https://doi.org/10.46389/rjd-2020-1048
www.rjdnmd.org

Rom J Diabetes Nutr Metab Dis
2020; volume 27, issue 4, pages 321-335

Original Research

The effects of exendin-4 on glucose homeostasis, pancreatic and duodenal homebox 1, and glucose transporter 2 gene expression disturbance induced by bisphenol A in male mice. The effects of exendin-4 on pancreatic gene expression

Akram Ahangarpour[1], Golshan Afshari[2]*, Seyyed Ali Mard[3], Ali Khodadadi[4], Mahmoud Hashemitabar[5]

[1] Department of Physiology, Health Research Institute, Diabetes Research Center, Faculty of Medical Sciences, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[2] Clinical Research Development Unit of Golestan Hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[3] Physiology Research Center, Research Institute for Infectious Diseases of Digestive System and Department of Physiology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[4] Cellular and Molecular Research Center, Department of Immunology science, Ahvaz Jundishapur University of Medical Sciences, Faculty of Medicine, Ahvaz, Iran
[5] Cellular and Molecular Research Center, Department of Anatomical Science, Ahvaz Jundishapur University of Medical Sciences, Faculty of Medicine, Ahvaz, Iran

*Correspondence to: Golshan Afshari, Golestan Hospital, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, E-mail: golshanafshari59@gmail.com & Afshari.g@ajums.ac.ir, Phone: +989166004225, +986133743369, +986133764246.

Received: 9 March 2020 / Accepted: 25 May 2020

Abstract

Introduction: Bisphenol A (BPA) is a substance used in the packaging of food and beverages. It can bind to estrogen receptors with destructive consequences. Exendin-4 is a 39-amino acid peptide that bonds with GLP-1 receptors and stimulates insulin secretion. In this study, we aimed to explore the effect of exendin-4 on resolving BPA side effects. **Materials and Method:** We tested the effects of five concentrations of exendin-4 alone in combination with BPA on insulin secretion from isolated islets in in-vitro assay. Following in-vivo part of an examination, both BPA and exendin-4 were prescribed alone. Then, in combination for 20 days, next blood glucose, plasma insulin level, and Pdx1 and GLUT2 gene expression were examined. **Result:** Studies showed that BPA increased the blood glucose level, whereas exendin-4 was useful in removing these symptoms. Quantitative real-time polymerase chain reaction results (PCR) showed that BPA decreased the expression level of Pdx1 and GLUT2 genes in pancreatic tissue, whereas exendin-4 had a preventive role. In an in-vitro experiment, BPA increased the percentage of apoptotic cells, whereas exendin-4 restrained it. **Conclusion:** We observed evidence, such as a decrease in apoptosis in pancreatic islet cells and increase in Pdx1 and GLUT2 gene expression in the pancreas tissue. This can be an indication of the protective and supportive effects of exendin-4 on the pancreatic tissue, as well as the prevention of hyperglycemia in this assay.

Keywords: Bisphenol A, exendin-4, GLUT2, Pdx1.

Introduction

Diabetes is a prevalent disorder characterized by high blood glucose and is now-a-days increasingly recognized as a serious public health concern worldwide. Several studies have reported that the biological function of estrogen mediated through two separate nuclear receptors, including estrogen receptor α (ERα) and estrogen receptor β (ERβ), which are a part of the super family of nuclear receptors [1, 2]. There are various literature evidences which recognizes the

importance of estrogen receptors as an impressive molecule in glucose homeostasis, health, and metabolic disorders [3–5], so that ERα knockout mice gets afflicted with obesity and insulin resistance [6]. Nowadays, humans are inadvertently exposed to phenolic estrogen substances and endocrine disrupting chemicals (EDC), which lead to endocrine disorders [7] Bisphenol A (BPA), also known as a phenolic estrogen, is one of the most widely used substances in polycarbonate plastics, lining of canned foods and beverage bottles [8, 9]. BPA has been an object of research since 1970. As a common EDC, BPA intervenes with classical and non-classical estrogen receptors and through inappropriate activation of estrogen receptors, interferes in problems, such as type II diabetes and metabolic disorders [9, 10]. A primary concern is an easy displacement of BPA from canned food linings and plastic containers to their contents, and finally to the human body [11]. Recently, researchers have shown a direct relation between high urinary levels of BPA (>4.2 ng/mL) and type 2 diabetes]. What is known about BPA is based largely upon its ability to bind to estrogen receptors. Because estrogen receptors (ERα and ERβ) exist in beta cells, exposure to BPA is considered as a cause of glucose homeostasis disturbance [13]. Details of BPA effects on glucose metabolism are not well clarified, but several pathways, such as irregularity in insulin secretion through the mitochondria, cause damage and induce insulin resistance associated with oxidative stress attributed to BPA [14, 15]. In addition, BPA contributes to the development of hyperglycemia, down regulation of insulin receptors and insulin resistance [9, 15].

Glucagon-like peptide-1 (GLP-1) is a peptide hormone primarily released from intestinal L cells. GLP-1 plays a significant role in post-prandial insulin secretion, promoting insulin sensitivity, inhibiting glucagon secretion, and enhancing beta cell mass and insulin expression. GLP-1 has a short half-life and is rapidly degraded by dipeptidyl peptidase-4 (DPP-4) (16). The agonists of GLP-1R are new classes of drugs for type 2 diabetes. Exendin-4 is a 39-amino acid peptide that is extracted from the saliva of the Gila, a large lizard of North America, and has a 53% homology to GLP-1. The effects of exendin-4 on the human body are like those of GLP-1, though there are some differences, such as longer half-life and greater strength. Exendin-4 has been proven to enhance proliferating properties of pancreatic beta cells and prevents beta cell death in hyperglycemia and endoplasmic reticulum stress. Based on this evidence, exendin-4 is not an analogue of GLP-1 and, in fact, the joint activities of these two proteins in glucose regulation relate to their common organs in the pancreas [17, 18]. Various studies confirm the glucose homeostasis properties, beta cell mass protection, and neogenesis of pancreatic duct cells by exendin-4 [19–21]. Pdx1 is a transcription factor essential for pancreatic evolution and beta cells maturation. In addition to contributing to the survival of beta cells, Pdx1 also plays a role in enhancing insulin secretion, somatostatin, and beta-cell responses, so that any mutation leads to a variety of pancreatic-related diseases [22, 23]. Glut2 is an effective sensor and vector for glucose and is expressed on the plasma membrane of beta cells of the pancreas, liver, small intestine, and hypothalamus. Any disorder in the function of this protein has a negative effect on glucose homeostasis and disrupts the production of insulin from the beta cells [24].

A study in 2015 by J P. Tiano demonstrated significant synergistic effects of estrogen (E2) and GLP-1 on GSIS betterment. The study also reported that conjugated E2-GLP-1, by synergistic effects can play a role in preventing type 2 diabetes. One of mechanisms attributed to this process was inhibition of glucose production by liver [25]. Other similar studies have reported that simultaneous treatment with estrogen receptor agonists and GLP-1 improves insulin sensitivity in the liver tissue [26–28]. Since bisphenol is a substance that can bind to estrogen receptors and exendin-4 is a strong agonist of the GLP-1 receptors, therefore, in the present study, we have chosen to prescribe these two substances together.

In this study, an effective insulin tropic concentration of exendin-4 on isolated islets was selected and the islet cell apoptosis rate was evaluated in vitro. Further, the preventive effects of exendin-4 on glucose homeostasis and Pdx1 (pancreatic and duodenal homebox 1) and GLUT2 (glucose transporter 2) gene expression disturbed by BPA were evaluated.

https://doi.org/10.46389/rjd-2020-1048

Materials and Method

NMRI male mice with 25–30 g body weight was purchased from the animal house at the Ahvaz Jundishapur University of Medical Sciences. Animals were accommodated in BPA-free cages at 22°C ± 2°C, under a standard 12 h light/12 hour dark cycle with ad libitum access to food and tap water. All protocols executed were compatible with standards of animal care, demonstrated by the ethics commission (CMRC-96) of the Ahvaz Jundishapur University of Medical Sciences (Ahvaz, Iran). The anesthesia and euthanasia methods used in the current experiment were based on the method described by Carter et al. in 2009 [29], and on AVMA Guidelines for the Euthanasia of Animals, 2013 edition. We used male mice in the present study to elude the disconcerting effect of circulating estrogens in females. It should be noted that many studies on BPA have been performed on male laboratory specimens [30, 31], and cases involving female animals, mostly have been studied in the field of fertility and embryos [32, 33].

