EFFECTS OF MATERNAL STREPTOZOTOCIN-DIABETES ON FETAL GROWTH, ENERGY RESERVES AND BODY COMPOSITION OF NEWBORN PIGS\textsuperscript{1,2}

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Summary

Two doses of Streptozotocin (50 and 100 mg/kg body weight) were administered to two groups of pregnant gilts at d 80 of gestation to determine the influence of two levels of maternal diabetes on the gilts, their developing progenies and the body composition of the pigs. All the experimental animals received 1.82 kg of gestation diet/day throughout gestation. Serum glucose concentration increased to hyperglycemic levels in low-dose and high-dose groups; insulin concentrations decreased (P<.01) in the high-dose, but not in the low-dose group (P>.05). Maternal free fatty acids (FFA) increased (P<.05) in both treatment groups when compared with the control. However, birth weight of the litter and litter size were not affected. The liver weight increased (P<.01) in the progeny of high-dose but not the low-dose group. Total liver DNA and RNA were not altered by the treatments, however; total liver protein and protein:DNA ratio increased (P<.01) in the progeny of high-dose gilts. Pigs from high-dose and low-dose groups showed increases (P<.01) in liver glycogen concentrations and percentage liver lipid. Body chemical composition data showed increases in percentage dry matter and percentage lipid (P<.05 and P<.01, respectively) in the progeny of high-dose but not in the low-dose group. It was concluded that streptozotocin administered to gestating gilts increased the maternal nutrient supply to the developing pigs, which resulted in higher energy status of the pigs at birth.

(Key Words: Maternal Diabetes, Liver, Glycogen, Lipid, Pig.)

Introduction

High mortality rate among newborn pigs should theoretically be reduced if increased energy storage is induced in developing pigs before birth. Studies have shown that maternal diabetes increased the body fat content and(or) liver glycogen concentrations of fetal pigs (Ezekwe and Martin, 1978, 1980; Kasser et al., 1981a,b). In these studies pregnant pigs were hysterectomized before birth and it was not known whether or not normal farrowing would be affected by diabetes in pigs. Before alteration of maternal glucose and(or) free fatty acids can be successfully used in swine production, the ability of the animals to farrow live pigs must be evaluated.

Alloxan and streptozotocin have been shown in a variety of experimental animals to induce a diabetic-like state by a cytotoxic effect on pancreatic beta cells. Alloxan was used to induce diabetes in pigs with its concomitant severe effects (O'Hea et al., 1971; Ezekwe and Martin, 1978, 1980; Kasser et al., 1981a). Streptozotocin, a diabetogenic drug often used to produce dose-related severe and mild forms of diabetes in other species (Pitkin and Van Orden, 1974) has not been used in porcine animals. Therefore, the objectives of the experiment were to determine the effects of mild and severe streptozotocin-diabetes on: 1) reproductive performance and blood glucose, FFA and insulin levels of pregnant gilts; 2) body weight, liver and...
TABLE 1. COMPOSITION OF THE GESTATION DIET

<table>
<thead>
<tr>
<th>Item</th>
<th>%a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn (IFN 4-02-931)</td>
<td>77.72</td>
</tr>
<tr>
<td>Soybean meal, 44% CP (IFN 5-04-604)</td>
<td>14.13</td>
</tr>
<tr>
<td>Alfalfa (IFN 1-00-025)</td>
<td>5.00</td>
</tr>
<tr>
<td>Dicalcium phosphate (IFN 6-01-080)</td>
<td>1.00</td>
</tr>
<tr>
<td>Limestone (IFN 6-01-069)</td>
<td>.90</td>
</tr>
<tr>
<td>Trace mineral saltb</td>
<td>.50</td>
</tr>
<tr>
<td>Salt (IFN 6-14-013)</td>
<td>.50</td>
</tr>
<tr>
<td>Vitamin-selenium premixc</td>
<td>.25</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100.00</strong></td>
</tr>
</tbody>
</table>

* Calculated to contain 14% crude protein.

b Contains (%): 17.5 Zn; 14 Mn; 8.8 Fe; 1.7 Cu; .35 I and .35 Co.

c Supplied (per kg of premix) 1.76 g riboflavin; 8.8 g pantothenic acid; 8.8 g niacin; 8.8 mg vitamin B12; 176 g choline chloride; 1,760,000 IU vitamin A; 176,000 IU vitamin D3; 4,400 IU vitamin E; 440 mg menadione dimethylprimidinol bisulfite; 88.2 mg biotin and 40 mg Se.

muscle DNA, RNA and protein, liver glycogen and lipid and serum insulin of newborn pigs; 3) the body chemical composition of the pigs at birth.

