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Associations between Levels of Serum Perfluorinated Chemicals and Adiponectin in a Young Hypertension Cohort in Taiwan

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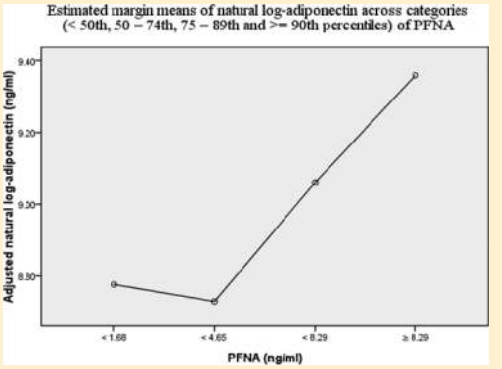
Supporting Information

ABSTRACT: In animals, perfluorinated chemicals (PFCs), specifically perfluorooctanoic acid (PFOA) and perfluorooctane sulfate (PFOS), function as peroxisome proliferator-activated receptor (PPAR) alpha agonists. However, the relevance of animal (primarily rodent) data to humans is unresolved. While plasma adiponectin level is very responsive to PPAR gamma agonist drugs, it has not been determined whether adiponectin level is related to serum PFCs concentrations. In the present study, 287 subjects (12−30 years of age) were recruited to determine the relationship between serum level of PFCs and serum level of adiponectin. The results showed males had higher serum PFOS concentrations than females and that those with metabolic syndrome had lower serum PFOA than controls. Besides, it showed regional elevations of the perfluoroundecanoic acid (PFUA) (median concentration: 7.11 ng/mL) in the study subjects. No relationship of PFOA, PFOS, PFUA, and the sum of all four PFCs was found to glucose homeostasis, adiponectin level, lipid profile, and inflammatory markers. The median and the range of perfluorononanoic acid (PFNA) concentration (in ng/mL; for four categories corresponding to the <50, 50−74, 75−89, and ≥90th percentiles) were 0.38 (0.38−1.68), 3.22 (1.73−4.65), 5.85 (4.75−8.29), 10.56 (8.40−25.40), respectively. After controlling for confounding factors, multiple linear regression analysis revealed that the mean natural log-transformed level of adiponectin increased significantly across categories of PFNA (in ng/mL; 8.78, 8.73, 9.06, 9.36; *P* for trend = 0.010 in the full model). In conclusion, higher serum PFNA concentration is associated with elevated serum adiponectin concentration.



INTRODUCTION

The toxicity of PFOS and PFOA has been linked to their functions as peroxisome proliferator-activated receptor (PPAR) agonists, including their ability to alter the expression of genes involved in peroxisome proliferation, cell cycle control, and apoptosis. In addition, other PFCs have also been shown to act as strong peroxisomal β-oxidation inducers.[1] Moreover, recent studies using advanced technologies in genomics and bioinformatics have shown that several categories of genes are commonly altered by some PFCs including those for peroxisome proliferation, fatty acid metabolism, lipid transport, cholesterol synthesis, proteosome activation and proteolysis, cell communication, and inflammation.[2] A recent study in mice found low-dose developmental exposure to PFOA led to greater weight in adulthood and increased serum leptin and insulin levels. Animals exposed to higher doses of PFOA, on the other hand, had decreased birth weight.[3]

Epidemiological studies in human beings, unlike studies in animals,[4] do not report hypocholesteremic effects. In occupational populations, several studies have failed to establish a definite association between exposure to PFCs and adverse

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health effects.[5−7] A few cross-sectional and longitudinal occupational studies have proposed a positive correlation between PFOA and serum lipid and liver enzymes levels but without clinical relevance.[8,9] Examination of a nonworker population versus the rest of the U.S. population after exposure to PFOA through contaminated drinking water found an association of elevated levels of PFOA and PFOS to increased cholesterol[10,11] and uric acid.[12] In the general U.S. population, analysis of data from the National Health and Nutrition Examination Survey (NHANES) also found a positive association between PFCs and cholesterol, despite much lower levels of exposure to PFOA.[13]

