ARTICLE

Perfluorooctanoic Acid, Perfluorooctanesulfonate, and Serum Lipids in Children and Adolescents

Results From the C8 Health Project

Stephanie J. Frisbee, MSc, MA; Anoop Shankar, MD, PhD; Sarah S. Knox, PhD; Kyle Steenland, PhD; David A. Savitz, PhD; Tony Fletcher, PhD; Alan M. Ducatman, MD, MS

Background: Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS) are man-made compounds with widespread presence in human sera. In previous occupational and adult studies, PFOA and PFOS were positively associated with serum lipid levels.

Objective: To interrogate associations between PFOA and PFOS and serum lipids in children and adolescents.

Design: Cross-sectional community-based study.

Setting: Mid–Ohio River Valley.

Participants: A total of 12 476 children and adolescents included in the C8 Health Project, which resulted from the pretrial settlement of a class action lawsuit pursuant to PFOA contamination of the drinking water supply.

Main Outcome Measures: Serum lipids (total, high-density lipoprotein [HDL-C], and low-density lipoprotein [LDL-C] cholesterol and fasting triglycerides).

Results: Mean (SD) serum PFOA and PFOS concentrations were 69.2 (111.9) ng/mL and 22.7 (12.6) ng/mL, respectively. In linear regression after adjustment for covariables, PFOA was significantly associated with increased total cholesterol and LDL-C, and PFOS was significantly associated with increased total cholesterol, HDL-C, and LDL-C. Using general linear model analysis of covariance, between the first and fifth quintiles of PFOA there was a 4.6-mg/dL and a 3.8-mg/dL increase in the adjusted mean levels of total cholesterol and LDL-C levels, respectively, and an 8.5-mg/dL and a 5.8-mg/dL increase in the adjusted mean levels of total cholesterol and LDL-C, respectively, between the first and fifth quintiles of PFOS. Increases were 10 mg/dL for some age- and sex-group strata. Observed effects were nonlinear, with larger increases in total cholesterol and LDL-C levels occurring at the lowest range, particularly of PFOA.

Conclusion: Although the epidemiologic and cross-sectional natures of this study limit causal inferences, the consistently observed associations between increasing PFOA and PFOS and elevated total cholesterol and LDL-C levels warrant further study.

Arch Pediatr Adolesc Med. 2010;164(9):860-869

Author Affiliations: Department of Community Medicine (Ms Frisbee and Drs Shankar, Knox, and Ducatman) and Center for Cardiovascular and Respiratory Sciences (Ms Frisbee and Dr Shankar), West Virginia University School of Medicine, Morgantown; Department of Environmental and Occupational Health, Emory University Rollins School of Public Health, Atlanta, Georgia (Dr Steenland); Department of Community and Preventive Medicine, Mount Sinai School of Medicine, New York, New York (Dr Savitz); and Public Health and Environmental Research Unit, London School of Hygiene and Tropical Medicine, London, England (Dr Fletcher).

PERFLUOROOCTANOIC ACID (PFOA) and perfluorooctanesulfonate (PFOS) are perfluoroalkyl acids: man-made compounds used as emulsifiers during the manufacture of fluoropolymers, which are chemicals that give nonstick heat resistance to cookware or breathable yet waterproof properties to fabrics and upholstery. Perfluoroalkyl acids may also result from the metabolism or breakdown of fluorinated telomers, which are compounds used as coatings for commercial food packaging, factory treatments for fabrics and carpets, and manufacturer pretreatment for "stain-resistant" clothing.

Recent reviews of the scientific literature[1-3] regarding PFOA and PFOS emphasize their environmental persistence and presence in a variety of marine and freshwater species. Human serum samples across myriad age groups and geographic areas are known to contain perfluoroalkyl acids generally and PFOA and PFOS specifically. Recent results from the National Health and Nutrition Examination Survey[4,5] reported detection of perfluoroalkyl acids in almost all samples, with a US population median for PFOA of 5.2 ng/mL and 3.9 ng/mL (1999-2000 and 2003-2004), respectively. Identified sources of human exposure to PFOA and PFOS include drinking water, dust, food packaging, breast milk, cord blood, microwave popcorn, ambient air, and occupational exposure,[1,6-8] although the relative contribution of each is unknown.

Animal studies have identified the liver as a primary target organ for perfluoroalkyl acid physiologic activity. Reported toxicological effects of exposure to PFOA and/or PFOS include hepatomegaly, reduction in serum triglycerides and cholesterol in some animal species, and alterations in coenzyme A activity. These alterations in he-

©2010 American Medical Association. All rights reserved.

patic metabolism and function have been attributed to perfluoroalkyl acid action as a peroxisome proliferator-activated receptor–α (PPAR-α) agonist with subsequent peroxisome proliferation.[1-3]

Compared with effects seen in animals, human studies have reported different associations between PFOA and lipid levels. In occupationally exposed employees, PFOA associations have included increased PFOA concentrations and increased total cholesterol (total-C) but not triglycerides or other lipids[9]; increased total-C and low-density lipoprotein cholesterol (LDL-C) but not high-density lipoprotein cholesterol (HDL-C)[10]; increased total-C but not triglycerides or HDL-C[11]; and increased triglycerides but no association with total-C or LDL-C.[12] A study of a community cohort with known environmental PFOA contamination reported no association between PFOA and total-C.[13] A recent, much larger study of adults from the same and adjacent environmentally exposed communities reported a robust, positive association between PFOA and total-C and LDL-C and a less clear association between PFOA and triglycerides.[14]

To our knowledge, no study has investigated potential associations between perfluoroalkyl acids and lipids in children and adolescents. The importance of such studies is 3-fold. First, if such associations are etiologic, exposure prevention would become important to reduce the long-term health consequences of elevations in known cardiovascular disease risk factors. Second, studying potential health consequences of an environmental exposure in children and adolescents may provide greater insight because these groups likely have fewer factors confounding underlying associations (eg, prevalent chronic or acute disease or medication use) compared with adults. Third, given possible differences in physiologic processes owing to developmental changes in children and adolescents, toxic effects may be different compared with those observed in adults. Thus, the purpose of this study is to interrogate associations between serum PFOA and PFOS and lipids in a large, community-based sample of children and adolescents.

METHODS

STUDY METHODS AND PARTICIPANTS

The participants in this study were 12 476 children and adolescents aged 1.0 to 17.9 years at their enrollment in the C8 Health Project (hereafter referred to as the Project). The Project has been more completely described elsewhere.[15] Briefly, it resulted from a pretrial settlement of the class action lawsuit *Leach v E.I. du Pont de Nemours & Co*,[16] filed in 2002 after PFOA from the DuPont Washington Works facility near Parkersburg, West Virginia, was found to have infiltrated several local drinking water supplies along the mid–Ohio River Valley. Project eligibility criteria included the ability to document consumption of contaminated drinking water (from 1 of 2 public water districts in West Virginia or 4 in Ohio, or from private water sources within the public water districts that contained ≥0.05 PFOA parts per billion) for at least 1 year between 1950 and December 3, 2004, at a primary residence, place of employment, or school. Participants were enrolled during a 13-month period between August 1, 2005, and August 31, 2006. Participation and enrollment included documentation of identity and eligibility; a self-reported survey of demographics, personal health history, and lifestyle habits; self-reported height and weight; and voluntary submission of a blood sample. As we have previously reported, approximately 60% of participants were residents in an eligible water district at the time of their enrollment in the Project, and an estimated 80% of people who resided in the eligible water districts at the time of the Project enrolled as participants.[15] With 69 030 total participants (12 476 children and adolescents), to our knowledge the Project represents the largest community-based study to date investigating potential associations between PFOA exposure and human health effects.

