Fetal and Placental Composition in Experimental Maternal Diabetes[1] (35852)

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(Introduced by J. T. Bradbury)
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Pregnancy complicated by diabetes mellitus is associated with profound influences on fetal growth, development, and metabolism. Of the many fetal alterations associated with maternal diabetes, macrosomia has probably been recognized for the longest time and is the most frequently observed. Initially, the excessive size of infants of diabetic women was attributed to increased water content but more recent investigations have established that the increase is primarily in fat.

The present report concerns determination of water, fat, protein, calcium, and deoxyribose nucleic acid (DNA) in fetuses and placentas of rats in which a diabetic-like state was induced prior to pregnancy. The agent used for induction of diabetes was streptozotocin, an antibiotic which has recently been shown to have a relatively specific cytotoxic action against pancreatic β-cells (1).

Methods. The streptozotocin utilized in the study (lot BVL 69-206) was dissolved in distilled water to make a solution containing in each milliliter: streptozotocin, 30 mg; and citric acid, 6 mg; with sodium hydroxide added to pH 3.8–4.2. All solutions were made immediately prior to injection into the tail vein of virgin female Sprague-Dawley rats weighing 200 to 250 g (Sprague-Dawley, Inc., Madison, Wisconsin). Injections were given following an overnight fast. The volume injected was 1 ml/kg of body weight, resulting in a streptozotocin dose of 30 mg/kg. Control animals received an appropriate volume of an acidic solution containing citric acid and sodium hydroxide in the above proportions. Urine obtained 24 hr after injection was tested for glucose with glucose oxidase paper (Tes-tape, Eli Lilly and Co., Indianapolis, Ind.) and, if positive, the animal was considered diabetic. Rats who received streptozotocin but did not exhibit glucosuria were excluded from the study.

Diabetic animals were randomly divided into untreated and treated groups and the latter received an injection sc of NPH insulin (Iletin, Eli Lilly and Co., Indianapolis, Ind.) late in the afternoon of each day. The insulin dosage varied from 1 to 2.5 units daily, as determined by 24-hr urinary glucose excretion. Insulin treatment was begun prior to breeding and continued throughout pregnancy.

Rats were kept two or three per cage and given Purina rat chow and water *ad libitum*. At the time of proestrus, as determined by daily vaginal smears, each female was put with a male overnight. If the vaginal smear the following morning contained sperm, that day was considered day one of pregnancy. The same male rat was used for all breeding. At weekly intervals during pregnancy, each animal was weighed and placed in an individual metabolic cage for 24 hr for determination of urine volume and glucose concentration.

Pregnant rats were killed by intraperitoneal pentobarbital injection on day 21 of pregnancy following an overnight fast. Fetuses and placentas were delivered by hysterotomy and weighed individually, after which the fetuses were killed by cervical dislocation. All

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fetuses from a particular litter were sealed in an airtight plastic bag and stored at —20° until analyzed. Placentas (including umbilical cord and membranes) were similarly pooled and stored.

The pooled specimens were thawed and reweighed immediately prior to composition studies. Water content was determined by lyophilization to constant weight (usually requiring 24 to 48 hr) with VirTis lyophilization equipment. The residue was ground to a fine powder and aliquots were taken for determination of protein, fat, DNA, and calcium. Nitrogen was measured on a Coleman nitrogen analyzer and the result was multiplied by 6.25 for protein. Fat content was determined by the method of Van de Kamer *et al.* (2) modified for tissue fat. DNA was measured by color development with diphenylamine (3) following multiple extractions with 5% trichloroacetic acid at cold, hot, and room temperatures. Calcium was determined with a Perkin-Elmer 303 atomic absorption spectrophotometer on an aliquot ashed and dissolved in hydrochloric acid.

Data were analyzed by Student's *t* test or analysis of variance followed by Tukey's test for comparing treatment means (4).

Results. Fifteen litters, equally divided between control, untreated diabetic, and treated diabetic animals, were studied in the manner outlined. Maternal weight gain and appearance during pregnancy did not differ between the three groups. Glucosuria was consistently negative in control animals while in untreated diabetics the mean glucose excretion was 215 mg/24 hr (range 60 to 400) and in treated diabetics the mean was 30 mg/24 hr (range 0 to 80).

