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Association Among Serum Perfluoroalkyl Chemicals, Glucose Homeostasis and Metabolic Syndrome in Adolescents and Adults

Running title: *Perfluoroalkyl chemicals, glucose homeostasis and metabolic syndrome*

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Objective: Perfluoroalkyl chemicals (PFCs) have been used worldwide in a variety of consumer products. The effect of PFCs on glucose homeostasis is not known.

Research design and methods: We examined 474 adolescents and 969 adults with reliable serum measures of metabolic syndrome (MS) profile from the National Health and Nutrition Examination Survey 1999-2000 and 2003-2004.

Results: In adolescents, increased serum perfluorononanoic acid (PFNA) concentrations were associated with hyperglycemia (OR = 3.16, 95% CI = 1.39-7.16, $P < 0.05$). Increased serum PFNA concentrations also have favorable associations with serum HDL-C (OR = 0.67, 95% CI = 0.45-0.99, $P < 0.05$). Overall, increased serum PFNA concentrations were inversely correlated with the prevalence of the MS (OR = 0.37, 95% CI = 0.21-0.64, $P < 0.005$). In adults, increased serum perfluorooctanoic acid (PFOA) concentrations were significantly associated with increased β cell function (βcoeff = 0.07±0.03, $P < 0.05$). Increased serum perfluorooctane sulfate (PFOS) concentrations were associated with increased blood insulin (βcoeff = 0.14±0.05, $P < 0.01$), HOMA-IR (βcoeff = 0.14±0.05, $P < 0.01$) and β cell function (βcoeff = 0.15±0.05, $P < 0.01$). Serum PFOS concentrations were also unfavorably correlated with serum HDL-C (OR = 1.61, 95% CI = 1.15-2.26, $P < 0.05$).

Conclusions: Serum PFCs were associated with glucose homeostasis and indicators of MS. Further clinical and animal studies are warranted to clarify putative causal relationships.

The perfluoroalkyl chemicals (PFCs) are a family of perfluorinated chemicals that consist of a carbon backbone typically 4-14 in length and a charged functional moiety [1]. PFCs have been used extensively since the 1950s in commercial applications, including as a component in surfactants, lubricants, paper and textile coatings, polishes, food packaging, and fire-retardant foams [2]. Some of these PFCs, including the most widely known examples of perfluorooctanoic acid (PFOA) and perfluorooctane sulfate (PFOS), persisted in humans and the environment and have been detected worldwide in wildlife [2]. The routes of human exposure to PFCs are currently being investigated. Possible exposure pathways that are being examined include drinking water, dust in homes, and food or migration from food packaging and cookware. Animal studies have shown that they are well absorbed orally, but poorly eliminated; they are not metabolized, and undergo extensive uptake from enterohepatic circulation and are distributed mainly to the serum, kidney and liver [1]. Although some PFCs have been voluntarily removed from the market by manufacturers, PFOA and PFOS and their derivatives are still produced commercially and its potential risk to humans continues to be evaluated.

In animal studies, exposure to PFOS and PFOA is associated with adverse health effects, including carcinogenicity [3, 4], hepatotoxicity [4, 5], and developmental and reproductive toxicity [4]. For human beings, PFC exposure has been shown to be associated with certain types of cancers [6]. Maternal exposure to PFOS and PFOA also has been linked to low birth weight [7].

The causal biochemical mechanisms leading to the adverse health outcomes after exposure to PFCs are largely unknown. However, recent studies using advanced technologies in genomics and bioinformatics have shown that several categories of genes are commonly altered by some PFCs including peroxisome proliferation, fatty acid metabolism, lipid transport, cholesterol synthesis, proteosome activation and proteolysis, cell communication, and inflammation [1]. The agonistic properties of PFCs on PPAR-α (peroxisome proliferator-activated receptors-α) are well supported and are thought to be a major mechanism leading to PFC-mediated liver damage [8, 9]. Since activation of PPAR-α can decrease serum triglycerides, normalize low-density lipoprotein cholesterol and increase HDL-C, we hypothesized that PFCs might have favorable effects on lipid homeostasis and may also be associated with reduced insulin resistance, improved serum lipid profile and lower prevalence of the metabolic syndrome (MS). The goal of the present study is to test this hypothesis by examining data from the National Health and Nutrition Examination Survey (NHANES) collected from 1999-2000 and 2003-2004.