BLOOD SAMPLE PROCESSING AND LABORATORY METHODS

Children and adolescents voluntarily submitted blood samples at a maximum of 26 mL. Samples were centrifuged, divided into aliquots, and refrigerated at community-based data collection sites until being shipped to laboratories for analysis.

Clinical laboratory tests were performed at an accredited clinical diagnostic laboratory (LabCorp Inc, Burlington, North Carolina). The lipid panel included total-C, HDL-C, LDL-C, and triglycerides. The LDL-C was calculated using the Friedewald formula for participants with triglycerides lower than 400 mg/dL (to convert to millimoles per liter, multiply by 0.0113). Although fasting was not a requirement for phlebotomy, the time of the last meal was reported.

The primary laboratory performing perfluorocarbon analysis (Exygen Research Inc, State College, Pennsylvania) was also used for an independent study of a smaller group of residents in 1 water district included in the Project.[17] Full perfluoroalkyl acid analytic techniques and quality assurance protocols for the Project have been published elsewhere.[15] Briefly, the analytic protocol was a modification of a previously described protocol that used a protein precipitation extraction together with reverse-phase high-performance liquid chromatography/tandem mass spectrometry.[18] Spectrometric detection was performed using a triple quadrupole mass spectrometer in selected reaction monitoring mode, monitoring for the individual m/z (mass-to-charge) transitions for the perfluoroalkyl acid and ^{13}C-PFOA surrogate.

CONSENTING PROCEDURES

Project data collection administered by Brookmar Inc, Parkersburg, West Virginia, was conducted with the authority and supervision of the Wood County, West Virginia, Circuit Court. Brookmar Inc used a consenting procedure approved by parties to the Settlement that included language specific to the Project's purpose and data collection procedures and the mandatory documentation requirements to demonstrate Class eligibility. All participants submitting a voluntary blood sample completed the standard consent and release forms of the clinical laboratory contracted for phlebotomy. The Project group at West Virginia University and the C8 Science Panel obtained institutional review board approval from their own academic institutions permitting access to anonymous Project data.

STATISTICAL ANALYSIS

The outcome variables were total-C, LDL-C, HDL-C, and triglycerides. For simplicity, the same covariables were considered for all analytic models: age, sex (except for models stratified by sex), body mass index (BMI) *z* score, duration of fast (in minutes), and whether the participant engaged in a regular exercise program. Age was included as a continuous variable and as 2 strata (aged 1.0-11.9 years and 12.0-17.9 years) to assess possible age and developmental confounding. For all analyses using quantiles, grouping was established within age group and sex, and so quantiles are age-group and sex specific. Engagement in a regular exercise program was self-reported as part

©2010 American Medical Association. All rights reserved.

of the survey ("Do you engage in an exercise program?" with a "Yes/No" response option), as were fasting status, height, and weight. Analyses including triglycerides were conducted for only those reporting a fast of 6 hours or more. The BMI *z* score was calculated using Epi Info software, 2000 reference data set (Centers for Disease Control and Prevention, Atlanta, Georgia).

Multiple linear regression was performed to assess for the presence of an overall linear association between PFOA or PFOS and lipids. Both PFOA or PFOS and the dependent variable were natural log transformed.

General linear model analysis of covariance was performed to estimate predicted lipids (estimated marginal mean [EMM]) after adjustment for covariables based on increasing PFOA or PFOS quintiles. The serum lipid was defined as the dependent variable, PFOA or PFOS quintile as a fixed factor, and the other independent variables (age, sex, BMI *z* score, exercise, and fasting status) as covariates. The covariable-adjusted EMM for each quintile is presented graphically for the overall population (all age groups and both sexes combined). The difference in the covariable-adjusted EMM between the fifth and first quintile was established for each age and sex strata. To interpret the statistical significance of the trend in the quintile-based change in the covariable-adjusted EMM, linear regression analysis was used to estimate the β coefficient (and corresponding *P* value) for the PFOA or PFOS quintile, which is reported as a β for trend.

To assess the linearity or nonlinearity of any association between PFOA or PFOS and serum lipid levels, 20-group quantiles were determined and the population median for each group was calculated. General linear model analysis of covariance was again used to determine the covariable-adjusted EMM for each quantile, which was plotted against its PFOA or PFOS median.

Binary logistic regression analysis was performed to assess the odds of abnormal lipids with increasing PFOA or PFOS quintile. For total-C, LDL-C, and triglycerides, categorization as abnormal was based on American Heart Association–endorsed cutoff values for "borderline" or "high" in children (total-C≥170 mg/dL, LDL-C≥110 mg/dL [to convert to millimoles per liter, multiply by 0.0259], and triglycerides ≥150 mg/dL.[19] For HDL-C, values lower than 40 mg/dL (to convert to millimoles per liter, multiply by 0.0259) were classified as abnormal. Logistic regression analysis was performed using PFOA or PFOS quintile dummy variables, in which the first quintile was considered the reference group.

Interaction between PFOA and PFOS was dually assessed. Logistic regression analysis, with models otherwise constructed as described previously, was performed with the PFOA quintile, the PFOS quintile, and the product of quintiles (interaction term) included in the analytic model. The statistical significance of the interaction term (*P* for interaction) is reported. Logistic regression analysis was also used to assess for the presence of multiplicative interaction using models as described previously. Four groups were created based on the PFOA and PFOS quintiles: group 1 (PFOA first to fourth quintile and PFOS first to fourth quintile), group 2 (PFOA fifth quintile and PFOS first to fourth quintile), group 3 (PFOA first to fourth quintile and PFOS fifth quintile), and group 4 (PFOA fifth quintile and PFOS fifth quintile). Logistic regression was then completed using 3 dummy variables, in which group 1 was considered the reference group.

Sensitivity analyses were conducted for the effects of fasting and socioeconomic status. Using models otherwise constructed as described previously, multiple linear regression was performed for models with and without household income (dichotomized at ≤$30 000/y or >$30 000/y) and models adjusted for fasting status or with only participants having completed a fast of 6 hours or longer. The magnitude and statistical significance of β coefficients for the different models were then compared. Both PFOA or PFOS and the dependent variable were natural log transformed. All analyses were performed using SPSS statistical software (SPSS Inc, Chicago, Illinois).

RESULTS

General characteristics of participants are reported in **Table 1**. The mean (SD) age was 11.1 (4.5) years, and participation was similar across both sexes (48.9% girls and 51.0% boys; data missing for 6 participants [0.1%]). Of the 12 476 children and adolescents included in this study, more than 10 000 (>80.0%) had perfluoroalkyl acid quantification and serum lipids available for analysis. Consistent with US Census Bureau estimates for the area, 11 894 (96.1%) reported their ethnicity as white, 4406 (39.7%) were classified as overweight or obese (≥85th BMI percentile), and 4576 (36.7%) reported having a regular exercise program. According to participant-reported residence at the time of enrollment, a slightly higher proportion of participants were from Ohio compared with West Virginia (6796 [54.5%] vs 5520 [44.2%], respectively).

Mean (SD) total-C was 160.7 (29.3) mg/dL, with 65.8% of values classified as acceptable (<170 mg/dL). Mean (SD) calculated LDL-C was 87.3 (25.2) mg/dL, with 83.7% of values classified as acceptable (<110 mg/dL). Mean (SD) HDL-C was 49.3 (11.3) mg/dL, with 92.2% of values classified as ideal (≥35 mg/dL) and 19.7% lower than 40 mg/dL. Finally, mean (SD) fasting triglycerides were 99.1 (56.0) mg/dL, with 85.6% of values classified as acceptable (≤150 mg/dL).

