

A Study of the Effect of Maternal Obesity on the Structure and Function of Pancreatic Islets in Newborn Rats

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Abstract. Obesity is a global problem with high risks of cardiovascular diseases, stroke, and Type 2 diabetes. The current study aimed to clarify effects of maternal obesity on functional and structural integrity of pancreatic islets in newborn rats. Pancreatic specimens were obtained from 1-day-old newborn rats of obese and non-obese mothers. After processing, sections were stained with anti-insulin antibodies. A morphometry was performed on pancreatic islets. Blood glucose and insulin levels were measured in newborns of obese and non-obese mothers. The immunohistological structure of newborn pancreatic islets of obese mothers was normal and didn't differ from that of controls. Volume density, percentage of B cells per total islet cells and diameter of newborn pancreatic islets of obese mothers didn't vary significantly from that of controls. Blood glucose level of newborns of obese mothers was not significantly different from that of controls. The serum insulin of newborns of obese mothers was significantly higher than that of the controls. Maternal obesity didn't affect volume density of B cells, percentage of B cells per total islet cells, diameter of pancreatic islets and blood glucose level of rat newborns. However, obesity during pregnancy resulted in an increase of serum insulin of rat newborns.

Keywords: Maternal obesity, Pancreatic islets, B cells, Rat newborn.

Introduction

Obesity during pregnancy has implications for morbidity and mortality in both mother and baby^[1,2]. It is believed that maternal obesity can affect the islet B cells of the fetuses and newborns. Maternal obesity was

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reported to induce neonatal hypoglycaemia^[3]. The serum insulin level of fetuses of obese mothers was found to be higher than that of fetuses of non-obese mothers^[4]. The offspring of obese mothers was reported to develop hyperinsulinemia^[5-7]. Maternal obesity can be one of the factors contributing to the alarming rise in the prevalence of diabetes. Maternal obesity can also increase the risk of developing diabetes mellitus in the offsprings^[8].

Unfortunately, a detailed morphometric, immunohistochemical and correlated functional study of the effects of maternal obesity on the fetal pancreatic B cells were rarely found in the literatures. The use of immunohistochemical stain would allow sensitive and specific localization of B cells. This accurate identification of B cells would allow the performance of an accurate morphometric study.

The aim of this study was to examine the effect of obesity during pregnancy on the volume density of B cells (V_{vb}), the percentage of B cells in relation to the total islet cells (B_p), and the islet diameter (D_i) of newborns. The effect of maternal obesity on the newborn blood glucose level and newborn serum insulin level were also assessed.

Materials and Methods

Animal and Tissue Preparation

Fifteen adult female and fifteen male Wister rats were used in the current study obtained from animal house of King Fahd Research Center in King Abdulaziz University (KAU) in Jeddah, Saudi Arabia. The principles of animal laboratory care under the guidelines of KAU Animal Care Committee were followed. The body weights and blood glucose levels of rat mothers were estimated before and after mating to exclude obese diabetic mothers.

Diet Preparation

High-fat diet (HFD) was prepared by mixing a powder from Sigma containing the following components: 21% casein, 15% sucrose, 18% cornstarch, 5% corn oil, 31% lard. This formula provides 60% energy from fat. All the previous components were mixed with little water and pellet. The pellets were air dried and then stored in air-tight plastic bags

at 2-4°C. An enough amount of diet was prepared before the beginning of the experiment for the entire period of the study.

The Animals were Divided into Two Groups

- *Group I* (Twenty newborn rats): they were the offspring's of ten adult obese female rats, which were mated with ten male rats. The female rats were made obese by feeding them a high-fat diet (HFD) previously described.
- *Group II* (Ten newborn rats) (control group): they were the offspring's of five adult non-obese female rats, which were mated with five male rats. Animals of this group were only fed on the non-purified commercial food.

The pancreata of offspring's were removed in the first day after delivery of both obese and non-obese mothers. They were fixed in neutral buffered formalin, dehydrated, embedded in paraplast (Sherwood Medical Co., St. Louis, MO., USA) and sectioned serially at 4 μ m.

