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Longitudinal Association of Biomarkers of Pesticide Exposure with Cardiovascular Disease Risk Factors in Youth with Diabetes

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abstract

Background: Cardiovascular disease (CVD) is the leading cause of death among individuals with diabetes, but little is known about the role of exposures to environmental chemicals such as pesticides in the early development of CVD risk in this population.

Objectives: To describe changes over time in concentrations of pesticide biomarkers among youth with diabetes in the United States and to estimate the longitudinal association between these concentrations and established risk factors for CVD.

Methods: Pesticide biomarkers were quantified in urine and serum samples from 87 youth with diabetes participating in the multi-center SEARCH cohort study. Samples were obtained around the time of diagnosis (baseline visit, between 2006 and 2010) and, on average, 5.4 years later (follow-up visit, between 2012 and 2015). We calculated geometric mean (95% CI) pesticide biomarker concentrations. Eight CVD risk factors were measured at these two time points: body mass index (BMI) z-score, HbA1c, insulin sensitivity, fasting C-peptide (FCP), LDL cholesterol, HDL cholesterol, total cholesterol, and triglycerides. Linear regression models were used to estimate the associations between each pesticide biomarker at baseline and each CVD risk factor at follow-up, adjusting for baseline health outcome, elapsed time between baseline and follow up, sex, age, race/ethnicity, and diabetes type.

Results: Participants were, on average, 14.2 years old at their baseline visit, and most were diagnosed with type 1 diabetes (57.5%). 4-nitrophenol, 3-phenoxybenzoic acid, 2,4-dichlorophenoxyacetic acid (2,4-D), 3,5,6-trichloro-2-pyridinol, 2,2-bis(4-chlorophenyl)-1,1-dichloroethene, and hexachlorobenzene were detected in a majority of participants at both time points. Participants in the highest quartile of 2,4-D and 4-nitrophenol at baseline had HbA1c levels at follow-up that were 1.05 percentage points (95% CI: −0.40, 2.51) and 1.27 percentage points (95% CI: −0.22, 2.75) higher, respectively, than participants in the lowest quartile of these pesticide biomarkers at baseline. These participants also had lower log FCP levels (indicating reduced beta-cell function) compared to participants in the lowest quartile at baseline: beta (95% CI) for log FCP of −0.64 (−1.17, −0.11) for 2,4-D and −0.39 (−0.96, 0.18) for 4-nitrophenol. In other words, participants in the highest quartile of 2,4-D had a 47.3% lower FCP level compared to participants in the lowest quartile, and those in the highest quartile of 4-nitrophenol had a 32.3% lower FCP level than those in the lowest quartile. Participants with trans-nonachlor concentrations in the highest quartile at baseline had HbA1c levels that were 1.45 percentage points (95% CI: −0.11, 3.01) higher and log FCP levels that were −0.28 (−0.84, 0.28) lower than participants in the lowest quartile at baseline, that is to say, participants in the highest quartile of trans-nonachlor had a 24.4% lower FCP level than those in the lowest quartile. While not all of these results were statistically significant, potentially due to the small same size, clinically, there appears to be quantitative differences. No associations were observed between any pesticide biomarker at baseline with BMI z-score or insulin sensitivity at follow-up.

Conclusions: Exposure to select pesticides may be associated with impaired beta-cell function and poorer glycemic control among youth with diabetes.

Keywords

HbA1c; C-peptide; Pesticides; Cohort Study; Vulnerable Populations

1. Introduction

Pesticide exposures, particularly organochlorine insecticides (Evangelou et al. 2016; Taylor et al. 2013; Thayer et al. 2012) but also organophosphate insecticides and chlorophenoxy herbicides (Schreinemachers 2010), have been linked to metabolic disruptions including diabetes, obesity, and abnormal lipids. Animal studies have shown that low-dose exposure to organophosphate insecticides induces gluconeogenesis and glycogenolysis in the liver (Abdollahi et al. 2004), resulting in an increase in blood glucose. Organophosphate insecticide exposure may also prevent insulin-induced suppression of free fatty acid release from adipose cells (Bomser et al. 2002); increase plasma triglycerides and LDL cholesterol (Ibrahim and EI-Gamal 2003); decrease HDL cholesterol (Ibrahim and EI-Gamal 2003); and cause oxidative stress-induced apoptosis of pancreatic beta-cells (Kamath and Rajini 2007).

Individuals with diabetes have an increased risk of cardiovascular disease (CVD); thus, the American Diabetes Association recommends regular screening of the “ABCs of diabetes”: glycated hemoglobin A1c (HbA1c), blood pressure, and cholesterol (American Diabetes Association 2018). While many studies have evaluated the role of nutritional exposures in the development of CVD risk among individuals with diabetes, few studies have evaluated the role of exposures to environmental chemicals such as pesticides. Biomarker quantification in urine or serum is considered the gold standard for assessing exposure to pesticides in epidemiological studies (Barr 2008).

The SEARCH for Diabetes in Youth Cohort Study is a multi-center, longitudinal study investigating the progression of type 1 and type 2 diabetes and associated complications among adolescents and young adults (Dabelea et al. 2017). SEARCH is, to our knowledge, the largest population-based registry of pediatric diabetes in the United States. The current study presents pesticide biomarkers in repeat serum and urine samples collected from a subset of this cohort. The aims were to describe concentrations of pesticide biomarkers among youth with diabetes in the United States and to estimate the longitudinal association between these concentrations and established CVD risk factors.

2. Materials and Methods

2.1 Study Population

SEARCH Cohort Study participants were from a population-based incidence registry network drawn from five U.S. states (California, Colorado, Ohio, South Carolina, and Washington) by the SEARCH for Diabetes in Youth Registry Study (Hamman et al. 2014). Patients receiving a diagnosis of type 1 or type 2 diabetes at younger than 20 years of age in 2002-2006 or 2008 were contacted to complete a baseline visit. Those who completed a baseline visit were asked to return for follow-up visits at 12, 24, and 60 months (2003-2010). Participants who had completed a follow-up visit and who were 10 years or older and had a

diabetes duration of at least 5 years were then recruited for an additional outcome visit between 2011 and 2015 (Dabelea et al. 2017). For the current ancillary study, we used the following inclusion criteria: diagnosed with type 1 or type 2 diabetes in 2006 or 2008, aged 10 years or older at diagnosis, attended at least one follow-up visit between 2011 and 2015, did not have missing outcome data at baseline or follow-up (i.e. fasting C-peptide [FCP] concentration, insulin sensitivity score, HbA1c, body mass index [BMI], and fasting blood lipids concentration), and had sufficient urine and serum samples in storage for pesticide biomarker assessment (n=87). For the selected sample, baseline visits occurred between 2006 and 2010 and follow-up visits between 2012 and 2015, on average, 5.4 years later (interquartile range, 1.5 years). There were no statistically significant differences in gender, race, ethnicity, estimated total annual household income, highest level of education of either parent, insurance coverage, BMI classification, age at baseline, or age at diagnosis between the subsample included in this study and the larger SEARCH sample from which they were drawn. Participants of the current study with type 1 diabetes (n=50) were less likely to come from a two-parent household (58.0% compared to 70.3% in the larger SEARCH sample of participants with type 1 diabetes, p=0.04) and those with type 2 diabetes in the current study had a lower baseline HbA1c, on average (6.51% compared to 7.32% in the larger SEARCH sample of participants with type 2 diabetes, p=0.05).

