Association of Organochlorine Pesticides with Peripheral Neuropathy in Patients with Diabetes or Impaired Fasting Glucose

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OBJECTIVE—Recent epidemiological studies have shown that background exposure to persistent organic pollutants (POPs)—xenobiotics accumulated in adipose tissue—is strongly associated with type 2 diabetes. Hyperglycemia is the cause of long-term complications of diabetes as well as diabetes itself, and POPs are well-known neurotoxicants. This study was performed to explore whether POPs are associated with peripheral neuropathy, a common long-term complication of diabetes, in people with glucose abnormalities.

RESEARCH DESIGN AND METHODS—We studied cross-sectional associations of peripheral neuropathy with 25 POPs, each of which were detectable in at least 60% of study subjects, in 246 subjects aged ≥40 years with diabetes or impaired fasting glucose (IFG) using National Health and Nutrition Examination Survey 1999–2002 datasets.

RESULTS—Among five subclasses of POPs, organochlorine pesticides showed a strong dose-response relation with prevalence of peripheral neuropathy; adjusted ORs were 1.0, 3.6, and 7.3 (P for trend <0.01), respectively, across three categories of serum concentrations of organochlorine pesticides. Furthermore, when we restricted the analyses to 187 participants with A1C <7%, the adjusted ORs were still 1.0, 3.9, and 6.7 (P for trend <0.01). Organochlorine pesticides were also strongly associated with the prevalence of A1C ≥7%; adjusted ORs were 1.0, 2.5, and 5.0 (P for trend <0.01). Specific POPs belonging to organochlorine pesticides showed similar positive associations.

CONCLUSIONS—This study suggests that background exposure to organochlorine pesticides may be associated with higher risk of peripheral neuropathic complications among those with glucose abnormalities, even beyond the influence of diabetes itself. ***Diabetes*** **57:3108–3111, 2008**

Recent epidemiological studies have found that background exposure to persistent organic pollutants (POPs)—xenobiotics accumulated in adipose tissue—is associated with type 2 diabetes, insulin resistance, and metabolic syndrome (1–3), suggesting that POPs may play a key role in their pathogenesis. Among various subtypes of POPs, organochlorine pesticides were most consistently and strongly associated with disturbances of glucose metabolism.

Hyperglycemia itself not only defines type 2 diabetes but also is the cause of its most characteristic symptoms and long-term complications (4). Epidemiological data indicate that the degree and duration of hyperglycemia is associated with the microvascular complications of diabetes (5,6). Peripheral neuropathy is a common long-term complication of diabetes (7).

A1C, a biological correlate of long-term control of blood glucose, is a recognized surrogate measure for the risk of diabetes complications (4). We hypothesized that POPs would be associated with poorer glycemic control and consequent excess risk of peripheral neuropathy among those with glucose abnormalities. Furthermore, exposure to chemicals such as POPs can increase the risk of neuron damage (8–12). Therefore, it is also possible that POPs increase the risk of peripheral neuropathy directly. This study was performed in people with diabetes or impaired fasting glucose (IFG) to explore whether serum concentrations of POPs were associated with A1C and peripheral neuropathy.

RESEARCH DESIGN AND METHODS

The 1999–2002 National Health and Nutrition Examination Survey (NHANES) was designed to be nationally representative of the U.S. noninstitutionalized civilian population. Details of the NHANES protocol are available elsewhere (13,14). Serum concentrations of various biologically important POPs or their metabolites were measured in subsamples of the NHANES 1999–2002 surveys (15).

Venous blood samples were collected and shipped weekly at −20°C. POPs were measured by high-resolution gas chromatography/high-resolution mass spectrometry using isotope dilution for quantification. All these analytes were measured using a modification of the method of Turner et al. (16). The POPs were reported on a lipid-adjusted basis using concentrations of serum total cholesterol and triglycerides. Although 49 POPs were measured in both NHANES 1999–2000 and 2001–2002, to avoid bias in estimation among those below the limit of detection, we selected the 25 POPs for which at least 60% of study subjects had concentrations more than the logarithm of odds, as follows: three polychlorinated dibenzo-*p*-dioxins (PCDDs), four polychlorinated dibenzofurans (PCDFs), five dioxin-like polychlorinated biphenyls (PCBs), seven non–dioxin-like PCBs, and six organochlorine pesticides.