Whether serum PFCs are associated with diabetes mellitus or glucose homeostasis in humans remains a mystery. A case-control study in a community whose members were exposed to PFOA-contaminated groundwater found no association of serum PFOA level and type II diabetes either self-reported or verified from medical records.[14] Using data from the NHANES database in 1999−2000 and 2003−2004, we found that serum PFCs concentrations were associated with glucose homeostasis and indicators of metabolic syndrome,[15] but a similar analysis by Nelson et al. of data from the 2003−2004 NHANES database found no such association.[13]

Adiponectin is a protein hormone that modulates a number of metabolic processes, including glucose regulation and fatty acid catabolism.[16] In addition, plasma adiponectin level is highly responsive to treatment with PPAR gamma agonist drugs, and the increase in plasma adiponectin level following pioglitazone treatment is much greater than would be predicted from the change in insulin sensitivity.[17]

On the basis of the animal study and humans studies mentioned above,[3,15] it is possible that PFCs might modulate glucose sensitivity or insulin tolerance. Moreover, some of the toxicity of PFCs has been linked to their functions as PPAR agonists. While plasma adiponectin is very responsive to PPARgamma agonist drugs, the relationship between serum PFCs and adiponectin has not yet been determined. We hypothesize that PFCs might be associated with serum adiponectin levels, glucose, and lipid homeostasis. We conducted a cross-sectional study in a community-based sample of adolescents and young adults in Taiwan selected on the basis of results from a mass urine screening for proteinuria, glucosuria, or hematuria.

MATERIALS AND METHODS

Participants and Study Design. From 1992 to 2000, the Chinese Foundation of Health in Taipei, Taiwan, conducted an annual urine screening of approximately 2 615 000 to 2 932 000 school age children in grades 1−12 in Taiwan. A urine strip was used for the screening. School-age children with positive results on two tests for proteinuria, glucosuria, or hematuria underwent a third urine screening test and a general health check-up following the same protocol. The check-up included anthropometric measures, fasting blood tests, and blood pressure (BP). Overall, 131 547 students who participated in the general check-up were referred to their physicians for further diagnosis and follow-up care. This campaign was described in a previous report.[18]

From 2006 to 2008 we established a cohort based on these hypertensive students and students without hypertension selected from the mass urine screening population. Letters of invitation were mailed to eligible students in the Taipei area and in Taichung City. After 3−5 days, 12 trained assistants and nurses conducted telephone interviews inviting these subjects to come in for a follow-up health examination. Normotensive students were not contacted by telephone. In all, 347 of the 707 subjects with elevated blood pressure (EBP) and 641 of the 6390 subjects with normal BP completed follow-up health questionnaires, for a response rate of 49% and 10%, respectively. The detailed information is available in a recent report.[19] In this current study, we selected 790 subjects who lived in the Taipei area whose serum samples were available for further analysis. The interview/check-up was conducted in National Taiwan University Hospital. The study protocol was approved by the ethics committee of National Taiwan University Hospital. Each participant gave her/his inform consent before she/he joined the cohort follow-up study.

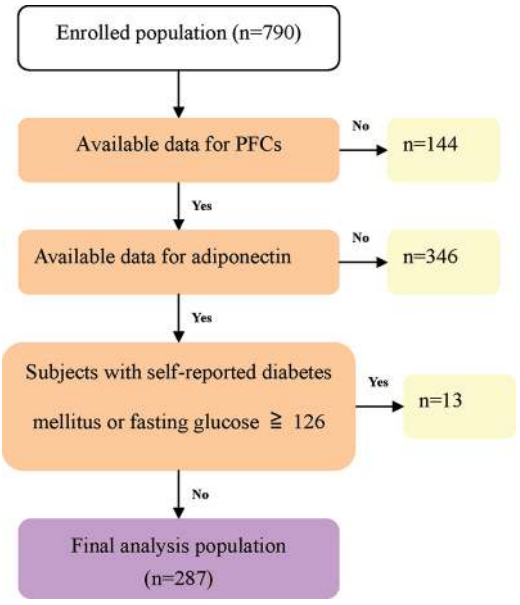

Figure 1. Algorithm used to select the participants with PFCs.

