Perinatal Exposure to Bisphenol A at Reference Dose Predisposes Offspring to Metabolic Syndrome in Adult Rats on a High-Fat Diet

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Bisphenol A (BPA), a widely used environmental endocrine disruptor, has been reported to disrupt glucose homeostasis. BPA exposure may be a risk factor for type 2 diabetes. In this study, we investigated the effects of early-life BPA exposure on metabolic syndrome in rat offspring fed a normal diet and a high-fat diet. Pregnant Wistar rats were exposed to BPA (50, 250, or 1250 μg/kg · d) or corn oil throughout gestation and lactation by oral gavage. Offspring were fed a normal diet or a high-fat diet after weaning. Body weight, parameters of glucose and lipid metabolism, morphology, and function of β-cells were measured in offspring. On a normal diet, perinatal exposure to 50 μg/kg · d BPA resulted in increased body weight, elevated serum insulin, and impaired glucose tolerance in adult offspring. On a high-fat diet, such detrimental effects were accelerated and exacerbated. Furthermore, severe metabolic syndrome, including obesity, dyslipidemia, hyperleptindemia, hyperglycemia, hyperinsulinemia, and glucose intolerance, was observed in high-fat-fed offspring perinatally exposed to 50 μg/kg · d BPA. No adverse effect of perinatal BPA exposure at 250 and 1250 μg/kg · d was observed no matter on a normal diet or a high-fat diet. These results suggest that perinatal exposure to BPA at reference dose, but not at high dose, impairs glucose tolerance in adult rat offspring on a normal diet and predisposes offspring to metabolic syndrome at adult on a high-fat diet. High-fat diet intake is a trigger that initiates adverse metabolic effects of BPA. **(*Endocrinology* 152: 3049–3061, 2011)**

Obesity and type 2 diabetes represent a serious threat to the health of the population of almost every country in the world, and a growing number of people are being diagnosed with obesity-related type 2 diabetes (1, 2). Also increasing is the incidence of metabolic syndrome, a complex condition linked to obesity that is characterized by a cluster of risk factors, including hyperinsulinemia, hyperlipidemia, hypertension, and glucose intolerance (rather than hyperinsulinemia alone) (3, 4). The potential role of early-life chemical exposure in metabolic syndrome gains much less interest than the factors such as heredity, diet, lifestyle, and socioeconomic status (5, 6). However, increasing evidence suggests that such early-life chemical exposure has a significant impact on the origins of metabolic disorders. As just one example, it has been proven that prenatal exposure to nicotine leads to obesity and alters the development of pancreas in the offspring (7, 8).

Attention also has been turned to the endocrine-disrupting chemicals (EDC), which can be found in food packages or insecticide residues on vegetable crops, *etc.* and to which many people are exposed (9). There are epidemiological evidence for the association between EDC

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Abbreviations: ANCOVA, Analysis of covariance; AUC, area under the curve; BPA, bisphenol A; EDC, endocrine-disrupting chemical; GD, gestational day; Glut2, glucose transporter 2; GSIS, glucose-stimulated insulin secretion; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OGTT, oral glucose tolerance test; ITT, insulin tolerance test; Pdx-1, pancreatic and duodenal homeobox-1; PND, postnatal day; TC, total cholesterol; TG, triglyceride; Ucp2, uncoupling protein 2.

exposure and metabolic disorders (10, 11). The subpopulations that have the highest rates of obesity or diabetes are also those that have greater exposure to EDC such as p,p′-dichlorodiphenyltrichloroethane, bisphenol A (BPA), or dioxins (12–14).

BPA, one of the highest volume EDC produced worldwide (9, 15), is used as the monomer to manufacture many food and beverage containers. It is reported that BPA is found in 92.6% of the urine samples from 2500 participants of the National Health and Nutrition Examination Survey (9). BPA exposure has been associated epidemiologically with type 2 diabetes, cardiovascular disease, and liver enzyme abnormalities (12). Animal studies are also mounting in support of the concept that BPA exposure disrupts pancreatic β-cells function and the whole-body blood glucose homeostasis. One and 10 nM BPA has been reported to induce increases of insulin content in isolated islets compared with vehicle-exposed islets (16). Significantly higher insulin secretion after the long-term exposure to BPA for 24 h in 16.7 mM glucose is also observed in isolated islets than that without exposure (17). Additionally, administration of 100 μg/kg · d BPA for 4 d in healthy male mice can lead to postprandial hyperinsulinemia and insulin resistance (18).

The relationship between BPA exposure and the impact on metabolism is more important when exposure occurs during the critical period of development. Male mice offspring exposed to 10 μg/kg · d BPA *in utero* [gestational day (GD) 9–16] show altered blood parameters, impaired glucose tolerance, and insulin resistance as well as enhanced insulin secretion, altered calcium signaling, and reduced incorporation of 5-bromo-2′-deoxyuridine, although β-cells mass is unaltered (19). Perinatal exposure to approximate 70 μg/kg · d BPA via drinking water has been shown to alter early adipogenesis and increase body weight (20). Perinatal exposure to BPA at 0.25 μg/kg · d via the mother's diet has also been reported to increase growth around the time of weaning in CD-1 mice offspring, but this effect does not translate to increase body weight and impair glucose regulation, even when the mice are fed a high-fat diet later in life (21).

In the present study, we address whether perinatal BPA exposure would contribute to metabolic syndrome in rat offspring and whether metabolic disrupt effects of BPA was exacerbated under high-fat feeding condition.

Materials and Methods

Maintenance and treatment of animals

All of the animal experiments in this study followed the guidelines for the care and use of animals established by Tongji Medical College (Huazhong University of Science and Technology, Wuhan, China) and were approved by the Ethics Committee of Tongji Medical College.

Virgin female (270–300 g) and male (350–400 g) genitor Wistar rats (Hubei Research Center of Laboratory Animal, China) were housed in special pathogen-free condition, with *ad libitum* access to food and water in an environmentally controlled room maintained on a 12-h light, 12-h dark cycle. Glass water bottles and polypropylene cages were used in this study. The morning when sperm-positive smears appeared was declared GD 0. Pregnant rats were assigned to four groups and were subjected to the following treatments from GD 0 to the weaning at postnatal day (PND) 21 by oral gavage: 1) corn oil (Sigma-Aldrich, St. Louis, MO; CAS no. 8001-30-7); 2) 50 μg/kg · d of BPA (Sigma-Aldrich; purity ≥ 99%, CAS no. 80-05-7) dissolved in corn oil; 3) 250 μg/kg · d of BPA dissolved in corn oil; and 4) 1250 μg/kg · d of BPA dissolved in corn oil. Dosages were adjusted daily for body weight changes of pregnant rats (2.0 ml/kg body weight).

Pregnant rats were monitored daily until spontaneous delivery. At delivery, offspring were culled to 10 per lactating dam (five male and five female when possible) and kept with their mothers until PND21. Offspring were weighed on PND 1, 5 10, 15, and 21. On PND 21, litters with less than five male pups or five female pups were excluded from the following study. The remaining pups were sexed and weaned. Thirty-two male and 32 female pups (four pups per litter) from each group (eight litters per group) were chosen randomly and received either a normal diet or a high-fat diet (two pups per litter per group). The normal diet contains 12.05% fat, 24.93% protein, and 63.02% carbohydrates, with energy of 14.43 kJ/g. The high-fat diet contains 28.53% fat, 22.33% protein, and 49.14% carbohydrates, with energy of 17.36 kJ/g. Body weight was measured from wk 3 to wk 26. Food intake was measured from wk 11 to wk 20.

To control for litter effects, we ensured that each experimental condition described below included offspring from different litters in each treatment group (except for the measurement of body weight, fasting blood glucose and serum insulin in which all animals are represented). Moreover, differences in litter size were accommodated by including it as a cofactor in all statistical analyses.

Determination of blood parameters

Blood parameters were determined after 16 h overnight fast using the handheld commercial glucose meter (ACCU-CHEK Active; Roche, Mannheim, Germany). Serum triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were determined with automatic blood analyzer (Hitachi 747 autoanalyzer; Tokyo, Japan). Insulin and leptin concentrations were determined by commercially available RIA kits (Linco Research, Millipore, Billerica, MA).

