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Fetal Brain Development in Diabetic Guinea Pigs

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Summary

Fetal brain development was investigated near term in guinea pigs rendered diabetic with streptozotocin. The liver and the placenta were used as reference organs. Compared to controls, those fetuses from diabetic animals had normal cerebrum and cerebellum weights, but higher liver and placenta weights in relation to fetal weights. Although liver and placenta cell number (DNA content) was unchanged, it was significantly increased in the fetal cerebrum and cerebellum of diabetics. Although the tissue protein concentration was decreased in the liver and the placenta, it was unchanged and even increased in the cerebrum and cerebellum, respectively. The concentration of myelin (cerebroside-sulfatide) was unchanged in the cerebrum, but it was increased in the cerebellum of diabetic animals.

These data suggest that diabetes has a growth-promoting effect on the fetal brain cell number. Furthermore, differences in the protein content between fetal organs may reflect abnormalities in protein metabolism which do not affect the brain during diabetic pregnancies.

Textbooks of pediatrics and neonatology have repeatedly reported low brain weights in relation to body weights in macrosomic babies of diabetic mothers (2, 11, 24, 27). Gruenwald (14) has reported that the rate of growth of human fetal brain is diminished in diabetic pregnancies past 30 weeks of gestation. Decreased fetal brain weight in diabetic pregnancies has also been reported by Driscoll *et al.* (10) suggesting that maternal diabetes may interfere with fetal brain development.

In a recent study, a decreased placental transfer of amino acids to the fetus was found in diabetic guinea pigs (22). Consequently, diabetic pregnancy has been proposed as a new model of fetal malnutrition (23). While it has been shown that those parameters such as brain weight, cell number, protein content, and myelination were affected in situations of fetal malnutrition (3, 6, 12, 29, 30) these characteristics of fetal brain development have not been investigated in diabetic pregnancies.

The goal of this study was to investigate fetal brain development during diabetic pregnancies, with regard to cerebral and cerebellar DNA, protein content, and myelination (cerebroside-sulfatide content). In order to detect any specific effects of diabetes on brain development, other organs such as the liver and placenta were also examined. The guinea pig was selected as the model in view of its brain maturity at birth which approaches the human situation more than other species (1, 5, 8, 9).

Female guinea pigs of the Hartley strain were fed a standard diet (guinea pig chow, Ralston Purina), with free access to water, and were maintained under normal laboratory conditions. Because insulin-induced hypoglycemia potentiates the diabetogenic activity of streptozotocin in this species (4, 15, 20), the animals were given streptozotocin (150 mg/kg) IV (diabetic animals) or an equivalent amount of a saline solution (control animals) 90 min after one IV injection of 15 units of crystalline insulin.

After mating, day 1 of gestation was determined by the presence of spermatozoa in the vaginal smear, at which time the animals were isolated in separate cages.

Oral glucose tolerance tests were performed on days 10, 30, and 50 of gestation by force feeding the animals with a 25% glucose solution, 2 g/kg, after 18 h of fasting. Blood samples were obtained by puncture of ear capillaries and serum glucose levels were determined by the glucose oxidase technique (Beckman automatic glucose analyzer).

Near term (day 60 of gestation), an arterial blood sample was withdrawn for blood gases determination and the animals were sacrificed with an overdose of pentobarbital (Nembutal). A laparotomy was immediately performed in order to observe fetal gasping to ensure that all fetuses of the litter were alive. After a few minutes, fetuses and placentas were removed from the uterus. Placenta, cerebrum (including the cerebral hemispheres and the brain stem), cerebellum, and liver were removed, weighed, and homogenized in 4 volumes of distilled water.

DNA and proteins were precipitated with trichloroacetic acid from the same aliquot of homogenate, and they were separated according to the technique of Schneider (25) using highly polarized DNA of veal thymus as standard. Proteins were estimated according to a modified method of Bradford (26). Cerebroside and sulfatide were extracted as reported by Folch *et al.* (13), separated by thin layer chromatography on silica gel precoated aluminum plates, and after elution (7) quantified by a spectrophotometric technique using anthrone (18).

Analysis of results. The statistical significance of differences in mean values for both groups was determined using Student's *t* test. Simple linear regression equations were calculated for different variables. The statistical significance of each regression coefficient and the linearity of each regression were investigated by analysis of variance. Differences in regression coefficient values were determined with a Student's *t* test comparing population slopes (31).

