POLYCHLORINATED BIPHENYLS, DIOXINS, AND DIABETES IN THE ANNISTON COHORT

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Introduction

Diabetes prevalence is increasing worldwide and currently affects more than 30 million Americans. During the last decade, numerous epidemiologic studies have suggested that exposure to polychlorinated biphenyls (PCBs), along with other endocrine modulating persistent organic pollutants (POPs), plays a role in disturbances of glucose metabolism and development of diabetes (Lind et al., 2018). Previous toxicological studies have explored insulin signaling disruption, glucose homeostasis, pancreas cell function, as well as the disruption of adipogenesis as potential mechanisms by which exposure to POPs may lead to the development of diabetes (Lee et al., 2014; Lind et al., 2018; Turyk et al., 2015; Wang et al., 2010; Zhang et al., 2015; Gadupudi et al., 2015). There has also been growing evidence for the role of inflammatory cytokines and adipokines in insulin resistance and the risk of type 2 diabetes (Liu et al., 2016).

In the Anniston Community Health Survey (ACHS I, 2005-2007), we found the residents' polychlorinated biphenyl (PCB) concentrations were about 3 times higher than in the general U.S. population due to the operation of a PCB production facility in Anniston from 1929-1971 (Pavuk et al., 2014). Non-ortho PCB TEQs were also specifically elevated in this cohort (Yang et al., 2018). Results from the ACHS I showed significant associations between non-dioxin-like PCBs and diabetes (Silverstone et al., 2012) and for the sum of ortho-substituted PCBs. In 2014, we conducted a follow-up study (ACHS II) which included measurements of PCBs and pesticides and added an additional component, measurements of dioxins (Birnbaum et al., 2016; Yang et al., 2018).

Results of statistical analyses with total dioxin toxic equivalency (TEQ) (Van den Berg et al., 2006), non-ortho PCB TEQ, p,p'-DDE and diabetic status are presented here. We also analyzed associations between POPs and cytokines among diabetic individuals using multivariate linear regression models.

Materials and methods

We described the methods for ACHS I and ACHS II in detail elsewhere (Birnbaum et al., 2016). Participants gave informed consent prior to initiation of data collection, and the CDC IRB approved the study protocol. ACHS II data collection occurred in 2014 with analytical testing completed mostly in 2015-2016. Participants provided a fasting blood sample for measurements of glucose, POPs, and lipid levels. We retested all health markers and chemicals measured in ACHS I in addition to PCDDs, PCDFs, and non-ortho PCBs, which had not been measured previously (Yang et al., 2018). CDC's National Center for Environmental Health, Division of Laboratory Sciences performed chemical analyses. They measured seven PCDD congeners, 10 PCDF congeners, and 3 non-ortho PCB congeners (81, 126, 169) using high-resolution gas chromatography/high-resolution mass spectrometry (pg/g lipid) (Turner et al., 1997) and eight mono-ortho PCB congeners (dioxin-like) and 27 non-dioxin-like PCBs (di-, tri-, tetra-ortho) by gas chromatography/isotope-dilution high-resolution mass spectrometry (ng/g lipid) (Sjödin et al., 2004). Cytokines included in this analysis were tumor necrosis factor alpha (TNFα), adiponectin, leptin, insulin, transforming growth factor (TGF-β1), and monocyte chemoattractant protein 1 (MCP-1). The lab measured cytokines using two separate multiplex bead arrays (HADK2MAG-61K and HADK1MAG-61K; EMD Millipore, Billerica, MA).

The 338 participants included in the current study met inclusion criteria: surviving ACHS I participants who still lived within the study area, were successfully contacted, and had valid dioxin measurements. During the office visit, the study nurse verified glycemic control medication. We defined diabetic status as self-report of physician-diagnosed diabetes, fasting glucose ≥125 mg/dL, or being on glycemic control medication. We used the enzymatic "summation" method to calculate serum total lipids using triglyceride and total cholesterol measurements (Bernet et al., 2007) and the 2005 WHO Toxic Equivalency Factors (TEF) to calculate the congeners' toxic equivalency (TEQ) and total dioxin TEQ (Van den Berg et al., 2006). For the sum of TEQs, we only used congener concentrations above the limit of detection.