Of the potential subjects, 144 were eliminated due to lack of sufficient serum sample volume. Another 346 subjects were eliminated due to budgetary limitations that prevented the measurement of serum adiponectin levels. An additional 13 subjects were eliminated because they were taking medication for diabetes. They were excluded because numerous studies demonstrated that various agents (e.g., thiazolidinediones and sulphonylureas) increase adiponectin levels in subjects with diabetes.[20,21] Therefore, a final number of 287 participants were selected for the final analysis. A flowchart of the selection process is shown in Figure 1.

Anthropometric and Biochemical Data. Sociodemographic information (i.e., age, gender, history of medication, and household income) was elicited by interview. The household income was dichotomized into above versus below 50 000 new Taiwan dollars (NTD) per month. The degree of alcohol intake (as determined by questionnaire) was dichotomized into current versus no consumption of alcohol. Smoking status was either active smoker, environmental tobacco smoke, or never smoked as determined by serum cotinine level and as described on the questionnaire.[22] Serum cotinine concentration was measured by the DRI Cotinine Assay for urine (Microgenics Corp., Fremont, CA) on a Dimension RXL analyzer (Siemens Healthcare Diagnostics, Inc., Tarrytown, NY). Weight and height were measured by standard methods, and BMI was calculated. Two seated blood pressure

and heart rate measurements were made at least 1 min apart after 3 min of rest by using a mercury manometer and the appropriate cuff size. Blood was drawn from all study participants after they had fasted more than 8 h. Levels of triglyceride (TG), plasma total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), and glucose were measured by an autoanalyzer (Technician RA 2000 Autoanalyzer; Bayer Diagnostic, Michawaka, IN), and their units are mg/dL .

Commercially available kits were used to measure levels of serum insulin (Immulite 2000; Siemens Healthcare Diagnostics), serum adiponectin (Human Adiponectin/Acrp30 Immunoassay; R&D Systems, Minneapolis, MN), and serum high sensitivity (hs)-C-reactive protein (CRP) (chemiluminescent enzyme-labeled immunometric assay, Immulite C-Reactive Protein; Diagnostic Products Co., Los Angeles, CA). The homeostasis model assessment (HOMA) of insulin resistance (HOMA-IR) index (the product of basal glucose and insulin levels divided by 22.5) is regarded as a simple, inexpensive, and reliable surrogate measure of insulin resistance.[23] Diabetes mellitus was defined as the presence of fasting serum glucose ≥126 mg/dL or a self-reported current use of oral hypoglycemic agents or insulin. Hypertension was defined as self-reported current use of antihypertensive medication or an average BP greater than 140/90 mmHg. Childhood EBP was defined as systolic blood pressure, diastolic blood pressure, or both greater than or equal to values meeting the modified sex- and age-specific standards.[24]

Measurement of PFCs Concentration. Plasma samples were stored at −80 °C before analysis. In all, 8 of the 12 PFCs analyzed in our study were undetectable using a Waters ACQUITY UPLC system (Waters Corporation, Milford, MA) coupled with a Waters Quattro Premier XE triple quadrupole mass spectrometer (Waters Corporation). Therefore, we measured plasma levels of only PFOA, PFOS, perfluorononanoic acid (PFNA), and perfluoroundecanoic acid (PFUA). Details of the analytical method have been described elsewhere.[25] Briefly, frozen samples were thawed at room temperature and vortexed for 30 s to ensure homogeneity. Each plasma sample (100 μL) was vortexed for 30 s with 100 μL of 1% formic acid (pH = 2.8), then mixed with 80 μL of methanol and 20 μL of 0.375 ng/mL internal standard ($^{13}C_8$-PFOA) in methanol, sonicated for 20 min, and then centrifuged at 14 000 × g for 20 min. The collected supernatant (about 150 μL) was then filtered through a 0.22 μm polyvinyl difluoride (PVDF) syringe filter before instrumental analysis. The standard solutions used for calibration were prepared in 100 μL of bovine plasma and went through the same procedure used for sample preparation; the concentrations of all analytes were equivalent to 0.05−300 ng/mL bovine plasma containing a fixed amount of internal standard (75 ng/mL).