Our previous published study of BPA and exendin-4 had been focused on body weight, triglyceride, total cholesterol, LDL-cholesterol (LDLc), VLDL-cholesterol (VLDL–c) in plasma and also catalase (CAT), glutathione peroxidase (GPX), and superoxide dismutase (SOD) activity in pancreas tissue [34].

In-vitro protocol

About eight hours fasted intact mice were euthanized with an IP injection of ketamine (60 mg/kg) and xylazine (10 mg/kg) mixture. To expose all peritoneal cavity organs, the abdomen was cut surgically in a V shape. The common bile duct (CBD) near the small intestine junction was clamped, and 5 mL collagenase-p (Roche, Germany) was dissolved in 1.4 mg/mL concentration of Hank's balanced salt solution which was injected in the CBD junction of cystic and left hepatic ducts. After the pancreas started swelling, it was removed and allowed to digest at 37°C for 8–11 min. Further, digested tissue was centrifuged for 2 min at 1200 rpm, and the supernatant was discarded and all tubes were filled with Hank's solution. The rinse procedure was repeated 3–4 times. The islets were separated by hand picking under a stereo microscope and incubated at RPMI 1640+L-glutamine (Gibco Company, Germany) that was supplemented with 5 mM D glucose, 10% fetal bovine serum (FBS) (Gibco Company, Germany), 100 u/mL penicillin, and 100 µg/mL streptomycin (Gibco Company, Germany). Based on a previous experiment, 100 µg/L concentration of BPA (Sigma Aldrich Company, Germany) was selected for the in-vitro study [8, 31, 35].

Glucose stimulating insulin secretion

To select an effective concentration of exendin-4, first, five different concentrations of exendin-4 (2, 4, 8, 16 and 32 nM) were examined in the in-vitro condition (Sigma Aldrich Company, Germany. Cat No 141758-74-9, more than 97% purity). During the in-vitro experiment, the islets of different mice were pooled, and then divided into 12 groups so that each group has seven replicates each with five islets (Table 1). All groups were incubated at 37°C with 95% O_2 and 5% CO_2 for 24 h. Next, the islets in different groups were washed with Hank's solution, and all groups were incubated in three concentrations of glucose (2.8, 5.6, and 16.7 mM) for 60 min. Finally, the supernatant was collected and insulin secretion assessed using enzyme-linked immunosorbent assay (ELISA) method (Insulin ELISA Kit, Monobind, Inc, USA, code: 8525–300).

Apoptosis assay

After a dose response experiment of exendin-4 at the beginning of current study, the 4 nM concentration of exendin-4 determined as the selective insulinotropic dose in the in-vitro part and 4 nmol/kg/d of exendin-4 prescribe in the in-vivo experiment. Further glibenclamide is one of the most widely used drug as positive control group [36, 37] To evaluate islet cells wellness, isolated islets were pooled in culture medium and divided into five groups so that each group has

Table 1: In vitro groups for insulin secretion

Number	Description
1.	Control (culture media)
2.	2 nM exe-4 (media with 2 nM concentration of exendin4)
3.	4 nM exe-4 group (media with 4 nM concentration of exendin4)
4.	8 nM exe-4 group (media with 8 nM concentration of exendin4)
5.	16 nM exe-4 group (media with 16 nM concentration of exendin-4)
6.	32 nM exe-4 group (media with 32 nM concentration of exendin-4)
7.	BPA group (media with 100 µg/l concentration of BPA)
8.	BPA + 2nM exe-4 group (media with 2 nM concentration of exendin-4 and 100 µg/L concentration of BPA)
9.	BPA + 4nM exe-4 group (media with 4 nM concentration of exendin-4 and 100 µg/L concentration of BPA)
10.	BPA + 8 nM exe-4 group (media with 8 nM concentration of exendin-4 and 100 µg/L concentration of BPA)
11.	BPA + 16nM exe-4 group (media with 16 nM concentration of exendin-4 and 100 µg/L concentration of BPA)
12.	BPA + 32nM exe-4 group (media with 32 nM concentration of exendin-4 and 100 µg/L concentration of BPA)

four replicates with 10 islet: 1) control group (culture media), 2) BPA group (culture media with 100 µg/L concentration of BPA [9, 31, 35]), 3) the BPA + glibenclamide (Gb) group (culture media with 3 mg/L concentration of Gb and 100 µg/L concentration of BPA), 4) the BPA + exe-4 group (culture media with 4 nM concentration of exendin-4 and 100 µg/L concentration of BPA), 5) the exe-4 group (culture media with 4 nM concentration of exendin-4). Further, 11 mM of glucose concentration was established for all groups because the minimum rate of apoptosis and maximum viability rate of rodent islet cell occurs at this concentration [38]. Islets were incubated at 37 ˚C with 95% O_2 and 5% CO_2 for 48 hour. The medium was replaced with a fresh one every 24 hour. After this period, the medium was removed and the cells washed with phosphate buffer saline. Then trypsin was used for intracellular junction destruction. Next, the percentages of apoptosis of islet cells were measured using Annexin V Apoptosis detection kit FITC (According to the instructions, eBioscience, Cat 88-8005) and flow cytometry. Finally, the data were analyzed using the Win Med 2.9 software.

In-vivo protocol

In total, 40 adults, 2.5–3 months aged NMRI male mice were acclimatized for one week in a standard room. The mice were divided into five experimental groups (Table 2). The control group received solvent every day, BPA group received 100 µg/kg/d BPA for 20 days, the BPA + Gb group received 100 µg/kg/d BPA for 10 days and co-administration of 3 mg/kg/d Gb and BPA in the last 10 days, BPA + exendin-4 group received 100 µg/kg/d BPA for 10 days and co-administration of 4 nmol/kg/d exendin-4 and BPA in the last 10 days, and exendin-4 group received 4 nmol/kg/d exendin-4 for 20 days. BPA solvent was ethyl alcohol with a final concentration of 0.1%, exendin-4 and the glibenclamide solvent was distilled water. To induce disturbance, BPA was injected subcutaneously in order to ensure better absorption and, according to previous studies, there was no significant difference in plasma levels of BPA with different prescribing methods [39]. Exendin-4 was administered by IP injection, and glibenclamide was administered orally once daily. Our treatment period was chosen based on our earlier studies [40].

https://doi.org/10.46389/rjd-2020-1048

Table 2: In vivo experimental groups

	Groups/Days	Days 1–10	Days 11–20
1	Control	Solvent	Solvent
2	BPA	BPA	BPA
3	BPA + glibenclamide	BPA	BPA + glibenclamide
4	BPA + exendin-4	BPA	BPA + exendin-4
5	Exendin-4	Exendin-4	Exendin-4

Blood collection and biochemical assay

After treatment duration, mice were anesthetized with intraperitoneal injections of ketamine (60–80 mg/kg) and xylazine (10 mg/kg) mixture. The blood glucose level was measured through blood sampling from the tail by glucometer after 8–9 hours of fasting. Next, a cardiac puncture was performed to get more blood, and then plasma was separated by centrifugation (4000 rpm, 10–12 min). Enzyme-linked immunosorbent assay kit (Insulin ELISA Kit, Monobind, Inc, USA, code: 8525–300) was used to assay the plasma insulin level. The within-assay and between-assay coefficients of variation were 4.3% and 9.5%, respectively. Blood samples from the heart and pancreas tissue were rapidly removed and frozen in liquid nitrogen.

Quantitative real-time polymerase chain reaction (PCR)

RNA was extracted from homogenized pancreas tissues of five different groups using Qiagen RNeasy Plus Mini Kit (Cat 74134 USA); Thermo Scientific kit (K1621 USA) was used for cDNA synthesis. To perform quantitative real-time PCR, Thermo Scientific Maxima SYBR Green/ROX qPCR Master Mix2X (K0221 USA) was used. For Pdx1, GLUT2, and β-actin mRNA expression, the following primer sequence was used, according to the Suzuki study (Table 3) [41]: Quantitative real-time PCR (95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing and extension at 60°C for 30 s) performed with ABI plus (7000 PCR instrument, Applied Biosystems US). The level of these gene expressions normalized to β-actin as a housekeeping gene. The results were based on the $2^{-\Delta\Delta CT}$ method and relative quantification. The mean expression value of the control group was considered as one.