There were 246 patients with diabetes or IFG aged ≥40 years who had information on serum concentrations of POPs. Participants were considered to have diabetes if *1*) their fasting plasma glucose was ≥126 mg/dl or their nonfasting plasma glucose was ≥200 mg/dl or *2*) they were taking insulin or an anti-diabetes oral agent. IFG was defined as fasting plasma glucose ≥100 mg/dl. Exclusion of nonfasting subjects did not greatly change the estimates. However, as their exclusion substantially limited power of statistical tests, we presented the results based on 246 participants with diabetes or glucose impairment. In one analysis, the 642 participants with normal fasting or nonfasting glucose (<100 mg/dl) were included.

Definition of peripheral neuropathy and poor glycemic control. Peripheral neuropathy was operationalized by testing foot sensation using a 5.07-gauge Semmes-Weinstein nylon monofilament. Three plantar metatarsal sites (hallux and first and fifth metatarsal heads) were tested on each foot in

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TABLE 1
Characteristics of participants

	Diabetes	IFG
n	136	110
Age (years)	62.5 ± 12.3	64.9 ± 12.9
Male	48.5	58.2
White race	39.0	53.6
BMI (kg/m^2)	30.8 ± 6.5	29.6 ± 5.3
Smoker	8.1	18.2
Exercise	41.9	48.1
A1C (%)	7.5 ± 1.9	5.8 ± 0.4
A1C ≥7%	41.9	1.8
Duration of diabetes (years)	10.9 ± 14.8	—
Insulin treatment	22.1	—

Data are means ± SD or percent.

random order. Peripheral neuropathy was defined as having one or more insensate sites. Poor glycemic control was defined as A1C ≥7.0%.

Statistical analyses. For analyses of POPs and peripheral neuropathy or poor glycemic control, we calculated cumulative measures of five subclasses of POPs by summing the ranks of specific POPs that belong to each subclass (three PCDDs, four PCDFs, five dioxin-like PCBs, seven non–dioxin-like PCBs, and the six organochlorine pesticides). Detectable values of each POP were individually ranked, and the rank orders of the individual POPs in each subclass were summed to arrive at the subclass value. All undetectable values were ranked as zero. The summary values were categorized in tertiles. For analyses of individual POPs, correlation coefficients between the ranked POP and various outcomes were presented.

Logistic regression models were used to calculate multivariable-adjusted odd ratios (ORs). Potential confounders were age, sex, race/ethnicity, poverty income ratio, duration of diabetes (years), hypertension (yes or no), BMI, cigarette smoking (never, former, or current), cotinine concentrations (in nanogramsg per milliliter), alcohol consumption (in grams per day), and leisure-time physical activity (vigorous, moderate, or none). A1C was added as a possible confounder when we examined the associations with peripheral neuropathy. Further adjustment for education or insulin medication had little effect on findings. We substituted median values of study subjects for missing poverty income ratio, BMI, cotinine concentrations, or alcohol consumption in 25 subjects.

All statistical analyses were performed with SAS 9.1 and SUDAAN 9.0. Estimates of main results were calculated accounting for stratification and clustering (17), adjusting for age, race/ethnicity, and poverty income ratio instead of using sample weights; this adjustment has been regarded as a good compromise between efficiency and bias (17,18). As results were very similar with SAS 9.1 and SUDAAN 9.0, we present the results based on SAS 9.1.

RESULTS

The characteristics of study subjects are summarized in Table 1. In total, there were 246 participants (136 with diabetes and 110 with IFG). Mean ± SD age was 62.5 ± 12.3 years in participants with diabetes and 64.9 ± 12.9 years in participants with IFG. In 41.9% of the patients with diabetes, A1C >7%. Mean duration of diabetes among diabetic subjects was 10.9 years, and 22.1% of participants were treated with insulin.