The SEARCH study protocol was approved by local institutional review boards. Consent was obtained from a parent/guardian of all participants under age 18 years and assent from participants according to the specific age requirement deemed by the local institutional review board. This secondary analysis was deemed “Not Human Subjects Research” by the Harvard T.H. Chan School of Public Health Office of Human Research Administration (protocol #: IRB16-1336). The involvement of the Centers for Disease Control and Prevention (CDC) did not constitute engagement in human subjects research.

2.2 Exposure Assessment

We quantified biomarkers for eight non-persistent pesticides in urine and nine persistent pesticides in serum. Urine and blood collection supplies were provided to study centers by the SEARCH Central Laboratory at Northwest Lipid Metabolism & Diabetes Research, University of Washington in Seattle, WA. Spot urine samples were collected into sterile urine collection cups and 5.0 mL was transferred using a disposable plastic pipette to a 10-mL screw-cap sample vial. At least 5 mL of fasting venous blood was collected into 8.5-mL SST tiger-top tubes and serum was transferred to 2-mL cryovials after centrifugation for 10 minutes at 3500 RPM. Urine and blood samples were shipped fresh in a Ziploc bag along with freezer packs to the SEARCH Central Laboratory for long-term storage at -800.

In urine, we quantified eight biomarkers of non-persistent pesticides at the National Center for Environmental Health of the CDC: the chlorophenoxy herbicide 2,4-dichlorophenoxyacetic acid (2,4-D); four organophosphate insecticide metabolites, malathion dicarboxylic acid (MDA, a malathion metabolite), 3,5,6-trichloro-2-pyridinol (TCPY, a metabolite of chlorpyrifos and chlorpyrifos methyl), 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPY, a diazinon metabolite), and 4-nitrophenol (a metabolite of parathion and methyl parathion); and three metabolites of pyrethroid insecticides, 4-

fluoro-3-phenoxybenzoic acid (4-F-3-PBA, a metabolite of cyfluthrin and flumethrin), 3-phenoxybenzoic acid (3-PBA, a non-specific metabolite of several pyrethroids including cyhalothrin, cypermethrin, deltamethrin, fenpropathrin, phenothrin permethrin, tralomethrin, and esfenvalerate), and trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (trans-DCCA, a metabolite of permethrin, cypermethrin, and cyfluthrin) (Davis et al. 2013). The limit of detection (LOD) was 0.1 ng/ml (TCPY, IMPY, 4-nitrophenol, 4-F-3-PBA, 3-PBA), 0.15 ng/ml (2,4-D), 0.5 ng/ml (MDA), and 0.6 ng/ml (trans-DCCA).

Nine persistent pesticide biomarkers were quantified in serum: hexachlorobenzene (HCB), beta-hexachlorocyclohexane (β-HCH), gamma-hexachlorocyclohexane (γ-HCH; also known as lindane), oxychlordane, trans-nonachlor, 2,2-bis(4-chlorophenyl)-1,1-dichloroethene (p,p-DDE), 2,2-bis(4-chlorophenyl-1,1,1-trichloroethane (p,p-DDT), 2-(4-chlorophenyl)-2-(2-chlorophenyl)-1,1,1-trichloroethane (o,p-DDT), and Mirex (Jones et al. 2012; Sjödin et al. 2004). The LODs for the persistent pesticides were calculated by adding a recovery standard to each sample. To calculate the sample-specific LOD, the instrumental LOD was adjusted for the absolute recovery of this standard and background noise for the sample. The mean LOD across all samples for HCB, γ-HCH, oxychlordane, trans-nonachlor, p,p-DDT, o,p-DDT, and mirex was 2.34 ng/ml; and for β-HCH and p,p-DDE it was 2.33 ng/ml.

Fasting blood lipids were measured at the Northwest Lipid Research Laboratory, University of Washington. Total cholesterol, triglycerides, and HDL cholesterol were measured using a Roche Modular-P autoanalyzer (Roche Diagnostics, Indianapolis, IN), and LDL cholesterol was calculated using the Friedewald equation (Friedewald et al. 1972) for individuals with triglyceride concentrations <400 mg/dL and by Lipid Research Clinics Beta Quantification (Hainline et al. 1983) for those with triglyceride concentrations ≥400 mg/dL. Total serum lipid content was calculated from the concentrations of total cholesterol and triglycerides using the Phillips formula (Phillips et al. 1989). The concentration of creatinine in urine was determined using the Creatinine Plus enzymatic Roche reagent on a Roche c501 Cobas chemistry autoanalyzer (Roche Diagnostics, Inc., Indianapolis, IN). The results of this procedure are traceable to the ID-MS reference method (Joint Committee for Traceability in Laboratory Medicine 2019). The reportable range of creatinine in urine samples was 0.03-1200.0 mg/dL and the inter-assay coefficients of variation obtained on urine quality control samples with low and high creatinine concentrations were systematically less than 2%.

We used creatinine-adjusted urinary concentrations for all analyses of biomarkers of non-persistent pesticides (in μg/g creatinine) and lipid-adjusted serum concentrations for all analyses of persistent pesticides (in ng/g lipid).

2.3 Outcome Assessment

Eight CVD risk factors were evaluated at both baseline and follow-up: BMI z-score, HbA1c, insulin sensitivity, FCP, LDL cholesterol, HDL cholesterol, total cholesterol, and triglycerides. Only four of these were evaluated as outcomes (BMI z-score, HbA1c, insulin sensitivity, and FCP) for the persistent pesticides given that they were lipid-adjusted. BMI z-scores were calculated from 2000 CDC Growth Charts (National Center for Health Statistics

2000). Standardized anthropometry protocols were used to measure height and weight. FCP and HbA1c were measured using existing laboratory methods at the Northwest Lipid Research Laboratory, University of Washington. A two-site immunoenzymetric assay with a sensitivity of 0.05 ng/mL was used to determine FCP concentration (Tosoh 1800; Tosoh Bioscience, San Francisco, CA). An automated nonporous ion-exchange HPLC system was used to measure HbA1c in whole blood (model G-7; Tosoh Bioscience, San Francisco, CA). Insulin sensitivity scores were estimated based on a validated equation that included waist circumference, HbA1c, and triglyceride levels (Dabelea et al. 2011).

2.4 Statistical Analysis

Descriptive statistics were used to summarize the concentrations of pesticide biomarkers and changes over time. Concentrations of pesticide biomarkers below the LOD were assigned a value of LOD divided by the square root of two (Hornung and Reed 1990). We investigated demographic covariates significantly associated with each pesticide biomarker at baseline using Fisher's exact test for categorical variables and Kruskal-Wallis non-parametric tests for continuous variables. Baseline demographic covariates included sex, age at baseline visit, age at diagnosis, ethnicity, race, estimated total annual household income, and highest level of education of either parent. We used histogram plots to evaluate the normality of the distributions of all outcome variables and natural log-transformed values of FCP and triglycerides.