RESEARCH DESIGN AND METHODS

Study design and population: Data were from NHANES 1999–2000 and 2003–2004. The NHANES is a population-based survey designed to collect information on the health and nutrition of the U.S. household population, and to obtain a representative sample of the non-institutionalized civilian US population. The survey data are released every 2 years. Detailed survey operations manuals, consent documents, and brochures of the NHANES 1999–2000 and 2003–2004 are available on the NHANES website [10].

We limited our analyses to the 3685 participants at least 12 years of age who had a blood test for PFCs. Among these subjects, only 1788 subjects had a morning examination and had fasting plasma glucose, insulin and triglyceride (TG) data available. Of these 1788 participants, we included the

1443 subjects without missing data for further analyses.

Anthropometric and biochemical data: According to the statements on the NHANES website, data were collected at all study sites by trained personnel according to standardized procedures. Sociodemographic information such as age, gender, and race/ethnicity, education level and household income was collected during the household interview. Alcohol intake was determined by the questionnaire "in any one year, have you had at least 12 drinks of any type of alcohol beverage?" and was dichotomized. For adolescents, since there were too many missing data, alcohol intake was not entered for analysis. Smoking status was categorized as active smoker, former/passive smoker and non-smoker by smoking questionnaire and serum cotinine levels as previously described [11]. Laboratory measurements were performed in a mobile examination center. Weight and height were measured using standard methods and digitally recorded. Three and sometimes 4 blood pressure (BP) determinations were collected by a physician using a mercury sphygmomanometer. BP was measured in the right arm unless otherwise specified. Averaged systolic BP (SBP) and diastolic BP (DBP) were obtained. Blood specimens were processed locally, and then stored and shipped to central laboratories for analysis. Levels of serum total cholesterol (TC) and TG were measured enzymatically. Levels of HDL-C were measured after precipitation of other lipoproteins on a Hitachi model 704 analyzer (Roche Diagnostics, Indianapolis, Ind). Serum CRP levels were measured by latex-enhanced nephelometry. Plasma insulin was determined by immunoenzymometric assay. Insulin resistance status (HOMA-IR) and β-cell function were estimated by the updated homeostasis model assessment (HOMA2) [12].

Definition of metabolic syndrome: For subjects above 18 years old, presence of the metabolic syndrome was calculated by sex as defined by the National Cholesterol Education Program Third Adult Treatment Panel (NCEP/ATP III) [13] guideline of presenting with at least 3 of the following qualifications: waist measurement greater than 88cm for women and greater than 102 cm for men; serum TG $\geqq$1.69mmol/L; serum HDL-C <1.03 mmol/L in men and <1.29 mmol/L in women; SBP $\geqq$130 mmHg or DBP $\geqq$85 mmHg or a self report of taking anti-hypertensive medications; and fasting glucose $\geqq$6.10 mmol/L or a self report of taking anti-hyperglycemic medications. To define the MS among the young participants aged between 12 to 17 years, we used a previously proposed modification of the definition proposed in the NCEP/ATP III. The participants had to meet 3 of the following 5 criteria: serum concentration of TG$\geqq$1.24 mmol/L, HDL-C$\leqq$1.04 mmol/L, WC $\geqq$sex specific 90th percentile [14], glucose concentration$\geqq$5.55 mmol/L or a self report of taking anti-hyperglycemic medications [15], and SBP or DBP$\geqq$age, height and sex-specific 90th percentile or a self-report of taking anti-hypertensive medications [16].

Assessment of serum PFCs: As part of NHANES, serum samples of PFOA, PFOS, perfluorohexane sulfonic acid (PFHS) and perfluorononanoic acid (PFNA) were collected for analysis. The analytical method has been described in detail [17]. Briefly, without protein precipitation, only dilution with 0.1M formic acid, one aliquot of 100 μL serum was injected into a commercial column switching system allowing for concentration of the analytes on a C18 solid-phase extraction column. This column was placed automatically in front of a C8 analytical high-performance liquid chromatography column for chromatographic separation of the analytes. Detection and quantification were

done using negative-ion TurbolonSpray ionization, tandem mass spectrometry. Isotope-labeled internal standards were used for quantification.