Materials and Methods

Twelve pregnant crossbred gilts with known breeding dates were assigned to three treatment groups. Gilts were allotted randomly to the control, low-dose and high-dose groups, respectively. Animals were fed 1.82 kg/d of corn-soybean meal diet containing 14% protein and adequately fortified with vitamins and minerals to meet the nutrient requirement for gestating swine (table 1). Diabetic gilts were given access to 5% glucose solution in water for 48 h after streptozotocin injection to minimize initial effects of streptozotocin. Diabetes was induced by iv (anterior vena cava) injection of streptozotocin at 80 d of gestation according to methods of Romsos et al. (1971) and modified by Ezekwe and Martin (1980). The low-dose group received 50 mg/kg body weight of streptozotocin (dissolved in citrate buffer, pH 4.0) and the high-dose group was given 100 mg/kg body weight. Control gilts received saline injections. All the treated animals exhibited glycosuria within 24 h after treatment. Maternal blood samples were taken before streptozotocin administration and 48 h after treatment. One animal died from the high-dose group and another from the low-dose group aborted. Causes of death and abortion appeared to be related to high environmental summer temperatures. Weekly blood samples were taken via anterior vena cava from all the animals. All animals were meal-fed and blood was sampled 1 to 2 h after each feeding. Serum was collected by centrifugation and frozen at −20°C until analyzed for glucose, FFA and insulin. Glucose was analyzed by the glucose oxidase method6. Serum free fatty acids were assayed according to methods of Dunoccombe (1963, 1964). The lower range of sensitivity for the assay was 10 μEq FFA/liter of serum. Serum insulin was determined by double antibody radioimmunoassay techniques7. Porcine insulin (First IRP 66-34 WHO) was used for radioiodination. Antisera to porcine insulin prepared in guinea pigs7 was used. The second antibody was produced in sheep9. The lower range of sensitivity for the assay was 2 μU/ml of serum. All samples were analyzed in a single assay.

All farrowings were attended. At parturition, pigs were cleaned and weighed and two pigs/litter randomly selected on the basis of the average body weight of the litter were killed at birth for tissue determinations. The liver was quickly removed and weighed, triplicate samples were digested in 30% KOH saturated with Na₂SO₄ for glycogen determination (Lo et al., 1970). The remainder of the liver tissue was frozen at −20°C for later biochemical determinations. The hind quarter was cut and similarly frozen. The frozen hind quarter was later thawed and gastrocnemius and semitendinosus muscles were dissected out, weighed and a representative sample, taken from the entire length of the muscle was used for DNA, RNA and protein analyses. Samples from liver and skeletal muscles were homogenized in ice-cold .4 N KCl in a Virtis homogenizer. Aliquots of homogenized liver and muscle were used for DNA and RNA determinations, according to Schmidt and Thannhouser (1945) methods as previously

6 Sigma Chemical Co., St. Louis, MO.
7 Roch Biomedical Laboratory, Columbus, OH.
8 Grand Island Biological Company, Grand Island, NY.
reported (Ezekwe and Martin, 1975). Protein was analyzed using methods of Lowry et al. (1951). Liver lipid was determined according to methods of Folch et al. (1957). A similar random sample of the piglets (two/litter) was killed by decapitation and frozen at −20°C and later used for body composition determinations. Carcasses for body composition were thawed and uniformly ground in a meat grinder and samples were taken for body composition determination according to the procedure described by Hartsook and Hersberger (1963) for small samples.

Statistical evaluation was performed by one-way analysis of variance. Significant differences between means were detected by Newman-Keuls procedures (Steel and Torrie, 1960).