We then evaluated the association of each pesticide biomarker at baseline against each CVD risk factor at follow-up in separate linear regression models. For all models, we categorized pesticide biomarkers as this approach does not assume a linear relationship. Concentrations of pesticide biomarkers detected in more than 40% of samples at either baseline or follow-up were categorized into quartiles according to the baseline distribution of creatinine-adjusted urinary concentrations (non-persistent pesticides) and lipid-adjusted serum concentrations (persistent pesticides). Concentrations below the LOD were included in the lowest quartile. Models were adjusted for baseline values of the CVD risk factor, time between baseline and follow-up, and demographic covariates significantly ($p<0.10$) associated with the pesticide biomarker. All *p*-values were two-sided. Data were analyzed using Stata version 14.0 (College Station, TX).

3. Results

Baseline characteristics of participants (n=87) are shown in Supplemental Material, Table 1. Participants were, on average, 14.2 years old at baseline, and over half (57.5%) were diagnosed with type 1 diabetes. The sample was fairly balanced in terms of sex: 51.7% female and 48.3% male. Most participants self-identified as ethnically non-Hispanic (85.1%) and racially white (59.8%).

3.1 Non-persistent pesticide biomarkers

The most frequently detected biomarkers of non-persistent pesticides at both baseline and follow-up were 4-nitrophenol and 3-PBA, detected in more than 90% of participants (Table 1). While detected in a lower proportion of samples (73.2% at baseline and 64.9% at follow-

up), the geometric mean (GM) of TCPY was highest at both time points: 1.74 (95% CI: 1.46, 2.06) μg/g creatinine at baseline, and 1.33 (1.08, 1.64) μg/g creatinine at follow up. 2,4-D was also detected in a majority of participants at both time points (81.6% at baseline and 75.9% at follow-up), but had the lowest GM (95% CI): 0.25 (0.21, 0.30) μg/g creatinine at both baseline and follow up. Four of the eight non-persistent pesticide biomarkers measured (MDA, IMPY, 4-F-3-PBA, and trans-DCCA) were detected in less than 40% of participants at both time points and were therefore excluded from subsequent analyses.

Comparing baseline to follow-up, 50.6% of participants had a decline in 2,4-D concentration (Supplemental Figure S1); 59.5% had a decline in TCPY concentration (Supplemental Figure S2); 54.8% had a decline in 4-nitrophenol concentration (Supplemental Figure S3); and 48.8% had a decline in 3-PBA concentration (Supplemental Figure S4). Baseline and follow-up values were highly correlated for all four of these pesticide biomarkers: Spearman correlation coefficient of 0.33 (p=0.002) for 2,4-D, 0.28 (p=0.02) for TCPY, 0.29 (p=0.008) for 4-nitrophenol, and 0.35 (p=0.001) for 3-PBA.

Supplemental Tables S2–S5 present baseline characteristics according to quartile of 2,4-D, TCPY, 4-nitrophenol, and 3-PBA. Participants with concentrations in the highest quartile of TCPY were more likely to have an annual household income of $75K+ (37% versus 30% in the lowest quartile), type 1 diabetes (80% versus 45% in the lowest quartile), and were younger at diagnosis and the baseline visit (all p<0.10, Supplemental Table S3).

Participants with 2,4-D concentrations in the highest quartile had HbA1c levels that were percentage points (95% CI: −0.40, 2.51) higher (though not statistically significant at p<0.05) than participants in the lowest quartile in models adjusted for baseline HbA1c, elapsed time between baseline and follow up, sex, age, race, and diabetes type (Table 2 and Figure 1). Similarly, participants with 4-nitrophenol concentrations in the highest quartile had HbA1c levels that were 1.27 percentage points (95% CI: −0.22, 2.75) higher (though not statistically significant at p<0.05) than participants in the lowest quartile (Table 2 and Figure 1). Participants with 2,4-D concentrations in the highest quartile had significantly (p<0.05) lower log FCP levels (indicating reduced beta-cell function) than participants in the lowest quartile (−0.64 [−1.17, −0.11]), and those in the highest quartile of 4-nitrophenol also had lower levels, though not statistically significant: −0.39 (−0.96, 0.18) (Table 2 and Figure 2). In other words, participants in the highest quartile of 2,4-D had a 47.3% lower FCP level than those in the lowest quartile of 2,4-D, and participants in the highest quartile of 4-nitrophenol had a 32.3% lower FCP level than those in the lowest quartile of 4-nitrophenol. No statistically significant associations were observed between any of these four biomarkers of non-persistent pesticides and BMI z-score or insulin sensitivity.

For the blood lipid outcomes, participants with 3-PBA concentrations in the highest quartile had significantly (p<0.05) higher LDL cholesterol levels than participants in the lowest quartile (15.98 mg/dL [95% CI: 1.81, 30.15]) and had non-significantly higher total cholesterol levels (16.52 mg/dL [95% CI: −2.41, 35.44]) (Table 2). Similarly, higher levels of LDL and total cholesterol were observed among participants in the highest quartile of 2,4-D and 4-nitrophenol compared to the lowest quartile, but the wide 95% CI precluded making strong conclusions regarding these associations (Table 2).

3.2 Persistent pesticide biomarkers

The most frequently detected biomarkers of persistent pesticides at both baseline and follow-up were p,p-DDE and HCB, detected in more than 95% of participants (Table 1). p,p-DDE had a notably high GM (95% CI) of 51.96 (44.97, 60.02) ng/g lipid at baseline and 50.39 (44.47, 57.09) ng/g lipid at follow-up compared to, for example, 5.95 (5.49, 6.46) ng/g lipid at baseline and 5.32 (4.97, 5.69) ng/g lipid at follow-up for HCB. Trans-nonachlor was detected in approximately half of participants at baseline and follow-up, whereas the other six biomarkers (p,p-DDT, o,p-DDT, β-HCH, γ-HCH, mirex, and oxychlordane) were detected in less than 40% of participants at both time points and were therefore excluded from subsequent analyses.

Comparing baseline to follow-up, 48.2% of participants had a decline in p,p-DDE concentration (Supplemental Figure S5); 60.9% had a decline in HCB concentration (Supplemental Figure S6); and 46.0% had a decline in trans-nonachlor concentration (Supplemental Figure S7). Baseline and follow-up values were highly correlated for all three of these pesticide biomarkers: Spearman correlation coefficient of 0.84 ($p<0.0001$) for p,p-DDE, 0.42 ($p=0.0001$) for HCB, and 0.77 ($p<0.0001$) for trans-nonachlor.

Supplemental Tables S6–S8 present baseline characteristics according to quartile of p,p-DDE, HCB, and trans-nonachlor. Participants with concentrations in the highest quartile of p,p-DDE were more likely to be male (67% versus 27% in the lowest quartile), Hispanic (48% versus 0% in the lowest quartile), have an annual household income of $75K+ (35% versus 9% in the lowest quartile), and have type 1 diabetes (62% versus 32% in the lowest quartile) (all $p<0.05$, Supplemental Table S6). Participants with concentrations in the highest quartile of HCB were more likely to be male (81% versus 18% in the lowest quartile) and have type 1 diabetes (90% versus 36% in the lowest quartile) (all $p<0.05$, Supplemental Table S7). Participants with concentrations in the highest quartile of trans-nonachlor were more likely to be white (82% versus 39% in the lowest quartile) and have type 1 diabetes (77% versus 30% in the lowest quartile) (all $p<0.05$, Supplemental Table S8). Participants in the highest quartile of trans-nonachlor were significantly younger at baseline and at diagnosis compared to participants in the lowest quartile.

