ORIGINAL ARTICLE

The effect of maternal diabetes on pancreatic islets in newborn rats: a quantitative and immunocytochemical study

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The effect of diabetes during pregnancy on the endocrine pancreas of the newborn has been studied before, but a detailed morphometric immunocytochemical study is not available. The aim of this study was to investigate the effect of maternal diabetes on the morphology of islets in newborn rats. Pancreatic sections were stained by the indirect immunoperoxidase method to localise the insulin-producing β-cells and were used for morphometric analysis. The islet volume density, diameter and volume and the absolute islet cell number were significantly greater in the diabetic group than in controls. The β-cell volume density was significantly lower in the diabetic group, although the β-cell nuclear diameter and volume did not differ significantly in this group. The results obtained from this investigation indicate that maternal diabetes induces islet hypertrophy in newborn offspring and causes an increase in the total islet cell number.

Key words: quantitative, β-cells. immunocytochemical

INTRODUCTION

The endocrine pancreata of foetuses and the newborn offspring of diabetic mothers were found to be affected by maternal hyperglycaemia. Maternal diabetes can lead to proliferation and hypertrophy of foetal β-cells [2, 3, 9, 14, 15] and stimulates the secretory function and hormonal synthesis of β-, α- and PP-cells [8, 12]. Glucose can stimulate the proliferation of β cells and insulin secretion in vivo and in vitro [4, 10, 11, 13, 16]. Infants of diabetic mothers show hypertrophy and proliferation of endocrine β-cells. Foetal exposure to maternal diabetes during pregnancy seems to have a long-lasting effect. The exposure of foetal rats to mild maternal diabetes induces a decrease in insulin secretion in later life [18].

Several studies of the effect of maternal diabetes on the endocrine pancreas of the newborn offspring have been found. However, no detailed morphometric immunocytochemical study has been reported. The use of immunocytochemical stain in this study would allow clear localisation of β-cells and allow the performance of an accurate morphometric study of the endocrine pancreas. The aim of this study was to examine the effect of maternal diabetes on islet volume density, diameter, cell number and β-cell volume density in the newborn. The effects of maternal diabetes on the nuclear diameter and volume in the newborn were also examined.

MATERIAL AND METHODS

Animal and tissue preparation

Adult male and female Lewis albino rats were used in this study. The rats were allowed free access

to food and water. They were kept at a constant temperature of 24°C and a light cycle of 12 h on/12 h off. Virgin female Lewis rats were made diabetic by intravenous injection of streptozotocin (Sigma, St. Louis, Mo., USA) at a dose of 50 mg/kg. Non-fasting blood glucose concentrations were measured to confirm the presence of diabetes. Diabetic female rats were mated with males and pregnancy was confirmed. The newborn rats were studied at 1 and 4 days after birth. The blood glucose concentrations of the newborn were measured. The pancreata were removed, fixed in buffered neutral formalin, dehydrated, embedded in paraplast (Sherwood Medical Co., St. Louis, Mo., USA) and sectioned serially at 5 μm. The pancreata of one-day-old and four-day-old newborn rats of non-diabetic mothers were obtained and used as controls. Six pancreata were examined per group, with a total of 24 pancreata. The sections studied were obtained from whole pancreata.

Immunocytochemical staining

Four sections, 20 sections apart were obtained from each specimen. The sections were stained by the indirect immunoperoxidase method [17] to localise the insulin-producing β-cells. 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