Statistics: Data were expressed as the mean ± standard error (S.E). Participants were divided into adolescents (12 to 20 years of age) and adult (greater than 20 years of age) groups for analysis. The strength of the associations between concentrations of various serum PFCs and blood glucose, insulin and HOMA-IR levels was tested using multiple linear regression models. Logistic regression analyses were conducted to examine the odds ratios of MS (yes or no for having at least 3 components of MS) and its components (yes/no for that component) associated with one unit increase in log-PFCs. Log transformation was performed for variables with significant deviation from normal distribution before further analyses. For linear regression, we used an extended model approach for covariates adjustment: Model 1 = age, gender, race; Model 2 = Model 1 + health behaviors (smoking status, alcohol intake and household income); Model 3 = Model 2 + measurement data (waist measurement, CRP, and insulin/glucose/HOMA) + current medications (anti-hypertensive, anti-hyperglycemic and anti-hyperlipidemic agents). For logistic regression, the models for adjustment were Model 4 = age, gender, race, health behaviors (smoking status, alcohol intake and household income), measurement data (CRP, HOMA/insulin) and current medications (anti-hypertensive, anti-hyperglycemic and anti-hyperlipidemic agents). Model 5 = Model 4 + other components of the MS. A $P<0.05$ was considered statistically significant. To avoid "model dependent association", the association was considered significant only when it remained statistically significant in all models.

Sampling weights that account for unequal probabilities of selection, oversampling, and nonresponse were applied for all analyses using the Complex Sample Survey module of SPSS Version 13.0 for Windows XP (SPSS Inc. Chicago, Illinois, U.S.A.).

RESULTS

The basic demography of the participants is summarized in Table 1. The study sample consisted of 474 adolescents (age between 12 to 20 years) and 969 adults (age above 20 years). The serum PFHS levels were significantly higher in adolescents than in adults (log-PFHS ng/mL, 0.95±0.10 vs. 0.60±0.04 respectively, $P < 0.001$) while the serum PFNA concentrations were lower in adolescents than in adults (log-PFNA ng/mL, -0.35±0.07 vs. -0.21±0.07, $P = 0.001$). The serum PFOA and PFOS concentrations were not different between these two groups.

The associations between the serum PFCs levels and glucose homeostasis markers are shown in Table 2. In adolescents, increased serum PFNA concentrations were associated with decreased blood insulin (βcoeff = -0.10±0.05, $P < 0.05$) and β cell function (βcoeff -0.12±0.06, $P < 0.05$) with borderline significant ($P = 0.05$-0.09 in all models and < 0.05 in final model). Other PFCs were not associated with the serum markers for glucose homeostasis. In adults, increased serum PFOA concentrations were significantly associated with increased β cell function (βcoeff = 0.07±0.03, $P < 0.05$). Increased serum PFOS concentrations were also associated with increased blood insulin (βcoeff = 0.14±0.05, $P < 0.01$), HOMA-IR (βcoeff = 0.14±0.05, $P < 0.01$) and β cell function (βcoeff = 0.15±0.05, $P < 0.01$).

The associations between the serum PFCs and the MS/MS components are summarized in Table 3. In adolescents, increased serum PFNA concentrations were associated with lower prevalence of the MS (OR = 0.37, 95% CI = 0.21-0.64, $P < 0.005$)

and HDL-C below the MS criteria (OR = 0.67, 95% CI = 0.45-0.99, $P < 0.05$). Increased serum PFNA concentrations were also correlated with higher prevalence of blood glucose above the MS definition (OR = 3.16, 95% CI = 1.39-7.16, $P < 0.05$). We also found that the serum PFOS (OR = 0.37, 95% CI = 0.16-0.82, $P < 0.05$) concentrations were inversely correlated with a lower prevalence of waist circumference below the MS definition. In adult subjects, among all the PFCs and MS components, only serum PFOS concentrations were associated with higher prevalence of HDL-C below MS definition (OR = 1.61, 95% CI = 1.15-2.26, $P < 0.05$).