RESULTS

Seven control animals (22 fetuses) and 10 diabetic animals (33 fetuses) were studied near term (day 60 of gestation). Gestational duration is 65 to 67 days in the guinea pig. All animals had normal arterial blood gases: pH, 7.48 ± 0.01 (SEM); P_{CO_2}, 35 ± 1 mm Hg; P_{O_2}, 76.5 ± 3.2 mm Hg; HCO_3^-, 25.5 ± 0.6 meq/liter; O_2 saturation, 95.3 ± 1%. No differences in the acid-base status or in the arterial oxygenation were observed between control and diabetic animals. Compared to the controls, streptozotocin-treated animals were glucose intolerant during gestation as reflected by their glucose tolerance tests (Table 1).

Although fetal weight ranged from 43 to 94 g, no differences were observed in the mean values of fetal weight between the two groups: 74.8 ± 2.4 g in the controls *versus* 72.8 ± 1.7 g in the diabetic animals (mean ± SEM).

Cerebrum values. No significant differences were found between the two groups with regard to cerebral weight, protein, and

Table 1. *Results of glucose tolerance tests during gestation**

	Serum glucose (mg/100 ml)						
	0 min	30 min	60 min	90 min	120 min	150 min	180 min
Control ($n = 7$)	88 ± 7	160 ± 14	214 ± 19	231 ± 21	198 ± 20	153 ± 17	122 ± 11
Diabetic ($n = 10$)	117 ± 12	218 ± 15	279 ± 18	302 ± 19	284 ± 17	250 ± 19	215 ± 20
P	NS	<0.01	<0.025	<0.02	<0.005	<0.001	<0.001

* Each control and streptozotocin-treated animal was studied three times during gestation. Values are mean ± SEM. NS, not significant.

cerebroside-sulfatide content (Table 2). However, cerebrum DNA content was increased ($P < 0.001$) in the fetuses of diabetic animals.

Cerebellum values. Although cerebellum weight, protein, and cerebroside-sulfatide values were comparable in both groups, the protein content was increased ($P < 0.05$), when expressed per tissue weight, but it was decreased ($P < 0.001$) when expressed per number of cells (DNA) in diabetic animals (Table 3). The cerebroside-sulfatide content per g of cerebellum was also increased ($P < 0.05$) in diabetic animals. As found in the cerebrum, cell number (DNA content) was increased ($P < 0.001$) in the fetal cerebellum of diabetic animals.

Liver and placenta values. No significant differences were observed in the weight or in the DNA content of the liver and the placenta between the two groups (Table 4). However, liver weight, when reported as a percentage of fetal weight, was increased in the diabetic animals 5.18 ± 0.11% (mean ± SEM), compared with control animals, 4.67 ± 0.13% ($P < 0.005$). In contrast to the cerebrum and cerebellum, the protein content was found to be decreased in the liver ($P < 0.005$) and in the placenta ($P < 0.001$) of the diabetic animals, either in total content, per g of tissue, or per DNA.

In order to search for any relationship between body weight and growth of each organ, regressions were calculated and compared between the two groups.

The cerebrum, liver, and placenta weights correlated linearly with fetal weights, within each group (Fig. 1). Furthermore, the weight of the cerebrum varied with fetal weight according to the same linear regression equation (same slope, same elevation) in the diabetic and control animals. No correlation was observed between the cerebellum weight and fetal weight within each group. In regard to liver and placenta weight, the slope of their regression line with fetal weight was higher ($P < 0.025$ and < 0.05) in the diabetic animals compared to controls. This suggests that in the fetuses of diabetic animals, liver and placenta weights in relation to fetal weights increase at a faster rate as fetal weights rise. The smallest fetus of the diabetic group (43 g) had a placental weight of 5.87 g, which differentiated it from the others of its group (Fig. 1*D*). Therefore, this fetus has been excluded from the analysis of the correlation between placental and fetal weight, and it will be discussed separately.