Fetuses of untreated diabetic rats were heavier than those of either control or treated groups, as indicated in Table I. Considering each fetus as an individual unit for statistical analysis, the fetuses of diabetics weighed significantly more than those of either of the other two groups ($p<0.05$) while placental weight did not differ significantly. However, the mean litter size was somewhat smaller in untreated diabetics than in controls (10.8 vs 13) and this fact could account for weight differences. On the other hand, mean fetal weight was significantly greater in untreated than in treated diabetic animals, in spite of essentially identical litter sizes.

TABLE I. Fetal and Placental Weight.

	Control	Diabetic	Treated
No. of litters	5	5	5
No. of fetuses	65	54	57
Fetal wt (g)			
Mean	5.45	5.85	5.41
SEM	0.05	0.06	0.04
Placental wt (g)			
Mean	0.87	0.93	0.93
SEM	0.06	0.06	0.07

Figure 1 contains data regarding proportions of water, fat, and protein in fetuses of each litter. Fetuses of untreated diabetic animals, in comparison with those of controls and treated diabetics, had less water and more fat. The differences in fat and water were consistent and significant ($p<0.05$). Protein, on the other hand, did not differ between the three groups.

As indicated in Fig. 2, DNA concentration was consistently greater in fetuses of untreated diabetics than in those of the other two groups and the differences were significant ($p<0.05$). The ratio of protein to DNA, while lower on the average in diabetics, exhibited considerable overlap and did not

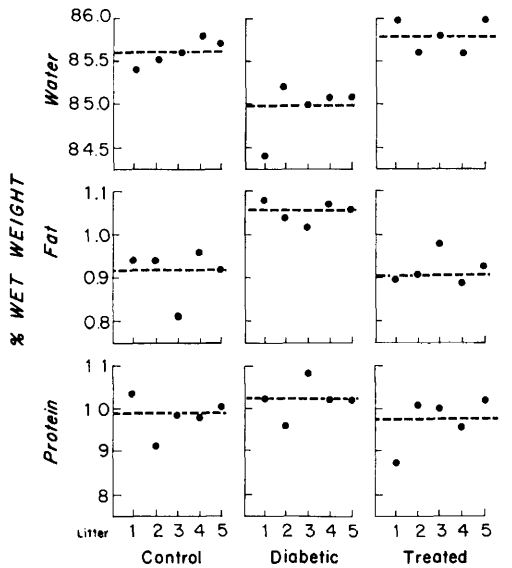

FIG. 1. Fetal water, fat, and protein as a proportion of wet weight: (- - -) means of each group.

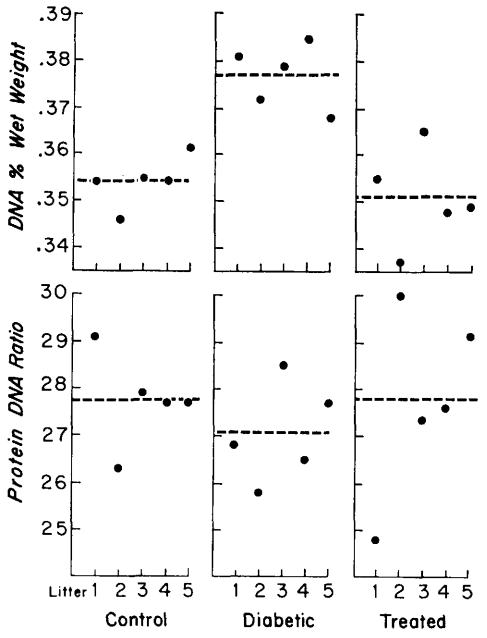

FIG. 2. Fetal DNA and protein–DNA ratio: (- - -) means of each group.

differ significantly among the three groups.

Fetal calcium levels varied from 220 to 262 mg/100 g of wet weight and had no relation to maternal diabetic state.

In contrast to fetal studies, placental composition exhibited no particular patterns. There was no significant difference between control, diabetic, and treated groups for any of the parameters studied. Mean values for placental composition are listed in Table II.

Comment. The long recognized clinical association of maternal diabetes, particularly of relatively mild degree, with increased fetal size has recently been clarified by body composition studies. Direct total body analysis of stillbirths and early neonatal deaths by Fee and Weil (5) established that the infant of the diabetic woman, in comparison with that of the nondiabetic, has more fat, less water, and unchanged protein. Though body water as a proportion of total weight was diminished, Fee and Weil found that water as a function of fat-free wet weight did not differ in infants of diabetics and nondiabetics. Osler's studies in living infants, using estimations of total body water by deuterium oxide studies (6) and of fat by caliper and X-ray measurements (7), are in agreement with these findings. Thus, it seems clear that the major feature of the infant of the diabetic woman is an increase in body fat.