**Results**

Polyuria and glycosuria were observed in all the streptozotocin-treated gilts after 24 h. Pretreatment serum glucose levels were 51.5 ± 3.9, 64.8 ± 5.1, 58.8 ± 6.6 mg/100 ml for high-dose, low-dose and control gilts, respectively (figure 1). At d 2 postinjection, the levels increased to 309.8 ± 8.9 and 210.1 ± 34.6 mg/100 ml (mean ± SE) for high-dose and low-dose groups, respectively. Control gilts maintained a constant and normal level of serum glucose throughout the experimental period. High-dose gilts had higher (P<.05) serum glucose throughout the postinjection period when compared with preinjection level. Differences (P<.05) were also observed between the pre- and post-treatment serum glucose level in the low-dose group.

Pretreatment insulin levels did not differ among the three treatment groups. Insulin concentrations decreased (P<.01) in the high-dose group after streptozotocin injection and remained low throughout the experimental period. The low-dose group maintained lower insulin concentrations, however, no significant differences were observed between the pre- and postinjection levels. Control gilts had fairly constant and higher insulin levels throughout gestation, with marked differences (P<.05) between the high-dose and control groups (figure 1). The concentration of serum free fatty acids (FFA) in the sows before and during experimental treatment is presented in figure 2. Pretreatment levels were 442.5 ± 55.5, 507.0 ± 19.81 and 570.0 ± 85.3 µEq/liter (mean ± SE) for the control, low-dose and high-dose groups, respectively. No significant differences were observed among the three groups. On d 83 of gestation, FFA (µEq/liter) increased (P<.05) in the low-dose (828.0 ± 33.1) and high-dose (1,870 ± 470.1) groups when compared to the control (493.7 ± 100.3). The same trend was maintained by the high-dose group throughout the experimental period. The control group maintained a fairly constant level of serum FFA while the levels in the low-dose group, for most part, remained higher than the control values.

Gestation length was shorter (P<.01) for gilts in the high-dose group than for gilts in low-dose and control groups, with no difference between the low-dose and control gilts. No differences were observed in placental weight, birth weight of litter and litter size among the treatment groups (table 2).

Liver weight was higher (P<.01) in the pigs from high-dose gilts, while no differences were observed between those of low-dose and control progenies (table 3). Total liver DNA and RNA in the pigs were similar among the progenies of the three treatment groups. Total liver protein and protein:DNA ratio were elevated (P<.01) in the pigs from high-dose group, with no differences between the progenies of low-dose and control gilts.

![Figure 1. Maternal serum glucose (mg/100 ml) and immunoreactive insulin (µU/ml) levels in pregnant gilts before and after streptozotocin and saline injections.](image-url)
The RNA:DNA ratio showed no differences among the three groups. Liver glycogen and percentage liver lipid were higher (P<.01) in the progenies of high-dose and low-dose groups when compared with the control. A slight depression of serum insulin was observed among the progeny of high-dose group. Gastrocnemius and semitendinosus muscle weight, muscle DNA, RNA and protein content of the progenies were not altered. Similar RNA:DNA and protein:DNA ratios in muscles from high-dose and low-dose progenies were comparable with those of the control muscles (data not shown). No differences were detected in progeny carcass weight, percentage protein and ash among the three groups at birth (table 4). However, percentage dry matter and lipid were higher (P<.05 and P<.01, respectively) for pigs from high-dose groups compared with the control and low-dose progenies. No differences were noted among pigs from low-dose and control groups.

**Discussion**

Maternal diabetes in pigs has been used as a model for the study of mechanisms controlling fetal energy storage (Ezekwe and Martin, 1978, 1980; Kasser et al., 1981a, 1982). In these studies, alloxan, a drug that induces severe diabetes was used. Streptozotocin has been used to induce mild diabetes in rats (Pitkin and Van Orden, 1974) and has been shown to produce a diabetic condition without the usual toxic effects of alloxan (Cheek, 1975). With a four- to eightfold increase in serum glucose during the treatment period, the high-dose group was regarded as severely diabetic. The low-dose group had 1.7- to threefold increase in serum glucose during the same period and were therefore mildly diabetic. The results (figure 1) showed that streptozotocin could be used to induce mild, as well as severe diabetes in pregnant gilts. The cytotoxic effect of streptozotocin on the beta cells of the pancreas was reflected by the serum insulin levels of the treated gilts. The lower (P<.05) post-treatment insulin concentrations in the high-dose gilts indicated a greater destruction of beta cells. The advantage of streptozotocin over alloxan is its ability to produce dose-related levels of hyperglycemia. It is easier, therefore, to establish a dose-response relationship suitable for the dam and her offspring.

