Decreased basal insulin secretion from pancreatic islets of pups in a rat model of maternal obesity

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Abstract

Maternal obesity (MO) is a deleterious condition that enhances susceptibility of adult offspring to metabolic diseases such as type 2 diabetes. The objective is to study the effect of MO on in vitro insulin secretion and pancreatic cellular population in offspring. We hypothesize that a harmful antenatal metabolic environment due to MO diminishes the basal glucose-responsive secretory function of pancreatic beta cells in offspring. Mothers were fed a control (C) or high-fat diet from weaning through pregnancy (120 days) and lactation. At postnatal days (PNDs) 36 and 110, pups were killed, peripheral blood was collected and pancreatic islets were isolated. Basal insulin secretion was measured in vitro in islets for 60 min. It was found that blood insulin, glucose and homeostasis model assessment (HOMA) index were unaffected by maternal diet and age in females. However, male MO offspring at PND 110 showed hyperinsulinemia and insulin resistance compared with C. Body weight was not modified by MO, but fat content was higher in MO pups compared with C pups. Triglycerides and leptin concentrations were higher in MO than in C offspring in all groups except in females at PND 36. Pancreatic islet cytoarchitecture was unaffected by MO. At PND 36, islets of male and female C and MO offspring responded similarly to glucose, but at PND 110, male and female MO offspring islets showed a 50% decrease in insulin secretion. It was concluded that MO impairs basal insulin secretion of offspring with a greater impact on males than females, and this effect mainly manifests in adulthood.

Introduction

As the primary regulator of blood glucose concentration, insulin inhibits hepatic glucose production and increases glucose uptake in muscle and fat tissue. Insulin also promotes cell growth and differentiation and synthesis of glycogen and protein, and it inhibits lipolysis and protein breakdown. Insulin resistance or deficiency alters the regulation of these processes and causes elevated fasting and postprandial levels of glucose and lipids. Maternal obesity (MO) is increasing worldwide and is an important risk factor contributing to type 2 diabetes in offspring (Wang & Lobstein 2006, Samuelsson et al. 2008, Gonzalez et al. 2013, Latouche et al. 2014). Type 2 diabetes is polygenic and may involve polymorphisms in multiple genes encoding proteins involved in
insulin signaling, insulin secretion and intermediary metabolism (Stern 2000, Kahn et al. 2006). Regardless, the detrimental impact of diet-induced MO on the long-term health, adiposity and metabolism of offspring is well established (Kirk et al. 2009). MO offspring are at an increased risk of obesity, impaired glucose tolerance, reduced whole-body insulin sensitivity and other components of metabolic syndrome (Taylor et al. 2005, Zambrano et al. 2010, Latouche et al. 2014). Adipose tissue has a special role in insulin resistance. Circulating free fatty acids derived from adipocytes are elevated in many insulin-resistant states and have been suggested to contribute to the insulin resistance of diabetes and obesity by inhibiting glucose uptake, glycogen synthesis and glucose oxidation and by increasing hepatic glucose output (Bergman & Ader 2000). Although insulin biosynthesis is controlled by multiple factors, glucose metabolism is the most important physiological event that stimulates insulin gene transcription and mRNA translation (Poitout et al. 2006).

Insulin content in beta cells is highly dynamic, accumulating in the presence of nutrients and decreasing in response to nutrient deprivation. This study aimed to separate the functions of the beta cells by challenging isolated pancreatic islets with physiological levels of glucose. We hypothesized that a MO diet administered to rats from weaning through pregnancy and lactation periods would alter the ability of pancreatic beta cells to respond to glucose at physiological levels.

Materials and methods

Animals and maternal diet

Female albino Wistar rats (Rattus norvegicus), age 15–17 weeks and weighing 220±20 g, were maintained in the animal facility of the Instituto Nacional de Ciencias Médicas y Nutrición (INCMNSZ), Mexico City, Mexico. All procedures were approved by the Animal Experimental Ethics Committee of INCMNSZ in accordance with the Official Mexican Guideline for the Care and Use of Laboratory Animals (NOM-062-ZOO-1999). Rats were maintained at a controlled temperature of 22–23°C and on a 12h light:12h darkness cycle. Animals had free access to water and were fed normal laboratory chow (Zeigler Rodent RQ 22-5, Gardner, PA, USA) containing 22.0% protein, 5.0% fat, 31.0% polysaccharide, 31.0% simple sugars, 4.0% fiber, 6.0% minerals, 1.0% vitamins (w/w) and 4.0 kcal g⁻¹ energy. Female rats were mated overnight with proven male breeders. To ensure the homogeneity of evaluated offspring, all litters studied were adjusted to ten pups per dam at postnatal day 2 with equal numbers of males and females wherever possible.