The limits of quantitation for PFOA, PFOS, PFNA, and PFUA were 1.5, 0.22, 0.75, and 1.5 ng/mL, respectively. In blank samples, trace background amounts of PFOA (up to 1.5 ng/mL), PFNA (up to 0.75 ng/mL), and PFUA (up to 3 ng/mL) but no PFOS was detected; consequently, the reported PFOA, PFNA, and PFUA concentrations are concentrations corrected by subtraction of background levels found in the blank. For concentrations below the detection limits (49.5% for PFOA, 1.7% for PFOS, 44.3% for PFNA, and 26.5% for PFUA), a proxy value of half the detection limit was used. All laboratory analyses were conducted by investigators blinded to the characteristics of study subjects. Total PFCs were determined by summing the four individual PFC congeners (raw or imputed) for each study subject.

Definition of Metabolic Syndrome. For subjects ≥20 years old, metabolic syndrome (MS) was defined according to the updated guidelines of the National Cholesterol Education Program Third Adult Treatment Panel (NCEP/ATP III).[26] At least three of the following criteria had to be satisfied: waist measurement ≥ 80 cm for women and ≥ 90 cm for men (Asian criteria); serum triglyceride ≥ 1.7 mmol/L; serum HDL-C < 1.03 mmol/L in men and < 1.29 mmol/L in women; systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg or a self-report of taking antihypertensive medications; fasting glucose ≥ 5.6 mmol/L or a self-report of taking antihyperglycemic medications. To define MS in participants (age, 12−19 years), we used a modification of the definition proposed in the NCEP/ATP III. The participants had to meet 3 of the following 5 criteria: serum concentration of triglyceride ≥ 1.24 mmol/L; HDL-C < 1.03 mmol/L; waist circumference ≥ 92nd percentile for males and 72nd percentile for females; SBP or DBP ≥ 92nd percentile for males and 72nd percentile for females or a self-report of taking antihypertensive medications; glucose concentration ≥ 5.6 mmol/L or a self-report of taking antihyperglycemic medications.[27]

Statistical Analysis. SPSS for Windows (version 16.0, SPSS Inc., Chicago, IL) was used for all statistical analyses. Because exposure was relatively low in most people and variance was considerably greater at the higher exposure end, PFC concentrations were expressed as the median with range (difference between the maximum and minimum value). The relation of PFC variables to categorical variables was tested using the Mann−Whitney U test or Kruskal−Wallis test (if 3 or more groups). We also divided PFCs in four categories, with cut points at the 50th (the reference category), 75th, and 90th percentiles in a linear regression model. Analyses were conducted using linear regression with adiponectin, glucose homeostasis, lipid profile, and inflammatory markers as the outcome variables. Natural log transformation was performed for adiponectin, CRP, HOMA-IR, triglyceride levels with significant deviation from the normal distribution before further analyses. All the log-transformed data in the study had a normal distribution (by Kolmogorov−Smirnov test). Because adiponectin concentration depends on many endogenous and exogenous factors, including age, gender, smoking, alcohol consumption, and renal function,[28−30] we used an extended model approach for covariates to adjust for potential confounders in multiple linear regression models. Model 1 adjusted for age and gender. Model 2 adjusted for age, gender, life-style factors (smoking status, drinking status, and household income). Model 3 = model 2 adjusted for measurement data (waist measurement, systolic blood pressure, total cholesterol, HOMA-IR, and creatinine). To avoid "model-dependent association", an association was considered significant only when it remained statistically significant in all models. Each PFC was modeled separately. When the independent variables were the derivatives of the plasma glucose and the serum insulin, no adjustment for either plasma glucose or serum insulin was made in the model. The HOMA-IR was adjusted in the final model if the independent variable was not an arithmetic combination of the plasma glucose and the serum insulin.