Mean (SD) serum PFOA and PFOS concentrations were 69.2 (111.9) ng/mL and 22.7 (12.6) ng/mL, respectively, with serum concentrations statistically significantly higher in boys and younger children for PFOA and PFOS, particularly the former. Consistent with the environmental (drinking water) contamination and as previously reported,[15] serum PFOA concentration for 12- to 19-year-olds in the Project population was substantially higher than the reported concentration for 12- to 19-year-olds in the 2003-2004 National Health and Nutrition Examination Survey,[5] whereas PFOS concentrations were similar (29.3 ng/mL vs 3.9 ng/mL and 19.1 ng/mL vs 19.3 ng/mL, respectively).

Results from regression analysis (not shown) demonstrated that after adjustment for covariables, total-C and LDL-C were linearly and positively associated with PFOA and PFOS ($P<.001$ for all models). Triglycerides were also linearly and positively associated with PFOA ($P=.02$) but not with PFOS. The HDL-C was not linearly associated with PFOA but was positively associated with PFOS. For linear regression models and other analyses reported in this article, virtually all covariables were statistically significantly associated with the dependent variable (not shown).

Associations between increasing PFOA and PFOS quintiles and the covariable-adjusted EMM of serum lipid levels are depicted in **Figure 1**. Total-C and LDL-C demonstrated a consistent increase for each increase in PFOA or PFOS quintile: a 4.6-mg/dL and 3.8-mg/dL increase in the covariable-adjusted EMM of total-C and LDL-C between the first and fifth quintiles of PFOA and an 8.5-mg/dL and 5.8-mg/dL increase in the covariable-adjusted EMM of total-C and LDL-C, respectively, between the first and fifth quintiles of PFOS. Overall associations between PFOA and PFOS and HDL-C and triglycerides were less clear, with no apparent association between PFOA quintile and covariable-adjusted EMM for HDL-C or between PFOS quintile and

©2010 American Medical Association. All rights reserved.

Table 1. Participant Characteristics[a]

Characteristic	Boys[b]	Girls[c]	Total[d]
Age, median/mean (SD), y	11.6/11.2 (4.4)	11.5/11.1 (4.5)	11.6/11.1 (4.5)
Age group, median/mean (SD), y			
1.0-11.9	3287 (51.7)	3249 (53.2)	6536 (52.4)
12.0-17.9	3072 (48.3)	2862 (46.8)	5934 (47.6)
Regular exercise			
Yes	2491 (39.1)	2085 (34.1)	4576 (36.7)
No	3872 (60.9)	4028 (65.9)	7900 (63.3)
BMI percentile, %			
<5.0 (underweight)	272 (4.8)	291 (5.4)	563 (5.1)
5.0-84.9 (normal)	2947 (51.7)	3168 (58.9)	6115 (55.2)
85.0-95.0 (overweight)	934 (16.4)	911 (16.9)	1845 (16.6)
>95.0 (obese)	1550 (27.2)	1011 (18.8)	2561 (23.1)
Missing data	660	732	1392
Time fasting, h			
<6	4591 (72.2)	4400 (72.0)	8991 (72.1)
≥6	1394 (21.9)	1346 (22.0)	2740 (22.0)
Not reported	378 (5.9)	367 (6.0)	745 (6.0)
Household income, $/y			
≤30 000	2471 (49.3)	2303 (47.6)	4774 (48.4)
>30 000	2540 (50.7)	2540 (52.4)	5080 (51.6)
Missing data	1352	1270	2622
Ethnicity			
White	6061 (96.3)	5833 (96.0)	11 894 (96.1)
Black	106 (1.7)	96 (1.6)	202 (1.6)
All other ethnicities	130 (2.1)	145 (2.4)	275 (2.2)
Missing data	51	34	85
State residence at time of enrollment			
Ohio	3476 (54.6)	3320 (54.3)	6796 (54.5)
West Virginia	2817 (44.3)	2703 (44.2)	5520 (44.2)
Other	70 (1.1)	90 (1.5)	160 (1.3)
Total-C, mg/dL			
<170	3729 (68.6)	3207 (62.8)	6936 (65.8)
≥170	1706 (31.4)	1901 (37.2)	3607 (34.2)
Missing data	928	1005	1933
LDL-C, mg/dL			
<110	4581 (85.3)	4158 (82.0)	8739 (83.7)
≥110	788 (14.7)	915 (18.0)	1703 (16.3)
Missing data	994	1040	2034
HDL-C, mg/dL			
<40	1313 (24.2)	760 (14.9)	2073 (19.7)
≥40	4122 (75.8)	4348 (85.1)	8470 (80.3)
Missing data and nonfasting participants	702	1231	1933
Fasting triglycerides, mg/dL			
<150	1244 (84.6)	1201 (86.7)	2445 (85.6)
≥150	227 (15.4)	185 (13.3)	412 (14.4)
Missing data	4892	4727	9619
PFOA, ng/mL, mean (SD)			
1.0-11.9 y	35.1/82.1 (129.2)	30.7/73.1 (120.1)	32.6/77.7 (124.9)
12.0-17.9 y	30.1/69.3 (107.1)	22.9/53.7 (88.1)	26.3/61.8 (98.8)
PFOS, ng/mL, mean (SD)			
1.0-11.9 y	21.7/24.6 (13.4)	19.9/22.6 (12.6)	20.7/23.6 (13.1)
12.0-17.9 y	20.3/23.2 (12.9)	18.2/20.5 (11.3)	19.3/21.9 (12.2)

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonate; total-C, total cholesterol.

SI conversion factors: To convert HDL-C, LDL-C, and total-C to millimoles per liter, multiply by 0.0259; fasting triglycerides to millimoles per liter, multiply by 0.0113.

[a] Data are presented as number (percentage) unless otherwise indicated. Denominators vary because of missing data. Percentages may not total 100 because of rounding, are based on category totals, and reflect the "valid percentage" (ie, missing data are not included in the denominator).

[b] n=6359.

[c] n=6111.

[d] N=12 470. Data reguarding sex were missing for 6 participants.

covariable-adjusted EMM for triglycerides. There was a small increase in the covariable-adjusted EMM of HDL-C and the first to third quintiles of PFOS, but not for the third to fifth quintiles of PFOS.

These associations are more fully characterized in **Table 2**, which reports differences between the fifth and first quintiles and β for trend for each of these age/sex groups. For PFOA and total-C and LDL-C, there was a

©2010 American Medical Association. All rights reserved.