Between 32 and 48 islets (8–12 per section) were examined for each newborn rat. Weibel's point-counting method [20] was used to calculate the volume density of the islets per pancreatic tissue (V_{vi}) and the volume density of β cells per islet (V_{vb}). The sections were examined at a magnification of $\times$ 400 to estimate V_{vi} and at a magnification of $\times$ 1000 to estimate V_{vb}. The number of cellular profiles per test area (N_{Ac}, no./μm^2) was calculated by dividing the number of cell nuclei profiles per islet profile by the area of the islet profile area. The nuclei were counted by the direct counting method at a magnification of x 1000. The numerical density of the cells per unit volume of islet (N_{vc}, no./μm^3) was calculated by a variant of the DeHoff and Rhines formula [21]

$$N_{vc} = \frac{N_{Ac}}{\bar{D}_n + t}$$

where N_{Ac} represents the number of nuclear profiles per unit area estimated in sections of thickness t and D_n represents the mean corrected nuclear diameter. The absolute number of total cells per islet was estimated by multiplying N_{vc} by islet volume.

A graticule of a calibrated linear scale was used to measure the major (a) and minor (b), at right angles to (a), axes of the islet. The islet profile diameter was calculated from the equation $d_i = {}^2\sqrt{ab}$ [20]. The mean axial ratio of the islets was calculated. On the assumption that the islets were spheroid structures, Fullman's formula [21] was used to calculate the mean islet diameter ($\bar{D}_i$).

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

where N represents the total profiles measured. The β-cell nuclear profile diameter (d_n) was measured by similar steps. Fifty nuclei were measured per slide at a magnification of $\times$ 1000. The Abercrombie method [21] was used to calculate the corrected mean nuclear diameter ($\bar{D}_n$).

$$\bar{D}_n = d_n \times 4/\pi$$

The mean islet volume and the mean β-cell nuclear volume were calculated from the mean corrected islet diameter ($\bar{D}_i$) and mean corrected nuclear diameter ($\bar{D}_n$) [21].

$$V = 4\,\pi/3 \times (\bar{D}/2)^3$$

Statistical analysis

Student's t-test was used to analyse the data. All the statistical computations were made using the statistical packages SPSS and Excel. The difference was considered significant when $p < 0.05$.

RESULTS

The blood glucose levels of control mothers of one-day-old and four-day-old newborn rats were 83.5 $\pm$ $\pm$ 3.03 mg/dl and 91.2 $\pm$ 3.88 mg/dl respectively. The blood glucose levels of one-day-old and four-day-old control newborn rats were 97 $\pm$ 3.65 mg/dl and 88.3 $\pm$ 3.86 mg/dl respectively. The blood glucose level of diabetic mothers of one-day-old newborn rats was 415 $\pm$ 17.2 mg/dl and the blood glucose level of diabetic mothers of four-day-old newborn rats was 347 $\pm$ 14.2 mg/dl. The blood glucose level of one-day-old

Table 1. Volume density of the islets per pancreatic tissue, volume density of β-cells per islet, islet diameter, islet volume and the absolute number of total cells per islet of one-day-old control and diabetic newborn rats (n = 6)

	Control group	Diabetic group
Volume density of the islets per pancreatic tissue (V_{vi})	0.0784 ± 0.09	0.118 ± 0.01*
Volume density of β-cells per islet (V_{vb})	0.504 ± 0.015	0.35 ± 0.01*
Islet diameter [μm]	87.1 ± 1.4	104 ± 5.12*
Islet volume [μm^3]	346285 ± 16557	607832 ± 89759*
Number of total cells/islet	381 ± 24.6	953 ± 101*

Values are presented as mean ± SEM; *p < 0.05, diabetic group vs. control group

Table 2. Volume density of the islets per pancreatic tissue, volume density of β-cells per islet, islet diameter, islet volume and absolute number of total cells per islet of four-day-old control and diabetic newborn rats (n = 6)

	Control group	Diabetic group
Volume density of the islets per pancreatic tissue (V_{vi})	0.109 ± 0.008	0.172 ± 0.02*
Volume density of stained β-cells per islet (V_{vb})	0.588 ± 0.028	0.325 ± 0.013*
Islet diameter [μm]	107 ± 3.16	126 ± 2.5*
Islet volume [μm^3]	644865 ± 57511	1041130 ± 64699*
Number of total cells/islet	763 ± 105	1458 ± 66.3*

