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The Offspring of the Female Diabetic "Nonobese Diabetic" (NOD) Mouse Are Large for Gestational Age and Have Elevated Pancreatic Insulin Content: A New Animal Model of Human Diabetic Pregnancy (42481)

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Abstract. Pregnancy in diabetic mothers is associated with intrauterine death, perinatal mortality, and birth weight greater than that of infants born of normal mothers. The use of rodents made diabetic by alloxan or streptozotocin as an animal model for human diabetic pregnancy has been controversial because of the severity of the diabetes as well as the direct effect of diabetogenic drugs on the developing organism. Among our female NOD (nonobese diabetic) mice, insulin-dependent diabetes occurs spontaneously in 9% by 12 weeks and in 80% by 29 weeks of age. Offspring born within 21 days of conception to mildly hyperglycemic NOD pregnant mice between 26 and 52 weeks of age, and prior to the onset of maternal ketonuria are macrosomic with an average of 31% increase in body weight and 44% increase in kidney weight, in comparison to controls. Besides organomegaly, the macrosomic offspring have significantly higher pancreatic insulin content which was elevated 80% when compared with that of controls, and litter sizes are significantly 50% smaller. These results suggest that the mildly hyperglycemic pregnant NOD mouse represents a promising model for the study of pregnancy complicated by diabetes.

Macrosomia and associated fetopathy are reported to occur in up to 40% of all human maternal diabetic pregnancies (1–4). However macrosomia is defined, population samples of infants of diabetic mothers above the 90th percentile for weight demonstrate increased morbidity and mortality. Unexplained death *in utero,* severe congenital anomalies, birth trauma, hypertrophic cardiomyopathy, vascular thrombosis, and neonatal hypoglycemia contribute to the excess morbidity and mortality. For these reasons, inquiries into the nature and causes of macrosomia are important in the hope that methods of prevention can be applied.

The use of rodents made diabetic by alloxan or streptozotocin prior to conception in attempts to create experimental models of human diabetic pregnancy has led to conflicting and controversial results (5–9). The BB rat is an adequately characterized rodent model of human insulin-dependent diabetes, but the offspring of diabetic BB dams have significantly lower birth weights in comparison to control rats and often display congenital abnormalities (10). The observation reported here that macrosomia occurs spontaneously and reliably in the offspring of the female diabetic NOD mouse is, therefore, of special interest.

Materials and Methods. The Sansum NOD (nonobese diabetic) mouse colony was established in 1985 from five breeding pairs shipped from Clea Japan Inc. (Tokyo, Japan) by brother–sister inbreeding, and comprises on the average 300 mice. Macrosomic offspring were born within 21 days of conception to NOD pregnant mice between 26 and 52 weeks of age prior to onset of maternal ketonuria and insulin dependency. Random postpartum maternal blood glucose levels immediately after birth were elevated when compared to controls (187 ± 5 mg/dl versus 145 ± 8 mg/dl, $P < 0.001$, $n = 10$), and this status was defined as mildly hyperglycemic. Offspring of young normoglycemic (blood glucose less than 145 mg/dl) NOD mice between 10 and 12 weeks of age were used as controls.

Liveborn offspring were taken for study immediately after birth before nursing or cannibalism could take place. Body, heart, and kidney weights were measured. The livers of the macrosomic pups also appeared enlarged, but could not be reliably excised. The pancreata were carefully dissected by teasing them away from the spleen and the greater curvature

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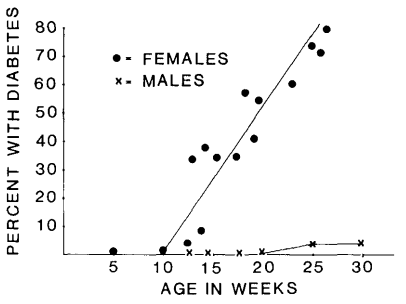

FIG. 1. Frequency of spontaneous diabetes (onset of glycosuria) in cohorts (12 to 20 mice) of female (0) (n = 267) and male (x) (n = 80) NOD mice.

of the stomach, followed by blunt dissection around the second portion of the duodenum and were placed in ice-cold (4°C) acid ethanol for insulin extraction. After 24 hr, insulin was determined by radioimmunoassay using rat insulin as standard (11). Total insulin was expressed in micrograms per pancreas. Plasma glucose was determined by a glucose oxidase method (Beckman glucose analyzer). All data were expressed as means ± SD. Student's unpaired t test was used for comparison of data.

Results. Insulin-dependent ketosis prone diabetes is observed among 9% of the female mice at about 12 weeks of age, and thereafter the number of mice with overt diabetes increases. The cumulative incidence reaches approximately 80% at 29 weeks of age. On the other hand, less than 10% of the males become diabetic by the age of 30 weeks (Fig. 1). The prevalence of diabetes in the Sansum NOD mouse strain is comparable to other NOD mouse strains reported in the literature (12, 13).

