quintile	56.9-123.0	0.84 (0.70-1.01)	53.9-118.1	0.87 (0.79-0.95)
5th quintile	123.1-5,994.8	0.99(0.83-1.19)	118.3-22,412	0.85 (0.78-0.93)
Tend		0.680		<0.001
PFNA		0.95 (0.87-1.02)		0.98 (0.95-1.02)
Ordinal regression		0.99 (0.93-1.07)		
1st quintile	0.25-0.8	Ref.	0.25-0.90	Ref.
2nd quintile	0.9-1.1	0.88 (0.73-1.06)	1.0-1.2	0.89 (0.82-0.97)
3rd quintile	1.2-1.4	0.81 (0.67-0.98)	1.3-1.4	0.94(0.85-1.04)
4th quintile	1.5-1.8	0.82 (0.68-0.99)	1.5-1.9	0.92 (0.85-1.01)
5th quintile	1.9-11.7	0.88 (0.73-1.07)	2.0-28.6	0.94 (0.86-1.03)
Trend		0.177		0.493
PFHxS		0.96 (0.91-1.01)		0.97 (0.94-0.99)
Ordinal regression		0.98 (0.93-1.02)		
1st quintile	0.25-1.9	Ref.	0.25-1.7	Ref.
2nd quintile	2.0-2.8	0.98 (0.82-1.18)	1.8-2.6	0.98 (0.90-1.07)
3rd quintile	2.9-3.9	0.95 (0.79-1.15)	2.7-3.6	1.03 (0.94-1.13)
4th quintile	4.0-6.0	0.98 (0.82-1.17)	3.7-5.6	0.96 (0.87-1.04)
5th quintile	6.1-232.6	0.86 (0.71-1.03)	5.7-232.6	0.89 (0.81-0.97)
Trend		0.139		0.010

[b]Model 2 includes age (one-year age bands), ethnicity, gender, and school level (categorical), household income (categorical), physical activity, alcohol consumption (categorical, none/<1drink/month, < 1 drink/week, few drinks/week, 1-3 drinks/day, >3 drinks/day, undetermined), and cigarette smoking (categorical, never, former, < 10 cig/day, 12-20 cig/day, 20+ cig/day)

* Sensitivity analysis including subjects aged 65 years or older only (N=7,097)

^ Sensitivity analysis using a more restrictive definition of memory impairment (those reporting frequent episode of short-term memory loss only, cases = 1,115)

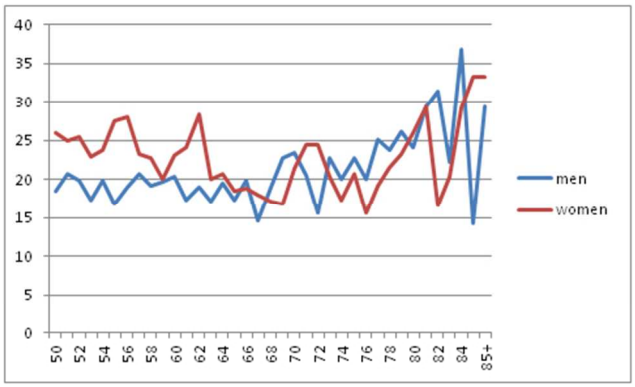

Figure 1: Prevalence of self-reported short-term memory impairment by age and sex in the study population
203x162mm (96 x 96 DPI)

Online Table 1: The association between PFAAs and self-reported memory impairment in logistic regression for a doubling PFAA concentration, by quintiles of PFAAs, and in ordinal regression (n=21,024).

	Range (ng/mL)	Model 1[a]	Model 2[b]	Model 3[c]
PFOS		0.92 (0.89-0.95)	0.93 (0.90-0.96)	0.93 (0.90-0.96)
Ordinal regression		0.95 (0.92-0.97)	0.95 (0.93-0.98)	0.96 (0.93-0.98)
1st quintile	0.25-14.4	Ref.	Ref.	Ref.
2nd quintile	14.5-20.4	0.95 (0.85-1.05)	0.96 (0.87-1.07)	0.97 (0.88-1.08)
3rd quintile	20.5-27.1	0.84 (0.76-0.93)	0.86 (0.78-0.96)	0.87 (0.79-0.97)
4th quintile	27.2-37.2	0.83 (0.75-0.93)	0.87 (0.78-0.96)	0.88 (0.79-0.97)
5th quintile	37.3-759.2	0.81 (0.73-0.91)	0.85 (0.76-0.94)	0.86 (0.77-0.96)
Trend		<0.001	<0.001	0.001
PFOA		0.95 (0.94-0.97)	0.96 (0.94-0.98)	0.96 (0.94-0.98)
Ordinal regression		0.97 (0.95-0.98)	0.97 (0.96-0.98)	0.97 (0.96-0.99)
1st quintile	0.25-14.0	Ref.	Ref.	Ref.
2nd quintile	14.1-27.0	0.86 (0.78-0.96)	0.88 (0.79-0.97)	0.88 (0.80-0.98)
3rd quintile	27.1-53.8	0.82 (0.74-0.91)	0.83 (0.75-0.92)	0.84 (0.76-0.93)
4th quintile	53.9-118.1	0.77 (0.70-0.86)	0.79 (0.71-0.88)	0.81 (0.73-0.90)
5th quintile	118.3-22,412	0.76 (0.69-0.85)	0.79 (0.71-0.88)	0.80 (0.72-0.90)
Tend		<0.001	<0.001	<0.001
PFNA		0.94 (0.90-0.98)	0.96 (0.91-1.00)	0.96 (0.92-1.01)
Ordinal regression		0.96 (0.93-0.99)	0.97 (0.94-1.01)	0.98 (0.94-1.01)
1st quintile	0.25-0.90	Ref.	Ref.	Ref.
2nd quintile	1.0-1.2	0.85 (0.77-0.94)	0.86 (0.78-0.96)	0.87 (0.78-0.96)
3rd quintile	1.3-1.4	0.85 (0.76-0.95)	0.87 (0.77-0.98)	0.88 (0.78-0.98)
4th quintile	1.5-1.9	0.83 (0.75-0.92)	0.86 (0.77-0.95)	0.86 (0.78-0.95)
5th quintile	2.0-28.6	0.85 (0.76-0.94)	0.89 (0.80-0.99)	0.90 (0.81-1.01)
Trend		0.004	0.053	0.079
PFHxS		0.95 (0.92-0.98)	0.96 (0.93-0.99)	0.97 (0.94-1.00)
Ordinal regression		0.96 (0.94-0.99)	0.97 (0.94-0.99)	0.97 (0.95-0.99)
1st quintile	0.25-1.7	Ref.	Ref.	Ref.
2nd quintile	1.8-2.6	1.00 (0.90-1.11)	1.01 (0.91-1.12)	1.02 (0.91-1.13)
3rd quintile	2.7-3.6	1.00 (0.90-1.11)	1.02 (0.91-1.13)	1.03 (0.93-1.15)
4th quintile	3.7-5.6	0.91 (0.82-1.02)	0.93 (0.84-1.04)	0.96 (0.86-1.06)
5th quintile	5.7-232.6	0.86 (0.77-0.96)	0.89(0.79-0.99)	0.92 (0.82-1.02)
Trend		0.001	0.009	0.053

Online Table 2: The association between PFAAs and self-report memory impairment for a doubling PFAA concentration and by tertiles of distribution by self-reported anti-diabetic treatment

	Range (ng/ML)	N	PFOS OR (95% CI)*	Range (ng/ML)	PFOA OR (95% CI)*	Range (ng/ML)	PFNA OR (95% CI)*	Range (ng/ML)	PFHxS OR (95% CI)*
Thiazolidinedione use		809	1.00 (0.86-1.16)		0.97 (0.88-1.07)		0.94 (0.74-1.19)		1.02 (0.87-1.20)
Ordinal regression			1.06 (0.93-1.20)		1.03 (0.95-1.11)		1.02 (0.84-1.25)		1.05 (0.92-1.20)
1st tertile	0.25-17.9		Ref.	1.1-17.5	Ref.	0.25-1.0	Ref.	0.25-1.9	Ref.
2nd tertile	18.0-29.9		0.76 (0.50-1.16)	17.6-49.7	0.72 (0.47-1.10)	1.1-1.5	0.83 (0.54-1.26)	2.0-3.5	1.56 (1.02-2.38)
3rd tertile	30.1-104.9		0.93 (0.61-1.42)	19.9-8,068	0.81 (0.53-1.24)	1.6-14.7	0.79 (0.51-1.23)	3.6-84.0	1.13 (0.72-1.77)
p-value for trend			0.737		0.333		0.309		0.628
Other medications		1,244	0.90 (0.80-1.01)		1.00 (0.93-1.07)		0.95 (0.79-1.15)		0.91 (0.81-1.03)
Ordinal regression			0.92 (0.83-1.01)		1.00 (0.93-1.07)		0.94 (0.81-1.10)		0.94 (0.86-1.04)
1st tertile	0.25-17.9		Ref.	0.25-20.5	Ref.	0.25-1.1	Ref.	0.25-2.1	Ref.
2nd tertile	18.0-29.8		0.75 (0.54-1.04)	20.6-63.2	0.99 (0.71-1.39)	1.2-1.6	0.72 (0.52-1.01)	2.2-3.6	0.99 (0.71-1.38)
3rd tertile	29.9-218.0		0.68 (0.48-0.95)	63.4-2,316.2	0.92 (0.66-1.29)	1.7-6.0	0.85 (0.61-1.20)	3.7-99.7	0.82 (0.58-1.16)
p-value for trend			0.023		0.644		0.341		0.259
No medication		1,390	0.95 (0.85-1.07)		1.00 (0.94-1.08)		1.03 (0.87-1.23)		1.01 (0.90-1.13)
Ordinal regression			0.94 (0.86-1.03)		1.00 (0.95-1.06)		0.98 (0.85-1.13)		0.99 (0.90-1.08)
1st tertile	0.25-18.3		Ref.	0.7-20.2	Ref.	0.25-1.0	Ref.	0.25-2.1	Ref.
2nd tertile	18.4-29.3		1.11 (0.81-1.52)	20.3-63.4	1.05 (0.77-1.44)	1.1-1.5	1.01 (0.72-1.40)	2.2-3.7	0.93 (0.68-1.28)
3rd tertile	29.4-272.0		1.02 (0.74-1.40)	63.5-22,412	0.99 (0.72-1.37)	1.6-14.5	1.12 (0.81-1.54)	3.8-43.3	0.99 (0.72-1.37)
p-value for trend			0.897		0.984		0.473		0.957

*using clinical record validated diagnosis of diabetes and self-reported use of medications, adjusted for age (one-year age bands), ethnicity, gender, school level (categorical), household income (categorical), physical activity, alcohol consumption (categorical, none/<1drink/month, < 1 drink/week, few drinks/week, 1-3 drinks/day, >3 drinks/day, undetermined), and cigarette smoking (categorical, never, former, < 10 cig/day, 12-20 cig/day, 20+ cig/day); † OR for doubling PFAA concentration

Table S1
(Gallo et al. [2011] PLoS Med; doi:10.1371/journal.pmed.1001117)

The Strengthening the Reporting Observational studies in Epidemiology – Molecular Epidemiology (STROBE-ME) Reporting Recommendations: Extended from STROBE statement

Item	Item number	STROBE Guidelines	Extension for Molecular Epidemiology Studies (STROBE-ME)
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	**ME-1** State the use of specific biomarker(s) in the title and/or in the abstract if they contribute substantially to the findings
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	
Introduction			
Background rationale	2	Explain the scientific background and rationale for the investigation being reported	**ME-2** Explain in the scientific background of the study how/why the specific biomarker(s) have been chosen, potentially among many others (e.g., others are studied but reported elsewhere, or not studied at all)
Objectives	3	State specific objectives, including any pre-specified hypotheses	**ME-3** *A priori* hypothesis: if one or more biomarkers are used as proxy measures, state the *a priori* hypothesis on the expected values of the biomarker(s)
Methods			
Study design	4	Present key elements of study design early in the paper	**ME-4** Describe the special study designs for molecular epidemiology (in particular nested case/control and case/cohort) and how they were implemented
Biological sample collection			**ME-4.1** Report on the setting of the biological sample collection; amount of sample; nature of collecting procedures; participant conditions; time between sample collection and relevant clinical or physiological endpoints.
Biological sample storage			**ME-4.2** Describe sample processing (centrifugation, timing, additives, etc).
Biological sample processing			**ME-4.3** Describe sample storage until biomarker analysis (storage, thawing, manipulation, etc).
Biomarker biochemical characteristics			**ME-4.4** Report the half-life of the biomarker, and chemical and physical characteristics (e.g., solubility).
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	
Participants	6	(a) Cohort study—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up Case-control study—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants	**ME-6** Report any habit, clinical conditions, physiological factor, or working or living condition that might affect the characteristics or concentrations of the biomarker
		(b) Cohort study—For matched studies, give matching criteria and number of exposed and unexposed Case-control study—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	
Data source/measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	**ME-8** Laboratory methods: report type of assay used, detection limit, quantity of biological sample used, outliers, timing in the assay procedures (when applicable) and calibration procedures or any standard used
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	**ME-12** Describe how biomarkers were introduced into statistical models
		(b) Describe any methods used to examine subgroups and interactions	
		(c) Explain how missing data were addressed	
		(d) Cohort study—If applicable, explain how loss to follow-up was addressed Case-control study—If applicable, explain how matching of cases and controls was addressed Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(e) Describe any sensitivity analyses	

Validity/reliability of measurement and internal/external validation			ME-12.1 Report on the validity and reliability of measurement of the biomarker(s) coming from the literature and any internal or external validation used in the study.
Results			
Participants	13	(a) Report the numbers of individuals at each stage of the study—e.g., numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	ME-13 Give reason for loss of biological samples at each stage
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14	(a) Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential confounders	
		(b) Indicate the number of participants with missing data for each variable of interest	
		(c) Cohort study—Summarise follow-up time (e.g., average and total amount)	
Distribution of biomarker measurement			ME-14.1 Give the distribution of the biomarker measurement (including mean, median, range, and variance)
Outcome data	15	Cohort study—Report numbers of outcome events or summary measures over time Case-control study—Report numbers in each exposure category, or summary measures of exposure Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included	
		(b) Report category boundaries when continuous variables were categorized	
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	ME-19 Describe main limitations in laboratory procedures
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	ME-20 Give an interpretation of results in terms of *a-priori* biological plausibility
Generalisability	21	Discuss the generalisability (external validity) of the study results	
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	
Ethics			ME-22.1 Describe informed consent and approval from ethical committee(s). Specify whether samples were anonymous, anonymised or identifiable

Serum perfluoroalkyl acids concentrations and memory impairment in a large cross-sectional study

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Key words: memory disorders [MeSH], prefluoroalkyl acids, perfluorooctanoic acid [MeSH], C8 Health Panel Study

Running title: perfluoroalkyl acids and memory impairment

Word count: 3,251~~160~~

Summary

Article focus

- Cross-sectional association between serum level of Perfluorooctanate (PFOA), perfuorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA) and perfluorohexane sulfonate (PFHxS) and self-reported memory impairment in a population exposed to high levels of PFOA
- Potential interaction between the association of perfluoroalkyl acids (PFAAs) ~~perfluoroclorinated compound~~ with memory impairment by diabetes status

Key Message

- Inverse associations between PFOS and PFOA and memory impairment were ~~highly~~ statistically significant ~~suggesting~~ perhaps due to a potential anti-inflammatory effect exerted through PPAR agonism. Comparable inverse associations with PFNA and PFHxS were of borderline statistical significance
- Inverse associations of PFAAs with memory impairment were weaker or non-existent in diabetics than overall in non-diabetics. Analysis further stratified by specific diabetes medication use showed no variation in odds ratios more than explicable by chance given the number of tests made.

Strengths and limitations

- Very large sample size including 21,024 adults with measured serum level of Perfluoroalkyl acids (PFAAs) ~~perfluorinated compounds~~ with a given geographical distribution allowing some multilevel modelling
- The cross-sectional nature of the design does not allow any causal inference and makes results particularly prone to reverse causality
- Self-reported is not an optimal method for estimating the degree of memory impairment in a population

ABSTRACT

Objectives – To examine the cross-sectional association between serum PFOA, PFOS, PFNA, and PFHxS concentrations with self-reported memory impairment in adults and the interaction of these associations with diabetes status

Design - Cross-sectional study

Setting – Population-based in Mid-Ohio Valley, West Virginia following contamination by a chemical plant

Participants - The C8 Health Project collected data and measured serum level of ~~prefluoroclorinated compounds~~ PFAAs of 21,024 adults aged 50+ years

Primary ~~and secondary~~ outcome measure~~s~~ – Self-reported memory impairment as defined by the question "have experienced short term memory loss?"

Results - A total of 4,057 subjects self-reported short-term memory impairment. Inverse associations between PFOS and PFOA and memory impairment were highly statistically significant with fully adjusted OR=0.93 (95% C.I. 0.90-0.96) for doubling PFOS and OR=0.96 (95% C.I. 0.94-0.98) for doubling PFOA concentrations. Comparable inverse associations with PFNA and PFHxS were of borderline statistical significance. Inverse associations of PFAAs with memory impairment were weaker or non-existent in diabetics than overall in non-diabetics.