Some studies have shown that in human diabetic pregnancies, gestation periods are

**TABLE 2. REPRODUCTIVE PERFORMANCE OF STREPTOZOTOCIN-DIABETIC GILTS**

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Low-dose</th>
<th>High-dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of gilts</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Gestation length, d</td>
<td>114.7 ± .2a</td>
<td>114.3 ± .5</td>
<td>111.0 ± 1.2b</td>
</tr>
<tr>
<td>Placental weight, kg</td>
<td>1.8 ± .4</td>
<td>2.0 ± .4</td>
<td>1.5 ± .1</td>
</tr>
<tr>
<td>Birth weight of litter, kg</td>
<td>1.1 ± .1</td>
<td>1.2 ± .1</td>
<td>1.0 ± .1</td>
</tr>
<tr>
<td>Total pigs/litterc</td>
<td>10.3 ± .7</td>
<td>11.0 ± .8</td>
<td>10.3 ± 1.4</td>
</tr>
<tr>
<td>Mean no. stillborn</td>
<td>.8</td>
<td>1.7</td>
<td>2.0</td>
</tr>
</tbody>
</table>

aMean ± SE.

bHigh-dose value differed (P<.01) from those of low-dose and control.

cNumber alive plus number stillborn.
usually longer than normal (Kyle, 1963), however, in the present studies, gestation length was shorter (P<.01) in the high-dose group and unaffected in the low-dose gilts (table 2). Although the birth weight of the litter was not affected significantly, the pigs from high-dose gilts appeared less mature at birth. Human maternal diabetes has been reported to result in heavier babies (Farquhar, 1966); however, in the present experiment, no such increases in body size were observed. Shorter gestation period in pigs, compared with the human, may not allow enough time for diabetes to affect fetal alterations in body size. A pig at birth has been compared with a 25-wk-old human fetus (Widdowson and Spray, 1951). The comparable litter weight among the treatment groups was in agreement with the reports of Ezekwe and Martin (1978) and Kasser et al. (1981a).

The elevation (P<.01) of fetal liver weight after maternal diabetes in high-dose groups (table 2) was similar to previous reports (Ezekwe and Martin, 1978; Kasser et al., 1981a). Increased maternal-fetal glucose levels might have been responsible for the stimulation of liver cell growth in order to accommodate increased glycogen synthesis. Elevated liver glycogen observed in high-dose as well as low-dose progenies suggested that mild diabetes offered adequate stimulation for the synthesis of liver glycogen, an important energy source for baby pigs. This increase in liver glycogen without the concomitant increase in liver size observed in low-dose progeny was unexpected. It is likely that the stimulatory influence of serum glucose on liver cell growth is regulated by glucose concentration in the fetal blood. The high-dose progeny responded to higher maternal and fetal glucose concentrations by an increase in liver size to maintain glucose homeostasis; in the low-dose progeny the demand for extracellular growth might not have existed because lower maternal glucose levels were maintained. Maternal diabetes has been shown to increase fetal serum glucose in pigs (Ezekwe and Martin, 1980; Kasser et al., 1981a).

The comparable liver DNA and RNA among the treatment groups, in spite of increased liver size in the progeny of high-dose gilts, indicate that cellular hyperplasia might have

### Table 3. Liver Weight, Liver Cellular Constituents and Immunoreactive Insulin Concentrations in Progeny of Diabetic and Normal Gilts at Birth

