Experimental BPA Exposure and Glucose-Stimulated Insulin Response in Adult Men and Women

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Context: Human cross-sectional and animal studies have shown an association of the chemical bisphenol A (BPA) with insulin resistance, type 2 diabetes, and other metabolic diseases, but no human experimental study has investigated whether BPA alters insulin/C-peptide secretion.

Design: Men and postmenopausal women (without diabetes) were orally administered either the vehicle or a BPA dose of 50 μg/kg body weight, which has been predicted by US regulators (Food and Drug Administration, Environmental Protection Agency) to be the maximum, safe daily oral BPA dose over the lifetime. Insulin response was assessed in two cross-over experiments using an oral glucose tolerance test (OGTT; experiment 1) and a hyperglycemic (HG) clamp (experiment 2). Main outcomes were the percentage change of BPA session measures relative to those of the control session.

Results: Serum bioactive BPA after experimental exposure was at levels detected in human biomonitoring studies. In the OGTT, a strong positive correlation was found between hemoglobin A1c (HbA1c) and the percentage change in the insulinogenic index (Spearman = 0.92), an indicator of early-phase insulin response, and the equivalent C-peptide index (Pearson = 0.97). In the HG clamp study, focusing on the later-phase insulin response to a stable level of glucose, several measures of insulin and C-peptide appeared suppressed during the BPA session relative to the control session; the change in insulin maximum concentration (Cmax) was negatively correlated with HbA1c and the Cmax of bioactive serum BPA.

Conclusions: This exploratory study suggests that BPA exposure to a dose considered safe by US regulators may alter glucose-stimulated insulin response in humans.

Freeform/Key Words: bisphenol A, C-peptide, HbA1c, hyperglycemic clamp, insulin, oral glucose tolerance test

Disruption of glucose homeostasis is a common feature of metabolic diseases that has become more prevalent in recent decades [1, 2]. Glucose-stimulated insulin secretion is modulated by hormones and can be altered by environmental endocrine-disrupting contaminants such as bisphenol A (BPA), which is the monomer commonly used to manufacture thermal paper

receipts, polycarbonate plastic, and a wide range of other products; BPA is one of the highest volume chemicals in global production (>7 billion metric tons per year).

Experiments with human and mouse pancreatic β cells have revealed the molecular mechanisms by which a low-dose exposure of estradiol or BPA, in the presence of 8 mM (144 mg/dL) glucose, triggers a rapid release of insulin within minutes [3–6]. In contrast to these rapid effects, an experiment with adult male mice showed that low-dose BPA exposure for 4 days caused insulin resistance, as indicated by intraperitoneal glucose and insulin tolerance tests [3, 7].

Because of these findings and the ubiquitous nature of BPA exposures in the general US population [8], a hypothesis has been proposed that in susceptible individuals, exposure to BPA could interact with genetic and lifestyle factors to promote the development of metabolic diseases [2]. This hypothesis is supported by data from cross-sectional and prospective epidemiological studies showing a relationship between BPA levels and metabolic diseases: obesity, insulin resistance, type 2 diabetes, hypertension, and both cardiovascular and nonalcoholic fatty liver disease [9–15], as well as a large literature showing that BPA causes similar effects in animals [1, 2, 16]. Although there has been controversy concerning whether human exposure to BPA is high enough to warrant concern, the exposure models used have been based on the assumption that BPA exposure is almost entirely from food sources, such that BPA would be subjected to extensive first-pass metabolism. However, we have shown that transdermal absorption from BPA-coated thermal paper, when enhanced by the use of hand sanitizer, can result in serum levels of bioactive (unconjugated) BPA within the range of levels reported in human biomonitoring studies [17, 18], and that these levels are similar to the ones showing effects *in vitro* and in animal studies.
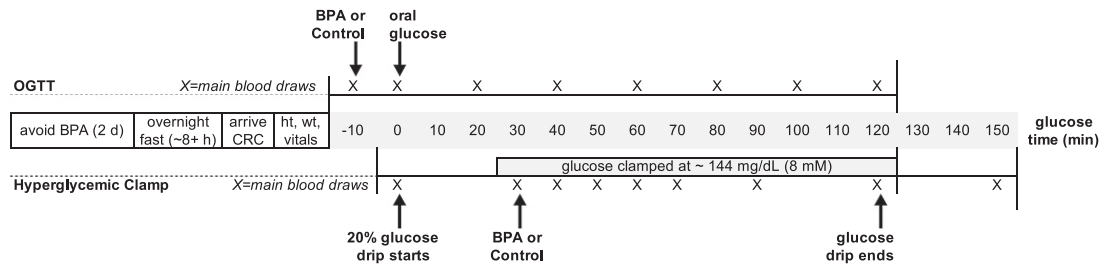
Resolving controversies about the safety for the general population of exposure to manmade chemicals is often hampered, appropriately, by ethical challenges to conducting human experiments with chemicals that are potentially harmful. However, we identified an ethical path to a controlled human experimental exposure using BPA. First, the BPA effect we were looking for is rapid and transient and should require only a single BPA exposure. BPA itself is not persistent and is cleared with a half-life of about 6 hours after oral administration [19]. There is evidence of repeated daily exposures to BPA [20], to which our study would only add one additional exposure. We thus exposed subjects to the oral BPA exposure believed by US regulators to be safe over a lifetime, known as the "reference dose," which is 50 µg BPA/kg body weight per day [21]. Finally, before we began the formal study, we conducted a pilot study on two of the authors (R.W.S. and F.S.v.S.) and found that the BPA reference dose produced internal bioactive levels that were not different from what we found in our thermal receipt paper study [18].

We conducted this experiment to test two competing hypotheses: US regulatory agencies hypothesize that there should be no measurable adverse effect on health-relevant outcomes in people in response to daily exposure to BPA at the reference dose. We hypothesized on the basis of a large literature from animal experiments and human epidemiology that there would be demonstrable effects in response to a single exposure to this dose of BPA.

We therefore conducted two exploratory cross-over studies, with each subject serving as his/her own control, by testing subjects with and without BPA exposure. We recruited adults without diabetes to investigate effects of oral BPA exposure on the insulin response to glucose. In experiment 1, we examined the effect of BPA on insulin response with an oral glucose tolerance test (OGTT) to examine both the initial and later phases of the insulin response to glucose. In experiment 2, we used the hyperglycemic (HG) clamp because it enabled us to stabilize glucose prior to BPA administration. The focus of the clamp experiment was thus only on the later phase of the insulin response to glucose.