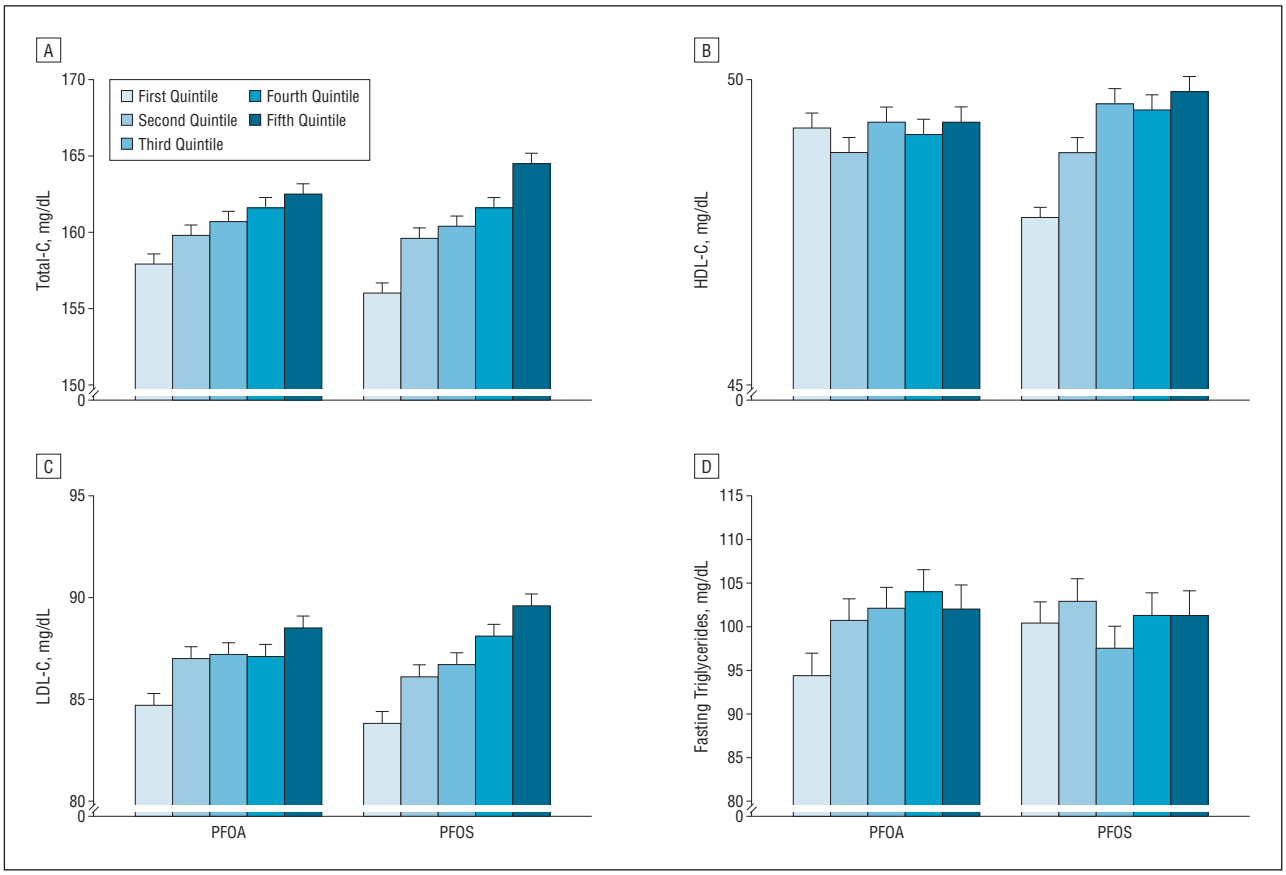

Figure 1. Changes in covariable-adjusted estimated marginal means (general linear model analysis) across perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS) quintiles. A, Total cholesterol (total-C). B, High-density lipoprotein cholesterol (HDL-C). C, Low-density lipoprotein cholesterol (LDL-C). D, Fasting triglycerides. Lipid values are presented as mean (SE). To convert total-C, HDL-C, and LDL-C to millimoles per liter, multiply by 0.0259; fasting triglycerides to millimoles per liter, multiply by 0.0113.

trend toward a larger increase in the covariable-adjusted EMM in the younger compared with the older age group (5.8 mg/dL vs 4.2 mg/dL, respectively, for total-C and 4.9 mg/dL vs 3.2 mg/dL, respectively, for LDL-C) and in boys compared with girls (within each age group). The β for trend for each of these age groups was statistically significant ($P < .05$). For PFOS, there was a trend toward a larger increase in the covariable-adjusted EMM in older compared with younger age groups (9.5 mg/dL vs 5.5 mg/dL, respectively, for total-C and 7.5 mg/dL vs 3.4 mg/dL, respectively, for LDL-C) and a trend toward larger increases for boys compared with girls (within each age group). The β for trend for each of these age groups was also statistically significant ($P < .05$).

In contrast, each β for trend for age group and sex strata for the associations between PFOA and HDL-C and triglycerides was not statistically significant (except in 1 group), making differences in the covariable-adjusted EMM difficult to interpret. For associations between PFOS and HDL-C, each β for trend was statistically significant for boys and both sexes combined (but not girls) for both age groups, although marginal increases in the covariable-adjusted EMM were small (1.1-2.6 mg/dL). Similarly, each β for trend for age group and sex strata for the associations between PFOS and triglycerides was not statistically significant (except in 1 group), making differences in the covariable-adjusted EMM difficult to interpret.

In **Figure 2** and **Figure 3**, the covariable-adjusted EMM for serum lipids for the 20-group quantiles of PFOA or PFOS are plotted against the median of PFOA or PFOS for the quantile. For PFOA and PFOS, the results demonstrated a nonlinear association between increasing PFOA or PFOS concentration and total-C and LDL-C. For PFOA, the largest increases in the covariable-adjusted EMM of total-C and LDL-C were seen at the lowest range of PFOA concentrations; the slope attenuated at higher serum concentrations. Although population exposure and corresponding serum concentrations of PFOS were lower, the relationship between increasing PFOS quantiles and the covariable-adjusted EMM of total-C and LDL-C was similar across the spectrum of serum PFOS concentrations. The HDL-C level also demonstrated a small, nonlinear association with increasing PFOS quantile.

Logistic regression results are given in **Table 3**. Increasing PFOA and PFOS quintiles were positively associated with an increased risk of abnormal total-C (adjusted odds ratio, 1.2 [95% confidence interval, 1.1-1.4] and 1.6 [1.4-1.9], respectively) and LDL-C (1.4 [1.2-1.7] and 1.6 [1.3-1.9], respectively). Increasing PFOS quintiles were also associated with decreased risk of low HDL-C (adjusted odds ratio, 0.7 [95% confidence interval, 0.6-0.9]). Neither PFOA nor PFOS was associated with an increased risk of abnormal triglycerides.

©2010 American Medical Association. All rights reserved.

Table 2. Differences in PFOA and PFOS Between First and Fifth Quintile EMMs (GLM Analysis) and Assessment of Quintile Trend (Regression Analysis)

		Total-C[b]			HDL-C[b]		
Age Group, y	No. (Fasting)[a]	Difference in EMM, mg/dL	β for Trend (SE)	P_β	Difference in EMM, mg/dL	β for Trend (SE)	P_β
PFOA							
1.0-11.9							
Both sexes	3857 (803)	5.8	1.3 (0.3)	<.001	<1.0	−0.02 (0.1)	.88
Girls	1886 (397)	5.8	1.1 (0.4)	<.001	<1.0	0.02 (0.2)	.90
Boys	1971 (406)	6.3	1.6 (0.4)	<.001	<1.0	−0.06 (0.2)	.70
12.0-17.9							
Both sexes	5293 (1428)	4.2	1.1 (0.3)	<.001	<1.0	0.1 (0.1)	.20
Girls	2520 (687)	3.9	1.0 (0.4)	.02	<1.0	0.3 (0.2)	.09
Boys	2773 (741)	4.8	1.1 (0.4)	.005	<1.0	0.03 (0.1)	.80
PFOS							
1.0-11.9							
Both sexes	3857 (803)	5.5	1.3 (0.3)	<.001	1.6	0.3 (0.1)	.007
Girls	1886 (397)	4.6	1.3 (0.5)	.004	<1.0	0.1 (0.2)	.50
Boys	1971 (406)	6.2	1.2 (0.5)	.01	2.6	0.5 (0.2)	.003
12.0-17.9							
Both sexes	5293 (1428)	9.5	2.1 (0.3)	<.001	1.5	0.4 (0.1)	.001
Girls	2520 (687)	9.4	1.9 (0.4)	<.001	1.8	0.3 (0.2)	.06
Boys	2773 (741)	9.3	2.1 (0.4)	<.001	1.1	0.4 (0.1)	.003

