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Use of Fetal Streptozotocin Injection To Determine the Role of Normal Levels of Fetal Insulin in Regulating Uteroplacental and Umbilical Glucose Exchange

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ABSTRACT. The present study was performed to determine the role of the normal fetal concentration of insulin in regulating placental-fetal glucose exchange. Fetal insulin deficiency was produced by streptozotocin injection into near term fetal sheep, and the effects of this insulin deficiency on net uteroplacental glucose uptake and net umbilical glucose uptake were measured. Each fetus received two or three doses of streptozotocin, 100 mg·kg^{-1}·dose^{-1}, given on separate days. This dosage of streptozotocin produced a 97.6% reduction in fetal pancreatic insulin content, a fall in fetal plasma insulin concentration (21 ± 2 to 10 ± 1 μU·ml^{-1}), a rise in fetal plasma glucagon concentration (57 ± 4 to 114 ± 19 pg·ml^{-1}), a rise in fetal blood glucose concentration (20.4 ± 0.9 to 33.4 ± 4.4 mg·dl^{-1}), and a failure of insulin secretion in response to glucose infusion. Fetal blood oxygen content and umbilical oxygen uptake were normal and did not change during the entire study. Umbilical glucose uptake was reduced by 66% (5.98 ± 0.38 to 2.02 ± 1.31 mg·min^{-1}·kg^{-1}) after the streptozotocin-induced hypoinsulinemia and hyperglycemia but was returned to the control level by an insulin infusion into the fetus that reestablished the control maternal to fetal glucose concentration gradient. Net uteroplacental glucose uptake (consumption) did not change throughout the study. Because glucose concentration and umbilical glucose uptake could be normalized by an insulin infusion, it is unlikely that direct or toxic effects of streptozotocin on fetal or placental glucose metabolism were primarily responsible for the hyperglycemia and the reduced rate of umbilical glucose uptake. Thus, changes in fetal glucose concentration primarily affected umbilical glucose uptake. Furthermore, the failure of the fetus to respond to a glucose infusion after streptozotocin injection with the same rate of exogenous glucose entry (umbilical uptake plus intravenous infusion) measured during the control period glucose infusion indicates that hypoinsulinemia contributed to the fetal hyperglycemia. These results indicate that the normal fetal insulin concentration determines net umbilical glucose uptake by regulating fetal glucose concentration. (*Pediatr Res* 24: 312–317, 1988)

Several recent investigations have indicated that insulin regulates fetal glucose metabolism. Increased concentrations of insulin produced in the fetal lamb by acute injection or chronic infusion have reduced glucose concentration (1, 2) and increased fetal uptake and use of glucose and oxygen (3, 4). Because these effects of insulin were observed with more than normal concentrations of insulin, however, the function of the "normal" concentration of insulin as a modulator of fetal metabolism remains speculative.

One approach to assess the function of the normal concentration of insulin has been to produce hypoinsulinemia and to compare measurements of potentially insulin-mediated metabolism during hypoinsulinemia with the same measurements made during normoinsulinemia. Experimental hypoinsulinemia has been produced with pancreatectomy (5), antiinsulin sera (6), and the diabetogenic drug streptozotocin (7, 8). These experiments have produced fetal hyperglycemia and reduced umbilical glucose uptake by the fetus. These results indicate that the normal level of insulin in the fetus plays a role in regulating fetal glucose concentration and fetal glucose supply. However, each of these experimental methods is not free of metabolic effects other than hypoinsulinemia. For example, pancreatectomy also removes pancreatic glucagon and its potential effects on fetal glucose metabolism. Antiinsulin serum may alter both insulin action and secretion and has only been studied in the fetal lamb in an acute, exteriorized, and presumably stressed state (6).