Statistical analysis

Before statistical analysis, normal distribution and homogeneity of the variances were evaluated using Levene's test, then by using one-way analysis of variance followed by Tukey's as post hoc test and was presented in figures as mean ± SEM. Differences were considered statistically significant at P values <0.05.

Table 3: Primers used for real-time PCR

Gene	Primer	Sequence (5′-3′)	PCR length
Pdx1	Pdx1 Forward	CCG AGA GAC ACA TCA AAA TCT GG	80 bp
	Pdx1 Reverse	CCC GCT ACT ACG TTT CTT ATC TTC C	
GLUT2	GLUT2 Forward	TTG ACT GGA GCC CTC TTG ATG	73 bp
	GLUT2 Reverse	CAC TTC GTC CAG CAA TGA TGA	
β-ACTIN	β-ACTIN Forward	GGC CAA CCG TGA AAA GAT GA	79 bp
	β-ACTIN Reverse	CAC AGC CTG GAT GGC TAC GT	

Result and Discussion

Results

In-vitro results

The effect of different concentration of exendin-4 alone and in combination with BPA on glucose-stimulated insulin secretion (GSIS), in vitro

Exendin-4 in five different concentrations and three various doses of glucose (Table 1) increased the insulin secretion from isolated islets, but the highest values were at 4 nM concentration ($p<0.001$ in comparison with the control group, Table 4). At higher values of exendin-4 (>4 nM of exendin-4), insulin secretion from isolated islets was less. BPA reduced by approximately 31% the amount of insulin secretion from isolated islets in comparison with the control group and this reduction, only at 16.7 mM concentration of glucose was significant ($p<0.01$). The concomitant use of exendin-4 with BPA elevated insulin secretion and could increase the level of insulin secretion to the same level of the control group (Table 4).

Islet cells apoptosis

Apoptosis will be detected initially by staining the cells with Annexin V and propidium Iodide solution followed by flow cytometry analysis. In the chart provided by flow cytometry (dot plot chart), the upper left quadrant displayed necrosis, whereas the left lower quadrant displayed the healthy cells. The upper right quadrant revealed dead cells and the lower right quadrant displayed early stage of apoptosis of cells, which in the current experiment are reported as apoptotic cell (Fig. 2). [42]. The evaluation of apoptosis results showed that in the BPA group, the percentage of apoptosis in islet cells was significantly increased ($p<0.001$) and the percentage of healthy cells were obviously decreased ($p<0.001$), compared to the control group. As expected, there are similarities between control group and exendin-4 group in the percentage of apoptosis, so that exendin-4 group showed the lowest percentage of apoptosis. The study of the percentage of normal cells by flow cytometry revealed that BPA significantly reduced the level of normal cells, and in co-administration of BPA and exendin-4, reduction in normal cell count did not occur (Fig. 1).

In-vivo results

Fasting blood glucose (FBG)

BPA increased fasting blood glucose compared with control ($p < 0.001$), and co-administration of exendin-4 with BPA modified blood glucose levels. The results of BPA+ exendin-4 group were similar to those of the BPA + Gb group, and using exendin-4 alone in a 20-day period had no effect on fasting blood glucose (Fig. 3).

Plasma insulin level

Plasma insulin level in the BPA group significantly reduced ($p<0.01$) in comparison with the control group, and co-administration of BPA with exendin-4 significantly increased the level of insulin in comparison with the control group ($p<0.01$) and BPA ($p<0.001$) group (Fig. 4).

Pdx1 and GLUT2 gene expression

Quantitative real-time PCR results of pancreatic tissue showed that BPA significantly decreased ($p<0.05$) the level of Pdx1/β-Actin expression in comparison with the control group. Conversely, in the group where BPA was administered in combination with exendin-4, the expression level of Pdx1/β-Actin showed a significant increase ($p<0.05$) in comparison with the BPA group. Moreover, in the exendin-4 group, the expression level of Pdx1/β-Actin increased significantly ($p<0.001$) in comparison with the control group (Fig. 5). QRTPCR results of GLUT2/β-Actin gene expression in different groups showed that BPA significantly ($p<0.01$) reduces the level of gene expression. The co-administration of BPA

https://doi.org/10.46389/rjd-2020-1048

Table 4: In vitro insulin secretion

	control	2nM exe-4	4nM exe-4	8nM exe-4	16nM exe-4	32nM exe-4	BPA	BPA+2nM exe-4	BPA+4nM exe-4	BPA+8nM exe-4	BPA+16nM exe-4	BPA+32nM exe-4
2.8mM Glucose	0.09 ± 0.004	0.11 ± 0.008	0.12 ± 0.007	0.12 ± 0.015	0.12 ± 0.025*	0.12 ± 0.013*	0.07 ± 0.005	0.07 ± 0.007	0.08 ± 0.01	0.12 ± 0.014*#	0.09 ± 0.007	0.07 ± 0.014
5.6mM Glucose	0.11 ± 0.004	0.11 ± 0.007	0.16 ± 0.01***	0.12 ± 0.008*	0.12 ± 0.014*	0.12 ± 0.009*	0.08 ± 0.002	0.09 ± 0.005	0.11 ± 0.023#	0.11 ± 0.004#	0.09 ± 0.013	0.09 ± 0.004
16.7mM glucose	0.13 ± 0.004	0.13 ± 0.011	0.2 ± 0.32***	0.12 ± 0.007	0.12 ± 0.009	0.12 ± 0.01	0.08 ± 0.005**	0.10 ± 0.007	0.12 ± 0.018#	0.11 ± 0.003#	0.10 ± 0.014	0.10 ± 0.004

Insulin secretion ng/ml/islet/60 min from isolated islet after 24 hour incubation in different concentration of exendin-4 and 100 µg/L BPA and then incubation in three doses of glucose for 60 min. All groups compared with its relative control group in terms glucose concentration. *p<0.05, **p<0.01, ***p<0.001 vs. control group. #p<0.05 vs. BPA group. Data are expressed as mean ± SEM.

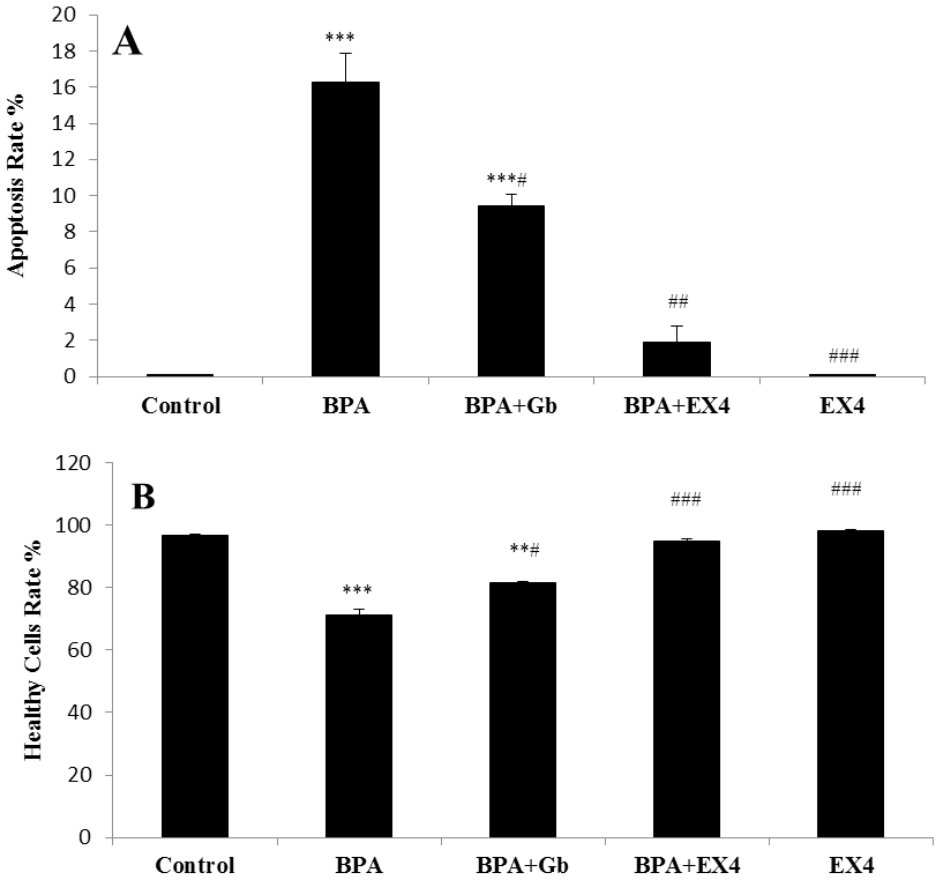

Figure 1: *Effect of BPA and exendin-4, alone and together, on pancreatic islet cell Apoptosis* (A) and healthy (B) after 48 h incubation of intact islets in five in vitro groups. **p<0.01, *** p<0.001 vs. control. #p<0.05, ##p<0.01, ### p<0.001 vs. BPA group. Data is expressed as mean ± SEM, based on the percentage provided by flow cytometry (n = 4).

and exendin-4 compensates for this reduction similar to the control group. In the exendin-4 group, there was a significant (p<0.001) increase in GLUT2/β-Actin gene expression than in the control and BPA groups (Fig. 6).

