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Diabetes in Pregnancy: A Rat Model for the Study of Fetal Complications

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INTRODUCTION

Previous studies have shown a decreased body weight and a greatly reduced pancreatic insulin content in the offspring of manifest diabetic rat mothers (1). These findings differ from those in human diabetic fetopathia which is characterized by an increased body weight, elevated levels of circulating insulin and an increased fetal pancreatic insulin reserve (2). It is nevertheless clear, that manifest diabetes in the pregnant rat gives rise to a variety of disturbances in fetal development. These alterations include not only those changes mentioned above, but also an arrest, rather than a stimulation of the pancreatic B-cell growth (3). The present study evaluates to what extent the disturbed development in the offspring can be prevented by insulin treatment of the mother during pregnancy.

METHODS

Diabetes was induced by a single i.v. injection of 40-50 mg streptozotocin (SZ) per kg body weight (SZ was a gift from Dr. W. Dulin, The Upjohn Co., Kalamazoo, U.S.A.) two weeks prior to mating in about 200 virgin female Sprague--Dawley rats (Anticimex AB, Sollentuna, Sweden). At the time of the experiment the animals were 3 months old, with an average body weight of 250 g. The drug at a concentration of 60 mg/ml, was dissolved in 0.01 M citrate buffer (pH 4.5) immediately before injection. All animals were allowed free access to tap water and laboratory chow (Ewos AB, Södertälje, Sweden) throughout the course of the experiment. Control rats (N) were not injected. The diabetic rats (exhibiting non-fasting serum glucose > 20 mmol/l one week after the SZ injection) were divided into a manifest diabetic (MD) and an insulin-treated (MDI) group. The latter animals were given 2-8 IU Novo Ultralente Insulin (Novo A/S, Copenhagen, Denmark) in one s.c. daily dose. The insulin treatment commenced one week after the SZ injection and was continued throughout the experiment. The non-fasting serum glucose concentration was determined twice a week using a Beckman Glucose Analyzer 2, (Beckman, Fullerton, U.S.A.). Mating was commenced two weeks after the SZ injection and the day of a sperm-containing vaginal smear was considered as day 0 of gestation. Pregnancy was interrupted by Caesarian section on day 20. Serum and pancreatic insulin determinations were performed according to Heding (4). Further details concerning the experimental protocol have been previously described (1). Probabilities (p) of chance differences between groups were calculated according to Student's two-tailed t-test.

RESULTS AND DISCUSSION

The serum glucose values during pregnancy in the three experimental groups are shown in Figure 1. In the MDI rats, the insulin dose was adjusted so that the glucose levels were lowered, without causing hypoglycemia, resulting in blood glucose concentrations within the range 4 - 14 mmol/l. With this regimen, the average insulin dose was about 5 IU/day throughout pregnancy. Towards the end of gestation, the serum glucose levels in the N and MDI groups decreased (Figure 1, $p < 0.001$ for day 5 vs. day 20 in both groups), whereas the opposite trend was found in the MD group ($p < 0.001$ for day 5 vs. day 20). The lowered levels of serum glucose in the MDI and N groups correspond to those reported in late human diabetic (5,6) and non-diabetic (7,8) pregnancy. An increased fetal-placental uptake of glucose might, at least partly, explain these findings (5). In the MD rats, the precise reason for the sustained rise in maternal serum glucose throughout pregnancy (cf. Figure 1) remains unclear.

Although complete normalization of the maternal glucose levels in the MDI group was neither achieved, nor attempted, the insulin treatment caused a significant increase in both the fetal body weight and the maternal weight gain (Table 1) compared to the MD group.