DISCUSSION

To our knowledge, this report is the first to systemically analyze the link among serum PFC concentration, glucose homeostasis, and the MS/MS components in a nationally representative sample. In this study, we showed that PFCs were differentially associated with glucose homeostasis in adolescents and adults. We should take into careful consideration about extrapolating and interpreting data between laboratory animal studies and the corresponding biological effects (at high ppm range) compared to general human populations (at low ppb range). We found that the concentrations reported for PFCs in the occupational studies have been two and three orders of magnitude higher than what has been measured in the general population. While there were several studies about the maternal exposure and child development, worker's exposure and health outcome, the relationship of serum PFCs levels to medical diseases and laboratory abnormality in a nationally representative survey has never been explored. Our results might suggest that low dose PFCs exposure might have effects on glucose metabolism in general population.

Our analysis showed that the serum PFOA and PFOS concentrations were not different between adolescents and adults. Unlike other lipophilic persistent pollutants that display increasing serum concentrations as people age, the lack of this general trend in PFOS and PFOA could be explained by intra-uterine transfer, exposure early in life, ongoing exposures being much higher than earlier historical exposures or a combination of these factors [18]. By contrast, the mean concentrations of PFHS were higher for adolescents than for adults, as previously reported [2, 19]. The higher concentrations of PHFS in children and adolescents could be related to their increased contact with carpeted floors containing PFHS, which is used for specific postmarket carpet-treatment applications [2, 19].

We showed that in adolescents, increased serum PFNA concentrations were associated with decreased blood insulin, impaired β cell function (borderline significance) and clinical hyperglycemia. On the other hand, we found that increased serum PFNA had a favorable correlation with serum HDL. Overall, increased serum PFNA concentrations were inversely associated with the prevalence of MS in adolescents. In adults, serum PFOS concentrations were independently associated with increases in both blood insulin and insulin resistance status (HOMA-IR). Interestingly, both serum PFOA and PFOS were also positively correlated withβ cell function. The balance between increased insulin resistance and β cell function has a neutral effect on blood glucose. Increased PFOS also showed an unfavorable association with serum HDL-C in adults. Overall, the PFCs in the present study had neutral effects on the prevalence of the MS in adults.

There has been a great deal of progress in the last few years in understanding the toxicology and distribution of PFCs in the environment, in wildlife, and in humans. However, there is a paucity of information pertaining to many specific PFCs [1]. Thus

far, there are no published reports of either in vitro or in vivo data pertaining to effects of PFCs on glucose homeostasis. The underlying mechanisms of this linkage are unknown and might partially be related to peroxisome activation. The liver toxicity of PFOS and PFOA has been linked to their PPAR-α agonist property [8, 9]. PFNA also has been shown to be a strong peroxisomal β oxidation inducer in animals [20, 21]. Fibrates, amphipathic carboxylic acids that activate PPAR-α, can decrease TG, normalize the low-density lipoprotein cholesterol profile, and increase HDL-C [22]. However, our results are not entirely consistent with previous animal findings, suggesting an alternative or even multiple pathways in association among PFCs, glucose and lipid metabolism. For example, Luebker et al. have demonstrated that both PFOS and PFOA can interfere with the binding affinity of L-FABP (liver-fatty acid binding protein) in rodents [23]. Interestingly, they also found that among the tested PFCs, PFOS exhibited the highest level of inhibition of L-FABP binding, which might partially explain the unfavorable association between increased serum PFOS and HDL-C.

CONCLUSION

In conclusion, we present the first report of a relationship between serum PFCs, glucose homeostasis and metabolic syndrome. Since PFCs have been widely used worldwide in a variety of consumer products, further longitudinal clinical and in vitro studies are urgently warranted to elucidate the putative casual relationships between PFCs and metabolism.

Study limitation: Our study has several limitations. First, the cross-sectional design does not permit any causal inference. Second, due to a number of missing data, drinking status was not included in our analyses of adolescents.

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Table 1 Basic demography and serum concentrations of PFCs of the sample subjects