		LDL-C[b,c]			Fasting Triglycerides[d]		
Age Group, y	No. (Fasting)[a]	Difference in EMM, mg/dL	β for Trend β (SE)	P_β	Difference in EMM, mg/dL	β for Trend β (SE)	P_β
PFOA							
1.0-11.9							
Both sexes	3857 (803)	4.9	1.0 (0.3)	.001	2.0	2.0 (1.3)	.10
Girls	1886 (397)	5.4	0.8 (0.4)	.04	16.2	4.0 (1.9)	.04
Boys	1971 (406)	4.8	1.1 (0.4)	.004	5.3	0.4 (1.9)	.80
12.0-17.9							
Both sexes	5293 (1428)	3.2	0.7 (0.2)	.004	3.8	1.5 (1.1)	.10
Girls	2520 (687)	3.2	0.7 (0.4)	.05	1.8	0.8 (1.4)	.60
Boys	2773 (741)	3.5	0.7 (0.3)	.03	5.9	2.4 (1.6)	.10
PFOS							
1.0-11.9							
Both sexes	3857 (803)	3.4	0.9 (0.3)	.002	2.8	0.1 (1.4)	.99
Girls	1886 (397)	2.6	0.8 (0.4)	.04	7.6	0.6 (1.9)	.70
Boys	1971 (406)	4.1	0.9 (0.4)	.03	−1.4	−0.3 (2.0)	.90
12.0-17.9							
Both sexes	5293 (1428)	7.5	1.7 (0.2)	<.001	<1.0	−0.1 (1.0)	.90
Girls	2520 (687)	6.9	1.5 (0.4)	<.001	−13.4	−3.0 (1.3)	.02
Boys	2773 (741)	7.9	1.8 (0.3)	<.001	11.1	2.2 (1.6)	.20

Abbreviations: EMM, estimated marginal mean; GLM, general linear model; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonate; total-C, total cholesterol.

SI conversion factors: To convert HDL-C, LDL-C, and total-C to millimoles per liter, multiply by 0.0259; fasting triglycerides to millimoles per liter, multiply by 0.0113.

[a] The first number is the total fasting and nonfasting participants and so is the sample size for the analyses of total-C, HDL-C, and LDL-C; the number in the parentheses is fasting participants only and so is the sample size for analysis of fasting triglycerides.

[b] Models were adjusted for age, estimated time of fasting, body mass index *z* score, sex, and regular exercise; sex-stratified models were not adjusted for sex.

[c] Calculated for participants with a triglyceride level <400 mg/dL regardless of fasting status.

[d] Defined as self-reported fasting ≥6 hours before phlebotomy.

Results of analyses assessing interaction appear in **Table 4**. For total-C, LDL-C, and triglycerides, results do not support an interaction between PFOA and PFOS in the prediction of these lipids. There is some evidence of multiplicative interaction between PFOA and PFOS in the reduction of risk of low HDL-C (the odds ratio for group 4 exceeds the product of the odds ratios for groups 2 and 3).

Sensitivity analyses were conducted to assess the stability of the association between PFOA or PFOS and lipid levels after the inclusion of household income and for fasting-only participants. Results (not shown) suggested that after adjustment for the same covariables, models were stable and associations were unaltered. The positive, statistically significant association between both PFOA and PFOS and total-C and LDL-C was not altered after the inclusion of household income (a proxy for socioeconomic status) or when analysis was performed only on the subset of participants who had completed a fast of 6 hours or more. Likewise, the positive, statistically significant association between PFOA and fasting triglyc-

©2010 American Medical Association. All rights reserved.

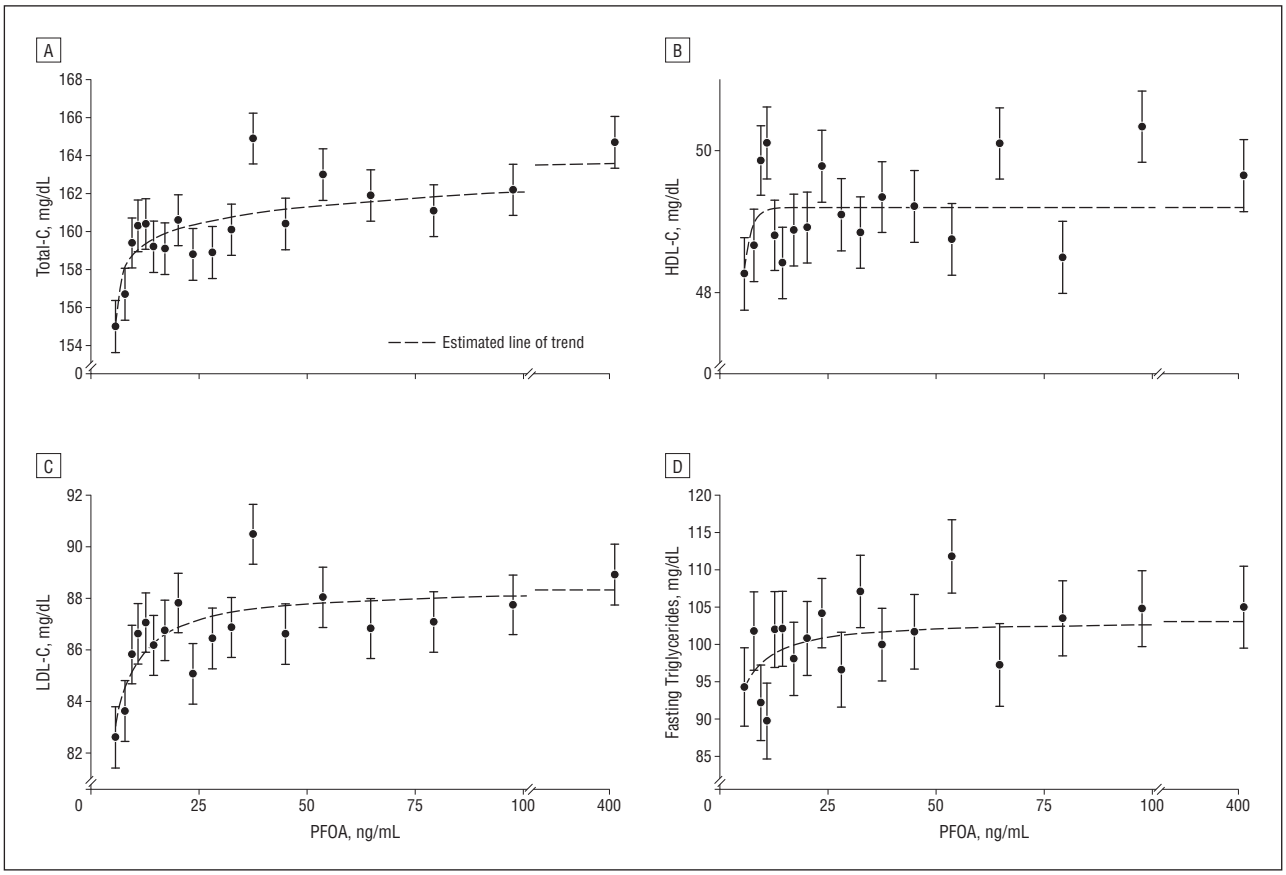

Figure 2. Nonlinear changes in covariable-adjusted estimated marginal means (general linear model analysis) across perfluorooctanoic acid (PFOA) 20-group quantiles. Models were adjusted for age, estimated time of fasting, body mass index *z* score, sex, and regular exercise. Lipid values are presented as mean (SE). Values for PFOA are presented as the median value for the quantile group in question (axis truncated because of very large range in median values ≠ PFOA serum concentration). A, Total cholesterol (total-C). B, High-density lipoprotein cholesterol (HDL-C). C, Low-density lipoprotein cholesterol (LDL-C). D, Fasting triglycerides. To convert total-C, HDL-C, and LDL-C to millimoles per liter, multiply by 0.0259; fasting triglycerides to millimoles per liter, multiply by 0.0113.

erides was not altered after the inclusion of household income. Perfluorooctanesulfonate was not associated with fasting triglycerides, with or without the inclusion of household income. The association between PFOA and HDL-C was not statistically significant, with or without the inclusion of household income or for the fasting or nonfasting participants. The positive, linear association between PFOS and HDL-C was statistically significant, with and without the inclusion of household income and for the fasting and nonfasting participants.

