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Letter to the Editor

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Department of Pediatrics "A" and Medical Genetic Unit, Sackler School of Medicine, Tel Aviv University and Beilinson Medical Center, Petah Tikva Israel (RS) and Neonatal and Pediatric Medicine Branch, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland USA (J.E.G. and R.E.V.)

Cuezva *et al.* (1) in a very comprehensive report described the effects of streptozotocin-induced maternal diabetes on alterations in the fetal plasma concentrations of glucose and insulin as well as many other parameters. According to this report "...the plasma insulin concentrations were significantly higher in the two groups of diabetic rats than in the normal pups at birth" and furthermore plasma insulin concentrations in the newborns of the diabetic mothers remained significantly higher than in the age-matched normal pups during 4 h of study postnatally. Recently we looked at the insulin receptors in the developing fetal lung (3) and conducted a comparative study of insulin receptors in the developing fetal lung of normal and streptozotocin induced diabetic pregnancies in rats (4, 5) (60 mg/kg streptozotocin injected intraperitoneally at 7 days of gestation). We measured plasma glucose and insulin concentrations during pregnancy period, birth and 10 days and 3 months after delivery. The relevant data are shown in Table 1. Our data as emerged from these experiments (4) oppose in a way Cuezva *et al.* (1) findings and add to the perplexity of drawing a parallel between newborn of human diabetic mother to streptozotocin-induced diabetic rats. In our experience fetal body weight was almost identical in the control and diabetic pregnancies

Table 1. Maternal, fetal, and newborn serum glucose and insulin concentration in streptozotocin-treated and control rats

	Control ¹	Streptozotocin-treated
Maternal²		
Blood glucose (mM)	5.4 ± 0.5	32.1 ± 2.8
Insulin (ng/ml)	1.2 ± 0.1	0.2 ± 0.07
Fetal³		
Blood glucose (mM)	2.2 ± 0.1	20.8 ± 2.2
Insulin (ng/ml)	2.5 ± 0.2	2.7 ± 0.3
Newborn⁴		
Blood glucose (mM)	4.8 ± 0.4	4.9 ± 0.4
Insulin (ng/ml)	1.5 ± 0.1	1.7 ± 0.2

¹ The results are the means ± SE of the number of examinees in each group.

² Ten pregnant rats and 14 streptozotocin-induced diabetic rats were compared at 21 days of gestational age.

³ Fetuses at 21 days of gestation were c-sectioned and 12 litters in control group vs 14 litters in experimental group compared.

⁴ At age of 10 days of life insulin and glucose compared in 4 litters in each group.

3.05 ± 0.6 versus 4.05 ± 0.5 g, respectively. The fetal lung net weights for 12 litters in the control group and 14 litters in diabetes group were 110 ± 52 versus 105 ± 27 mg, respectively. Our results are similar to those reported by Kervran *et al.* (2) who found that

the rat fetuses from severely diabetic pregnancies were incapable of producing insulin at level commensurate with the high blood glucose level. The same patterns of behavior was observed between the two groups in a prolonged postnatal follow-up of 3 months. Once again we looked for differences in the plasma concentrations of insulin and glucose. The results were almost identical: in the control group at age of 3 months glucose level was 5.05 ± 0.22 versus 5.44 ± 0.27 mM for streptozotocin-treated group of newborn. Insulin levels were 1.2 ± 0.2 ng/ml versus 1.1 ± 0.2 ng/ml for control and diabetic group, respectively. Our findings imply that there was no functional damage to the fetal pancreas as confirmed histologically and immunocytochemically study of fetal pancreases by Cuezva *et al.* (1). Unfortunately, our findings add some more confusion to the already existing conflicting data and call for further investigations on the streptozotocin induced maternal diabetes in rats and the newborn of the diabetic rats in

order to come to a better model which will be as similar as possible to the human infants of diabetic mothers.

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Response

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As discussed in our recent publication (1), there are conflicting reports on the effects of chemically induced maternal diabetes in rats on newborn weight and concentrations of plasma and pancreatic insulin. The use of experimental protocols differing in important details as: (i) the dose of streptozotocin or alloxan administered to pregnant rats; (ii) the time after the onset of pregnancy when the agent is administered; (iii) the careful documentation of hyperglycemia throughout gestation; (iv) the timing of delivery; (v) litter size differences; and (vi) insulin treatment schedules have made it difficult to compare data derived from various laboratories.