	Unweighted No. adolescent/adult	Adolescents (≧12 yrs, <20 yrs)	Adults 20 yrs
Age, years	474/969	15.5±0.2	46.2±0.8
Gender, %			
Male	266/476	56.6±3.1	50.2±1.7
Female	208/493	43.4±3.1	49.8±1.7
Race, %			
Mexican American	182/273	11.7±2.5	8.7±1.7
Non-Hispanic White	123/510	71.7±3.5	80.8±2.5
Non-Hispanic Black	169/186	16.6±2.6	10.5±1.7
Smoking, %			
Active smoker	66/197	19.1±2.1	25.3±2.5
Former/passive smoker	246/144	49.6±3.6	14.5±1.4
Non-smoker	162/628	31.4±3.8	60.2±2.5
Alcohol drinking status, %			
≧12 drinks last year	/659	N/A	73.3±2.3
<12 drinks last year	/310	N/A	26.7±2.3
Annual household income, %			
<25000	186/352	27.3±3.1	25.2±2.4
25000-55000	163/320	33.8±3.6	33.4±2.7
>55000	125/297	38.9±3.9	41.4±2.2
MS, %	38/382	8.6±2.1	36.2±2.0
MS waist, %	124/786	26.4±2.7	81.2±1.8
MS glucose, %	35/212	7.3±2.1	15.7±1.5
MS HDL-C, %	96/313	23.8±3.1	32.6±2.3
MS triglyceride, %	86/364	21.8±2.8	34.6±2.2
MS blood pressure, %	49/470	8.3±1.6	42.0±2.2
DM medication	0/79	0	4.8±0.7
Hypertension medication	0/245	0	19.5±1.5
Hyperlipidemia medication	0/118	0	9.7±1.0
Log-CRP, mg/dL	474/969	-2.94±0.07	-1.54±0.05
Log-Insulin, pmol/L	474/969	4.05±0.04	3.99±0.04
Log-PFHS, ng/mL	474/969	0.95±0.10	0.60±0.04
Log-PFNA, ng/mL	474/969	-0.35±0.07	-0.21±0.07
Log-PFOA, ng/mL	474/969	1.51±0.05	1.48±0.04
Log-PFOS, ng/mL	474/969	3.11±0.05	3.19±0.04

Abbreviations. MS, metabolic syndrome; HDL-C, high density lipoprotein cholesterol; CRP, C-reactive protein; PFHS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfate; N/A, not assessment

Table 2 Linear regression coefficients with one unit increase in log-PFCs in adolescents and adults

	Log-PFHS βcoeff	Log-PFNA βcoeff	Log-PFOA βcoeff	Log-PFOS βcoeff
Adolescent				
Glucose				
Model 1	-0.02±0.03	0.04±0.04	-0.04±0.05	-0.03±0.06
Model 2	-0.02±0.03	0.05±0.05	-0.04±0.05	-0.04±0.06
Model 3	-0.01±0.03	0.07±0.04	-0.03±0.05	-0.03±0.06
Log-insulin				
Model 1	0.02±0.04	-0.09±0.05	0.05±0.08	0.06±0.07
Model 2	0.03±0.04	-0.10±0.05	0.07±0.09	0.07±0.07
Model 3	0.06±0.03	-0.10±0.05*	0.08±0.07	0.15±0.08
Log-HOMA-IR				
Model 1	0.02±0.04	-0.09±0.05	0.04±0.08	0.05±0.07
Model 2	0.02±0.05	-0.09±0.05	0.06±0.09	0.07±0.07
Model 3	0.05±0.03	-0.08±0.04	0.08±0.05	0.15±0.07
Log-β cell function				
Model 1	0.03±0.04	-0.12±0.07	0.06±0.10	0.06±0.08
Model 2	0.03±0.04	-0.12±0.06	0.08±0.10	0.08±0.08
Model 3	0.05±0.03	-0.12±0.06*	0.08±0.08	0.13±0.09
Adult				
Glucose				
Model 1	-0.07±0.09	-0.05±0.04	-0.11±0.10	-0.03±0.08
Model 2	-0.05±0.09	-0.02±0.05	-0.11±0.11	-0.23±0.09
Model 3	-0.02±0.06	0.00±0.04	-0.09±0.08	-0.03±0.07
Log-insulin				
Model 1	-0.04±0.05	-0.06±0.04	0.08±0.04	0.13±0.05*
Model 2	-0.04±0.05	-0.05±0.04	0.08±0.04	0.13±0.05*
Model 3	0.01±0.03	-0.04±0.03	0.07±0.03*	0.14±0.05†
Log-HOMA-IR				
Model 1	-0.05±0.05	-0.06±0.04	0.06±0.05	0.12±0.05*
Model 2	-0.04±0.05	-0.06±0.05	0.07±0.05	0.12±0.05*
Model 3	0.00±0.04	-0.04±0.04	0.06±0.04	0.14±0.05†
Log-β cell function				
Model 1	-0.02±0.04	-0.05±0.03	0.09±0.04*	0.14±0.06*
Model 2	-0.02±0.04	-0.05±0.04	0.09±0.04*	0.14±0.06*
Model 3	0.01±0.03	-0.04±0.03	0.07±0.03*	0.15±0.05†