Participants with trans-nonachlor concentrations in the highest quartile had HbA1c levels that were 1.45 percentage points (95% CI: −0.11, 3.01) higher (though not statistically significant at $p<0.05$) and log FCP levels that were −0.28 (−0.84, 0.28) lower (though not statistically significant at $p<0.05$) than participants in the lowest quartile of trans-nonachlor (Table 3, Figures 1 and 2). In other words, participants in the highest quartile of trans-nonachlor had a 24.4% lower FCP level than those in the lowest quartile of trans-nonachlor. Participants with HCB concentrations in the third quartile had HbA1c levels that were 1.50 percentage points (95% CI: 0.08, 2.92) higher and log FCP levels that were −0.28 (−0.83, 0.28) lower (though not statistically significant at $p<0.05$) than participants in the lowest quartile of HCB (Table 3, Figures 1 and 2). In other words, participants in the third quartile of HCB had a 24.4% lower FCP level than those in the lowest quartile of HCB. Similar to observed effects of non-persistent pesticide biomarkers, no statistically significant associations were observed between persistent pesticide biomarkers and BMI z-score or insulin sensitivity.

4. Discussion

In this prospective cohort of youth with type 1 and type 2 diabetes in the United States, the GM concentration of TCPY, a biomarker of the organophosphate insecticide chlorpyrifos, was the highest of all non-persistent pesticide biomarkers quantified in urine, and the GM concentration of p,p-DDE, a metabolite of the organochlorine insecticide DDT, was the highest of all persistent pesticide biomarkers analyzed in serum. Six of the 17 pesticide biomarkers analyzed (4-nitrophenol, 3-PBA, 2,4-D, TCPY, p,p-DDE, and HCB) were detected in a majority of participants at both time points-more than 90% of samples in most cases. Participants with the highest concentrations of 2,4-D, 4-nitrophenol, and trans-nonachlor tended to have higher HbA1c levels and lower FCP levels. While not all of these results were statistically significant, potentially due to the small sample size, clinically, there appears to be quantitative differences, suggesting that exposure to pesticides may be associated with impaired beta-cell function and poorer glycemic control among youth with diabetes.

4-nitrophenol and 3-PBA were detected in nearly all participants, with GM (95% CI) concentrations of 0.45 (0.39, 0.51) and 0.80 (0.61, 1.05) μg/g creatinine, respectively. These concentrations are higher than those in a representative sample of U.S. youth aged 12-19 years (2009-2010): 0.368 (0.319, 0.426) and 0.347 (0.302, 0.399) μg/g creatinine, respectively (Centers for Disease Control and Prevention 2019). The GM (95% CI) concentration of TCPY was similarly higher in this sample of youth with diabetes compared to the general U.S. youth population: 1.74 (1.46, 2.06) versus 0.757 (0.675, 0.850) μg/g creatinine, respectively (Centers for Disease Control and Prevention 2019). However, similar to the general youth population in the United States, we saw a decline in the GM of this insecticide biomarker from baseline to follow up. The GM (95%) concentration of the herbicide biomarker, 2,4-D, in our sample was similar to the U.S. general population of youth: 0.25 (0.21, 0.30) versus 0.258 (0.212, 0.314) μg/g creatinine, respectively (Centers for Disease Control and Prevention 2019).

In contrast to the non-persistent pesticide biomarkers, the concentrations of p,p-DDE and HCB in this sample of youth with diabetes were lower than those reported for the general U.S. youth population (12-19 years, 2003-2004): p,p-DDE, 51.96 (44.97, 60.02) versus 105 (84.7, 129) ng/g lipid; and HCB, 5.95 (5.49, 6.46) versus 13.3 (12.5, 14.1) ng/g lipid (Centers for Disease Control and Prevention 2019). For trans-nonachlor, the proportion of samples for youth in the general U.S. population with detectable concentrations was too low to report a GM (Centers for Disease Control and Prevention 2019), suggesting that for this persistent pesticide biomarker, concentrations were higher in our sample of youth with diabetes: detected in 45% of participants with a GM (95% CI) of 4.83 (3.81,6.11) ng/g lipid. As expected, given their persistent nature, the baseline and follow-up concentrations of the persistent pesticide biomarkers were substantially more correlated than those for the non-persistent pesticide biomarkers with Spearman correlation coefficients ranging from 0.42 for HCB to 0.84 for p,p-DDE.

To date, there have been no epidemiological studies that evaluate the association of pesticides with CVD risk factors in youth with diabetes. A previous study in Denmark

reported that adolescents (10-16 years; excluding those with type 1 or type 2 diabetes) born to mothers with occupational exposure to pesticides had an HbA1c 5.0 percentage points (95% CI: 1.8, 8.2) higher than adolescents born to mothers with no occupational exposure to pesticides (Andersen et al. 2018). However, they did not observe a significant difference in either FCP or insulin sensitivity.

The association between pesticide biomarkers and FCP, and lack of association with insulin sensitivity, observed in this study of youth with diabetes is consistent with several previous studies of organochlorine pesticides that concluded that the increased risk of diabetes is the result of modulation of beta-cell function as opposed to reduced insulin-stimulated glucose uptake (Faerch et al. 2012; Grandjean et al. 2011; Jørgensen et al. 2008). For example, a study of the highly-exposed Greenland Inuit population found that persistent organic pollutants including organochlorine pesticides were associated with HOMA-B (homeostatic model assessment beta-cell function), but not HOMA-IR (homeostatic model assessment insulin resistance) (Jørgensen et al. 2008).

Only a handful of studies have evaluated the effects of 2,4-D on metabolic disruption. An analysis of data from ~700 adults aged 20-59 years from the U.S. National Health and Nutrition Examination Survey (NHANES) spanning 1988-1994 found that the associations of 2,4-D with insulin and FCP were more pronounced among individuals with HbA1c greater than 5.1% (Schreinemachers 2010). In addition, having detectable urinary concentrations of 2,4-D was associated with an overall 5-9% decrease in HDL cholesterol compared to non-detectable concentrations, and associations of 2,4-D with triglycerides, insulin, and FCP were only present among individuals with low HDL (i.e., HDL-dependent effects) (Schreinemachers 2010).

In contrast with that NHANES analysis, a 2014 study of farmers' wives enrolled in the Agricultural Health Study who reported ever personally applying or mixing pesticides at recruitment (13,637 women aged 17 to 88 years) found no association between the ever-use of 2,4-D and incident diabetes (Starling et al. 2014). Ever-use of chlorpyrifos (a precursor of TCPY), diazinon (a parent chemical of IMPY), and malathion (a parent chemical of MDA) had no association with incident diabetes (Starling et al. 2014). However, consistent with our findings, parathion, a precursor of 4-nitrophenol, was found to be associated with incident diabetes (hazard ratio [95% CI], 1.61 [1.05, 2.46]) (Starling et al. 2014). A 2008 study also used Agricultural Health Study data to investigate associations between pesticide exposure and incident diabetes among pesticide applicators (33,457 non-Hispanic white adult males) (Montgomery et al. 2008). They found no significant associations between ever using 2,4-D, chlorpyrifos, diazinon, malathion, parathion, or permethrin after adjustment for age, state, and BMI (Montgomery et al. 2008). However, in a dose-response analysis, they found that participants who used chlorpyrifos and diazinon for more than 100 days per year were significantly more likely to develop diabetes compared to participants who never used these pesticides (odds ratio [95% CI], 1.24 [1.02, 1.52] and 1.59 [1.09, 2.31], respectively) (Montgomery et al. 2008).