Oral glucose tolerance test (OGTT) and insulin tolerance test (ITT)

For the OGTT test, offspring were fasted for 16 h and administered 2.0 g/kg body weight glucose by oral gavage. Blood glucose and serum insulin concentration were determined at various time points (0, 15, 30, 60, and 120 min). Glucose and insulin responses during the OGTT were calculated by the area under the curve (AUC) using the trapezoidal method. For the ITT test, offspring were fasted for 6 h and injected ip with 0.75 U/kg body

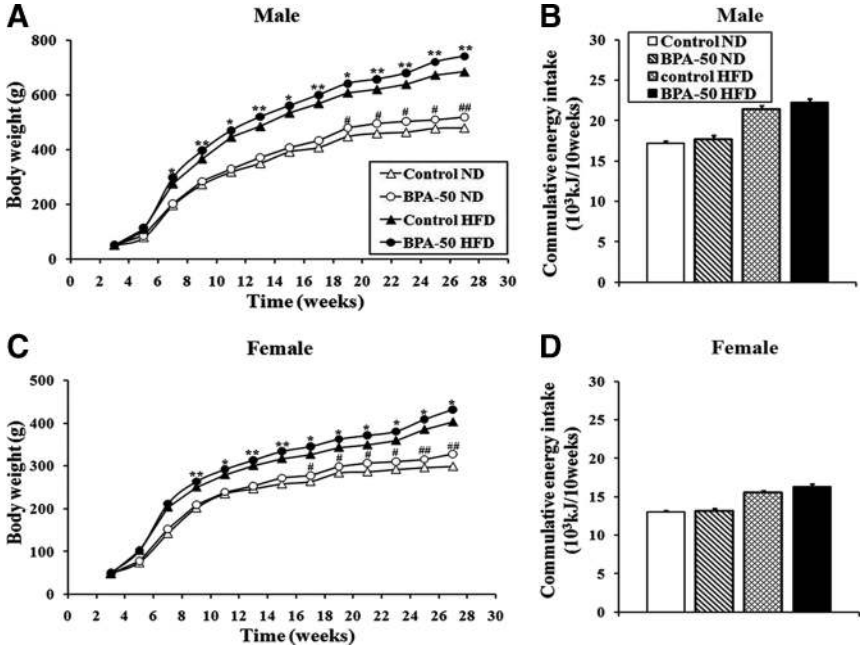

FIG. 1. Body weight and food intake of male (A and B) and female offspring perinatally exposed to 50 μg/kg · d BPA. A, Body weight in male offspring [repeated-measure (RM) ANCOVA: *P* (litter size) > 0.05]. B, Body weight in female offspring [RM ANCOVA: *P* (litter size) > 0.05] (n = 16, 3–12 wk; n = 11, 13–20 wk; n = 6, 21–27 wk per group). C, Food intake in male offspring [ANCOVA: *P* (litter size) > 0.05]. D, Food intake in female offspring [ANCOVA: *P* (litter size) > 0.05] (n = 10 per group). Results are expressed as mean ± SEM. HFD, High-fat diet; ND, normal diet. *, $P < 0.05$ compared with control HFD group; **, $P < 0.01$compared with control HFD group; #, $P < 0.05$ compared with control ND group; ##, $P < 0.01$compared with control ND group.

weight biosynthetic human insulin (Novolin R; Novo Nordisk AIS, Bagsvaerd, Denmark). Blood glucose was measured at various time points as described above.

Transmission electron microcopy

At wk 3 and 27, portions of the splenic region of pancreas were collected and processed for electron microscopy as described (22, 23). Sections were examined with FEI Tecnai 12G^2 transmission electron microscope (FEI, Eindhoven, Netherlands). β-Cells were identified by the presence of a round or spherical core of the vesicles with the surrounding the limiting membrane (24). Secretory granules in β-cells were classified as filled (dense core), immature (light gray core), or empty (22, 25) and were manually quantified. Manual quantifications were performed on 15 sections from eight islets per rat (n = 3 per group). Mitochondrial swelling was assessed by the average mitochondrial area and OD, which were determined by manually circling a minimum of 100 mitochondria within the β-cells per rat (n = 3 per group) and was analyzed using Image Pro Plus version 6.0 software (Media Cybernetics, Silver Spring, MD).

Light microscopy and immunocytochemistry

At wk 27, the whole pancreas was removed from offspring and weighed. For immunohistochemistry, part of the splenic region of the pancreas (n = 3 per group) were fixed in 4% paraformaldehyde and embedded in paraffin. Sections of 5 μm thick were prepared and incubated with antiinsulin (Millipore, Billerica, MA) and antiglucagon (Abcam, Cambridge, UK) antibodies. β-Cell areas were calculated by insulin-positive cells in sections (26). β-Cells mass was calculated by multiplying the corresponding pancreatic weight by the percentage of islet area as described (27). Detection was performed in 10 sections (5 μm) separated by 200 μm per rat and was also analyzed using Image Pro Plus version 6.0 software (Media Cybernetics).

At wk 27, white adipose tissue from gonadal fat pads were collected and weighed and then fixed in 4% paraformaldehyde and embedded in paraffin (n = 3 per group). Five-micrometer sections were mounted and stained with hematoxylin and eosin. Morphometric measurements were performed on five serial sections per rat in each group and about 30–50 adipocytes were measured in each section.

Glucose-stimulated insulin secretion (GSIS)

Islets were isolated from pancreas of offspring by collagenase type V (Sigma-Aldrich) digestion and Ficoll step density gradient separation as described (28–30). Groups of 20 islets of similar size were cultured in media containing 3.0, 5.8, or 16.7 mmol/liter glucose for 1 h. Insulin secreted in the media was assayed by RIA (31).

RNA preparation and mRNA quantification

Total RNA was extracted from isolated rat islets using Trizol reagent (Invitrogen, Carlsbad, CA). The expression of the mRNA was determined by quantitative real-time PCR using an Applied Biosystems model 7900HT fast real-time PCR system. Relative gene expression was calculated by the $2^{-\triangle\triangle CT}$ method with 36B4 as an endogenous reference gene. The primers are listed in Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://endo.endojournals.org.

Statistical analysis

Data were expressed as mean ± SEM and were analyzed using SPSS 13.0 (SPSS, Chicago, IL). Litter size, sex ratio, and the body weight of the offspring during lactation were analyzed using one-way ANOVA followed by least significant differences and Dunnett's T_3 test. The remaining data were analyzed using the appropriate analysis of covariance (ANCOVA) model, with litter size as the covariate, followed by the Bonferroni test. Differences between the two groups were analyzed using an independent two-tailed *t* test. A $P < 0.05$ was considered significant.

Results

Effects of BPA on body weight and energy intake

Offspring perinatally exposed to BPA at 50 μg/kg · d had more weight gain than the control starting in wk 19

(male) and 17 (female) (Fig. 1, A and B), although no difference was observed in body weight, litter size, sex ratio at birth, and weight gain during lactation between controls and BPA-exposed offspring (Supplemental Table 2 and Supplemental Fig. 1). On a high-fat diet, male and female offspring exposed to BPA weighed more than the control as early as wk 7 and 9 (Fig. 1, A and B). Cumulative energy intake was comparable between BPA-treated groups and controls during growth (Fig. 1, C and D). Litter size was not associated with body weight and energy intake in either sex ($P > 0.05$).

Effects of BPA on glucose homeostasis

On a normal diet, no difference in fasting blood glucose levels was observed between controls and 50 μg/kg · d BPA-treated offspring (Fig. 2, A and B). Serum insulin was elevated at wk 15 in male offspring (Fig. 2C) and at wk 26 in females (Fig. 2D). On a high-fat diet, fasting blood glucose and serum insulin levels were elevated by 50 μg/kg · d BPA exposure as early as wk 9 in male offspring (Fig. 2, A and C) and wk 15 in females (Fig. 2, B and D).

Glucose tolerance in offspring perinatally exposed to 50 μg/kg · d BPA was tested at wk 3, 6, 9, 15, and 26, and no significant difference was observed between controls and BPA-treated offspring from wk 3 to wk 9 (data not shown). On a normal diet, no significant effect of BPA on glucose clearance was detected in male offspring at wk 15 (Fig. 3A), but insulin AUC was higher in BPA-treated male offspring compared with controls (Fig. 3B). There was no difference in OGTT between female BPA-treated offspring and controls on a normal diet (Fig. 4, A and B) at wk 15. At the age of 26 wk, both male and female BPA-treated offspring on a normal diet had greater glucose and insulin AUC compared with controls (Figs. 3, D and E, and 4, D and E). On a high-fat diet, BPA exposure evoked glucose intolerance at wk 15 in both male and female offspring, characterized by significantly higher blood glucose and serum insulin levels in response to OGTT (Figs. 3, A and B, and 4, A and B). In male BPA-treated offspring fed a high-fat diet, blood glucose levels was also significantly elevated throughout the 120-min OGTT (Fig. 3D). Serum insulin was significantly decreased at 15 min after glucose loading, although the elevated insulin level during basal period was still evident (Fig. 3E). Blood glucose and serum insulin level at each time point retained significantly higher in female BPA exposed offspring on a high-fat diet at wk 26 (Fig. 4, D and E).