COMMENT

To our knowledge, this study reports the first assessment of associations between PFOA and PFOS and serum lipids in children and adolescents from the largest community-based study of the effects of PFOA exposure to date. Across several types of analyses, results consistently provided evidence for a positive association between PFOA and PFOS and serum lipids, specifically an increase in total-C and LDL-C with increasing PFOA and PFOS serum concentrations. Dose-response relationships were nonlinear, with larger increases in lipids at the lower range of PFOA concentration in particular. Additionally, results suggested that the magnitude of association between PFOS and total-C and LDL-C was higher than that between PFOA and total-C and LDL-C. Finally, there was a statistically significantly increased risk of abnormal total-C and LDL-C with increasing PFOA and PFOS serum concentrations.

Results reported in this article are consistent with those of previous studies in adults, which have generally shown a trend toward a positive association between PFOA and cholesterol. Our observations are also consistent with a study of adults (≥18 years) from the same Project population, in which authors reported an 11- to 12-mg/dL increase in total-C from the lowest to highest decile of serum PFOA (vs our finding of a 4.6-mg/dL increase from the lowest to highest quintile) and a corresponding 8- to 9-mg/dL increase in LDL-C (vs 3.8 mg/dL reported in this article), and a 10- to 12-mg/dL increase in total-C from the lowest to highest decile of serum PFOS (vs our finding of an 8.5-mg/dL increase from the lowest to highest quintile) and a corresponding 11- to 12-mg/dL increase in LDL-C (vs 5.8 mg/dL reported in this article).[14]

Evidence from animal studies has suggested that activation of PPAR-α is an important mechanism through which PFOA and PFOS exert biological effects, although it is unclear whether this animal-based evidence can be extrapolated to humans. The elimination time for PFOA and PFOS in humans is substantially longer than in rodents, resulting in a longer duration to reach a steady-

©2010 American Medical Association. All rights reserved.

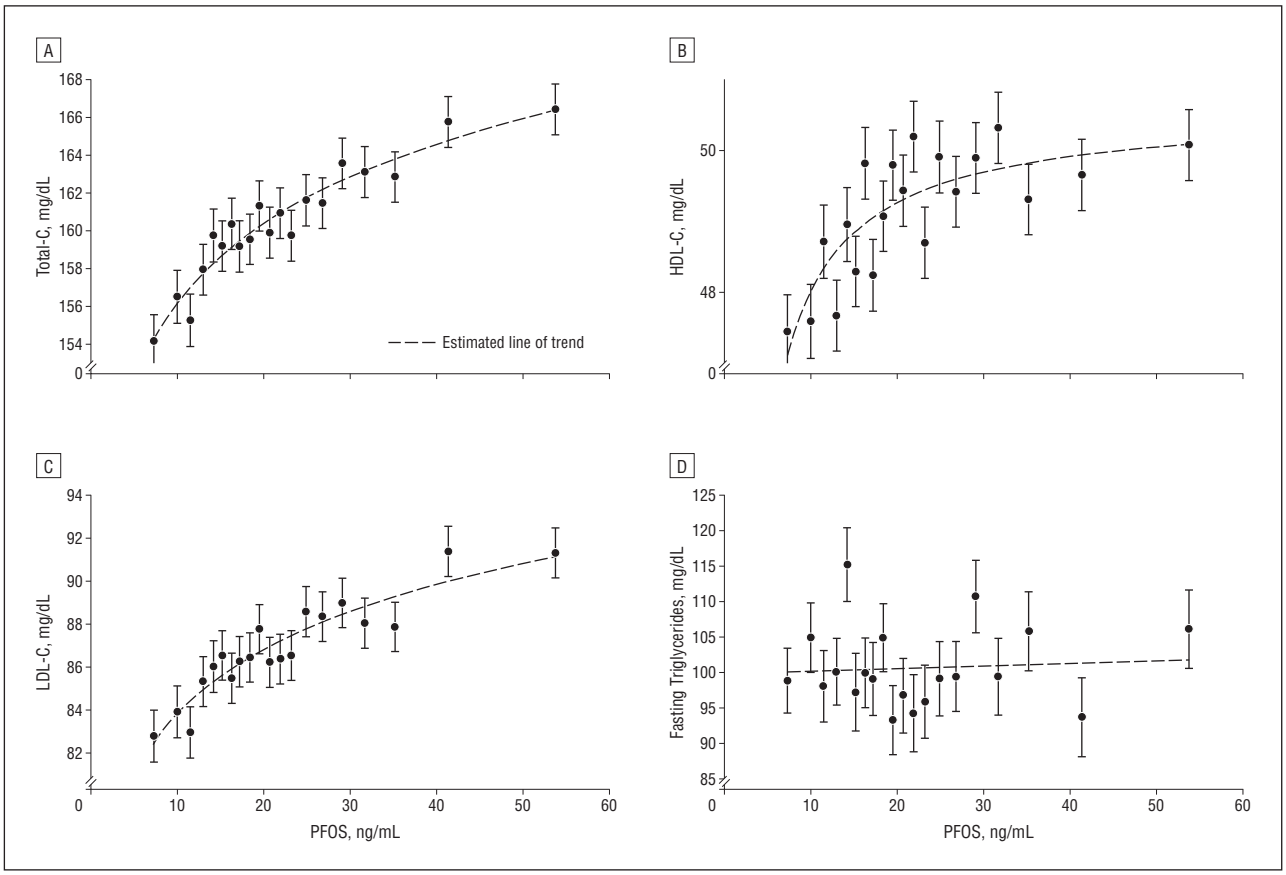

Figure 3. Nonlinear changes in covariable-adjusted estimated marginal means (general linear model analysis) across perfluorooctanesulfonate (PFOS) 20-group quantiles. Models were adjusted for age, estimated time of fasting, body mass index *z* score, sex, and regular exercise. Lipid values are presented as mean (SE). Values for PFOS are presented as the median valve for the quantile group in question. A, Total cholesterol (total-C). B, High-density lipoprotein cholesterol (HDL-C). C, Low-density lipoprotein cholesterol (LDL-C). D, Fasting triglycerides. To convert total-C, HDL-C, and LDL-C to millimoles per liter, multiply by 0.0259; fasting triglycerides to millimoles per liter, multiply by 0.0113.