Conclusion - An inverse association between PFAA serum levels and self-reported memory impairment has been observed in this large population-based, cross-sectional study stronger and more statistically significant for PFOA and PFOS. The associations can be potentially explained by preventive anti-inflammatory effect exerted by a PPAR agonist effect of these PFAAs, but confounding or even reverse causation cannot be excluded as an alternative explanation.

INTRODUCTION

Perfluoroalkyl acids (PFAAs) are man-made compounds used during the manufacture of fluoropolymers including non-stick cookware and breathable, yet waterproof, fabrics. They can also result from the metabolism of fluorinated telomers, compounds used for food package coatings, carpet treatments, and stain-resistant fabric treatment. Perfluorooctanate (PFOA), perfuorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA) and perfluorohexane sulfonate (PFHxS) persist in the environment and are found in the blood of humans and many animal species throughout the world [1 2]. Potential sources of exposure to PFAAs in humans include drinking water, dust, breast milk, fish and other foods, food packaging, ambient air, and occupational exposure [3-6].

In animal models, perfluoroalkyl chemicals can activate peroxisome proliferator-activated receptor alpha (PPARα), a ligand-activated transcription factor that regulates gene expression, lipid modulation, glucose homeostasis, cell proliferation and inflammation [7 8]. The PPAR receptor has been involved in the ageing process: PPARα null mice showed a decreased longevity compared with the wild-type due to non-neoplastic spontaneous ageing lesions which occurred with a higher incidence and a short latency in the PPARα null mice [9]. Also PPARγ variants were reported to be associated with longevity in humans with low insulin resistance [10 11]. Activation of the PPARγ receptor *in vitro* and *in vivo* also prevents the expression of inflammatory cytokines and other inflammatory mediators in brains of Alzheimer disease animal models [12 13]. In addition, PPARγ agonists have been demonstrated to suppress the Aβ-mediated activation of microglia *in vitro* and prevent cortical or hippocampal neuronal cell death [14-16]. PPARγ is also deeply involved in diabetes, given its ability to orchestrate the expression of genes involved in lipid metabolism, adipogenesis, and inflammation. It is activated by endogenous ligands (such as fatty acids and prostaglandins) or drugs such as thiazolidinedione. It is most highly expressed in adipocytes where it acts as the master regulator of adipogenesis via induction of adipogenic genes [17]. However, a study *in vitro* showed that PFOA and PFOS activate differentially PPARα and PPARγ receptors, but it is not possible to directly extrapolate these results to toxicity studies *in vivo* [18]. Therefore, in line with what was recently observed by Power et al[19], we hypothesised that increased exposure to PFAA could be associated with a better cognitive function.

The positive association between diabetes and cognitive impairment is well established [20]; some studies investigating the association between PFOA exposure and diabetes suggested the presence of an inverse association: a negative trend in diabetes occurrence by increasing serum PFOA deciles

was found in a case–control study nested in a previous study based on the population investigated here [21 22]; but not in others [23 24].

From 1950-2005, a chemical plant in the Mid-Ohio Valley, West Virginia was responsible for emitting PFOA into the surrounding environment. In 2001, a group of residents from the nearby West Virginia and Ohio communities filed a class action lawsuit alleging health damage from drinking water supplies drawing on PFOA-contaminated groundwater [25]. Part of the pre-trial settlement of the class action lawsuit included a baseline survey, the C8 Health Project, conducted in 2005-2006, that gathered data from over 69,000 people from six contaminated water districts surrounding the plant [25]. In this population, PFOA concentrations ranged from US background levels to very high; overall PFOA levels are much higher in this population (geometric mean 42.6.0 ng/mL, 95% C.I. 41.8-43.3) than in the corresponding US population surveys (NHANES in same year mean 3.95 ng/mL, 95% C.I. 3.65-4.27)~~)~~ [1 25]. The mean PFOS (geometric mean 22.4, 95% C.I. 22.2-22.6), PFNA (1.37, 95% C.I. 1.36-1.38), and PFHXs (3.18, 95% C.I. 3.15-3.22) closely resembled values from a nationally representative US sample form a similar time frame (mean PFOS 20.7, 95% C.I. 19.2-22.3; mean PFNA 0.97, 95% C.I. 0.82-1.14; and PFHXs 1.93, 95% C.I. 1.73-2.16) [1].

The present study uses these data to examine the cross-sectional association between serum PFOA, PFOS, PFNA, and PFHxS concentrations with self-reported memory impairment in adults, and its potential interaction with diabetes status.

METHODS

The Study population

This study is one of the C8 Science Panel Studies and uses information from questionnaires and blood tests collected in the C8 Health Project, supplemented by further information on classification by water district developed in a companion C8 Science Panel Study.

The C8 Health Project enrolled eligible subjects between August 2005 and August 2006. Individuals were eligible to participate in the C8 Health Project if they had consumed water for at least one year between 1950 and December 3, 2004 while living, working, or going to school in one of the following six water districts: Little Hocking Water Association of Ohio; City of Belpre, Ohio; Tupper Plains–Chester District of Ohio; Village of Pomeroy, Ohio; Lubeck Public Service District of West Virginia; Mason County Public Service District of West Virginia; or private water sources within

aforementioned districts and areas of documented PFOA contamination. Details of the study enrolment process, including consenting procedures, have been described elsewhere [25].

The C8 Health Project collected data on 69,030 people. ~~While it is not possible to estimate the~~ The participation rate for the C8 Health Project ~~as it is not possible to estimate the number of total possible participants over 50 years of environmental contamination, a participation rate,~~ based on US census counts of residents in the affected water districts during Project enrolment, have been estimated at around 80% [25]. In this population, the strongest predictor of PFOA serum concentration was residence in one of the contaminated water districts [26]; serum levels of other PFAAs do not show such geographic variation. Of the population, 21,724 older adults (aged ≥50 years) were considered for this analysis, and a total of 21,024 (96.8%) were included in the final analysis after exclusion of subjects with missing data on ethnicity, education level, socio-economic status, cigarette smoking, or BMI measurements.

Memory impairment definition

During the survey (2005-2006), all participants were asked if they "had experienced short term memory loss", the possible answers being "frequently", "sometimes", "rarely", and "never". The principle analyses assessed m~~M~~emory impairment ~~was~~ defined as reporting short-term memory loss frequently or sometimes, compared to rarely and never. ~~Severe m~~Memory impairment ever was also considered, defined as reporting any memory loss and compared to the never category.~~frequent episodes of short term memory loss.~~

Laboratory analysis

Blood samples were obtained and processed at individual data collection sites. Samples were drawn into four tubes per participant, with a maximum 35 mL. Tubes were spun, aliquoted, and refrigerated until shipping. Samples were shipped on dry ice daily from each data collection site to the laboratory daily [25]. Participants were not asked to fast before blood sample withdrawal, but fasting status was recorded.

Laboratory analysis of PFAAs used an automated solid-phase extraction combined with reverse-phase high-performance liquid chromatography [27]. Analyses were conducted by the Exygen Research Inc., State College, PA, USA; an intra-laboratory quality assurance program was carried out by analysis of duplicate samples at AXYS Analytical Service Ltd., Sidney, BC, Canada [25]. The intra-laboratory coefficient of variation for ~~both PFOA and PFOS~~ all PFAAs measurements was 0.1; the inter-laboratory comparison coefficient of variation was 0.2 for PFOA and PFNA, ~~and~~ 0.1 for PFOS,

and not applicable for PFHxA as all in the second lab measurement values were below level of detection[25]. The detection limit for all PFAAS ~~PFOA and PFOS~~ was 0.5 ng/mL and observations below this limit were assigned a value of 0.25 ng/mL (*n*=~~32~~16, ~~and~~ *n*=~~230~~101, n=532, and n=387 ~~for~~ PFOA, ~~and~~ PFOS, PFNA, and PFHxS, respectively, for this study population). All PFAAs ~~Both PFOA and PFOS~~ concentration distributions were skewed to the right. Methods and results are reported according to STROBE-ME recommendations [28].

Statistical analysis

Associations between exposure (serum concentration of PFOA, PFOS, PFNA, and PFHxS) and memory impairment were studied using logistic regression. Minimally adjusted models included age, in one year age-band, race (white, black, and others), gender, and educational level (high school diploma or general educational development (GED), some college, bachelor degree or higher) (Model 1). Further adjusted models additionally included average household income (≤$10,000, $10,001-20,000, $20,001-30,000, $30,001-40,000, $40,001-50,000, $50,001-60,000, $60,001-70,000, >$70,000), physical activity, alcohol consumption (none, <1 drink/month, <1 drink/week, few drinks/week, >1 drink/day) and cigarette smoking (never smoker, former smoker, current smoker <10 cigarettes/day, current smoker 10-19 cigarettes/day, current smoker ≥20 cigarettes/day) (Model 2). Fully adjusted models included also body mass index (BMI) (underweight/normal weight; overweight; and obese class I, II, and III), and diabetes (Model 3). PFAA concentrations were log-transformed to reduce skewness. For each model the association between PFAAs and self-reported memory impairment was calculated for a doubling in PFAA concentration in a model with PFAA entered as ~~numerical~~ continuous covariate, ~~and~~ for quintile groups of the PFAA distribution, and by ordinal regression analysis with the outcome variable comprising the four original levels of slef-reported frequency of episodes of memory loss, again in relation to a doubling of PFAAs. To explore possible differential effect of PFAA in sub-groups, analyses were further stratified by diabetes status and, among diabetics, by type of medications.

The following f~~F~~our sensitivity analyses were carried out: firstly one analysis restricting the sample to 7,097 subjects aged 65 years and older. Secondly, an analysis conducted on the entire sample, but using as outcome definition ~~only~~ those reporting any ~~frequent episodes of~~ memory loss (frequently, sometimes, and rarely). ~~Third, an ordinal regression analysis with the outcome variable comprising the four original levels of self-reported frequency of episodes of memory loss~~. ~~Fourth, we also considered the possibility that mobility (i.e. moving house measured as number of address during lifetime) might be associated with both memory loss and C8 and hence confound the association.~~

~~Finally~~Our final sensitivity analysis utilises ~~,~~ the geographical clustering of PFOA exposure by ~~in~~ water districts which allowed use to decompose the overall estimate of association of PFOA with memory impairment into within and between water district components, by including as explanatory variables both water district mean logged PFOA serum concentration and ~~potential~~ the deviations of individual's values from their district mean [29]. These two associations are subject to different potential biases, so help interpretation.

Role of funding

Funding for this work, the "C8 Science Panel Community Study at London School of Hygiene and Tropical Medicine - LSHTM", comes from the C8 Class Action Settlement Agreement (Circuit Court of Wood County, WV, USA) between DuPont and plaintiffs, which resulted from releases of ~~perfluorooctanoate (~~PFOA~~,~~ (or C8) into drinking water. It is one of the C8 Science Panel Studies undertaken by the Court-approved C8 Science Panel established under the same Settlement Agreement. The task of the C8 Science Panel, of which Tony Fletcher is a member, is to undertake research in the Mid-Ohio Valley, and subsequently evaluate the results along with other available information to determine if there are any probable links between PFOA and disease. Funds were administered by the Garden City Group (Melville, NY) that reports to the Court. The authors of this manuscript declare that their ability to design, conduct, interpret, or publish research was unimpeded by and fully independent of the court and/or settling parties. In addition, they declare no competing financial interests. The LSHTM Ethics Committee approved this study.

RESULTS

A total of 4,462 subjects (21.2% of the entire population of 21,024 individuals aged 50 years or older) self-reported short-term memory impairment (**Error! Reference source not found.**~~**Error! Reference source not found.**~~Figure 1): episodes of short-term memory loss were reported frequently by 1,115 subjects (5.3%); sometimes by 3,347 (15.9%); rarely by 4,283 (20.4%) and never by 12,279 (58.4%). Many personal characteristics were associated individually with memory loss,

including higher age, lower socio-economic status, smoking, and diagnosis of diabetes (Table 1), though to what extent these reflected independent risk factors was not investigated.

Results from the logistic regression of association between PFAAs and memory impairment are shown in Table 2. Results for minimally, further and fully adjusted models were similar, so we show only further adjusted results in this table, but results for all models are in the on-line Table 1. Inverse associations between PFOS and PFOA and memory impairment were highly statistically significant with adjusted OR=0.93 (0.90-0.96) for PFOS and OR=0.96 (0.94-0.98) for PFOA for doubling PFAA concentrations. Inverse associations of similar magnitude with PFNA and PFHxS but of borderline statistical significance were found: OR=0.96 (0.92-1.02) for PFNA and OR=0.97 (0.94-1.00) for PFHxS. The analysis by PFAA quintile groups shows similar patterns.

Inverse associations of PFAAs with memory impairment were weaker or non-existent in diabetics than ~~overall in~~ for non-diabetics (Table 3), though odds ratios were imprecise, and the difference by diabetes status ~~so this pattern~~ was only significant for PFOA (p-value for interaction = 0.014). Analysis further stratified by specific diabetes medication use showed no variation in odds ratios more than explicable by chance given the number of tests made (on-line Table 2).

In the sensitivity analysis on subjects older than 65 years, the substantial reduction of the sample size resulted in loss of precision in odds ratios. However, the points estimates of ~~there were~~ associations with memory impairment were of comparable magnitude ~~in memory impairment with~~ for all PFAAs except ~~for~~ PFOA for which the association with memory impairment was close to null ~~virtually disappears~~ (OR= 0.99 (0.97-1.03)) (Table 4).

The analysis carried out on the entire sample, ~~but~~ comparing those with any memory impairment against those with no memory problems~~restricting the definition of memory impairment to those who report frequent short-term memory loss episodes~~ shows slightly weaker associations for each PFAAs ~~substantially unaltered associations for PFOA and PFNA, and somewhat reduced inverse associations for PFOS and PFHxS,~~ but precision was reduced (Table 4). Analyses using ordinal regression in place of binary logistic regression ~~gave~~ yielded similar results to the logistic regressions (Table 2, Table 3, and Table 4).

~~Mobility as indicated by count of addresses was not appreciably associated with C8, so results changed very little on inclusion this variable in our regression analysis and are not shown.~~

The analysis separating the PFOA-memory impairment association into within and between water district components found that within water districts there was an inverse association between PFOA ~~individuals with high PFOA tended to have less~~ and memory impairment, as in the overall

association (OR 0.94, 95%CI 0.91-0.98, scale and adjustments as before). However there was no association between geometric mean concentration by ~~tendency for water districts with high PFOA on average to a lower proportion of persons with~~ and memory ~~loss~~ impairment (OR 1.00, 95%CI 0.97-1.03, per doubling in geometric mean PFOA by district).

Extra data is available upon request by emailing ~~Valentina Gallo~~ Tony Fletcher (~~v.gallo@qmul.ac.uk~~tony.fletcher@lshtm.ac.uk).

DISCUSSION

An inverse association between PFAAs serum levels (including PFOS, PFOA, PNA, and PFHxS) and self-reported memory impairment has been observed in this large population-based, cross-sectional study. This association is ~~stronger~~ more clearly monotonic with increasing exposure, and more statistically significant for PFOA and PFOS. However, the consistent decrement for all PFAAs suggests a common mechanism.

It is plausible that PFAAs could have an effect on cognitive function via PPAR agonism. Although it is not clear to what extent PFAAs act differentially on PPAR receptors α and γ[18], ~~i~~it could be speculated that this ~~effect~~ association might be mediated by the activation of the PPAR receptor by PFAAs. Activation of the PPARγ receptors has been shown to decrease the secretion of proinflamatory cytokines and possibly increase phagocytosis of Aβ inclusions, probably through activation of microglia[30]. However there was suggestion that this effect of suppression of the activation of microglia was age-dependent or disease stage-dependent being not significant in patients with advanced Alzheimer's disease (AD)[31 32]. PPAR agonist drugs, such as non-steroidal anti-inflammatory drugs (NSAID) and some anti-diabetics (i.e. thiazolodinedione or pioglitazone) have been proposed as preventive drugs for neurodegenerative conditions, including Alzheimer's dementia[30 33].

In a previous published study an inverse association between PFAAs and memory impairment was observed specifically among non-medicated diabetics[19]. In the present study, this pattern was not replicated, with the inverse association between PFAAs and cognitive impairment ~~was~~ being more evident in those without ~~than with~~ diabetes~~.~~; among diabetics, the association was not present, irrespective of treatment status. This finding could be ~~at least partially due to~~ explained by the fact that in diabetics PPAR receptors are more phosphorylated with a consequent reduced transcriptional activity [34 35], and the balance between PPARγ expression and activity levels is altered [34 36]. It is therefore ~~reasonable to assume~~ possible – based on the present data – that the PPAR-

agonist effect of PFAAs is different in subjects with and without PPAR-mediated metabolic changes such as diabetes. Also, it has been reported that PFAAs have a PPAR agonist effect, more prominently PPAR-α [37]; animal models suggest that PFOA has a stronger agonistic effect than PFOS [37]. Our findings of ~~Taken all together these results are compatible with~~ an inverse association between PFAA and memory impairment among non-diabetics, ~~and~~ would ~~be~~ therefore be compatible with a possible anti-inflammatory role exerted by PFAA on early symptoms of cognitive impairment.