Table 2. *Fetal values for the cerebrum**

	Control ($n = 22$)	Diabetic ($n = 33$)	P
Weight (g)	2.26 ± 0.03	2.22 ± 0.02	NS
DNA (mg)	3.01 ± 0.26	5.47 ± 0.52	<0.001
DNA/g	1.34 ± 0.12	2.46 ± 0.23	<0.001
Protein (mg)	62.2 ± 5.8	75.3 ± 4.6	NS
Protein/g	27.6 ± 2.5	34.1 ± 2.2	NS
Protein/DNA	24.2 ± 3.6	17.2 ± 1.8	NS
Cerebroside-sulfatide (mg)	7.94 ± 1.41	8.32 ± 1.15	NS
Cerebroside-sulfatide/g	3.46 ± 0.59	3.71 ± 0.50	NS

* Values are mean ± SEM. NS, not significant.

Table 3. *Fetal values for the cerebellum**

	Control ($n = 22$)	Diabetic ($n = 33$)	P
Weight (g)	0.25 ± 0.01	0.22 ± 0.01	NS
DNA (mg)	1.69 ± 0.21	3.85 ± 0.18	<0.001
DNA/g	7.15 ± 0.84	18.77 ± 1.10	<0.001
Protein (mg)	8.70 ± 0.83	9.53 ± 0.66	NS
Protein/g	35.5 ± 3.5	48.5 ± 4.5	<0.05
Protein/DNA	6.26 ± 1.04	2.70 ± 0.24	<0.001
Cerebroside-sulfatide (mg)	2.39 ± 0.32	3.73 ± 0.65	NS
Cerebroside-sulfatide/g	9.71 ± 1.28	18.0 ± 3.0	<0.05

* Values are mean ± SEM. NS, not significant.

Table 4. *Fetal values for the liver and the placenta**

	Control ($n = 22$)	Diabetic ($n = 33$)	P
Liver			
Weight (g)	3.49 ± 0.14	3.79 ± 0.14	NS
DNA (mg)	19.2 ± 1.7	22.0 ± 1.0	NS
DNA/g	5.40 ± 0.32	5.99 ± 0.29	NS
Protein (mg)	342 ± 53	207 ± 16	<0.01
Protein/g	101 ± 17	55.5 ± 3.8	<0.005
Protein/DNA	16.5 ± 2.7	9.62 ± 0.64	<0.005
Placenta			
Weight (g)	4.17 ± 0.16	4.45 ± 0.16	NS
DNA (mg)	13.1 ± 0.9	16.2 ± 1.2	NS
DNA/g	3.17 ± 0.21	3.63 ± 0.23	NS
Protein (mg)	221 ± 17	149 ± 9	<0.001
Protein/g	53.6 ± 3.9	33.5 ± 1.8	<0.001
Protein/DNA	18.1 ± 1.6	9.8 ± 0.6	<0.001

*Values are mean ± SEM. NS, not significant.

DISCUSSION

This study raises important issues with regard to fetal brain development during diabetic pregnancy. Whereas decreased fetal brain weights have been previously reported in diabetic pregnancy (10, 14), our results do not support these findings. Fetal cerebrum and cerebellum weights were found to be similar in diabetic and control guinea pigs, when comparisons were made at the same time of gestation, in animals with similar fetal weights. It is well known that diabetes may affect fetal growth and predisposes to prematurity. Therefore, it is essential to consider these two variables together, especially since maturity and nutritional status affect fetal brain development (3, 6, 12, 29, 30). Accordingly, positive correlations were found in this study between cerebrum weights and fetal weights over a wide range of fetal weight values. Furthermore, the linear regression between these two variables was identical in the diabetic and control animals, clearly indicating that brain growth was not affected by diabetes once fetal weight and gestational age were taken into account.

However, higher liver and placenta weights, in relation to fetal weight changes, were found in diabetic animals. This finding corroborates other studies which reported an increased placental/fetal weight ratio in diabetic women and in streptozotocin-treated rats (17, 28). In this regard, the smallest of all the fetuses came from the diabetic group and it had a high placental weight. This fetus may have been growth-retarded secondary to maternal

