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Retrospective Cohort Study

Association between urinary concentrations of bisphenol A substitutes and diabetes in adults

Moreno-Gómez-Toledano R *et al*. Urinary BPA substitutes and diabetes

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Abstract

BACKGROUND

Due to new restrictions on the use of bisphenol A (BPA), industries are beginning to replace it with derived molecules such as bisphenol S and F (BPS and BPF). There is extensive evidence in the academic literature on the potential health effects of BPA, which is known to be a diabetogenic molecule. However, there are few publications related to new compounds derived from BPA.

AIM

The objective of the work is the epidemiological study of urinary BPS and BPF in the American National Health and Nutrition Examination Survey (NHANES) cohort, analyzing its possible relationship with diabetes mellitus.

METHODS

NHANES datasets from 2013 to 2016 were used due to the urinary BPF and BPS availability. Data from 3658 adults were analyzed to perform regression models exploring the possible relationship between BPA-derived compounds and diabetes.

RESULTS

Descriptive statistics, linear regression modeling, and logistic regression models have found a significant relationship between urinary BPS, but not BPF, with diabetes risk. Additionally, a relationship has been observed between both compounds and hypertension and a slight relationship between BPF and dyslipidemia.

CONCLUSION

In the present study, a strong relationship between urinary BPS and diabetes risk has been determined, not observed with BPF. BPA substitute molecules do not exempt the population from potential health risks.

Key Words: Bisphenol S; Bisphenol F; Diabetes mellitus; National Health and Nutrition Examination Survey; Urine

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Core Tip: Molecules derived from bisphenol A (increasing use in the plastic industry and the production of heat-sensitive tickets) could be related to pathologies such as diabetes (bisphenol S) and hypertension (bisphenol S and F).

INTRODUCTION

In the last decades, the demand and production of plastic polymers have increased substantially. Both its production and recycling involve the release of pollutants, xenobiotic compounds that should not be found in the air, rivers, or the human population[1,2]. One of the most important, due to its wide distribution and variety of biological effects, is bisphenol A (BPA)[3-6]. The European Chemicals Agency has recently included BPA within the Candidate List of substances of very high concern due to its properties as an endocrine disruptor and its potentially harmful effect on reproduction[7]. Furthermore, the European Union has restricted the use of this substance in thermal paper due to its potential danger to the health of exposed workers[8].

Therefore, the need to seek alternatives to BPA is a fact of vital importance for modern industry. Currently, two known compounds are bisphenol S (BPS) and bisphenol F (BPF), which can already be found in BPA-free packaging and thermal tickets regulated by European legislation[9,10]. The use of these derivatives does not imply a reduction in possible adverse effects *per se*; it only indicates the use of new materials whose safety has not yet been tested. Comparative studies between BPA substitutes have shown that both BPF and BPS are as hormonally active as BPA[9], so they could also be included in the category of endocrine disruptors.

Diabetes mellitus (DM), and its associated complications, are a medical catastrophe of global dimensions[11]. The number of people affected has risen from 108 million affected in 1980[12] to almost 500 million today[13]. The latest estimates suggest that it could rise to 578 million in 2030 and 700 million in 2045[13]. Risk causes for the disease include a combination of genetic and metabolic factors. There are non-modifiable factors, such as ethnicity or age, and modifying factors, such as diet, obesity, or smoking[14]. The multiplicity of factors that influence the development of the disease implies that environmental pollutants could also affect it. There is evidence that BPA exposure correlates with the risk of developing DM[15].

However, new alternative compounds to BPA have only been used in modern industry for a short time. For this reason, few academic publications study its possible relationship with diabetes. The first pieces of evidence have been detected in a cellular experimental model[16], in males (but not females) of a murine experimental model[17], and in human cohorts from China[18,19] and France[20]. However, it has not yet been studied in one of the world's largest urinary bisphenol cohorts, the American National Health and Nutrition Examination Survey (NHANES). Studies in this cohort have demonstrated the presence of BPF and BPS in urine, observing positive and statistically significant relationships with disorders such as asthma[21], obesity[22-24], or depression[25]. Obesity is closely related to diabetes[26-28], so studying its relationship with new environmental pollutants is coherent and necessary. The present work aims to correlate, for the first time in the NHANES cohort, diabetes with the urinary concentration of BPA substitutes using regression models.

MATERIALS AND METHODS

NHANES 2013-2016 population

The NHANES datasets from 2013 to 2016 were used in the present statistical model due to the urinary BPF and BPS availability. In the first phase, the data of all the study participants were extracted through the official website of the Centers for Disease Control and Prevention[29] (accessed December 01, 2021), obtaining 20146 individuals. Subsequently, the individuals with available BPS and BPF were selected, obtaining 5333 subjects, of which 3699 were adults (over 18 years of age). Data from 3658 patients could be used for regression models (complete data). Subsequently, two classifications were made: Group 1 was performed with individuals with and without diabetes. Groups 2 and 3 were performed by analyzing the individuals based on the concentration of urinary phenols (BPS for group 2 and BPF for group 3).