There is some evidence of detrimental effects of PFAAs in neurodevelopment of mice affecting the cholinergic system and cognitive function[38-40], thus timing of exposure may also be relevant in order for the PFAAs to exert this hypothesised anti-dementing role.

~~That the association with PFOA was entirely within water districts, and not present at all between water districts despite large differences in (geometric) mean PFOA between districts (range 15.7 – 405.1) helps shed light on which biases the results are most vulnerable to. The between district estimate is not vulnerable to reverse causation and related biases at individual level, making this a more plausible explanation of the results. This association is, however, subject to bias by "ecologic" confounding by unmeasured factors differing across districts. This suggests that either the association documented at individual level could be confounded (e.g. by a common genetic variant related to both dementia risk and some excretion pathways); or that the association at the district level is biased towards the null (e.g. by confounding by socio-economic status). The notion that the association estimates found for PFOA are in the same direction of those found for PFNA and PFHXs, and in the majority of cases very consistent with those found for PFOS, however, tend to reinforce the notion of an inverse association between PFOA (and other PFAAs) and memory impairment at individual level. This suggests a common biological mechanism behind the findings.~~

~~Another alternative explanation of these findings is that the association between PFAAs and memory impairment is confounded by drinking water as inversely related to drinking artificially sweetened soft drinks. Fructose, currently the most used sweetener used in drinks as well as in a wide range of packaged food, has been associated with higher risk of dementia [40]. Assuming that the consumption of water (contaminated by PFOA in this population case) is inversely related to the consumption of soft drinks, this would lead to an artificial association between PFOA and memory impairment. However, indirect evidences gathered mainly during intervention trials among adolescents suggest that soft drink consumption is independent from the amount of water consumed by individuals [41-42]. Also, if this was true one would expect that the pattern observed for PFOA to be substantially different from those observed for the other PFAAs, which is not in this case.~~

However, these findings should ~~also~~ be interpreted cautiously given the ~~several~~ limitations of the study. Firstly, given the cross-sectional nature of the study, reverse causality must be considered~~cannot be ruled out~~: subjects suffering from memory impairment could have ~~drunk~~ consumed less of these compounds via water and food sources, though ~~less water resulting in average lower levels of PFAA, although~~ this is not a likely explanation given the consistency of the association across various PFAAs which have substantially different routes of exposure. Host characteristics such as genotype could be correlated with both some mechanism predisposing these symptoms and variation in PFAA excretion rates, thus leading to a confounded association with serum levels. Further~~Secondly~~, self-report~~ed~~ is not a very accurate method for ascertaining memory impairment, although errors in classification would be expected to be ~~are likely to result in~~ non-differential misclassification, biasing the estimate of association towards the null. ~~Thirdly, t~~The effects of PFAA have been mostly studied in relation to PPARα [7], while the receptor mostly implicated in metabolic changes and diabetes and in dementia PPARγ [30]; however, these two belong to the same receptor family and some degree of cross-activation cannot be excluded, and the knowledge of their pleiotropic effects is currently advancing [41]. Lastly, the classification into ~~analysis of~~ different anti-diabetic medications is uncertain as ~~particularly hampered by the fact that~~ these were self-reported and not prompted by interviewers. However, we consider it very unlikely that any misreporting would be confounded with serum PFAAs. This would tend to ~~has likely led to~~ low specificity and thus bias of the association (if any) towards the null.

On the other hand, strengths of this study include the fact that all showed estimates were adjusted for numerous potential confounders, including age in one-year age bands, making the effect of PFAA on memory impairment not likely to be confounded by lifestyle characteristics. Furthermore, these results are based on a very large population representative of the general population in West Virginia and Ohio [25], thus estimates are solid; and ~~. Finally,~~ the 21% prevalence of memory impairment is compatible and consistent with figures on prevalence of dementia reported for North America (Ferri et al, 2005).

Individual serum levels reflect the contributions of both intake and retention/excretion rates. While we have no direct data on either of these components, the large differences in drinking water contamination and associated average population serum levels for PFOA in the 6 water districts, allow an estimate of the effect of exposure. That the association with PFOA was entirely within water districts, and not present at all between water districts despite large differences in (geometric) mean PFOA between districts (range 15.7 – 405.1) is suggestive of a bias operating at one or both of these levels. The between district estimate is not vulnerable to reverse causation or confounding at

individual level, though some ecological confounding may operate if it happens to correlate with exposure level. Conversely the within district estimate but not between district estimate could reflect such individual confounding if present. Thus either the association documented at individual level could be confounded (e.g. by a common genetic variant related to both dementia risk and some excretion pathways); or that the association at the district level is biased towards the null (e.g. by confounding by socio-economic status). This sensitivity analysis cannot prove the presence of confounding at either level, but if the association had been consistent at both individual and district level that would have been more convincing of the association being due to PFAAs.

The size of the associations observed has both strong and weak aspects. The strong statistical significance suggests chance is an unlikely explanation. However, the odds ratios are only modestly different from one, 0.75 at the most extreme, so that biases are a more plausible explanation than they would be with more extreme ratios.

In conclusion, these data show an inverse association between PFOA and PFOS exposure and self-reported memory-impairment, particularly in non diabetics. This can be potentially explained by preventive anti-inflammatory effect exerted by a PPAR agonist effect of these ~~perfluoroclorinated compounds~~PFAAs, but confounding or even reverse causation cannot be excluded as an alternative explanation.

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Table 1: Participant Characteristics, Mid-Ohio Valley, 2005-2006 (N=21,024)

	All N=21,024*	Memory impaired N=4,462^
Males, n (%)	10,353 (49.2)	2,040 (19.7)
Females, n (%)	10,671 (50.8)	2,422 (22.7)
Age, median/mean (SD)	60.5/62.3 (9.0)	59.9/62.3 (9.4)
Age groups		
50-54 years	5,381 (25.6)	1,185 (22.0)
55-59 years	4,831 (23.0)	1,055 (21.8)
60-64 years	3,715 (17.7)	740 (19.9)
65-69 years	2,930 (13.9)	535 (18.3)
70-74 years	1,979 (9.4)	419 (21.2)
75-79 years	1,251 (6.0)	269 (21.5)
80+ years	937 (4.5)	259 (27.6)
Regular exercise, n (%)	6,774 (32.2)	1,306 (19.3)
BMI, n (%)		
Normal weight	5,100 (24.3)	1,051 (20.6)
Overweight	8,194 (39.0)	1,612 (19.7)
Obese class I	4,789 (22.8)	1,028 (21.5)
Obese class II	1,805 (8.6)	457 (25.3)
Obese class III	1,136 (5.4)	314 (27.6)
Household income, $/y n (%)		
≤10,000	1,486 (7.1)	448 (30.2)
10,001-20,000	3,059 (14.6)	757 (24.8)
20,001-30,000	3,281 (15.6)	751 (22.9)
30,001-40,000	2,936 (14.0)	572 (19.5)
40,001-50,000	2,135 (10.2)	422 (19.8)
50,001-60,000	1,815 (8.6)	359 (19.8)
60,001-70,000	1,367 (6.5)	268 (19.6)
>70,000	2,882 (13.7)	480 (16.7)
Undetermined	2,063 (9.8)	405 (19.6)
Education, n (%)		
< 12 years	3,310 (15.7)	845 (25.5)
HS diploma or GED	9,704 (46.2)	1,979 (20.4)
Some college	5,612 (26.7)	1,204 (21.5)
Bachelor degree or higher	2,398 (11.4)	434 (18.1)
Race, n (%)		
White	20,514 (97.6)	4,349 (21.2)
Black	213 (1.0)	38 (17.8)
Other	297 (1.4)	75 (25.3)
Alcohol consumption, n (%)		
None	13,276 (63.2)	2,848 (21.5)
< 1 drink/month	2,589 (12.3)	597 (23.1)
< 1 drink/week	1,530 (7.3)	309 (20.2)
Few drinks/week	2,087 (9.9)	397 (19.0)
1-3 drinks/day	805 (3.8)	142 (17.6)
>3 drinks/day	310 (1.5)	66 (21.3)
Undetermined	427 (2.0)	103 (24.1)
Smoking status, n (%)		
Never smoker	9,804 (46.6)	1,906 (19.4)
Former smoker	7,555 (35.8)	1,693 (22.5)
Current smoker < 10 cig/day	1,212 (5.8)	256 (21.1)
Current smoker 10-19 cig/day	1,260 (6.0)	310 (24.6)
Current smoker 20+ cig/day	1,213 (5.8)	297 (24.5)
Diabetes, n (%)	3,443 (16.4)	875 (25.4)
Thiazolidinedion use~	809 (23.5)	202 (25.0)
Other medications~	1,244 (36.1)	321 (25.8)
No medication~	1,390 (40.4)	352 (25.3)

*percentages refer to the proportion with respect to the entire population; ^percentages reflect the proportion of memory impaired in each category; percentages among diabetics only

Table 2: The association between PFAAs and self-report memory impairment in logistic regression for a doubling PFAA concentration, by quintiles of PFAAs, and in ordinal regression (n=21,024)

	Range (ng/mL)	Adjusted OR and 95% C.I.*
PFOS		0.93 (0.90-0.96)
1st quintile	0.25-14.4	Ref.
2nd quintile	14.5-20.4	0.96 (0.87-1.07)
3rd quintile	20.5-27.1	0.86 (0.78-0.96)
4th quintile	27.2-37.2	0.87 (0.78-0.96)
5th quintile	37.3-759.2	0.85 (0.76-0.94)
Trend		<0.001
Ordinal regression		0.95 (0.93-0.98)
PFOA		0.96 (0.94-0.98)
1st quintile	0.25-14.0	Ref.
2nd quintile	14.1-27.0	0.88 (0.79-0.97)
3rd quintile	27.1-53.8	0.83 (0.75-0.92)
4th quintile	53.9-118.1	0.79 (0.71-0.88)
5th quintile	118.3-22,412	0.79 (0.71-0.88)
Tend		<0.001
Ordinal regression		0.97 (0.96-0.98)
PFNA		0.96 (0.91-1.00)
1st quintile	0.25-0.90	Ref.
2nd quintile	1.0-1.2	0.86 (0.78-0.96)
3rd quintile	1.3-1.4	0.87 (0.77-0.98)
4th quintile	1.5-1.9	0.86 (0.77-0.95)
5th quintile	2.0-28.6	0.89 (0.80-0.99)
Trend		0.053
Ordinal regression		0.97 (0.94-1.01)
PFHxS		0.96 (0.93-0.99)
1st quintile	0.25-1.7	Ref.
2nd quintile	1.8-2.6	1.01 (0.91-1.12)
3rd quintile	2.7-3.6	1.02 (0.91-1.13)
4th quintile	3.7-5.6	0.93 (0.84-1.04)
5th quintile	5.7-232.6	0.89(0.79-0.99)
Trend		0.009
Ordinal regression		0.97 (0.94-0.99)

*Model adjusted for age (one-year age bands), ethnicity, gender, and school level (categorical), household income (categorical), physical activity, alcohol consumption (categorical, none/<1drink/month, < 1 drink/week, few drinks/week, 1-3 drinks/day, >3 drinks/day, undetermined), and cigarette smoking (categorical, never, former, < 10 cig/day, 12-20 cig/day, 20+ cig/day)

Table 3: The association between PFAAs and self-report memory impairment in logistic regression for a doubling PFAA concentration, by quintiles of distribution, and in ordinal regression by diabetes status (validated by clinical records)

	Range (ng/mL)	OR (95% CI)*	p for inter	N	OR (95% CI)*	p for inter
	PFOS N=17,832			**PFOA N=17,832**		
Non-diabetics		0.93 (0.90-0.96)†	-		0.95 (0.93-0.97) †	-
Ordinal regression		0.96 (0.93-0.99)			0.96 (0.95-0.98)	
1st quintile	0.25-14.5	Ref.		0.25-14.3	Ref.	
2nd quintile	14.6-20.5	0.96 (0.86-1.08)		14.4-27.2	0.85 (0.76-0.95)	
3rd quintile	20.6-27.0	0.90 (0.80-1.01)		27.3-54.3	0.82 (0.73-0.92)	
4th quintile	27.1-37.1	0.88 (0.78-0.99)		54.4-119.1	0.76 (0.68-0.86)	
5th quintile	37.2-759.2	0.85 (0.76-0.96)		119.2-8,416	0.75 (0.67-0.84)	
Trend		0.002			<0.001	
	N=3,192			**N=3,192**		
Diabetics		0.94 (0.88-1.02) †	0.698		1.02 (0.97-1.06) †	0.014
Ordinal regression		0.95 (0.90-1.01)			1.00 (0.97-1.04)	
1st quintile	0.25-13.6	Ref.		0.25-12.6	Ref.	
2nd quintile	13.7-20.0	1.06 (0.82-1.36)		12.7-25.4	1.04 (0.80-1.34)	
3rd quintile	20.1-27.3	0.82 (0.63-1.06)		25.5-48.0	0.88 (0.67-1.14)	
4th quintile	27.4-37.3	0.87 (0.67-1.13)		48.1-102.1	1.04 (0.80-1.35)	
5th quintile	37.4-272.0	0.90 (0.69-1.17)		102.4-22,412	1.09 (0.84-1.42)	
		0.162			0.543	
	PFNA			**PFHxS**		
	N=17,832			**N=17,832**		
Non-diabetics		0.95 (0.90-0.99) †	-		0.96 (0.93-0.99) †	-
Ordinal regression		0.97 (0.93-1.01)			0.97 0.94-0.99)	
1st quintile	0.25-0.9	Ref.		0.25-1.8	Ref.	
2nd quintile	1.0-1.2	0.86 (0.77-0.97)		1.9-2.6	0.98 (0.87-1.10)	
3rd quintile	1.3-1.5	0.85 (0.76-0.95)		2.7-3.7	0.99 (0.89-1.11)	
4th quintile	1.6-1.9	0.83 (0.73-0.93)		3.8-5.7	0.93 (0.82-1.05)	
5th quintile	2.0-28.6	0.88 (0.78-0.99)		5.8-232.6	0.88 (0.79-0.99)	
Trend		0.031			0.029	
	N=3,192			**N=3,192**		
Diabetics		1.01 (0.90-1.13) †	0.259	3,192	0.99 (0.92-1.06) †	0.683
Ordinal regression		0.99 (0.91-1.09)				
1st quintile	0.25-0.8	Ref.		0.25-1.6	Ref.	
2nd quintile	0.9-1.1	1.06 (0.80-1.40)		1.7-2.3	1.06 (0.81-1.38)	
3rd quintile	1.2-1.4	0.88 (0.66-1.17)		2.4-3.2	1.10 (0.85-1.42)	
4th quintile	1.5-1.8	1.03 (0.77-1.36)		3.3-5.0	1.02 (0.79-1.33)	
5th quintile	1.9-14.5	1.08(0.82-1.43)		5.1-99.7	1.00 (0.77-1.31)	
		0.620			0.942	

*using clinical record validated diagnosis of diabetes and self-reported use of medications, adjusted for age (one-year age bands), ethnicity, gender, school level (categorical), household income (categorical), physical activity, alcohol consumption (categorical, none/<1drink/month, < 1 drink/week, few drinks/week, 1-3 drinks/day, >3 drinks/day, undetermined), and cigarette smoking (categorical, never, former, < 10 cig/day, 12-20 cig/day, 20+ cig/day); † OR for doubling PFAA concentration

Table 4: Sensitivity analysis of the association between PFAAs and self-report memory impairment for a doubling PFAA concentration, by quintiles of PFAAs, and in ordinal regression on subjects aged 65 years and older (n=7,097), and using ~~severe~~ any memory impairment as outcome measure (n=21,024).