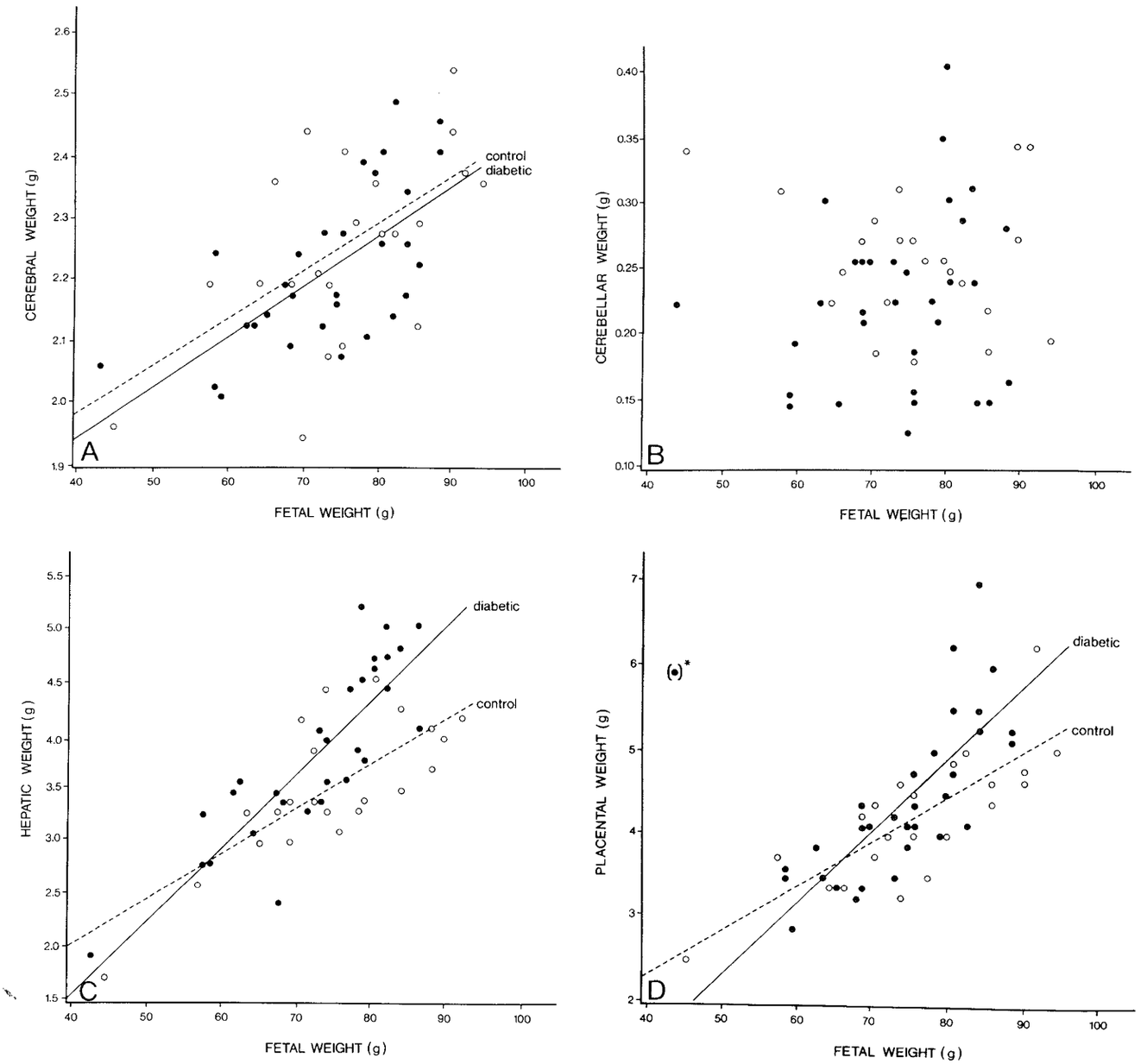

Fig. 1. Linear regressions between fetal parameters and fetal weight in control (○) and diabetic (●) guinea pigs. *A*, Cerebral weight *versus* fetal weight in controls, $y = 1.66 + 0.008x \pm 0.13$ (SE), $r = 0.58$ ($P < 0.005$) and in diabetics, $y = 1.60 + 0.008x \pm 0.1$, $r = 0.65$ ($P < 0.001$); regression lines are identical ($P > 0.05$). *B*, Cerebellar weight *versus* fetal weight in controls, $y = 0.32 - 0.0009x \pm 0.05$, $r = -0.22$ ($P > 0.05$) and in diabetics, $y = 0.13 + 0.0012x \pm 0.063$, $r = 0.19$ ($P > 0.05$); regression lines are not drawn, because they are not significant. *C*, Hepatic weight *versus* fetal weight in controls, $y = 0.28 + 0.043x \pm 0.45$, $r = 0.74$ ($P < 0.001$) and in diabetics, $y = -1.31 + 0.07x \pm 0.43$, $r = 0.85$ ($P < 0.001$); slopes of the regression lines are different ($P < 0.025$). *D*, Placental weight *versus* fetal weight in controls, $y = 0.24 + 0.053x \pm 0.47$, $r = 0.79$ ($P < 0.001$) and in diabetics, $y = -1.99 + 0.087x \pm 0.58$, $r = 0.79$ ($P < 0.001$); slopes of the regression lines are different ($P < 0.05$). *, Diabetic value excluded for the calculation of the regression equation.