RESULTS

The basic demography of the sample population (121 males and 166 females) is summarized in Table 1. Males had a significantly higher median serum PFOS level than females ($P < 0.001$), and subjects with metabolic syndrome had signficantly lower

Table 1. Basic Demographics of the Sample Subjects Including Median (range) of PFC Concentrations

	no.	PFOA (ng/mL)	PFOS (ng/mL)	PFNA (ng/mL)	PFUA (ng/mL)
total	287	2.39 (27.38)	8.93 (67.14)	1.68 (25.03)	7.11 (83.96)
Sex					
males	121	0.75 (27.38)	11.82 (67.14)[a]	1.52 (19.26)	6.07 (65.30)
females	166	2.45 (22.76)	8.10 (28.34)[a]	1.81 (25.03)	7.49 (83.96)
Age in Years					
12−19	78	0.75 (19.62)	8.85 (28.67)	1.76 (19.26)	6.83 (58.91)
20−30	209	2.67 (28.38)	8.93 (67.14)	1.64 (25.03)	7.20 (83.96)
Household Income					
<50 000 NT dollar per month	123	0.75 (13.35)	8.54 (67.14)	1.68 (25.03)	6.74 (83.96)
≥50 000 NT dollar per month	164	3.02 (27.38)	9.08 (32.14)	1.69 (19.26)	7.22 (65.30)
Smoking Status					
never smoked	162	0.75 (27.38)	8.94 (67.14)	1.64 (25.03)	7.69 (83.96)
environmental smoker	24	4.07 (13.35)	11.71 (27.45)	2.43 (19.26)	7.60 (52.84)
active smoker	101	2.60 (22.76)	8.51 (33.05)	1.68 (13.04)	5.93 (44.90)
Current Alcohol Consumption					
yes	25	3.66 (9.48)	8.41 (67.14)	0.38 (13.25)	5.02 (44.90)
no	262	0.75 (27.38)	9.03 (33.05)	1.75 (25.03)	7.11 (83.96)
Body Mass Index (kg/m^2)					
<24	206	2.78 (22.76)	8.48 (67.14)	1.65 (25.03)	6.51 (84.00)
≥24	81	0.75 (27.38)	10.91 (30.4)	1.78 (13.08)	8.63 (65.30)
Current Hypertension					
yes	17	0.75 (10.00)	9.16 (26.81)	0.38 (6.55)	8.31 (44.91)
no	270	2.55 (27.38)	8.86 (67.14)	1.71 (25.03)	6.86 (83.96)
Metabolic Syndrome					
yes	15	0.75 (7.23)[b]	8.28 (26.81)	0.38 (11.61)	8.31 (65.30)
no	272	2.67 (27.38) [b]	8.93 (67.14)	1.71 (25.03)	7.10 (37.16)

[a] Males have a higher median concentration of PFOS than females ($P < 0.001$). [b] PFOA levels were significantly lower in subjects with metabolic syndrome ($P = 0.009$).

serum PFOA level ($P = 0.009$). Concentrations of the four PFCs were similar in other groups. Some of the PFCs were not correlated with one another (see Supporting Information Table S1). PFNA and PFUA were strongly correlated (Spearman correlation coefficient, 0.62; $P < 0.001$).

Glucose homeostasis, adiponectin level, lipid profile, and level inflammatory markers were not significantly associated with categories of serum PFOA, PFOS, PFUA, and total PFCs in the simple model (Supporting Information Tables S2−5). The association of categories of serum PFNA concentration with glucose homeostasis, adiponectin level, lipid profile, and levels of inflammatory markers after the adjustment for other potential covariates is summarized in Table 2. The log transformed mean adiponectin level (ng/mL) was significantly increased across categories of PFNA in all three models (8.78, 8.73, 9.06, and 9.36; P for trend = 0.010 in the full model). Insulin level and HOMA-IR were significantly decreased with PFNA in model 1 and model 2 but not in the full model. The PFNA concentration was not associated with levels of glucose, HDL, triglyceride, or CRP.

Linear regression coefficients (SEs) of the relationship of serum adiponectin level with categories of PFNA defining different subpopulations of the sample are shown in Table 3. The association between serum adiponectin and PFNA levels was significant in subjects with male gender, age 20−30 years old, and higher HOMA-IR. The relationship between serum adiponectin level and quartiles of BMI in different subpopulations of the sample is also shown (Supporting Information Table S6).