With regard to streptozotocin, it has been suggested that it may reduce insulin secretion separate from insulin concentration (8) and that it may inhibit tissue glucose uptake (fetal or uteroplacental) and/or uteroplacental glucose transport directly in addition to producing metabolic changes specific to hypoinsulinemia. If streptozotocin is to be used as an agent to induce fetal hypoinsulinemia, more definitive information about its effects on fetal and uteroplacental tissues is needed. Therefore, we conducted the experiments described in this report to more fully characterize the effect of fetal intravenous injections of streptozotocin on uteroplacental glucose uptake, net uteroplacental glucose consumption, and net umbilical glucose uptake by the fetus, as well as on fetal pancreatic insulin content, fetal pancreatic insulin secretion, and fetal plasma insulin concentration. Control experiments were conducted during normoinsulinemia followed by comparative experiments during hypoinsulinemia

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produced by fetal streptozotocin injection. Additional experiments with fetal glucose infusion and fetal insulin infusion were conducted to test if the capacity of the streptozotocin-treated fetus and its uteroplacenta to respond to changes in glucose and insulin concentrations had been altered by the streptozotocin injection per se.

MATERIALS AND METHODS

Animals. Eleven Columbia-Rambouillet ewes were used. Each ewe carried a single fetus. The ewes were fasted for 2 days before surgery but were allowed water and a mineral block *ad libitum.*

Operative procedures. Surgery was conducted at 126 ± 3 days of gestation (term = 145 days) under intravenous pentobarbital sedation and spinal anesthesia (6 mg pontocaine in 5 ml of 10% dextrose in water). Vascular catheters were placed according to the following scheme using techniques described previously (9). Twenty-gauge polyvinyl catheters were placed into the maternal femoral artery through a groin incision and after exposure of the uterus through a midline abdominal incision into the uterine vein draining the uterine horn containing the study fetus. Hysterotomy was performed and 20-gauge polyvinyl catheters were placed into the fetal abdominal aorta via a pedal artery, two fetal femoral veins via pedal veins, and the common umbilical vein. Ampicillin (250 mg) was placed into the amniotic cavity, and the ewe was given 500 mg of penicillin and 500 mg of streptomycin intramuscularly. The catheters were tunneled subcutaneously to the ewe's flank and kept in a plastic pouch secured to the ewe's skin.

The catheters were filled and flushed daily with 0.9% NaCl solution containing 30 U sodium heparin per ml. The ewes were given food (alfalfa pellets) and water *ad libitum* and were kept in carts until the studies were completed. At the conclusion of experiments for each animal, the ewe and fetus were killed with an intravenous injection of T-61 euthanasia solution (Taylor Pharmaceutical Co., Decatur, IL).

Experimental procedures. The ewes were allowed to recover for 5–7 days after surgery at which time they were consuming a normal amount of food and water, appeared healthy, and had normal and relatively constant arterial blood glucose and oxygen concentrations.

Control period studies. On the 6th or 7th postoperative day (132 ± 3 days gestation) a control period study was performed. At time zero a 10% solution of antipyrine was infused at about 0.10 $ml \cdot min^{-1}$ (10 $mg \cdot min^{-1}$) into the fetus via a saphenous vein catheter to measure umbilical blood flow. Between 120 and 150 min of infusion four sets of blood samples were drawn at 10-min intervals from the umbilical vein and artery catheters simultaneously. Blood from these samples was used to measure arterial plasma insulin and glucagon concentrations, whole blood glucose and antipyrine concentrations, and blood oxygen capacity, percent oxygen saturation, and oxygen content. After the initial 120 to 150-min basal sampling period glucose was infused into each fetus via a separate femoral vein catheter to raise and maintain arterial blood glucose concentration about 20 $mg \cdot dl^{-1}$ above the mean basal period glucose concentration. After 60 min at the higher glucose concentration, four arterial blood samples were drawn at 10-min intervals to measure plasma insulin and blood glucose concentrations. The purpose of this glucose infusion was to test the ability of the fetus to respond with insulin secretion to hyperglycemia.

Streptozotocin injection. After the control study, each fetus was injected with streptozotocin. Streptozotocin was purchased as a lyophilized powder from the Upjohn Company (Kalamazoo, MI) made up in citric acid buffered saline (5:1 ratio of streptozotocin to citric acid, pH of final solution about 4.5) and injected over 2 min into the fetus via a saphenous vein catheter. Each fetus received two ($n = 8$) or three ($n = 3$) injections of streptozotocin (one injection per day) each of 100 $mg \cdot kg^{-1}$ estimated fetal weight per gestational age. In preliminary trials, single doses of less than 40 $mg \cdot kg^{-1}$ fetal weight did not consistently produce any measurable change in fetal glucose or insulin concentration whereas single doses of 300 $mg \cdot kg^{-1}$ or more resulted in a 20% mortality rate (three of four fetuses that died were less than 115 days gestation, and two of these were small for gestational aged twins). Each fetus was sampled daily for measurement of arterial blood glucose concentration, arterial plasma insulin concentration, and blood oxygen content.

