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Associations between urinary phthalate metabolites and diabetes in the general US adult population

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Abstract

Associations between urinary phthalate metabolites and diabetes in the general adult US population

By Michael Essien, MBChB

OBJECTIVE: Phthalates are ubiquitous endocrine-disrupting chemicals found in the environment and have been thought to alter adipocyte differentiation which leads to obesity and increase in insulin resistance. We investigated whether urinary phthalate metabolites are associated with diabetes in a cross-sectional subset of the adult US population

RESEARCH DESIGN AND METHODS: A total of 2,993 subjects aged 20 to 70 years who met all the inclusion criteria were investigated using NHANES 2-year cycle datasets (2003 – 2014). This is an ongoing survey by the National Center for Health Statistics at the Centers for Disease Control and Prevention. Exposure variable was urinary phthalate metabolites with diabetes and prediabetes as outcome variables. Demographic variables, physical activity, body mass index and dietary factors were considered as potential confounders. Using multivariate logistic regression, we estimated the prevalence odds ratios (ORs) and 95% confidence intervals (CIs) adjusting for urinary creatinine and the potential confounders.

RESULTS: A total of 162 subjects representing 5.41% of the study population had diabetes. Following adjustment for potential confounders, MnBP was shown to be statistically significantly associated with increased odds of prevalent diabetes. Higher than median levels of MEP, MBzP and ∑DEHP were associated with increased odds of diabetes with uniform increases in ORs observed across all quartiles for MCPP and MEP.

DISCUSSION: Our findings show that urinary phthalate metabolites are associated with diabetes across the subset of the adult US population. Future longitudinal studies are needed to understand this association further.

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AIMS: To determine if an association exists between urinary phthalate concentrations and diabetes in a cross-sectional subset of the adult US population

HYPOTHESIS: Higher urinary phthalate levels are associated with increased odds of diabetes in the general adult US population.

INTRODUCTION

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The classification and diagnostic criteria for diabetes have evolved over the past years but its importance in society has greatly increased. The American Diabetes Association classifies diabetes into four (4) categories: Type 1 (due to autoimmune beta cell destruction), type 2 (due to progressive loss of beta cell insulin secretion on the background of insulin resistance), gestational diabetes mellitus (diabetes diagnosed from the second trimester of pregnancy in a previously non-diabetic woman) and diabetes due to other causes (such as dug/chemical-induced diabetes, neonatal diabetes). The two main types are type 1 and type 2 diabetes with the latter being the most common, accounting for about 90% to 95% of cases. (CDC National Diabetes Statistics Report, 2017). A complex interaction between genetic and environmental factors underlie the etiology of diabetes. Well known major risk factors include weight greater than 120% of desirable body weight, family history of diabetes, sedentary lifestyle, hypertension, dyslipidemia and a history of gestational diabetes. (https://emedicine.medscape.com/article/117853). Despite these known risk factors, there are concerns that environmental exposures may play a role and chemicals of interest in these environmental exposures include phthalates, persistent organic pollutants, tobacco smoke constituents among others. (Kuo, Moon, Thayer, & Navas-Acien, 2013).

The prevalence of diabetes among people of all ages in the United States in the year 2015 was 30.3 million, representing 9.4% of the US population. This is of great concern given that diabetes is a major risk factor for myocardial infarction, stroke, renal failure, blindness and peripheral vascular diseases. Out of this number, a shocking 23.8% were unaware of their diagnosis. (CDC National Diabetes Statistics Report, 2017). Diabetes is taking a toll on healthcare expenditure in the United States as the estimated disease burden cost in 2017 was reported to be $327 billion.("Economic Costs of Diabetes in the U.S. in 2017," 2018).

Phthalates are ubiquitous endocrine-disrupting chemicals found in the environment. They are used as plasticizers to impart flexibility and transparency, to improve their longevity, and in personal care and consumer products, to hold color, shine and fragrance. According to the Centers for Disease Control and Prevention's (CDC) National Biomonitoring Program, these chemicals are used in several products including detergents, lubricating oils, vinyl flooring, automatic plastics, plastic raincoats and personal-care products such as soaps, shampoos, hair sprays and nail polishes. (www.cdc.gov/biomonitoring/Phthalates_FactSheet.html). Whether knowingly or unknowingly, we are exposed to these chemicals in our daily routines. Women and ethnic minorities have been known to have higher levels of urinary phthalate metabolites than do men and Non-Hispanic whites. (Manori J. Silva et al., 2004). Differences in lifestyle and behavioral patterns may account for these differences. Current phthalate exposure, however, may not be strongly correlated with historic exposure because of inter- and intra-person variation in exposure, their short biological half-lives, the temporality of exposure and the changing nature of the chemicals used in manufacturer over time.