DISCUSSION

In this study, increased serum PFNA concentration was associated with elevated serum adiponectin concentration. Studying the potential health consequences of an environmental exposure in adolescents and young adults rather than in older adults might provide greater insight because the number of underlying factors confounding the associations is likely to be smaller.

In this study, the median concentration of PFUA was 6.07 ng/mL in males and 7.49 ng/mL in females. Our findings are compatible with those of another study that analyzed perfluorinated chemicals in umbilical cord blood in Taiwan.[25] These concentrations are 10 times higher than those previously reported for PFUA while concentrations of PFOA, PFOS, and PFNA are comparable to those of another report in a different country.[31] Taiwan is the only country thus far reported with high PFUA serum concentrations, possibly from an environmental source. One study which investigated the influence of discharge by the semiconductor and electronics industries on contamination of river water with PFCs in Taiwan detected PFUA in wastewater but not the

Table 2. Mean and Standard Error of Adiponectin, Glucose Homeostasis, Lipid Profile, and Inflammatory Marker across Different Categories of Serum PFNA Level in Linear Regression Models[a]

PFNA percentile %[b]	log-adiponectin (ng/mL)	glucose (mg/dL)	log-insulin (pmol/L)	log-HOMA-IR	HDL (mg/dL)	log-TG (mg/dL)	log-CRP (mg/L)
Model 1							
<50th	8.76 (0.08)	84.44 (0.54)	1.07 (0.08)	−0.50 (0.08)	48.38 (0.77)	4.33 (0.04)	1.59 (0.09)
50th−74th	8.64 (0.12)	84.88 (0.77)	1.09 (0.11)	−0.48 (0.12)	49.23 (1.10)	4.35 (0.06)	1.70 (0.13)
75th−89th	9.14 (0.16)	84.03 (1.00)	0.70 (0.14)	−0.87 (0.15)	48.80 (1.42)	4.38 (0.07)	1.27 (0.17)
≥90th	9.51 (0.19)	84.20 (1.23)	0.75 (0.18)	−0.82 (0.18)	48.89 (1.75)	4.29 (0.09)	1.21 (0.21)
P for trend	<0.001	0.913	0.051	0.057	0.936	0.887	0.138
Model 2							
<50th	8.75 (0.14)	84.51 (0.86)	0.97 (0.13)	−0.60 (0.13)	47.53 (1.26)	4.31 (0.07)	1.49 (0.15)
50th−74th	8.64 (0.16)	85.07 (1.03)	0.99 (0.15)	−0.58 (0.16)	48.38 (1.50)	4.34 (0.08)	1.63 (0.18)
75th−89th	9.16 (0.19)	84.05 (1.21)	0.59 (0.18)	−0.99 (0.18)	48.01 (1.75)	4.35 (0.09)	1.16 (0.21)
≥90th	9.48 (0.22)	83.84 (1.38)	0.67 (0.21)	−0.91 (0.21)	47.92 (2.01)	4.26 (0.10)	1.11 (0.25)
P for trend	<0.001	0.787	0.054	0.057	0.937	0.981	0.068
Model 3							
<50th	8.78 (0.10)	84.77 (0.58)	0.98 (0.08)	−0.66 (0.11)	48.64 (0.89)	4.28 (0.04)	1.47 (0.10)
50th−74th	8.73 (0.12)	85.33 (0.75)	0.87 (0.10)	−0.77 (0.13)	50.20 (1.14)	4.25 (0.05)	1.46 (0.12)
75th−89th	9.06 (0.16)	85.51 (0.98)	0.73 (0.13)	−0.90 (0.15)	48.20 (1.48)	4.37 (0.07)	1.28 (0.16)
≥90th	9.36 (0.19)	85.18 (1.15)	0.88 (0.15)	−0.76 (0.17)	47.70 (1.75)	4.31 (0.08)	1.27 (0.19)
P for trend	0.010	0.852	0.328	0.363	0.504	0.479	0.549