1. General Methods

In this exploratory study, we assessed the insulin response to a glucose challenge using the OGTT in experiment 1 and the HG clamp in experiment 2 (Fig. 1). Two investigators (R.W.S., F.S.v.S.) piloted the OGTT protocol. Both experiments used a cross-over design, with participating subjects attending two separate sessions, BPA and control, such that each subject

Figure 1. Experimental design summary. We performed two experiments; 8 subjects in each experiment (16 total). To assess insulin response, we used the OGTT in the first experiment and the HG clamp in the second experiment. For the OGTT, the oral glucose dose was 100 g. In the clamp study, glucose was measured every 5 minutes, and the rate of the 20% glucose IV was changed according to a spreadsheet-based algorithm to achieve a stable ("clamped") glucose level of 144 mg/dL (8 mM) by a time of ~30 minutes. This glucose level was chosen based upon published *in vitro* studies that showed a rapid release of insulin was triggered in human and mouse β cells by estradiol or BPA at a glucose concentration of 8 mM. Main blood draw times (insulin, C-peptide, BPA) are indicated by "X"; the events marked with arrows (*e.g.*, BPA or control dosing, oral glucose administration) occurred just after the blood draw at the specified time.

was his/her own control. As a result, the main outcome variables were not the session-specific results but instead were the percentage difference between the subject's BPA and control visit results [$100\% \times (X_{BPA} - X_{control})/X_{control}$]. This environmental exposure study was not registered at Clinicaltrials.gov because it does not meet the definition of an "applicable drug clinical trial" according to federal regulations. As part of this assessment, we requested and received an exemption from the relevant Investigational New Drug requirements of the US Food and Drug Administration. The University of Missouri Health Sciences Institutional Review Board approved the protocols. There were no adverse events.

A. Subject Visits

Subjects were scheduled for two visits to the University of Missouri Clinical Research Center (CRC), allowing at least 1 week of washout time between visits. The subjects were randomly assigned by author J.A.T., via a software-generated sequence, to receive BPA at either the first or second visit. Other investigators, the subject, and nurses were blinded as to treatment order. For 48 hours prior to a visit, subjects were asked to avoid canned food and beverage and to minimize handling of cash register receipts to reduce background BPA levels. The subjects were instructed to fast overnight before the study visit, and to report to the CRC in the morning (typically 8 AM).

B. BPA/Control Solutions and Administration

BPA (purchased from Sigma-Aldrich, St. Louis, MO; >99% pure) was dissolved in Everclear (95% food-grade ethanol Luxco, St. Louis, MO) to form a BPA stock solution (10 mg BPA/mL). Tonic water was mixed with Everclear (Luxco, St. Louis, MO) to create a tonic water and 15% ethanol stock solution. Tonic water helped maintain blinding by masking the slightly bitter

taste of BPA. All solutions were stored at 4°C. The concentration of the BPA in the BPA stock solution was measured by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) prior to use as previously described [18]. For control sessions, subjects were given 1 mL of the tonic water/15% ethanol solution. For the BPA visits, the body weight measured at the CRC for that visit was used to calculate the BPA dose. The volume of the BPA stock solution required to give that dose was added to the tonic water/ethanol stock solution to give a final volume of 1 mL. When administered, the subjects were directed to hold the BPA or control solution under their tongues for 60 seconds before swallowing to increase sublingual absorption of BPA. Based on the subjects' BPA visit weights, the total BPA administered ranged from 3.3 to 5.4 mg and ethanol volume from 0.41 to 0.58 mL to achieve a final dose of 50 μg/kg body weight in a 1-mL ethanol-tonic water solution.

C. Insulin and C-Peptide (β Cell) Response Assessment

In both experiments we measured glucose (plasma), insulin (serum), and C-peptide (serum) at multiple time points. These data were corrected for within-subject differences between control and BPA sessions so that the starting point of each outcome measure was equal to the average of the starting point data from subjects' control and BPA sessions, with other session values adjusted accordingly. The starting point for the OGTT experiment was glucose time zero (10 minutes after BPA administration), and for the HG clamp experiment, at the time of BPA/control administration (clamp time, 30 minutes) when blood glucose was stabilized. For BPA and the metabolic measurements, we identified the maximum concentration (Cmax) of each analyte and calculated the area under the concentration time curve (AUC) using the trapezoidal rule.

D. Metabolic Measurements

Serum was analyzed at the University of Missouri Diabetes Diagnostic Laboratory for hemoglobin A1c (HbA1c) (using the Tosoh G8 variant mode ion-exchange HPLC method; Tosoh Biosciences, San Francisco, CA). Insulin and C-peptide were measured using the Tosoh AIA-PACK IRI and ST AIA-PACK C-peptide II reagents on the Tosoh AIA-900 (Tosoh Biosciences, San Francisco, CA). In the OGTT experiment, glucose was measured in fluoride plasma using the Roche GLUC3 hexokinase reagents on a Roche Cobas 311 (Roche Diagnostics, Indianapolis, IN). In the HG clamp experiment, plasma glucose was measured every 5 minutes at the bedside in the CRC using the YSI 2300 Stat Plus (YSI Inc., Yellow Springs, OH).

Table 1. OGTT Experimental Subjects: Descriptive

Subject ID	Ethnicity	Age	BMI	HbA1c	Fasting Glucose	Fasting Insulin	HOMA-IR
M1	White	25	25.9	4.9	96.5	6.5	1.54
M2	White	31	25.0	5.0	101.5	2.9	0.71
M3	Chinese	26	19.4	5.1	92.5	5.0	1.14
M4	White	27	23.8	5.1	84.0	3.4	0.70
M5	Arab American	21	19.8	5.2	90.5	1.9	0.42
M6	Indian[a]	37	28.9	5.2	95.5	11.4	2.68
M7	White	28	25.7	5.3	102.5	5.2	1.32
M8	White	30	27.7	5.5	102.0	7.9	1.99
Mean		28.1	24.5	5.2	95.6	5.5	1.31
Median		27.5	25.3	5.2	96.0	5.1	1.23

Subject IDs are ordered by rank of their HbA1c (M1 to M8 = low to high). BMI, fasting glucose, insulin, and HOMA-IR are the average of each subject's BPA and control visit values. Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) is an insulin resistance estimate, calculated as HOMA-IR = [fasting glucose (mM) × fasting insulin (μU)]/22.5.
[a]From India (not American Indian/Native American).