Immunohistochemical staining: Five sections, 20 sections apart, were obtained from each pancreas. The sections were stained by indirect immunoperoxidase method to identify the insulin-producing B cells^[9]. The primary antibody used was guinea pig anti-swine insulin serum (optimal dilution 1:500). The secondary antibody used was rabbit anti-guinea pig immunoglobulin conjugated with peroxidase (dilution 1:200). All sera and antisera were obtained from Dako Corporation, Carpinteria, CA, USA. The chromogen substrate used was 3, 3-Diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO., USA). The sections were counterstained with Harris' haematoxylin to facilitate nuclear identification.

Morphometric Analysis

The morphometric study was performed on the immunohistochemical stained slides at a magnification of $\times 1000$. The volume density of B cells per islet (V_{vb}) was calculated by the point counting method of Weibel^[10]. A graticule of a calibrated linear scale was used to measure the major (a) and minor (b), at right angle to (a), axes of the islet. The islet profile diameter was calculated from the equation $d_i = 2\sqrt{ab}$ ^[11]. The mean axial ratio of the islets was calculated. Assuming that

the islets were spheroid structures, the formula of Fullman was used to calculate the mean islet diameter (D_i)^[11].

$$D_i = \frac{\pi}{2} \times \frac{N}{1/d1 + 1/d2 - 1/dN}$$

Where N represents number of the total profiles measured.

The B cell percentage (B_p) per total islet cells was calculated by using the nucleus as the counting base. The nuclei of the stained B cells (B_n) and the nuclei of total islet cells (I_n) per islet profile were counted. The ratio of B cells nuclei to the total islet nuclei was expressed as the B cell percentage per islet cells (B_p). The following equation was used to calculate B_p .

$$B_p = (B_n / I_n) \times 100.$$

Functional Study

The blood sugar and insulin levels of the sacrificed newborn rats were measured at the time of obtaining the pancreatic samples. The serum insulin level was measured by Coat - A - Coat Insulin, radioimmunoassay kits obtained from DPC, Los Angeles, CA, USA.

Statistical Analysis

“Student’s” *t*-test was used for statistical analysis of the results. The difference was considered as significance when $p < 0.05$. All the statistical computations were made using the statistical packages SPSS (version 10) and Excel.

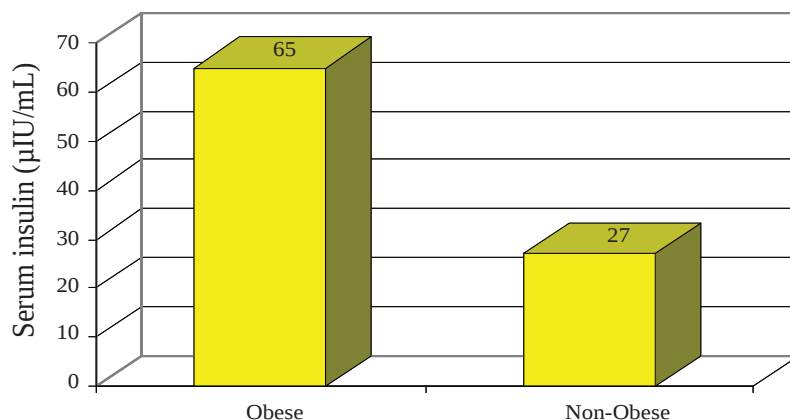
Results

The weight of obese pregnant rats was 18.3% higher than that of non-obese pregnant rats. The blood glucose levels of both obese and non-obese mothers were recorded in Table 1 to confirm exclusion of diabetes.

Table 1. The blood glucose level (mg/dl) of non-obese and obese mothers.

Blood glucose level of obese mothers Mean $\pm$ SD	Blood glucose level of non-obese mothers Mean $\pm$ SD
90.1 $\pm$ 5	70 $\pm$ 2.2

The serum insulin levels of newborns of obese and non-obese mothers are shown in Histogram 1. The blood glucose level of newborns of obese mothers was not significantly different from that of newborns of non-obese mothers ($p > 0.05$). The serum insulin level of newborns of obese mothers was significantly higher than that of newborns of non-obese mothers ($p < 0.05$).