[a] Model 1: adjusted for age, gender. Model 2: adjusted for age, gender, lifestyle (smoking status, drinking status, household income). Model 3: adjusted for age, gender, lifestyle (smoking status, drinking status, household income) and measurement data (SBP, waist, HOMA-IR, total cholesterol, creatinine). [b] The median and the range concentrations of the PFNA for the cut points used (<50th, 50−74th, 75−89th, and ≥90th percentiles) were listed below: PFNA 0.38 (0.38−1.68) ng/mL, 3.22 (1.73−4.65) ng/mL, 5.85 (4.75−8.29) ng/mL, 10.56 (8.40−25.40) ng/mL, respectively.

Table 3. Adjusted Mean and Standard Error of Natural Log Adiponectin (ng/mL) across Different Serum Levels of PFNA in Sub-Populations of the Sample Subjects[a]

	no.	<50th	50th−74th	75th−89th	≥90th	*P* for trend
Sex						
males	121	8.59 (0.16)	8.43 (0.21)	9.01 (0.25)	9.51 (0.23)	0.001
females	166	8.92 (0.20)	8.85 (0.23)	9.04 (0.27)	9.05 (0.35)	0.864
Age in Years						
12−19	78	8.70 (0.31)	8.36 (0.36)	8.95 (0.40)	8.81 (0.46)	0.504
20−30	209	8.82 (0.14)	8.86 (0.17)	9.14 (0.19)	9.41 (0.23)	0.032
Body Mass Index (kg/m^2)						
≤24	206	9.05 (0.16)	9.00 (0.20)	9.32 (0.23)	9.61 (0.25)	0.073
>24	81	8.42 (0.19)	8.19 (0.21)	8.55 (0.26)	8.70 (0.35)	0.356
Smoking Status						
never smoked	162	8.78 (0.22)	8.73 (0.28)	8.97 (0.30)	9.45 (0.29)	0.084
has smoked	125	8.84 (0.13)	8.72 (0.16)	9.09 (0.20)	9.26 (0.29)	0.148
HOMA-IR						
≤0.57	144	9.02 (0.22)	9.19 (0.24)	9.33 (0.25)	9.52 (0.31)	0.310
>0.57	143	8.58 (0.15)	8.27 (0.19)	8.83 (0.26)	9.22 (0.26)	0.005

[a] Adjusted for full model.

major PFCs.[32] The source of exposure is not clear and needs to be further investigated.

Like the eight-carbon PFOA, the nine-carbon PFNA is a developmental toxicant and an immune system toxicant in animals.[33] However, longer chain PFCs are considered more bioaccumulative and toxic. PFNA has been shown to be a strong peroxisome β-oxidation inducer in animals.[1] Fibrates (amphipathic carboxylic acids that activate PPAR alpha) can decrease the triglycerides level, normalize the LDL cholesterol profile, and increase HDL cholesterol.[34] However, most epidemiological studies in humans[10−12] unlike studies in animals[4] report hypercholesteremic effects. Duration of exposure, dose (concentration), and

interspecies differences may account for the inconsistent cholesterol findings between animal and human studies. Moreover, human liver tissue expresses PPAR alpha at approximately 10% of rodent levels, and the liver tissue of humans and other primates (compared with that of rodents) is refractory or less responsive to PPAR alpha agonists.[35]

In a cross-sectional study of 2003−2004 NHANES samples, the authors observed a positive association of PFOS, PFOA, and PFNA concentrations with total and non-high-density cholesterol concentrations, and the opposite for perfluorohexane sulfonic acid (PFHxS).[13] The strongest, most consistent cholesterol results were seen for PFNA, despite lower serum concentrations versus the other PFCs in the NHANES population. However, no significant correlation between lipid profile and serum PFCs concentration was found in the current study. Race/ethnicity differences between studies and the existence of a higher frequency of aberrant serum lipid profiles in adults than in younger people (children and adolescents) may account for this discrepancy between studies.