E. BPA Measurement

Unconjugated BPA (uBPA) and its primary metabolites, bisphenol glucuronide (BPA-G) and bisphenol monosulfate, were measured in the vom Saal laboratory by author J.A.T. as
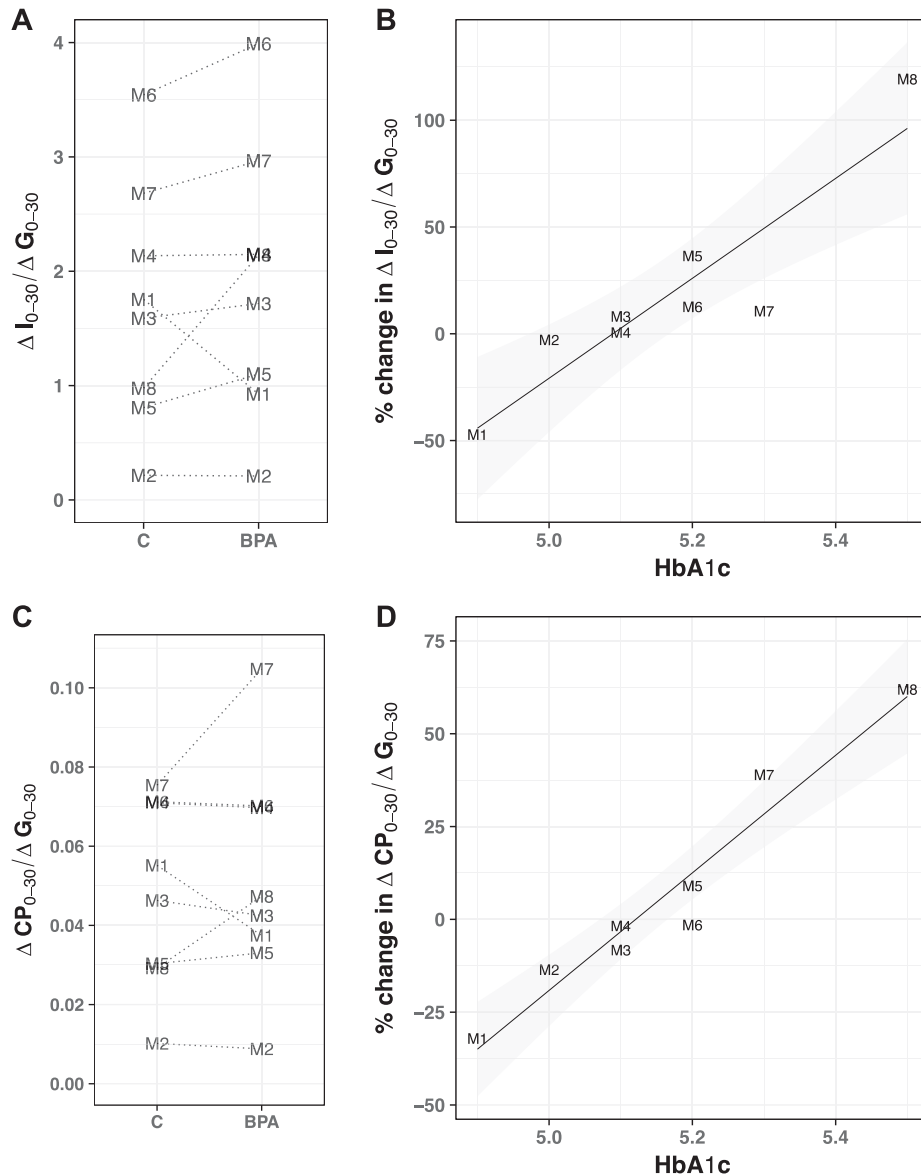

Figure 2. OGTT study insulinogenic index and its relationship to HbA1c by subject. Panels A and B plot the insulinogenic index, an indicator of first-phase insulin release, which is calculated as Δ insulin from 0 to 30 minutes)/(Δ glucose from 0 to 30 minutes) ($\Delta I_{0\text{-}30}/\Delta G_{0\text{-}30}$). Panels C and D plot the equivalent index using C-peptide ($\Delta CP_{0\text{-}30}/\Delta G_{0\text{-}30}$). Panels A and C show two versions of the insulinogenic index, $\Delta I_{0\text{-}30}/\Delta G_{0\text{-}30}$ and $\Delta CP_{0\text{-}30}/\Delta G_{0\text{-}30}$, by session (control, BPA). Panels B and D show the percentage change in outcomes between the BPA and control sessions, plotted against HbA1c, where the percentage change in the insulinogenic index or the C-peptide equivalent was calculated as the percentage change in outcome (%Δ X) between BPA and control sessions = 100% $\times$ ($X_{BPA} - X_{control}$)/$X_{control}$. HbA1c was measured from the baseline blood draw of each subject's first visit. A linear regression line is included in the scatterplots with shaded 95% CI. The correlations of these variables with HbA1c are (B) $\Delta I_{0\text{-}30}/\Delta G_{0\text{-}30}$ Spearman = 0.92, $P = 0.0014$ and (D) $\Delta CP_{0\text{-}30}/\Delta G_{0\text{-}30}$ Pearson = 0.97, $P = 0.0001$. Points are labeled with the subject IDs, which correspond to the rank ordering of their HbA1c (M1 to M8 = low to high). Shapiro-Wilk normality test was used to select Pearson or Spearman correlations, using P value cutoff of >0.3 as "normal."