At weaning (postnatal day 21), offspring females were randomly assigned to either a control (C; n=6) group that received the laboratory chow or to a maternal obesity group (MO; n=6) that received a high-energy obesogenic diet containing 23.5% protein, 20.0% animal lard, 5.0% corn oil, 21% polysaccharide, 21% simple sugars, 5.0% fiber, 5.0% mineral mix, 1.0% vitamin mix (w/w) and 4.9 kcal g⁻¹ energy (Bautista et al. 2016). At 120 days, obese (Novelli et al. 2007) and control females were mated with control males, and after delivery, all pups were placed on the control diet (Fig. 1). Six male and six female offspring from different litters were studied at 36 days (around puberty) and 110 days (young adult). Rats were killed by decapitation (Leary et al. 2013).

Measurement of body weight and fat content in offspring at postnatal days 36 and 110

Offspring body weight was determined and adipose tissue from visceral and retroperitoneal areas was collected and weighed. Fat content per rat was determined as the percentage of total fat (intra-abdominal and retroperitoneal) to body weight.

Figure 1

Design of experimental groups. Female rats were fed with control (CTR) or obesogenic diet (MO) before and during the gestational and lactation periods. Male and female offspring were assessed at postnatal days (PND) 36 and 110. n=6.
Blood measurements
Fasting serum glucose and triglyceride concentrations were determined enzymatically by a Synchron CX auto analyzer (Beckman Coulter Co, Brea, CA, USA). Insulin and leptin concentrations were determined using rat double-antibody radioimmunoassay (Millipore). Homeostasis model assessment (HOMA) was calculated as HOMA = glucose (mmolL⁻¹) × insulin (U mL⁻¹)/22.5 (Zambrano et al. 2006).

Pancreatic tissue and immunohistochemistry
Pancreatic samples were collected from offspring and fixed in 4% w/v formaldehyde in phosphate-buffered saline (PBS). Paraffin-embedded pancreatic sections (5-µm thickness) were dewaxed and rehydrated in graded ethanol solutions. Slides were heated by microwave radiation for 10 min in 0.01 M citrate buffer (pH 6.0) for antigen retrieval. Endogenous peroxidase activity was blocked with 0.3% H₂O₂ in methanol for 30 min. To prevent nonspecific antibody binding, sections were blocked with bovine serum albumin (BSA) (Sigma-Aldrich) for 60 min. Slides were then incubated with primary antibodies to anti-insulin (H-86 sc-9168, 1:100; Santa Cruz Biotechnology) and anti-glucagon (G2654, 1:8000; Sigma-Aldrich) overnight at 4°C. After three washes with PBS for 5 min each, the primary antibody was detected with the appropriate secondary antibody (1:100 at 37°C, for 60 min). The slides were washed for 5 min and incubated using 3,3'-diaminobenzidine-tetra-hydrochloride (DAB) as chromogen (Zymed/ Invitrogen), rinsed in distilled water and counterstained with hematoxylin. The sections were mounted and coverslipped with a synthetic mounting medium (Entellan; Merck). A negative control was performed on pancreatic tissue without the addition of primary antibodies to anti-insulin and anti-glucagon. All slides represented a random sampling of the tissue stained.

Analysis of immunoreactivity
Images were visualized using a Nikon Eclipse E600 microscope using a 20X objective and were captured with a CoolSNAP-Pro cf digital camera (Roper Scientific, Inc, Tucson, AZ, USA). The area of positive insulin or glucagon staining was calculated based on the total area of each islet and reported as % of beta or alpha cells.