Phthalates are produced by reacting phthalic anhydride with varying chain lengths of linear or branched alcohols. The molecular weight of phthalates thus varies from 194 amu (dimethyl phthalate) to 531 amu (ditridecyl phthalate). (https://pubchem.ncbi.nlm.nih.gov/compound/phthalate). Phthalates are grouped as either low molecular weight (LMW) phthalates or high molecular weight

(HMW) phthalates. In general, LMW compounds are used in personal care products (PCPs), pharmaceutical coatings and medical devices whereas HMW phthalates are commonly in PVC products such as wires and cables, flooring, synthetic leather, coated fabrics and roofing and automobile applications. (https://phthalates.americanchemistry.com/). Phthalates are used as additives during the manufacturing process and since they are not covalently bound to the product produced, these chemicals tend to leach into the environment, surfaces, food or water with which they contact. Exposure to phthalates is, thus, multi-pathway (e.g., food, water, air, PCPs) and multi-route (e.g., ingestion, dermal, inhalation, injection). (Swan, 2008). Phthalates have relatively short half-lives, so measurement of urinary phthalate metabolites are indicative of current exposures (e.g., typically the past 24-48 hours). Phthalate undergo phase I and phase II metabolism in the liver to produce glucuronide-bound monoester metabolites that are excreted in the urine. The free monoesters (i.e., not glucuronide-bound) are thought to be responsible for all the endocrine-disrupting health effects associated with phthalate exposures. (Frederiksen, Skakkebaek, & Andersson, 2007). Phthalate metabolites can also be measured in urine, pre-treated serum, breast milk and saliva (Hines, Calafat, Silva, Mendola, & Fenton, 2009); amniotic fluid (M. J. Silva et al., 2004); semen (Wang et al., 2016); and ovarian follicular fluid (Du et al., 2019), but urine is the most preferred matrix.

Existing literature identifies linkages between phthalates exposure and diabetes. Phthalates are thought to be associated with diabetes through several pathways including obesity and increased resistance. These endocrine disruptors are thought to bind to the nuclear peroxisome proliferator-activated receptor gamma that are involved in adipose tissue and lipid homeostasis. (Lind, Zethelius, & Lind, 2012). This results in alteration of adipocyte differentiation. The effect is therefore upregulation of adipocyte production which leads to obesity with resultant increase in insulin resistance. (Desvergne, Feige, & Casals-Casas, 2009). Phthalates are also thought to modulate hormones and

inflammatory pathways (Ferguson et al., 2014) with a resultant increase in inflammatory profile and insulin resistance (Wisse, 2004).

Several studies have tried to study the association between various phthalate metabolites and diabetes with mixed results. A study of Swedish elderly people conducted by (Lind et al., 2012) reported that higher levels of serum monomethyl phthalate, monoisobutyl phthalate and monoethyl phthalate were associated with an increased prevalence of diabetes. The metabolites found to be associated with diabetes among the elderly in this study were monoesters of LMW phthalates found in body care products. The metabolite mono-(2-ethylhexyl) phthalate which is a metabolite of HMW phthalate di-(2-ethylhexyl) phthalate was not found to be associated with diabetes. Diabetes is thus associated with certain monoesters of phthalates as compared to others. It could be therefore be hypothesized that the most frequent routes of exposure to these phthalates by the general population places a role in determining the phthalate metabolite(s) associated with diabetes. Study of urinary phthalate metabolite concentrations and diabetes among women in the National Health and Nutrition Examination Survey (NHANES) by (James-Todd et al., 2012) established an association between several urinary phthalate metabolites and prevalent diabetes. In the study, diabetes was associated with higher levels of monoisobutyl phthalate, monobenzyl phthalates, mono-(3-carboxypropyl) phthalates and summation of four di-(2-ethylhexyl) phthalate metabolites as compared to lower levels. The results of the study compared to that of (Svensson et al., 2011) that showed higher levels of di-(2-ethylhexyl) phthalate metabolites to be associated with diabetes among a cross-sectional subset of Mexican women. A cross-sectional study of the Canadian Health Measures Survey (CHM) (2009 – 2011), conducted by (Dales, Kauri, & Cakmak, 2018) reported that increased phthalate metabolite concentrations are associated with reduced glycemic blood control, with di-(2-ethylhexyl) phthalate metabolites also associated with increased fasting glucose concentrations.

RESEARCH DESIGN AND METHODS

Study design

This was cross-sectional study using six National Health and Nutrition Examination Survey (NHANES) 2-year cycle datasets (2003 – 2014). These data are publicly available at www.cdc.gov/nhanes.

Study population

The National Center for Health Statistics at the CDC conducts the National Health and Nutrition Examination Survey (NHANES). This is an ongoing survey that is a representative sample of the US non-institutionalized, civilian population. Certain subgroups of the population including older adults, those of lower socioeconomic status, blacks and Mexican Americans were oversampled in this survey by using complex multi-stage probability sampling strategy. Each year, about 7500 individuals participate in NHANES, and the data are reported in 2-year cycles. Thorough in-home questionnaires, demographic, dietary and behavioral information are collected, while trained assistants collect anthropometric and biological samples using mobile exams unit. Most environmental chemicals including phthalates were measured in a random 1/3 subset which maintains the representativeness of the original sample. (CDC NHANES Sample Design).