	Range (ng/mL)	OR (95% C.I.)[b] N=7,097 Restricted to those aged 65+*	Range (ng/mL)	OR (95% C.I.)[b] N=21,024 ~~Severely memory impaired~~Any memory impairment^
PFOS		0.95 (0.90-1.00)		0.96 (0.~~90~~94-~~1.02~~0.99)
Ordinal regression		0.98 (0.94-1.03)		
1st quintile	0.25-15.3	Ref.	0.25-14.4	Ref.
2nd quintile	15.4-22.0	0.99 (0.83-1.20)	14.5-20.4	~~1.13~~ 0.96 (0.~~94~~88-1.~~3~~05)
3rd quintile	22.1-28.9	0.95 (0.79-1.14)	20.5-27.1	0.~~92~~ 90 (0.~~76~~82-~~1.11~~0.98)
4th quintile	29.0-4.0	0.97 (0.81-1.16)	27.2-37.2	0.~~92~~ 94 (0.~~75~~86-1.~~12~~03)
5th quintile	40.1-759.2	0.84 (0.70-1.01)	37.3-759.2	0.~~92~~ 93 (0.~~75~~85-1.~~12~~02)
Trend		0.079		0.~~09~~4121
PFOA		0.99 (0.97-1.03)		0.9~~75~~ (0.9~~2~~6-0.9~~8~~9)
Ordinal regression		1.00 (0.97-1.03)		
1st quintile	0.25-15.0	Ref.	0.25-14.0	Ref.
2nd quintile	15.1-29.6	0.91 (0.75-1.09)	14.1-27.0	0.~~84~~ 90 (0.~~70~~82-~~1.01~~0.98)
3rd quintile	29.7-56.8	0.90 (0.75-1.08)	27.1-53.8	0.~~85~~86 (0.~~71~~79-~~1.02~~0.94)
4th quintile	56.9-123.0	0.84 (0.70-1.01)	53.9-118.1	0.~~79~~ 87 (0.~~66~~79-0.~~96~~95)
5th quintile	123.1-5,994.8	0.99(0.83-1.19)	118.3-22,412	0.~~75~~ 85 (0.~~61~~78-0.~~91~~93)
Tend		0.680		~~0.003~~<0.001
PFNA		0.95 (0.87-1.02)		0.9~~8~~2 (0.~~85~~95-1.~~00~~02)
Ordinal regression		0.99 (0.93-1.07)		
1st quintile	0.25-0.8	Ref.	0.25-0.90	Ref.
2nd quintile	0.9-1.1	0.88 (0.73-1.06)	1.0-1.2	0.89 (0.~~74~~82-~~1.07~~0.97)
3rd quintile	1.2-1.4	0.81 (0.67-0.98)	1.3-1.4	0.~~82~~ 94(0.~~66~~85-1.~~01~~04)
4th quintile	1.5-1.8	0.82 (0.68-0.99)	1.5-1.9	0.~~85~~ 92 (0.~~71~~85-1.~~02~~01)
5th quintile	1.9-11.7	0.88 (0.73-1.07)	2.0-28.6	0.~~79~~ 94 (0.~~65~~86-~~0.97~~1.03)
Trend		0.177		0.~~02~~3493
PFHxS		0.96 (0.91-1.01)		0.9~~7~~8 (0.9~~4~~3-~~1.04~~0.99)
Ordinal regression		0.98 (0.93-1.02)		
1st quintile	0.25-1.9	Ref.	0.25-1.7	Ref.
2nd quintile	2.0-2.8	0.98 (0.82-1.18)	1.8-2.6	~~1.10~~0.98 (0.~~91~~90-1.~~33~~07)
3rd quintile	2.9-3.9	0.95 (0.79-1.15)	2.7-3.6	1.~~04~~ 03 (0.~~86~~94-

				1.~~27~~13)
4th quintile	4.0-6.0	0.98 (0.82-1.17)	3.7-5.6	0.~~91~~ 96 (0.~~75~~87-1.~~1~~204)
5th quintile	6.1-232.6	0.86 (0.71-1.03)	5.7-232.6	0.~~98~~ 89 (0.~~80~~81-~~1.19~~0.97)
Trend		0.139		~~0.283~~0.010

[b]Model 2 includes age (one-year age bands), ethnicity, gender, and school level (categorical), household income (categorical), physical activity, alcohol consumption (categorical, none/<1drink/month, < 1 drink/week, few drinks/week, 1-3 drinks/day, >3 drinks/day, undetermined), and cigarette smoking (categorical, never, former, < 10 cig/day, 12-20 cig/day, 20+ cig/day)

* Sensitivity analysis including subjects aged 65 years or older only (N=7,097)

^ Sensitivity analysis using a more restrictive definition of memory impairment (those reporting frequent episode of short-term memory loss only, cases = 1,115)

Serum perfluoroalkyl acids concentrations and memory impairment in a large cross-sectional study

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Serum perfluoroalkyl acids concentrations and memory impairment in a large cross-sectional study

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Summary

Article focus

- Cross-sectional association between serum level of Perfluorooctanate (PFOA), perfuorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA) and perfluorohexane sulfonate (PFHxS) and self-reported memory impairment in a population exposed to high levels of PFOA
- Potential interaction between the association of perfluoroalkyl acids (PFAAs) with memory impairment by diabetes status

Key Message

- Inverse associations between PFOS and PFOA and memory impairment were statistically significant perhaps due to a potential anti-inflammatory effect exerted through PPAR agonism. Comparable inverse associations with PFNA and PFHxS were of borderline statistical significance
- Inverse associations of PFAAs with memory impairment were weaker or non-existent in diabetics than overall in non-diabetics. Analysis further stratified by specific diabetes medication use showed no variation in odds ratios more than explicable by chance given the number of tests made.

Strengths and limitations

- Very large sample size including 21,024 adults with measured serum level of Perfluoroalkyl acids (PFAAs) with a given geographical distribution allowing some multilevel modelling
- The cross-sectional nature of the design does not allow any causal inference and makes results particularly prone to reverse causality
- Self-reported is not an optimal method for estimating the degree of memory impairment in a population

ABSTRACT

Objectives – To examine the cross-sectional association between serum PFOA, PFOS, PFNA, and PFHxS concentrations with self-reported memory impairment in adults and the interaction of these associations with diabetes status

Design - Cross-sectional study

Setting – Population-based in Mid-Ohio Valley, West Virginia following contamination by a chemical plant

Participants - The C8 Health Project collected data and measured serum level of PFAAs of 21,024 adults aged 50+ years

Primary outcome measure – Self-reported memory impairment as defined by the question "have experienced short term memory loss?"

Results - A total of 4,057 subjects self-reported short-term memory impairment. Inverse associations between PFOS and PFOA and memory impairment were highly statistically significant with fully adjusted OR=0.93 (95% C.I. 0.90-0.96) for doubling PFOS and OR=0.96 (95% C.I. 0.94-0.98) for doubling PFOA concentrations. Comparable inverse associations with PFNA and PFHxS were of borderline statistical significance. Inverse associations of PFAAs with memory impairment were weaker or non-existent in diabetics than overall in non-diabetics.

Conclusion - An inverse association between PFAA serum levels and self-reported memory impairment has been observed in this large population-based, cross-sectional study stronger and more statistically significant for PFOA and PFOS. The associations can be potentially explained by preventive anti-inflammatory effect exerted by a PPAR agonist effect of these PFAAs, but confounding or even reverse causation cannot be excluded as an alternative explanation.

INTRODUCTION

Perfluoroalkyl acids (PFAAs) are man-made compounds used during the manufacture of fluoropolymers including non-stick cookware and breathable, yet waterproof, fabrics. They can also result from the metabolism of fluorinated telomers, compounds used for food package coatings, carpet treatments, and stain-resistant fabric treatment. Perfluorooctanate (PFOA), perfuorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA) and perfluorohexane sulfonate (PFHxS) persist in the environment and are found in the blood of humans and many animal species throughout the world [1 2]. Potential sources of exposure to PFAAs in humans include drinking water, dust, breast milk, fish and other foods, food packaging, ambient air, and occupational exposure [3-6].

In animal models, perfluoroalkyl chemicals can activate peroxisome proliferator-activated receptor alpha (PPARα), a ligand-activated transcription factor that regulates gene expression, lipid modulation, glucose homeostasis, cell proliferation and inflammation [7 8]. The PPAR receptor has been involved in the ageing process: PPARα null mice showed a decreased longevity compared with the wild-type due to non-neoplastic spontaneous ageing lesions which occurred with a higher incidence and a short latency in the PPARα null mice [9]. Also PPARγ variants were reported to be associated with longevity in humans with low insulin resistance [10 11]. Activation of the PPARγ receptor *in vitro* and *in vivo* also prevents the expression of inflammatory cytokines and other inflammatory mediators in brains of Alzheimer disease animal models [12 13]. In addition, PPARγ agonists have been demonstrated to suppress the Aβ-mediated activation of microglia *in vitro* and prevent cortical or hippocampal neuronal cell death [14-16]. PPARγ is also deeply involved in diabetes, given its ability to orchestrate the expression of genes involved in lipid metabolism, adipogenesis, and inflammation. It is activated by endogenous ligands (such as fatty acids and prostaglandins) or drugs such as thiazolidinedione. It is most highly expressed in adipocytes where it acts as the master regulator of adipogenesis via induction of adipogenic genes [17]. However, a study *in vitro* showed that PFOA and PFOS activate differentially PPARα and PPARγ receptors, but it is not possible to directly extrapolate these results to toxicity studies *in vivo* [18]. Therefore, in line with what was recently observed by Power et al[19], we hypothesised that increased exposure to PFAA could be associated with a better cognitive function.

The positive association between diabetes and cognitive impairment is well established [20]; some studies investigating the association between PFOA exposure and diabetes suggested the presence of an inverse association: a negative trend in diabetes occurrence by increasing serum PFOA deciles

was found in a case–control study nested in a previous study based on the population investigated here [21 22]; but not in others [23 24].

From 1950-2005, a chemical plant in the Mid-Ohio Valley, West Virginia was responsible for emitting PFOA into the surrounding environment. In 2001, a group of residents from the nearby West Virginia and Ohio communities filed a class action lawsuit alleging health damage from drinking water supplies drawing on PFOA-contaminated groundwater [25]. Part of the pre-trial settlement of the class action lawsuit included a baseline survey, the C8 Health Project, conducted in 2005-2006, that gathered data from over 69,000 people from six contaminated water districts surrounding the plant [25]. In this population, PFOA concentrations ranged from US background levels to very high; overall PFOA levels are much higher in this population (geometric mean 42.6.0 ng/mL, 95% C.I. 41.8-43.3) than in the corresponding US population surveys (NHANES in same year mean 3.95 ng/mL, 95% C.I. 3.65-4.27) [1 25]. The mean PFOS (geometric mean 22.4, 95% C.I. 22.2-22.6), PFNA (1.37, 95% C.I. 1.36-1.38), and PFHXs (3.18, 95% C.I. 3.15-3.22) closely resembled values from a nationally representative US sample form a similar time frame (mean PFOS 20.7, 95% C.I. 19.2-22.3; mean PFNA 0.97, 95% C.I. 0.82-1.14; and PFHXs 1.93, 95% C.I. 1.73-2.16) [1].

The present study uses these data to examine the cross-sectional association between serum PFOA, PFOS, PFNA, and PFHxS concentrations with self-reported memory impairment in adults, and its potential interaction with diabetes status.

METHODS

The Study population

This study is one of the C8 Science Panel Studies and uses information from questionnaires and blood tests collected in the C8 Health Project, supplemented by further information on classification by water district developed in a companion C8 Science Panel Study.

The C8 Health Project enrolled eligible subjects between August 2005 and August 2006. Individuals were eligible to participate in the C8 Health Project if they had consumed water for at least one year between 1950 and December 3, 2004 while living, working, or going to school in one of the following six water districts: Little Hocking Water Association of Ohio; City of Belpre, Ohio; Tupper Plains–

Chester District of Ohio; Village of Pomeroy, Ohio; Lubeck Public Service District of West Virginia; Mason County Public Service District of West Virginia; or private water sources within aforementioned districts and areas of documented PFOA contamination. Details of the study enrolment process, including consenting procedures, have been described elsewhere [25].

The C8 Health Project collected data on 69,030 people. The participation rate for the C8 Health Project based on US census counts of residents in the affected water districts during Project enrolment, have been estimated at around 80% [25]. In this population, the strongest predictor of PFOA serum concentration was residence in one of the contaminated water districts [26]; serum levels of other PFAAs do not show such geographic variation. Of the population, 21,724 older adults (aged ≥50 years) were considered for this analysis, and a total of 21,024 (96.8%) were included in the final analysis after exclusion of subjects with missing data on ethnicity, education level, socio-economic status, cigarette smoking, or BMI measurements.

Memory impairment definition

During the survey (2005-2006), all participants were asked if they "had experienced short term memory loss", the possible answers being "frequently", "sometimes", "rarely", and "never". The principle analyses assessed memory impairment defined as reporting short-term memory loss frequently or sometimes, compared to rarely and never. Memory impairment ever was also considered, defined as reporting any memory loss and compared to the never category.

Laboratory analysis

Blood samples were obtained and processed at individual data collection sites. Samples were drawn into four tubes per participant, with a maximum 35 mL. Tubes were spun, aliquoted, and refrigerated until shipping. Samples were shipped on dry ice daily from each data collection site to the laboratory daily [25]. Participants were not asked to fast before blood sample withdrawal, but fasting status was recorded.

Laboratory analysis of PFAAs used an automated solid-phase extraction combined with reverse-phase high-performance liquid chromatography [27]. Analyses were conducted by the Exygen Research Inc., State College, PA, USA; an intra-laboratory quality assurance program was carried out by analysis of duplicate samples at AXYS Analytical Service Ltd., Sidney, BC, Canada [25]. The intra-laboratory coefficient of variation for all PFAAs measurements was 0.1; the inter-laboratory comparison coefficient of variation was 0.2 for PFOA and PFNA, 0.1 for PFOS, and not applicable for PFHxA as all in the second lab measurement values were below level of detection[25]. The detection

limit for all PFAAS was 0.5 ng/mL and observations below this limit were assigned a value of 0.25 ng/mL (*n*=16, *n*=101, n=532, and n=387for PFOA, PFOS, PFNA, and PFHxS, respectively, for this study population). All PFAAs concentration distributions were skewed to the right. Methods and results are reported according to STROBE-ME recommendations [28].

Statistical analysis

Associations between exposure (serum concentration of PFOA, PFOS, PFNA, and PFHxS) and memory impairment were studied using logistic regression. Minimally adjusted models included age, in one year age-band, race (white, black, and others), gender, and educational level (high school diploma or general educational development (GED), some college, bachelor degree or higher) (Model 1). Further adjusted models additionally included average household income (≤$10,000, $10,001-20,000, $20,001-30,000, $30,001-40,000, $40,001-50,000, $50,001-60,000, $60,001-70,000, >$70,000), physical activity, alcohol consumption (none, <1 drink/month, <1 drink/week, few drinks/week, >1 drink/day) and cigarette smoking (never smoker, former smoker, current smoker <10 cigarettes/day, current smoker 10-19 cigarettes/day, current smoker ≥20 cigarettes/day) (Model 2). Fully adjusted models included also body mass index (BMI) (underweight/normal weight; overweight; and obese class I, II, and III), and diabetes (Model 3). PFAA concentrations were log-transformed to reduce skewness. For each model the association between PFAAs and self-reported memory impairment was calculated for a doubling in PFAA concentration in a model with PFAA entered as continuous covariate, for quintile groups of the PFAA distribution, and by ordinal regression analysis with the outcome variable comprising the four original levels of slef-reported frequency of episodes of memory loss, again in relation to a doubling of PFAAs. To explore possible differential effect of PFAA in sub-groups, analyses were further stratified by diabetes status and, among diabetics, by type of medications.

The following four sensitivity analyses were carried out: firstly one analysis restricting the sample to 7,097 subjects aged 65 years and older. Secondly, an analysis conducted on the entire sample, but using as outcome definition those reporting any memory loss (frequently, sometimes, and rarely). Our final sensitivity analysis utilises the geographical clustering of PFOA exposure by water districts which allowed use to decompose the overall estimate of association of PFOA with memory impairment into within and between water district components, by including as explanatory variables both water district mean logged PFOA serum concentration and the deviations of individual's values from their district mean [29]. These two associations are subject to different potential biases, so help interpretation.

Role of funding

Funding for this work, the "C8 Science Panel Community Study at London School of Hygiene and Tropical Medicine - LSHTM", comes from the C8 Class Action Settlement Agreement (Circuit Court of Wood County, WV, USA) between DuPont and plaintiffs, which resulted from releases of PFOA (or C8) into drinking water. It is one of the C8 Science Panel Studies undertaken by the Court-approved C8 Science Panel established under the same Settlement Agreement. The task of the C8 Science Panel, of which Tony Fletcher is a member, is to undertake research in the Mid-Ohio Valley, and subsequently evaluate the results along with other available information to determine if there are any probable links between PFOA and disease. Funds were administered by the Garden City Group (Melville, NY) that reports to the Court. The authors of this manuscript declare that their ability to design, conduct, interpret, or publish research was unimpeded by and fully independent of the court and/or settling parties. In addition, they declare no competing financial interests. The LSHTM Ethics Committee approved this study.

RESULTS

A total of 4,462 subjects (21.2% of the entire population of 21,024 individuals aged 50 years or older) self-reported short-term memory impairment (**Error! Reference source not found.**): episodes f short-term memory loss were reported frequently by 1,115 subjects (5.3%); sometimes by 3,347 (15.9%); rarely by 4,283 (20.4%) and never by 12,279 (58.4%). Many personal characteristics were associated individually with memory loss, including higher age, lower socio-economic status, smoking, and diagnosis of diabetes (Table 1), though to what extent these reflected independent risk factors was not investigated.

Results from the logistic regression of association between PFAAs and memory impairment are shown in Table 2. Results for minimally, further and fully adjusted models were similar, so we show only further adjusted results in this table, but results for all models are in the on-line Table 1. Inverse associations between PFOS and PFOA and memory impairment were highly statistically significant with adjusted OR=0.93 (0.90-0.96) for PFOS and OR=0.96 (0.94-0.98) for PFOA for doubling PFAA concentrations. Inverse associations of similar magnitude with PFNA and PFHxS but of borderline statistical significance were found: OR=0.96 (0.92-1.02) for PFNA and OR=0.97 (0.94-1.00) for PFHxS. The analysis by PFAA quintile groups shows similar patterns.