One previous analysis of adults (≥40 years) with diabetes or impaired fasting glucose in NHANES found that organochlorine pesticides were strongly associated with odds of having

an HbA1c ≥7%: odds ratio (95% CI) comparing highest and lowest tertiles of total organochlorine pesticide levels 5.0 (1.8, 13.4), with the strongest effects observed for β-HCH and heptachlor epoxide (Lee et al. 2008). Several other studies have reported substantial associations between organochlorine pesticides and prevalent or incident type 2 diabetes. A meta-analysis of 11 cross-sectional studies and 6 prospective studies reported an overall relative risk (95% CI) of 2.30 (1.81, 2.93) for organochlorine pesticides and type 2 diabetes (Song et al. 2016). A more recent meta-analysis concluded that of all pesticides evaluated, the strongest evidence for a link with type 2 diabetes exists for p,p-DDE (Lind and Lind 2018). Our results in a sample of youth largely with type 1 diabetes did not indicate an association between p,p-DDE and HbA1c, FCP, or insulin sensitivity. We did, however, find an association as hypothesized with two other organochlorine pesticides: trans-nonachlor and HCB. More research is needed to understand potential modification of these effects by age (e.g., adolescents versus adults) and diabetes type.

Additional studies have analyzed the association between pesticides and lipoproteins. A 2018 study of 214 conventional farmers and 222 organic farmers in Thailand found that conventional farmers had significantly higher LDL cholesterol and total cholesterol levels compared to organic farmers (Kongtip et al. 2018). A study on the effects of 2,4-D on lipoprotein concentrations in rats found that all groups treated with 2,4-D experienced significant increases in LDL cholesterol, total cholesterol, and triglycerides levels and decreases in HDL cholesterol levels when compared to the control group (Tayeb et al. 2013). Similarly, we found higher levels of LDL cholesterol and total cholesterol among participants in the highest quartile of 2,4-D compared to the lowest quartile, though our results were not statistically significant. A 2008 study of allethrin and prallethrin (pyrethroids) among 36 male volunteers ages 35 to 45 years (12 subjects in each of the following study arms: control subjects, allethrin-exposed subjects, and prallethrin-exposed subjects) found that while HDL cholesterol levels were not significantly different between groups, triglycerides and very low-density lipoprotein cholesterol levels were significantly higher among the allethrin and prallethrin-exposed subjects (Narendra et al. 2008). This is consistent with our study in which we found that 3-PBA, a biomarker of the pyrethroid chemical class, was significantly associated with LDL cholesterol and not significantly associated with HDL cholesterol. However, in contrast with this 2008 study, we found no association between 3-PBA and triglycerides. A 2005 study found that neonatal male rats exposed to chlorpyrifos had elevated cholesterol in adulthood (Slotkin et al. 2005), but we did not find any consistent association between TCPY, a metabolite of chlorpyrifos, and blood lipids in this epidemiological study. More research is needed to understand the potential effects of pesticide exposures on blood lipid levels in humans.

Our study did not find any associations between the analyzed pesticide biomarkers and BMI. In contrast, a study of Chinese factory workers found that workers exposed to pyrethroid insecticides had significantly higher BMIs and waist circumferences (Wang et al. 2011). A prospective study of 90 young adults ages 18 to 30 found that p,p-DDE concentrations at baseline were significantly associated with BMI 18 years later (Lee et al. 2011). Similarly, a prospective birth cohort study of 138 mother-infant pairs in Belgium found that DDE concentration had a small effect on the BMI standard deviation scores of children of nonsmoking mothers (3 years of age), but had a much larger effect on the BMI standard

deviation scores of children of smoking mothers (3 years of age): difference in BMI standard deviation scores for DDE concentrations between the 10th and 90th percentiles, 0.13 and 0.76, respectively (Verhulst et al. 2009).

This study has several limitations. The small sample size – just 87 youth with diabetes – prevented stratification according to diabetes type, among other factors, and also limited our power to detect an effect. However, a priori calculations determined that a sample size of n=40 with a standard deviation of HbA1c of 1.7% or 2.7% (Petitti et al. 2007) achieves 80% power to detect a change in slope from 0.00 under the null hypothesis to 0.22 and 0.35, respectively, and under the alternative hypothesis when the two-sided significance level is 0.05 and the standard deviation of the concentration is 3.20 ng/g lipid. Of note, the average standard deviation of persistent organic pollutants reported in NHANES 2007-2008 for 12-19 year-olds was 3.3 ng/g lipid (Sjödin et al. 2013). Another limitation of the small sample size is that we estimated the effects of each pesticide biomarker individually, and the correlations among pesticide biomarkers make it difficult to identify the effect of a single exposure. Future research in larger samples may facilitate disentangling the effects of exposure to complex mixtures of pesticides. Major strengths of the study are the prospective design and the objective assessment of exposure using pesticide biomarkers rather than relying on participants' self-reported use of pesticides. Nonetheless, exposure biomonitoring is not without its limitations. In particular, the non-persistent pesticide biomarkers reflect recent environmental exposure (e.g., hours or days) whereas the persistent pesticide biomarkers reflect cumulative, longer-term exposures (Barr 2008). Because the non-persistent pesticide biomarkers were measured in a single baseline spot urine, there is the potential for misclassification of average exposure to these chemicals, especially if participants were not chronically exposed. Moreover, the reliance on metabolites may lead to an overestimation of exposure to the parent compound. For example, individuals may be exposed to both chlorpyrifos and its metabolite, TCPY, in their environments (Morgan 2005), and urinary TCPY excretion will represent both exposures, not just environmental exposure to the parent compound (chlorpyrifos).

To the best of our knowledge, this is the first study to quantify concentrations of a wide range of pesticide biomarkers among youth with diabetes, and to evaluate their association with CVD risk factors. Participants with the highest levels of 2,4-D, 4-nitrophenol, and trans-nonachlor tended to have higher HbA1c levels and lower FCP levels, suggesting that exposure to select pesticides may be associated with increased CVD risk. Additional studies are warranted to inform whether these observations reflect causal pathways.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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- Prospective associations of pesticides with clinical outcomes in youth with diabetes
- 2,4-D and 4-nitrophenol were associated with poorer glycemic control
- 2,4-D and 4-nitrophenol were associated with impaired beta-cell function
- Trans-nonachlor was associated with poorer glycemic control
- Trans-nonachlor was associated with impaired beta-cell function