Measurement of phthalate metabolites

Phthalate metabolites were measured by CDC's National Center for Environmental Health using previously published methods. (Manori J. Silva et al., 2004). Briefly, urine samples were spiked with isotopically labeled analogues of the target phthalate metabolites then subjected to an enzyme deconjugation to liberate glucuronide-bound metabolites. The samples were analyzed using either off- or in-line solid phase extraction and liquid chromatography-tandem mass spectrometry. The concentrations were quantified using isotope dilution calibration. Appropriate quality control and

assurance methods were incorporated into the analyses. The phthalate metabolites measured in NHANES study participants varied by year, therefore metabolites that were measured in all the years from 2003 – 2014 with > 60% of sample concentrations above the limit of detection (LOD) were chosen for the analysis. (James-Todd et al., 2012). Due to the correlation among di(2-ethylhexyl) phthalate (DEHP) metabolites, the molar sum of four DEHP metabolites that satisfied the above criteria [mono-(2-ethyl)-hexyl phthalate, mono-(2-ethyl-5-hydroxyhexyl) phthalate, mono-(2-ethyl-5-oxohexyl) phthalate and mono-2-ethyl-5-carboxypentyl phthalate) were calculated as one entity (∑DEHP) and included in this analysis.

However, eighteen phthalate metabolites were measured in NHANES at various points from the year 2003 – 2014. These were: mono-n-butyl phthalate, mono-cyclohexyl phthalate, mono-ethyl phthalate, mono-(2-ethyl)-hexyl phthalate, mono-isononyl phthalate, mono-n-octyl phthalate, mono-benzyl phthalate, mono-n-methyl phthalate, mono-(3-carboxypropyl) phthalate, mono-(2-ethyl-5-hydroxyhexyl) phthalate, mono-(2-ethyl-5-oxohexyl) phthalate, mono-isobutyl phthalate, mono-2-ethyl-5-carboxypentyl phthalate, mono(carboxyoctyl) phthalate, mono(carboxynonyl) phthalate, mono(carboxyisononyl) phthalate, mono(carboxyisoctyl) phthalate, cyclohexane 1,2-dicarboxylic acid monohydroxy isononyl ester.

Exposure and outcome variables

Urinary phthalate metabolite concentrations were the exposure variable of interest, with diabetes and prediabetes as the outcome variables.

As a result of the criteria set forth, the urinary phthalate metabolites investigated were mono-n-butyl phthalate (MnBP), mono-(3-carboxypropyl) phthalate (MCPP), mono-ethyl phthalate (MEP), mono-isobutyl phthalate (MiBP), mono-benzyl phthalate (MBzP) and the defined ∑DEHP.

Diabetes status was defined as ever diagnosed with diabetes, other than during pregnancy? (Yes or No), fasting blood glucose (FBG) ≥126mg/dL or glycated hemoglobin ≥6.5%. Prediabetes was defined as FBG levels between 100mg/dL and 125mg/dL, or glycated hemoglobin between 5.7% to 6.4%.("Standards of Medical Care in Diabetes. American Diabetes Association," 2018).

Covariates

Covariates assessed in the study included age, race/ethnicity, sex, body mass index (BMI), physical activity, total caloric and fat intakes, socioeconomic status and educational status.

Respondents age were grouped as 20 – 29, 30 – 39, 40 – 49, 50 – 59, 60 – 69 or 70 – 79. The lower adult age limit was restricted to 20 years because under the NHANES demographic dataset, educational level was grouped into 2 sets (Children/Youth 6 – 19; Adults 20+). It was therefore more convenient to use age 20 as the lower limit for age since educational status was also considered a covariate in the analysis. The upper age limit was restricted to less than 80 years since NHANES classifies participants greater than 80 years as one age group, either 80 or 85 years for confidentiality issues. Race was classified as non-Hispanic white (reference), non-Hispanic black, Mexican American or other. Sex was clinically classified as either male or female. BMI was classified as either underweight (< 18.5 kg/m^2), normal weight [18.5 – 24.9 kg/m^2 (reference)], overweight (25 kg/m^2 – 29.9 kg/m^2) or obese (≥ 30 kg/m^2). Physical activity was classified as self-reported vigorous or moderate activity [Yes or No (reference)] over past 30 days as documented in NHANES. The total caloric and fat intakes for first obtained from dietary interview were used in the analysis. These values were grouped into quartiles, with the lowest quartile used as reference. To assess socioeconomic status, poverty-income ratio (PIR) in the demographic dataset was used and classified as PIR < 1 or ≥ 1 (reference) based on household income information and federal poverty threshold. (Ali et al., 2011). Educational status was classified as

less than or equal to high school graduate (reference), some college and college graduate or more education. (James-Todd et al., 2012).