Inverse associations of PFAAs with memory impairment were weaker or non-existent in diabetics than for non-diabetics (Table 3), though odds ratios were imprecise, and the difference by diabetes status was only significant for PFOA (p-value for interaction = 0.014). Analysis further stratified by specific diabetes medication use showed no variation in odds ratios more than explicable by chance given the number of tests made (on-line Table 2).

In the sensitivity analysis on subjects older than 65 years, the substantial reduction of the sample size resulted in loss of precision in odds ratios. However, the points estimates of associations with memory impairment were of comparable magnitude for all PFAAs except PFOA for which the association with memory impairment was close to null (OR= 0.99 (0.97-1.03)) (Table 4).

The analysis carried out on the entire sample, comparing those with any memory impairment against those with no memory problems shows slightly weaker associations for each PFAAs but precision was reduced (Table 4). Analyses using ordinal regression in place of binary logistic regression yielded similar results to the logistic regressions (Table 2, Table 3, and Table 4).

The analysis separating the PFOA-memory impairment association into within and between water district components found that within water districts there was an inverse association between PFOA and memory impairment, as in the overall association (OR 0.94, 95%CI 0.91-0.98, scale and adjustments as before). However there was no association between geometric mean concentration by and memory impairment (OR 1.00, 95%CI 0.97-1.03, per doubling in geometric mean PFOA by district).

Extra data is available upon request by emailing Tony Fletcher (tony.fletcher@lshtm.ac.uk).

DISCUSSION

An inverse association between PFAAs serum levels (including PFOS, PFOA, PNA, and PFHxS) and self-reported memory impairment has been observed in this large population-based, cross-sectional study. This association is more clearly monotonic with increasing exposure, and more statistically significant for PFOA and PFOS. However, the consistent decrement for all PFAAs suggests a common mechanism.

It is plausible that PFAAs could have an effect on cognitive function via PPAR agonism. Although it is not clear to what extent PFAAs act differentially on PPAR receptors α and γ[18], it could be speculated

that this association might be mediated by the activation of the PPAR receptor by PFAAs. Activation of the PPARγ receptors has been shown to decrease the secretion of proinflamatory cytokines and possibly increase phagocytosis of Aβ inclusions, probably through activation of microglia[30]. However there was suggestion that this effect of suppression of the activation of microglia was age-dependent or disease stage-dependent being not significant in patients with advanced Alzheimer's disease (AD)[31 32]. PPAR agonist drugs, such as non-steroidal anti-inflammatory drugs (NSAID) and some anti-diabetics (i.e. thiazolodinedione or pioglitazone) have been proposed as preventive drugs for neurodegenerative conditions, including Alzheimer's dementia[30 33].

In a previous published study an inverse association between PFAAs and memory impairment was observed specifically among non-medicated diabetics[19]. In the present study, this pattern was not replicated, with the inverse association between PFAAs and cognitive impairment being more evident in those without diabetes; among diabetics, the association was not present, irrespective of treatment status. This finding could be explained by the fact that in diabetics PPAR receptors are more phosphorylated with a consequent reduced transcriptional activity [34 35], and the balance between PPARγ expression and activity levels is altered [34 36]. It is therefore possible – based on the present data – that the PPAR-agonist effect of PFAAs is different in subjects with and without PPAR-mediated metabolic changes such as diabetes. Also, it has been reported that PFAAs have a PPAR agonist effect, more prominently PPAR-α [37]; animal models suggest that PFOA has a stronger agonistic effect than PFOS [37]. Our findings of an inverse association between PFAA and memory impairment among non-diabetics, would therefore be compatible with a possible anti-inflammatory role exerted by PFAA on early symptoms of cognitive impairment.

There is some evidence of detrimental effects of PFAAs in neurodevelopment of mice affecting the cholinergic system and cognitive function[38-40], thus timing of exposure may also be relevant in order for the PFAAs to exert this hypothesised anti-dementing role. However, these findings should be interpreted cautiously given the limitations of the study. Firstly, given the cross-sectional nature of the study, reverse causality must be considered: subjects suffering from memory impairment could have consumed less of these compounds via water and food sources, though this is not a likely explanation given the consistency of the association across various PFAAs which have substantially different routes of exposure. Host characteristics such as genotype could be correlated with both some mechanism predisposing these symptoms and variation in PFAA excretion rates, thus leading to a confounded association with serum levels. Further, self-report is not a very accurate method for ascertaining memory impairment, although errors in classification would be expected to be non-differential misclassification, biasing the estimate of association towards the null. The effects of

PFAA have been mostly studied in relation to PPARα [7], while the receptor mostly implicated in metabolic changes and diabetes and in dementia PPARγ [30]; however, these two belong to the same receptor family and some degree of cross-activation cannot be excluded, and the knowledge of their pleiotropic effects is currently advancing [41]. Lastly, the classification into different anti-diabetic medications is uncertain as these were self-reported and not prompted by interviewers. However, we consider it very unlikely that any misreporting would be confounded with serum PFAAs. This would tend to low specificity and thus bias of the association (if any) towards the null.

On the other hand, strengths of this study include the fact that all showed estimates were adjusted for numerous potential confounders, including age in one-year age bands, making the effect of PFAA on memory impairment not likely to be confounded by lifestyle characteristics. Furthermore, these results are based on a very large population representative of the general population in West Virginia and Ohio [25], thus estimates are solid; and the 21% prevalence of memory impairment is compatible and consistent with figures on prevalence of dementia reported for North America (Ferri et al, 2005).

Individual serum levels reflect the contributions of both intake and retention/excretion rates. While we have no direct data on either of these components, the large differences in drinking water contamination and associated average population serum levels for PFOA in the 6 water districts, allow an estimate of the effect of exposure. That the association with PFOA was entirely within water districts, and not present at all between water districts despite large differences in (geometric) mean PFOA between districts (range 15.7 – 405.1) is suggestive of a bias operating at one or both of these levels. The between district estimate is not vulnerable to reverse causation or confounding at individual level, though some ecological confounding may operate if it happens to correlate with exposure level. Conversely the within district estimate but not between district estimate could reflect such individual confounding if present. Thus either the association documented at individual level could be confounded (e.g. by some unmeasured individual characteristic); or that the association at the district level is confounded to obscure association (for example socio-economic status). This sensitivity analysis cannot prove the presence of confounding at either level, but if the association had been consistent at both individual and district level that would have been more convincing of the association being due to PFAAs.

The size of the associations observed has both strong and weak aspects. The strong statistical significance suggests chance is an unlikely explanation. However, the odds ratios are only modestly different from one, 0.75 at the most extreme, so that biases are a more plausible explanation than they would be with more extreme ratios. In conclusion, these data show an inverse association

between PFOA and PFOS exposure and self-reported memory-impairment, particularly in non diabetics. This can be potentially explained by preventive anti-inflammatory effect exerted by a PPAR agonist effect of these PFAAs, but confounding or even reverse causation cannot be excluded as an alternative explanation.

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Table 1: Participant Characteristics, Mid-Ohio Valley, 2005-2006 (N=21,024)

	All N=21,024*	Memory impaired N=4,462^
Males, n (%)	10,353 (49.2)	2,040 (19.7)
Females, n (%)	10,671 (50.8)	2,422 (22.7)
Age, median/mean (SD)	60.5/62.3 (9.0)	59.9/62.3 (9.4)
Age groups		
50-54 years	5,381 (25.6)	1,185 (22.0)
55-59 years	4,831 (23.0)	1,055 (21.8)
60-64 years	3,715 (17.7)	740 (19.9)
65-69 years	2,930 (13.9)	535 (18.3)
70-74 years	1,979 (9.4)	419 (21.2)
75-79 years	1,251 (6.0)	269 (21.5)
80+ years	937 (4.5)	259 (27.6)
Regular exercise, n (%)	6,774 (32.2)	1,306 (19.3)
BMI, n (%)		
Normal weight	5,100 (24.3)	1,051 (20.6)
Overweight	8,194 (39.0)	1,612 (19.7)
Obese class I	4,789 (22.8)	1,028 (21.5)
Obese class II	1,805 (8.6)	457 (25.3)
Obese class III	1,136 (5.4)	314 (27.6)
Household income, $/y n (%)		
≤10,000	1,486 (7.1)	448 (30.2)
10,001-20,000	3,059 (14.6)	757 (24.8)
20,001-30,000	3,281 (15.6)	751 (22.9)
30,001-40,000	2,936 (14.0)	572 (19.5)
40,001-50,000	2,135 (10.2)	422 (19.8)
50,001-60,000	1,815 (8.6)	359 (19.8)
60,001-70,000	1,367 (6.5)	268 (19.6)
>70,000	2,882 (13.7)	480 (16.7)
Undetermined	2,063 (9.8)	405 (19.6)
Education, n (%)		
< 12 years	3,310 (15.7)	845 (25.5)
HS diploma or GED	9,704 (46.2)	1,979 (20.4)
Some college	5,612 (26.7)	1,204 (21.5)
Bachelor degree or higher	2,398 (11.4)	434 (18.1)
Race, n (%)		
White	20,514 (97.6)	4,349 (21.2)
Black	213 (1.0)	38 (17.8)
Other	297 (1.4)	75 (25.3)
Alcohol consumption, n (%)		
None	13,276 (63.2)	2,848 (21.5)
< 1 drink/month	2,589 (12.3)	597 (23.1)
< 1 drink/week	1,530 (7.3)	309 (20.2)
Few drinks/week	2,087 (9.9)	397 (19.0)
1-3 drinks/day	805 (3.8)	142 (17.6)
>3 drinks/day	310 (1.5)	66 (21.3)
Undetermined	427 (2.0)	103 (24.1)
Smoking status, n (%)		
Never smoker	9,804 (46.6)	1,906 (19.4)
Former smoker	7,555 (35.8)	1,693 (22.5)
Current smoker < 10 cig/day	1,212 (5.8)	256 (21.1)
Current smoker 10-19 cig/day	1,260 (6.0)	310 (24.6)
Current smoker 20+ cig/day	1,213 (5.8)	297 (24.5)
Diabetes, n (%)	3,443 (16.4)	875 (25.4)
Thiazolidinedion use~	809 (23.5)	202 (25.0)
Other medications~	1,244 (36.1)	321 (25.8)
No medication~	1,390 (40.4)	352 (25.3)

*percentages refer to the proportion with respect to the entire population; ^percentages reflect the proportion of memory impaired in each category; percentages among diabetics only

Table 2: The association between PFAAs and self-report memory impairment in logistic regression for a doubling PFAA concentration, by quintiles of PFAAs, and in ordinal regression (n=21,024)

	Range (ng/mL)	Adjusted OR and 95% C.I.*
PFOS		0.93 (0.90-0.96)
1st quintile	0.25-14.4	Ref.
2nd quintile	14.5-20.4	0.96 (0.87-1.07)
3rd quintile	20.5-27.1	0.86 (0.78-0.96)
4th quintile	27.2-37.2	0.87 (0.78-0.96)
5th quintile	37.3-759.2	0.85 (0.76-0.94)
Trend		<0.001
Ordinal regression		0.95 (0.93-0.98)
PFOA		0.96 (0.94-0.98)
1st quintile	0.25-14.0	Ref.
2nd quintile	14.1-27.0	0.88 (0.79-0.97)
3rd quintile	27.1-53.8	0.83 (0.75-0.92)
4th quintile	53.9-118.1	0.79 (0.71-0.88)
5th quintile	118.3-22,412	0.79 (0.71-0.88)
Tend		<0.001
Ordinal regression		0.97 (0.96-0.98)
PFNA		0.96 (0.91-1.00)
1st quintile	0.25-0.90	Ref.
2nd quintile	1.0-1.2	0.86 (0.78-0.96)
3rd quintile	1.3-1.4	0.87 (0.77-0.98)
4th quintile	1.5-1.9	0.86 (0.77-0.95)
5th quintile	2.0-28.6	0.89 (0.80-0.99)
Trend		0.053
Ordinal regression		0.97 (0.94-1.01)
PFHxS		0.96 (0.93-0.99)
1st quintile	0.25-1.7	Ref.
2nd quintile	1.8-2.6	1.01 (0.91-1.12)
3rd quintile	2.7-3.6	1.02 (0.91-1.13)
4th quintile	3.7-5.6	0.93 (0.84-1.04)
5th quintile	5.7-232.6	0.89(0.79-0.99)
Trend		0.009
Ordinal regression		0.97 (0.94-0.99)

*Model adjusted for age (one-year age bands), ethnicity, gender, and school level (categorical), household income (categorical), physical activity, alcohol consumption (categorical, none/<1drink/month, < 1 drink/week, few drinks/week, 1-3 drinks/day, >3 drinks/day, undetermined), and cigarette smoking (categorical, never, former, < 10 cig/day, 12-20 cig/day, 20+ cig/day)

Table 3: The association between PFAAs and self-report memory impairment in logistic regression for a doubling PFAA concentration, by quintiles of distribution, and in ordinal regression by diabetes status (validated by clinical records)

	Range (ng/mL)	OR (95% CI)*	p for inter	N	OR (95% CI)*	p for inter
	PFOS N=17,832			**PFOA N=17,832**		
Non-diabetics		0.93 (0.90-0.96)†	-		0.95 (0.93-0.97) †	-
Ordinal regression		0.96 (0.93-0.99)			0.96 (0.95-0.98)	
1st quintile	0.25-14.5	Ref.		0.25-14.3	Ref.	
2nd quintile	14.6-20.5	0.96 (0.86-1.08)		14.4-27.2	0.85 (0.76-0.95)	
3rd quintile	20.6-27.0	0.90 (0.80-1.01)		27.3-54.3	0.82 (0.73-0.92)	
4th quintile	27.1-37.1	0.88 (0.78-0.99)		54.4-119.1	0.76 (0.68-0.86)	
5th quintile	37.2-759.2	0.85 (0.76-0.96)		119.2-8,416	0.75 (0.67-0.84)	
Trend		0.002			<0.001	
	N=3,192			**N=3,192**		
Diabetics		0.94 (0.88-1.02) †	0.698		1.02 (0.97-1.06) †	0.014
Ordinal regression		0.95 (0.90-1.01)			1.00 (0.97-1.04)	
1st quintile	0.25-13.6	Ref.		0.25-12.6	Ref.	
2nd quintile	13.7-20.0	1.06 (0.82-1.36)		12.7-25.4	1.04 (0.80-1.34)	
3rd quintile	20.1-27.3	0.82 (0.63-1.06)		25.5-48.0	0.88 (0.67-1.14)	
4th quintile	27.4-37.3	0.87 (0.67-1.13)		48.1-102.1	1.04 (0.80-1.35)	
5th quintile	37.4-272.0	0.90 (0.69-1.17)		102.4-22,412	1.09 (0.84-1.42)	
		0.162			0.543	
	PFNA			**PFHxS**		
	N=17,832			**N=17,832**		
Non-diabetics		0.95 (0.90-0.99) †	-		0.96 (0.93-0.99) †	-
Ordinal regression		0.97 (0.93-1.01)			0.97 0.94-0.99)	
1st quintile	0.25-0.9	Ref.		0.25-1.8	Ref.	
2nd quintile	1.0-1.2	0.86 (0.77-0.97)		1.9-2.6	0.98 (0.87-1.10)	
3rd quintile	1.3-1.5	0.85 (0.76-0.95)		2.7-3.7	0.99 (0.89-1.11)	
4th quintile	1.6-1.9	0.83 (0.73-0.93)		3.8-5.7	0.93 (0.82-1.05)	
5th quintile	2.0-28.6	0.88 (0.78-0.99)		5.8-232.6	0.88 (0.79-0.99)	
Trend		0.031			0.029	
	N=3,192			**N=3,192**		
Diabetics		1.01 (0.90-1.13) †	0.259	3,192	0.99 (0.92-1.06) †	0.683
Ordinal regression		0.99 (0.91-1.09)				
1st quintile	0.25-0.8	Ref.		0.25-1.6	Ref.	
2nd quintile	0.9-1.1	1.06 (0.80-1.40)		1.7-2.3	1.06 (0.81-1.38)	
3rd quintile	1.2-1.4	0.88 (0.66-1.17)		2.4-3.2	1.10 (0.85-1.42)	
4th quintile	1.5-1.8	1.03 (0.77-1.36)		3.3-5.0	1.02 (0.79-1.33)	
5th quintile	1.9-14.5	1.08(0.82-1.43)		5.1-99.7	1.00 (0.77-1.31)	
		0.620			0.942	

*using clinical record validated diagnosis of diabetes and self-reported use of medications, adjusted for age (one-year age bands), ethnicity, gender, school level (categorical), household income (categorical), physical activity, alcohol consumption (categorical, none/<1drink/month, < 1 drink/week, few drinks/week, 1-3 drinks/day, >3 drinks/day, undetermined), and cigarette smoking (categorical, never, former, < 10 cig/day, 12-20 cig/day, 20+ cig/day); † OR for doubling PFAA concentration

Table 4: Sensitivity analysis of the association between PFAAs and self-report memory impairment for a doubling PFAA concentration, by quintiles of PFAAs, and in ordinal regression on subjects aged 65 years and older (n=7,097), and using any memory impairment as outcome measure (n=21,024).