All individuals whose doctor had diagnosed them with diabetes, those taking blood glucose medication, and individuals with fasting glucose values ≥ 126 mg/dL or hemoglobin A1c ≥ 6.5% were included in the diabetic group. The individuals classified

according to the concentration of phenols were divided into four quartiles for BPS and BPF (Q1-Q4). BPS and BPF values were corrected for urinary creatinine to normalize variations due to hydration or glomerular filtration capacity[30].

Binary logistic regression models were corrected for factors such as age, sex, body mass index (BMI), smoking, hypertension, or dyslipidemia. All those patients diagnosed by their doctor, those with medication for hypertension, individuals with systolic pressure ≥ 140 mmHg or systolic ≥ 90 mmHg were considered hypertensive. The patients included in dyslipidemia were those with diagnosed cholesterol disorders, with prescribed medication or fasting total cholesterol ≥ 240 mg/dL[31]. For smoking, all individuals who answered affirmatively to the question "have you smoked more than 100 cigarettes in your life?" or individuals with serum cotinine values greater than 10 mg/dL [32] were included.

Statistical analysis

The IBM SPSS Statistics 27 program was used for the statistical analyses to carry out linear regression and logistic regression models, and the GraphPad Prism 7.0 program was used for basic descriptive statistics and comparative analysis. In the comparative analysis of the diabetes subgroup, the Mann-Whitney test was used. In the case of classification based on the phenol quartile, the Kruskal-Wallis test was used. The linear regression analysis used the R-squared coefficient of determination to define the percentage of change in the dependent variable affected by the independent variable. The ANOVA test was used to validate the statistical significance of the coefficient. Finally, the β coefficients and their statistical significance were calculated.

Since the diabetes variable is dichotomous, a binary logistic regression model was performed. BPF and BPS values were analyzed with the corresponding correction with urinary creatinine, using their logarithmic transformation to normalize the non-parametric distribution. Three different regression analyzes were performed for each parameter: individual (1), corrected for age, sex, and BMI (2), corrected for the above parameters and smoking, hypertension, and dyslipidemia (3).

In the study of groups 2 and 3, a multinomial logistic regression model was used. As in the previous statistical model, age, sex, BMI, smoking, hypertension, and dyslipidemia were also included. In all cases, those results whose p-value was less than 0.05 were interpreted as statistically significant.

RESULTS

Implant data

Descriptive statistical analyzes showed, in addition to the expected differences related to blood glucose, interesting changes in BPS levels, significantly higher in diabetic patients. However, the BPF values did not show significant variations. In addition, diabetic patients had higher age, BMI, and systolic pressure and lower total cholesterol (Table 1).

Descriptive analyzes of group 2 (distributed according to BPS quartile) showed that individuals with a higher concentration of BPS (Q4) had a significant increase in BMI, fasting glucose, and BPF than individuals with a lower level of BPS (Q1) (Table 2). In addition, the percentages showed a positive and dose-dependent relationship between the BPA quartile and the number of patients with diabetes, hypertension, and dyslipidemia. Interestingly, the percentage of men showed a negative trend with urinary BPS concentration, and a positive trend was observed between the percentage of individuals with diabetes, hypertension, dyslipidemia, and urinary BPS concentration.

On the other hand, the descriptive analyzes of group 3 (distributed according to the BPF quartile) showed significant age differences (in quartiles 2, 3, and 4), BMI (Q2 and Q3), and cotinine (the quartile 4 had a significantly higher concentration than the other three quartiles) (Table 3). In this group, no significant differences were observed in the parameters related to diabetes, but an interesting positive relationship was observed in the percentage of individuals with hypertension.

Simple linear regression

Linear regression analyses were performed using fasting glucose and hemoglobin A1c (HbA1c) values to explore the relationship between diabetes and phenols (Table 4). As shown in Table 5, the results were significant in the BPS group, while BPF did not show a statistical relationship with these parameters.

Binary logistic regression

The subsequent binomial logistic regression models performed on the dichotomous dependent variable diabetes confirmed the data observed in the linear regression. Thus, it was observed that the urinary concentration of BPS, both individually and corrected with other factors, is an independent factor related to diabetes mellitus. However, this relationship could not be determined in the urinary concentration of BPF.

Multinomial logistic regression

This statistical analysis model showed a significant relationship between diabetes and BPS, but not BPF (Table 6). However, statistically significant data were only observed in the first two models (individual and corrected for sex, age, and BMI). Although it did not become significant when corrected with all the parameters, the resulting p-value was 0.063.