Table 1. *Maternal weight gain up to day 20 of pregnancy, number of viable fetuses and their body weights at gestational day 20 in normal (N), manifest diabetic (MD) and insulin-treated diabetic (MDI) pregnancies. Figures in parentheses denote the numbers of rat mothers or numbers of litters, respectively. Means ± S.E.M. a) = $p < 0.01$ vs. N, b) = $p < 0.01$ vs. MD.*

	maternal weight gain (g)		number of viable fetuses/litter		fetal body weight (g)	
N	131 ± 4	(41)	13.5 ± 0.4	(65)	3.5 ± 0.1	(41)
MD	66 ± 5	(41)[a]	9.4 ± 0.5	(44)[a]	2.6 ± 0.1	(24)[a]
MDI	114 ± 5	(36)[ab]	11.0 ± 0.7	(23)[a]	2.9 ± 0.05	(23)[ab]

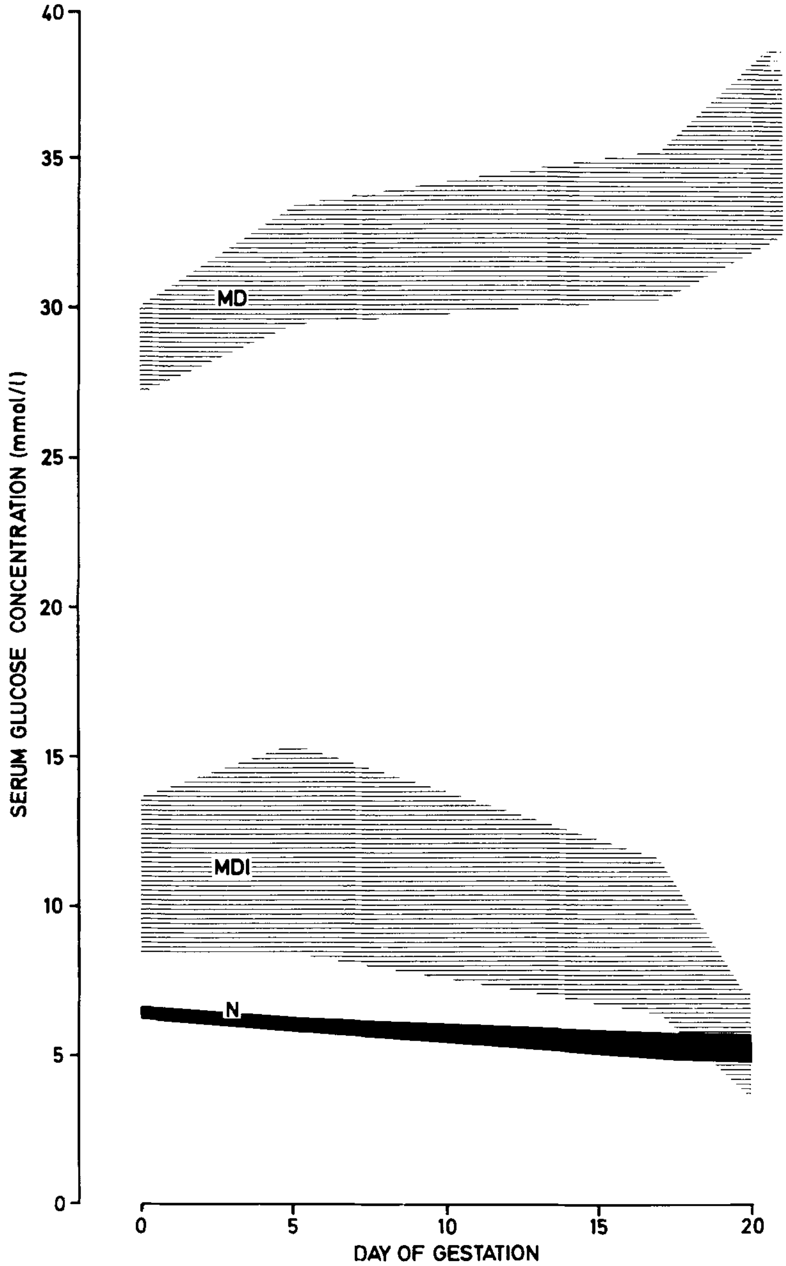

Figure 1. *Non-fasting morning serum glucose concentrations of non-pregnant (denoted as day 0) and pregnant rats at gestational days 5, 17 and 20. The values are expressed as 95% confidence intervals. The number of glucose determinations in the normal (N, lower interval) group varied between 34-41 per time point. In the manifest diabetic (MD, upper interval) and insulin-treated diabetic (MDI, middle interval) groups the ranges were 28-72 and 30-37 observations per time point, respectively.*

A trend towards increased numbers of viable fetuses per litter in the MDI compared to the MD group was also evident although the probability for a chance difference was still high ($0.05 < p < 0.1$). On the other hand, the N animals exhibited significantly increased values compared to both the MD and MDI groups regarding both fetal weight and viability and the growth of the mother (Table 1). As seen in Table 3, this was true also for fetal pancreatic weight, which tended to be normalized by insulin treatment.