	Range (ng/mL)	OR (95% C.I.)[b] N=7,097 Restricted to those aged 65+*	Range (ng/mL)	OR (95% C.I.)[b] N=21,024 Any memory impairment^
PFOS		0.95 (0.90-1.00)		0.96 (0.94-0.99)
Ordinal regression		0.98 (0.94-1.03)		
1st quintile	0.25-15.3	Ref.	0.25-14.4	Ref.
2nd quintile	15.4-22.0	0.99 (0.83-1.20)	14.5-20.4	0.96 (0.88-1.05)
3rd quintile	22.1-28.9	0.95 (0.79-1.14)	20.5-27.1	0.90 (0.82-0.98)
4th quintile	29.0-4.0	0.97 (0.81-1.16)	27.2-37.2	0.94 (0.86-1.03)
5th quintile	40.1-759.2	0.84 (0.70-1.01)	37.3-759.2	0.93 (0.85-1.02)
Trend		0.079		0.121
PFOA		0.99 (0.97-1.03)		0.97 (0.96-0.99)
Ordinal regression		1.00 (0.97-1.03)		
1st quintile	0.25-15.0	Ref.	0.25-14.0	Ref.
2nd quintile	15.1-29.6	0.91 (0.75-1.09)	14.1-27.0	0.90 (0.82-0.98)
3rd quintile	29.7-56.8	0.90 (0.75-1.08)	27.1-53.8	0.86 (0.79-0.94)
4th quintile	56.9-123.0	0.84 (0.70-1.01)	53.9-118.1	0.87 (0.79-0.95)
5th quintile	123.1-5,994.8	0.99(0.83-1.19)	118.3-22,412	0.85 (0.78-0.93)
Tend		0.680		<0.001
PFNA		0.95 (0.87-1.02)		0.98 (0.95-1.02)
Ordinal regression		0.99 (0.93-1.07)		
1st quintile	0.25-0.8	Ref.	0.25-0.90	Ref.
2nd quintile	0.9-1.1	0.88 (0.73-1.06)	1.0-1.2	0.89 (0.82-0.97)
3rd quintile	1.2-1.4	0.81 (0.67-0.98)	1.3-1.4	0.94(0.85-1.04)
4th quintile	1.5-1.8	0.82 (0.68-0.99)	1.5-1.9	0.92 (0.85-1.01)
5th quintile	1.9-11.7	0.88 (0.73-1.07)	2.0-28.6	0.94 (0.86-1.03)
Trend		0.177		0.493
PFHxS		0.96 (0.91-1.01)		0.97 (0.94-0.99)
Ordinal regression		0.98 (0.93-1.02)		
1st quintile	0.25-1.9	Ref.	0.25-1.7	Ref.
2nd quintile	2.0-2.8	0.98 (0.82-1.18)	1.8-2.6	0.98 (0.90-1.07)
3rd quintile	2.9-3.9	0.95 (0.79-1.15)	2.7-3.6	1.03 (0.94-1.13)
4th quintile	4.0-6.0	0.98 (0.82-1.17)	3.7-5.6	0.96 (0.87-1.04)
5th quintile	6.1-232.6	0.86 (0.71-1.03)	5.7-232.6	0.89 (0.81-0.97)
Trend		0.139		0.010

[b]Model 2 includes age (one-year age bands), ethnicity, gender, and school level (categorical), household income (categorical), physical activity, alcohol consumption (categorical, none/<1drink/month, < 1 drink/week, few drinks/week, 1-3 drinks/day, >3 drinks/day, undetermined), and cigarette smoking (categorical, never, former, < 10 cig/day, 12-20 cig/day, 20+ cig/day)

* Sensitivity analysis including subjects aged 65 years or older only (N=7,097)

^ Sensitivity analysis using a more restrictive definition of memory impairment (those reporting frequent episode of short-term memory loss only, cases = 1,115)

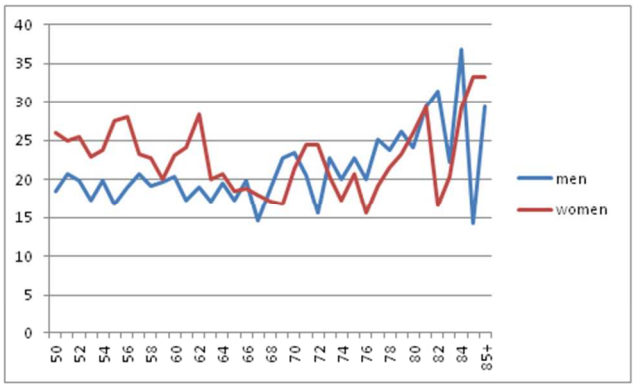

Figure 1: Prevalence of self-reported short-term memory impairment by age and sex in the study population
203x162mm (96 x 96 DPI)

Online Table 1: The association between PFAAs and self-reported memory impairment in logistic regression for a doubling PFAA concentration, by quintiles of PFAAs, and in ordinal regression (n=21,024).

	Range (ng/mL)	Model 1[a]	Model 2[b]	Model 3[c]
PFOS		0.92 (0.89-0.95)	0.93 (0.90-0.96)	0.93 (0.90-0.96)
Ordinal regression		0.95 (0.92-0.97)	0.95 (0.93-0.98)	0.96 (0.93-0.98)
1st quintile	0.25-14.4	Ref.	Ref.	Ref.
2nd quintile	14.5-20.4	0.95 (0.85-1.05)	0.96 (0.87-1.07)	0.97 (0.88-1.08)
3rd quintile	20.5-27.1	0.84 (0.76-0.93)	0.86 (0.78-0.96)	0.87 (0.79-0.97)
4th quintile	27.2-37.2	0.83 (0.75-0.93)	0.87 (0.78-0.96)	0.88 (0.79-0.97)
5th quintile	37.3-759.2	0.81 (0.73-0.91)	0.85 (0.76-0.94)	0.86 (0.77-0.96)
Trend		<0.001	<0.001	0.001
PFOA		0.95 (0.94-0.97)	0.96 (0.94-0.98)	0.96 (0.94-0.98)
Ordinal regression		0.97 (0.95-0.98)	0.97 (0.96-0.98)	0.97 (0.96-0.99)
1st quintile	0.25-14.0	Ref.	Ref.	Ref.
2nd quintile	14.1-27.0	0.86 (0.78-0.96)	0.88 (0.79-0.97)	0.88 (0.80-0.98)
3rd quintile	27.1-53.8	0.82 (0.74-0.91)	0.83 (0.75-0.92)	0.84 (0.76-0.93)
4th quintile	53.9-118.1	0.77 (0.70-0.86)	0.79 (0.71-0.88)	0.81 (0.73-0.90)
5th quintile	118.3-22,412	0.76 (0.69-0.85)	0.79 (0.71-0.88)	0.80 (0.72-0.90)
Tend		<0.001	<0.001	<0.001
PFNA		0.94 (0.90-0.98)	0.96 (0.91-1.00)	0.96 (0.92-1.01)
Ordinal regression		0.96 (0.93-0.99)	0.97 (0.94-1.01)	0.98 (0.94-1.01)
1st quintile	0.25-0.90	Ref.	Ref.	Ref.
2nd quintile	1.0-1.2	0.85 (0.77-0.94)	0.86 (0.78-0.96)	0.87 (0.78-0.96)
3rd quintile	1.3-1.4	0.85 (0.76-0.95)	0.87 (0.77-0.98)	0.88 (0.78-0.98)
4th quintile	1.5-1.9	0.83 (0.75-0.92)	0.86 (0.77-0.95)	0.86 (0.78-0.95)
5th quintile	2.0-28.6	0.85 (0.76-0.94)	0.89 (0.80-0.99)	0.90 (0.81-1.01)
Trend		0.004	0.053	0.079
PFHxS		0.95 (0.92-0.98)	0.96 (0.93-0.99)	0.97 (0.94-1.00)
Ordinal regression		0.96 (0.94-0.99)	0.97 (0.94-0.99)	0.97 (0.95-0.99)
1st quintile	0.25-1.7	Ref.	Ref.	Ref.
2nd quintile	1.8-2.6	1.00 (0.90-1.11)	1.01 (0.91-1.12)	1.02 (0.91-1.13)
3rd quintile	2.7-3.6	1.00 (0.90-1.11)	1.02 (0.91-1.13)	1.03 (0.93-1.15)
4th quintile	3.7-5.6	0.91 (0.82-1.02)	0.93 (0.84-1.04)	0.96 (0.86-1.06)
5th quintile	5.7-232.6	0.86 (0.77-0.96)	0.89(0.79-0.99)	0.92 (0.82-1.02)
Trend		0.001	0.009	0.053

Online Table 2: The association between PFAAs and self-report memory impairment for a doubling PFAA concentration and by tertiles of distribution by self-reported anti-diabetic treatment

	Range (ng/ML)	N	PFOS OR (95% CI)*	Range (ng/ML)	PFOA OR (95% CI)*	Range (ng/ML)	PFNA OR (95% CI)*	Range (ng/ML)	PFHxS OR (95% CI)*
Thiazolidinedione use		809	1.00 (0.86-1.16)		0.97 (0.88-1.07)		0.94 (0.74-1.19)		1.02 (0.87-1.20)
Ordinal regression			1.06 (0.93-1.20)		1.03 (0.95-1.11)		1.02 (0.84-1.25)		1.05 (0.92-1.20)
1st tertile	0.25-17.9		Ref.	1.1-17.5	Ref.	0.25-1.0	Ref.	0.25-1.9	Ref.
2nd tertile	18.0-29.9		0.76 (0.50-1.16)	17.6-49.7	0.72 (0.47-1.10)	1.1-1.5	0.83 (0.54-1.26)	2.0-3.5	1.56 (1.02-2.38)
3rd tertile	30.1-104.9		0.93 (0.61-1.42)	19.9-8,068	0.81 (0.53-1.24)	1.6-14.7	0.79 (0.51-1.23)	3.6-84.0	1.13 (0.72-1.77)
p-value for trend			0.737		0.333		0.309		0.628
Other medications		1,244	0.90 (0.80-1.01)		1.00 (0.93-1.07)		0.95 (0.79-1.15)		0.91 (0.81-1.03)
Ordinal regression			0.92 (0.83-1.01)		1.00 (0.93-1.07)		0.94 (0.81-1.10)		0.94 (0.86-1.04)
1st tertile	0.25-17.9		Ref.	0.25-20.5	Ref.	0.25-1.1	Ref.	0.25-2.1	Ref.
2nd tertile	18.0-29.8		0.75 (0.54-1.04)	20.6-63.2	0.99 (0.71-1.39)	1.2-1.6	0.72 (0.52-1.01)	2.2-3.6	0.99 (0.71-1.38)
3rd tertile	29.9-218.0		0.68 (0.48-0.95)	63.4-2,316.2	0.92 (0.66-1.29)	1.7-6.0	0.85 (0.61-1.20)	3.7-99.7	0.82 (0.58-1.16)
p-value for trend			0.023		0.644		0.341		0.259
No medication		1,390	0.95 (0.85-1.07)		1.00 (0.94-1.08)		1.03 (0.87-1.23)		1.01 (0.90-1.13)
Ordinal regression			0.94 (0.86-1.03)		1.00 (0.95-1.06)		0.98 (0.85-1.13)		0.99 (0.90-1.08)
1st tertile	0.25-18.3		Ref.	0.7-20.2	Ref.	0.25-1.0	Ref.	0.25-2.1	Ref.
2nd tertile	18.4-29.3		1.11 (0.81-1.52)	20.3-63.4	1.05 (0.77-1.44)	1.1-1.5	1.01 (0.72-1.40)	2.2-3.7	0.93 (0.68-1.28)
3rd tertile	29.4-272.0		1.02 (0.74-1.40)	63.5-22,412	0.99 (0.72-1.37)	1.6-14.5	1.12 (0.81-1.54)	3.8-43.3	0.99 (0.72-1.37)
p-value for trend			0.897		0.984		0.473		0.957

*using clinical record validated diagnosis of diabetes and self-reported use of medications, adjusted for age (one-year age bands), ethnicity, gender, school level (categorical), household income (categorical), physical activity, alcohol consumption (categorical, none/<1drink/month, < 1 drink/week, few drinks/week, 1-3 drinks/day, >3 drinks/day, undetermined), and cigarette smoking (categorical, never, former, < 10 cig/day, 12-20 cig/day, 20+ cig/day); † OR for doubling PFAA concentration

Table S1
(Gallo et al. [2011] PLoS Med; doi:10.1371/journal.pmed.1001117)

The Strengthening the Reporting Observational studies in Epidemiology – Molecular Epidemiology (STROBE-ME) Reporting Recommendations: Extended from STROBE statement

Item	Item number	STROBE Guidelines	Extension for Molecular Epidemiology Studies (STROBE-ME)
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	**ME-1** State the use of specific biomarker(s) in the title and/or in the abstract if they contribute substantially to the findings
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	
Introduction			
Background rationale	2	Explain the scientific background and rationale for the investigation being reported	**ME-2** Explain in the scientific background of the study how/why the specific biomarker(s) have been chosen, potentially among many others (e.g., others are studied but reported elsewhere, or not studied at all)
Objectives	3	State specific objectives, including any pre-specified hypotheses	**ME-3** *A priori* hypothesis: if one or more biomarkers are used as proxy measures, state the *a priori* hypothesis on the expected values of the biomarker(s)
Methods			
Study design	4	Present key elements of study design early in the paper	**ME-4** Describe the special study designs for molecular epidemiology (in particular nested case/control and case/cohort) and how they were implemented
Biological sample collection			**ME-4.1** Report on the setting of the biological sample collection; amount of sample; nature of collecting procedures; participant conditions; time between sample collection and relevant clinical or physiological endpoints.
Biological sample storage			**ME-4.2** Describe sample processing (centrifugation, timing, additives, etc).
Biological sample processing			**ME-4.3** Describe sample storage until biomarker analysis (storage, thawing, manipulation, etc).
Biomarker biochemical characteristics			**ME-4.4** Report the half-life of the biomarker, and chemical and physical characteristics (e.g., solubility).
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	
Participants	6	(a) Cohort study—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up Case-control study—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants	**ME-6** Report any habit, clinical conditions, physiological factor, or working or living condition that might affect the characteristics or concentrations of the biomarker
		(b) Cohort study—For matched studies, give matching criteria and number of exposed and unexposed Case-control study—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	
Data source/measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	**ME-8** Laboratory methods: report type of assay used, detection limit, quantity of biological sample used, outliers, timing in the assay procedures (when applicable) and calibration procedures or any standard used
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	**ME-12** Describe how biomarkers were introduced into statistical models
		(b) Describe any methods used to examine subgroups and interactions	
		(c) Explain how missing data were addressed	
		(d) Cohort study—If applicable, explain how loss to follow-up was addressed Case-control study—If applicable, explain how matching of cases and controls was addressed Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(e) Describe any sensitivity analyses	

Validity/reliability of measurement and internal/external validation			**ME-12.1** Report on the validity and reliability of measurement of the biomarker(s) coming from the literature and any internal or external validation used in the study.
Results			
Participants	13	(a) Report the numbers of individuals at each stage of the study—e.g., numbers potentially eligible, examined for eligibility, con-firmed eligible, included in the study, completing follow-up, and analysed	**ME-13** Give reason for loss of biological samples at each stage
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14	(a) Give characteristics of study participants (e.g., demographic, clinical, social) and information on exposures and potential con-founders	
		(b) Indicate the number of participants with missing data for each variable of interest	
		(c) Cohort study—Summarise follow-up time (e.g., average and total amount)	
Distribution of biomarker measurement			**ME-14.1** Give the distribution of the biomarker measurement (including mean, median, range, and variance)
Outcome data	15	Cohort study—Report numbers of outcome events or summary measures over time Case-control study—Report numbers in each exposure category, or summary measures of exposure Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included	
		(b) Report category boundaries when continuous variables were categorized	
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	**ME-19** Describe main limitations in laboratory procedures
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	**ME-20** Give an interpretation of results in terms of *a-priori* biological plausibility
Generalisability	21	Discuss the generalisability (external validity) of the study results	
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	
Ethics			**ME-22.1** Describe informed consent and approval from ethical committee(s). Specify whether samples were anonymous, anonymised or identifiable

Serum perfluoroalkyl acids concentrations and memory impairment in a large cross-sectional study

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Running title: perfluoroalkyl acids and memory impairment

Word count: 3,24151

Summary

Article focus

- Cross-sectional association between serum level of Perfluorooctanate (PFOA), perfuorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA) and perfluorohexane sulfonate (PFHxS) and self-reported memory impairment in a population exposed to high levels of PFOA
- Potential interaction between the association of perfluoroalkyl acids (PFAAs) with memory impairment by diabetes status

Key Message

- Inverse associations between PFOS and PFOA and memory impairment were statistically significant perhaps due to a potential anti-inflammatory effect exerted through PPAR agonism. Comparable inverse associations with PFNA and PFHxS were of borderline statistical significance
- Inverse associations of PFAAs with memory impairment were weaker or non-existent in diabetics than overall in non-diabetics. Analysis further stratified by specific diabetes medication use showed no variation in odds ratios more than explicable by chance given the number of tests made.