Data sets

Data set drawn from NHANES and used in the analysis included:

- Demographic dataset (prefix Demo) which provides information on demographic data that were considered covariates in the study
- Physical Activity (prefix PAQ) which is based on the Global Physical Activity Questionnaire (GPAQ) and includes questions related to daily activities, leisure time activities and sedentary activities
- Dietary Interview – Total Nutrient Intakes, First Day (prefix DR1TOT). This data provides the types and amounts of foods and beverages and the determines their energy and nutrients consumed 24 hours prior to the interview
- Body mass index dataset (prefix BMX) which provides information on weight, height and calculated body mass indexes of participants
- Consumer Behavior questionnaire (prefix CBQ) which provides interview data on food expenditure at the family level
- Phthalate dataset (prefix PHTHE) which provides information on the fifteen (15) urinary phthalate metabolites and their comments, as well as urinary creatinine information
- Diabetes section dataset (prefix DIQ) which provides personal interview data on diagnosis of diabetes and prediabetes, age at which one was diagnosed with diabetes/prediabetes, insulin or oral hypoglycemic agent usage
- Glucose dataset (prefix Glu) which provides information on fasting blood glucose

- Glycated hemoglobin dataset (prefix GHb) which provides information on glycated hemoglobin levels of participants

  (https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx)

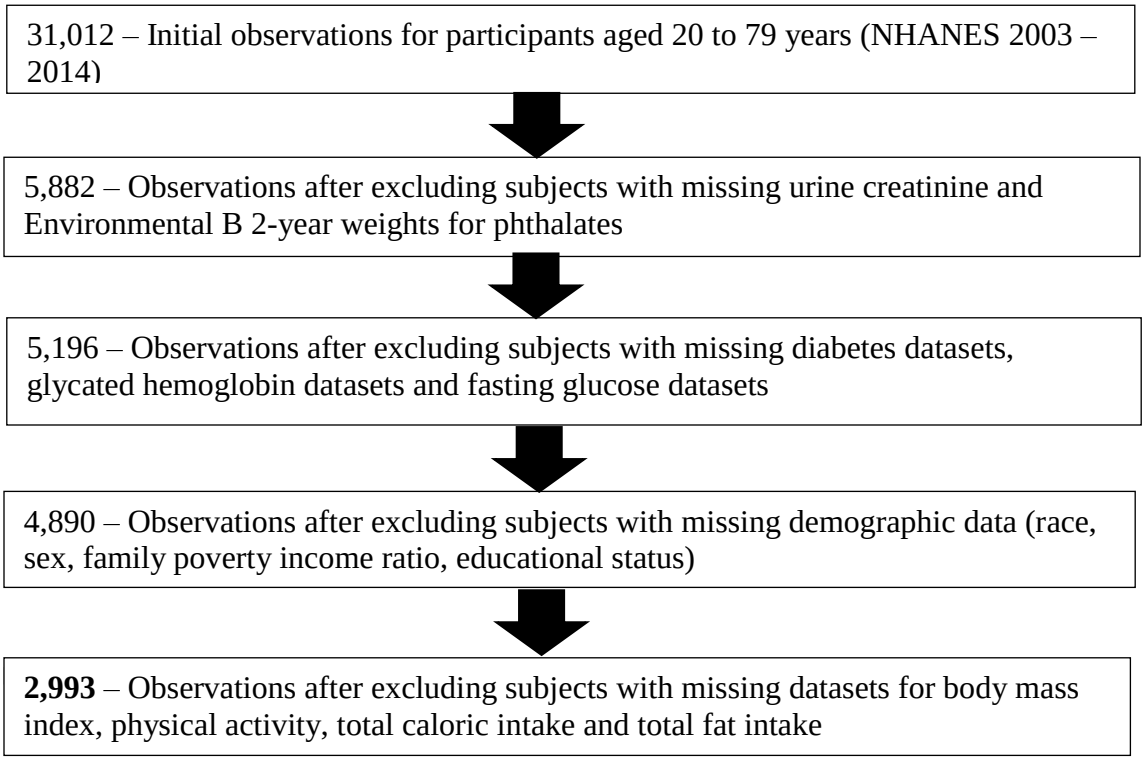
Statistical analysis

The association between urinary phthalate metabolites and the outcomes (prediabetes and diabetes) was assessed using multivariate logistic regression analysis, considering the strata, cluster and sample weight variables. Observations with missing covariate variables of interest were excluded in the analysis. The urinary phthalate metabolite concentrations were not normally distributed, so their log transformed values were used in the analysis. Descriptive analyses of all variables were done and the associations between all the categorical covariates and outcome variables were evaluated. Each phthalate metabolite was evaluated in quartiles, with the lowest quartile, Q1 used as reference group. 2 separate models were constructed to estimate the association between each phthalate metabolite. Model 1 assessed the crude association between each phthalate and prevalent diabetes whilst model 2 evaluated the association between the metabolites with all covariates inclusive. In both models, adjustment for urinary creatinine was done to account for variations that may result from the varied dilutions of urine that can influence measured urinary concentrations of phthalate metabolites. This is a more favorable alternative to the historically used creatinine-corrected phthalate levels. (Barr et al., 2005).