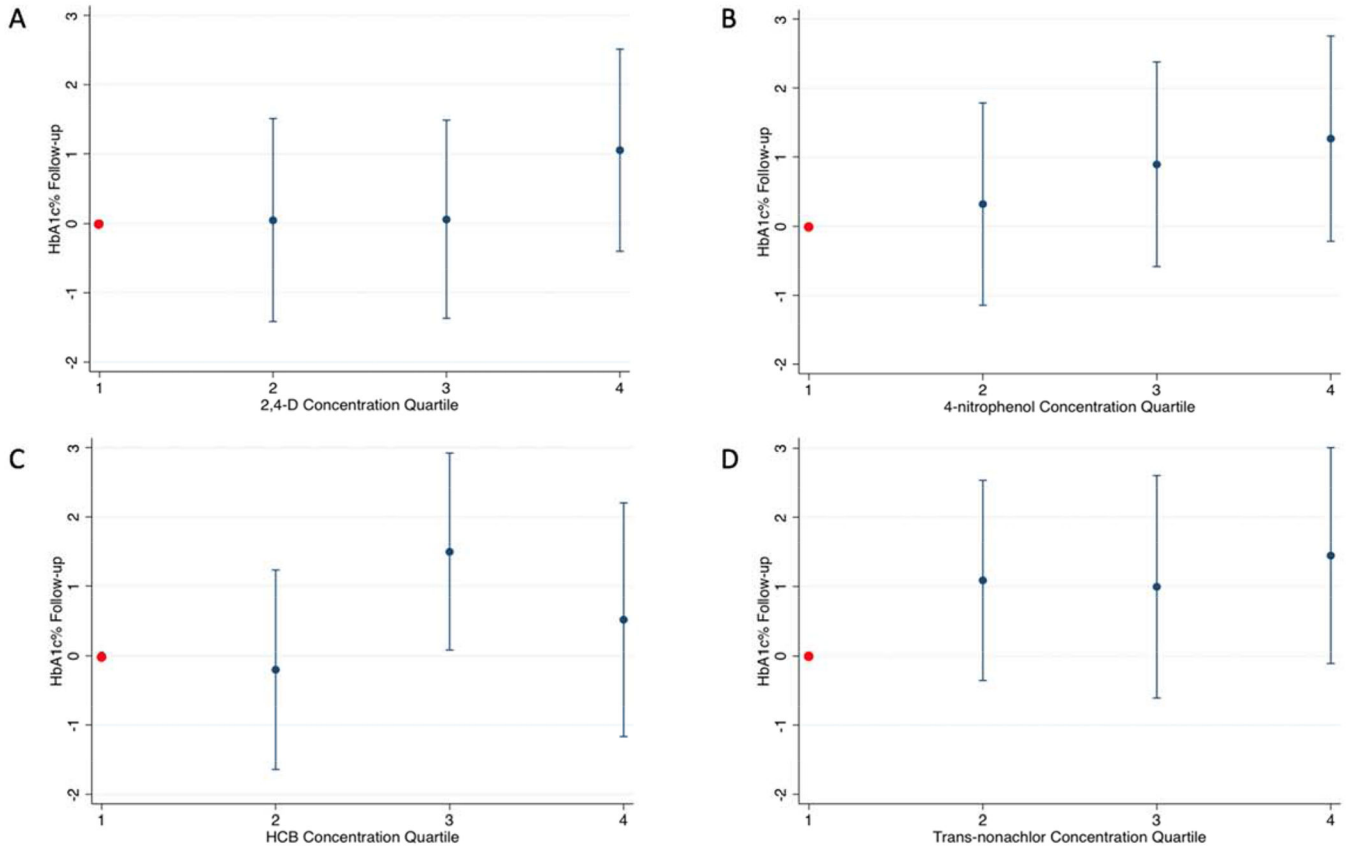

Figure 1.

Associations of HbA1c (%) with (A) 2,4-dichlorophenoxyacetic acid (2,4-D), (B) 4-nitrophenol, (C) hexachlorobenzene (HCB), and (D) trans-nonachlor concentration quartiles. Note: regression model was adjusted for baseline HbA1c (%), elapsed time between baseline and follow-up, sex (male versus female), age (years), race (white versus non-white), and diabetes type (type 1 versus type 2). Bars represent 95% confidence intervals. Red dot represents reference group.

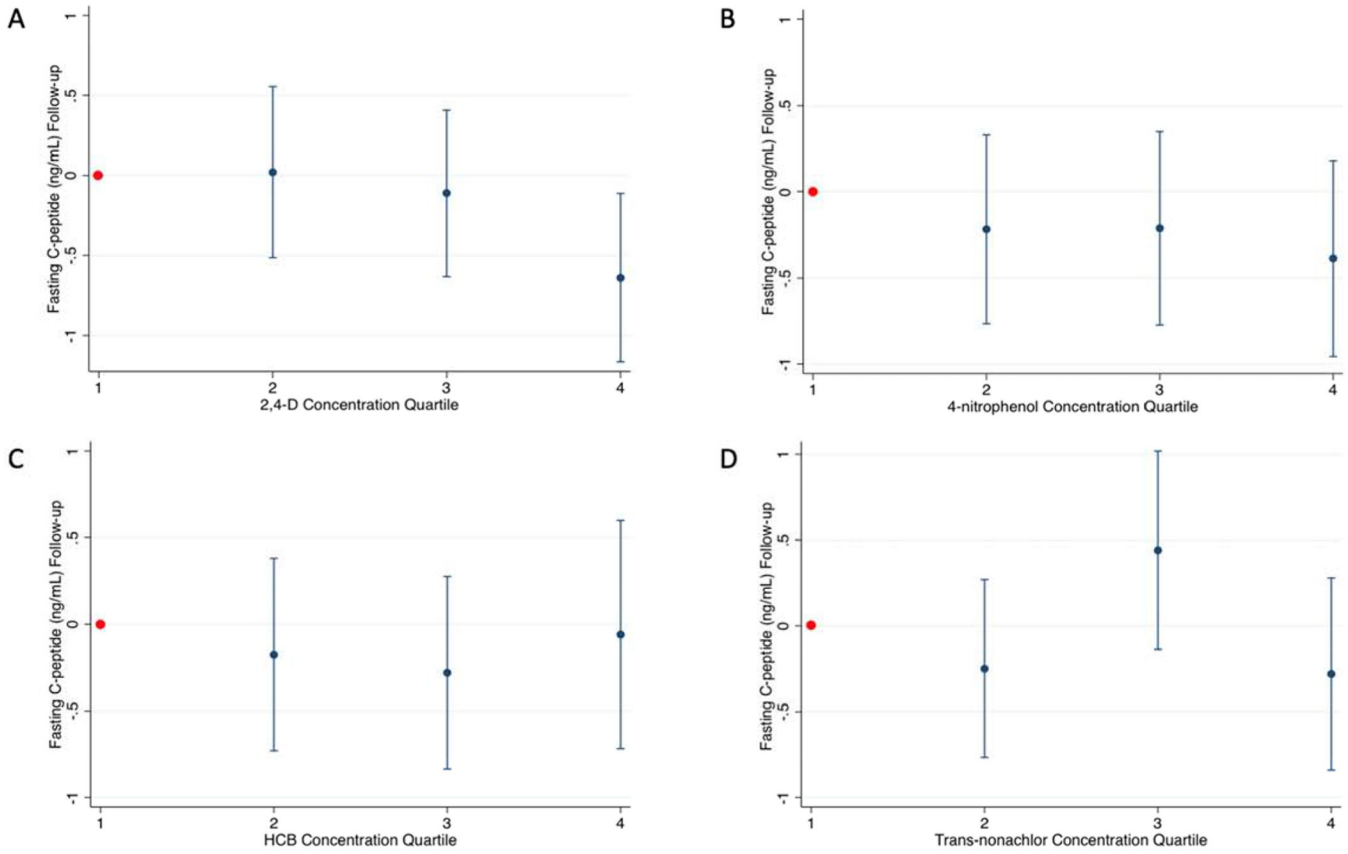

Figure 2.