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>Low-dose</th>
<th>High-dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver weight, g</td>
<td>3.58 ± 1.6a</td>
<td>33.3 ± 2.7</td>
<td>54.8 ± 3.9b</td>
</tr>
<tr>
<td>Total liver DNA, mg</td>
<td>131.8 ± 9.2</td>
<td>134.5 ± 16.5</td>
<td>163.7 ± 9.5</td>
</tr>
<tr>
<td>Total liver RNA, mg</td>
<td>216.9 ± 17.3</td>
<td>194.4 ± 13.1</td>
<td>225.2 ± 14.5</td>
</tr>
<tr>
<td>Total liver protein, mg</td>
<td>2,936.2 ± 257.8</td>
<td>2,289.2 ± 250.9</td>
<td>4,534.9 ± 338.1b</td>
</tr>
<tr>
<td>RNA/DNA</td>
<td>1.0 ± 1.0</td>
<td>1.3 ± 1.1</td>
<td>1.2 ± 1.1</td>
</tr>
<tr>
<td>Protein/DNA</td>
<td>13.9 ± 1.0</td>
<td>15.2 ± 1.2</td>
<td>24.5 ± 1.7b</td>
</tr>
<tr>
<td>Liver glycogen, mg/g</td>
<td>98.3 ± 12.0c</td>
<td>197.2 ± 36.1d</td>
<td>291.7 ± 43.2c</td>
</tr>
<tr>
<td>Percentage liver lipid</td>
<td>2.4 ± 1.0c</td>
<td>3.4 ± 2.0d</td>
<td>3.4 ± 2.0d</td>
</tr>
<tr>
<td>Serum insulin, uU/ml</td>
<td>7.3 ± 2.1</td>
<td>7.5 ± 8.8</td>
<td>4.8 ± 6.6</td>
</tr>
</tbody>
</table>

aMean ± SE for six animals.
bDiffered (P<.01) from low-dose and control values.
c,d,eMeans in the same row with different superscripts differ (P<.01).

differed from control progeny and low-dose progeny (P<.05).

differed from control progeny and low-dose progeny (P<.01).
been halted in the pigs from high-dose group. However, liver cell protein and protein:DNA ratio (cell size) were elevated (P<.01). This would indicate that hypertrophic growth was initiated in the liver of the progeny of high-dose gilts. Liver and adipose lipogenesis were not stimulated in offspring of diabetic pigs (Ezekwe and Martin, 1980; Kasser et al., 1981a). Therefore, the higher (P<.01) percentage liver lipid in pigs from high-dose and low-dose gilts might be due to placental transfer of maternal fatty acids shown to be abundant in the blood of diabetic pigs (Romso et al., 1971). An increase in maternal FFA levels can raise the rate of transport to the fetus (Sabasta et al., 1968). Fetal triglyceride synthesis appears to be the major source of the elevated liver and carcass lipid in low-dose as well as high-dose progeny.

Progeny insulin concentrations among the three groups were not affected by the treatments; however, there was a nonsignificant depression of serum insulin among the progeny of the high-dose group. Although Kasser et al. (1982) reported increased insulin release in fetal pigs from diabetic gilts, it is doubtful whether the classical hyperglycemia-hyperinsulinemia theory (Pedersen and Osler, 1961) can be applied to porcine animals. Insulin levels were reported to be undetectable in neonatal pigs (Swiatek et al., 1968; Kasser et al., 1981b) and in pigs from diabetic dams (Ezekwe and Martin, 1980). Using histological techniques, Erikson and Swenne (1982) showed that newborn rats from manifest diabetic dams had markedly retarded B-cell development as well as fewer insulin-positive tissues indicating that maternal diabetes is not always followed by fetal hyperinsulinemia.

The lack of significant differences in carcass weight, percentage ash and protein among the three groups (table 4) was in agreement with previous reports (Ezekwe and Martin, 1980; Kasser et al., 1981a). Increased availability of maternal supply of FFA to high-dose progeny may have been utilized for triglyceride synthesis, resulting in a threefold elevation of body lipid component (table 4). Body fat increase in pigs at birth would be expected to provide a source of energy and(or) insulation against heat loss during the neonatal period because pigs are born with only 1% body fat (Widdowsson, 1950). Higher energy status in pigs at birth would improve the chances of survival. Pigs from alloxan-diabetic dams have been shown to survive a 60-h fast better than control pigs (Kasser et al., 1982).

In conclusion, the data presented show that streptozotocin induced dose-related diabetes in pregnant gilts without adverse effects on the ability of the gilts to farrow live pigs. Energy reserves in the form of liver glycogen and lipids were elevated in the baby pigs and reflected the levels of maternal nutrient supply. Progeny carcass lipid was elevated in the severely diabetic group and unaffected by mild diabetes in the low-dose group.

**Literature Cited**