Values are presented as mean ± SEM; * p < 0.05, diabetic group vs. control group

newborn rats of diabetic mothers was 275 ± 15.8 mg/dl and the blood glucose level of four-day-old newborn rats of diabetic mothers was 113 ± 6.06. The control mothers and newborn rats were normoglycaemic. The mean islet axial ratios of one-day-old and four-day-old newborn rats were 1.24 ± 0.039 and 1.25 ± 0.042 respectively. The mean nuclear axial ratios of one-day-old and four-day-old newborn rats were 1.18 ± 0.037 and 1.15 ± 0.04 respectively.

Table 1 shows the mean volume density of the islets per pancreatic tissue (V_{vi}), the mean volume density of β-cells per islet (V_{vb}), the mean islet diameter, the mean islet volume and the mean absolute number of total cells per islet in the one-day-old control and diabetic groups. It was observed that the mean volume density of the islets per pancreatic tissue (V_{vi}), the mean islet diameter, the mean islet volume and the mean absolute number of total cells per islet of the diabetic group were significantly greater than the corresponding values of the control group (p < 0.05). The volume density of β-cells per islet (V_{vb}) of the diabetic group was significantly lower than that of the control group (p < 0.05).

The mean volume density of the islets per pancreatic tissue (V_{vi}), the mean islet diameter, the mean islet volume and the mean absolute number of total cells per islet of the four-day-old diabetic group were significantly greater than the corresponding values of the control group (p < 0.05). The volume density of β-cells per islet (V_{vb}) of the diabetic group was significantly lower than that of the control group (p < 0.05). These results are expressed in Table 2.

The mean β-cell nuclear diameter and volume of the one-day-old and four-day-old diabetic group did not vary significantly from the corresponding values

Table 3. β-cell nuclear diameter and volume of one-day-old control and diabetic newborn rats (n = 6)

	Control group	Diabetic group
Nuclear diameter [μm]	7.36 ± 0.25	7.08 ± 0.24 (NS)
Nuclear volume [μm^3]	210 ± 21.5	187 ± 19.9 (NS)

Values are presented as mean ± SEM; NS: p > 0.05, diabetic group vs. control group

Table 4. β-cell nuclear diameter and volume of four-day-old control and diabetic newborn rats (n = 6)

|	Control group	Diabetic group
Nuclear diameter [μm]	7.1 ± 0.25	7.07 ± 0.18 (NS)
Nuclear volume [μm^3]	211 ± 21.6	185 ± 13.3 (NS)

Values are presented as mean ± SEM; NS: p > 0.05, diabetic group vs. control group

of the control group (p > 0.05). These values are expressed in Tables 3 and 4.

The islets of the one-day-old and four-day-old diabetic and control groups were well defined and nearly rounded and the b-cells occupied the central part of the islet (Fig. 1–4).