Streptozotocin studies. On the 5th to 7th day after completing the streptozotocin injections (138 ± 3 days of gestation), a second study was conducted identical to the control study including both the basal and the hyperglycemic periods. In six of the fetuses at the end of the second study the glucose infusion was stopped, and insulin was infused at 2–3 $mU \cdot min^{-1} \cdot kg^{-1}$ estimated fetal weight until the fetal arterial blood glucose concentration and the maternal-fetal glucose concentration gradient reached the control study basal period levels. At this point blood samples from the maternal artery, uterine vein, umbilical vein, and fetal artery were drawn at 10-min intervals over 30 min. These samples were analyzed for whole blood antipyrine and glucose concentrations and were used to calculate uterine, uteroplacental, and fetal glucose uptakes to test if streptozotocin had affected directly the placental transfer capacity for glucose or the capacity for fetal glucose uptake.

On the day after the streptozotocin period studies (139 ± 3 days of gestation) the ewe and fetus were killed by a 10-s injection of T-61 intravenous euthanasia solution. The fetus was extracted from the uterus within 1 min and weighed. The entire fetal pancreas was removed quickly, placed immediately into 2.0 ml of 0.01 N HCl, and frozen to −70°C for insulin content analysis at a later date. Pieces of each pancreas (from both proximal and distal portions) were fixed in Bouin's solution for insulin and glucagon immunohistochemical analysis while pieces of fetal liver, kidney, and pancreas were fixed in formalin for routine histological examination. Similar autopsies were performed on six normal, noninstrumented fetal sheep. The entire fetal pancreas was removed by visual inspection and more than 75% (including both proximal and distal portions) was used for determination of insulin content.

Analytical methods. Blood for oxygen analysis was drawn into glass capillaries lined with dried sodium fluoride and heparin. Blood for insulin, glucagon, glucose, and antipyrine was drawn into plastic syringes lined with EDTA powder. Whole blood antipyrine was measured using a Technicon autoanalyzer. Whole blood glucose was determined using a glucose oxidase method (Sigma Chemical Co., St. Louis, MO). Blood oxygen capacity, percent saturation, and content were measured with a Radiometer OSM2 hemoximeter calibrated with sheep blood. Blood for glucagon was placed into a separate plastic centrifuge tube that contained 1.2 mg EDTA and 50 U of aprotinin (Trasylol) per ml of blood. Plasma for insulin and glucagon was separated in a refrigerated centrifuge and stored at −70° C until analysis. Plasma insulin and glucagon were measured with double antibody methods using kits from Serono. Ovine insulin and glucagon standards were provided by the Eli Lilly Company (Indianapolis, IN). Pancreatic insulin content was measured using the technique reported by Brinsmead and Thorburn (7) and a Serono double antibody insulin kit.

Calculations. All calculations were made using equations appropriate for steady-state kinetics. Umbilical and uterine blood flows were calculated using the antipyrine steady-state diffusion technique of Meschia *et al.* (10). Net umbilical and uterine uptake rates for glucose and oxygen were calculated by the Fick principle as the product of umbilical or uterine blood flow times the umbilical venoarterial or uterine arteriovenous glucose concentration and oxygen content differences, respectively. Net uteroplacental glucose and oxygen uptakes were calculated as the difference between the net uterine and fetal uptakes. During the

glucose infusion period, the exogenous glucose infusion rate was calculated as the product of the pump infusion rate and the infusate glucose concentration (the latter was measured directly), and total exogenous glucose entry into the fetus was calculated as the sum of the exogenous glucose infusion and net umbilical glucose uptake.

Statistical analysis. Comparisons of results were made with paired and unpaired *t* tests and by two-way analysis of variance. Results are expressed as mean ± SEM (unless otherwise noted). Differences were considered significant at $p < 0.05$. Glucose and oxygen flux rates are expressed per kg of fetal weight at autopsy.