The logistic regression using PROC SURVEYLOGISTICS, and including strata, cluster and weights from the phthalate dataset as per NCHS/CDC analytic guidelines commands was used to estimate the prevalence odds ratios (OR) and 95% confidence intervals as a measure of the association between the urinary phthalate metabolites and outcomes (prediabetes and diabetes). (CDC 2011e). The SAS software version 9.4 was used for all the analyses.

RESULTS

Exclusion criteria



Descriptive analysis

A total of 2,993 study participants were evaluated in this study, after excluding missing values in variables used for the analysis. Extreme observations detected through descriptive studies were also excluded from the final analysis. A diagnosis of diabetes was made in 162 participants (5.41%). Prediabetes was defined in only 2 participants and as such were added to the non-diabetic group. The population under study could be classified as a younger adult population since 57.6% are aged less than 50. Table 1 summarizes the characteristics of the study population and includes the geometric means with 95% confidence interval of the selected urinary phthalate metabolites among the various subgroups.

Associations between covariates and diabetes

The Rao-Scott modified chi-square test of association between the individual covariates and diabetes was calculated, with results shown in Table 2. The table also shows the distribution of the outcome variable diabetes among the various subgroups of the covariates. In summary, there were no associations found between any of the covariates and diabetes (all p values > 0.05).

Associations between urinary phthalate metabolites and diabetes

The results of the logistic regression to determine the association between each phthalate metabolite (evaluated in quartiles) and diabetes are presented in Table 3. For model 1, depicting the crude associations, MnBP was found to be statistically significantly associated with prevalent diabetes, with the fourth quartile (Q4) producing 2.52 increased odds of diabetes (95% CI: 1.39, 4.57). The other metabolites, MCPP, MEP, MiBP, MBzP and ∑DEHP were not found to have statistically significant crude association with diabetes. For MCPP, MEP, MBzP and ∑DEHP, it was noted that values of these metabolites greater than the median value were associated with increased odds of diabetes, even though the associations were not statistically significant. Although MCPP and MEP did not show statistically significant association with diabetes, they depict uniform increases in OR and associations across the quartiles from Q2 to Q4 whilst MnBP, MiBP, MBzP and ∑DEHP showed a non-uniform increase in association with diabetes across the same quartiles.

Inclusion of the covariates in model 2 did not affect the already established crude association between the phthalate metabolites and diabetes. MnBP still remained the only phthalate metabolite that was statistically significantly associated with diabetes. The same pattern and trend seen in model 1 were repeated in model 2.

Table 1. Study population characteristics showing geometric means with 95% confidence interval of adults aged 20 – 79 years (NHANES 2003 – 2014)