A 120-min pilot ITT was performed to evaluate insulin sensitivity at wk 15 and 26. On a normal diet, no apparent

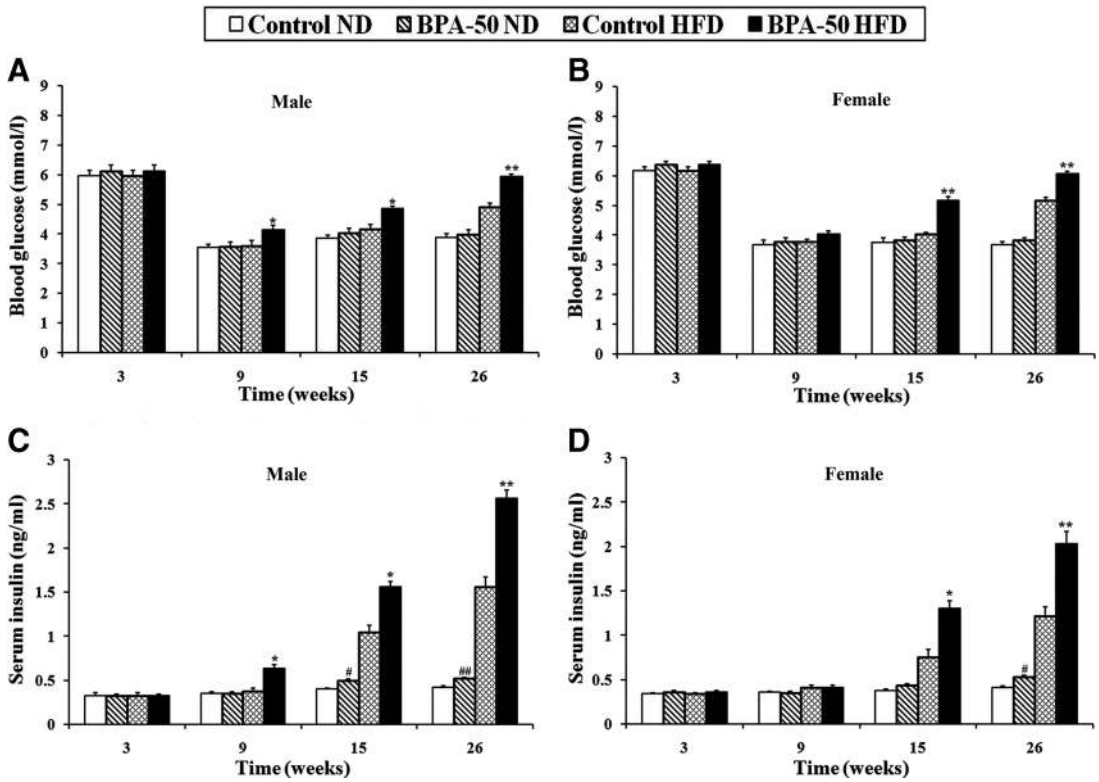

FIG. 2. Effects of perinatal exposure to 50 μg/kg · d BPA on fasting blood glucose and fasting serum insulin in male (A and C) and female (B and D) offspring. A and B, Blood glucose [ANCOVA: P (litter size) > 0.05]. C and D, Serum insulin [ANCOVA: P (litter size) > 0.05] (n = 16, 3–12 wk; n = 11, 13–20 wk; n = 6, 21–26 wk per group). Results are expressed as mean ± SEM. HFD, High-fat diet; ND, normal diet. *, $P < 0.05$ compared with control HFD group; **, $P < 0.01$compared with control HFD group; #, $P < 0.05$ compared with control ND group; ##, $P < 0.01$ compared with control ND group.

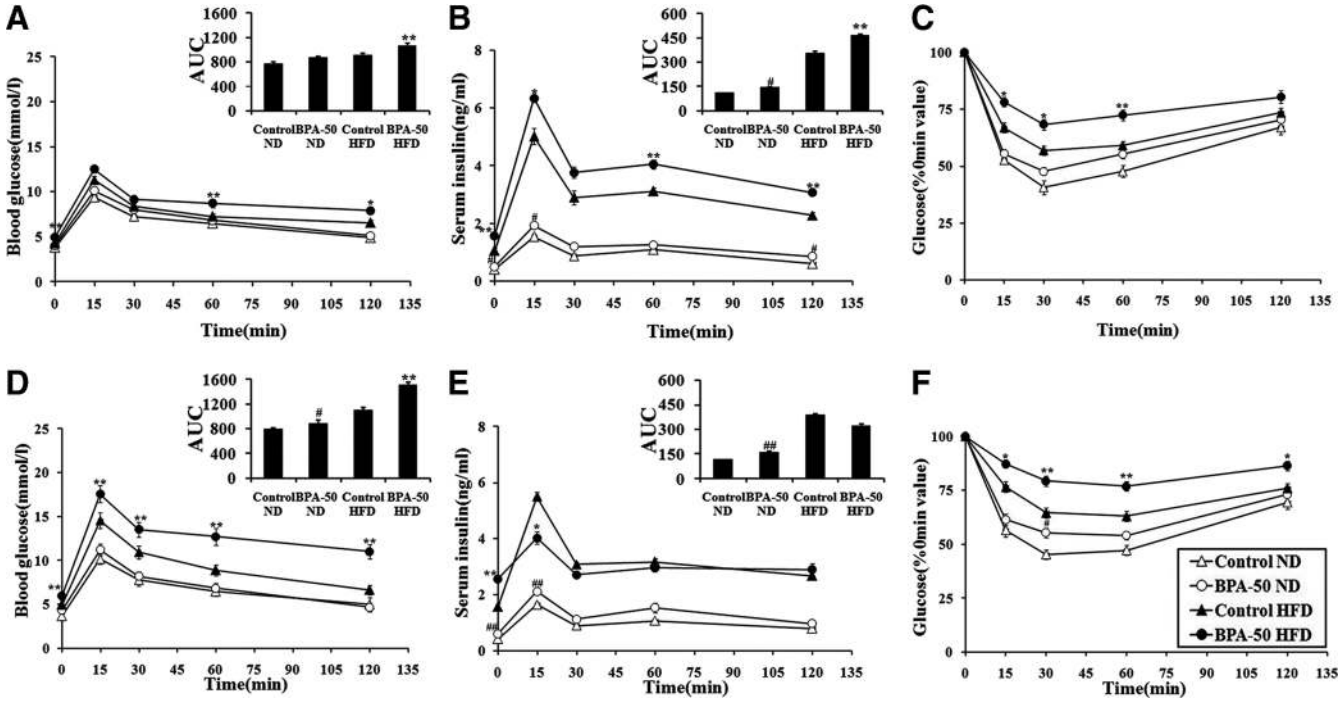

FIG. 3. Glucose tolerance test (2.0 g/kg, oral gavage) and ITT (0.75 IU/kg, ip) for male offspring exposed to 50 μg/kg · d BPA at wk 15 (A–C) and wk 26 (D–F). A and D, Blood glucose before and after oral administration of glucose [repeated-measure (RM) ANCOVA: P (litter size) > 0.05]. *Insets* (A and D) represent the mean total glucose AUC [ANCOVA: P (litter size) > 0.05]. B and E, Serum insulin before and after oral administration of glucose [RM ANCOVA: P (litter size) > 0.05]. *Insets* (B and E) represent the mean total insulin AUC. The integrated AUC was determined using the trapezoidal method [ANCOVA: P (litter size) > 0.05]. C and F, Blood glucose levels before and after ip injection of insulin [RM ANCOVA: P (litter size) > 0.05]. Blood glucose levels were normalized to those at $t = 0$ min (100%). Results are expressed as mean ± SEM. HFD, High-fat diet; ND, normal diet. *, $P < 0.05$ compared with control HFD group; **, $P < 0.01$compared with control HFD group; #, $P < 0.05$ compared with control ND group; ##, $P < 0.01$compared with control ND group.

difference was displayed between 50 μg/kg · d BPA-treated male offspring and controls at wk 15 in the whole-body insulin sensitivity (Fig. 3C). At wk 26, blood glucose decrease was less pronounced in BPA-treated male offspring at 30 min after administration of insulin than that in controls (Fig. 3F), confirming a mild reduction of insulin sensitivity in BPA-treated male offspring. Response during the ITT was unaltered in BPA-treated female offspring up to wk 26 (Fig. 4, C and F). On a high-fat diet, the glucose-lowering effect of insulin was significantly attenuated in BPA-treated male and female offspring compared with controls starting in wk 15, characterized by much lower fractional fall in blood glucose after insulin administration (Figs. 3, C and F, and 4, C and F). Litter size was not associated with blood glucose, serum insulin, glucose tolerance, and insulin tolerance in either sex ($P > 0.05$).

Effects of BPA on mitochondrial structure and insulin granule characteristics in β-cells

Relative to controls (Fig. 5A), swollen mitochondria and dilated of rough endoplasmic reticulum were observed in β-cells of 50 μg/kg · d BPA-treated male offspring at weaning (Fig. 5B). The average area and OD of the mitochondria in β-cells of BPA-treated male offspring were increased compared with controls (Fig. 5, C and D). A mild increase in empty granules was observed in male BPA-treated offspring (Fig. 5E).

At wk 27, structure abnormalities including hypertrophy mitochondria and rough endoplasmic reticulum were also evident in β-cells of BPA-treated male offspring (Fig. 5G) compared with the control (Fig. 5F) on a normal diet. Average area and OD of the mitochondria in BPA-exposed β-cells remained significantly higher than controls (Fig. 5, J and K). Reduction in filled granules was observed in BPA-treated male offspring fed a normal diet (Fig. 5L). On a high-fat diet, markedly degenerative β-cells were observed in male offspring, characterized by almost completely absent of secretory granules, well-dilated mitochondria, hypertrophied rough endoplasmic reticulum, and karyopyknosis (Fig. 5I). There was no difference in the average area of mitochondria, but the density of the mitochondria was significantly higher in β-cells of BPA-treated male offspring fed a high-fat diet (Fig. 5, J and K). A significant reduction in the number of filled granules and a significant increasing in the number of empty granules were exhibited in β-cells of BPA-treated male offspring (Fig. 5L). Litter size was not associated with the area and the density of mitochondria ($P > 0.05$). Adverse effect on glucose homeostasis in BPA-treated female offspring was