RESULTS

Control studies were conducted at 132 ± 3 (range) days gestational age; streptozotocin studies were conducted 6–7 days later. At autopsy [139 ± 3 (range) days] fetal weight was 3.48 ± 0.14 kg, which was normal for gestational age (3).

Blood glucose values increased significantly ($p < 0.05$) and plasma insulin values decreased significantly ($p < 0.05$) by the 2nd to 3rd day after completing the streptozotocin injections (Fig. 1). At the time of the streptozotocin basal period studies (Table 1) glucose values were 64.2 ± 2.2% higher ($p < 0.01$), plasma insulin values were 50 ± 1% lower ($p < 0.01$), and glucagon values were 100.7% higher ($p < 0.001$) than mean control study, basal period values. Blood oxygen capacity, saturation, and content were normal and did not change significantly throughout the entire study.

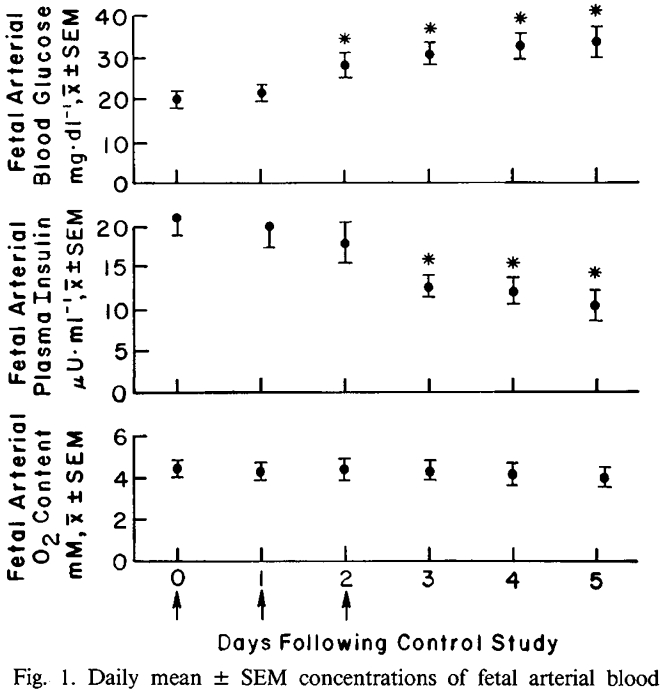

Fig. 1. Daily mean ± SEM concentrations of fetal arterial blood glucose (*top*), plasma insulin (*middle*), and blood oxygen content (*bottom*) after the once-daily streptozotocin injections (*arrows*). *Asterisks* indicate significant differences ($p < 0.05$) from the control study mean values on day 0.

Calculated flux rates in each sampling period are shown in Table 2. Umbilical blood flow did not change significantly from the basal period to the glucose infusion period in each study ($p > 0.2$), but umbilical blood flow during the streptozotocin studies was significantly reduced by 16.3% from the basal period in the control period to the basal period in the streptozotocin study ($p < 0.05$). Also, umbilical glucose uptake was reduced by 66.2% ($p < 0.001$) from the basal period in the control study to the basal period in the streptozotocin study. Umbilical oxygen uptake was not different between the basal periods in the control and the streptozotocin studies ($p > 0.5$).

During the glucose infusion period of the control study, fetal arterial blood glucose averaged 100.4% more than the mean value for the basal period of the control study, a mean increment of 20.4 mg·dl^{-1} ($p < 0.001$). At the same time fetal arterial plasma insulin concentrations increased by 15 uU·ml^{-1} or 71.4% ($p < 0.001$). During the glucose infusion period of the streptozotocin study, blood glucose averaged 44.8% more the mean value for the basal period of the streptozotocin study, a mean increment of 15.0 mg·dl^{-1} ($p < 0.001$). Insulin concentration did not change significantly from the mean value of the basal period of the streptozotocin study ($p > 0.5$).