*, p<0.05; †, p<0.01; ‡, p<0.005; Model 1 adjusted for age, gender, race; Model 2 adjusted for Model 1 + health behaviors (smoking status, alcohol intake and household income); Model 3 adjusted for Model 2 + measurement data (waist circumference, CRP, and insulin/glucose/HOMA) + medications

Table 3 Odds ratios of metabolic syndrome and its components associated with one unit increase in log-PFCs in adolescents and adults

	Log-PFHS OR (95% CI)	Log-PFNA OR (95% CI)	Log-PFOA OR (95% CI)	Log-PFOS OR (95% CI)
Adolescent				
MS				
Model 4	0.56 (0.22-1.45)	0.37 (0.21-0.64) ‡	0.79 (0.30-2.12)	0.49 (0.18-1.30)
MS Waist				
Model 4	0.72 (0.48-1.09)	0.99 (0.59-1.63)	0.61 (0.32-1.13)	0.41 (0.21-0.83)*
Model 5	0.64 (0.45-0.91)*	1.09 (0.61-1.95)	0.58 (0.34-1.00)*	0.37 (0.16-0.82)*
MS Gluocse				
Model 4	1.10 (0.46-2.62)	3.15 (1.39-7.12)*	0.46 (0.25-0.85)*	0.58 (0.31-1.10)
Model 5	0.98 (0.44-2.17)	3.16 (1.39-7.16)*	0.55 (0.24-1.25)	0.58 (0.28-1.14)
MS HDL-C				
Model 4	0.93 (0.58-1.47)	0.59 (0.42-0.83)†	1.20 (0.60-2.39)	0.89 (0.51-1.55)
Model 5	0.93 (0.60-1.43)	0.67 (0.45-0.99)*	1.50 (0.67-3.36)	1.38 (0.61-3.14)
MS Triglyceride				
Model 4	1.07 (0.76-1.52)	0.68 (0.40-1.15)	1.64 (0.72-3.73)	0.95 (0.50-1.80)
Model 5	1.08 (0.83-1.40)	0.71 (0.37-1.34)	1.15 (0.54-2.47)	0.78 (0.41-1.49)
Adult				
MS				
Model 4	0.93 (0.73-1.19)	0.92 (0.69-1.24)	1.07 (0.73-1.57)	1.25 (0.86-1.82)
MS Waist				
Model 4	0.73 (0.53-0.99)*	1.25 (0.88-1.74)	0.95 (0.63-1.45)	0.89 (0.59-1.34)
Model 5	0.80 (0.58-1.10)	1.34 (0.93-1.92)	0.97 (0.65-1.46)	0.91 (0.59-1.41)
MS Gluocse				
Model 4	0.79 (0.53-1.16)	0.81 (0.62-1.07)	0.89 (0.63-1.26)	0.83 (0.64-1.08)
Model 5	0.76 (0.54-1.07)	0.86 (0.66-1.12)	0.87 (0.61-1.26)	0.81 (0.62-1.05)
MS HDL-C				
Model 4	0.90 (0.69-1.18)	0.80 (0.65-0.99)*	1.14 (0.84-1.55)	1.47 (1.07-2.00)*
Model 5	1.00 (0.73-1.37)	0.81 (0.65-1.00)	1.22 (0.86-1.71)	1.61 (1.15-2.26)*
MS Triglyceride				
Model 4	0.80 (0.64-0.99)*	0.98 (0.82-1.16)	0.91 (0.69-1.20)	0.97 (0.73-1.27)
Model 5	0.78 (0.60-1.02)	0.99 (0.81-1.19)	0.86 (0.65-1.13)	0.86 (0.65-1.16)

*, $p<0.05$; †, $p<0.01$; ‡, $p<0.005$; Model 4 adjusted for age, gender, race, health behaviors (smoking status, alcohol intake and household income), measurement data (CRP, HOMA/insulin) and medications; Model 5 adjusted for Model 4 + other components of the metabolic syndrome