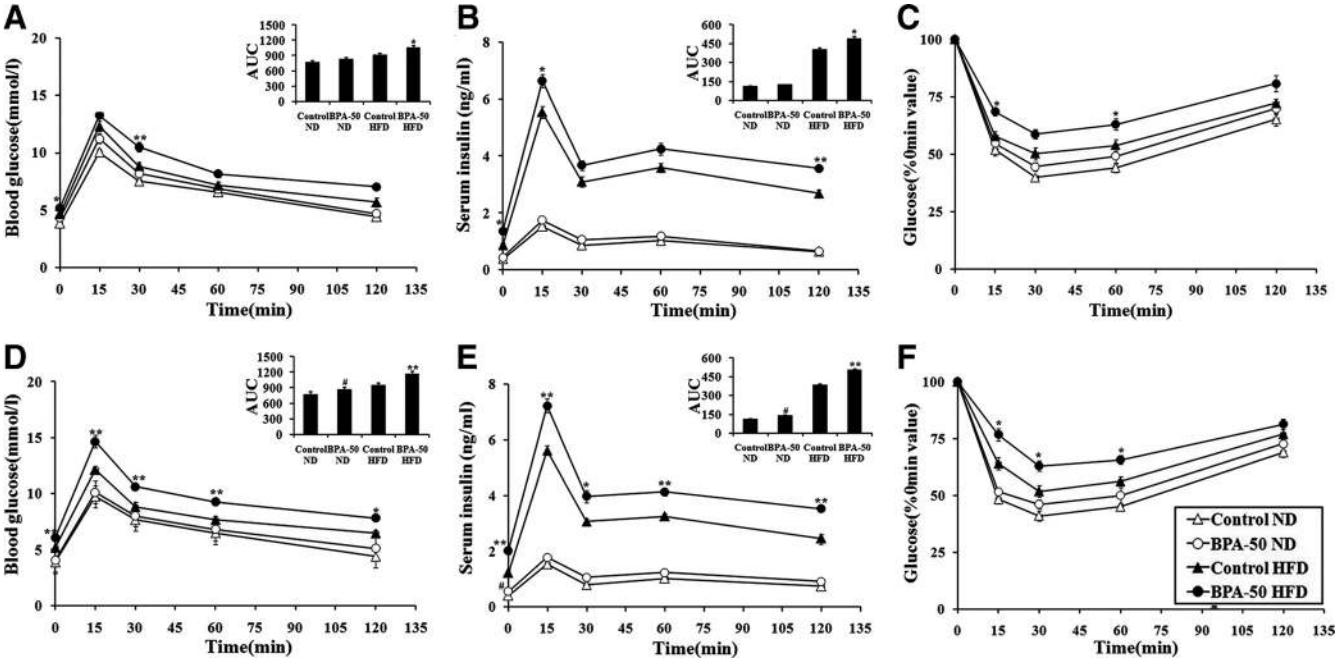

FIG. 4. Glucose tolerance test (2.0 g/kg, oral gavage) and ITT (0.75 IU/kg, ip) for female offspring exposed to 50 μg/kg · d BPA at wk 15 (A–C) and wk 26 (D–F). A and D, Blood glucose before and after oral administration of glucose [repeated-measure (RM) ANCOVA: *P* (litter size) > 0.05]. *Insets* (A and D) represent the mean total glucose AUC [ANCOVA: *P* (litter size) > 0.05]. B and E, Serum insulin before and after oral administration of glucose [RM ANCOVA: *P* (litter size) > 0.05]. *Insets* (B and E) represent the mean total insulin AUC. The integrated AUC was determined using the trapezoidal method [ANCOVA: *P* (litter size) > 0.05]. C and F, Blood glucose levels before and after ip injection of insulin [RM ANCOVA: *P* (litter size) > 0.05]. Blood glucose levels were normalized to those at $t = 0$ min (100%). Results are expressed as mean ± SEM. HFD, High-fat diet; ND, normal diet. *, $P < 0.05$ compared with control HFD group; **, $P < 0.01$compared with control HFD group; #, $P < 0.05$ compared with control ND group; ##, $P < 0.01$compared with control ND group.

present but less severe than males; thus, analysis was shown only in males (data for females was present in Supplemental Fig. 2).

Effects of BPA on β-cells morphometry and mass

On a normal diet, 50 μg/kg · d BPA-treated offspring had mild dilated or large elongated islets (Fig. 6A). When the islets were separated by area into large (>10,000 μm^2), medium (1000–10000 μm^2), or small islets (<1,000 μm^2), the frequency of medium and large islets were higher in BPA-treated offspring (Fig. 6, B and C). The calculated mean area and mass of β-cells in male BPA-exposed offspring were larger than that in controls, and no differences was observed in females (Fig. 6, D and E). In offspring fed a high-fat diet, islets were markedly hypertrophied and less organized characterized by dispersed staining (Fig. 6A). Consequent to the significant increases in large islets (Fig. 6, B and C), significantly higher mean area of β-cells were observed in BPA-treated male and female offspring (Fig. 6D). β-Cell mass was increased in female BPA-treated offspring on a high-fat diet. In males, β-cell mass tended to be lower after BPA exposure but did not reach statistical significance (Fig. 6E). Litter size was not associated with the average area and the mass of β-cells in either sex ($P > 0.05$).

Effects of BPA on β-cells functions islets function was measured by GSIS

On a normal diet, islets of offspring perinatally exposed to 50 μg/kg · d BPA showed greater insulin secretion than the control when stimulated by 16.7 mmol/liter glucose in either sex (Fig. 6, F and G). On a high-fat diet, BPA exposure induced insulin secretory in response to glucose was quite different between male and female offspring. Islets isolated from male offspring tended to release more insulin in response to 3.0 or 5.8 mmol/liter glucose stimulus but exhibited a significantly diminished ability to secrete insulin in response to 16.7 mmol/liter glucose stimulus (Fig. 6F). On the other hand, BPA exposure significantly increased insulin secretion in females when islets exposed to 5.8 and 16.7 mmol/liter glucose (Fig. 6G). Litter size was not associated with GSIS in either sex ($P > 0.05$).

Effects of BPA on the expression of mRNA in islets

Expression of islet-associated transcription factors were dramatically reduced, including pancreatic and duodenal homeobox-1 (*Pdx-1*) and *Nkx6.1*, in 50 μg/kg · d BPA-treated offspring, no matter whether fed a normal or a high-fat diet. Similarly, reduced levels of glucose transporter 2 (*Glut2*; also known as *Slc2a2*) and *Gck* were also