DISCUSSION

The results found in this study show that the islet diameters and volumes of one-day-old and four-day-old diabetic newborn rats were significantly greater than the corresponding values of the control group. The increase in β-cells may be a mechanism to increase insulin production in response to hyperglycaemia. This may also suggest that maternal diabetes during pregnancy stimulates the hypertrophy and proliferation of foetal endocrine
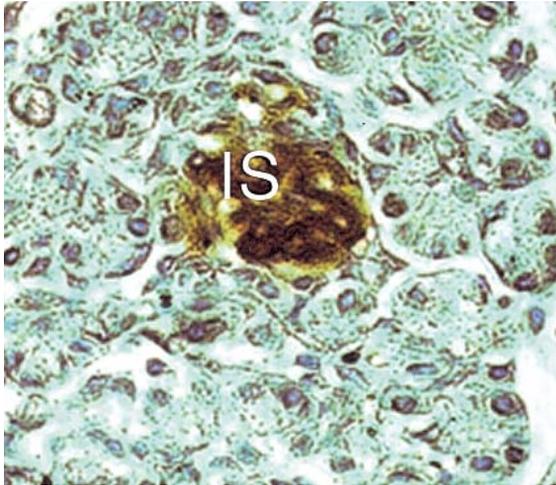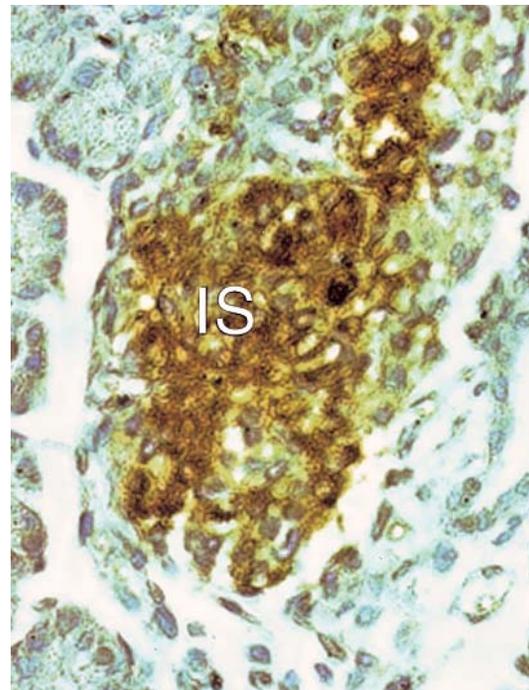
Figure 1. Light micgrograph of pancreatic islet (IS) of one-day-old control newborn rat stained with the indirect immunoperoxidase method to demonstrate insulin-producing β-cells; $\times$ 200.
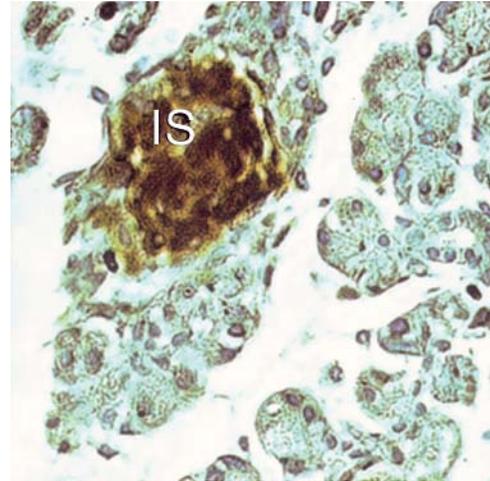
Figure 3. Light micgrograph of pancreatic islet (IS) of four-day-old control newborn stained with the indirect immunoperoxidase method to demonstrate insulin-producing β-cells; $\times$ 200.
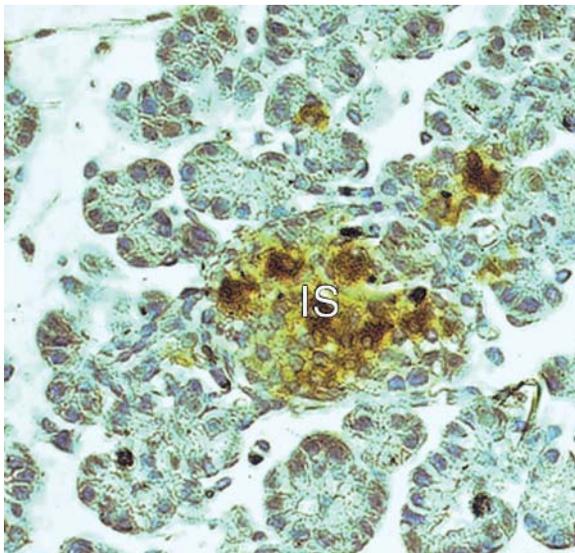
Figure 2. Light micrograph of pancreatic islet (IS) of one-day-old diabetic newborn rat stained with indirect immunoperoxidase method to demonstrate insulin-producing β-cells; $\times$ 200.