Strengths and limitations

- Very large sample size including 21,024 adults with measured serum level of Perfluoroalkyl acids (PFAAs) with a given geographical distribution allowing some multilevel modelling
- The cross-sectional nature of the design does not allow any causal inference and makes results particularly prone to reverse causality
- Self-reported is not an optimal method for estimating the degree of memory impairment in a population

ABSTRACT

Objectives – To examine the cross-sectional association between serum PFOA, PFOS, PFNA, and PFHxS concentrations with self-reported memory impairment in adults and the interaction of these associations with diabetes status

Design - Cross-sectional study

Setting – Population-based in Mid-Ohio Valley, West Virginia following contamination by a chemical plant

Participants - The C8 Health Project collected data and measured serum level of PFAAs of 21,024 adults aged 50+ years

Primary outcome measure – Self-reported memory impairment as defined by the question "have experienced short term memory loss?"

Results - A total of 4,057 subjects self-reported short-term memory impairment. Inverse associations between PFOS and PFOA and memory impairment were highly statistically significant with fully adjusted OR=0.93 (95% C.I. 0.90-0.96) for doubling PFOS and OR=0.96 (95% C.I. 0.94-0.98) for doubling PFOA concentrations. Comparable inverse associations with PFNA and PFHxS were of borderline statistical significance. Inverse associations of PFAAs with memory impairment were weaker or non-existent in diabetics than overall in non-diabetics.

Conclusion - An inverse association between PFAA serum levels and self-reported memory impairment has been observed in this large population-based, cross-sectional study stronger and more statistically significant for PFOA and PFOS. The associations can be potentially explained by preventive anti-inflammatory effect exerted by a PPAR agonist effect of these PFAAs, but confounding or even reverse causation cannot be excluded as an alternative explanation.

INTRODUCTION

Perfluoroalkyl acids (PFAAs) are man-made compounds used during the manufacture of fluoropolymers including non-stick cookware and breathable, yet waterproof, fabrics. They can also result from the metabolism of fluorinated telomers, compounds used for food package coatings, carpet treatments, and stain-resistant fabric treatment. Perfluorooctanate (PFOA), perfuorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA) and perfluorohexane sulfonate (PFHxS) persist in the environment and are found in the blood of humans and many animal species throughout the world [1 2]. Potential sources of exposure to PFAAs in humans include drinking water, dust, breast milk, fish and other foods, food packaging, ambient air, and occupational exposure [3-6].

In animal models, perfluoroalkyl chemicals can activate peroxisome proliferator-activated receptor alpha (PPARα), a ligand-activated transcription factor that regulates gene expression, lipid modulation, glucose homeostasis, cell proliferation and inflammation [7 8]. The PPAR receptor has been involved in the ageing process: PPARα null mice showed a decreased longevity compared with the wild-type due to non-neoplastic spontaneous ageing lesions which occurred with a higher incidence and a short latency in the PPARα null mice [9]. Also PPARγ variants were reported to be associated with longevity in humans with low insulin resistance [10 11]. Activation of the PPARγ receptor *in vitro* and *in vivo* also prevents the expression of inflammatory cytokines and other inflammatory mediators in brains of Alzheimer disease animal models [12 13]. In addition, PPARγ agonists have been demonstrated to suppress the Aβ-mediated activation of microglia *in vitro* and prevent cortical or hippocampal neuronal cell death [14-16]. PPARγ is also deeply involved in diabetes, given its ability to orchestrate the expression of genes involved in lipid metabolism, adipogenesis, and inflammation. It is activated by endogenous ligands (such as fatty acids and prostaglandins) or drugs such as thiazolidinedione. It is most highly expressed in adipocytes where it acts as the master regulator of adipogenesis via induction of adipogenic genes [17]. However, a study *in vitro* showed that PFOA and PFOS activate differentially PPARα and PPARγ receptors, but it is not possible to directly extrapolate these results to toxicity studies *in vivo* [18]. Therefore, in line with what was recently observed by Power et al[19], we hypothesised that increased exposure to PFAA could be associated with a better cognitive function.

The positive association between diabetes and cognitive impairment is well established [20]; some studies investigating the association between PFOA exposure and diabetes suggested the presence of an inverse association: a negative trend in diabetes occurrence by increasing serum PFOA deciles

was found in a case–control study nested in a previous study based on the population investigated here [21 22]; but not in others [23 24].

From 1950-2005, a chemical plant in the Mid-Ohio Valley, West Virginia was responsible for emitting PFOA into the surrounding environment. In 2001, a group of residents from the nearby West Virginia and Ohio communities filed a class action lawsuit alleging health damage from drinking water supplies drawing on PFOA-contaminated groundwater [25]. Part of the pre-trial settlement of the class action lawsuit included a baseline survey, the C8 Health Project, conducted in 2005-2006, that gathered data from over 69,000 people from six contaminated water districts surrounding the plant [25]. In this population, PFOA concentrations ranged from US background levels to very high; overall PFOA levels are much higher in this population (geometric mean 42.6.0 ng/mL, 95% C.I. 41.8-43.3) than in the corresponding US population surveys (NHANES in same year mean 3.95 ng/mL, 95% C.I. 3.65-4.27) [1 25]. The mean PFOS (geometric mean 22.4, 95% C.I. 22.2-22.6), PFNA (1.37, 95% C.I. 1.36-1.38), and PFHXs (3.18, 95% C.I. 3.15-3.22) closely resembled values from a nationally representative US sample form a similar time frame (mean PFOS 20.7, 95% C.I. 19.2-22.3; mean PFNA 0.97, 95% C.I. 0.82-1.14; and PFHXs 1.93, 95% C.I. 1.73-2.16) [1].

The present study uses these data to examine the cross-sectional association between serum PFOA, PFOS, PFNA, and PFHxS concentrations with self-reported memory impairment in adults, and its potential interaction with diabetes status.

METHODS

The Study population

This study is one of the C8 Science Panel Studies and uses information from questionnaires and blood tests collected in the C8 Health Project, supplemented by further information on classification by water district developed in a companion C8 Science Panel Study.

The C8 Health Project enrolled eligible subjects between August 2005 and August 2006. Individuals were eligible to participate in the C8 Health Project if they had consumed water for at least one year between 1950 and December 3, 2004 while living, working, or going to school in one of the following six water districts: Little Hocking Water Association of Ohio; City of Belpre, Ohio; Tupper Plains–

Chester District of Ohio; Village of Pomeroy, Ohio; Lubeck Public Service District of West Virginia; Mason County Public Service District of West Virginia; or private water sources within aforementioned districts and areas of documented PFOA contamination. Details of the study enrolment process, including consenting procedures, have been described elsewhere [25].

The C8 Health Project collected data on 69,030 people. The participation rate for the C8 Health Project based on US census counts of residents in the affected water districts during Project enrolment, have been estimated at around 80% [25]. In this population, the strongest predictor of PFOA serum concentration was residence in one of the contaminated water districts [26]; serum levels of other PFAAs do not show such geographic variation. Of the population, 21,724 older adults (aged ≥50 years) were considered for this analysis, and a total of 21,024 (96.8%) were included in the final analysis after exclusion of subjects with missing data on ethnicity, education level, socio-economic status, cigarette smoking, or BMI measurements.

Memory impairment definition

During the survey (2005-2006), all participants were asked if they "had experienced short term memory loss", the possible answers being "frequently", "sometimes", "rarely", and "never". The principle analyses assessed memory impairment defined as reporting short-term memory loss frequently or sometimes, compared to rarely and never. Memory impairment ever was also considered, defined as reporting any memory loss and compared to the never category.

Laboratory analysis

Blood samples were obtained and processed at individual data collection sites. Samples were drawn into four tubes per participant, with a maximum 35 mL. Tubes were spun, aliquoted, and refrigerated until shipping. Samples were shipped on dry ice daily from each data collection site to the laboratory daily [25]. Participants were not asked to fast before blood sample withdrawal, but fasting status was recorded.

Laboratory analysis of PFAAs used an automated solid-phase extraction combined with reverse-phase high-performance liquid chromatography [27]. Analyses were conducted by the Exygen Research Inc., State College, PA, USA; an intra-laboratory quality assurance program was carried out by analysis of duplicate samples at AXYS Analytical Service Ltd., Sidney, BC, Canada [25]. The intra-laboratory coefficient of variation for all PFAAs measurements was 0.1; the inter-laboratory comparison coefficient of variation was 0.2 for PFOA and PFNA, 0.1 for PFOS, and not applicable for PFHxA as all in the second lab measurement values were below level of detection[25]. The detection

limit for all PFAAS was 0.5 ng/mL and observations below this limit were assigned a value of 0.25 ng/mL (*n*=16, *n*=101, n=532, and n=387for PFOA, PFOS, PFNA, and PFHxS, respectively, for this study population). All PFAAs concentration distributions were skewed to the right. Methods and results are reported according to STROBE-ME recommendations [28].

Statistical analysis

Associations between exposure (serum concentration of PFOA, PFOS, PFNA, and PFHxS) and memory impairment were studied using logistic regression. Minimally adjusted models included age, in one year age-band, race (white, black, and others), gender, and educational level (high school diploma or general educational development (GED), some college, bachelor degree or higher) (Model 1). Further adjusted models additionally included average household income (≤$10,000, $10,001-20,000, $20,001-30,000, $30,001-40,000, $40,001-50,000, $50,001-60,000, $60,001-70,000, >$70,000), physical activity, alcohol consumption (none, <1 drink/month, <1 drink/week, few drinks/week, >1 drink/day) and cigarette smoking (never smoker, former smoker, current smoker <10 cigarettes/day, current smoker 10-19 cigarettes/day, current smoker ≥20 cigarettes/day) (Model 2). Fully adjusted models included also body mass index (BMI) (underweight/normal weight; overweight; and obese class I, II, and III), and diabetes (Model 3). PFAA concentrations were log-transformed to reduce skewness. For each model the association between PFAAs and self-reported memory impairment was calculated for a doubling in PFAA concentration in a model with PFAA entered as continuous covariate, for quintile groups of the PFAA distribution, and by ordinal regression analysis with the outcome variable comprising the four original levels of slef-reported frequency of episodes of memory loss, again in relation to a doubling of PFAAs. To explore possible differential effect of PFAA in sub-groups, analyses were further stratified by diabetes status and, among diabetics, by type of medications.

The following four sensitivity analyses were carried out: firstly one analysis restricting the sample to 7,097 subjects aged 65 years and older. Secondly, an analysis conducted on the entire sample, but using as outcome definition those reporting any memory loss (frequently, sometimes, and rarely). Our final sensitivity analysis utilises the geographical clustering of PFOA exposure by water districts which allowed use to decompose the overall estimate of association of PFOA with memory impairment into within and between water district components, by including as explanatory variables both water district mean logged PFOA serum concentration and the deviations of individual's values from their district mean [29]. These two associations are subject to different potential biases, so help interpretation.

Role of funding

Funding for this work, the "C8 Science Panel Community Study at London School of Hygiene and Tropical Medicine - LSHTM", comes from the C8 Class Action Settlement Agreement (Circuit Court of Wood County, WV, USA) between DuPont and plaintiffs, which resulted from releases of PFOA (or C8) into drinking water. It is one of the C8 Science Panel Studies undertaken by the Court-approved C8 Science Panel established under the same Settlement Agreement. The task of the C8 Science Panel, of which Tony Fletcher is a member, is to undertake research in the Mid-Ohio Valley, and subsequently evaluate the results along with other available information to determine if there are any probable links between PFOA and disease. Funds were administered by the Garden City Group (Melville, NY) that reports to the Court. The authors of this manuscript declare that their ability to design, conduct, interpret, or publish research was unimpeded by and fully independent of the court and/or settling parties. In addition, they declare no competing financial interests. The LSHTM Ethics Committee approved this study.

RESULTS

A total of 4,462 subjects (21.2% of the entire population of 21,024 individuals aged 50 years or older) self-reported short-term memory impairment (**Error! Reference source not found.**): episodes of short-term memory loss were reported frequently by 1,115 subjects (5.3%); sometimes by 3,347 (15.9%); rarely by 4,283 (20.4%) and never by 12,279 (58.4%). Many personal characteristics were associated individually with memory loss, including higher age, lower socio-economic status, smoking, and diagnosis of diabetes (Table 1), though to what extent these reflected independent risk factors was not investigated.

Results from the logistic regression of association between PFAAs and memory impairment are shown in Table 2. Results for minimally, further and fully adjusted models were similar, so we show only further adjusted results in this table, but results for all models are in the on-line Table 1. Inverse associations between PFOS and PFOA and memory impairment were highly statistically significant with adjusted OR=0.93 (0.90-0.96) for PFOS and OR=0.96 (0.94-0.98) for PFOA for doubling PFAA concentrations. Inverse associations of similar magnitude with PFNA and PFHxS but of borderline statistical significance were found: OR=0.96 (0.92-1.02) for PFNA and OR=0.97 (0.94-1.00) for PFHxS. The analysis by PFAA quintile groups shows similar patterns.

Inverse associations of PFAAs with memory impairment were weaker or non-existent in diabetics than for non-diabetics (Table 3), though odds ratios were imprecise, and the difference by diabetes status was only significant for PFOA (p-value for interaction = 0.014). Analysis further stratified by specific diabetes medication use showed no variation in odds ratios more than explicable by chance given the number of tests made (on-line Table 2).

In the sensitivity analysis on subjects older than 65 years, the substantial reduction of the sample size resulted in loss of precision in odds ratios. However, the points estimates of associations with memory impairment were of comparable magnitude for all PFAAs except PFOA for which the association with memory impairment was close to null (OR= 0.99 (0.97-1.03)) (Table 4).

The analysis carried out on the entire sample, comparing those with any memory impairment against those with no memory problems shows slightly weaker associations for each PFAAs but precision was reduced (Table 4). Analyses using ordinal regression in place of binary logistic regression yielded similar results to the logistic regressions (Table 2, Table 3, and Table 4).

The analysis separating the PFOA-memory impairment association into within and between water district components found that within water districts there was an inverse association between PFOA and memory impairment, as in the overall association (OR 0.94, 95%CI 0.91-0.98, scale and adjustments as before). However there was no association between geometric mean concentration by and memory impairment (OR 1.00, 95%CI 0.97-1.03, per doubling in geometric mean PFOA by district).

Extra data is available upon request by emailing Tony Fletcher (tony.fletcher@lshtm.ac.uk).

DISCUSSION

An inverse association between PFAAs serum levels (including PFOS, PFOA, PNA, and PFHxS) and self-reported memory impairment has been observed in this large population-based, cross-sectional study. This association is more clearly monotonic with increasing exposure, and more statistically significant for PFOA and PFOS. However, the consistent decrement for all PFAAs suggests a common mechanism.

It is plausible that PFAAs could have an effect on cognitive function via PPAR agonism. Although it is not clear to what extent PFAAs act differentially on PPAR receptors α and γ[18], it could be speculated

that this association might be mediated by the activation of the PPAR receptor by PFAAs. Activation of the PPARγ receptors has been shown to decrease the secretion of proinflamatory cytokines and possibly increase phagocytosis of Aβ inclusions, probably through activation of microglia[30]. However there was suggestion that this effect of suppression of the activation of microglia was age-dependent or disease stage-dependent being not significant in patients with advanced Alzheimer's disease (AD)[31 32]. PPAR agonist drugs, such as non-steroidal anti-inflammatory drugs (NSAID) and some anti-diabetics (i.e. thiazolodinedione or pioglitazone) have been proposed as preventive drugs for neurodegenerative conditions, including Alzheimer's dementia[30 33].

In a previous published study an inverse association between PFAAs and memory impairment was observed specifically among non-medicated diabetics[19]. In the present study, this pattern was not replicated, with the inverse association between PFAAs and cognitive impairment being more evident in those without diabetes; among diabetics, the association was not present, irrespective of treatment status. This finding could be explained by the fact that in diabetics PPAR receptors are more phosphorylated with a consequent reduced transcriptional activity [34 35], and the balance between PPARγ expression and activity levels is altered [34 36]. It is therefore possible – based on the present data – that the PPAR-agonist effect of PFAAs is different in subjects with and without PPAR-mediated metabolic changes such as diabetes. Also, it has been reported that PFAAs have a PPAR agonist effect, more prominently PPAR-α [37]; animal models suggest that PFOA has a stronger agonistic effect than PFOS [37]. Our findings of an inverse association between PFAA and memory impairment among non-diabetics, would therefore be compatible with a possible anti-inflammatory role exerted by PFAA on early symptoms of cognitive impairment.