Interestingly, in the BPS study, Q4 individuals showed a positive and significant relationship with gender, with an OR (95%CI) of 1.94 (1.61-2.35) for women. An important relationship was also observed in the risk of suffering hypertension, with an OR of 1.26 (1.01-1.57). In the BPF study, the same significant relationship was observed in gender, with an OR of 2.13 (1.75-2.58) for women. Finally, a positive relationship was observed with smoking [OR, 95%CI of 1.78 (1.47-2.17)] and slightly negative with the BMI [0.98 (0.97-0.998)].

Complementary study of significant pathologies in regression models

Due to the results observed in the regression models and the trends observed in the descriptive statistics, a binomial logistic regression model was carried out, using

hypertension or dyslipidemia as the dependent variable, in order to relate the risk of suffering from any of them depending on the concentration of urinary phenols. As shown in Table 7, urinary BPS is an independent factor related to hypertension. BPF, on the other hand, showed a statistically significant relationship when analyzed individually with both hypertension and dyslipidemia. This relationship held when correcting for age, sex, and BMI for hypertension, but not for dyslipidemia. Finally, no significant relationship was determined after correction with the rest of the parameters.

DISCUSSION

In the present work, it has been demonstrated, for the first time in the NHANES cohort, that BPS, but not BPF, is related to diabetes. The academic literature includes few publications that explore the BPS-diabetes or BPF-diabetes paradigm. There are only three relevant epidemiological studies; two studied type 2 diabetes[18,20], and the remaining investigated gestational diabetes[19].

Duan *et al*[18] considered that all individuals with fasting glucose ≥ 7.0 mmol/L or HbA1c ≥ 6.5% had type 2 diabetes mellitus. After performing the logistic regression analysis, they determined an OR (95%CI) of 1.73 (1.37-2.18) for the urinary BPS, analogous to the results observed in this study.

Rancière *et al*[20] conducted a longitudinal study analyzing the cases of type 2 diabetes developed over nine years in the DESIR cohort. Due to the low rate of detection of urinary BPS (less than 15%), the statistical model was carried out comparing individuals with detectable levels of BPS with those in whom the compound had not been detected, obtaining a higher (significant) risk of developing type 2 diabetes in those individuals with detectable levels of BPS. The detection rate in the NHANES cohort was 57.1% and 88.4% for BPF and BPS, respectively[21]. The results also support and reaffirm those obtained in the present work, although the difference in the detection ratio is very striking. In the case of Völkel *et al*[33,34] exclusively quantified BPS-glucuronide, the main metabolized form according to human pharmacokinetic models. In the case of the NHANES cohort, the total concentration of bisphenol was analyzed after previously

deconjugating the metabolized forms (glucuronide and sulfate) with Helix pomatia enzymes.

Lastly, Zhang et al[19] analyzed BPS and BPF in a cohort of Chinese pregnant women to study their possible relationship with gestational diabetes mellitus. Interestingly, quantitative analyses detected BPS and BPF in most urine samples from pregnant women (greater than 90% in both cases). However, the regression models did not show significant relationships with either compound. They only determined a slight but significant increase in glucose related to urinary BPS concentration. Finally, when studying the relationship between blood glucose and urinary BPS according to fetal sex, they observed that the relationship was more significant in the case of female fetuses.

The present manuscript has determined that there is a significant relationship between urinary BPS and diabetes. However, such a relationship was not observed with urinary BPF. From a molecular point of view, it is interesting to note that BPF, like BPA, has carbon and hydrogen atoms, while BPS also contains sulfur atoms[19]. There is conflicting evidence in the academic literature between BPA and diabetes. Thus, some studies observe a positive and significant relationship with diabetes mellitus[35,36] or prediabetes[37], while others do not find a significant relationship[38]. In addition, there are even works, such as Wang et al[39], in which they determined that pregnant women with higher levels of urinary BPA had a lower risk of developing gestational diabetes.

Interestingly, both BPS and BPF (like BPA) have been shown to have pro-estrogenic and anti-androgenic activity[40]. In pancreatic cell cultures, it has been observed that both BPS and BPF can negatively affect insulin secretion and ion channels through a signaling mechanism that includes estrogen receptor beta[16]. A recent animal study developed by Qiu et al[41] observed that BPF and BPS produced similar effects on the immune system in zebrafish. In an experimental non-obese diabetic mouse model, it has been observed that BPS could negatively affect glucose homeostasis in males, while a protective effect was observed in females[17]. From a mechanistic point of view, bisphenols have the potential to affect the development of diabetes through different pathways. In addition to the classical estrogen receptors (ER-α, ER-β, G protein-coupled

receptor 30), BPA has been shown to have increased binding capacity to the estrogen-related receptor (ERR-γ)[43]. The ERR-γ is important in diabetes since it plays an essential role in correctly maturing pancreatic β cells[44] and insulin secretion[45]. This receptor also plays a vital role in coordinating metabolic and endocrine signals, regulating hepatic glucose metabolism[46]. Previous work by our group demonstrated that this receptor participates in the loss of podocyte adhesion induced by BPA and is directly related to diabetic nephropathy[3]. On the other hand, it has been observed that both BPA and BPS can affect insulin cell signaling in skeletal muscle and adipose tissue (reducing the expression of insulin receptor substrate 1 and Akt phosphorylation)[43].