Figure 2 is a photograph of a pup born of a mildly hyperglycemic mouse compared to a pup born of a normoglycemic mouse. In litters from mildly hyperglycemic mice, all the pups were macrosomic and litter sizes were significantly smaller than in the control mice (5.4 ± 1.5, n = 8) versus 10.9 ± 2.2 (n = 9, P < 0.001). As shown in Table I, the infants of the mildly hyperglycemic NOD mice were 31% heavier than the controls. There was no significant difference in the weight of the heart between the two groups and consequently the heart-to-body weight was significantly lower in the macrosomic pups. The weight of the kidneys was significantly higher in the macrosomic newborns than in the controls, whereas the difference between the kidney-to-body weight ratios was not statistically significant.

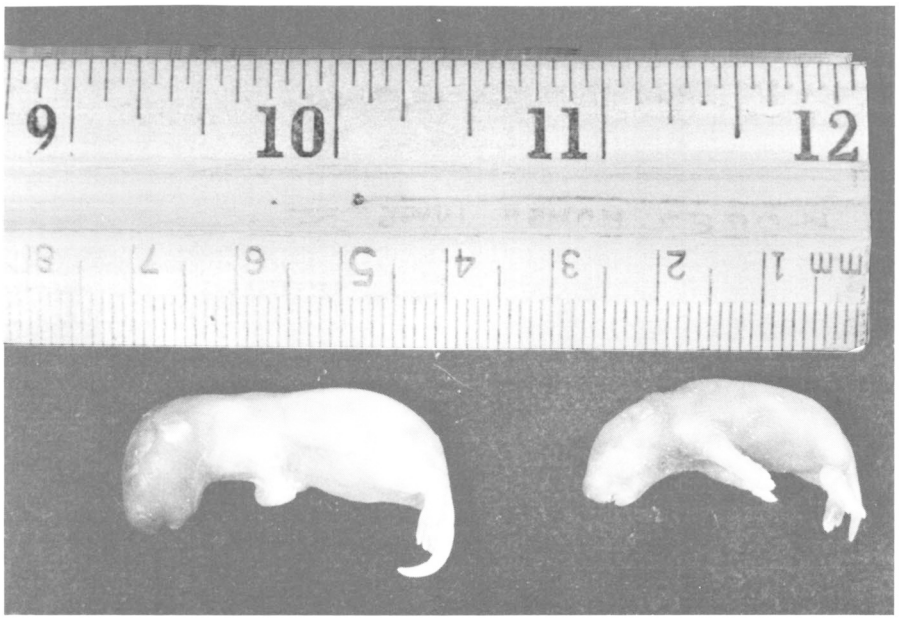

FIG. 2. Photograph showing a pup born of a mildly hyperglycemic mouse compared to a pup born of a normoglycemic mouse. Both pups are less than 1 hr old.

TABLE I. POSTPARTUM PROFILES OF MACROSOMIC NEWBORN OF MILDLY HYPERGLYCEMIC NOD MICE

	Total body wt (g)	Total heart wt (mg)	Relative heart wt (mg/g of body wt)	Total kidney wt (mg)	Relative kidney wt (mg/g of body wt)	Total pancreas insulin content (μg/ pancreas)	Relative pancreas insulin content (μg/g of body wt)
Macrosomics	1.82 ± 0.09	16.26 ± 4.09	9.02 ± 2.33	18.94 ± 2.28	10.44 ± 0.97	1.28 ± 0.29	0.72 ± 0.14
Controls	1.37 ± 0.08	16.45 ± 2.86	11.98 ± 1.69	13.17 ± 1.68	9.56 ± 1.27	0.71 ± 0.04	0.53 ± 0.05
p	<0.0001	NS	<0.005	<0.001	NS	<0.001	<0.001
n	19	19	19	19	19	14	14

Both total pancreatic insulin content and pancreatic insulin content per gram of body weight were significantly higher in the macrosomic offspring than in the controls.

Discussion. The classic criteria characterizing diabetic fetopathy in human beings include increased birth weight, adiposity, organomegaly, increased pancreatic insulin content, and hyperinsulinemia, accompanied by hypoglycemia (14–18). The precise pathogenesis of this fetal macrosomia is relatively unknown, partly because of the lack of satisfactory animal models.

The present study documents that the NOD mouse provides a useful model and that macrosomia of the offspring of mildly hyperglycemic mothers is accompanied by specific organomegaly and elevated pancreatic insulin content. Other similarities between this animal model and the macrosomia occurring in the infants of the diabetic woman include the fact that macrosomic offspring were born to older animals which were mildly hyperglycemic and all eventually developed diabetes and ketosis with increasing age.

Of interest is the fact that heart weight was not increased in the macrosomic offspring. Studies in human beings have confirmed cardiomegaly in macrosomic infants of diabetic women (15–18). Further pathological studies of the heart are warranted in this model, especially in view of the fact that a decrease (as reported here) in litter size is considered a form of congenital malformation in rodents (10). By contrast, the increased kidney weights seen in the macrosomic offspring appears to reflect a compensatory response appropriate to the increase in body mass.

Further studies are necessary to determine whether this unique rodent model parallels the human condition in terms of other congenital anomalies, hypoglycemia, hypocalcemia, hypomagnesemia, hyperbilirubinemia, erythremia, body composition, respiratory problems, or fetal wastage.

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