Associations of fasting C-peptide (log-transformed ng/mL) with (A) 2,4-dichlorophenoxyacetic acid (2,4-D), (B) 4-nitrophenol, (C) hexachlorobenzene (HCB), and (D) trans-nonachlor concentration quartiles. Note: regression model was adjusted for baseline fasting C-peptide (log-transformed ng/mL), elapsed time between baseline and follow-up, sex (male versus female), age (years), race (white versus non-white), and diabetes type (type 1 versus type 2). Bars represent 95% confidence intervals. Red dot represents reference group.

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Table 1.

Concentrations of biomarkers of pesticides among youth with type 1 and type 2 diabetes in the United States (n=87).

	Baseline			Follow-up		
Exposure biomarker	**>LOD, n (%)[a]**	**Missing, n (%)**	**Geometric Mean (95% CI)[b]**	**>LOD, n (%)[a]**	**Missing, n (%)**	**Geometric mean (95% CI)[b]**
Non-persistent pesticide biomarkers quantified in urine (μg/g creatinine)						
2,4-D	71 (81.6)	0 (0.0)	0.25 (0.21, 0.30)	66 (75.9)	0 (0.0)	0.25 (0.21, 0.30)
MDA	17 (19.5)	0 (0.0)	-	21 (24.1)	0 (0.0)	-
TCPY	60 (73.2)	5 (5.8)	1.74 (1.46, 2.06)	50 (64.9)	10 (11.5)	1.33 (1.08, 1.64)
IMPY	25 (29.1)	1 (1.2)	-	20 (23.5)	2 (2.3)	-
4-nitrophenol	84 (97.7)	1 (1.2)	0.45 (0.39, 0.51)	82 (96.5)	2 (2.3)	0.46 (0.40, 0.52)
4-F-3-PBA	8 (9.2)	0 (0.0)	-	10 (11.5)	0 (0.0)	-
3-PBA	79 (92.9)	2 (2.3)	0.80 (0.61, 1.05)	84 (98.8)	2 (2.3)	0.78 (0.64, 0.97)
trans-DCCA	20 (23.5)	2 (2.3)	-	16 (19.5)	5 (5.8)	-
Persistent pesticide biomarkers quantified in serum (ng/g lipid)						
p,p-DDT	18 (20.7)	0 (0.0)	-	20 (23.0)	0 (0.0)	-
o,p-DDT	1 (1.2)	0 (0.0)	-	0 (0.0)	0 (0.0)	-
p,p-DDE	86 (100)	1 (1.2)	51.96 (44.97, 60.02)	85 (100)	2 (2.3)	50.39 (44.47, 57.09)
β-HCH	3 (3.5)	1 (1.2)	-	3 (3.5)	1 (1.2)	-
γ-HCH	1 (1.6)	24 (27.6)	-	0 (0.0)	24 (27.6)	-
HCB	85 (97.7)	0 (0.0)	5.95 (5.49, 6.46)	85 (97.7)	0 (0.0)	5.32 (4.97, 5.69)
Mirex	0 (0.0)	0 (0.0)	-	0 (0.0)	0 (0.0)	-
Oxychlordane	27 (31.0)	0 (0.0)	-	23 (26.4)	0 (0.0)	-
Trans-nonachlor	39 (44.8)	0 (0.0)	4.83 (3.81,6.11)	40 (46.0)	0 (0.0)	4.73 (3.89, 5.74)

Abbreviations: beta-hexachlorocyclohexane (β-HCH), 2,2-Bis(4-chlorophenyl)-1,1-dichloroethene (p,p-DDE), 2,2-Bis(4-chlorophenyl-1,1,1-trichloroethane (p,p-DDT), 2-(4-chlorophenyl)-2-(2-chlorophenyl)-1,1,1-trichloroethane (o,p-DDT), 2,4-Dichlorophenoxyacetic acid (2,4-D), gamma-hexachlorocyclohexane (γ-HCH), hexachlorobenzene (HCB), 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPY), malathion dicarboxylic acid (MDA), 4-fluoro-3-phenoxybenzoic acid (4-F-3-PBA), 3-phenoxybenzoic acid (3-PBA), 3,5,6-trichloro-2-pyridinol (TCPY), trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (trans-DCCA).

[a]LOD was 0.1 ng/ml (TCPY, IMPY, 4-nitrophenol, 4-F-3-PBA, 3-PBA), 0.15 ng/ml (2,4-D), 0.5 ng/ml (MDA), and 0.6 ng/ml (trans-DCCA). The LODs for the persistent pesticides were calculated by adding a recovery standard to each sample. To calculate the sample-specific LOD, the instrumental LOD was adjusted for the absolute recovery of this standard and background noise for the sample. The mean LOD across all samples for HCB, γ-HCH, oxychlordane, trans-nonachlor, p,p-DDT, o,p-DDT, and mirex was 2.34 ng/ml; and for β-HCH and p,p-DDE it was 2.33 ng/ml.

[b]Geometric mean calculated among participants with detectable concentrations. Not reported for pesticide biomarkers detected in fewer than 40% of samples at either baseline or follow-up.

Table 2.

Prospective associations between baseline urinary concentrations of non-persistent pesticide biomarkers and cardiovascular disease risk factors at follow-up, on average, 5.4 years later, among youth with type 1 and type 2 diabetes in the United States (n=87).

Exposure biomarker GM (95% CI) for each quartile	BMI z-score	HbA1c (%)	Insulin Sensitivity	Fasting C-peptide (log-transformed ng/mL)	LDL cholesterol (mg/dL)	Total cholesterol (mg/dL)	HDL cholesterol (mg/dL)	Triglycerides (log-transformed mg/dL)
2,4-D								
1 0.09 (0.08-0.10) μg/g creatinine	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
2 0.18 (0.17-0.20) μg/g creatinine	−0.25 (−0.66, 0.15)	0.05 (−1.42, 1.51)	0.07 (−1.11, 1.25)	0.02 (−0.51, 0.55)	−3.35 (−17.88, 11.19)	2.66 (−16.64, 21.96)	2.39 (−3.46, 8.24)	0.25 (−0.11, 0.61)
3 0.30 (0.28-0.33) μg/g creatinine	0.12 (−0.26, 0.49)	0.06 (−1.37, 1.49)	0.16 (−0.99, 1.31)	−0.11 (−0.63, 0.41)	6.46 (−7.72, 20.65)	0.01 (−18.82, 18.84)	6.64 (0.96, 12.32)	−0.33 (−0.69, 0.02)
4 0.72 (0.57-0.89) μg/g creatinine	0.09 (−0.30, 0.47)	1.05 (−0.40, 2.51)	−0.64 (−1.81, 0.53)	−0.64 (−1.17, −0.11)	7.63 (−6.76, 22.02)	10.44 (−8.66, 29.54)	3.85 (−1.91, 9.62)	0.00 (−0.36, 0.36)
TCPY								
1 0.06 (0.05-0.08) μg/g creatinine	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
2 0.83 (0.70-0.99) μg/g creatinine	−0.17 (−0.56, 0.23)	−0.49 (−2.00, 1.02)	0.48 (−0.72, 1.69)	0.09 (−0.48, 0.67)	5.37 (−9.38, 20.11)	11.27 (−7.95, 30.49)	1.96 (−4.15, 8.07)	−0.02 (−0.41, 0.36)
3 1.64 (1.51-1.78) μg/g creatinine	0.13 (−0.25, 0.52)	0.09 (−1.45, 1.63)	0.01 (−1.22, 1.24)	0.22 (−0.36, 0.81)	5.46 (−9.65, 20.58)	3.14 (−16.59, 22.88)	1.78 (−4.56, 8.13)	−0.17 (−0.56, 0.23)
4 3.63 (3.10-4.24) μg/g creatinine	0.16 (−0.25, 0.57)	0.07 (−1.57, 1.70)	−0.46 (−1.75, 0.83)	0.23 (−0.41, 0.87)	−2.33 (−18.34, 13.67)	−0.55 (−21.39, 20.28)	4.76 (−1.94, 11.47)	−0.11 (−0.53, 0.30)
4-nitrophenol								
1 0.21 (0.19-0.23) μg/g creatinine	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
2 0.34 (0.33-0.36) μg/g creatinine	−0.27 (−0.65, 0.11)	0.32 (−1.15, 1.78)	0.55 (−0.61, 1.71)	−0.22 (−0.76, 0.33)	7.50 (−7.08, 22.08)	13.84 (−5.53, 33.21)	4.03 (−1.75, 9.80)	−0.03 (−0.41, 0.35)