Table 3. Risk of Abnormal Blood Serum Lipids (Logistic Regression Analysis) Based on Increasing PFOA and PFOS Quintiles

	Odds Ratio (95% Confidence Interval)			
Quintile	**Total-C[a]**	**HDL-C[a]**	**LDL-C[a,b]**	**Fasting Triglycerides[a,c]**
PFOA				
First	1.0 [Reference]	1.0 [Reference]	1.0 [Reference]	1.0 [Reference]
Second	1.1 (1.0-1.3)	1.0 (0.8-1.2)	1.2 (1.0-1.5)	1.0 (0.7-1.5)
Third	1.2 (1.0-1.4)	1.0 (0.8-1.2)	1.2 (1.0-1.4)	1.3 (0.9-1.9)
Fourth	1.2 (1.1-1.4)	1.0 (0.9-1.2)	1.2 (1.0-1.4)	1.6 (1.1-2.3)
Fifth	1.2 (1.1-1.4)	0.9 (0.8-1.1)	1.4 (1.2-1.7)	1.0 (0.7-1.6)
PFOS				
First	1.0 [Reference]	1.0 [Reference]	1.0 [Reference]	1.0 [Reference]
Second	1.3 (1.1-1.4)	0.9 (0.8-1.1)	1.2 (1.0-1.5)	1.3 (0.9-1.8)
Third	1.3 (1.2-1.5)	0.8 (0.7-1.0)	1.2 (1.0-1.5)	1.0 (0.7-1.4)
Fourth	1.3 (1.2-1.6)	0.8 (0.7-0.9)	1.3 (1.1-1.6)	1.1 (0.7-1.6)
Fifth	1.6 (1.4-1.9)	0.7 (0.6-0.9)	1.6 (1.3-1.9)	1.2 (0.8-1.5)

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonate; total-C, total cholesterol.

[a] Models were adjusted for age, estimated time of fasting, body mass index *z* score, sex, and regular exercise.

[b] Calculated for participants with a triglyceride level <400 mg/dL (to convert to millimoles per liter, multiply by 0.0113) regardless of fasting status.

[c] Defined as self-reported fasting ≥6 hours before phlebotomy.

state dose.[20] In addition, PPAR-α is expressed in human liver tissue at approximately 10% of rodent levels, and humans and other primates are refractory or less responsive to PPAR-α agonists compared with rodents.[21] Thus, associations reported in this article are etiologically plausible if a non-PPAR-α mechanistic pathway operates in humans in addition to or instead of a PPAR-α pathway; some studies of humans and other primates have re-

©2010 American Medical Association. All rights reserved.

Table 4. Assessment of Interaction (Logistic Regression Analysis) in Coincident PFOA and PFOS Quintile Groups

Quintile	Odds Ratio (95% Confidence Interval)			
	Total-C[a]	HDL-C[a]	LDL-C[a,b]	Fasting Triglycerides[c]
Group 1[d]	1.0 [Reference]	1.0 [Reference]	1.0 [Reference]	1.0 [Reference]
Group 2[e]	1.1 (0.9-1.2)	1.0 (0.8-1.1)	1.2 (1.0-1.4)	0.9 (0.6-1.3)
Group 3[f]	1.3 (1.2-1.5)	0.9 (0.8-1.0)	1.3 (1.1-1.6)	1.2 (0.8-1.8)
Group 4[g]	1.4 (1.2-1.7)	0.7 (0.5-0.9)	1.2 (0.8-1.8)	0.8 (0.4-1.7)
P for interaction	>0.2	>0.2	>0.2	>0.2

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PFOA, perfluorooctanoic acid; PFOS, perfluorooctanesulfonate; total-C, total cholesterol.

[a] Models were adjusted for age, estimated time of fasting, body mass index *z* score, sex, and regular exercise.

[b] Calculated for participants with triglycerides <400 mg/dL (to convert to millimoles per liter, multiply by 0.0113) regardless of fasting status.

[c] Defined as self-reported fasting ≥6 hours before phlebotomy.

[d] PFOA first to fourth quintile and PFOS first to fourth quintile.

[e] PFOA fifth quintile and PFOS first to fourth quintile.

[f] PFOA first to fourth quintile and PFOS fifth quintile.

[g] PFOA fifth quintile and PFOS fifth quintile.

ported lipidemic effects through non-PPAR-α–dependent mechanisms.[22]

The nonlinear nature of the observed associations, particularly for PFOA, suggests a possible saturation point in an underlying physiologic mechanism. Furthermore, whereas serum PFOA levels in this study population exceeded levels from a nationally representative sample, PFOS levels were similar (as explained previously). Regardless, the magnitude of the observed associations between PFOS and total-C and LDL-C were similar and in some instances larger compared with those observed with PFOA. Thus, PFOA and PFOS specifically, and possibly perfluoroalkyl acids as a general class, appear to be associated with serum lipids, and the association seems to exist at levels of PFOA and PFOS exposure that are in the range characterized by nationally representative studies.

The strengths of this study include the large sample size and participation rate (and consequent ability to examine associations in different age groups and in both sexes) and the replication in children and adolescents of observations made in adult populations. The replication of observations in a sample free from some factors that can confound associations in adult samples contributes additional strength to our observations. Confounding on the basis of sex or developmental effects was controlled through stratification, but the prevalent design of the Project did not permit assessment of the effects of cumulative exposure nor of the residual or persistent effects of prenatal exposure. The long-term health consequences of elevated serum lipid levels in the ranges observed in this study (3.0-10.0 mg/dL) are unclear.

The cross-sectional nature of this study is an acknowledged weakness; thus, causal inference is limited. Additional potential limitations include self-reported survey data, limited availability of covariables known to be associated with lipids, and uncertainty of fasting status for the analysis of triglycerides.

Studying the susceptibility of children and adolescents to these chemicals has been identified as a particular data need and research priority.[23] We have previously reported a U-shaped pattern in serum concentrations of PFOA in boys and girls and of PFOS in girls in this population (ie, serum concentrations were higher in younger age groups, decreased into early to middle adulthood, and increased again through adulthood).[15] Furthermore, the effects of perfluoroalkyl acids on developing physiologic systems or of sustained, long-term exposure are unknown. The large sample size in this study and findings consistent with results from adult occupational and larger community studies support a strong need to prioritize research regarding the effects of perfluoroalkyl acids in children and adolescents to assess whether the clear associations reported in this article are etiologic.

In conclusion, although the epidemiologic and cross-sectional nature of this study inherently limits causal inference, the consistently observed associations between increasing PFOA and PFOS serum concentrations and elevated total-C and LDL-C warrant further study. Should the association prove to be etiologic, the cumulative effects of such an elevation in cholesterol on long-term cardiovascular health are unclear given the early age at which these associations were observed.

Accepted for Publication: February 9, 2010.

Correspondence: Stephanie J. Frisbee, MSc, MA, Department of Community Medicine, West Virginia University School of Medicine, Robert C. Byrd Health Sciences Center, 1 Medical Center Dr, PO Box 9105, Morgantown, WV 26506-9105 (sfrisbee@hsc.wvu.edu).

Author Contributions: Ms Frisbee and Drs Shankar, Knox, Steenland, Savitz, Fletcher, and Ducatman had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. *Study concept and design:* Frisbee, Knox, and Ducatman. *Acquisition of data:* Frisbee and Ducatman. *Analysis and interpretation of data:* Frisbee, Shankar, Knox, Steenland, Savitz, Fletcher, and Ducatman. *Drafting of the manuscript:* Frisbee and Ducatman. *Critical revision of the manuscript for important intellectual content:* Frisbee, Shankar, Knox, Steenland, Savitz, Fletcher, and Ducatman. *Statistical analysis:* Frisbee, Shankar, Knox, Steenland, Savitz, and Fletcher. *Obtained funding:* Ducatman. *Administrative, technical, and material support:* Frisbee and Ducatman.