We used unconditional logistic regression models to contrast diabetes status (diabetic, non-diabetic) with the exposure variables: total dioxin TEQ, non-ortho PCB TEQ, and p,p'-DDE. We analyzed quintiles of exposure and whole weight exposure variables adjusted for log-transformed total lipids, age, race (African-American or White), sex (female or male), BMI (continuous), current smoking status (ever or never), and family history of diabetes (yes or no) in logistic models. Odds ratios (OR) and 95% confidence intervals (CI) are presented. We studied associations between measured cytokines and exposure variables among diabetic individuals using multivariate linear regression models (data not shown for non-diabetics).

Results and Discussion

Participants with diabetes were older by almost 4 years and had significantly higher BMI than non-diabetics (32.8 vs 30.9, p=0.036), although both groups were obese (Table 1). African Americans constituted a significantly higher proportion of diabetics than non-diabetics (61% vs 44%). Females represented the majority of participants (72%); however, there was no major difference in the proportion of females among diabetics and non-diabetics. Smoking status and total lipids had no substantial difference across diabetic status.

Table 1. Demographics* and exposure variables of ACHS II participants (2014).

Demographics/Exposure	Diabetics (n=135)	Non-diabetics (n=203)	p-value
Age (years)	65.06 ± 1.02	61.11 ± 0.95	0.003
BMI (kg/m^2)	32.78 ± 0.78	30.89 ± 0.52	0.036
African Americans	82 (60.74%)	90 (44.33%)	0.004
Females	101 (74.81%)	144 (70.94%)	0.074
Total lipid (mg/dL)	618.81 ± 14.71	625.61 ± 10.10	0.69
Smoking Status (ever/never)	24 (17.78%)	47 (23.15%)	0.28
Family History of Diabetes	105 (77.78%)	152 (74.88%)	0.23
Non-ortho PCB TEQ	11.52 ± 1.58	6.74 ± 0.80	0.00034
Total dioxin TEQ	32.49 ± 2.56	22.46 ± 1.43	0.0003
pp'-DDE	4548.97 ± 484.27	2551.57 ± 239.96	<0.0001

*Demographic results are presented as Mean ± Standard Error for continuous variables and Number Count (%) for categorical variables. We adjusted TEQ exposure variables for total lipids. We calculated p-values for continuous variables from a t-test and from a chi-square test for categorical variables.

We observed elevated odds ratios for diabetes in relation to chemical exposure groups in the model adjusted for standard risk factors of diabetes. The pesticide p,p'-DDE had the strongest association (OR 2.37 [95% CI 1.27, 4.41], followed by dioxin TEQ [OR 2.06 (95% CI 0.82, 5.16)]; non-ortho PCB TEQ exhibited a weak association [OR 1.22 (95% CI 0.69, 2.16)] (data not shown). In Table 2, we show odds ratios for diabetes across quintiles of exposure. Odds ratios for p,p'-DDE ranged from 1.75 to 4.12 (in the fifth quintile) while total dioxin TEQ were consistently elevated with odds ratios greater than 2 noted from the second to fifth quintiles.

Linear regression analyses of cytokines among diabetic participants (n=135) are shown in Table 3, and include TNFα, leptin, insulin, and TGF-β1. Insulin showed consistent inverse associations across all exposure groups, with the strongest noted for total dioxin TEQ. TGF-β1 showed positive association with non-ortho PCB TEQ while TNF-α demonstrated a positive association with total dioxin TEQ. We did not observe strong associations with adiponectin; leptin concentrations showed only weak negative associations with total dioxin TEQ. When we applied the same models to non-diabetics, there were no observed statistically significant associations (data not shown).