Environ Res. Author manuscript; available in PMC 2021 February 01.

Exposure biomarker GM (95% CI) for each quartile	BMI z-score	HbA1c (%)	Insulin Sensitivity	Fasting C-peptide (log-transformed ng/mL)	LDL cholesterol (mg/dL)	Total cholesterol (mg/dL)	HDL cholesterol (mg/dL)	Triglycerides (log-transformed mg/dL)
3 0.53 (0.49-0.57) μg/g creatinine	0.01 (−0.38, 0.41)	0.90 (−0.59, 2.38)	−0.01 (−1.20, 1.18)	−0.21 (−0.77, 0.35)	10.60 (−3.91, 25.12)	14.44 (−4.63, 33.51)	4.00 (−1.91, 9.91)	−0.14 (−0.52, 0.25)
4 1.07 (0.90-1.27) μg/g creatinine	−0.14 (−0.53, 0.25)	1.27 (−0.22, 2.75)	−0.32 (−1.53, 0.88)	−0.39 (−0.96, 0.18)	5.94 (−8.45, 20.33)	6.73 (−12.20, 25.65)	0.09 (−5.80, 5.98)	−0.11 (−0.49, 0.27)
3-PBA								
1 0.18 (0.14-0.23) μg/g creatinine	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
2 0.44 (0.39-0.49) μg/g creatinine	0.03 (−0.37, 0.43)	0.41 (−1.11, 1.94)	0.27 (−0.89, 1.42)	0.25 (−0.31, 0.80)	14.17 (−0.49, 28.84)	5.54 (−13.82, 24.91)	2.55 (−3.43, 8.52)	−0.31 (−0.68, 0.06)
3 0.97 (0.88-1.06) μg/g creatinine	−0.04 (−0.44, 0.35)	−0.09 (−1.57, 1.39)	0.12 (−1.00, 1.25)	−0.28 (−0.84, 0.27)	4.10 (−10.03, 18.23)	1.02 (−17.89, 19.94)	8.57 (2.89, 14.25)	−0.41 (−0.77, −0.05)
4 3.54 (2.35-5.33) μg/g creatinine	−0.05 (−0.45, 0.35)	−0.09 (−1.57, 1.40)	−0.11 (−1.25, 1.03)	−0.11 (−0.66, 0.44)	15.98 (1.81, 30.15)	16.52 (−2.41, 35.44)	4.31 (−1.33, 9.96)	−0.04 (−0.40, 0.32)

Note: values are beta coefficients (95% confidence intervals) from multivariable linear regression, adjusting for baseline health outcome (continuous), elapsed time between baseline and follow up, sex (male versus female), age (years), race (white versus non-white), and diabetes type (type 1 versus type 2). Abbreviations: body mass index (BMI), 2,4-dichlorophenoxyacetic acid (2,4-D), 3,5,6-trichloro-2-pyridinol (TCPY), 3-phenoxybenzoic acid (3-PBA).

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Table 3.

Prospective associations between baseline serum concentrations of persistent pesticide biomarkers and cardiovascular disease risk factors at follow-up, on average, 5.4 years later, among youth with type 1 and type 2 diabetes in the United States (n=87).

Exposure biomarker GM (95% CI) for each quartile	BMI z-score	HbA1c (%)	Insulin Sensitivity	Fasting C-peptide (log-transformed ng/mL)
p,p-DDE				
1 22.93 (20.85-25.20) ng/g lipid	Ref	Ref	Ref	Ref
2 39.23 (36.65-41.99) ng/g lipid	−0.04 (−0.45, 0.36)	−0.74 (−2.24, 0.76)	0.50 (−0.73, 1.72)	−0.02 (−0.59, 0.54)
3 65.44 (59.37-72.14) ng/g lipid	−0.05 (−0.50, 0.40)	0.83 (−0.73, 2.38)	−0.43 (−1.72, 0.86)	−0.15 (−0.75, 0.45)
4 127.32 (111.67-145.17) ng/g lipid	0.08 (−0.36, 0.53)	−0.50 (−2.08, 1.09)	0.04 (−1.31, 1.39)	0.13 (−0.48, 0.73)
HCB				
1 3.55 (3.22-3.93) ng/g lipid	Ref	Ref	Ref	Ref
2 5.08 (4.93-5.25) ng/g lipid	−0.21 (−0.60, 0.19)	−0.20 (−1.64, 1.23)	0.60 (−0.58, 1.79)	−0.18 (−0.73, 0.38)
3 6.60 (6.33-6.89) ng/g lipid	−0.15 (−0.54, 0.25)	1.50 (0.08, 2.92)	0.02 (−1.16, 1.20)	−0.28 (−0.83, 0.28)
4 9.86 (9.06-10.74) ng/g lipid	0.09 (−0.41, 0.58)	0.52 (−1.17, 2.20)	0.19 (−1.22, 1.59)	−0.06 (−0.72, 0.60)
Trans-nonachlor				
1 1.32 (1.23-1.42) ng/g lipid	Ref	Ref	Ref	Ref
2 1.96 (1.88-2.04) ng/g lipid	−0.07 (−0.43, 0.30)	1.09 (−0.36, 2.53)	−0.27 (−1.39, 0.85)	−0.25 (−0.77, 0.27)
3 2.82 (2.63-3.02) ng/g lipid	−0.49 (−0.90, −0.07)	0.99 (−0.61, 2.60)	0.97 (−0.29, 2.22)	0.44 (−0.14, 1.02)

Exposure biomarker GM (95% CI) for each quartile	BMI z-score	HbA1c (%)	Insulin Sensitivity	Fasting C-peptide (log-transformed ng/mL)
4 7.78 (6.00-10.08) ng/g lipid	−0.15 (−0.57, 0.28)	1.45 (−0.11, 3.01)	−0.40 (−1.63, 0.83)	−0.28 (−0.84, 0.28)

Note: values are beta coefficients (95% confidence intervals) from multivariable linear regression, adjusting for baseline health outcome (continuous), elapsed time between baseline and follow-up, sex (male versus female), age (years), race (white versus non-white), and diabetes type (type 1 versus type 2). Abbreviations: body mass index (BMI), 2,2-bis(4-chlorophenyl)-1,1-dichloroethene (p,p-DDE), hexachlorobenzene (HCB).