Table 2. OGTT Experimental Subjects: BPA Exposure and Outcome Variables

| Subject ID | HbA1c | Serum uBPA | | | $\Delta I_{0-30}/\Delta G_{0-30}$ | | |
		Baseline[a] Time: –10 Min	Time: 0 to 40 Min	T: 60 to 120 Min	Control	BPA	% Change
M1	4.9	0.0	2.4	1.1	1.75	0.93	−47.2
M2	5.0	0.0	2.7	5.3	0.22	0.21	−2.9
M3	5.1	0.2	1.9	3.0	1.59	1.72	8.1
M4	5.1	1.1	5.7	3.8	2.14	2.15	0.6
M5	5.2	NS	3.8	4.6	0.81	1.10	36.4
M6	5.2	0.0	8.2	1.0	3.54	3.99	12.6
M7	5.3	0.0	1.6	4.5	2.68	2.97	10.6
M8	5.5	0.9	2.9	4.9	0.98	2.14	119.3
Mean	5.2	0.3	3.7	3.5	1.71	1.90	17.2
Median	5.2	0.0	2.8	4.2	1.67	1.93	9.4
Pearson correlation HbA1c							
Spearman correlation HbA1c							0.92[d]

(Continued)

previously described [18]. The on-column limit of detection for uBPA, BPA-G, and bisphenol monosulfate was 0.12, 0.08, and 0.15 ng/mL, respectively.

F. Statistical Analyses

For data analysis we used the R statistical system, version 3.4.0 [22], and the RStudio programming environment, version 1.0.143 [23]. All plots were made using the R graphics package ggplot2 [24]. Our main results were descriptive statistics, plots, correlations, and within-subject comparisons between BPA and control session results. We added linear regression lines to some plots. To choose between parametric (Pearson correlations, *t* tests) and nonparametric methods (Spearman correlations and Wilcoxon rank tests), we tested the distribution of each variable with the Shapiro-Wilk normality test. Due to our small sample size, we conservatively required a normality test *P* value >0.3 to treat data as normally distributed. We bootstrapped 95% confidence intervals for significant correlations by sampling with replacement 10,000 times. Because this is an exploratory study, we conducted many statistical tests to maximize the information extracted from these first human experimental data. As discussed by Rothman [25], correction for multiple comparisons in this scenario increases the chance of false negatives, which in an exploratory study should be reduced.

2. Experiment 1: OGTT

A. Methods

In 2014 we consented eight male subjects with body mass index (BMI) <30, who reported being a nonsmoker, without prescription medications, diabetes, or prediabetes. Each subject was weighed, had vital signs collected, and was placed in a hospital bed, with a saline lock (IV access port) placed for blood draws. Ten minutes before the glucose drink was administered, a blood sample was collected for fasting glucose, insulin, and C-peptide. At the first visit, this sample was also measured for HbA1c. The BPA or control solution was then given as described above. Ten minutes after the BPA/control administration, a second baseline was collected (glucose time = 0 minutes), after which the subject consumed a glucose tolerance test drink (100 g dextrose) over 1 to 3 minutes. Blood was subsequently collected at 10, 20, 40, 60, 80, 100, and 120 minutes after glucose administration.

Table 2. OGTT Experimental Subjects: BPA Exposure and Outcome Variables (Continued)

Subject ID	$\Delta I_{0\text{-}30}/\Delta G_{0\text{-}30}$				% Change in Disposition Index
	Control	BPA	% Change	%Change in ISI_{OGTT}	
M1	0.055	0.037	−32.1	6.9	−43.6
M2	0.010	0.009	−13.6	2.9	0.0
M3	0.046	0.042	−8.3	−5.5	2.2
M4	0.071	0.070	−1.8	30.8	31.6
M5	0.030	0.033	9.0	−60.9	−46.7
M6	0.071	0.070	−1.5	−0.1	12.6
M7	0.075	0.105	38.9	1.0	11.7
M8	0.029	0.047	62.0	−28.1	57.7
Mean	0.049	0.052	6.6	−6.6	3.2
Median	0.051	0.045	−1.7	0.5	7.0
Pearson correlation HbA1c			0.97^b		0.64^c
Spearman correlation HbA1c				-0.63^c	

The percentage change in outcome X (%Δ X) = 100% × ($X_{BPA} - X_{control}$)/$X_{control}$. Times are in minutes after oral glucose administration. The BPA or control solution was administered at time = −10 min. The BPA dose was 50 μg BPA per kg body weight. The uBPA baseline values equal to 0.0 are baseline samples without detectable BPA. The disposition index refers to insulinogenic index ($\Delta I_{0\text{-}30}/\Delta G_{0\text{-}30}$) × ISI_{OGTT}.

Abbreviation: NS, blood volume not sufficient.

[a]Baseline uBPA in serum collected just before BPA administration = 0.0 for samples without detectable BPA.

[b]$P = 0.0001$.

[c]$P < 0.10$.

[d]$P = 0.0014$.

A-1. Calculations

In addition to the AUC and Cmax for insulin and C-peptide, the insulinogenic index, an indicator of early-phase insulin release [26], was calculated as the change in insulin divided by the change in glucose from time 0 to 30 minutes ($\Delta I_{0\text{-}30}/\Delta G_{0\text{-}30}$). The equivalent index was also calculated for C-peptide ($\Delta CP_{0\text{-}30}/\Delta G_{0\text{-}30}$). Values at 30 minutes were estimated as the mean of the 20- and 40-minute values. The Matsuda insulin sensitivity index (ISI_{OGTT}) was calculated as 10,000/square root (fasting insulin × fasting glucose × glucose mean$_{(time\ 0\text{-}120)}$ × insulin mean$_{(time\ 0\text{-}120)}$) [27].The disposition index was insulinogenic index × ISI_{OGTT}. Units for glucose, insulin, and C-peptide were mg/dL, μU/mL, and pmol/mL, respectively).

B. Results

Our subjects are described in Table 1. Glucose, insulin, and C-peptide vs time are plotted in Supplemental Figure 1 and BPA serum levels in Supplemental Figure 2. At baseline at the BPA exposure session, three subjects had detectable levels of uBPA (0.7 to 1.1 ng/mL), which indicates that these subjects had some BPA exposure prior to arrival at the CRC.

HbA1c was a strong predictor of the BPA-associated change in insulin response in the first 30 minutes after oral glucose challenge (Fig. 2; Table 2). Specifically, there was a strong correlation between HbA1c and the within-subject percentage change between control and BPA sessions in the insulinogenic index: Spearman = 0.92 (95% CI: 0.48, 1.0), $P = 0.0014$. The equivalent C-peptide–based index was similar: Pearson = 0.97 (95% CI: 0.86, 1.0), $P = 0.0001$. Excluding the subjects M3, M4, and M8 who had detectable uBPA at baseline, as well as subject M5 who did not have sufficient serum for the baseline analysis, did not substantially change the relationship of HbA1c and the insulinogenic index (Spearman = 0.80) or the C-peptide equivalent (Pearson = 0.94). HbA1c was moderately, but not statistically significantly, associated with the percentage change between BPA and control in the insulin sensitivity index (Spearman = −0.63, $P = 0.10$) and the disposition index (Pearson = 0.64, $P = 0.09$).