Discussion

This study aimed to evaluate the effects of exendin-4 on glucose homeostasis and gene expression complicated by BPA. This paper first reported about exendin-4 effects on GSIS improvement, and also highest values of insulin secretion at doses of 5.6 and 16.7 mM of glucose and the finding is in agreement with Niu B et al. findings [43]. Consistent with our study, Padmasekar et al. observed that exendin-4 increased insulin secretion from INS-1E cells and isolated islets of mouse in a dose-dependent manner [44]. Several studies have shown that phenolic estrogen induces morphological changes in isolated islets and impairs the amount and content of insulin secretion [45–47]. In addition, BPA disrupts the endocrine system through interaction with the ER and through other pathways, including those of oxidative stress [40, 46, 48], insulin signaling disturbance [49], and beta cell apoptosis [13, 40]. A recent literature has emerged that the effect of BPA on insulin secretion has an inverse U shape in a dose-dependent manner. At doses as low as 100 pM–1 nM of BPA, insulin secretion increases, but higher doses of exendin-4 induced decrease in the content and secretion of insulin from islets [50]. It is interesting to note that, an increase in the amount of insulin released in response to glucose stimulation in the presence of BPA, as seen in some literature, is due to the depletion of high content of insulin in beta cells [51]. As mentioned in the prior studies, just because there is no significant reduction in insulin secretion in BPA group, we cannot ignore its

https://doi.org/10.46389/rjd-2020-1048

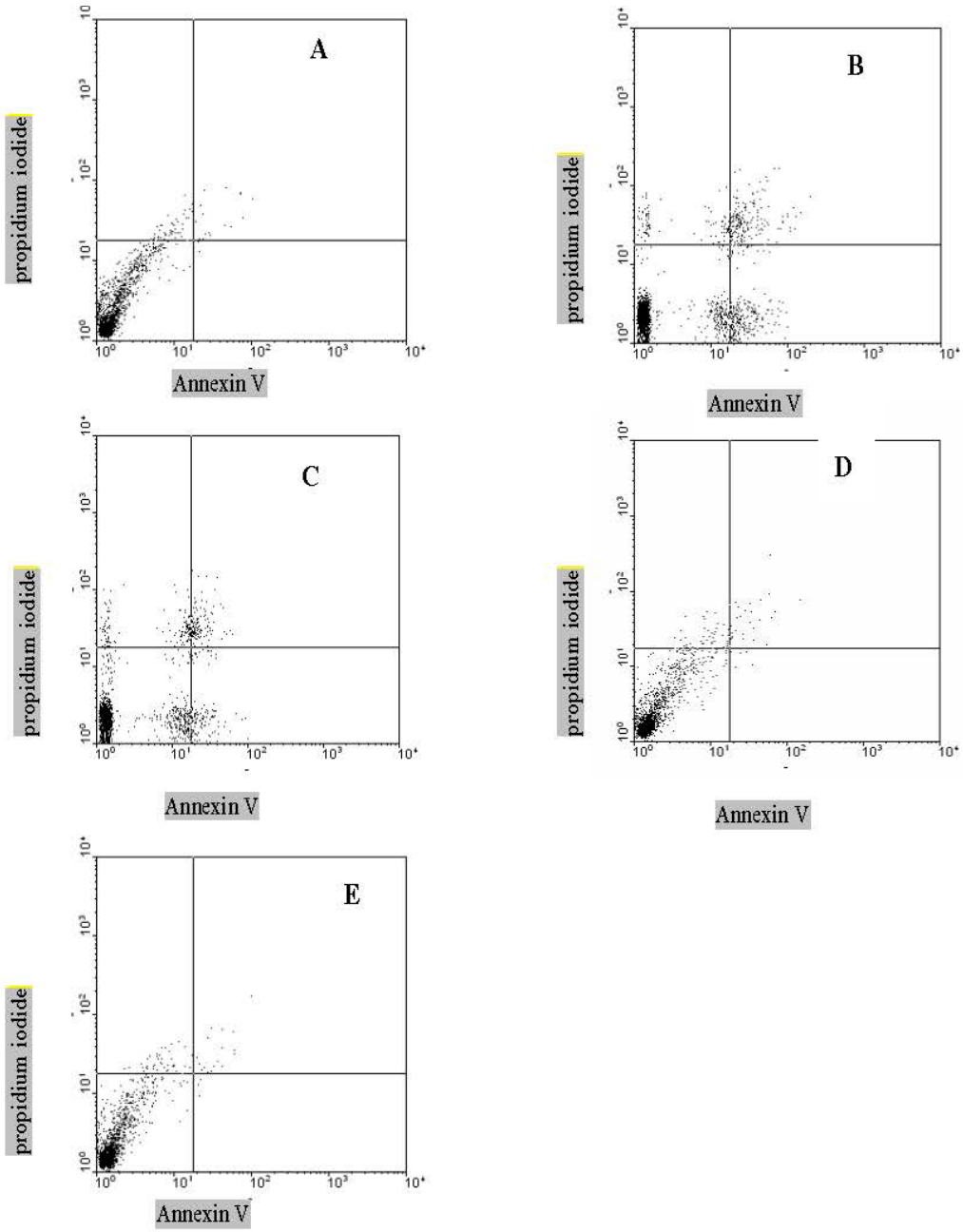

Figure 2: *Apoptosis dot plot*. (A) Control; (B) BPA; (C) BPA + Gb; (D) BPA + EX4; (E) EX4. X axis indicates the cells bind to annexin V and Y axis indicates the cells bind to propidium iodide (PI). The upper left quadrant display necrosis, whereas the left lower quadrant displays the healthy cells. The upper right quadrant revealed dead cells and the lower right quadrant display early stage of apoptosis cells.

damaging effects on GSIS. Further, in current examination insulin secretion showed a decreasing trend at doses of exendin-4 increased >4 nM/L. This chosen concentration of exendin-4 in our study is in agreement with those of the others studies on exendin-4 and its effects on insulin resistance, serum glucose and insulin, lipid profile and antioxidant level [52, 53].

Another important finding was that BPA induced a high percentage of apoptosis in pancreatic islet cells and, it has been said that the amount of healthy cells in the islands has been significantly reduced in BPA group. Also, in this study, the exendin-4 recipient groups showed a very little percentage of apoptosis and the amount of healthy cell was significantly higher. In the study of the effects of bisphenol on pancreatic function, in 2013, Liu et al. demonstrated that functional abnormality occurred in beta cells, could be attributed to BPA impacts on beta