Pancreatic islet isolation
The method used for pancreatic islet isolation has been described in detail previously (Morimoto et al. 2001). Briefly, a bile duct catheter was introduced, and, following collagenase digestion, pancreatic islets were isolated and collected individually using a microscope. Islets were cultured overnight with RPMI-1640 medium at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. Islets were washed twice with a buffer solution (pH 7.4) containing 20 mmol L⁻¹ HEPES, 115 mmol L⁻¹ NaCl, 5 mmol L⁻¹ NaHCO₃, 5 mmol L⁻¹ KCl, 2.6 mmol L⁻¹ CaCl₂, 1.2 mmol L⁻¹ KH₂PO₄, 1.2 mmol L⁻¹ MgSO₄, 3 mmol L⁻¹ d-glucose and 1% (w/v) bovine serum albumin (Sigma-Aldrich). As a methodological observation, the isolation of pancreatic islets was considerably more difficult in the MO group. The tissue was exceptionally fatty and fragile in comparison with the tissue obtained from the C group.

Islet insulin secretory response
In vitro insulin release was measured in groups of ten isolated islets/well from an individual animal in 1 mL of buffer solution and in the presence of 5 and 11 mmol L⁻¹ glucose. The experiments with higher glucose concentration (11 mmol L⁻¹) were very difficult to reproduce due to the fragility of the islets. Therefore, we decided to evaluate the insulin secretory response only with 5 mmol L⁻¹ glucose for 1 h as reported previously (Morimoto et al. 2012). After 1 h, the medium was collected and stored at −70°C until analyzed for insulin concentration.

Statistical analysis
All data are presented as mean±s.e.m. To avoid skewed effects from a single litter, only one male and one female per litter were studied, equaling six offspring per group. We evaluated the differences between the groups at the same age and within the same groups at different ages. Data were analyzed using one-way or two-way analysis of variance (ANOVA), and all pairwise multiple comparisons were determined by the Holm–Šidák or Tukey’s post hoc tests. P<0.05 was considered significant.
Results

Body weight, fat content and blood measurements

Maternal obesity did not affect pup body weight, but the percentage of fat in MO offspring was higher compared with C offspring for both ages and sexes and was lower in female offspring. Triglyceride and leptin levels were higher in MO than in C offspring in all cases except in females at PND 36. At PND 110, serum leptin concentrations were higher for both sexes and groups compared with PND 36. Triglyceride levels were lower in female MO offspring at both ages compared with male offspring. Leptin levels were lower in both female C and MO offspring at PND 110 compared with males at the same age (Table 1). In both male and female offspring, blood glucose was unaffected by age, gender or maternal diet. Insulin levels of offspring at PND 36 were similar among offspring from all groups tested. In the MO male subjects, we found both increased insulin and HOMA values at PND 110 compared with the females (Fig. 2).

Islet morphology

Our results showed no differences in morphology or cytoarchitecture of individual pancreatic islets related to sex, age or maternal diet. In Fig. 3, representative microphotographs show the beta cells in the core of the islets and the alpha cells aligned in the periphery. Maternal obesity did not affect the distribution of beta and alpha cells within pancreatic islets.

Cellular composition of pancreatic islets

Beta cells The cellular composition in the pancreatic islets had a similar pattern; both male and female MO offspring presented a decreased proportion of beta cells at PND 36 compared with C offspring. However, at PND 110, maternal obesity inverted this pattern. As shown in Fig. 4, we observed a considerably lower proportion of beta cells at PND 36 compared with PND 110 (P<0.05). These changes were also significantly different when we compared the C vs MO subjects by sex and age. Interestingly, MO males were more affected than females at PND 110.

Alpha cells The observed alpha cell distribution in all subjects was consistent regardless of age or sex. Both male and female MO offspring at PND 36 presented an increased percentage of alpha cells compared with C.