Beyond the initial 30 minutes after glucose administration, the results for the later phase of insulin and C-peptide release relative to glucose levels (AUC for insulin or C-peptide, divided by glucose AUC) were highly variable and not significantly correlated with HbA1c or pooled serum uBPA levels (data not shown). Ignoring HbA1c, there were no significant within-subject differences between either the treatment conditions (BPA, control) or the pooled serum uBPA values and the percentage change in any of the outcome measures. Measurement of pooled serum samples for uBPA in this experiment (Table 2) prevented us from examining associations with uBPA Cmax.

3. Experiment 2: HG Clamp

A. Methods

We used this experiment to examine the effect of BPA on the later phase of insulin/C-peptide release against relatively constant plasma glucose, rather than the highly variable later-phase
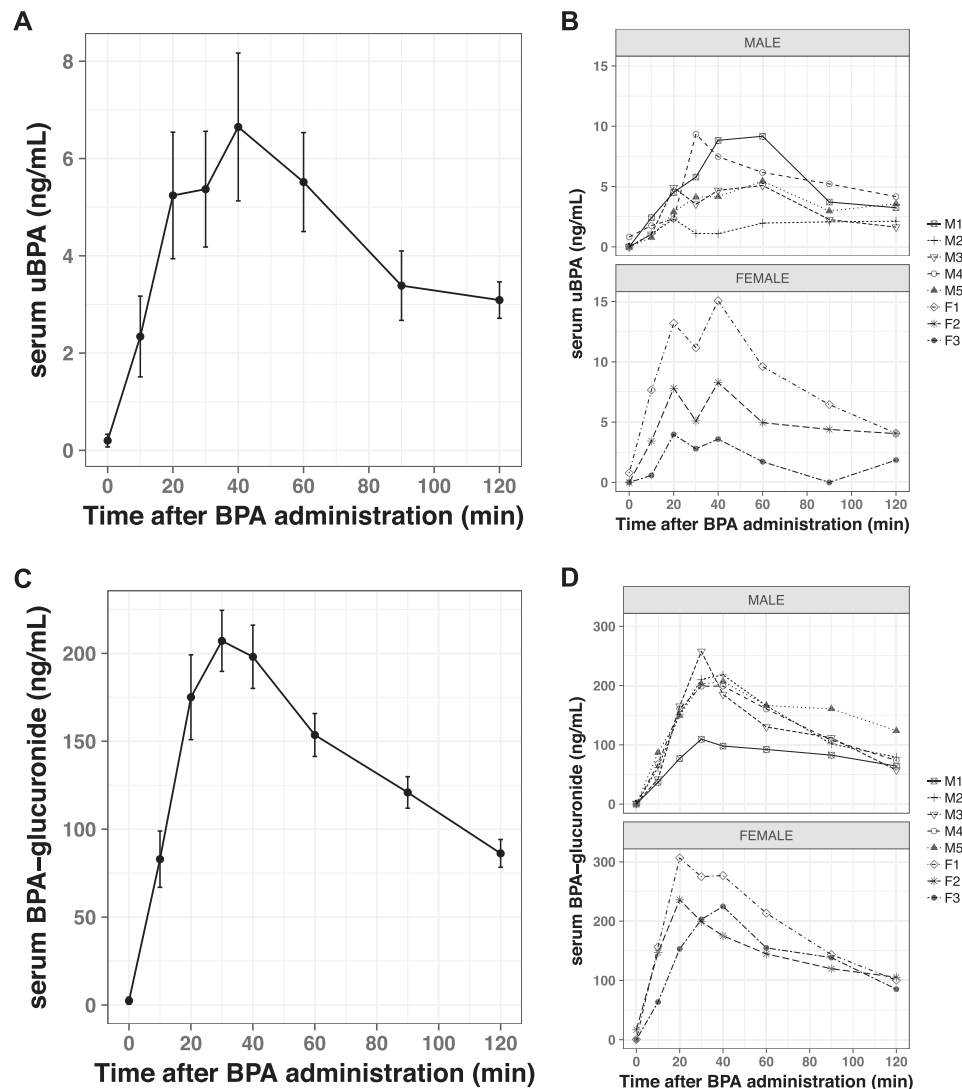

Figure 3. HG clamp study BPA pharmacokinetics. (A and B) Serum uBPA and (C and D) serum BPA-glucuronide vs time after oral/sublingual administration of BPA 50 μg/kg. BPA was administered at 30 minutes into the clamp (BPA time = clamp time − 30). Panels A and C are mean ± SE. Panels B and D show individual subjects.

glucose in the OGTT study. Figure 1 shows the experimental design: subjects could be older, female (postmenopausal), with obesity and prediabetes by HbA1c. Our first subject (M1) was author R.W.S. Females were required to be postmenopausal to eliminate estrogen cycling and any fetal risk of exposure to BPA during pregnancy. Subjects reported no history of cancers that could be estrogen responsive. Ten subjects were originally consented (2016 to 2017); however, two females were unable to complete both sessions due to IV access problems.

An IV was placed in one arm and a saline lock in the other arm. The IV was used to administer 20% glucose, and the saline lock to draw blood. The saline lock was typically placed in a dorsal hand vein of the blood draw arm; however, the antecubital fossa was used in a few cases. The hand of the blood draw arm was then placed in a warming box maintained at 50°C to help arterialize the venous blood.

The glucose infusion rate was calculated using a computerized algorithm provided to us by Thomas Solomon, PhD [28], which was based on the original description of the glucose clamp protocol [29]. The infusion rate for the first 15 minutes was set by the algorithm based on baseline data. Thereafter, the algorithm used plasma glucose measurements, made every 5 minutes using the YSI glucose analyzer, to adjust the 20% glucose infusion rate to stabilize (clamp) plasma glucose at 8 mM (144 mg/dL) by clamp time ~30 minutes, and then to maintain this level for the next 90 minutes (total time, 120 minutes). Blood draws for BPA and metabolic measurements other than glucose were taken at clamp times 0, 30, 40, 50, 60, 70, 90, and 120 minutes. The first blood draw (clamp time, 0) was taken just before the glucose infusion began and was used to determine HbA1c and the fasting values for glucose, insulin, and C-peptide. The 30-minute blood draw was taken just before BPA or vehicle control administration.