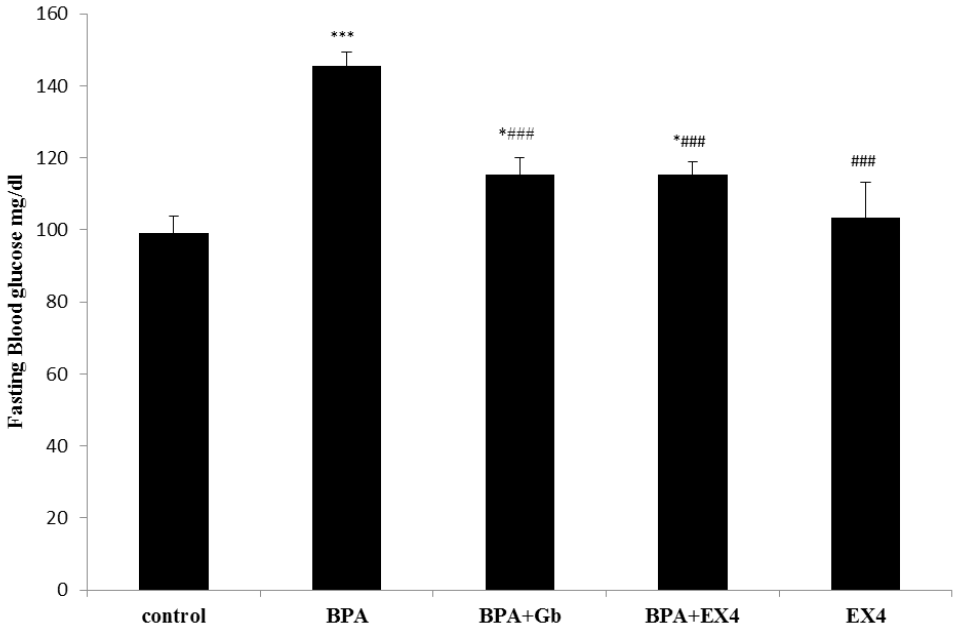

Figure 3: *Effect of BPA and exendin-4, singly and together, on fasting blood glucose in five in vivo groups.* *p<0.05, *** p<0.001 vs. control. ### p<0.001 vs. BPA group. Data is expressed as mean ± SEM.

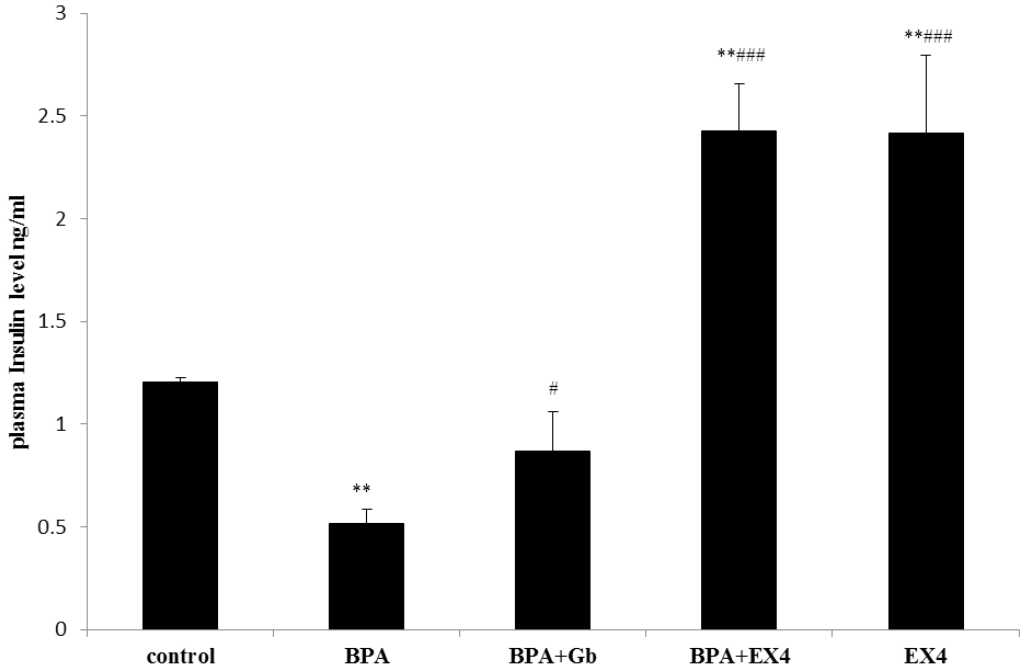

Figure 4: *Effect of BPA and exendin-4, singly and together, on plasma insulin level in five in vivo groups.* ** p<0.01 vs. control. # p<0.05, ### p<0.001 vs. BPA group. Data is expressed as mean ± SEM.

cell apoptosis [54]. Several pathways have been demonstrated as intermediaries for cell apoptosis. Studies by Song et al. have shown that BPA by coupling with ER, induces the stimulatory effect on insulin secretion, and increasing demand for insulin can cause mitochondrial dysfunction in pancreatic beta cells, [46] and over-repetition of these stages resulted in apoptosis of beta cells [55]. Previous studies have reported that exendin-4 by inhibiting mitogen-activated protein kinase kinase 7 and 4 (MKK7 & 4) and reducing G-protein-coupled receptor 40 (GPR40) expression can prevent from apoptosis of beta cells [56]. MKK isoforms are involved in signal transduction

https://doi.org/10.46389/rjd-2020-1048

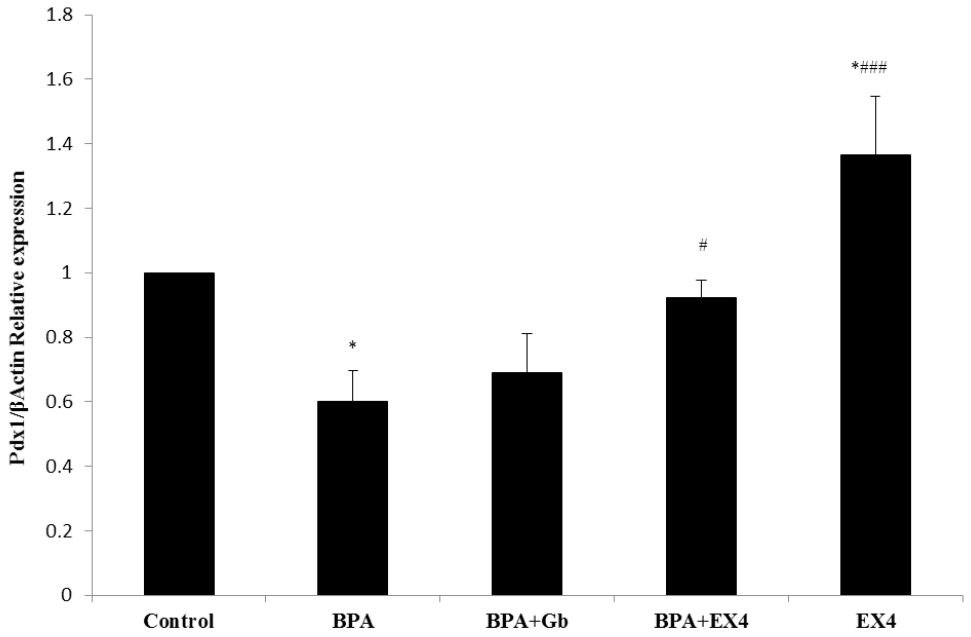

Figure 5: *Effect of BPA and exendin-4, singly and together on Pdx1/βActin relative expression ratio in five in vivo groups.* * p<0.05, vs. control. # p<0.05, ### p<0.001 vs. BPA group. Data is expressed as mean ± SEM.

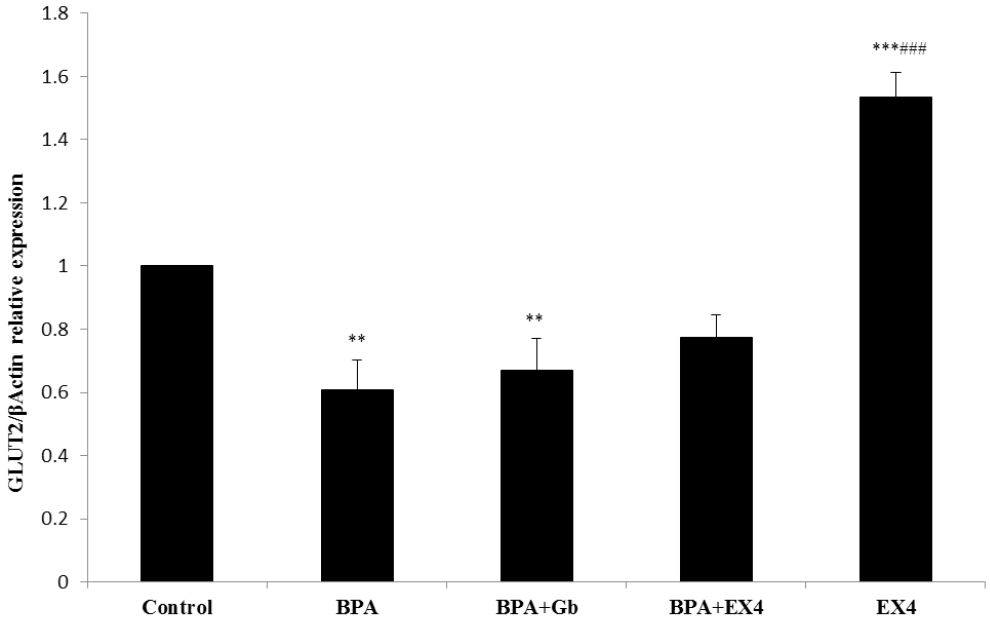

Figure 6: *Effect of BPA and exendin-4, alone and together, on GLUT2/βActin relative expression ratio in five in vivo groups.* ** p<0.01, *** p<0.01 vs. control. ### p<0.001 vs. BPA group. Data is expressed as mean ± SEM.

mediating the cellular responses to proinflammatory cytokines, and environmental stresses [57]. GPR40 plays an important role in obesity and type 2 diabetes, and also in over expression of GPR40 in beta cells leading to diabetes [58]. Carlessi R, et al. in 2015 noted the effects of exendin-4 on improving the health of beta cell by reducing the pancreatic inflammation and oxidative stress, which leads to reduction in ER stress and likelihood of cell death [59].