The glucose infusion in each study completely blocked umbilical glucose uptake (Table 2). During the glucose infusion of the control study, umbilical glucose uptake (−0.39 ± 0.55 mg·min^{-1}·kg^{-1}) and intravenous glucose infusion (9.92 ± 0.71 mg·min^{-1}·kg^{-1}) totaled 9.53 ± 0.38 mg·min^{-1}·kg^{-1} of exogenous glucose entry. During the glucose infusion of the streptozotocin study, umbilical glucose uptake (−0.76 ± 0.70 mg·min^{-1}·kg^{-1}) and intravenous glucose infusion (5.25 ± 0.79 mg·min^{-1}·kg^{-1}) totaled 4.49 ± 0.78 mg·min^{-1}·kg^{-1} of exogenous glucose entry. The total exogenous glucose entry rate during the glucose infusion of the streptozotocin study was significantly less than during the glucose infusion of the control study by 5.4 ± 0.59 mg·min^{-1}·kg^{-1}, $p < 0.001$.

During the insulin infusion at the end of the streptozotocin studies (Table 3) fetal arterial blood glucose concentration decreased to 19.89 ± 0.96 mg·dl^{-1}, not different from the value of 18.84 ± 0.93 mg·dl^{-1} during the basal period of the control studies ($p > 0.1$) (Fig. 2). At the same time the maternal-fetal arterial plasma glucose concentration gradient increased to 42.10 ± 3.31 mg·dl^{-1}, not different ($p > 0.5$) from the value of 44.4 ± 3.6 mg·dl^{-1}. During the basal period of the control studies, umbilical glucose uptake by the fetus was not different between these two periods ($p > 0.5$), and uteroplacental glucose uptake and net uteroplacental glucose uptake (consumption) were not different among the basal period of the control study, the basal period of the streptozotocin study, or the streptozotocin plus insulin study ($p > 0.5$). Values for umbilical glucose uptake by the fetus are shown in Figure 3 and are compared with the range

Table 1. *Fetal glucose, insulin, glucagon, and oxygen values [mean (SEM) n = 11]**

	Control				Streptozotocin			
	Basal		Glucose infusion		Basal		Glucose infusion	
Fetal arterial plasma insulin (μU·ml^{-1})	21	(2)	36	(4)[a]	10	(1)[b]	10	(1)[e]
Fetal arterial plasma glucagon (pg·ml^{-1})	57	(4)			114	(19)[b]		
Umbilical arterial blood glucose (mg·dl^{-1})	20.36	(0.88)	40.80	(1.46)[a]	33.43	(4.40)[b]	48.40	(3.47)[d]
Umbilical venous blood glucose (mg·dl^{-1})	23.49	(0.88)	40.48	(1.45)[a]	34.49	(4.03)[b]	47.97	(3.41)[d]
Umbilical arterial blood O_2 content (mM)	4.27	(0.20)	3.79	(0.16)[a]	3.99	(0.16)	3.47	(0.20)[d]
Umbilical venous blood O_2 content (mM)	5.95	(0.23)	5.66	(0.23)	5.96	(0.19)	5.41	(0.21)
Umbilical arterial blood O_2 capacity (mM)	6.9	(0.2)	6.8	(0.2)	7.1	(0.3)	6.9	(0.4)
Umbilical arterial O_2 saturation (%)	61.9	(2.0)	63.4	(2.2)	56.2	(2.0)[c]	50.1	(2.1)[d]

* Control period hyperglycemia different from control basal; [a] $p < 0.01$. Streptozotocin period basal different from control basal [b] $p < 0.05$, [c] $p < 0.01$, Streptozotocin period hyperglycemia different from streptozotocin basal, [d] $p < 0.05$; [e] $p < 0.01$.

Table 2. *Values for umbilical blood flow and glucose and oxygen flux rates [mean (SEM) n = 11]**

	Control				Streptozotocin			
	Basal		Glucose infusion		Basal		Glucose infusion	
Umbilical blood flow ($ml \cdot min^{-1} \cdot kg^{-1}$)	209	(18)	201	(16)	175	(12)[b]	167	(10)
Umbilical glucose uptake ($mg \cdot min^{-1} \cdot kg^{-1}$)	5.98	(0.38)	−0.39	(0.55)[a]	2.02	(1.31)[c]	−0.76	(0.70)[e]
Fetal glucose infusion ($mg \cdot min^{-1} \cdot kg^{-1}$)			9.92	(0.71)			5.25	(0.79)
Exogenous fetal glucose entry† ($mg \cdot min^{-1} \cdot kg^{-1}$)	5.98	(0.38)	9.53	(0.38)	2.02	(1.31)[c]	4.49	(0.78)[d]
Umbilical O_2 uptake ($mmol \cdot min^{-1} \cdot kg^{-1}$)	0.351	(0.026)	0.379	(0.032)	0.345	(0.019)	0.307	(0.013)[d]

* Control hyperglycemia different from control basal, [a] $p < 0.01$. Streptozotocin basal different from control basal, [b] $p < 0.05$; [c] $p < 0.01$. Streptozotocin hyperglycemia different from streptozotocin basal, [d] $p < 0.05$; [e] $p < 0.01$.

