POLYCHLORINATED BIPHENYLS, INFLAMMATORY BIOMARKERS, AND DIABETES STATUS IN THE ANNISTON COMMUNITY HEALTH SURVEY

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Introduction

Anniston, Alabama, is the site of a former Monsanto Chemical Company production facility that manufactured polychlorinated biphenyls (PCBs). Between 1932 and 1971, the facility produced all commercial and experimental Aroclor® mixtures in the U.S., each containing different individual PCB congeners, accounting for more than half of the total PCB production in the country. High concentrations of PCBs have been reported historically in people[1] and environmental media[2]. The present cross-sectional study was conducted by the Anniston Environmental Health Research Consortium in 2005-2007 in response to community concerns about health effects of PCB exposure. We have reported previously on PCB exposure in Anniston residents, as well as on associations between PCBs and diabetes, blood pressure, and serum lipid concentrations[3-6].

Associations between exposure to PCBs, along with other persistent organic pollutants, and diabetes have been reported widely[7-8]. Meanwhile, associations between diabetes and indicators of inflammation have been observed, including associations of tumor necrosis factor (TNF)-α and interleukin (IL)-6 levels with diabetes and insulin resistance[9-10]. IL-6 level also has been observed in an association with diabetes, apparently independently of insulin resistance and obesity[11]. Toxicological studies have explored mechanisms by which exposure to PCBs may lead to production of inflammatory cytokines[12], insulin resistance, and insulin signaling disruption[13-14]. In mice exposed to doses approximating the maximum serum PCB levels reported for the Anniston cohort, Aroclor 1260 exposure increased serum cytokines as well as hepatic expression of IL-6 and TNFα[15]. While evidence exists for a role for IL-1β in disruption of insulin signaling[16] and severity of type 2 diabetes[17-18], the causal relationship between diabetes and inflammation still is not evident[19]. To further investigate possible pathways from PCB exposure to inflammation and diabetes, we examined the relationships between PCBs and inflammatory biomarkers by diabetes status among Anniston residents.

Materials and methods

Study Design and Population

Anniston residents aged 18 years or older were eligible and were selected through a stratified random sample. A pool of 3,320 eligible addresses was randomly selected from a commercial list of all residential properties in Anniston, with oversampling (two-thirds of all eligible) in west Anniston. One adult resident was randomly selected from each of the 1,823 successfully contacted households. Out of 1,110 respondents, 774 visited the study office and provided a fasting blood sample for measurements of glucose, PCBs and lipid levels, and had their height, weight, waist circumference and blood pressure measured using a standardized protocol. Demographic information, medical and family history, as well as self-reported health behaviors, health conditions, and individual medications were recorded. The study was reviewed and approved by the appropriate Institutional Review Boards.

Laboratory and Statistical Analyses

The 35 major ortho-substituted PCB congeners were measured by the Division of Laboratory Sciences at the Centers for Disease Control and Prevention's National Center for Environmental Health using high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry[20]. Study specimens were analyzed in batches of 24 intermixed with quality control (QC, n=3) and method blank (n=3) samples. Values below the detection limit were substituted with the congener-specific limit of detection divided by the square root of 2. Serum total lipids were calculated using the enzymatic "summation" method using triglyceride and total cholesterol measurements[21]. Serum glucose was measured as well[6]. Inflammatory biomarkers IL-1β, IL-6, IL-8, TNFα, and plasminogen activator inhibitor (PAI)-1 were measured by multi-analyte chemiluminescent detection (Luminex) and commercially available ELISA kits[22-24]. The biomarker results were available for 741 participants, which were included in the statistical analyses.

Diabetes was defined as self-report of physician-diagnosed diabetes or fasting glucose > 125 mg/dL; prediabetes was defined as fasting glucose 100–125 mg/dL, absence of previously diagnosed diabetes, and absence of glycemic control medications; normoglycemia was defined as individuals with a negative diabetes self-report, fasting glucose < 100 mg/dL, and the absence of glycemic control medications[6]. We compared least square geometric means (LSGM) for serum concentrations of the sum of 35 PCB congeners (ΣPCBs, whole-weight), IL-1β, IL-6, IL-8, TNFα, and PAI-1 by diabetes status. We also used linear regression models to examine relationships between ΣPCBs and measured biomarkers (IL-1β, IL-6, IL-8, TNFα, PAI-1), stratified by diabetes status. ΣPCBs were modeled as the logarithm to base 10 ($\log_{10}$) of the sum of 35 PCB congeners (ng/g whole weight), adjusting for log-transformed total lipids as well as for age (centered at mean of 55 years), race (African-American or White), sex (female or male), BMI (centered at mean of 31.2 kg/m^2), and current smoking (yes or no). The measured biomarkers were also log-transformed. Effect estimates are displayed in terms of multiplicative factors for the biomarkers (i.e., 10^{β} where β is the slope from the regression model for $\log_{10}$ of each biomarker).