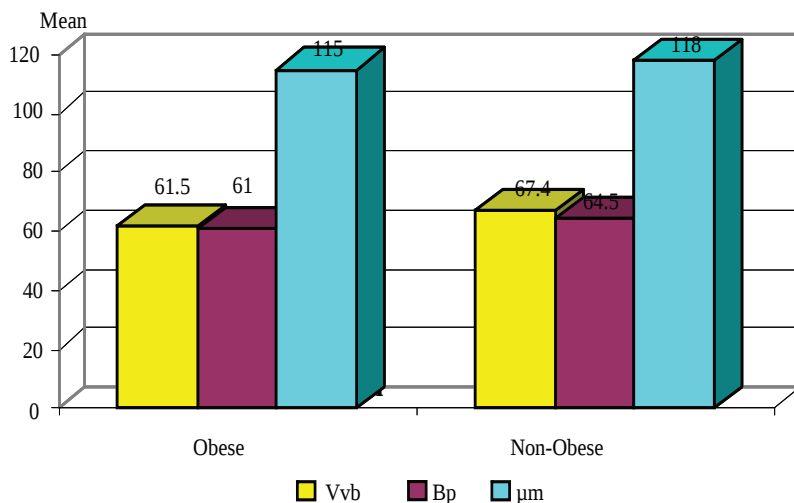


Histogram 1. The serum of insulin level (μIU/mL) of newborns to those of non-obese and obese mothers.

The microscopic examination of immunostained sections of all groups revealed the following: The mean axial ratio of newborn pancreatic islet was 1.23 ± 0.036 , this indicates that the islets could be considered as spheroid structures. The volume density (V_{vb}) of B cells per islet, the B cell percentage per total islet cells (B_p) and the islet diameter (D_i) of newborns of obese and non-obese mothers were shown in Table 2 and Histogram 2. The volume density of B cells per islet; the B cell percentage per total islet cells and the islet diameter of newborns of obese mothers were not significantly different from that of newborns of non-obese mothers ($p > 0.05$).

Table 2. Volume density of B cells per islet, percentage of B cells per total islet cells (%) and islet diameter of pancreatic islets (μm) of newborns of non-obese and obese mothers (n = 5).

Newborns of obese mothers Mean ± SD	Newborns of non-obese mothers Mean ± SD	Measure parameters
67.4 ± 2.01	61.5 ± 1.64	Volume density of B cells per islet (V_{vb})
64.5 ± 0.96	61 ± 1.39	Percentage of B cells per total islet cells (B_p)
118 ± 5.7	115 ± 1.76	Islet diameter (μm)



Histogram 2. Volume density of B cells per islet (Vvb), percentage of B cells per total islet cells (Bp) and islet diameter of pancreatic islets (μm) of newborns of non-obese and obese mothers.

The pancreatic islets of newborns of obese mothers showed normal histological structure. The islets were nearly rounded and well defined. The insulin secreting cells or β -cells could be demonstrated in immunohistochemically stained sections with anti-insulin antibodies. The β -cells composed the major cell population of the islets. They occupied mainly the central zone. The anti-insulin antibody reaction was seen as brown granules occupying the cytoplasm of the β -cells (Fig. 1). The histological structure of islet of Langerhan's in newborns of obese mothers did not differ from that of newborns of non-obese mothers (Fig. 2)

Discussion

Maternal obesity may be an important factor contributing to the overall rise of Type 2 diabetes in the population. The results obtained in this study showed that the volume density of islet B cells of newborns of obese mothers did not vary significantly from that of newborns of non-obese mothers ($p > 0.05$). The B cell volume density represents the volume of B cells per islets. Subsequently, these results may suggest that maternal obesity did not affect the volume of B cells per islets in newborns.

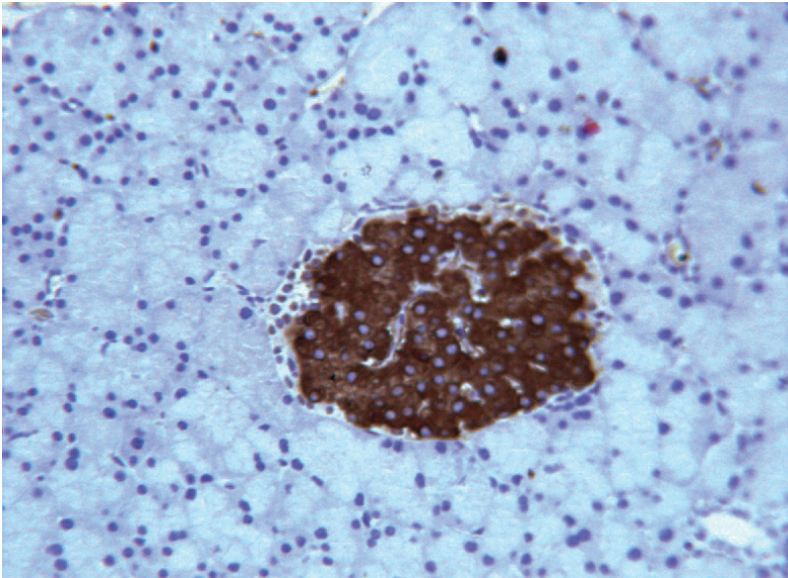


Fig. 1. Light micrograph of pancreatic islet (IS) of newborn of non-obese mothers. The islet is stained with indirect immunoperoxidase method to show B cells ($\times 400$).