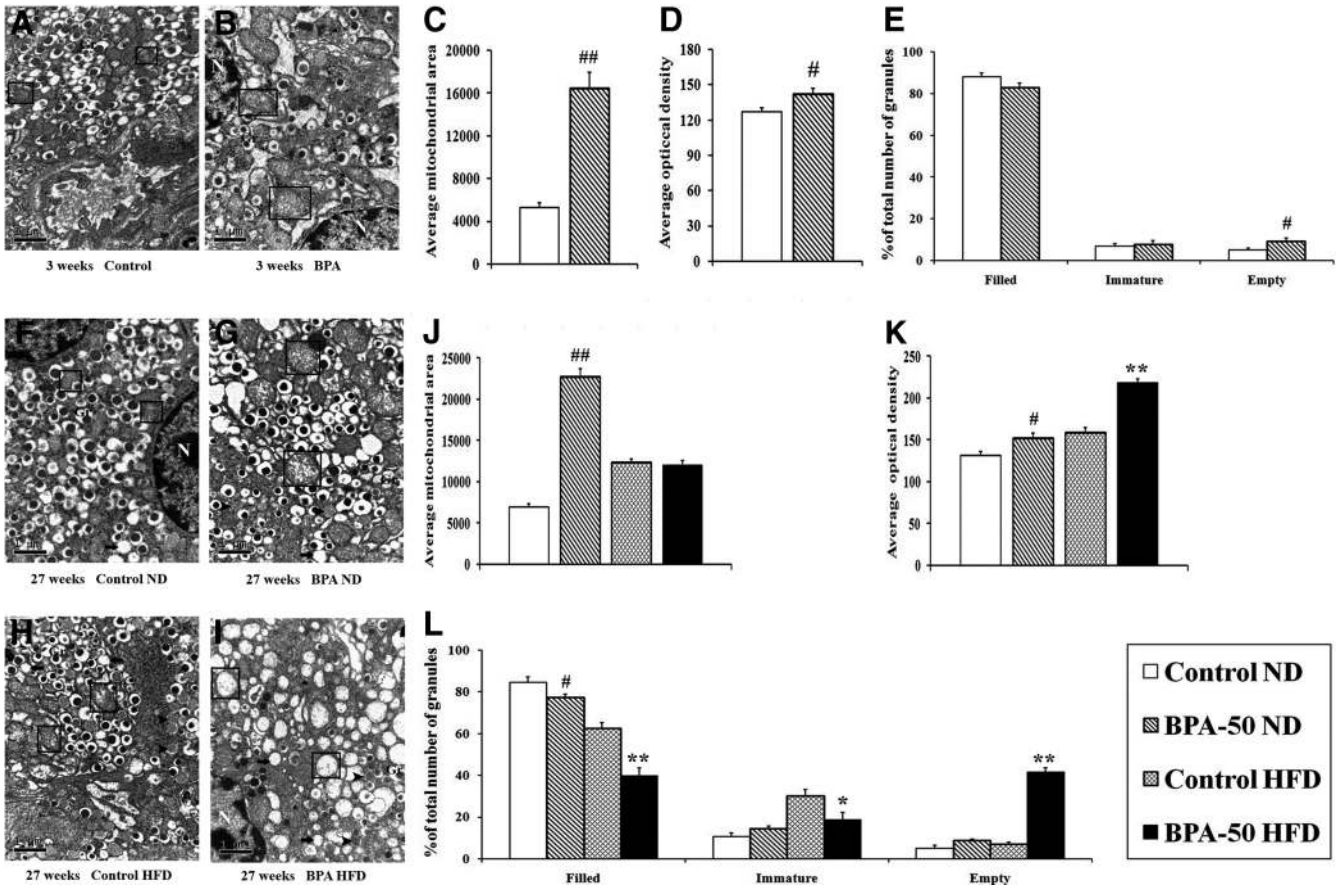

FIG. 5. β-Cell morphological analysis in male offspring perinatal exposure to 50 μg/kg · d BPA (n = 3 per group). Representative electron microscopy photomicrographs of control (A, F, and H) and BPA-exposed β-cells (B, G, and I) at wk 3 (A and B) and wk 27 (F–I) illustrated the typical insulin granules and mitochondria pattern. N, Nucleus; Gr, secrete granulation. Swollen mitochondria were indicated by *black boxes*. *Black arrowheads* showed immature secretory granules, and *black arrows* showed empty granules. Photographs were taken at ×13,500 magnification. Mitochondria were quantified within the β-cells and presented as the average mitochondrial area (C and J) and the average mitochondrial OD (D and K) at wk 3 (C and D) and wk 27 (J and K) [ANCOVA: P (litter size) > 0.05]. Higher OD values (a measure of brightness) were an indication of organelle swelling. E and L, Manual quantifications of the percentage of total number of granules (filled, immature, and empty) at 3 (E) and 27 (L) wk of age [ANCOVA: P (litter size) > 0.05]. Results are expressed as mean ± SEM. HFD, High-fat diet; ND, normal diet. *, $P < 0.05$ compared with control HFD group; **, $P < 0.01$compared with control HFD group; #, $P < 0.05$ compared with control ND group; ##, $P < 0.01$compared with control ND group.

observed in BPA-treated offspring. Additionally, expression of *Ldha*, a gene involved in lactate metabolism and transport, was markedly increased in BPA-treated offspring fed a high-fat diet. Levels of uncoupling protein 2 (*Ucp2*) were significantly increased in BPA-treated offspring fed either a high-fat diet or a normal diet (Table 1). Litter size was not associated with the expression of mRNA in either sex ($P > 0.05$).

Effects of BPA on lipid homeostasis

As shown in Fig. 7, A–D, BPA exposure resulted in a higher body fat percentage and significantly greater size of adipocytes in normal-diet or high-fat fed offspring of both genders. Litter size was significantly associated with the size of adipocytes in the high-fat-fed female offspring ($P < 0.05$) but was not significantly related to that in males ($P > 0.05$). Among both males and females, the litter size did not predict the body fat percentage ($P > 0.05$).

Higher TG level and lower HDL, but comparable TC and LDL, were observed in BPA-treated male offspring fed a normal diet (Fig. 7, E–H). No significant difference in these parameters was displayed in females. On a high-fat diet, BPA exposure resulted in significantly higher serum TC, TG, LDL levels and dramatically lower HDL levels in both male and female offspring (Fig. 7, E–H). Serum leptin levels were significantly higher in BPA-treated offspring of both genders than that in controls, no matter whether fed a normal or a high-fat diet (Fig. 7I). Litter size was not associated with the serum leptin, TC, TG, LDL, and HDL in either sex ($P > 0.05$).

Effects of BPA exposure at 250 and 1250 μg/kg · d

Perinatal BPA exposures at 250 and 1250 μg/kg · d did not have any impact on body weight, blood parameters, glucose tolerance, and insulin sensitivity in the offspring,

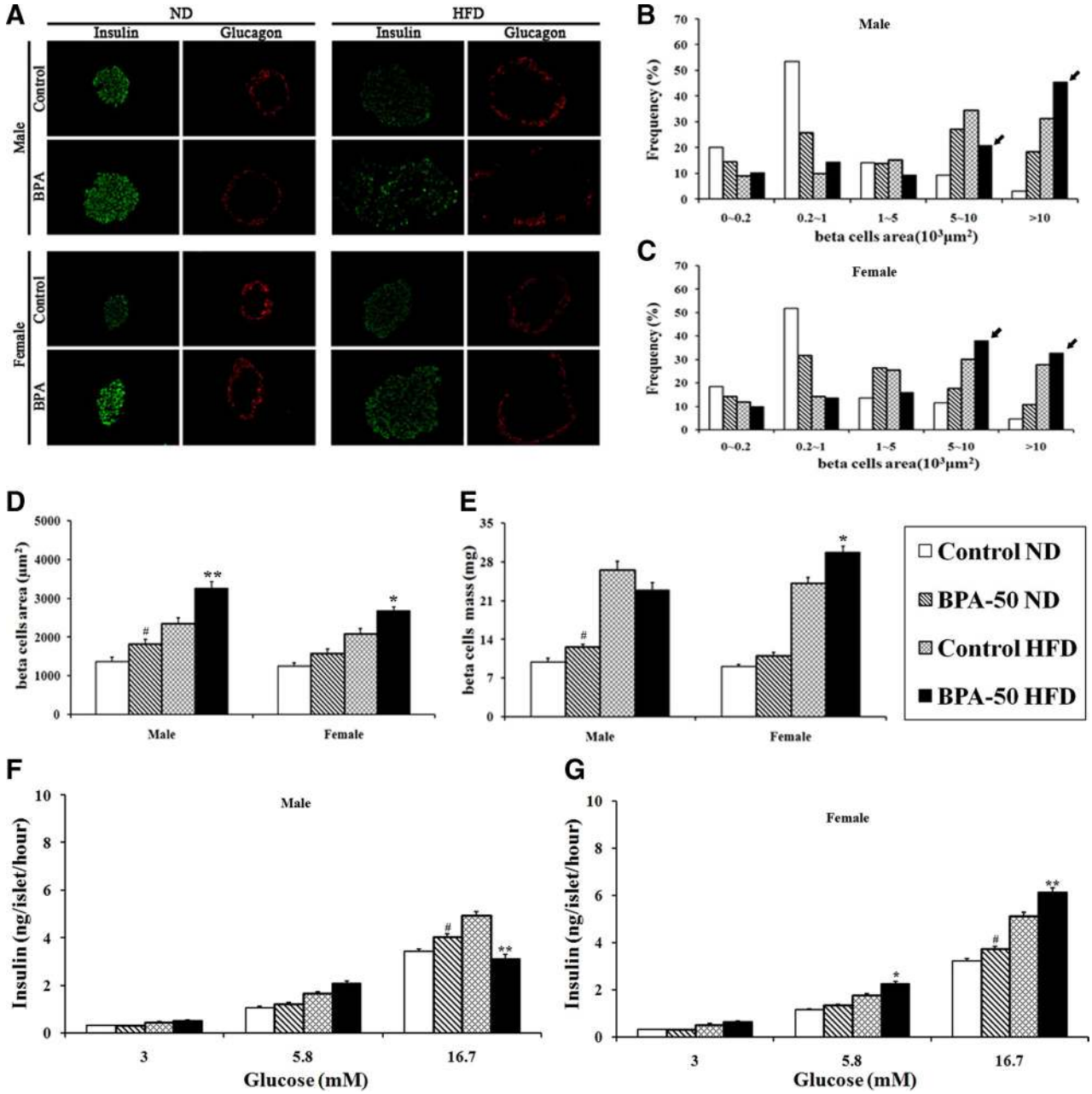

FIG. 6. Immunofluorescent staining of pancreas-stimulated insulin secretion and GSIS *ex vivo* in offspring at wk 27 (n = 3 per group). A, Representative pancreatic sections immunostained with antiinsulin (*green*) and antiglucagon (*red*) antibodies at ×20 magnification. B and C, The distribution of β-cell area in male (B) and female (C) offspring [ANCOVA: *P* (litter size) > 0.05]. *Black arrows* highlight the preponderance of larger islet areas in BPA-exposed offspring. D, Measurement of mean β-cell area for male and female offspring [ANCOVA: *P* (litter size) > 0.05]. E, Measurement of β-cell mass for male and female offspring [ANCOVA: *P* (litter size) > 0.05]. Results are expressed as mean ± SEM. Islets from male (F) and female (G) offspring exposed to 50 μg/kg · d BPA were incubated with the indicated glucose concentrations for 1 h, and the accumulated amount of insulin was determined. Results are expressed as mean ± SEM of six replicates from three independent isolations. HFD, High-fat diet; ND, normal diet. *, $P < 0.05$ compared with control HFD group; **, $P < 0.01$compared with control HFD group; #, $P < 0.05$ compared with control ND group; ##, $P < 0.01$compared with control ND group.

no matter whether fed a normal diet or a high-fat diet (Supplemental Figs. 3–10).

Discussion

The potential role of BPA in increasing the risk of metabolic abnormalities has been indicated in some epidemiological and animal studies (12, 18, 19). Here we report that perinatal BPA exposure at 50 μg/kg · d results in impaired glucose tolerance and insulin sensitivity in adult rat offspring; moreover, male offspring develop insulin resistance in adulthood. In addition, we first found that the ultrastructure of β-cells in BPA-treated offspring had been damaged at weaning before the onset of glucometabolic disorders, which got progressively worse as the offspring became older.