A-1. Calculations

We calculated the AUC for insulin, C-peptide, and uBPA. We split these AUCs at clamp times 30 to 70 and 70 to 120 minutes because, as shown in Figure 3A, BPA peaked on average at clamp time 70 minutes (40 minutes after BPA administration). A "late insulin response" was calculated as the mean insulin from 90 to 120 minutes minus fasting insulin, and insulin sensitivity was computed as 100 times the mean glucose infusion rate (mg/kg/min) from clamp time 90 to 120 minutes divided by the mean insulin level (μU/mL) from 90 to 120 minutes [30].

B. Results

Eight subjects completed the experiment (three females, five males); their age, BMI, and HbA1c are shown in Table 3. By chance, the HbA1c ranges for males and females did not

Table 3. HG Clamp Subjects: Descriptive

Subject ID	Ethnicity	Age	BMI	HbA1c	Fasting Glucose	Fasting Insulin	HOMA-IR
M1	White	60	22.2	4.9	88.6	3.3	0.73
M2	White	33	34.4	5.2	88.6	15.5	3.39
M3	White	59	32.4	5.5	95.2	8.0	1.88
M4	Indian[a]	41	30.4	5.6	91.6	13.6	3.08
M5	White	58	30.6	5.6	87.6	10.8	2.34
F1	White	63	35.7	5.7	95.7	19.8	4.69
F2	White	60	33.2	6.1	98.5	10.8	2.62
F3	White	58	29.8	6.3	88.1	6.3	1.36
Mean		54.0	31.0	5.6	91.7	11.0	2.51
Median		58.6	31.5	5.6	90.1	10.8	2.48

Subject IDs (males M1 to M5 and females F1 to F3) are in order of HbA1c from low to high. BMI, fasting glucose, and fasting insulin are the average of each subject's BPA and control visit values. HbA1c was measured during screening prior to the main study visits. Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) is an insulin resistance estimate, calculated as [fasting glucose (mM) × fasting insulin (μU)]/22.5.
[a]From India (not Native American).

Table 4. HG Clamp Subjects: BPA Exposure and Outcome Variables

Subject ID	HbA1c	uBPA t0[b]	Cmax	Tmax[c]	AUC 30–70 Min	AUC 70–120 Min
				uBPA (Serum)		
M1	4.9	0.0	9.2	60	171.3	373.4
M2	5.2	0.0	2.3	20	50.5	91.4
M3	5.5	0.1	5.1	60	118.3	208.0
M4	5.6	1.0	9.3	30	175.8	307.3
M5	5.6	0.2	5.5	60	98.7	222.5
F1	5.7	0.7	15.1	40	399.4	487.6
F2	6.1	0.0	8.3	40	204.8	272.5
F3	6.3	0.0	4.0	20	91.4	78.8
Mean	5.6	0.3	7.3	41	163.8	255.2
Median	5.6	0.1	6.9	40.0	144.8	247.5
t test						
Wilcoxon rank sum						

(*Continued*)

overlap. Based upon subject weights for their BPA sessions, the total BPA administered ranged from 3.5 to 5.4 mg (mean, 4.6 mg). The Cmax for serum uBPA ranged from 2.3 to 15.1 ng/mL (mean, 7.3 ng/mL), with the uBPA peak [time of Cmax (Tmax)] occurring between 20 and 60 minutes post-BPA administration (mean, 41 minutes; Fig. 3). Five of 8 subjects showed a temporary decrease in uBPA concentration at 30 minutes postadministration, suggestive of enterohepatic recirculation. The correlation between the serum uBPA Cmax and AUC_{30-120} measures was high (Pearson = 0.96; $P < 0.001$). Plots by subject of uBPA, insulin, and C-peptide vs time are shown in Supplemental Figure 3. Summary plots of mean serum insulin, plasma glucose, and glucose infusion rates are shown in Supplemental Figure 4.

For within-subject comparison of BPA vs control sessions, several C-peptide and insulin measures were at or near significance (Table 4). The late-phase insulin measure for the BPA session averaged 17.4% less than the control session (95% CI: –30% to –4.8%; *t* test, $P = 0.042$; Fig. 4A). The C-peptide Cmax during the BPA session averaged 6.4% less than that of the control session (95% CI: –11% to –1.3%; *t* test, $P = 0.046$; Fig. 4B). The insulin sensitivity measure was not statistically different between BPA and control sessions ($P = 0.24$).

The HG clamp results did not show a significant association between HbA1c and the percentage change in insulin response between BPA and control sessions (specifically, insulin or C-peptide AUC from clamp time 30 to 120 minutes). Of these measures, the strongest associations were between the percentage change in C-peptide AUC_{70-120} (*t* test, $P = 0.06$; Fig. 4C) and between HbA1c with the percentage change in C-peptide AUC_{70-120} (Spearman = –0.66, $P = 0.076$; Fig. 4D).

There was some evidence for a negative association between serum uBPA and insulin response. Serum uBPA Cmax was significantly negatively associated with percentage change in insulin Cmax (Supplemental Fig. 5; Pearson = –0.74; $P = 0.037$). However, the correlation of uBPA Cmax with C-peptide Cmax was not significant (Pearson = –0.46, $P = 0.25$).

The serum uBPA AUC from 30 to 120 minutes was positively correlated with age (Spearman = 0.71, $P = 0.047$; Supplemental Fig. 6), although the fact that six of eight subjects tightly cluster around age 60 years weakens this evidence.