In the in-vivo conditions, it was observed that BPA had significantly (p<0.001) increased fasting blood glucose levels in the groups receiving it. Several studies investigating that, BPA causes insulin resistance and hyperglycemia by down regulating insulin receptors, glucose

transporter and reducing their function (9, 50, 60). As noted in the results, exendin-4 was able to prevent hyperglycemia induced by BPA, and in this case was equal to glibenclamide. Data from several sources have identified that exendin-4 differentiates pancreatic duct cells into insulin producing cells [61–63]. Conversely, a different study concluded that the glucose lowering effect of exendin-4 in normal, non-diabetic mice appeared did not correlate with increased beta cell mass or insulin secretion [64].

Contrary to what we found, in a different work by Angle et al., BPA caused an increase in plasma insulin level [65] but there were differences in dose and duration of that treatment in our protocol, whereas the findings of D'Cruz et al. were similar to our findings in the current and in our previous experiment [40, 66]. Current knowledge about BPA is equivocal and the duration and dose of exposure are important factors in establishing the type and severity of complications. Further, in the current experiment, alone exendin-4 significantly increased plasma insulin levels. Consistent with our findings, several studies indicated that exendin-4 improved glucose tolerance by elevating plasma insulin levels [67–69].

GLUT2 plays an essential role in GSIS in pancreas β-cells by facilitating the entrance of glucose into the cells [70]. Another study by Moshtagh et al. on adipose-derived tissue stem cells showed that exendin-4 induced expression of Pdx1 and GLUT2 in differentiated cells [71]. In a different work, Chen et al. observed that activation of some cascade pathways in pancreatic β-cells may be important for GLUT2 gene transcription induction by exendin-4, indicating that exendin-4 by activating the CaMKK/CaMKIV cascade (calcium/calmodulin-dependent (CaM) kinase cascade) plays an important role in GK and GLUT2 expression and improvement of insulin secretion in pancreatic β-cells [72]. Recent evidence documented that exendin-4 progress the expression of some important transcription factors, such as Pdx1 and Glut2, suggesting that exendin-4 facilitates differentiation of R1 embryonic stem cells into insulin-producing cells during regeneration [73]. Based on Johnson's studies, the over expression of GLUT2 may be secondary to the up regulation of Pdx1, because Pdx1is a β-cell master gene that has been shown to regulate GLUT2 transcription in β-cells [74, 75]. After increasing of GLUT2 expression in the pancreas and subsequently increasing the glucose transport from beta cells, a signal will be produced, and consequently, insulin secretion will be increased and blood glucose level will be decreased to an extent that glucose homeostasis will be established. Conversely, if it is assumed that GLUT2 also shows over expressed in the intestinal lumen and other tissues, such as kidney tubules; the increase in the absorption and reabsorption of glucose in the blood will also cause more release of insulin from pancreatic beta cells. This can be taken into account in the dramatic increase in insulin levels in groups that received exendin-4.

Conclusions

The purpose of the current study was to determine the effective insulin tropic amount of exendin-4 in the In-vitro and its generalization to the In-vivo, as well as healing effects of exendin-4 on the biochemical and genetic parameters in terms of our existing conditions. These finding suggest that BPA causes increase in the blood glucose level, and also decrease the Pdx1 and GLUT2 gene expression in pancreatic tissue, whereas exendin-4 revealed a preventive role in these cases. This research provides a framework for future study in more potential mechanistic insights of the signaling pathways and more protein and gene evaluation.

Authorship

Authors' contributions: Golshan Afshari and Akram Ahangarpour designed the study, collected the data, conducted the statistical analysis and wrote the manuscript. Golshan Afshari contributed to the statistical analysis, contributed to the discussion, and reviewed the manuscript. Seyyed Ali Mard, Ali Khodadadi, and Mahmoud Hashemitabar contributed to the study design and reviewed the manuscript. All authors read and approved the final version.

https://doi.org/10.46389/rjd-2020-1048

Conflict of interest

The authors declare no conflict of interest.

Acknowledgment

This study is a part of the Ph.D. thesis of Golshan Afshari and financially supported by vice chancellor of research affairs of the Ahvaz Jundishapur University of Medical Sciences (AJUMS) (Grant No.CMRC-96). The authors also thank the experienced personnel of the Cellular and Molecular Research Center of AJUMS.