Table 1 Body weight, fat content and blood levels of triglycerides and leptin in offspring from mothers fed control (C) or high-fat diet (MO) at postnatal days (PNDs) 36 and 110.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
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<tbody>
<tr>
<td></td>
<td>36 PND</td>
<td>110 PND</td>
</tr>
<tr>
<td></td>
<td>36 PND</td>
<td>110 PND</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>139.19±7.98</td>
<td>141.79±2.93</td>
</tr>
<tr>
<td>MO</td>
<td>141.79±12.97</td>
<td>471.85±12.97</td>
</tr>
<tr>
<td>Fat content (%)</td>
<td>1.07±0.07</td>
<td>1.49±0.18</td>
</tr>
<tr>
<td>C</td>
<td>117.14±5.53</td>
<td>15.90±0.82</td>
</tr>
<tr>
<td>MO</td>
<td>122.05±3.20</td>
<td>22.94±0.91</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>55.62±4.29</td>
<td>63.42±10.69</td>
</tr>
<tr>
<td>MO</td>
<td>121.57±9.49</td>
<td>154.57±6.11</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.58±0.21</td>
<td>5.93±0.65</td>
</tr>
<tr>
<td>MO</td>
<td>3.21±0.21</td>
<td>5.93±0.65</td>
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aP<0.05 compared with C; bP<0.05 compared with 36 PND; cP<0.05 compared with males.
There was a higher percentage of alpha cells at PND 36 compared with PND 110. Similar to the findings for beta cells in the C group, there was also a larger number of alpha cells at younger ages in both sexes. As depicted in Fig. 4, MO caused a significantly higher percentage of alpha cells in offspring than C by age. Comparing the MO data at PND 110, we found that the female offspring had a smaller proportion of alpha cells than their male counterparts ($P<0.05$).

**Basal insulin secretion from isolated pancreatic islets**

In males, basal insulin response increased with age regardless of maternal diet. However, the absolute concentration was lower in MO offspring at PND 110 compared with C offspring. In females, C offspring showed the same pattern as the C males (lower at PND 36 than at PND 110), but in MO offspring, we observed an inverted pattern of higher basal insulin output at PND 36 compared with PND 110. The concentration of insulin secreted in response to basal glucose concentration was higher in females than in males regardless of maternal diet (Fig. 5).

**Discussion**

There is substantial evidence that maternal obesity (MO) and a high-fat diet in animal models produce several metabolic abnormalities in the fetus, neonate and adult offspring (Srinivasan et al. 2006, Elahi et al. 2009). In utero exposure to excess maternal lipids could affect a number of pathways in developing organs, such as the liver, skeletal muscle, adipose tissue, brain and pancreas (Bringhenti et al. 2013). Maternal high-fat diet during pregnancy and lactation induced insulin resistance and deterioration of pancreatic beta cell function in adult offspring in mice (Yokomizo et al. 2014), increased adult body weight and fat mass, increased blood glucose and cholesterol levels, and increased lipid deposition (Srinivasan et al. 2006, Elahi et al. 2009, Bringhenti et al. 2013). Our results agree partially with this. In this work, we found no variations in body weight; however, we found increased fat content, triglycerides and leptin in offspring from the MO group. These discrepancies could be due to differences in the studied species, duration of high-fat exposure, presence/absence of maternal obesity and diet composition. In our study, maternal high-fat diet was administered during the whole life of the mothers (after weaning and during growth, pregnancy and lactation); thus, the results reported here are the consequences of maternal obesity (Bautista et al. 2016). It is very important to consider that the effects of the obese maternal phenotype and the effects of the diet associated with that phenotype per se produce differential or summative effects; this must be taken into account when considering differences in results between different studies.

Previous work has shown that hyperinsulinemia and hyperglycemia observed in female adult offspring of lard-fed rat dams is accompanied by reduced whole-body insulin sensitivity, impaired pancreatic beta cell insulin secretion and pancreatic ultrastructural changes; these results suggest that islet cell exhaustion occurs due to high insulin demand secondary to skeletal muscle insulin insensitivity (Taylor et al. 2005). In this study, we observed that MO produced hyperinsulinemia with normoglycemia and insulin resistance (HOMA) with more marked effects in adult males. Male offspring appear to be more susceptible than females to the effect of MO (Vega et al. 2015). It has been previously reported that female mice are protected against insulin resistance and progression to diabetes by a maternal high-fat diet (HFD) (Riant et al. 2009). Results from several studies in both human and animal models indicate gender specificity in
the degree and type of metabolic alteration observed across tissues and species (McCormick et al. 1995, Zambrano et al. 2005). The sex differences in the effects of MO on beta cells may be partially related to increases in oxidative stress in male islets (Plata et al. 2014) and protection by estrogens (estradiol) in female pancreatic tissue, as shown by Yokomizo and colleagues (2014).