C. Discussion

In our OGTT experiment, we found a strong positive relationship in healthy adult young men between serum HbA1c and the within-subject percentage change between BPA and control

Table 4. HG Clamp Subjects: BPA Exposure and Outcome Variables (Continued)

| | %Δ of BPA vs Control[a] | | | | | | | |
| | C-Peptide | | | Insulin | | | | |
Subject ID	Cmax	AUC 30–70 Min	AUC 70–120 Min	Cmax	AUC 30–70 Min	AUC 70–120 Min	Late-Phase Insulin	Insulin Sensitivity
M1	−2.7	−1.5	−1.9	8.2	3.5	7.0	−5.9	45.4
M2	7.8	6.0	9.9	23.2	8.0	12.4	−6.7	−19.2
M3	−2.6	−5.6	−2.1	−5.7	−0.4	−8.2	−44.7	18.8
M4	−8.5	−4.9	−12.3	−18.3	−10.8	−21.0	−25.4	−0.2
M5	−12.2	−12.3	−13.3	−12.9	−17.7	−20.1	−34.4	−20.9
F1	−9.1	−7.2	−10.6	−24.6	−18.4	−27.1	7.8	18.5
F2	−17.1	−6.5	−13.4	−10.0	−9.1	−19.3	−34.4	2.4
F3	−6.5	−1.1	−4.4	2.5	−3.0	−4.3	4.8	53.1
Mean	−6.4	−4.1	−6.0	−4.7	−6.0	−10.1	−17.4	12.2
Median	−7.5	−5.3	−7.5	−7.8	−6.1	−13.8	−16.0	10.4
t test	0.046	0.07		0.42	0.12	0.09	0.042	0.24
Wilcoxon rank sum	0.06							

Times are minutes from start of clamp glucose infusion, except for uBPA Tmax, which is the number of minutes post-BPA administration that the Cmax occurred. C-peptide and insulin columns are the percentage difference between BPA and control sessions [%Δ X = 100% × (X$_{BPA}$ − X$_{control}$)/X$_{control}$]. uBPA AUCs were split at 70 minutes (30 to 70 and 70 to 120) to align with the uBPA Tmax (clamp time = 70 minutes). Late-phase insulin response = (mean insulin 90 to 120 minutes) – fasting insulin [30]. Insulin sensitivity = 100 × mean glucose infusion rate (mg/kg/min) from 90 to 120 minutes/mean insulin 90 to 120 minutes. The BPA dose was 50 μg BPA per kg body weight. Shapiro-Wilk normality test was used to select t test or Wilcoxon rank sum, using P value cutoff of >0.3 as "normal."
Abbreviation: t0, time 0.
[a]%Δ X = 100% × (X$_{BPA}$ − X$_{control}$)/X$_{control}$.
[b]Baseline uBPA in serum collected before the start of glucose drip at t0. = 0.0 for samples without detectable BPA.
[c]Minutes after BPA administration.

sessions in the insulinogenic index and the C-peptide equivalent (Fig. 2; Table 2). Given that the insulinogenic index is an important indicator of early-phase insulin release [26], and HbA1c is an indicator of glycemic control and a predictor of type 2 diabetes [31], this strong association was striking, especially considering the narrow range of HbA1c (4.9% to 5.5%) over which it was seen. A possible hypothesis is that even a mild impairment in glucose control (as indicated by increasing HbA1c) increases the likelihood of a stronger BPA effect on insulin/C-peptide release, or, alternatively, that people with elevated A1c are elevated because they are more sensitive to existing estrogenic exposures.

In the HG clamp experiment in which glucose was stabilized prior to BPA or vehicle administration, our subjects showed a different response to BPA: we observed a significant percentage decrease between control and BPA sessions in late-phase insulin and the C-peptide Cmax (Table 4), as well as a weak inverse relationship between the percentage change in later-phase C-peptide AUC with HbA1c (Fig. 4D). These results, which are opposite to the early-phase OGTT insulin findings, could indicate that BPA has different effects on the mechanisms of the early response (release of stored insulin) vs later insulin response (*de novo* insulin synthesis). This finding could also be influenced by our wider subject inclusion criteria in the HG clamp experiment (*e.g.*, inclusion of obese, older subjects with prediabetes, including postmenopausal females). Hyperinsulinemia, which could be aggravated by acute BPA exposure, decreases second-phase insulin release in humans [32]. Increased levels of palmitate in obese children and adolescents are also associated with initial hyperinsulinemia and then a later decrease in β-cell function [33].

Although our study results do not demonstrate that these effects of BPA are related to metabolic disease, they could have clinical and regulatory implications. Clinically, they
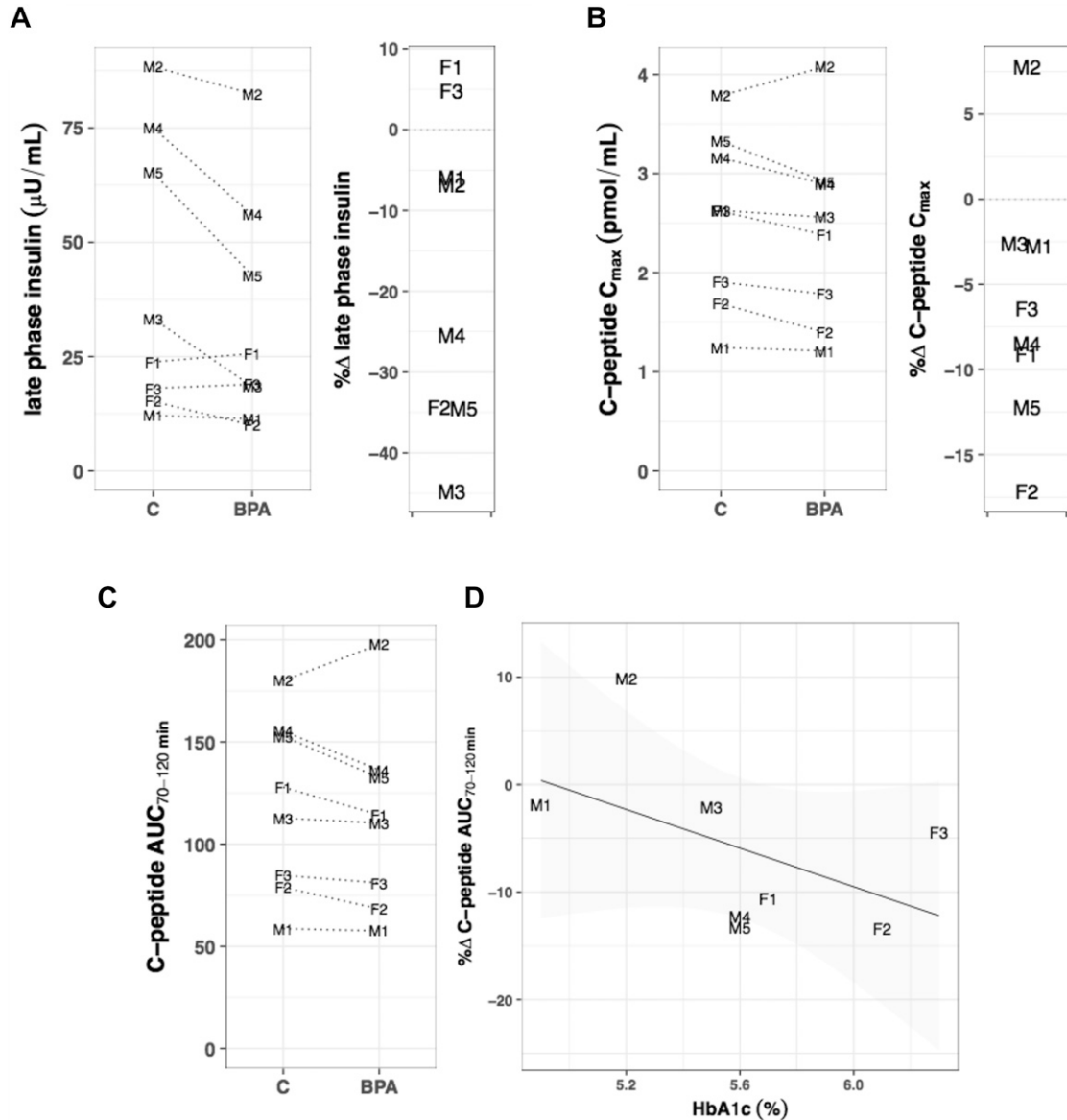
Figure 4. HG clamp study change in insulin release measures between control and BPA sessions. Percentage change in outcome (%Δ X) between BPA and control sessions = 100% × $(X_{BPA} - X_{control})/X_{control}$. Points are labeled with the subject IDs, which correspond to the rank ordering of their HbA1c levels (M1 to M5, F1 to F3 = low to high). The Shapiro-Wilk normality test was used to select parametric vs nonparametric tests and correlations, using P value >0.3 as "normal." (A) Late-phase insulin = mean insulin from clamp time 90 to 120 minutes minus fasting insulin [30]. Six of 8 values for %Δ in late-phase insulin are less than zero (t test, $P = 0.042$). (B) C-peptide Cmax. Seven of 8 values for %Δ C-peptide Cmax are less than zero (t test, $P = 0.046$). (C) C-peptide AUC from 70 to 120 minutes. Seven of 8 values for %Δ C-peptide $AUC_{70\text{-}120 \text{ min}}$ are less than zero (t test, $P = 0.06$). (D) The Spearman correlation of %Δ C-peptide $AUC_{70\text{-}120 \text{ min}}$ with HbA1c was –0.66, $P = 0.076$.