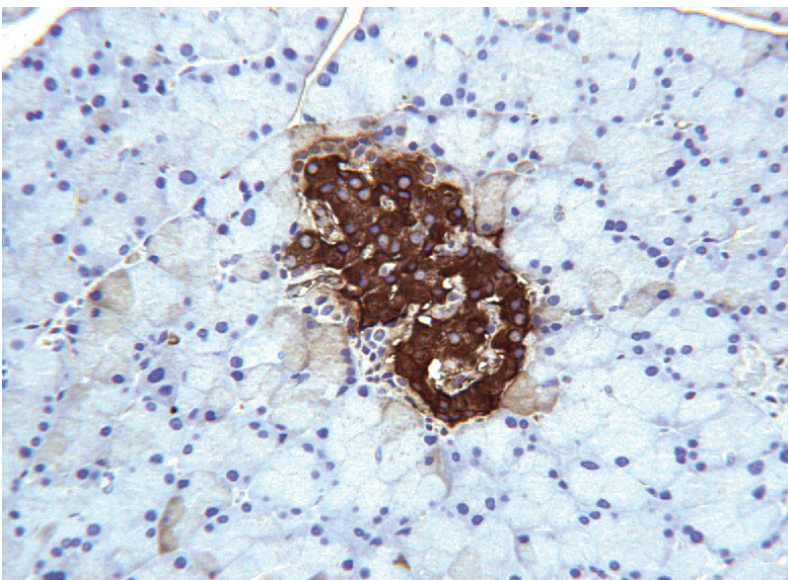


Fig. 2. Light micrograph of pancreatic islet (IS) of newborn of obese mothers. The islet is stained with indirect immunoperoxidase method to show B cells (X 400).

The percentage of B cells per total islet cells of newborns of obese mothers did not vary significantly from that of the controls ($p > 0.05$). This may also suggest that maternal obesity did not have any effect on the total number of B cells in the newborns. The islet diameter of newborns of obese mothers was not significantly different from that of the controls ($p > 0.05$). This result indicates that the total number of the islet cells, which includes B cells and other islet cell types, was not affected by maternal obesity. This also confirms the previous results in this study regarding the volume density and the percentage of B cells.

The histological structure of pancreatic islets of newborns of obese mothers was normal. The B cells were present in the central part of the islet and the islets were nearly rounded and well defined. The islet histological structure of newborn pancreatic islets of obese mothers did not differ from that of newborns of non-obese mothers. These findings indicate that obesity during pregnancy did not affect the structural integrity of newborn pancreatic islets.

The blood glucose level of newborns of obese mothers did not vary significantly from that of newborns of non-obese mothers ($p > 0.05$). This result may indicate that the endocrine pancreatic functional integrity of newborns of obese mothers is not affected by maternal obesity. This result is in agreement with the previous results in our study, which found that maternal obesity during pregnancy did not induce changes in the structure of the endocrine pancreas. However, the effect of maternal obesity on the endocrine pancreas of their offspring may appear later in life during childhood or adult period^[8]. They reported that maternal obesity during pregnancy might lead to the occurrence of diabetes mellitus in their offspring during the childhood or adult period.

In this study the serum insulin level of newborns of obese mothers was significantly higher than that of newborns of non-obese mothers ($P < 0.05$). In accordance with these results, it was reported that the serum insulin level of fetuses of obese human mothers is higher than that of fetuses of non-obese human control mothers^[4]. Another study recorded that offspring of obese mothers developed hyperinsulinemia⁽⁵⁾. The siblings of obese mothers developed chronic hyperinsulinemia and adult-onset obesity in another two studies^[6,7]. In contrary to the current results, maternal obesity has a long-term effect on the beta cells of female, but not of male offspring, and leads to increased risk of gestational diabetes and Type 2 diabetes in the offspring's later lives^[12].