pancreas and this effect continues to appear in the early days after birth. Previous studies have reported similar findings. Aerts and Van Assche [2] reported that foetuses and newborn rats of diabetic mothers showed islet hypertrophy and β-cell hyperplasia. They also reported that foetal β-cells of diabetic mothers showed degranulation. Milner et al. [13] reported that maternal diabetes during pregnancy stimulated the proliferation of foetal islet β-, α- and PP-cells. They added that foetal hyperglycaemia has a trophic effect on the stem cells, which develop into β-, α- or PP-cells. Kaung [11] reported that glu-

Figure 4. Light micrograph of pancreatic islet (IS) of four-day-old diabetic newborn stained with the indirect immunoperoxidase method to demonstrate insulin-producing β-cells; $\times$ 200.

cose stimulates foetal and neonatal β-cell proliferation in organic culture. Reusens-Billen et al. [14] found that diabetes during pregnancy in rats causes foetal and newborn β-cell hyperplasia and hypertrophy in vivo and in vitro. Badawoud [3] reported that maternal diabetes during pregnancy causes an increase in foetal islet diameter and volume. To prevent any effect of streptozotocin on foetal endo-

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crine pancreas the drug was injected one week before mating.

The islet volume density, which represents the total amount of pancreatic endocrine tissue in one-day-old and four-day-old diabetic newborn rats was significantly greater than that of the control group. This finding may suggest that maternal diabetes stimulates pancreatic endocrine tissue proliferation. It may also suggest that the endocrine pancreas of diabetic foetuses starts to appear earlier than that of the control group. This result is in agreement with that of Aerts and Van Assche [2], Fu et al. [7], Gollin et al. [8] and Badawoud [3]. The β-cell volume density of one-day-old and four-day-old diabetic newborn rats was significantly lower than that of the control group. This result may be caused by the increase in islet volume while the β-cell number remained unchanged. It may also be caused by degranulation of islet β-cells due to hyperglycaemia. These findings are in agreement with those of Aerts and Van Assche [2], Eriksson and Swenne [5], Verhaeghe et al. [19], Abdel-Rahman et al. [1] and Fu et al. [7].

The absolute number of total cells per islet of one-day-old and four-day-old diabetic newborns was significantly greater than that of the control group. This is in agreement with the increase in islet volume and diameter of the diabetic group.

The islet volume density, islet volume and number of islet cells were increased markedly from 1 to 4 days in both the control and the diabetic groups. These results are in agreement with those of Freie et al. [6]. They found that the islet mass, β-cell number index and islet density showed a marked increase and were nearly doubled from day 1 to day 5 in newborn rats. The growth rate of the pancreatic acini decreases in the last period of gestation and during the newborn period, while islet growth continues at a high rate during these periods [6]. This could explain the marked increase in islet volume density, islet volume and the number of islet cells found in this study.

The β-cell nuclear volume and diameter of one-day-old and four-day-old diabetic newborns were not significantly different from those of the control group. It seems that maternal diabetes does not have any effect on β-cell nuclear volume or diameter.

In conclusion, the results found in this study may suggest that maternal diabetes induces newborn islet hypertrophy and causes an increase in total islet cell number. They further demonstrate that maternal diabetes does not seem to affect the nuclear diameter or volume of β-cells.