TABLE 1. Comparison of islet-associated transcription factor and β-cell function mRNA levels in islets from offspring perinatally exposed to 50 μg/kg · d BPA

	Male (n = 3)		Female (n = 3)	
	ND	HFD	ND	HFD
Pdx-1	0.794 ± 0.011[a]	0.379 ± 0.011[b]	0.810 ± 0.017[a]	0.574 ± 0.025[b]
Nkx6.1	0.783 ± 0.013[a]	0.440 ± 0.022[b]	0.735 ± 0.021[a]	0.601 ± 0.014[b]
Glut2	0.714 ± 0.018[a]	0.385 ± 0.018[b]	0.840 ± 0.014[a]	0.494 ± 0.019[b]
Gck	0.706 ± 0.019[a]	0.386 ± 0.020[b]	0.826 ± 0.028[a]	0.531 ± 0.020[b]
Ldha	1.234 ± 0.023	2.105 ± 0.047[b]	1.168 ± 0.019	1.741 ± 0.027[b]
Ucp-2	1.519 ± 0.029[a]	1.967 ± 0.051[b]	1.447 ± 0.023[a]	1.645 ± 0.031[b]

Results are expressed as mean ± SEM determined by three replicates (n = 3 per group). Each gene was normalized to the 36B4 internal control, and gene expression values were normalized to control islet values [ANCOVA: P (litter size) > 0.05]. ND, Normal diet; HFD, high-fat diet.

[a] $P < 0.05$ compared with control ND group; [b] $P < 0.01$compared with control HFD group.

We chose three maternal doses of BPA, and the metabolic reprogramming effects occurred just at 50 μg/kg · d but not at 250 or 1250 μg/kg · d. This intriguing finding is consistent with an excellent study demonstrating that offspring exposed to 100 μg/kg · d BPA present milder glucose intolerance than that exposed to 10 μg/kg · d BPA (19). Perinatal exposure to approximate 70 μg/kg · d BPA has also been reported to alter early adipogenesis and elevate body weight but not impair glucose tolerance in rat offspring (20). These reports and our results propose a nonmonotonic dose response that low doses of BPA are more effective than high doses in altering metabolic homeostasis. In a well-designed study, Ryan *et al.* (21) reported that CD-1 mice offspring perinatally exposed to approximately 0.25 μg/kg · d BPA via the mother's diet do not accelerate body weight or impair glucose regulation in adulthood, even when exposed to a high-fat diet later in life. This study included large numbers of animals from a large number of litters and controlled for the litter effects by including only one animal of each sex from each litter to participate in each experimental condition, which proves the negative findings technically and raises the possibility that 0.25 μg/kg · d BPA may be considered as a dose lower than that elicits adverse effects on glucose metabolism.

To mimic the most common route of exposure to BPA in human and wildlife, we chose an oral route of BPA administration and continued BPA exposure throughout the period of lactation because both fetal and neonatal life is critical to pancreas development in rodents. In addition, consumption of high-energy foods is prevalent all over the world, so we evaluate the effects of switching the offspring to a high-fat diet after weaning, looking specifically for whether early-life BPA exposure would initiate type 2 diabetes in rats weaned onto a high-fat diet. As anticipated, we confirmed that the postweaning high-fat diet accelerates and exacerbates the development of metabolic syndrome induced by perinatal BPA exposure. A recent study reported that mice offspring perinatally exposed to 0.25 μg/kg · d BPA increase growth around the time of weaning, but BPA-exposed offspring do not develop to metabolic syndrome in adulthood when they eat a high-fat diet beginning at 9 wk of age (21). It would be therefore interesting to address whether mice offspring perinatally exposed to BPA at 0.25 μg/kg · d would contribute to metabolic disorders in adulthood if they eat a high-fat diet as soon as weaning.

The postweaning body weight curves of perinatal BPA-exposed offspring were steeper than those of the control in this study, which is in line with previous results demonstrating increased body weight in rat offspring perinatally exposed to 100 μg/kg · d BPA (32). We also observed that the increased body weight in adult rat offspring was associated with an increase of white adipose tissue size as well as an increase of body fat percentage. All these findings are in agreement with a prior paper in which early adipogenesis is altered and body weight in adulthood is increased in Sprague Dawley rat offspring exposed to 70 μg/kg · d BPA from GD6 throughout the period of lactation (20) but is distinct from other works reporting no alteration in body weight in mice offspring exposed to 10 μg/kg · d BPA only during pregnancy (19). One possible explanation for the difference in the alteration of body weight is that different periods of administration of BPA influence the results observed in these studies. White adipose tissue formation begins before birth, but its differentiation or expansion mainly takes place in postnatal life (33); as a result, the combination of prenatal BPA exposure together with postnatal exposure are more likely to increase body weight in adult offspring.

In this study, serum leptin was elevated in BPA-treated offspring at wk 26, which is different from a published data showing no alterations in serum leptin levels in 10 or 100 μg/kg · d BPA-exposed male offspring at 6 months of age. Leptin is secreted by mature adipocytes, and serum leptin concentrations are most closely correlated with the percentage of body fat (34). Data reported here indicate that increases in serum leptin is, in part, attributable to

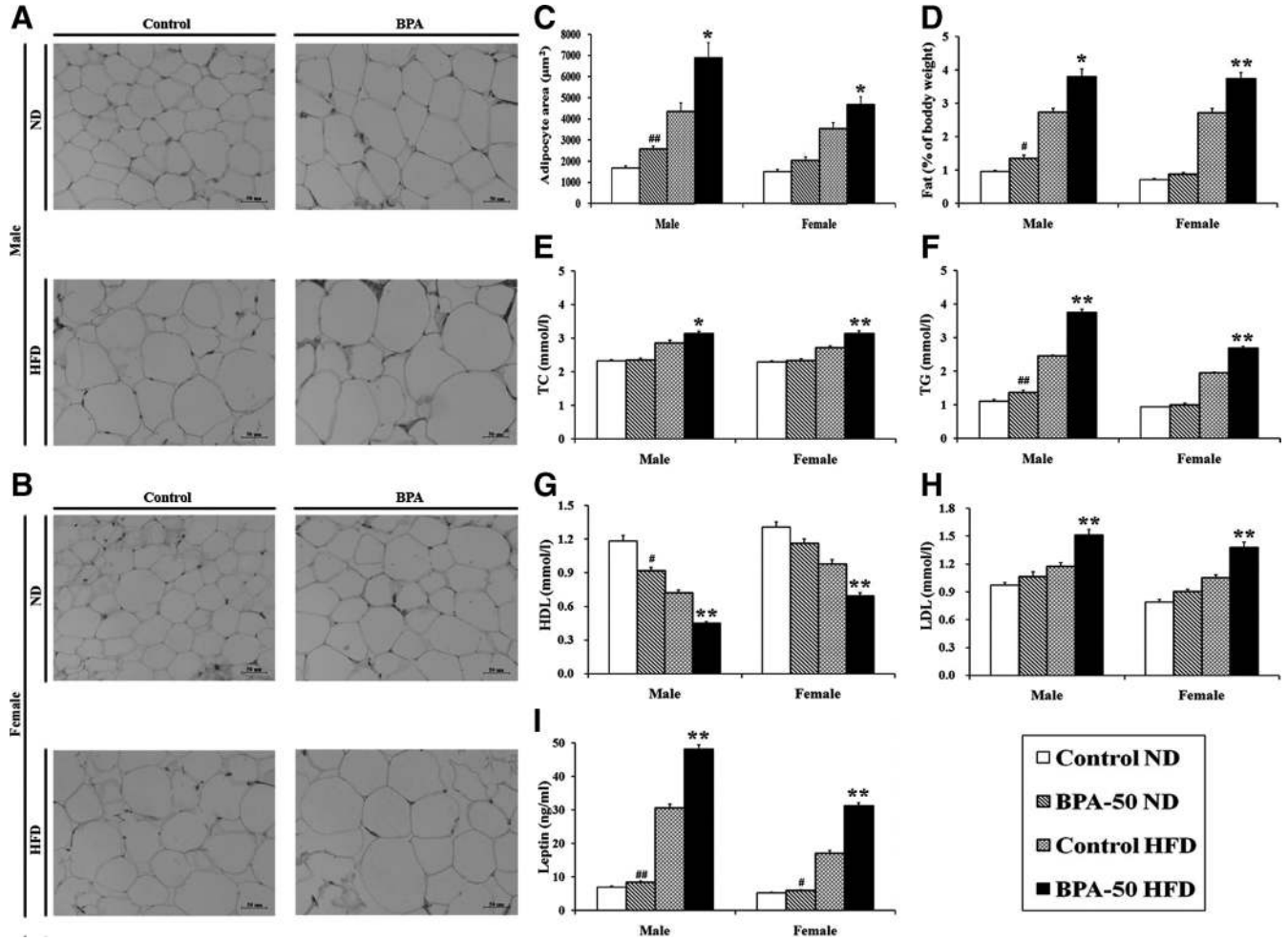

FIG. 7. Effects of perinatal exposure to 50 μg/kg · d BPA on lipid homeostasis in offspring. A and B, Representative histologic sections of adipocytes from male (A) and female (B) offspring at wk 27 (n = 3 per group). C, Body fat percentage measured at wk 27 [ANCOVA: *P* (litter size) > 0.05]. D, Average adipocyte area measured at wk 27 [ANCOVA: among males, *P* (litter size) > 0.05; among females, *P* (litter size) < 0.05]. E, Serum TC measured at wk 27 [ANCOVA: *P* (litter size) > 0.05]. F, Serum TG measured at wk 27 [ANCOVA: *P* (litter size) > 0.05]. G, Serum HDL measured at wk 27 [ANCOVA: *P* (litter size) > 0.05]. H, Serum LDL measured at wk 27 [ANCOVA: *P* (litter size) > 0.05]. I, Serum leptin measured at wk 27 [ANCOVA: *P* (litter size) > 0.05] (n = 6 per group). Results are expressed as mean ± SEM. HFD, High-fat diet; ND, normal diet. *, $P < 0.05$ compared with control HFD group; **, $P < 0.01$compared with control HFD group; #, $P < 0.05$ compared with control ND group; ##, $P < 0.01$compared with control ND group.

neonatal life BPA exposure-associated up-regulation of adipocyte synthesis in addition to the presence of insulin resistance or hyperinsulinemia. Notably, leptin in turn inhibits the production of insulin in the pancreas (35); thus, the significantly increased leptin may contribute to the subsequent development of diabetes in perinatal BPA-exposed individuals.