Although diverse studies have demonstrated that insulin resistance states are related to compensatory changes in pancreatic cell mass and function, the adaptive mechanism remains controversial. An increase in body fat, particularly in white adipose tissue, is an early indicator of obesity that precedes the development of insulin resistance (Bringhenti et al. 2013). Accordingly, we found increased fat content and blood triglycerides in MO offspring. Based on these data, we hypothesize that the stress produced by high-fat content and circulating levels of triglycerides generates insulin resistance.

**Figure 3**
Representative microphotography of pancreatic tissue in male offspring (A–D) at postnatal day (PND) 36 and (I–L) at PND 110 and female offspring (E–H) at PND 36 and (M–P) at PND 110. Positive insulin beta cells are visible in the core and positive glucagon alpha cells in the periphery of pancreatic islets. Scale bar 100 µm.
Although we found no alterations in islet cytoarchitecture induced by MO in any offspring (young or adult, male or female), cellular programming at early developmental stages affects pancreatic function later in life. This effect is evidenced by decreased basal insulin secretion by pancreatic islets in both female and male adults and by an increasing proportion of beta cells, which likely serves as a compensatory mechanism for the former (Gonzalez et al. 2013).

In humans, umbilical cord blood samples obtained from obese mothers showed increased HOMA-IR (an index of fetal insulin resistance), which was associated with increased fetal adiposity and leptin levels relative to lean control mothers (Catalano et al. 2009). It is known that beta cells are vulnerable to oxidative stress. Hydroxyl radicals are particularly dangerous due to their ability to cross the nuclear membrane and exert mutagenic effects (Robertson et al. 2003). Oxidative phosphorylation generates ROS (Baynes 1991), as do other pathways for glucose that are activated when glycolytic enzyme activity becomes saturated. These metabolic changes are likely mediated through oxidative activity in the mitochondria and abnormal protein folding in the endoplasmic reticulum. The importance of ROS in beta-cell pathology is supported by the observation that 8-hydroxy-2'-deoxyguanosine (8-OHdG), an oxidative stress marker, is elevated in beta cells from diabetic Goto-Kakizaki (GK) rats (Ihara et al. 1999) and that the insulin (Matsuoka et al. 1997) and glucokinase (Kajimoto et al. 1999) promoters are sensitive to glycation and the presence of ROS such as superoxide, hydrogen peroxide, nitric oxide and hydroxyl radicals. We have previously reported that oxidative stress has a deleterious effect on MO offspring testes as demonstrated by decreased fertility and numbers of spermatogonia and spermatocytes (Rodríguez-González et al. 2015). Therefore, it is likely that in this study using the same experimental model, other organs are subject to oxidative stress and their functions could be jeopardized.

Perinatal adverse conditions, including fetal exposure to a high-fat maternal diet, are associated with an increased susceptibility to adult-onset metabolic disorders such as diabetes. A primary mechanism accounting for perinatal adaptation is the epigenetic modification of chromatin, which is thought to occur in response to a perinatal insult in an effort to modulate gene expression and maximize fetal survival (Heerwagen et al. 2010, Joss-Moore et al. 2010, Sosa-Larios et al. 2015, Panchenko et al. 2016).

With the results obtained in the present investigation, we conclude that (a) MO is a deleterious condition for pancreatic beta cell functionality in offspring and (b) the
differences observed in the response of insulin to basal glucose concentration in isolated islets of MO offspring are sexually dimorphic.

To our knowledge, this is the first observation of the in vitro responsiveness of islets from a model of maternal obesity in the rat. These results contribute to the understanding of the programming of metabolic dysfunction in offspring as a result of MO.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Authors’ contribution statement
E Z and S M designed the study. T S, L C and C I performed the experiments. S M, C A M and A M analyzed the data and contributed to the statistical analysis. E Z, A M and S M wrote the paper. All authors read and approved the final version of the manuscript.

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