The prevalences of peripheral neuropathy were 13.6% among participants with IFG and 19.9% among those with diabetes. Among five subclasses of POPs, only organochlorine pesticides were strongly associated with the risk of peripheral neuropathy, with adjusted ORs of 1.0, 3.6, and 7.3, respectively, across tertiles of serum concentrations of organochlorine pesticides (P for trend <0.01) (Table 2). This strong association was obtained even after adjustment for A1C. Furthermore, when we restricted the analyses to 187 participants with A1C <7%, the adjusted ORs were still1.0, 3.9, and 6.7 (P for trend <0.01). The association of organochlorine pesticides with peripheral neuropathy persisted in participants both younger and older than 65 years; adjusted ORs were respectively 1.0, 3.1, and 12.3 among subjects aged <65 years and 1.0, 3.3, and 10.3 among those aged ≥65 years (P for trend <0.01). Among other subclasses of POPs, PCDD and dioxin-like PCBs tended to show positive associations but failed to reach statistical significance. However, when we repeated the same analyses in 642 participants with normal glucose tolerance, in whom peripheral neuropathy prevalence was 13.1%, organochlorine pesticides were not associated with peripheral neuropathy; adjusted ORs were respectively 1.0, 1.3, and 0.7 (P for interaction <0.01).

Organochlorine pesticides were also strongly associated with the risk of A1C ≥7% (Table 2). Across the tertiles of organochlorine pesticides, adjusted ORs of having A1C ≥7% were respectively 1.0, 2.5, and 5.0 (P for trend <0.01). Dioxin-like PCBs also showed statistically nonsignificant trends. In Table 3, we present adjusted correlation coefficients of specific POPs with peripheral neuropathy and A1C. In general, the trends of specific POPs were similar to those of the subclass to which they belong; most of the specific POPs among organochlorine pesticides were positively associated with peripheral neuropathy and A1C. Some POPs belonging to PCBs also showed positive trends.

DISCUSSION

This study raises the possibility that background exposure to some POPs may increase the risk of peripheral neuropathy among patients with diabetes or IFG. In this study, organochlorine pesticides were the only subclass of POPs that was associated with the prevalence of peripheral neuropathy and was also strongly associated with poor glycemic control. In fact, organochlorine pesticides were the class of POPs that was most consistently associated with type 2 diabetes, insulin resistance, and metabolic syndrome in our previous studies (1–3). However, organochlorine pesticides were not associated with peripheral neuropathy among participants with normal glucose tolerance, suggesting that an interaction between organochlorine pesticides and hyperglycemia affects the risk of peripheral neuropathy.

There can be a question whether statistical adjustment for age and BMI is sufficient to fully eliminate their confounding effects. Although organochlorine pesticides are typically stored in adipose tissue, the association of BMI with organochlorine pesticides is not strong (1–3), and statistical adjustment should work well. However, serum concentrations of organochlorine pesticides are strongly associated with age (1–3), and age is also an important risk factor for peripheral neuropathy. To examine whether adjustment for age was sufficient, we considered two sensitivity analyses. First, we stratified by age and found the same associations of organochlorine pesticides with peripheral neuropathy in both younger and older age-groups. Second, if our finding were due to age confounding, there should have been similar associations between organochlorine pesticides and peripheral neuropathy among subjects with normal glucose tolerance. As organochlorine pesticides were associated with peripheral neuropathy only among subjects with abnormal glucose tolerance, any residual confounding effect due to age may not explain the current findings.