Characteristic	N (%)	MnBP	McPP	MEP	MiBP	MBzP	∑DEHP
Total	2,993 (100)	15.7 (14.7, 16.8)	2.5 (2.4, 2.7)	82.1 (76.1, 88.5)	6.0 (5.7, 6.4)	7.1 (6.7, 7.5)	54.1 (50.6, 57.7)
Age (years)							
20 – 29 (Ref.)	630 (21.05)	15.0 (13.4, 16.9)	2.4 (2.2, 2.7)	79.9 (67.3, 94.8)	6.0 (5.3, 6.8)	7.1 (6.0, 8.3)	52.7 (45.9, 60.4)
30 – 39	548 (18.31)	16.4 (14.7, 18.3)	2.6 (2.3, 2.9)	73.8 (62.9, 86.5)	6.4 (5.5, 7.5)	7.1 (6.3, 7.9)	55.8 (49.5, 62.9)
40 – 49	546 (18.24)	16.1 (13.3, 19.6)	2.8 (2.4, 3.2)	79.4 (67.2, 93.8)	6.4 (5.5, 7.4)	7.2 (6.2, 8.3)	52.3 (45.3, 60.3)
50 – 59	428 (14.30)	14.8 (12.8, 17.2)	2.4 (2.0, 2.9)	89.2 (74.0, 107.5)	5.5 (4.8, 6.4)	6.4 (5.4, 7.6)	53.4 (44.1, 64.7)
60 – 69	479 (16.00)	15.8 (14.0, 17.8)	2.3 (2.0, 2.6)	93.3 (75.8, 114.8)	5.7 (5.0, 6.4)	7.4 (6.5, 8.4)	54.3 (47.5, 62.1)
70 – 79	362 (12.09)	16.6 (14.1, 19.5)	2.7 (2.2, 3.2)	79.6 (69.4, 91.2)	6.1 (5.2, 7.2)	7.3 (6.0, 8.8)	57.0 (47.8, 68.0)
Race/Ethnicity							
Mexican American	612 (20.45)	15.2 (13.1, 17.6)	2.4 (2.1, 2.7)	86.4 (77.0, 96.9)	5.8 (5.2, 6.6)	6.3 (5.5, 7.2)	48.7 (42.1, 56.3)
Other	208 (6.95)	19.9 (16.1, 24.7)	2.5 (2.1, 2.9)	110.4 (80.8, 150.8)	6.1 (5.0, 7.4)	8.5 (6.8, 10.6)	63.1 (48.0, 83.1)
Non-Hispanic white (Ref.)	1,486 (49.65)	15.8 (14.7, 17.1)	2.5 (2.3, 2.7)	79.6 (70.1, 90.3)	6.0 (5.7, 6.3)	7.2 (6.8, 7.7)	54.5 (48.6, 61.1)
Non-Hispanic black	687 (22.95)	15.1 (13.3, 17.0)	2.7 (2.4, 2.9)	77.0 (67.5, 87.9)	6.3 (5.7, 6.9)	7.2 (6.4, 8.1)	56.2 (47.5, 66.4)
Sex/Gender							
Male (Ref.)	1408 (47.04)	15.8 (14.4, 17.5)	2.5 (2.3, 2.7)	83.2 (75.0, 92.3)	5.9 (5.4, 6.5)	7.0 (6.5, 7.7)	55.3 (50.3, 60.7)
Female	1585 (52.96)	15.7 (14.6, 16.8)	2.5 (2.3, 2.8)	81.1 (73.5, 89.4)	6.1 (5.7, 6.5)	7.1 (6.5, 7.8)	53.1 (48.4, 58.1)
Education							
≤ High school (Ref.)	1,582 (52.86)	15.7 (14.0, 17.6)	2.5 (2.2, 2.8)	80.5 (73.2, 88.6)	6.1 (5.5, 6.8)	7.0 (6.2, 7.8)	54.2 (48.8, 60.2)
Some college	830 (27.73)	16.4 (14.8, 18.1)	2.7 (2.4, 2.9)	79.9 (67.5, 94.6)	6.2 (5.5, 6.9)	7.3 (6.6, 8.0)	57.4 (52.2, 63.1)
≥ College graduate	581 (19.41)	15.1 (13.1, 17.3)	2.4 (2.1, 2.7)	90.0 (75.5, 107.2)	5.6 (5.0, 6.4)	7.0 (6.1, 8.1)	49.0 (42.7, 56.3)
Socioeconomic status (PIR)							
Below poverty (< 1)	587 (19.61)	15.3 (13.7, 17.2)	2.6 (2.2, 2.9)	73.0 (62.1, 85.9)	6.0 (5.2, 6.8)	6.5 (5.6, 7.4)	55.2 (48.1, 63.5)
Below poverty (≥ 1) (Ref.)	2,406 (80.39)	15.8 (14.8, 17.0)	2.5 (2.3, 2.7)	84.4 (77.5, 91.9)	6.0 (5.7, 6.4)	7.2 (6.8, 7.6)	53.8 (49.6, 58.3)
Moderate or vigorous							