There is some evidence of detrimental effects of PFAAs in neurodevelopment of mice affecting the cholinergic system and cognitive function[38-40], thus timing of exposure may also be relevant in order for the PFAAs to exert this hypothesised anti-dementing role. However, these findings should be interpreted cautiously given the limitations of the study. Firstly, given the cross-sectional nature of the study, reverse causality must be considered: subjects suffering from memory impairment could have consumed less of these compounds via water and food sources, though this is not a likely explanation given the consistency of the association across various PFAAs which have substantially different routes of exposure. Host characteristics such as genotype could be correlated with both some mechanism predisposing these symptoms and variation in PFAA excretion rates, thus leading to a confounded association with serum levels. Further, self-report is not a very accurate method for ascertaining memory impairment, although errors in classification would be expected to be non-differential misclassification, biasing the estimate of association towards the null. The effects of

PFAA have been mostly studied in relation to PPARα [7], while the receptor mostly implicated in metabolic changes and diabetes and in dementia PPARγ [30]; however, these two belong to the same receptor family and some degree of cross-activation cannot be excluded, and the knowledge of their pleiotropic effects is currently advancing [41]. Lastly, the classification into different anti-diabetic medications is uncertain as these were self-reported and not prompted by interviewers. However, we consider it very unlikely that any misreporting would be confounded with serum PFAAs. This would tend to low specificity and thus bias of the association (if any) towards the null.

On the other hand, strengths of this study include the fact that all showed estimates were adjusted for numerous potential confounders, including age in one-year age bands, making the effect of PFAA on memory impairment not likely to be confounded by lifestyle characteristics. Furthermore, these results are based on a very large population representative of the general population in West Virginia and Ohio [25], thus estimates are solid; and the 21% prevalence of memory impairment is compatible and consistent with figures on prevalence of dementia reported for North America (Ferri et al, 2005).

Individual serum levels reflect the contributions of both intake and retention/excretion rates. While we have no direct data on either of these components, the large differences in drinking water contamination and associated average population serum levels for PFOA in the 6 water districts, allow an estimate of the effect of exposure. That the association with PFOA was entirely within water districts, and not present at all between water districts despite large differences in (geometric) mean PFOA between districts (range 15.7 – 405.1) is suggestive of a bias operating at one or both of these levels. The between district estimate is not vulnerable to reverse causation or confounding at individual level, though some ecological confounding may operate if it happens to correlate with exposure level. Conversely the within district estimate but not between district estimate could reflect such individual confounding if present. Thus either the association documented at individual level could be confounded (e.g. by some unmeasured individual characteristic ~~a common genetic variant related to both dementia risk and some excretion pathways~~); or that the association at the district level is confounded to obscure association ~~biased towards the null~~ (~~e.g. by confounding by~~ for example socio-economic status). This sensitivity analysis cannot prove the presence of confounding at either level, but if the association had been consistent at both individual and district level that would have been more convincing of the association being due to PFAAs.

The size of the associations observed has both strong and weak aspects. The strong statistical significance suggests chance is an unlikely explanation. However, the odds ratios are only modestly different from one, 0.75 at the most extreme, so that biases are a more plausible explanation than

they would be with more extreme ratios. In conclusion, these data show an inverse association between PFOA and PFOS exposure and self-reported memory-impairment, particularly in non diabetics. This can be potentially explained by preventive anti-inflammatory effect exerted by a PPAR agonist effect of these PFAAs, but confounding or even reverse causation cannot be excluded as an alternative explanation.

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Table 1: Participant Characteristics, Mid-Ohio Valley, 2005-2006 (N=21,024)

	All N=21,024*	Memory impaired N=4,462^
Males, n (%)	10,353 (49.2)	2,040 (19.7)
Females, n (%)	10,671 (50.8)	2,422 (22.7)
Age, median/mean (SD)	60.5/62.3 (9.0)	59.9/62.3 (9.4)
Age groups		
50-54 years	5,381 (25.6)	1,185 (22.0)
55-59 years	4,831 (23.0)	1,055 (21.8)
60-64 years	3,715 (17.7)	740 (19.9)
65-69 years	2,930 (13.9)	535 (18.3)
70-74 years	1,979 (9.4)	419 (21.2)
75-79 years	1,251 (6.0)	269 (21.5)
80+ years	937 (4.5)	259 (27.6)
Regular exercise, n (%)	6,774 (32.2)	1,306 (19.3)
BMI, n (%)		
Normal weight	5,100 (24.3)	1,051 (20.6)
Overweight	8,194 (39.0)	1,612 (19.7)
Obese class I	4,789 (22.8)	1,028 (21.5)
Obese class II	1,805 (8.6)	457 (25.3)
Obese class III	1,136 (5.4)	314 (27.6)
Household income, $/y n (%)		
≤10,000	1,486 (7.1)	448 (30.2)
10,001-20,000	3,059 (14.6)	757 (24.8)
20,001-30,000	3,281 (15.6)	751 (22.9)
30,001-40,000	2,936 (14.0)	572 (19.5)
40,001-50,000	2,135 (10.2)	422 (19.8)
50,001-60,000	1,815 (8.6)	359 (19.8)
60,001-70,000	1,367 (6.5)	268 (19.6)
>70,000	2,882 (13.7)	480 (16.7)
Undetermined	2,063 (9.8)	405 (19.6)
Education, n (%)		
< 12 years	3,310 (15.7)	845 (25.5)
HS diploma or GED	9,704 (46.2)	1,979 (20.4)
Some college	5,612 (26.7)	1,204 (21.5)
Bachelor degree or higher	2,398 (11.4)	434 (18.1)
Race, n (%)		
White	20,514 (97.6)	4,349 (21.2)
Black	213 (1.0)	38 (17.8)
Other	297 (1.4)	75 (25.3)
Alcohol consumption, n (%)		
None	13,276 (63.2)	2,848 (21.5)
< 1 drink/month	2,589 (12.3)	597 (23.1)
< 1 drink/week	1,530 (7.3)	309 (20.2)
Few drinks/week	2,087 (9.9)	397 (19.0)
1-3 drinks/day	805 (3.8)	142 (17.6)
>3 drinks/day	310 (1.5)	66 (21.3)
Undetermined	427 (2.0)	103 (24.1)
Smoking status, n (%)		
Never smoker	9,804 (46.6)	1,906 (19.4)
Former smoker	7,555 (35.8)	1,693 (22.5)
Current smoker < 10 cig/day	1,212 (5.8)	256 (21.1)
Current smoker 10-19 cig/day	1,260 (6.0)	310 (24.6)
Current smoker 20+ cig/day	1,213 (5.8)	297 (24.5)
Diabetes, n (%)	3,443 (16.4)	875 (25.4)
Thiazolidinedion use~	809 (23.5)	202 (25.0)
Other medications~	1,244 (36.1)	321 (25.8)
No medication~	1,390 (40.4)	352 (25.3)

*percentages refer to the proportion with respect to the entire population; ^percentages reflect the proportion of memory impaired in each category; percentages among diabetics only

Table 2: The association between PFAAs and self-report memory impairment in logistic regression for a doubling PFAA concentration, by quintiles of PFAAs, and in ordinal regression (n=21,024)

	Range (ng/mL)	Adjusted OR and 95% C.I.*
PFOS		0.93 (0.90-0.96)
1st quintile	0.25-14.4	Ref.
2nd quintile	14.5-20.4	0.96 (0.87-1.07)
3rd quintile	20.5-27.1	0.86 (0.78-0.96)
4th quintile	27.2-37.2	0.87 (0.78-0.96)
5th quintile	37.3-759.2	0.85 (0.76-0.94)
Trend		<0.001
Ordinal regression		0.95 (0.93-0.98)
PFOA		0.96 (0.94-0.98)
1st quintile	0.25-14.0	Ref.
2nd quintile	14.1-27.0	0.88 (0.79-0.97)
3rd quintile	27.1-53.8	0.83 (0.75-0.92)
4th quintile	53.9-118.1	0.79 (0.71-0.88)
5th quintile	118.3-22,412	0.79 (0.71-0.88)
Tend		<0.001
Ordinal regression		0.97 (0.96-0.98)
PFNA		0.96 (0.91-1.00)
1st quintile	0.25-0.90	Ref.
2nd quintile	1.0-1.2	0.86 (0.78-0.96)
3rd quintile	1.3-1.4	0.87 (0.77-0.98)
4th quintile	1.5-1.9	0.86 (0.77-0.95)
5th quintile	2.0-28.6	0.89 (0.80-0.99)
Trend		0.053
Ordinal regression		0.97 (0.94-1.01)
PFHxS		0.96 (0.93-0.99)
1st quintile	0.25-1.7	Ref.
2nd quintile	1.8-2.6	1.01 (0.91-1.12)
3rd quintile	2.7-3.6	1.02 (0.91-1.13)
4th quintile	3.7-5.6	0.93 (0.84-1.04)
5th quintile	5.7-232.6	0.89(0.79-0.99)
Trend		0.009
Ordinal regression		0.97 (0.94-0.99)

*Model adjusted for age (one-year age bands), ethnicity, gender, and school level (categorical), household income (categorical), physical activity, alcohol consumption (categorical, none/<1drink/month, < 1 drink/week, few drinks/week, 1-3 drinks/day, >3 drinks/day, undetermined), and cigarette smoking (categorical, never, former, < 10 cig/day, 12-20 cig/day, 20+ cig/day)

Table 3: The association between PFAAs and self-report memory impairment in logistic regression for a doubling PFAA concentration, by quintiles of distribution, and in ordinal regression by diabetes status (validated by clinical records)

	Range (ng/mL)	OR (95% CI)*	p for inter	N	OR (95% CI)*	p for inter
	PFOS N=17,832			**PFOA N=17,832**		
Non-diabetics		0.93 (0.90-0.96)†	-		0.95 (0.93-0.97) †	-
Ordinal regression		0.96 (0.93-0.99)			0.96 (0.95-0.98)	
1st quintile	0.25-14.5	Ref.		0.25-14.3	Ref.	
2nd quintile	14.6-20.5	0.96 (0.86-1.08)		14.4-27.2	0.85 (0.76-0.95)	
3rd quintile	20.6-27.0	0.90 (0.80-1.01)		27.3-54.3	0.82 (0.73-0.92)	
4th quintile	27.1-37.1	0.88 (0.78-0.99)		54.4-119.1	0.76 (0.68-0.86)	
5th quintile	37.2-759.2	0.85 (0.76-0.96)		119.2-8,416	0.75 (0.67-0.84)	
Trend		0.002			<0.001	
	N=3,192			**N=3,192**		
Diabetics		0.94 (0.88-1.02) †	0.698		1.02 (0.97-1.06) †	0.014
Ordinal regression		0.95 (0.90-1.01)			1.00 (0.97-1.04)	
1st quintile	0.25-13.6	Ref.		0.25-12.6	Ref.	
2nd quintile	13.7-20.0	1.06 (0.82-1.36)		12.7-25.4	1.04 (0.80-1.34)	
3rd quintile	20.1-27.3	0.82 (0.63-1.06)		25.5-48.0	0.88 (0.67-1.14)	
4th quintile	27.4-37.3	0.87 (0.67-1.13)		48.1-102.1	1.04 (0.80-1.35)	
5th quintile	37.4-272.0	0.90 (0.69-1.17)		102.4-22,412	1.09 (0.84-1.42)	
		0.162			0.543	
	PFNA			**PFHxS**		
	N=17,832			**N=17,832**		
Non-diabetics		0.95 (0.90-0.99) †	-		0.96 (0.93-0.99) †	-
Ordinal regression		0.97 (0.93-1.01)			0.97 0.94-0.99)	
1st quintile	0.25-0.9	Ref.		0.25-1.8	Ref.	
2nd quintile	1.0-1.2	0.86 (0.77-0.97)		1.9-2.6	0.98 (0.87-1.10)	
3rd quintile	1.3-1.5	0.85 (0.76-0.95)		2.7-3.7	0.99 (0.89-1.11)	
4th quintile	1.6-1.9	0.83 (0.73-0.93)		3.8-5.7	0.93 (0.82-1.05)	
5th quintile	2.0-28.6	0.88 (0.78-0.99)		5.8-232.6	0.88 (0.79-0.99)	
Trend		0.031			0.029	
	N=3,192			**N=3,192**		
Diabetics		1.01 (0.90-1.13) †	0.259	3,192	0.99 (0.92-1.06) †	0.683
Ordinal regression		0.99 (0.91-1.09)				
1st quintile	0.25-0.8	Ref.		0.25-1.6	Ref.	
2nd quintile	0.9-1.1	1.06 (0.80-1.40)		1.7-2.3	1.06 (0.81-1.38)	
3rd quintile	1.2-1.4	0.88 (0.66-1.17)		2.4-3.2	1.10 (0.85-1.42)	
4th quintile	1.5-1.8	1.03 (0.77-1.36)		3.3-5.0	1.02 (0.79-1.33)	
5th quintile	1.9-14.5	1.08(0.82-1.43)		5.1-99.7	1.00 (0.77-1.31)	
		0.620			0.942	

*using clinical record validated diagnosis of diabetes and self-reported use of medications, adjusted for age (one-year age bands), ethnicity, gender, school level (categorical), household income (categorical), physical activity, alcohol consumption (categorical, none/<1drink/month, < 1 drink/week, few drinks/week, 1-3 drinks/day, >3 drinks/day, undetermined), and cigarette smoking (categorical, never, former, < 10 cig/day, 12-20 cig/day, 20+ cig/day); † OR for doubling PFAA concentration

Table 4: Sensitivity analysis of the association between PFAAs and self-report memory impairment for a doubling PFAA concentration, by quintiles of PFAAs, and in ordinal regression on subjects aged 65 years and older (n=7,097), and using any memory impairment as outcome measure (n=21,024).

	Range (ng/mL)	OR (95% C.I.)[b] N=7,097 Restricted to those aged 65+*	Range (ng/mL)	OR (95% C.I.)[b] N=21,024 Any memory impairment^
PFOS		0.95 (0.90-1.00)		0.96 (0.94-0.99)
Ordinal regression		0.98 (0.94-1.03)		
1st quintile	0.25-15.3	Ref.	0.25-14.4	Ref.
2nd quintile	15.4-22.0	0.99 (0.83-1.20)	14.5-20.4	0.96 (0.88-1.05)
3rd quintile	22.1-28.9	0.95 (0.79-1.14)	20.5-27.1	0.90 (0.82-0.98)
4th quintile	29.0-4.0	0.97 (0.81-1.16)	27.2-37.2	0.94 (0.86-1.03)
5th quintile	40.1-759.2	0.84 (0.70-1.01)	37.3-759.2	0.93 (0.85-1.02)
Trend		0.079		0.121
PFOA		0.99 (0.97-1.03)		0.97 (0.96-0.99)
Ordinal regression		1.00 (0.97-1.03)		
1st quintile	0.25-15.0	Ref.	0.25-14.0	Ref.
2nd quintile	15.1-29.6	0.91 (0.75-1.09)	14.1-27.0	0.90 (0.82-0.98)
3rd quintile	29.7-56.8	0.90 (0.75-1.08)	27.1-53.8	0.86 (0.79-0.94)
4th quintile	56.9-123.0	0.84 (0.70-1.01)	53.9-118.1	0.87 (0.79-0.95)
5th quintile	123.1-5,994.8	0.99(0.83-1.19)	118.3-22,412	0.85 (0.78-0.93)
Tend		0.680		<0.001
PFNA		0.95 (0.87-1.02)		0.98 (0.95-1.02)
Ordinal regression		0.99 (0.93-1.07)		
1st quintile	0.25-0.8	Ref.	0.25-0.90	Ref.
2nd quintile	0.9-1.1	0.88 (0.73-1.06)	1.0-1.2	0.89 (0.82-0.97)
3rd quintile	1.2-1.4	0.81 (0.67-0.98)	1.3-1.4	0.94(0.85-1.04)
4th quintile	1.5-1.8	0.82 (0.68-0.99)	1.5-1.9	0.92 (0.85-1.01)
5th quintile	1.9-11.7	0.88 (0.73-1.07)	2.0-28.6	0.94 (0.86-1.03)
Trend		0.177		0.493
PFHxS		0.96 (0.91-1.01)		0.97 (0.94-0.99)
Ordinal regression		0.98 (0.93-1.02)		
1st quintile	0.25-1.9	Ref.	0.25-1.7	Ref.
2nd quintile	2.0-2.8	0.98 (0.82-1.18)	1.8-2.6	0.98 (0.90-1.07)
3rd quintile	2.9-3.9	0.95 (0.79-1.15)	2.7-3.6	1.03 (0.94-1.13)
4th quintile	4.0-6.0	0.98 (0.82-1.17)	3.7-5.6	0.96 (0.87-1.04)
5th quintile	6.1-232.6	0.86 (0.71-1.03)	5.7-232.6	0.89 (0.81-0.97)
Trend		0.139		0.010

[b]Model 2 includes age (one-year age bands), ethnicity, gender, and school level (categorical), household income (categorical), physical activity, alcohol consumption (categorical, none/<1drink/month, < 1 drink/week, few drinks/week, 1-3 drinks/day, >3 drinks/day, undetermined), and cigarette smoking (categorical, never, former, < 10 cig/day, 12-20 cig/day, 20+ cig/day)

* Sensitivity analysis including subjects aged 65 years or older only (N=7,097)

^ Sensitivity analysis using a more restrictive definition of memory impairment (those reporting frequent episode of short-term memory loss only, cases = 1,115)