We determined the effects of streptozotocin on maternal diabetes at varying dosages (*i.e.*, 30–70 mg/kg body weight). A dose of 30 mg/kg was not effective in inducing maternal diabetes in our hands. Streptozotocin in doses of 60–70 mg/kg produced hyperglycemia (mean of 22 mM glucose) but resulted in a high maternal mortality before day 16 of gestation from diabetic ketoacidosis. Therefore we settled on a dose of 45 mg/kg of streptozotocin administered on day 5 of gestation. In contrast, Steiner *et al.* (2) injected 60 mg/kg of streptozotocin in pregnant rats on day 7 of gestation. The mean blood glucose in their animals was 32.1 ± 2.8 mM (576 mg/dl). How many of their animals were in mild ketoacidosis? They provide no data on the effects of such severe hyperglycemia on maternal weight gain during gestation or on fetal weights on different days of gestation. They found no differences in body weights of 21-day-old fetuses of diabetic compared to normal rats (4.05 ± 0.5 g versus 3.05 ± 0.6 g). The body weights of the 21-day-old fetuses of normal rats in their study appear to be very low to us and may reflect variability in the actual duration of gestation in their animals. Furthermore, the mean body weight of a litter depends upon the number of fetuses in the litter. In our study the mean body weight of 20-day-old fetuses of normal rats was 2.70 ± 0.12 g. On day 22 of gestation our normal newborns had a mean weight of 5.50 ± 0.06 g compared with 5.25 ± 0.11 g ($P < 0.05$) for the untreated diabetic offspring (1). Our finding of a reduction in the mean body weight

of fetuses of untreated diabetic rats is consistent with previous reports (3–5). Severe maternal diabetes in rats significantly increases the length of gestation and hence influences the body weight of the newborns at the time of natural delivery (6). Untreated diabetic pregnant rats do not resemble the human condition. We found that the body weights of the newborns of insulin treated diabetic rats was significantly higher than that of the offspring of normal rats. This finding mimics the human condition. Steiner *et al.* (2) did not study insulin treated animals.

Our objective was to measure the hormonal and metabolic changes in the immediate postnatal period, *i.e.*, from delivery to 6 h of age. Steiner *et al.* (2) measured insulin concentrations at one time only on day 21 of gestation. Plasma insulin concentrations in the fetuses of normal rats in our study decreased from 188.3 ± 29.6 μ U/ml on day 21 of gestation to 59.9 ± 5.7 μ U/ml on day 22 of gestation (Table 1). This observed decline in the concentration of plasma insulin concentrations in normal fetuses on day 22 of gestation confirms the reports of others (7, 8). The plasma insulin concentrations measured in 21- and 22-day-old fetuses of untreated and insulin treated diabetic rats are shown in Table 1. In contrast to our findings, the plasma insulin concentrations in the 21-day-old fetuses of normal and diabetic rat were 60.0 ± 4.8 and 64.8 ± 7.2 μ U/ml in the study of Steiner *et al.* (2). They did not measure the decline in plasma insulin concentrations during the early postnatal period.

Table 1. Effect of maternal hyperglycemia on fetal glucose and insulin concentrations in fetuses during day 21 and 22 of gestation.¹

Mother's status	Gestational age (days)	Fetus	
		Plasma glucose (mM)	Plasma insulin (μ U/ml)
Normal	21	2.57 ± 0.19	188.3 ± 29.6
Untreated diabetic	21	17.40 ± 0.54^4	116.0 ± 15.4^2
Insulin treated diabetic	21	2.35 ± 0.13	153.1 ± 11.9
Normal	22	3.94 ± 0.33	59.9 ± 5.7
Untreated diabetic	22	20.00 ± 0.49^4	103.8 ± 5.1^4
Insulin treated diabetic	22	2.46 ± 0.22^3	139.2 ± 9.2^4

¹ The results are the means $\pm$ S.E. for fetuses from three to six mothers.

² $P < 0.01$ compared with normal.

³ $P < 0.005$ compared with normal.

⁴ $P < 0.001$ compared with normal.

Steinherz *et al.* (2) present insulin concentrations measured in the offspring of diabetic rats at 10 days of life. They find no differences in plasma insulin concentrations between the offspring of normal and diabetic rats. In another study, we recently reported that the concentrations of plasma glucose and insulin in 1-day-old newborns of untreated diabetic rats were undistinguishable from those of age-matched pups of normal rats (9, 10). Furthermore, plasma glucose and insulin did not vary significantly in the offspring of diabetic and normal rats 7 and 80 days after delivery. The findings of Steinherz *et al.* (2) at 10 days of life are consistent with these findings; however, the studies presented in reference 1 measured hormonal and metabolic changes in the newborns of untreated and insulin treated pregnant diabetic rats during the first 6 postnatal h of life. Steinherz *et al.* did a different study with other objectives and their data cannot be compared to ours.

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