support the relevance to human health of the large experimental literature (animal and *in vitro*) demonstrating numerous BPA effects on metabolic outcomes [2]. From a regulatory perspective, identifying any physiological response in humans to BPA at the presumed "safe" daily BPA dose would indicate that key assumptions in the regulatory process are incorrect, as discussed in prior reviews [34–36]. Guideline toxicology studies conducted by regulatory agencies use relatively crude measures such as organ weights, histology, and tumor incidence (often in relatively short-term studies) to determine the doses at which overtly toxic

effects are seen, and from there, the human "safe" dose is extrapolated, but typically not tested directly. Within that scientific framework, the results we report here should not have been found. Regulatory agency guideline testing methods cannot be expected to detect alterations in metabolism that, although not overtly toxic, can initiate or exacerbate metabolic disorders. Despite these limitations, the absence of such experimental data demonstrating causality has been considered adequate for regulatory decision-making about the safety of BPA, while numerous findings from human epidemiology cross-sectional and prospective studies have been ignored [16].

This study is an initial step toward investigation of an intriguing hypothesis that exposure to estrogenic chemicals such as BPA may contribute to insulin resistance by triggering an innate insulin resistance mechanism. We thus assume that other estrogenic endocrine-disrupting chemicals, such as other bisphenols [37], could interact with BPA to impact glucose homeostasis. In late pregnancy, women develop a temporary insulin resistance, which enables increased glucose transfer across the placenta and rapid fetal growth [1]. This insulin resistance is believed to be caused by increases in steroid and other hormones during pregnancy [38]. Although estrogens at typical premenopausal levels appear to promote insulin sensitivity and protect the endocrine pancreas, estrogen excess appears to cause impairment of glucose control and create insulin resistance [39]. In male mice, repeated exposure to a low dose of BPA for 4 days produced insulin resistance [3]. If humans are similarly responsive, and the effect is primarily mediated through extranuclear estrogen receptors that respond to BPA at 100 pM to 1 nM (228 pg/mL) concentrations [4, 6], this would predict that insulin resistance and type 2 diabetes can be exacerbated by BPA exposures and therefore be improved by reducing such exposures.

Given these implications, verification of our findings in this exploratory study is clearly required. The findings from our OGTT experiment were strong (Fig. 2), but the effects of BPA on glucose control are likely far more complex than a simple function of HbA1c and BPA exposure. Background endogenous estrogen levels may matter, and these vary by age in men and in premenopausal females by monthly cycling or use of hormonal contraceptives, as well as in relation to BMI [40]. The serum uBPA levels are important as well, because hormones and hormonally active chemicals like BPA may have nonmonotonic dose-response relationships whereby very high chemical concentrations shut down the systems that are stimulated at lower concentrations, and entirely different responses are activated [6, 35]. There was considerable between-subject variability in the serum levels of uBPA (Fig. 3; Table 4), which could be related to differences in the degree to which different subjects absorbed BPA sublingually vs enterally [41] as well as other factors such as age (Supplemental Fig. 6).

In summary, the results of our two experiments suggest that oral exposure to the ubiquitous environmental contaminant BPA at the Environmental Protection Agency–estimated "safe" daily (reference) dose alters the insulin/C-peptide response to a glucose challenge in adults. Importantly, the effect of BPA on insulin and C-peptide occurred at an internal dose of serum uBPA that could plausibly result from real-world exposures, which for some persons might occur multiple times each day [18, 20]. A growing experimental literature suggests that BPA and other endocrine-disrupting chemicals may meaningfully contribute to the etiology of metabolic diseases [1, 2]. If true, it implies that susceptible people might improve with reduced exposure to BPA and other bisphenols that are functionally similar [37]. These findings suggest new research directions into the causes and treatment of glucose dysregulation.

Acknowledgments

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