Alterations in body weight and body fat percentage also led to elevated levels of TG, TC, and LDL as well as declined levels of HDL in our study. These adverse effects of BPA exposure are exacerbated when offspring are fed a high-fat diet. Considerable evidence has been accumulated that both LDL and HDL affect the function and survival of pancreatic β-cells (36, 37). Furthermore, additional evidence is emerging that HDL regulates fat storage in adipocytes, energy expenditure, and insulin sensitivity (38, 39). Hence, our study confirms that perinatal BPA exposure is involved in the development of obesity and the compromises of lipid metabolism, thereby initiating a vicious cycle, which further aggravates insulin resistance and favors β-cell failure.

Obesity is usually associated with other comorbid conditions, such as insulin resistance or type 2 diabetes. In our study, the first observed that alteration related to glucose homeostasis is abnormal β-cell ultrastructure in offspring at weaning, characterized by swollen mitochondria, dilated rough endoplasmic reticulum, and a modest degranulation of β-cells, whereas at that time, the fasted blood glucose, serum insulin, and glucose tolerance in offspring were not changed. Mitochondria are critical for regulation of β-cell mass and maintenance of β-cell function through the coupling of a glucose stimulus to insulin release (40–42). Thus, we suggest that BPA-induced mitochondria and rough endoplasmic reticulum damage would have further

consequences for β-cell function and play an important role in the development of glucose intolerance or type 2 diabetes. In addition, the weaning period is a critical window for pancreatic development because offspring change from a constant milk diet to an omnivorous diet (43). BPA-treated offspring weaned onto a high-fat diet would further impair pancreatic development and thereby alter β-cell function, which lasts into the adulthood and has harmful lifetime consequences.

As anticipated, observed mitochondria defects progressively got worse, even though BPA exposure was discontinued at weaning. Offspring perinatally exposed to BPA on a normal diet exhibited increased serum insulin and β-cell mass and glucose intolerance at adulthood, which suggests that compensatory changes occurring in β-cells induce increased insulin production. But dilatate and hyperactive β-cells would entrain β-cells to fail in later life, when type 2 diabetes occurs. When offspring were weaned onto a high-fat diet, an adverse impact of BPA on glucose homeostasis was much more pronounced, characterized by elevated blood glucose and insulin and a decrease in glucose use. Insulin insensitivity was clear in BPA-treated offspring fed a high-fat diet, indicating peripheral insulin resistance would underline glucose intolerance. Female offspring exposed to BPA exhibited insulin hypersecretion during an OGTT, which suggests that the decreased in glucose use is due to an alteration of peripheral insulin sensitivity rather than a defect in insulin production or secretion. Significantly higher blood glucose but lower insulin was detected at 15 min after glucose loading in male BPA-treated offspring on a high-fat diet. The reduced first-phase insulin secretion could have been due to the mild reduction of β-cell mass or to the evident degranulation of β-cells. *Ex vivo* analysis of insulin secretion also demonstrated that, on a high-fat diet, islets of BPA-exposed male offspring showed higher insulin secretion at low glucose concentrations (3.0–5.8 mmol/liter) and moreover significantly reduced insulin secretion in response to 16.7 mmol/liter glucose compared with controls. All these data suggest that compensatory changes in islets of male BPA-treated offspring could not overcome the requirement of insulin according to the continuous hyperglycemia. Metabolic phenotype of adult male BPA-treated offspring is highly similar to the prediabetic situation (44), a pathological status associated with a mild reduction in β-cells mass and during which hypersecretion of insulin is not sufficient to compensate for peripheral insulin resistance. In addition, male offspring tend to be more sensitive than females to BPA exposure, which may be partly due to the protective effect of physiological estrogens in females against diabetes (45).

In this study, we also show that BPA-induced β-cell dysfunction is associated with a global disruption of gene expression with the induction of several normally suppressed genes and decreased expression of genes that optimize β-cell function. *Pdx-1* is a key component that is essential for normal glucose-induced insulin secretion and is correlated with β-cell mass (46, 47). The reduction in *Pdx-1* seems to be a critical threshold for causing a decrease in insulin, *Glut2*, and glucokinase genes, which are also important for normal β-cell function (48). In addition to the transactivation of genes, *Pdx-1* also regulates mitochondrial function in mature β-cells (16). Therefore, BPA exposure-induced β-cell dysfunction and mitochondrial damage are partly due to the decreased expression of *Pdx-1*. Additionally, *Ucp2* is considered to have negative influence on β-cell function (49, 50). Hyperinsulinemia, hyperglycemia, and impaired GSIS exhibited in BPA-treated offspring are associated with increased *Ucp2* mRNA levels.

In conclusion, our results overwhelmingly point to metabolic dysregulation as a consequence of perinatal BPA exposure at reference dose (50 μg/kg · d) in adulthood, characterized by an impairment in glucose tolerance in normal diet-fed offspring and moreover metabolic syndrome in high-fat-fed offspring. These detrimental effects on metabolism become more severe as the animals become older. Interestingly, data in this study suggest that a low dose of BPA (50 μg/kg · d) is more effective than a high dose, such as 250 and 1250 μg/kg · d, in disrupting glucose homeostasis in offspring and predisposing offspring to metabolic syndrome. Perinatal exposure to BPA at the reference dose represents a new risk factor in offspring for developing metabolic syndrome in adulthood.