Until now, the strongest risk factors for diabetes complications have been poor glycemic control and diabetes duration (4,5). In addition, other risk factors such as age,

TABLE 2
Adjusted ORs and 95% CIs of prevalence of peripheral neuropathy or A1C ≥7% by tertiles of POPs

	Tertile 1	Tertile 2	Tertile 3	P_{trend}
Peripheral neuropathy				
PCDDs				
n cases/subjects	6/82	16/82	20/82	
OR (95% CI)	Referent	2.5 (0.9–7.2)	2.4 (0.8–6.9)	0.15
PCDFs				
n cases/subjects	11/81	12/83	19/82	
OR (95% CI)	Referent	0.7 (0.3–1.9)	1.0 (0.4–2.5)	0.96
Dioxin-like PCBs				
n cases/subjects	6/82	12/82	24/82	
OR (95% CI)	Referent	1.5 (0.5–4.4)	2.2 (0.7–6.8)	0.15
Non–dioxin-like PCBs				
n cases/subjects	7/82	11/82	24/82	
OR (95% CI)	Referent	1.0 (0.3–3.0)	1.7 (0.6–5.1)	0.22
Organochlorine pesticides				
n cases/subjects	4/82	14/82	24/82	
OR (95% CI)	Referent	3.6 (1.1–12.2)	7.3 (2.1–25.3)	<0.01
A1C ≥7%				
PCDDs				
n cases/subjects	21/82	16/82	22/82	
OR (95% CI)	Referent	1.0 (0.5–2.4)	1.3 (0.5–3.0)	0.58
PCDFs				
n cases/subjects	20/81	17/83	22/82	
OR (95% CI)	Referent	0.9 (0.4–2.1)	1.4 (0.6–3.2)	0.42
Dioxin-like PCBs				
n cases/subjects	19/82	21/82	19/82	
OR (95% CI)	Referent	2.0 (0.9–4.7)	2.1 (0.8–5.4)	0.13
Non–dioxin-like PCBs				
n cases/subjects	20/82	20/82	19/82	
OR (95% CI)	Referent	1.4 (0.6–3.3)	1.4 (0.6–3.7)	0.46
Organochlorine pesticides				
n cases/subjects	12/82	19/82	28/82	
OR (95% CI)	Referent	2.5 (1.0–6.5)	5.0 (1.8–13.4)	<0.01

ORs adjusted for age, sex, race, poverty income ratio, duration of diabetes, hypertension, BMI, cigarette smoking, serum cotinine, exercise, alcohol consumption, and A1C (A1C not included as a covariate in the analyses with the outcome of A1C ≥7%). Detectable values of each POP were individually ranked and the rank orders of the individual POPs in each subclass were summed to arrive at the subclass value. All undetectable values were ranked as 0. The summary values were categorized by tertiles of the sum of ranks.

race, hypertension, hyperlipidemia, and smoking also contribute in developing diabetes complications (4,5). In this study, these conventional risk factors appeared to be associated with the prevalence of peripheral neuropathy, although most of them failed to reach statistical significance, consistent with small sample sizes. Compared with the degree of association between peripheral neuropathy and conventional risk factors, serum concentrations of organochlorine pesticides were very strongly associated with peripheral neuropathy. Tight glycemic control in diabetes through diet and medication is one of the cornerstones of management of diabetes. However, if environmental exposure to organochlorine pesticides is involved in disturbance of glucose metabolism, the current therapeutic approach may be insufficient for glucose control. Because people are exposed to organochlorine pesticides through food consumption, it could be helpful to identify foods containing organochlorine pesticides so that diabetic patients can avoid them.

At least two mechanisms exist that could lead to a causal link between organochlorine pesticides and peripheral neuropathy. First, poor glycemic control caused by organochlorine pesticides may increase the risk of diabetes complications. The positive association between organochlorine pesticides and A1C supports this possibility. In addition, organochlorine pesticides themselves could directly increase the risk of diabetes complications. It is well known that various pesticides have a direct toxic effect on nervous tissue (11). However, it is still unclear whether, with chronic environmental exposure, low concentrations of a mixture of pesticides can also act as neurotoxicants (11). Diabetic patients and those with IFG may be more susceptible than those with normal glucose tolerance to chronic exposure to low concentrations of organochlorine pesticides because of an already higher risk of peripheral neuropathy due to hyperglycemia. Importantly, these two mechanisms, hyperglycemia and organochlorine pesticides, may act synergistically to increase the risk of peripheral neuropathy among diabetic patients.