The mechanisms by which maternal obesity impairs islet function in offspring are unknown. The fact that the change can be seen after 50 weeks of delivery indicates that maternal obesity induces a change in developmental programming of the islet with permanent consequences for beta-cell function^[13]. Maternal non-esterified fatty acids can cross the placenta, and placental lipoprotein lipase hydrolyses triglycerides to fatty acids that can cross the placenta^[14]. Fatty acids are known to have detrimental effects on the adult beta cell^[15,16]. So, they may affect beta cell development as well. Many studies reported that oxidative stress happens in the embryo during diabetic pregnancy and also in the placenta of obese women^[17,18]. Therefore, beta cell oxidative stress that disrupts normal programming can occur in obese pregnancy.

The increase in serum insulin level of newborns of obese mothers found in the current study may be caused by the increase in insulin production of pancreatic B cells without the increase in the total number of the B cells. It could also be caused by the increase in the total number of B cells or by both.

In the present study, the morphometric study of the islets revealed that the total number of pancreatic B cells of newborns of obese mothers was not significantly different from that of the controls. Subsequently, the increase in the serum insulin level of newborns of obese mothers is most probably caused by the increase in the insulin production by B cells, without change in their total numbers. In contrary, another study reported low serum insulin levels in offspring's of mothers on high fat diet. They said that it is unclear whether the impact of high-fat feeding was due to the need for beta cells to compensate for prolonged insulin resistance induced by high fat, or to a direct toxic effect of elevated serum lipids on the beta cell^[15,19]. So, maternal obesity must have a different mode of damage to beta cells.

In conclusion, the volume density (V_{vb}) of B cells, the percentage of B cells per total islet cells (B_p) and the diameter (Φ_i) of newborn pancreatic islets of obese mothers did not vary significantly from that of the controls. The histological structure of newborn pancreatic islets of obese mothers was normal and did not differ from that of the controls. The blood glucose level of newborns of obese mothers was not significantly different from that of the controls. However, the serum insulin level of newborns of obese mothers was significantly higher than that of the controls. Further studies concentrating on the morphometric

study of islet of Langerhans will be needed to confirm the present results, as this type of studies is very deficient.

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دراسة عن تأثير بدانة الأم على تركيب ووظيفة جزر البنكرياس في الفئران حديثة الولادة

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المستخلص . إن السمنة مشكلة ذات مخاطر كبيرة، في أمراض القلب والصدمة والسكر، والهدف من الدراسة الحالية هو دراسة تأثير بدانة الأم على الوظيفة والتركيب النسيجي لجزر البنكرياس في الفئران حديثة الولادة. تم الحصول على بنكرياس الفئران حديثة الولادة للأمهات البدينات وغير البدينات بعد يوم واحد من الولادة. صبغت الشرائح هستومناغيا للأجسام المضادة للأنسولين، وتمت قياسات على خلايا البيتا باستخدام الميكروسكوب الضوئي. وكذلك تم قياس مستويات سكر الدم والأنسولين. وقد أظهرت الدراسة أن التركيب الهستولوجي ظهر طبيعيا في جزر البنكرياس في الفئران حديثي الولادة للأمهات بدينات يشبه المجموعة الضابطة. وكذلك لم تتغير حجم الكثافة والنسبة المئوية لخلايا البيتا في إجمالي عدد خلايا جزر البنكرياس، وقطر الجزر البنكرياسية في الفئران حديثي الولادة في الأمهات البدينات بشكل واضح من مثيلتها في المجموعة الضابطة. بالنسبة لمستوى السكر بالدم، وجد أنه لا يختلف بوضوح عن المجموعة الضابطة. بينما كان الأنسولين بالسيرم في الفئران حديثي الولادة من أمهات بدينات مرتفعاً بشكل ملحوظ عنه في المجموعة الضابطة. ونستنتج من الدراسة الحالية أن بدانة الأم لم تؤثر في التركيب الهستولوجي لجزر البنكرياس، وحجم كثافة خلايا

البيتا، والنسبة المئوية لخلايا البيتا لإجمالي عدد خلايا جزر البنكرياس، وقطر الجزر البنكرياسية، ومستوى السكر بالدم، في الفئران حديثي الولادة. ومع ذلك أدت السمنة مع الحمل إلى ارتفاع الأنسولين بالسيرم في الفئران حديثي الولادة.