The linear regression model of the present work showed very significant values only with the BPS. However, the R-squared value was low (0.005) despite being significant. This data implies that the relationship between both variables is low; urinary BPS could only explain a tiny part of diabetes cases. Subsequent binomial and multinomial logistic regression models confirmed and reinforced the relationship between BPS and diabetes while ruling out the statistical relationship with urinary BPF. Nowadays, the vision of "one factor-one disease" could be considered obsolete. Numerous pathologies, such as diabetes, cannot be explained by the action of a single element since they are multifactorial. Therefore, the main idea extracted from the results is that BPS is an environmental factor related to diabetes.

On the other hand, complementary studies on hypertension and dyslipidemia have shown interesting evidence. First, both derived compounds show interesting significant relationships with the risk of hypertension, especially BPS. As with diabetes, few works study the relationship between BPS or BPF and these diseases. Jiang et al[42] found a positive and significant relationship between individuals with higher levels of urinary BPS, but not BPF, with hypertension. On the other hand, the works of Liu et al[22] and Jacobson et al[24] found a significant relationship between urinary BPF[22] or both phenolic derivatives[24] with obesity in children and adolescents.

Due to the differences observed in the risks of predisposition to diseases, it could be stated that the compounds derived from BPA (despite having similar hormonal

activity) could act on different cell signaling mechanisms, promoting the development or progression of different diseases.

CONCLUSION

The present study has determined a strong relationship between urinary BPS and diabetes risk, not observed with BPF. In the case of hypertension, both molecules could be involved in pathophysiological mechanisms, which, in the case of dyslipidemia, would be exclusive to BPF. Future studies will be necessary to delve into the paradigm and explore the relationship of the new BPA-derived molecules with other related diseases, such as kidney disease. BPA substitute molecules do not exempt the population from potential health risks.

ARTICLE HIGHLIGHTS

Research background

New restrictions on the use of bisphenol A (BPA) have conditioned the use of new derivative compounds by the plastics industry. The small number of evidence of its possible effects on human health shows its need, especially in diseases such as diabetes, whose incidence has increased substantially in recent years.

Research motivation

The study of the urinary excretion of the new bisphenols and their possible relationship with human health is of particular importance. The present work aims to provide new evidence that supports the need for restriction in using new molecules derived from BPA.

Research objectives

The work's objective is to analyze the relationship between urinary bisphenols and diabetes in one of the largest global cohorts, National Health and Nutrition Examination Survey (NHANES). The possible results could support the need to explore

the signaling pathways involved in the pancreatic pathophysiology potentially induced by this class of molecules.

Research methods
By applying descriptive statistics, simple linear regressions, and logistic regression models, this manuscript aims to analyze the data from the NHANES cohort in a novel way in a context that has been little studied in the academic literature.

Research results
After using all the tools and statistical models, the results have consistently pointed to bisphenol S as a risk factor for diabetes, excluding bisphenol F. On the other hand, the relationships observed with hypertension and dyslipidemia maintain the need to evaluate both molecules in the human health context.

Research conclusions
In a novel way in the NHANES cohort, the present manuscript has shown that exposure to new bisphenols is directly related to diabetes.

Research perspectives
Future research should explore the causal relationship through longitudinal studies and evaluate the potential deleterious effects on other pathologies, such as kidney disease.

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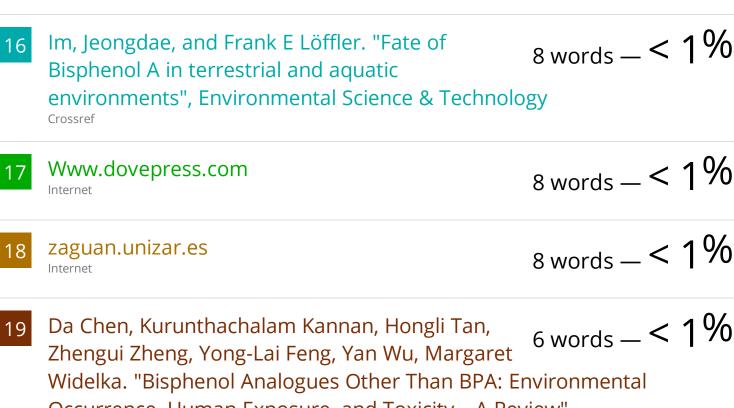   Internet   18 words — 1%

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