This study has several limitations. First, the cross-sectional study design in NHANES does not allow inferences regarding the causal relationship between POPs and diabetes complications. However, as neurotoxicity of some POPs is a well-known phenomenon, our results are biologically highly plausible. Second, the sample size was small. Thus, despite consistency of findings, we could not rule out a possibility of chance in some associations. Third, there would be misclassification in defining diabetes and impaired glucose tolerance, and loss of vibration sensation on a single occasion does not fully characterize neuropathy clinically.

TABLE 3
Adjusted correlation coefficients of 25 POPs with peripheral neuropathy or A1C ≥7% (N = 246)

	Peripheral neuropathy	A1C ≥7%
PCDDs		
D03	0.12	0.02
D05	0.04	0.08
D07	0.05	−0.02
PCDFs		
F03	−0.05	0.01
F04	0.03	0.10
F05	0.12	0.06
F08	0.04	0.05
Dioxin-like PCBs		
PCB074	0.01	0.09
PCB118	0.06	0.13*
PCB126	0.14*	0.15*
PCB156	0.05	0.08
PCB169	−0.02	0.06
Non–dioxin-like PCBs		
PCB099	0.07	0.06
PCB138	0.05	0.11
PCB146	0.03	0.11
PCB153	0.05	0.09
PCB170	0.01	0.08
PCB180	−0.02	0.09
PCB187	0.01	0.11
OC pesticides		
OXY	0.13	0.14*
TNA	0.17†	0.12
PDE	0.14*	0.10
PDT	0.11	0.14*
BHC	0.14*	0.23†
HPE	0.20†	0.22†

Correlation coefficients adjusted for age, sex, race, poverty income ratio, duration of diabetes, hypertension, BMI, cigarette smoking, serum cotinine, exercise, alcohol consumption, and A1C (A1C not included as a covariate in the analyses with the outcome of A1C≥7%). NHANES abbreviations: BHC: Beta-hexachlorocyclohexane; D03: 1,2,3,6,7,8,-Hexachlorodibenzo-p-dioxin; D05: 1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin; D07: 1,2,3,4,6,7,8,9-octachlorodibenzo-p-dioxin; F03: 2,3,4,7,8-Pentachlorodibenzofuran; F04: 1,2,3,4,7,8-Hexachlorodibenzofuran; F05: 1,2,3,6,7,8-Hexachlorodibenzofuran; F08: 1,2,3,4,6,7,8-Heptachlorodibenzofuran; HPE: Heptachlor epoxide; OXY: Oxychlordane; PCB074: 2,4,4',5-Tetrachlorobiphenyl; PCB118: 2,3',4,4',5-Pentachlorobiphenyl; PCB126: 3,3',4,4',5-Pentachlorobiphenyl; PCB156: 2,3,3',4,4',5-Hexachlorobiphenyl; PCB169: 3,3',4,4',5,5'-Hexachlorobiphenyl; PCB099: 2,2',4,4',5-Pentachlorobiphenyl; PCB138: 2,2',3,4,4',5-Hexachlorobiphenyl; PCB146: 2,2',3,4,5',5-Hexachlorobiphenyl; PCB153: 2,2',4,4',5,5'-Hexachlorobiphenyl; PCB170: 2,2',3,3',4,4',5-Heptachlorobiphenyl; PCB180: 2,2',3,4,4',5,5'-Heptachlorobiphenyl; PCB187: 2,2',3,4',5,5',6-Heptachlorobiphenyl; PDE: p,p'-Dichlorodiphenyldichloroethylene; PDT: p,p'-Dichlorodiphenyltrichloroethane; TNA: Trans-Nonachlor. *$P < 0.05$, †$P < 0.01$.

In summary, there were positive associations between serum concentrations of organochlorine pesticides and prevalence of peripheral neuropathy and poor glycemic control among patients with diabetes or IFG. If these findings are confirmed in prospective studies, new therapeutic approaches such as avoiding POPs or an increased excretion of POPs from the human body can be developed for the management of type 2 diabetes.

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