©2010 American Medical Association. All rights reserved.

Financial Disclosure: Ms Frisbee and Drs Shankar, Knox, and Ducatman were engaged in the Project pursuant to a contractual relationship between Brookmar Inc and West Virginia University. This study was funded in part by that contract. Drs Steenland, Savitz, and Fletcher are members of the separate C8 Science Panel, whose Wood County Circuit Court–appointed obligation is to determine "probable links" between PFOA exposure and health outcomes. Panel membership was jointly agreed to by the Plaintiffs and DuPont and then approved by the Court. As such, these authors are in receipt of funding from the C8 Class Action Settlement Agreement.

Funding/Support: The C8 Health Project was funded by the Settlement Agreement in the case of *Leach v E.I. du Pont de Nemours & Co., Civil Action No. 01-C-608 (W. Va. Circuit Court of Wood County, 2004)*, which resulted from drinking water contamination by the chemical perfluorooctanoic acid (PFOA, or C8). The Court authorized the creation of Brookmar Inc, an independent company created solely to design, publicize, and implement the C8 Health Project, including all enrollment and data collection. Brookmar Inc received funding exclusively from the Settlement, administered through a Court-approved financial professional (the Health Project Administrator). Brookmar Inc made data from the C8 Health Project available to the C8 Science Panel, also created pursuant to the Settlement Agreement, and to a group at West Virginia University, pursuant to a contractual relationship with Brookmar Inc. The design of the Project was developed in consultation with, although not subject to, the wishes of the settling parties. All authors declare that their ability to design, conduct, interpret, and publish this research study was unimpeded and fully independent of the Court and settling parties, who had no role in preparing, reviewing, or approving the manuscript.

Additional Contributions: We gratefully acknowledge the contributions of Cathy Lally, MPH, and Jessica MacNeil, MPH, from the Emory University Rollins School of Public Health for their expertise and assistance in data cleaning and the personnel at Exygen Research Corporation and Axys Analytics for ensuring accurate descriptions of laboratory methods. Finally, because of the scale of the C8 Health Project, many individuals provided valuable assistance during its development and implementation. We gratefully acknowledge these contributions and thank all individuals who supported the execution of the project.

REFERENCES

1. Lau C, Anitole K, Hodes C, Lai D, Pfahles-Hutchens A, Seed J. Perfluoroalkyl acids: a review of monitoring and toxicological findings. *Toxicol Sci.* 2007;99(2):366-394.
2. Kennedy GL Jr, Butenhoff JL, Olsen GW, et al. The toxicology of perfluorooctanoate. *Crit Rev Toxicol.* 2004;34(4):351-384.
3. Kudo N, Kawashima Y. Toxicity and toxicokinetics of perfluorooctanoic acid in humans and animals. *J Toxicol Sci.* 2003;28(2):49-57.
4. Calafat AM, Kuklenyik Z, Reidy JA, Caudill SP, Tully JS, Needham LL. Serum concentrations of 11 polyfluoroalkyl compounds in the US population: data from the National Health and Nutrition Examination Survey (NHANES). *Environ Sci Technol.* 2007;41(7):2237-2242.
5. Calafat AM, Wong L-Y, Kuklenyik Z, Reidy JA, Needham LL. Polyfluoroalkyl chemicals in the US population: data from the National Health and Nutrition Examination Survey (NHANES) 2003-2004 and comparisons with NHANES 1999-2000. *Environ Health Perspect.* 2007;115(11):1596-1602.
6. Kärrman A, Ericson I, van Bavel B, et al. Exposure of perfluorinated chemicals through lactation: levels of matched human milk and serum and a temporal trend, 1996-2004, in Sweden. *Environ Health Perspect.* 2007;115(2):226-230.
7. Inoue K, Okada F, Ito R, et al. Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: assessment of PFOS exposure in a susceptible population during pregnancy. *Environ Health Perspect.* 2004;112(11):1204-1207.
8. Olsen GW, Logan PW, Hansen KJ, et al. An occupational exposure assessment of a perfluorooctanesulfonyl fluoride production site: biomonitoring. *AIHA J (Fairfax, Va).* 2003;64(5):651-659.
9. Sakr CJ, Leonard RC, Kreckmann KH, Slade MD, Cullen MR. Longitudinal study of serum lipids and liver enzymes in workers with occupational exposure to ammonium perfluorooctanoate. *J Occup Environ Med.* 2007;49(8):872-879.
10. Sakr CJ, Kreckmann KH, Green JW, Gillies PJ, Reynolds JL, Leonard RC. Cross-sectional study of lipids and liver enzymes related to a serum biomarker of exposure (ammonium perfluorooctanoate or APFO) as part of a general health survey in a cohort of occupationally exposed workers. *J Occup Environ Med.* 2007;49(10):1086-1096.
11. Costa G, Sartori S, Consonni D. Thirty years of medical surveillance in perfluooctanoic acid production workers. *J Occup Environ Med.* 2009;51(3):364-372.
12. Olsen GW, Zobel LR. Assessment of lipid, hepatic, and thyroid parameters with serum perfluorooctanoate (FPOA) concentrations in fluorochemical production workers. *Int Arch Occup Environ Health.* 2007;81(2):231-246.
13. Emmett EA, Zhang H, Shofer FS, et al. Community exposure to perfluorooctanoate: relationships between serum levels and certain health parameters. *J Occup Environ Med.* 2006;48(8):771-779.
14. Steenland K, Tinker S, Frisbee S, Ducatman A, Vaccarino V. Association of perfluorooctanoic acid and perfluorooctane sulfonate with serum lipids among adults living near a chemical plant. *Am J Epidemiol.* 2009;170(10):1268-1278.
15. Frisbee SJ, Brooks AP Jr, Maher A, et al. The C8 Health Project: design, methods, and participants. *Environ Health Perspect.* 2009;117(12):1873-1882.
16. *Jack Leach et al* v *EI du Pont de Nemours and Company*, 01C-608 (WV 2002).
17. Emmett EA, Shofer FS, Zhang H, Freeman D, Desai C, Shaw LM. Community exposure to perfluorooctanoate: relationships between serum concentrations and exposure sources. *J Occup Environ Med.* 2006;48(8):759-770.
18. Flaherty JM, Connolly PD, Decker ER, et al. Quantitative determination of perfluorooctanoic acid in serum and plasma by liquid chromatography tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2005;819(2):329-338.
19. American Heart Association. American Heart Association scientific position: cholesterol and atherosclerosis in children. http://www.americanheart.org/presenter.jhtml?identifier=4499. Accessed October 19, 2009.
20. Hundley SG, Sarrif AM, Kennedy GL Jr. Absorption, distribution, and excretion of ammonium perfluorooctanoate (APFO) after oral administration to various species. *Drug Chem Toxicol.* 2006;29(2):137-145.
21. Klaunig JE, Babich MA, Baetcke KP, et al. PPARα agonist-induced rodent tumors: modes of action and human relevance. *Crit Rev Toxicol.* 2003;33(6):655-780.
22. Okochi E, Nishimaki-Mogami T, Suzuki K, Takahashi A. Perfluorooctanoic acid, a peroxisome-proliferating hypolipidemic agent, dissociates apolipoprotein B48 from lipoprotein particles and decreases secretion of very low density lipoproteins by cultured rat hepatocytes. *Biochim Biophys Acta.* 1999;1437(3):393-401.
23. Public Health Service, Agency for Toxic Substances and Disease Registry. *Draft Toxicological Profile for Perfluoroalkyls.* Atlanta, GA: Department of Health and Human Services; 2009.

©2010 American Medical Association. All rights reserved.