diabetes, and not to a placental mass deficiency. This would corroborate Pitkin's findings (17) who reported high placental/fetal weight ratio in growth-retarded fetuses of severely diabetic rats. The weight increase in different organs of newborns of diabetic mothers has also been associated by some authors with increased glycogen content (16, 17, 24).

In contrast to the cerebrum, no correlation was observed in diabetic or control animals between cerebellum weight and fetal weight, despite a wide range of fetal weight values. This is inconsistent with previous studies, which showed in the human and the guinea pig that cerebellum weight was more affected than the cerebrum by changes in fetal weight (5, 6). In these studies, although the DNA content was found to be lower in the cerebellum than in the cerebrum of human fetuses with growth retardation, it was equally decreased in both parts of the brain, in the guinea pig model (5, 6). This may be due to differences in brain maturation between species. For instance, approximately 25% of the adult brain weight is present at birth in the human, compared with 70% in the guinea pig (5). In addition, the total brain DNA content at birth represents 75% of the adult value in the human, compared with 100% in the guinea pig (5). Furthermore, different regions of the brain may have different rates of maturation *in utero*, and the patterns may be different from one species to another. For example, in the human, the cerebrum matures at a faster rate prenatally than does the cerebellum, whereas at birth, both cerebrum and cerebellum have achieved

the same maturity in the guinea pig (5, 9). Therefore, it is possible that such conditions as fetal malnutrition or diabetic pregnancy may have different effects upon brain development depending upon the species under study. Despite these differences between the human and the guinea pig, these two species still offer more similarities with regard to fetal brain development, than other animals such as mouse, rat and rabbit (1, 5, 8, 9).

One of the most unexpected findings of this study was the increased DNA content of the cerebrum and the cerebellum in diabetic animals. Organ DNA content is a reliable index of its cell number, without providing any information concerning the cell types. Many growth factors have been recently identified. Among them, the nerve growth factor, which has been implicated in fetal nervous tissue development, presents many structural homologies with proinsulin (21). Since infants of diabetic mothers and fetuses of streptozotocin-treated rats have high insulin blood levels (16, 17), it is possible that insulin, or other factors, in some way promotes brain cell number in these fetuses. Any further research to clarify this hypothesis in the guinea pig model will have to take into consideration the particulars of guinea pig insulin which has been reported to be different from porcine, bovine, or human insulin in terms of physical and biological properties (32, 33).

The decreased protein content found in the liver and the placenta might be related to the impaired placental transfer of essential amino acids previously reported in diabetic pregnancies (23). However, the brain seems in some way to be spared from the protein deprivation observed in the liver and placenta. In fact, the protein content per g of cerebellum was actually increased in diabetic animals. The extent to which fetal amino acid supply or protein synthesis in the brain is affected by diabetic pregnancy remains to be established.

Fetal malnutrition has been associated with decreased myelin content in the brain (5, 6, 12). In this study, this effect has not been observed in diabetic animals. On the contrary, cerebroside-sulfatide concentrations were increased in the cerebellum. Proteins represent 30% of the myelin composition (19). The fact that both are increased in the fetal cerebellum, per g of tissue, in diabetic animals, may not be coincidental. However, protein content was not increased to the same extent as cellularity in the cerebellum, as suggested by the decreased protein/DNA ratio.

It remains difficult to ascertain whether these observations made on fetal brain development in guinea pigs rendered diabetic with streptozotocin apply to the human situation. For instance, we were not able, in the present study, to demonstrate an increased fetal weight in the diabetic group compared to the control one, which may be due to the high range in fetal weight values observed within each litter in both groups. In addition this study does not investigate the causes or clinical implications of the effects of diabetes upon fetal brain development. These are important issues to evaluate, since in the human as in the guinea pig, the fetal brain is much more mature than any other organ at birth. It is always a possibility that any adverse condition affecting brain development during pregnancy may have long term postnatal effects.

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