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References

1. **Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM** 2006 Prevalence of overweight and obesity in the United States, 1999–2004. JAMA 295:1549–1555
2. **Zimmet P, Alberti KG, Shaw J** 2001 Global and societal implications of the diabetes epidemic. Nature 414:782–787
3. **Alexander CM, Landsman PB, Teutsch SM, Haffner SM** 2003 NCEP-defined metabolic syndrome, diabetes, and prevalence of coronary heart disease among NHANES III participants age 50 years and older. Diabetes 52:1210–1214
4. **Meigs JB, D'Agostino Sr RB, Wilson PW, Cupples LA, Nathan DM, Singer DE** 1997 Risk variable clustering in the insulin resistance syndrome. The Framingham Offspring Study. Diabetes 46:1594–1600
5. **Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW, Shields B, Harries LW, Barrett JC, Ellard S, Groves CJ, Knight B, Patch AM, Ness AR, Ebrahim S, Lawlor DA, Ring SM, Ben-Shlomo Y, Jarvelin MR, Sovio U, Bennett AJ, Melzer D, Ferrucci L, Loos RJ, Barroso I, Wareham NJ, Karpe F, Owen KR, Cardon LR, Walker M, Hitman GA, Palmer CN, Doney AS, Morris AD, Smith GD, Hattersley AT, McCarthy MI** 2007 A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 316:889–894
6. **Wang Y, Beydoun MA** 2007 The obesity epidemic in the United States—gender, age, socioeconomic, racial/ethnic, and geographic characteristics: a systematic review and meta-regression analysis. Epidemiol Rev 29:6–28
7. **Borowitz JL, Isom GE** 2008 Nicotine and type 2 diabetes. Toxicol Sci 103:225–227
8. **Somm E, Schwitzgebel VM, Vauthay DM, Camm EJ, Chen CY, Giacobino JP, Sizonenko SV, Aubert ML, Hüppi PS** 2008 Prenatal nicotine exposure alters early pancreatic islet and adipose tissue development with consequences on the control of body weight and glucose metabolism later in life. Endocrinology 149:6289–6299
9. **Calafat AM, Ye X, Wong LY, Reidy JA, Needham LL** 2008 Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003–2004. Environ Health Perspect 116:39–44
10. **Ukropec J, Radikova Z, Huckova M, Koska J, Kocan A, Sebokova E, Drobna B, Trnovec T, Susienkova K, Labudova V, Gasperikova D, Langer P, Klimes I** 2010 High prevalence of prediabetes and diabetes in a population exposed to high levels of an organochlorine cocktail. Diabetologia 53:899–906
11. **Wang SL, Tsai PC, Yang CY, Leon Guo Y** 2008 Increased risk of diabetes and polychlorinated biphenyls and dioxins: a 24-year follow-up study of the Yucheng cohort. Diabetes Care 31:1574–1579
12. **Lang IA, Galloway TS, Scarlett A, Henley WE, Depledge M, Wallace RB, Melzer D** 2008 Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. JAMA 300:1303–1310
13. **Codru N, Schymura MJ, Negoita S, Rej R, Carpenter DO** 2007 Diabetes in relation to serum levels of polychlorinated biphenyls and chlorinated pesticides in adult Native Americans. Environ Health Perspect 115:1442–1447
14. **Uemura H, Arisawa K, Hiyoshi M, Satoh H, Sumiyoshi Y, Morinaga K, Kodama K, Suzuki T, Nagai M, Suzuki T** 2008 Associations of environmental exposure to dioxins with prevalent diabetes among general inhabitants in Japan. Environ Res 108:63–68
15. **Lee YJ, Ryu HY, Kim HK, Min CS, Lee JH, Kim E, Nam BH, Park JH, Jung JY, Jang DD, Park EY, Lee KH, Ma JY, Won HS, Im MW, Leem JH, Hong YC, Yoon HS** 2008 Maternal and fetal exposure to bisphenol A in Korea. Reprod Toxicol 25:413–419
16. **Alonso-Magdalena P, Ropero AB, Carrera MP, Cederroth CR, Baquié M, Gauthier BR, Nef S, Stefani E, Nadal A** 2008 Pancreatic insulin content regulation by the estrogen receptor ERα. PLoS One 3:e2069
17. **Adachi T, Yasuda K, Mori C, Yoshinaga M, Aoki N, Tsujimoto G, Tsuda K** 2005 Promoting insulin secretion in pancreatic islets by means of bisphenol A and nonylphenol via intracellular estrogen receptors. Food Chem Toxicol 43:713–719
18. **Alonso-Magdalena P, Morimoto S, Ripoll C, Fuentes E, Nadal A** 2006 The estrogenic effect of bisphenol A disrupts pancreatic β-cell function *in vivo* and induces insulin resistance. Environ Health Perspect 114:106–112
19. **Alonso-Magdalena P, Vieira E, Soriano S, Menes L, Burks D, Quesada I, Nadal A** 2010 Bisphenol A exposure during pregnancy disrupts glucose homeostasis in mothers and adult male offspring. Environ Health Perspect 118:1243–1250
20. **Somm E, Schwitzgebel VM, Toulotte A, Cederroth CR, Combescure C, Nef S, Aubert ML, Hüppi PS** 2009 Perinatal exposure to bisphenol a alters early adipogenesis in the rat. Environ Health Perspect 117:1549–1555
21. **Ryan KK, Haller AM, Sorrell JE, Woods SC, Jandacek RJ, Seeley RJ** 2010 Perinatal exposure to bisphenol-a and the development of metabolic syndrome in CD-1 mice. Endocrinology 151:2603–2612
22. **Bruin JE, Petre MA, Raha S, Morrison KM, Gerstein HC, Holloway AC** 2008 Fetal and neonatal nicotine exposure in Wistar rats causes progressive pancreatic mitochondrial damage and β cell dysfunction. PLoS One 3:e3371
23. **Bruin JE, Gerstein HC, Morrison KM, Holloway AC** 2008 Increased pancreatic β-cell apoptosis following fetal and neonatal exposure to nicotine is mediated via the mitochondria. Toxicol Sci 103:362–370
24. **Mythili MD, Vyas R, Akila G, Gunasekaran S** 2004 Effect of streptozotocin on the ultrastructure of rat pancreatic islets. Microsc Res Tech 63:274–281
25. **Wijesekara N, Dai FF, Hardy AB, Giglou PR, Bhattacharjee A, Koshkin V, Chimienti F, Gaisano HY, Rutter GA, Wheeler MB** 2010 β Cell-specific Znt8 deletion in mice causes marked defects in insulin processing, crystallisation and secretion. Diabetologia 53:1656–1668
26. **Bernal-Mizrachi E, Wen W, Stahlhut S, Welling CM, Permutt MA** 2001 Islet β cell expression of constitutively active Akt1/PKB α induces striking hypertrophy, hyperplasia, and hyperinsulinemia. J Clin Invest 108:1631–1638
27. **Garofano A, Czernichow P, Bréant B** 1998 β-Cell mass and proliferation following late fetal and early postnatal malnutrition in the rat. Diabetologia 41:1114–1120
28. **Gotoh M, Maki T, Kiyoizumi T, Satomi S, Monaco AP** 1985 An improved method for isolation of mouse pancreatic islets. Transplantation 40:437–438
29. **Li DS, Yuan YH, Tu HJ, Liang QL, Dai LJ** 2009 A protocol for islet isolation from mouse pancreas. Nat Protoc 4:1649–1652
30. **Carter JD, Dula SB, Corbin KL, Wu R, Nunemaker CS** 2009 A practical guide to rodent islet isolation and assessment. Biol Proced Online 11:3–31
31. **Cherif H, Reusens B, Ahn MT, Hoet JJ, Remacle C** 1998 Effects of taurine on the insulin secretion of rat fetal islets from dams fed a low-protein diet. J Endocrinol 159:341–348
32. **Rubin BS, Murray MK, Damassa DA, King JC, Soto AM** 2001 Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. Environ Health Perspect 109:675–680
33. **Gregoire FM, Smas CM, Sul HS** 1998 Understanding adipocyte differentiation. Physiol Rev 78:783–809
34. **Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, Caro JF** 1996 Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 334:292–295
35. **Seufert J, Kieffer TJ, Leech CA, Holz GG, Moritz W, Ricordi C, Habener JF** 1999 Leptin suppression of insulin secretion and gene

expression in human pancreatic islets: implications for the development of adipogenic diabetes mellitus. J Clin Endocrinol Metab 84: 670–676
36. **von Eckardstein A, Sibler RA** 2011 Possible contributions of lipoproteins and cholesterol to the pathogenesis of diabetes mellitus type 2. Curr Opin Lipidol 22:26–32
37. **Kruit JK, Brunham LR, Verchere CB, Hayden MR** 2010 HDL and LDL cholesterol significantly influence β-cell function in type 2 diabetes mellitus. Curr Opin Lipidol 21:178–185
38. **Drew BG, Duffy SJ, Formosa MF, Natoli AK, Henstridge DC, Penfold SA, Thomas WG, Mukhamedova N, de Courten B, Forbes JM, Yap FY, Kaye DM, van Hall G, Febbraio MA, Kemp BE, Sviridov D, Steinberg GR, Kingwell BA** 2009 High-density lipoprotein modulates glucose metabolism in patients with type 2 diabetes mellitus. Circulation 119:2103–2111
39. **Van Linthout S, Foryst-Ludwig A, Spillmann F, Peng J, Feng Y, Meloni M, Van Craeyveld E, Kintscher U, Schultheiss HP, De Geest B, Tschöpe C** 2010 Impact of HDL on adipose tissue metabolism and adiponectin expression. Atherosclerosis 210:438–444
40. **Maechler P, de Andrade PB** 2006 Mitochondrial damages and the regulation of insulin secretion. Biochem Soc Trans 34:824–827
41. **Lowell BB, Shulman GI** 2005 Mitochondrial dysfunction and type 2 diabetes. Science 307:384–387
42. **MacDonald PE, Joseph JW, Rorsman P** 2005 Glucose-sensing mechanisms in pancreatic β-cells. Philos Trans R Soc Lond B Biol Sci 360:2211–2225
43. **Aguayo-Mazzucato C, Sanchez-Soto C, Godinez-Puig V, Gutiérrez-Ospina G, Hiriart M** 2006 Restructuring of pancreatic islets and insulin secretion in a postnatal critical window. PLoS One 1:e35
44. **Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC** 2003 β-Cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. Diabetes 52:102–110
45. **Liu S, Mauvais-Jarvis F** 2010 Minireview: estrogenic protection of β-cell failure in metabolic diseases. Endocrinology 151:859–864
46. **Arantes VC, Teixeira VP, Reis MA, Latorraca MQ, Leite AR, Carneiro EM, Yamada AT, Boschero AC** 2002 Expression of PDX-1 is reduced in pancreatic islets from pups of rat dams fed a low protein diet during gestation and lactation. J Nutr 132:3030–3035
47. **Shih DQ, Heimesaat M, Kuwajima S, Stein R, Wright CV, Stoffel M** 2002 Profound defects in pancreatic β-cell function in mice with combined heterozygous mutations in Pdx-1, Hnf-1α, and Hnf-3β. Proc Natl Acad Sci USA 99:3818–3823
48. **Reimer MK, Ahren B** 2002 Altered β-cell distribution of pdx-1 and GLUT-2 after a short-term challenge with a high-fat diet in C57BL/6J mice. Diabetes 51(Suppl 1):S138–S143
49. **Knauf C, Cani PD, Ait-Belgnaoui A, Benani A, Dray C, Cabou C, Colom A, Uldry M, Rastrelli S, Sabatier E, Godet N, Waget A, Pénicaud L, Valet P, Burcelin R** 2008 Brain glucagon-like peptide 1 signaling controls the onset of high-fat diet-induced insulin resistance and reduces energy expenditure. Endocrinology 149:4768–4777
50. **Laybutt DR, Sharma A, Sgroi DC, Gaudet J, Bonner-Weir S, Weir GC** 2002 Genetic regulation of metabolic pathways in β-cells disrupted by hyperglycemia. J Biol Chem 277:10912–10921