Unlike PFOA and PFOS, which do not activate mouse or human PPAR gamma,[36] PFNA activates both PPAR-alpha and PPAR-gamma.[33] In the latter study,[33] the PFNA (0, 1, 3, or 5 mg/kg/day) was administered via gastric gavage for 2 weeks. PPAR gamma (along with PPAR alpha) was upregulated in the thymus by 1 mg but not by 3 or 5 mg/kg/day, suggesting maximal transcriptional activity occurs at a dose no higher than 1 mg/kg/day. Maximal PPAR gamma activity may be induced in mice by a much lower level than the environmental levels to which humans are exposed. Unlike PPAR alpha receptor, PPAR gamma receptor is highly expressed in rodents and humans. PPAR gamma1 is highly expressed in the adipose tissue of adult humans.[37] PPAR gamma ligands increases the number of small adipocytes, thereby increasing the adiponectin level and insulin sensitivity and reducing the levels of tumor necrosis factor alpha, which induce insulin resistance.[16] The evidence from the above animal study and the present study suggests that PFNA may be involved in the pathway of PPAR-gamma activation. Nonetheless, interaction with insulin and other modulators may also be involved in this mechanism since adiponectin knockout mice are known to develop moderate insulin resistance and glucose intolerance.

In this study, increased serum PFNA concentration was associated with elevated serum adiponectin. An approximately 10 ng/mL PFNA difference in concentration between the 50th percentile and 90th percentile resulted in a 7 μg/mL difference in mean adiponectin concentration in the present study. In a recent meta-analysis of 13 studies, average adiponectin level ranged from 5 to 15 μg/mL.[38] Our study shows the magnitude of the effect if the association between PFNA and adiponectin has etiologic implications,.

A nonstatistically significant trend was observed with insulin and HOMA-IR with decreased PFNA. This association, however, is similar to the association we found between PFCs and glucose homeostasis in our previous study using 1999−2000 and 2003−2004 NHANES data.[15] In that study, increased serum PFNA concentration in adolescents was associated with decreased blood insulin. However, this was not found by Nelson et al. using the 2003−2004 NHANES databases. Although there were isolated suggestive trends, such as a significant positive trend between PFNA level and HOMA-IR in adult females, they found no association between PFNA concentration and HOMA-IR on the whole study population.[13] It is difficult to compare the two studies, inasmuch as we analyzed two additional years of data and used different criteria for inclusion of subjects. PFNA may increase serum adiponectin level and decrease serum insulin level by acting as an agonist to PPAR gamma, and thereby yielding correlations of PFNA level with serum HDL and MS. However, we found only the association between PFNA and adiponectin but not between PFNA and decreased insulin and insulin resistance. Insulin resistance, type II diabetes, obesity, and myocardial infarction are inversely related to increasing plasma adiponectin level.[38] The data presented would suggest that increased PFNA levels in the study population might be inversely associated with these pathological conditions because it causes plasma adiponectin level to increase.

In subgroup analysis, the association between serum PFNA and adiponectin was more evident in subjects with male gender, older age, and high insulin resistance. Serum adiponectin concentrations reveal a sexual dimorphism developed during the progression of puberty, with females having higher levels than males.[39] BMI was also significantly related to circulating levels.[39] It is possible that the relationship between PFNA and adiponectin was also influenced by gender and age. Whether these results are a statistical artifact or have a true underlying biological basis needs further study.

Our study had several limitations. First, the cross-sectional design does not permit any causal inference. Second, we did not include other environmental chemicals which may be important covariates or explanatory variables affecting the outcomes of our study. Third, since our study population was adolescents and young adults, the same association might not be found in older adults. Finally, a common physiology could influence both serum PFCs and adiponectin levels rather than exposure affecting outcome.

In conclusion, higher serum PFNA concentration was associated with higher serum adiponectin concentration in a sample of adolescents and young adults in Taiwan. Although the relationship between PFNA and adiponectin has little biological significance and the effect of this relationship on health is uncertain in the Taiwan population, our data suggest that it would be prudent to monitor the adiponectin level of people with low, environmentally relevant exposure to PFNA. Further studies are needed to confirm these findings and to clarify whether these associations are causal.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Additional tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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