Table 2. *Serum concentrations of glucose and insulin in the offspring of normal (N), manifest diabetic (MD) and insulin-treated diabetic (MDI) rats at gestational day 20. Figures in parentheses denote number of litters. Means ± S.E.M. a) = p < 0.01 vs. N, b) = p < 0.05 vs. MD.*

	serum glucose (mmol/l)		serum insulin (ng/ml)		glucose/insulin ratio
N	1.7 ± 0.1	(52)	10.8 ± 1.1	(14)	0.2
MD	27.5 ± 1.1	(33)[a]	8.4 ± 1.1	(13)	3.3
MDI	3.7 ± 1.3	(15)[ab]	12.2 ± 2.7	(17)	0.3

In Table 2 the concentrations, in fetal serum, of glucose and insulin are also shown. The MD offspring exhibited grossly abnormal glucose values on gestational day 20, greatly exceeding both the N and MDI groups with the values of the latter group being slightly higher than the normal group. On the other hand, no differences could be demonstrated between the serum insulin levels of the three groups on gestational day 20. Therefore, the increased glucose-to-insulin ratio in the MD group was almost normalized in the MDI offspring. Since insulin is not particularly antigenic in the rat and the duration of the insulin treatment was less than 8 weeks, the influence of maternal antibodies on the serum insulin determinations was considered negligable. As we have shown previously (1), the impact of manifest maternal diabetes on the pancreatic accumulation of insulin resulted in a marked decrease of both the concentration in the pancreas and the total insulin content of the MD fetal gland (Table 3). The insulin treatment normalized both the concentration and the content of fetal pancreatic insulin in the MDI group.

Separate studies have indicated a decreased B-cell volume in the MD group, which may at least partly explain the decreased pancreatic insulin content (3). The finding of a diminished B-cell growth together with a retarded somatic growth in the MD fetuses suggests a major disturbance of intrauterine supply or utilization of nutrients in the fetus. Although this idea may look surprising in view of the greatly increased fetal blood glucose levels, it could suggest either that a primary B-cell deficiency prevented the utilization of glucose at the cellular level or that other nutrients, essential for growth, were lacking. At any rate, however, the insulin treatment rectified many of these disturbances.

Table 3. *Pancreatic weights and accumulation of insulin in the pancreas of the offspring of normal (N), manifest diabetic (MD) and insulin-treated diabetic (MDI) rats at gestational day 20. Figures in parentheses denote number of litters. Means $\pm$ S.E.M. a) = $p < 0.01$ vs. N, b) = $p < 0.05$ vs. MD.*

	pancreatic weight (mg)		pancreatic insulin concentration (ng/mg)		pancreatic insulin content (ng/pancreas)	
N	14.7 $\pm$ 0.4	(27)	72 $\pm$ 7	(27)	1029 $\pm$ 98	(27)
MD	10.9 $\pm$ 0.5	$(15)^{a}$	27 $\pm$ 5	$(15)^{a}$	306 $\pm$ 56	$(15)^{a}$
MDI	12.7 $\pm$ 0.5	$(15)^{ab}$	69 $\pm$ 6	$(15)^{b}$	870 $\pm$ 81	$(15)^{b}$

The failure of the rat fetus to develop signs of hyperinsulinism and macrosomia does not seem to be related to the degree of maternal hyperglycemia during pregnancy. Thus neither severely diabetic, nor mildly hyperglycemic rats, as depicted in the present and a previous study (1), produced any offspring with this ailment. Although the precise reason for this remains to be clarified, it is suggested that the relatively short gestation in the rat is not compatible with the development of typical diabetic fetopathia.

This hypothesis is supported by recent reports of early growth retardation in human diabetic pregnancy (9). Therefore the offspring of diabetic rats may serve as a model for a study of early human fetal development in a diabetic environment. This animal model may also provide a tool to investigate other fetal disturbances in diabetic pregnancy, e.g. congenital malformations (10) and delayed pulmonary maturation (11).

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