physical activity over past 30 days							
Yes	2,041 (68.19)	15.6 (14.5, 16.9)	2.5 (2.3, 2.7)	84.1 (76.1, 92.9)	6.0 (5.6, 6.4)	7.0 (6.5, 7.6)	55.9 (51.6, 60.6)
No	952 (31.81)	15.9 (14.4, 17.7)	2.5 (2.3, 2.8)	77.7 (68.5, 88.1)	6.1 (5.5, 6.8)	7.1 (6.4, 8.0)	50.1 (44.7, 56.1)
BMI (kg/m^2)							
Underweight (< 18.50) (Ref.)	592 (19.78)	14.0 (12.1, 16.3)	2.4 (2.1, 2.8)	65.2 (55.1, 77.1)	6.0 (5.3, 6.8)	6.7 (5.6, 8.0)	50.5 (42.8, 59.5)
Normal (18.50 – 24.99)	1,028 (34.35)	16.4 (14.5, 18.5)	2.7 (2.4, 3.0)	85.2 (75.8, 95.9)	5.9 (5.3, 6.6)	7.0 (6.3, 7.9)	57.7 (50.8, 65.6)
Overweight (25.00 – 29.99)	696 (23.25)	15.6 (13.5, 18.0)	2.5 (2.1, 2.8)	74.8 (64.9, 86.1)	6.5 (5.8, 7.3)	7.3 (6.3, 8.4)	55.0 (47.0, 64.4)
Obese (≥ 30)	677 (22.62)	16.5 (15.0, 18.1)	2.4 (2.2, 2.7)	104.2 (90.4, 120.0)	5.7 (5.2, 6.3)	7.3 (6.5, 8.1)	50.9 (45.7, 56.8)
Total energy (kcal)							
< 1,430 (Ref.)	743 (24.82)	15.9 (13.9, 18.2)	2.5 (2.2, 2.7)	85.1 (73.6, 98.4)	6.2 (5.4, 7.1)	7.1 (6.1, 8.3)	50.0 (44.0, 56.7)
1,430 to <1,910	749 (25.03)	16.6 (14.5, 18.9)	2.7 (2.4, 3.0)	83.6 (71.9, 97.2)	6.4 (5.6, 7.4)	7.7 (6.7, 9.0)	56.3 (49.1, 64.5)
1,910 to < 2569	750 (25.06)	15.6 (13.9, 17.4)	2.6 (2.3, 2.8)	79.6 (69.8, 90.9)	5.6 (5.1, 6.3)	7.1 (6.3, 8.0)	56.0 (49.1, 63.8)
≥ 2569	751 (25.09)	15.0 (13.6, 16.5)	2.4 (2.1, 2.7)	80.1 (69.7, 92.1)	5.8 (5.2, 6.5)	6.4 (5.8, 7.1)	54.2 (48.2, 60.9)
Total fat (g)							
< 48.01 (Ref.)	746 (24.92)	15.5 (13.8, 17.3)	2.4 (2.2, 2.6)	81.2 (71.3, 92.6)	5.9 (5.2, 6.5)	6.9 (6.0, 7.9)	50.1 (43.9, 57.3)
48.01 to <69.76	746 (24.92)	15.7 (13.9, 17.9)	2.6 (2.3, 3.0)	87.2 (73.4, 103.5)	6.0 (5.4, 6.7)	7.4 (6.6, 8.2)	55.0 (47.9, 63.1)
69.76 to < 99.10	750 (25.06)	16.9 (15.2, 18.7)	2.7 (2.4, 3.0)	79.1 (67.5, 92.8)	6.5 (5.8, 7.2)	7.6 (6.6, 8.7)	56.7 (50.1, 64.3)
≥ 99.10	751 (25.09)	14.9 (13.7, 16.3)	2.4 (2.1, 2.6)	81.1 (70.8, 92.8)	5.7 (5.1, 6.4)	6.4 (5.8, 7.1)	54.6 (50.4, 59.3)
Outcome							
Diabetes	162 (5.41)	20.3 (15.9, 25.9)	2.8 (1.9, 4.1)	101.0 (77.9, 131.0)	7.0 (5.5, 8.7)	9.4 (6.5, 13.5)	64.1 (44.6, 92.1)
No diabetes	2,831 (94.59)	15.5 (14.6, 16.6)	2.5 (2.3, 2.7)	81.2 (74.8, 88.2)	6.0 (5.7, 6.3)	7.0 (6.6, 7.4)	53.6 (50.2, 57.1)

Table 2. Associations between covariates and diabetes of adults aged 20 – 79 years. The p-value results indicate that there were not statistically significant associations between each of the covariates and the outcome of interest, diabetes at the alpha level of 0.05 (all p-values > 0.05). (NHANES 2003 – 2014).

Characteristic	Diabetes 154 (5.34)	No Diabetes 2,721 (94.66)	Rao-Scott F Modified Chi-Square Test with p-value
Age (years)			0.5446 (p value = 0.7422)
20 – 29 (Ref.)	30	576	
30 – 39	27	500	
40 – 49	29	503	
50 – 59	25	387	
60 – 69	29	424	
70 – 79	14	331	
Race/Ethnicity			0.7805 (p value = 0.509)
Mexican American	26	578	
Other	9	185	
Non-Hispanic white (Ref.)	73	1,342	
Non-Hispanic black	46	616	
Sex/Gender			0.0103 (p value = 0.9199)
Male (Ref.)	75	1,270	
Female	79	1,451	
Education			1.7379 (p value = 0.1846)
≤ High school (Ref.)	85	1,433	
Some college	37	763	
≥ College graduate	32	525	
Socioeconomic status (PIR)			0.7079 (p value = 0.4068
Below poverty (< 1)	26	542	
Below poverty (≥ 1) (Ref.)	128	2,179	
Moderate or vigorous physical activity over past 30 days			0.0160 (p value = 0.9003)
Yes	103	1,847	
No	51	874	
BMI (kg/m^2)			1.5546 (p value = 0.2060)
Underweight (< 18.50) (Ref.)	23	541	

Normal (18.50 – 24.99)	53	935	
Overweight (25.00 – 29.99)	38	630	
Obese (≥ 30)	40	615	
Total energy (kcal)			0.7411 (p value = 0.5303)
< 1,430 (Ref.)	35	680	
1,430 to <1,910	48	662	
1,910 to < 2569	34	692	
≥ 2569	37	687	
Total fat (g)			0.5224 (p value = 0.6680)
< 48.01 (Ref.)	39	670	
48.01 to <69.76	38	679	
69.76 to < 99.10	41	682	
≥ 99.10	36	690	

Table 3. Association [OR (95% CI)] between each urinary phthalate metabolite and diabetes of adults aged 20 – 79 years. Model 1 represents the crude association adjusting for only urinary creatinine. Model 2 represents adjusted model, adjustment for urinary creatinine and all the covariates. (NHANES 2003 – 2014)