REFERENCES

1. Abdel-Rahman M, Elrakhawy F, Iskander F (1992) Endocrine pancreas in the postnatal offspring of alloxan diabetic rats. Toxicol Lett, 62 (2–3): 263–274.
2. Aerts L, Van Assche F (1977) Rat fetal endocrine pancreas in experimental diabetes. J Endocrinol, 73: 339–346.
3. Badawoud M (2003) Immunohistochemical and morphometric study of the effect of maternal diabetes on rat foetal pancreatic islets. Folia Morphol, 62: 19–24.
4. Bernard C, Berthault M, Saulnier C, Ktorza A (1999) Neogenesis vs. apoptosis as main components of pancreatic beta cell mass changes in glucose-infused normal and mildly diabetic adult rats. FASEB J, 13: 1195–1205.
5. Eriksson U, Swenne I (1982) Diabetes in pregnancy: growth of the fetal pancreatic B cells in the rat. Biol Neonate, 42 (5–6): 239–248.
6. Freie H, Pasma A, Bouman P (1975) Quantitative analysis of pancreatic islet development and insulin storage in the foetal and newborns rat. Acta Endocrinologica, 80: 657–666.
7. Fu Q, Honda M, Ohgawara H, Igarashi N, Toyada C, Omori Y, Kobayashi M (1996) Morphological analysis of pancreatic endocrine cells in newborn animals delivered by experimental diabetic rats. Diabetes Res Clin Pract, 31 (1–3): 57–62.
8. Gollin Y, Gracia C, Gollin G, Marks C, Marks W, Papandonatos G (1999) Effect of maternal diabetes on the fetal exocrine pancreas. Early Hum Dev, 53: 179–183.
9. Hellerstrom C, Swenne I (1991) Functional maturation and proliferation of fetal pancreatic beta-cells. Diabetes, 40 (Suppl 2): 89–93.
10. Hue-Lee C (1983) Effect of glucose on beta cell proliferation size in organic culture of fetal and neonatal rat pancreases. J Embryol Exp Morph, 74: 303–312.
11. Kaung H (1983) Effect of glucose on beta cell proliferation and population size in organ culture of foetal and neonatal rat pancreases. J Embryol Exp Morphol, 75: 303–312.
12. Li L, Wang Y, Feng Y (1999) Influences of gestational hyperglycemia on the function of fetal rat pancreatic islets. Zhonghua Fu Chan Ke Za Zhi, 34: 525–527.
13. Milner R, Wirdnam P, Tsanakas J (1981) Quantitative morphology of B, A, D, and PP cells in infants of diabetic mothers. Diabetes, 30: 271–274.
14. Reusens-Billen B, Remacle C, Daniline J, Hoet J (1983) Cell proliferation in pancreatic islets of rat fetuses and neonates from normal and diabetic mothers. An in vitro and in vivo study. Horm Metab Res, 16: 565–571.
15. Salvesen D, Brudenell J, Proudler A, Crook D, Nicolaides K (1993) Fetal pancreatic beta-cell function in pregnancies complicated by maternal diabetes mellitus: relationship to fetal acidemia and macrosomia. Am J Obstet Gynecol, 168: 1363–1369.
16. Scharfmann R, Basmaciogullari A, Czernichow P (1990) Effect of growth hormone and glucose on rat islet cells replication using 5-bromo-2-deoxyuridine incorporation. Diabetes Res, 15: 137–141.
17. Sternberger L (1979) The unlabelled antibody peroxidase-antiperoxidase (PAP) method. In: Cohen S, McClusky RT ed. Immunocytochemistry, John Wiley and Sons, New York, pp: 104–169.
18. Van Assche F, Aerts L, Holemans K (1991) The effects of maternal diabetes on the offspring. Baillieres Clin Obstet Gynaecol, 5: 485–492.
19. Verhaeghe J, Peeters T, Vandeputte M, Rombauts W, Bouillon R, Van Assche F (1989) Maternal and fetal endocrine pancreas in the spontaneously diabetic BB rat. Biol Neonate, 55 (4–5): 298–308.
20. Weibel E (1963) Principles and methods for the morphometric study of the lung and other organs. Lab Invest, 12: 131–155.
21. Williams M (1977) Quantitative methods in Biology. In: Glauert AM ed. Practical methods in electron microscopy, Vol. 6. North-Holland Publishing Company, Amsterdam, pp: 48–62.