In these analyses, we focused on total dioxin TEQ, non-ortho PCBs TEQ, and the pesticide p,p'-DDE, all of which have shown associations with diabetes previously (Lee 2014; Lind et al., 2018, Taylor et al., 2013; Turyk et al., 2015; Zong et al., 2018). We found positive associations between total dioxin TEQ and prevalent diabetes in this aging Anniston cohort. The odds ratios of total dioxin TEQs in quintile analyses were elevated across all quintiles (ORs 2.17; 2.90, 2.94; 2.63, respectively, p-for trend = 0.09). Furthermore, we observed an elevated odds ratio for p,p'-DDE (OR=2.22; 95% CI 1.20-4.13). Non-ortho PCBs did not show a strong association with prevalent diabetes in this follow up study (OR=1.22). The associations we observed provide additional support to earlier epidemiological findings for diabetes associations with dioxin-like compounds and p,p'-DDE.

Table 2. Odds ratios and (95% CI) for diabetes prevalence in ACHS II participants by chemical group quintiles.

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	p-value*
Non-ortho PCB TEQ	14/67 1.00 (Ref.)	25/68 1.99 (0.88, 4.51)	26/67 1.80 (0.77, 4.21)	**37/68** **3.12 (1.27, 7.65)**	33/68 1.49 (0.57, 3.92)	0.32
Total Dioxin TEQ	13/67 1.00 (Ref.)	25/69 2.17 (0.93, 5.06)	**30/67** **2.90 (1.19, 7.06)**	**30/67** **2.94 (1.15, 7.50)**	37/68 2.63 (0.94, 7.32)	0.099
p,p'-DDE	14/67 1.00 (Ref.)	25/67 2.05 (0.89, 4.72)	25/68 1.75 (0.73, 4.19)	29/67 2.16 (0.86, 5.41)	**42/69** **4.12 (1.53, 11.14)**	0.01

*Adjusted for age, race, sex, BMI, family history of diabetes, and smoking status. All TEQs and chemicals in pg/g

Table 3. Linear Regression Parameter Estimates of Cytokines vs. Chemical Groups (Whole Weight TEQs) among diabetic individuals (n=135).

	Non-ortho PCB TEQ		Total Dioxin TEQ		p,p'-DDE	
	β±SE	P	β±SE	P	β±SE	P
TNFα	0.1135 ± 0.0612	0.066	**0.253 ± 0.09798**	**0.011**	**0.2225 ± 0.0717**	**0.002**
IL-1β	0.0541 ± 0.1055	0.61	0.1902 ± 0.1684	0.26	0.131 ± 0.122	0.29
Leptin	0.0282 ± 0.0806	0.73	-0.0916 ± 0.1261	0.47	0.0274 ± 0.0933	0.77
TGF-β1	**0.0821 ± 0.0413**	**0.049**	0.0896 ± 0.0686	0.195	0.0459 ± 0.0507	0.37
Insulin	-0.0881 ± 0.06999	0.21	**-0.0213 ± 0.1103**	**0.049**	-0.1461 ± 0.0826	0.076
HOMA-B	-0.0439 ± 0.09299	0.64	-0.1707 ± 0.1487	0.25	**-0.226 ± 0.105**	**0.034**
Adiponectin	-0.04797 ± 0.0649	0.46	0.0025 ± 0.1031	0.98	-0.0000984 ± 0.0751	0.999
MCP-1	0.041 ± 0.048	0.400	0.038 ± 0.076	0.62	0.057 ± 0.056	0.31

**Models account for age, sex, race, BMI, family history of diabetes, and smoking status.

Several mechanisms have biological plausibility for the suggested impact that PCBs, dioxins and other organochlorines have on diabetes. These include changes in the insulin resistance/signaling pathways or insulin production (Lin et al. 2016; Lee 2014). The observed inverse associations with insulin in this analysis and the analyses of Anniston subjects with suspected toxicant associated steatohepatitis support these changes (Clair et al., 2018). Higher TGF-β1 in diabetic subjects observed here may be a factor in the progression and development of diabetic nephropathy and renal damage as reported elsewhere (Wang et al. 2014; Liang et al., 2018; Sun et al., 2018). In addition, TNF-α, which showed consistent positive associations with dioxins and PCBs in this study, has also been implicated in diabetic nephropathy/fibrosis (Lin et al., 2006; Liu et al., 2007) and diabetes risk (Liu et al. 2016). Future investigation into additional markers of systemic and tissue inflammation and potential diabetic complications are warranted and could provide further insight into these findings from the Anniston cohort.

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