TABLE II. Mean Values of Placental Composition (% wet wt).

	Control	Diabetic	Treated
Water	85.24	85.42	85.64
Fat	1.05	1.08	1.03
Protein	12.22	11.32	11.20
DNA	0.20	0.21	0.20
Calcium	0.014	0.012	0.012

Experimentally, induction of a diabetic-like state by any of several means has been described in a number of reports. Although increased fetal weight has been found in several of these studies of experimental diabetes in pregnancy, particularly when the diabetes is of mild degree (8, 9), the precise nature of the augmentation has received scant attention. In a study of alloxan-diabetes induced during pregnancy, Solomon (10) reported significantly greater birth weights in mildly diabetic rats than in normals without apparent differences in water, protein, or fat. However, body analysis was done on only two litters each of normals and diabetics. Induction of maternal hyperglycemia in otherwise normal rats by constant intravenous glucose infusion during the last two days of pregnancy was reported by Harding *et al.* (11) to cause increased fetal weight and greater water content but no difference in protein, fat, or ash.

In the present study, induction of a diabetic-like state prior to pregnancy was found to produce fetuses containing an increase in fat, a decrease in water, and unaltered protein proportions. These data, while different from the limited previous reports of animal experiments, are quite compatible with results of human studies. The degree of diabetes induced was relatively mild, at least as judged by maternal weight gain and glucosuria, since the dose of streptozotocin employed is near the lowest level of the diabetogenic dose (12). The occurrence of macrosomia with mild, as opposed to severe, degree of diabetes correlates well with clinical observations.

The finding of significantly greater DNA levels in fetuses of diabetic animals, as compared with controls and treated animals, has

not been previously reported in either experimental or human studies. It indicates the greater numbers of cells in the fetuses of diabetics, while the lack of a difference in protein–DNA ratios suggests that individual cell size does not vary.

In studies of cellular growth during prenatal and postnatal periods, Winick and Noble (13) have described three sequential phases: hyperplasia (*i.e.*, cell division), hyperplasia with hypertrophy, and finally hypertrophy (*i.e.*, cell enlargement) alone. Their detailed investigation in the rat indicated that, while the duration of each phase varies considerably from organ to organ, the animal as a whole shows a relatively constant cell size during early prenatal growth followed by an increasing cell size due to diminished rate of cell division during the last week of fetal life. Thus, the timing of an environmental influence on cell growth is critical in determining its effect. Stimuli acting during the period of hyperplasia will affect cell number while those acting during hypertrophy will affect cell size. The finding of the present study that maternal diabetes is associated with increased numbers of cells indicates that the environmental influence of maternal diabetes causing increased fetal growth, whatever it may be, has an action beginning early in intrauterine life.

The explanation of the many fetal alterations accompanying maternal diabetes mellitus is uncertain. A number of hypotheses, including fetal hyperadrenocorticism, excessive elaboration of growth hormone by the maternal pituitary or growth hormone-like substance by the placenta, and enhanced placental transmission of fatty acids, have been proposed. Probably the most commonly-held postulate is the hyperglycemia-fetal hyperinsulinism theory advanced mainly by Pedersen and Osler (14). The findings of the present study, while not necessarily incompatible with any of these hypotheses, generally tend to lend support to the hyperglycemia-hyperinsulinism theory, since control of maternal glucose levels by insulin treatment so effectively prevented the fetal alterations seen in untreated diabetic pregnancies. On the other hand, there are hazards inherent in any simplistic explanation, for if fetal growth depends on growth hormone for increase in cell number and on insulin for increase in cell size, as has been demonstrated in adults (15), simple hyperinsulinemia would not account for the changes observed.

Summary. A mild diabetic-like state was induced with streptozotocin in rats prior to breeding and fetal and placental composition was examined on day 21 of pregnancy. Fetuses of untreated diabetic rats were larger than those of controls and had significantly more fat, less water, and more DNA. These alterations in fetal size and composition were prevented by maternal insulin treatment during pregnancy.

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