References

1. Soltysik K, Czekaj P. Membrane estrogen receptors - is it an alternative way of estrogen action? *J Physiol Pharmacol*. 64(2):129–42, 2013.
2. Duong V, Licznar A, Margueron R, Boulle N, Busson M, Lacroix M, et al. ERalpha and ERbeta expression and transcriptional activity are differentially regulated by HDAC inhibitors. *Oncogene*. 25(12):1799–806, 2006.
3. Kim JH, Cho HT, Kim YJ. The role of estrogen in adipose tissue metabolism: insights into glucose homeostasis regulation. *Endocrine journal*. 61(11):1055–67, 2014.
4. Guillaume M, Montagner A, Fontaine C, Lenfant F, Arnal JF, Gourdy P. Nuclear and Membrane Actions of Estrogen Receptor Alpha: Contribution to the Regulation of Energy and Glucose Homeostasis. *Advances in experimental medicine and biology*. 1043:401–26, 2017.
5. Barros RP, Machado UF, Warner M, Gustafsson JA. Muscle GLUT4 regulation by estrogen receptors ERbeta and ERalpha. Proceedings of the National Academy of Sciences of the United States of America. 103(5):1605–8, 2006.
6. Heine PA, Taylor JA, Iwamoto GA, Lubahn DB, Cooke PS. Increased adipose tissue in male and female estrogen receptor-alpha knockout mice. *Proceedings of the National Academy of Sciences of the United States of America*. 97(23):12729–34, 2000.
7. Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, et al. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. *Endocrine reviews*.30(4):293–342, 2009.
8. Alonso-Magdalena P, Morimoto S, Ripoll C, Fuentes E, Nadal A. The Estrogenic Effect of Bisphenol A Disrupts Pancreatic β-Cell Function In Vivo and Induces Insulin Resistance. Environ Health Perspect. 114(1):106–12, 2005.
9. Alonso-Magdalena P, Morimoto S, Ripoll C, Fuentes E, Nadal A. The estrogenic effect of bisphenol A disrupts pancreatic beta-cell function in vivo and induces insulin resistance. *Environ Health Perspect*. 114(1):106–12, 2006.
10. Alonso-Magdalena P, Ropero AB, Soriano S, Quesada I, Nadal A. Bisphenol-A: a new diabetogenic factor? *Hormones (Athens)*. 9(2):118–26, 2010.
11. Brotons JA, Olea-Serrano MF, Villalobos M, Pedraza V, Olea N. Xenoestrogens released from lacquer coatings in food cans. *Environ Health Perspect*. 103(6):608–12, 1995.
12. Shankar A, Teppala S. Relationship between urinary bisphenol A levels and diabetes mellitus. *The Journal of clinical endocrinology and metabolism*. 96(12):3822–6, 2011.
13. Nadal A, Alonso-Magdalena P, Soriano S, Ripoll C, Fuentes E, Quesada I, et al. Role of estrogen receptors alpha, beta and GPER1/GPR30 in pancreatic beta-cells. *Frontiers in bioscience* (Landmark edition). 16:251–60, 2011.
14. Khan S, Beigh S, Chaudhari BP, Sharma S, Aliul Hasan Abdi S, Ahmad S, et al. Mitochondrial dysfunction induced by Bisphenol A is a factor of its hepatotoxicity in rats. *Environmental toxicology*. 31(12):1922–34, 2016.
15. Lin Y, Sun X, Qiu L, Wei J, Huang Q, Fang C, et al. Exposure to bisphenol A induces dysfunction of insulin secretion and apoptosis through the damage of mitochondria in rat insulinoma (INS-1) cells. *Cell death & disease*. 4:e460, 2013.
16. Lee CY. Glucagon-Like Peptide-1 Formulation--the Present and Future Development in Diabetes Treatment. *Basic & clinical pharmacology & toxicology*. 118(3):173–80, 2016.
17. Parkes DG, Pittner R, Jodka C, Smith P, Young A. Insulinotropic actions of exendin-4 and glucagon-like peptide-1 in vivo and in vitro. *Metabolism: clinical and experimental*. 50(5):583–9, 2001.
18. Goke R, Fehmann HC, Linn T, Schmidt H, Krause M, Eng J, et al. Exendin-4 is a high potency agonist and truncated exendin-(9-39)-amide an antagonist at the glucagon-like peptide 1-(7-36)-amide receptor of insulin-secreting beta-cells. *J Biol Chem*. 268(26):19650–5, 1993.
19. Baggio L, Adatia F, Bock T, Brubaker PL, Drucker DJ. Sustained expression of exendin-4 does not perturb glucose homeostasis, beta-cell mass, or food intake in metallothionein-preproexendin transgenic mice. *J Biol Chem*. 275(44):34471–7, 2000.
20. Wei Q, Sun YQ, Zhang J. Exendin-4, a glucagon-like peptide-1 receptor agonist, inhibits cell apoptosis induced by lipotoxicity in pancreatic beta-cell line. *Peptides*. 37(1):18–24, 2012.
21. Ah Kim H, Lee S, Park JH, Lee S, Lee BW, Ihm SH, et al. Enhanced protection of Ins-1 beta cells from apoptosis under hypoxia by delivery of DNA encoding secretion signal peptide-linked exendin-4. *Journal of drug targeting*. 17(3):242–8, 2009.
22. D'Amour KA, Bang AG, Eliazer S, Kelly OG, Agulnick AD, Smart NG, et al. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nature biotechnology*. 24(11):1392–401, 2006.
23. Ma J, Chen M, Wang J, Xia HH, Zhu S, Liang Y, et al. Pancreatic duodenal homeobox-1 (PDX1) functions as a tumor suppressor in gastric cancer. *Carcinogenesis*. 29(7):1327–33, 2008.
24. Stolarczyk E, Le Gall M, Even P, Houllier A, Serradas P, Brot-Laroche E, et al. Loss of sugar detection by GLUT2 affects glucose homeostasis in mice. *PloS one*. 2(12):e1288, 2007.
25. Tiano JP, Tate CR, Yang BS, DiMarchi R, Mauvais-Jarvis F. Effect of targeted estrogen delivery using glucagon-like peptide-1 on insulin secretion, insulin sensitivity and glucose homeostasis. *Scientific reports*. 5:10211, 2015.
26. Ahmed-Sorour H, Bailey CJ. Role of ovarian hormones in the long-term control of glucose homeostasis. Interaction

with insulin, glucagon and epinephrine. *Hormone research*. 13(6):396–403, 1980.
27. Lee YS, Shin S, Shigihara T, Hahm E, Liu MJ, Han J, et al. Glucagon-like peptide-1 gene therapy in obese diabetic mice results in long-term cure of diabetes by improving insulin sensitivity and reducing hepatic gluconeogenesis. *Diabetes*. 56(6):1671–9, 2007.
28. Zhu L, Brown WC, Cai Q, Krust A, Chambon P, McGuinness OP, et al. Estrogen treatment after ovariectomy protects against fatty liver and may improve pathway-selective insulin resistance. *Diabetes*. 62(2):424–34, 2013.
29. Carter JD, Dula SB, Corbin KL, Wu R, Nunemaker CS. A practical guide to rodent islet isolation and assessment. *Biol Proced Online*. 11:3–31, 2009.
30. Bodin J, Kocbach Bolling A, Wendt A, Eliasson L, Becher R, Kuper F, et al. Exposure to bisphenol A, but not phthalates, increases spontaneous diabetes type 1 development in NOD mice. *Toxicology reports*. 2:99–110, 2015.
31. Bodin J, Bolling AK, Samuelsen M, Becher R, Lovik M, Nygaard UC. Long-term bisphenol A exposure accelerates insulitis development in diabetes-prone NOD mice. *Immunopharmacology and immunotoxicology*. 35(3):349–58, 2013.
32. Alonso-Magdalena P, Garcia-Arevalo M, Quesada I, Nadal A. Bisphenol-A treatment during pregnancy in mice: a new window of susceptibility for the development of diabetes in mothers later in life. *Endocrinology*. 156(5):1659–70, 2015.
33. Ziv-Gal A, Flaws JA. Evidence for bisphenol A-induced female infertility: a review (2007-2016). *Fertility and sterility*. 106(4):827–56, 2016.
34. Ahangarpour A, Afshari G, Mard SA, Khodadadi A, Hashemitabar M. Alteration Effect of Exendin-4 on Oxidative Stress and Metabolic Disorders Induced by Bisphenol A in Adult Male Mice. *Jentashapir J Health Res*. 7(5):e37836 2016.
35. Adachi T, Yasuda K, Mori C, Yoshinaga M, Aoki N, Tsujimoto G, et al. Promoting insulin secretion in pancreatic islets by means of bisphenol A and nonylphenol via intracellular estrogen receptors. *Food Chem Toxicol*. 43(5):713–9, 2005.
36. Zhang H, Yi Y, Feng D, Wang Y, Qin S. Hypoglycemic Properties of Oxovanadium (IV) Coordination Compounds with Carboxymethyl-Carrageenan and Carboxymethyl-Chitosan in Alloxan-Induced Diabetic Mice. *Evidence-based complementary and alternative medicine: eCAM*.2011:691067, 2011.
37. Li F, Zhang Y, Zhong Z. Antihyperglycemic effect of ganoderma lucidum polysaccharides on streptozotocin-induced diabetic mice. *International journal of molecular sciences*. 12(9):6135–45, 2011.
38. Robertson RP, Harmon J, Tran PO, Tanaka Y, Takahashi H. Glucose toxicity in beta-cells: type 2 diabetes, good radicals gone bad, and the glutathione connection. *Diabetes*. 52(3):581–7, 2003.
39. Taylor JA, Welshons WV, Vom Saal FS. No effect of route of exposure (oral; subcutaneous injection) on plasma bisphenol A throughout 24h after administration in neonatal female mice. *Reproductive toxicology* (Elmsford, NY). 25(2):169–76, 2008.
40. Ahangarpour A, Afshari G, Mard SA, Khodadadi A, Hashemitabar M. Preventive effects of procyanidin A2 on glucose homeostasis, pancreatic and duodenal homebox 1, and glucose transporter 2 gene expression disturbance induced by bisphenol A in male mice. *J Physiol Pharmacol*. 67(2):243–52, 2016.
41. Suzuki R, Tobe K, Terauchi Y, Komeda K, Kubota N, Eto K, et al. Pdx1 expression in Irs2-deficient mouse beta-cells is regulated in a strain-dependent manner. *J. Biol Chem*. 278(44):43691–8, 2003.
42. Rieger AM, Nelson KL, Konowalchuk JD, Barreda DR. Modified annexin V/propidium iodide apoptosis assay for accurate assessment of cell death. *Journal of visualized experiments: JoVE*. (50):2597, 2011.
43. Niu B, Li C, Su H, Li Q, He Q, Liu L, et al. Glucagon-like peptide-1 receptor agonist exendin-4 protects against interleukin-1beta-mediated inhibition of glucose-stimulated insulin secretion by mouse insulinoma beta cells. *Experimental and therapeutic medicine*. 14(3):2671–6, 2017.
44. Padmasekar M, Lingwal N, Samikannu B, Chen C, Sauer H, Linn T. Exendin-4 protects hypoxic islets from oxidative stress and improves islet transplantation outcome. *Endocrinology*. 154(4):1424–33, 2013.
45. Alonso-Magdalena P, Vieira E, Soriano S, Menes L, Burks D, Quesada I, et al. Bisphenol A exposure during pregnancy disrupts glucose homeostasis in mothers and adult male offspring. *Environmental health perspectives*. 118(9):1243–50, 2010.
46. Song L, Xia W, Zhou Z, Li Y, Lin Y, Wei J, et al. Low-level phenolic estrogen pollutants impair islet morphology and beta-cell function in isolated rat islets. *The Journal of endocrinology*. 215(2):303–11, 2012.
47. Casey MF, Neidell M. Disconcordance in statistical models of bisphenol A and chronic disease outcomes in NHANES 2003-08. *PloS one*. 8(11):e79944, 2012.
48. Hassan ZK, Elobeid MA, Virk P, Omer SA, ElAmin M, Daghestani MH, et al. Bisphenol A induces hepatotoxicity through oxidative stress in rat model. Oxid Med Cell Longev. 2012:194829, 2012.
49. Batista TM, Alonso-Magdalena P, Vieira E, Amaral ME, Cederroth CR, Nef S, et al. Short-term treatment with bisphenol-A leads to metabolic abnormalities in adult male mice. *PloS one*. 7(3):e33814, 2012.
50. Alonso-Magdalena P, Ropero AB, Carrera MP, Cederroth CR, Baquie M, Gauthier BR, et al. Pancreatic insulin content regulation by the estrogen receptor ER alpha. *PloS one*. 3(4):e2069, 2008.
51. Alonso-Magdalena P, Quesada I, Nadal A. Endocrine disruptors in the etiology of type 2 diabetes mellitus. *Nature reviews Endocrinology*. 7(6):346–53, 2011.
52. Ahangarpour A, Oroojan AA, Badavi M. Exendin-4 protects mice from D-galactose-induced hepatic and pancreatic dysfunction. *Pathobiology of aging & age related diseases*. 8(1):1418593, 2018.
53. Egido EM, Silvestre RA, Hernandez R, Marco J. Exendin-4 dose-dependently stimulates somatostatin and insulin secretion in perfused rat pancreas. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme*. 36(9):595–600, 2004.
54. Liu J, Yu P, Qian W, Li Y, Zhao J, Huan F, et al. Perinatal bisphenol A exposure and adult glucose homeostasis: identifying critical windows of exposure. *PloS one*. 8(5):e64143, 2013.
55. Nadal A, Alonso-Magdalena P, Soriano S, Quesada I, Ropero AB. The pancreatic beta-cell as a target of estrogens and xenoestrogens: Implications for blood glucose homeostasis and diabetes. *Mol Cell Endocrinol*. 304(1–2):63–8, 2009.
56. Natalicchio A, Labarbuta R, Tortosa F, Biondi G, Marrano N, Peschechera A, et al. Exendin-4 protects pancreatic beta cells