Urinary phthalate metabolite	Model 1	Model 2
MnBP		
Q1	Ref.	Ref.
Q2	1.20 (0.49, 2.92)	1.14 (0.46, 2.84)
Q3	1.11 (0.50, 2.48)	1.16 (0.50, 2.67)
Q4	2.52 (1.39, 4.57) *	2.53 (1.40, 4.61) *
MCPP		
Q1	Ref.	Ref.
Q2	0.88 (0.52, 1.50)	0.88 (0.51, 1.52)
Q3	1.04 (0.51, 2.14)	1.07 (0.51, 2.23)
Q4	1.09 (0.46, 2.60)	1.09 (0.47, 2.52)
MEP		
Q1	Ref.	Ref.
Q2	1.23 (0.78, 1.94)	1.27 (0.79, 2.03)
Q3	1.31 (0.74, 2.31)	1.40 (0.74, 2.63)
Q4	1.44 (0.86, 2.43)	1.50 (0.84, 2.69)
MiBP		
Q1	Ref.	Ref.
Q2	1.26 (0.79, 2.00)	1.23 (0.79, 1.94)
Q3	1.15 (0.63, 2.09)	1.14 (0.64, 2.02)
Q4	1.38 (0.80, 2.38)	1.38 (0.82, 2.32)
MBzP		
Q1	Ref.	Ref.
Q2	0.58 (0.28, 1.22)	0.57 (0.27, 1.20)

Q3	1.46 (0.77, 2.77)	1.41 (0.74, 2.67)
Q4	1.28 (0.66, 2.49)	1.26 (0.64, 2.48)
∑DEHP		
Q1	Ref.	Ref.
Q2	0.81 (0.37, 1.77)	0.81 (0.38, 1.71)
Q3	1.34 (0.71, 2.51)	1.37 (0.72, 2.59)
Q4	1.14 (0.61, 2.13)	1.18 (0.62, 2.24)

*denotes statistical significance at alpha=0.05.

DISCUSSION

The upper quartile level of urinary MnBP, which is a metabolite of the parent phthalate diesters di-n-butyl phthalate (DBP) and benzylbutyl phthalate (BzBP), was statistically significantly associated with increased odds (2.5x) of prevalent diabetes. The other five phthalate metabolites were not statistically significantly associated with prevalent diabetes even though the highest association was also reported at the highest quartile level for three of the metabolites. From our study, it was observed that exposures to higher than median levels of phthalate metabolites MEP, MiBP, MBzP and ∑DEHP are associated with diabetes even though not statistically significant. The finding of the association between MnBP and diabetes in the crude and adjusted models of our study is consistent with the findings of (James-Todd et al., 2012). Also, a linear trend of association was noted between the phthalate metabolites MCPP and MEP, and diabetes in which increasing quantiles were associated with increasing odds of prevalent diabetes. Our results are also comparable to (Svensson et al., 2011) that reported no statistically significant association between MEP, MiBP, MBzP metabolites and diabetes both in the crude and adjusted models.

The NHANES dataset is large and robust and accounts for the disparity in population dynamics in the United States. The use of these data for analysis therefore gives a better representation of the population under study and allows for objective, albeit simultaneously collected, measures of exposure and outcome variables to be used in the analysis. In this study, diabetes was not only classified

based on self-reported diagnosis, but the fasting blood glucose and glycated hemoglobin values of subjects were considered in defining and coding diabetes status. This is a more consistent approach considering that diabetes is a laboratory diagnosis with the new guidelines for diagnosis defined in ("Standards of Medical Care in Diabetes. American Diabetes Association," 2018).

Our study has several limitations. The cross-sectional design of the study does not allow for causal inference and interpretation can be further complicated by the potential for reverse causality. LMW phthalates are used in personal care products, pharmaceutical coatings and medical devices. It is possible that individuals with diabetes and on medications with coatings contain these LMW phthalates are more likely to have higher levels of these compounds in their bodies compared to non-diabetes who may not be taking medications. Diabetics are also more likely to have a hospital admission compared to non-diabetics and therefore may be exposed to higher levels of phthalates through infusion bags, blood-giving IV sets and other medical devices employed in the management of diabetics in the hospital. Also, single urine spot urine samples were used to measure phthalate metabolites, and this does not account for temporal changes in exposure which we know are occurring. Also, current phthalate exposure may not be strongly correlated with historic exposure due to variation in individual exposure and the changing nature of the chemicals by manufacturers over time period.

Longitudinal studies with more exposure measured to capture temporality in exposures are proposed for future research to assess the association between phthalate exposure and diabetes. Proposed future study should also look at distinguishing the association between phthalates and the two main types of diabetes (type 1 and type 2) to determine which type is more likely associated with phthalate exposure.

In conclusion, urinary phthalate metabolites are associated with prevalent diabetes in this cross-sectional study, with the LMW phthalate MnBP showing a statistically significant association.

Higher than median levels of phthalates are also associated with higher association among the general population.

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