Results and discussion

Forty-seven percent of participants self-identified as African-American (and the rest as White), and 70% were female. The mean age was 54.7 years, ranging from 18 to 93 years. Approximately 31% of participants had less than a high school education and 40% were high school graduates without a college degree. Most (51%) had an elevated BMI (≥30 kg/m^2). Based on aforementioned criteria, 50.7% of participants were classified with normoglycemia, 21.5% with prediabetes, and 27.8% with diabetes. A Chi-square test of proportions showed higher prevalence of prediabetes in men (30.2%) than in women (17.6%, p=0.0006), but there was no statistically significant difference in diabetes prevalence between men (23.6%) and women (29.7%). A higher percentage of African-Americans than Whites had diabetes (31.8% vs. 24.3%), but the difference was not statistically significant.

As shown in Table 1, LSGM of ΣPCBs among diabetic participants was 67% higher than among normoglycemic participants (p=0.0005) and 40% higher than the prediabetic group (p=0.041). LSGM of PAI-1 was higher in the diabetic (p=0.0014) and prediabetic (p=0.0001) groups than in the normoglycemic group. LSGMs for the other measured biomarkers, however, did not vary consistently by diabetes status (Table 1). For IL-1β, IL-6 and TNFα, LSGM in the normoglycemic group was generally the lowest among the three groups, although the differences were not statistically different. LSGM IL-8 was the highest in the normoglycemic group, followed by the prediabetic and diabetic groups, although none of these differences were significant.

Table 1: Least square geometric means (LSGM) with 95% confidence intervals for inflammatory cytokines and serum concentrations of the sum of 35 PCB congeners (whole-weight), by diabetes status.

	Diabetes (n=206)	Prediabetes (n=159)	Normoglycemic (n=376)
IL-1β, pg/mL	9.707 (1.478-17.94)	8.768 (-0.576-18.11)	5.271 (-0.813-11.36)
IL-6, pg/mL	11.50 (2.425-20.57)	20.67 (10.31-31.03)	10.34 (3.612-17.08)
IL-8, pg/mL	77.66 (44.56-110.8)	87.34 (49.67-125.0)	98.83 (74.30-123.4)
TNFα, pg/mL	8.719 (6.340-11.10)	9.191 (6.483-11.90)	6.724 (4.962-8.485)
PAI-1, ng/mL	55.16 (52.30-58.01)**	56.87 (53.62-60.12)**	49.33 (47.22-51.45)
ΣPCBs, ng/g whole weight	9.064 (7.423-10.71)**	6.473 (4.605-8.341)*	5.442 (4.227-6.657)

** p<0.05. LSGM ΣPCBs was higher in the diabetic group than in the prediabetic group (p=0.041).*
***p<0.01. LSGM ΣPCBs was higher in the diabetic group than in the normoglycemic group (p=0.0005). LSGM PAI-1 was higher in the diabetic (p=0.0014) and prediabetic (p=0.0001) groups than in the normoglycemic group.*

Table 2 gives the linear regression model results for associations between serum concentrations of ΣPCBs and the measured inflammatory biomarkers. There appeared to be an inverse association between ΣPCBs and IL-1β in the prediabetic participants and a positive association in the normoglycemic group, both approaching statistical significance (p=0.06). All other regression results were not statistically significant. However, the results suggest potential positive associations between serum ΣPCBs and the measured biomarkers among diabetic and normoglycemic participants, while potential inverse associations are suggested in the prediabetic group.

Table 2: Linear regression results (10^{β} (p-value)) for inflammatory cytokines and ΣPCBs, by diabetes status.

	Diabetes (n=206)	Prediabetes (n=159)	Normoglycemic (n=376)
Log_{10}(IL-1β, pg/mL)	1.183 (0.62)	0.517 (0.06)	1.562 (0.06)
Log_{10}(IL-6, pg/mL)	1.314 (0.17)	0.839 (0.52)	1.261 (0.22)
Log_{10}(IL-8, pg/mL)	1.093 (0.76)	0.901 (0.75)	1.228 (0.39)
Log_{10}(TNFα, pg/mL)	1.222 (0.25)	0.929 (0.64)	1.121 (0.27)
Log_{10}(PAI-1, ng/mL)	0.888 (0.22)	0.903 (0.19)	1.054 (0.40)

** Models were adjusted for log_{10}(total lipid concentration, mg/dL), age (centered at mean of 55 years), race (African-American or White), sex (female or male), BMI (centered at mean of 31.2 kg/m^2), and current smoking (yes or no).*

In conclusion, we found significantly higher levels of serum PCBs in diabetic participants than in prediabetic and normoglycemic participants. PAI-1, a main inhibitor of the plasminogen activators, was higher in diabetic and prediabetic participants than in normoglycemic participants. Our findings are also in agreement with the evidence for involvement of IL-1β and the related inflammosome NLRP3 in type 2 diabetes development[18], suggesting PCB exposure may induce inflammatory responses that could contribute to development of a prediabetic status in those who are normoglycemic and progression of the disease in those with diabetes. Results of these exploratory models must be interpreted with caution; the exact mechanism of action is not known and the variation in associations across diabetes status could also be an indication that changes in metabolism may alter the immune response to pollutants such as PCBs. Further analyses are warranted to examine these possible patterns in more detail.

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