 https://doi.org/10.46389/rjd-2020-1048

from palmitate-induced apoptosis by interfering with GPR40 and the MKK4/7 stress kinase signalling pathway. *Diabetologia*. 56(11):2456–66, 2013.
57. Widenmaier SB, Ao Z, Kim SJ, Warnock G, McIntosh CH. Suppression of p38 MAPK and JNK via Akt-mediated inhibition of apoptosis signal-regulating kinase 1 constitutes a core component of the beta-cell pro-survival effects of glucose-dependent insulinotropic polypeptide. *J Biol Chem*. 284(44):30372–82, 2009.
58. Zhang Y, Xu M, Zhang S, Yan L, Yang C, Lu W, et al. The role of G protein-coupled receptor 40 in lipoapoptosis in mouse beta-cell line NIT-1. *Journal of molecular endocrinology*. 38(6):651–61, 2007.
59. Carlessi R, Lemos NE, Dias AL, Oliveira FS, Brondani LA, Canani LH, et al. Exendin-4 protects rat islets against loss of viability and function induced by brain death. *Molecular and cellular endocrinology*. 412:239–50, 2015.
60. Sakurai K, Kawazuma M, Adachi T, Harigaya T, Saito Y, Hashimoto N, et al. Bisphenol A affects glucose transport in mouse 3T3-F442A adipocytes. *British journal of pharmacology*. 141(2):209–14, 2004.
61. Xu G, Stoffers DA, Habener JF, Bonner-Weir S. Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. *Diabetes*. 48(12):2270–6, 1999.
62. Kim HS, Hong SH, Oh SH, Kim JH, Lee MS, Lee MK. Activin A, exendin-4, and glucose stimulate differentiation of human pancreatic ductal cells. *The Journal of endocrinology*. 217(3):241–52, 2013.
63. Zhou J, Pineyro MA, Wang X, Doyle ME, Egan JM. Exendin-4 differentiation of a human pancreatic duct cell line into endocrine cells: involvement of PDX-1 and HNF3beta transcription factors. *Journal of cellular physiology*. 192(3):304–14, 2002.
64. Fan R, Kang Z, He L, Chan J, Xu G. Exendin-4 improves blood glucose control in both young and aging normal non-diabetic mice, possible contribution of beta cell independent effects. *PloS one*. 6(5):e20443, 2011.
65. Angle BM, Do RP, Ponzi D, Stahlhut RW, Drury BE, Nagel SC, et al. Metabolic disruption in male mice due to fetal exposure to low but not high doses of bisphenol A (BPA): evidence for effects on body weight, food intake, adipocytes, leptin, adiponectin, insulin and glucose regulation. *Reproductive toxicology*. 42:256–68, 2013.
66. D'Cruz SC, Jubendradass R, Jayakanthan M, Rani SJ, Mathur PP. Bisphenol A impairs insulin signaling and glucose homeostasis and decreases steroidogenesis in rat testis: an in vivo and in silico study. Food and chemical toxicology: an international journal published for the British *Industrial Biological Research Association*. 50(3–4):1124–33, 2012.
67. Kwon DY, Kim YS, Ahn IS, Kim da S, Kang S, Hong SM, et al. Exendin-4 potentiates insulinotropic action partly via increasing beta-cell proliferation and neogenesis and decreasing apoptosis in association with the attenuation of endoplasmic reticulum stress in islets of diabetic rats. *Journal of pharmacological sciences*. 111(4):361–71, 2009.
68. Zhou J, Wang X, Pineyro MA, Egan JM. Glucagon-like peptide 1 and exendin-4 convert pancreatic AR42J cells into glucagon- and insulin-producing cells. *Diabetes*. 48(12):2358–66, 1999.
69. Greig NH, Holloway HW, De Ore KA, Jani D, Wang Y, Zhou J, et al. Once daily injection of exendin-4 to diabetic mice achieves long-term beneficial effects on blood glucose concentrations. *Diabetologia*. 42(1):45–50, 1999.
70. Wang X, Zhou J, Doyle ME, Egan JM. Glucagon-like peptide-1 causes pancreatic duodenal homeobox-1 protein translocation from the cytoplasm to the nucleus of pancreatic beta-cells by a cyclic adenosine monophosphate/protein kinase A-dependent mechanism. *Endocrinology*. 142(5):1820–7, 2001.
71. Moshtagh PR, Emami SH, Sharifi AM. Differentiation of human adipose-derived mesenchymal stem cell into insulin-producing cells: an in vitro study. *Journal of physiology and biochemistry*. 69(3):451–8, 2013.
72. Chen K, Yu X, Murao K, Imachi H, Li J, Muraoka T, et al. Exendin-4 regulates GLUT2 expression via the CaMKK/CaMKIV pathway in a pancreatic beta-cell line. *Metabolism: clinical and experimental*. 60(4):579–85, 2011.
73. Zhao Q, Yang Y, Hu J, Shan Z, Wu Y, Lei L. Exendin-4 enhances expression of Neurod1 and Glut2 in insulin-producing cells derived from mouse embryonic stem cells. *Archives of medical science: AMS*. 12(1):199–207, 2016.
74. Johnson JD, Ahmed NT, Luciani DS, Han Z, Tran H, Fujita J, et al. Increased islet apoptosis in Pdx1+/- mice. *The Journal of clinical investigation*. 111(8):1147–60, 2003.
75. Johnson JD et al. Insulin protects islets from apoptosis via Pdx1 and specific changes in the human islet proteome. *Proceedings of the National Academy of Sciences of the United States of America*. 103(51):19575–80, 2006.