† Exogenous glucose entry equals umbilical glucose uptake plus fetal intravenous glucose infusion.

Table 3. *Uteroplacental glucose exchange n = 6*

	Blood glucose concentration			Net glucose uptake		
	$mg \cdot dl^{-1}$, mean (SEM)			$mg \cdot min^{-1}$, mean (SEM)		
	Maternal artery	Fetal artery	Maternal artery–fetal artery	Uterus	Fetus	Uteroplacenta
Control	49.01	18.84	30.50	31.50	12.91	18.91
	(2.04)	(0.93)	(2.57)	(1.82)	(0.86)	(1.95)
Streptozotocin	50.83	29.46*	21.33*	24.65*	4.95*	19.70
	(1.90)	(1.79)	(2.56)	(2.71)	(1.17)	(2.96)
Streptozotocin + insulin	48.29	19.89	28.40	30.13	13.03	17.68
	(1.73)	(0.96)	(2.31)	(2.18)	(1.65)	(1.19)

* Value significantly different from control ($p < 0.05$).

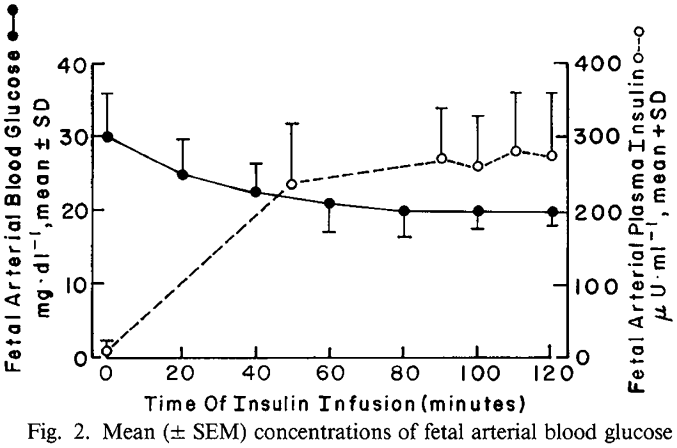

Fig. 2. Mean (± SEM) concentrations of fetal arterial blood glucose (●) and plasma insulin (○) in six animals during the insulin infusion at the end of the streptozotocin period studies. By 60 min of insulin infusion a new steady state was achieved for glucose and insulin concentrations. Blood sampling for measurement of umbilical glucose uptake by fetus and net uteroplacental glucose uptake (consumption) were made between 90 and 120 min of insulin infusion.

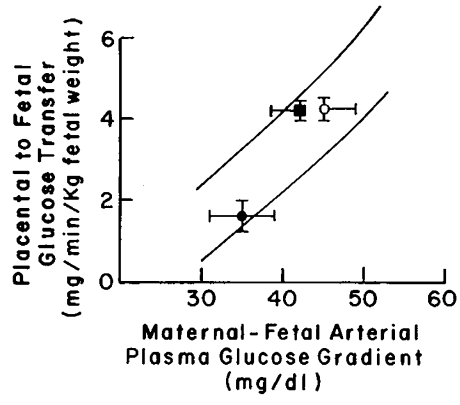

Fig. 3. Umbilical glucose uptake in six animals in the control (○) and streptozotocin (●) basal periods and in the streptozotocin plus insulin period (■) are compared with the normal range (*solid lines*: 95th percentile upper, 5th percentile lower) of umbilical glucose uptake at different maternal-fetal glucose concentration gradients in our laboratory (adapted from data published in Ref. 9).

of values for these measurements found in eight normal animals studied at the same gestational age in our laboratories.

At autopsy pancreatic insulin concentrations were markedly reduced in the streptozotocin-injected fetuses (0.08 ± 0.02 $U \cdot g^{-1}$) averaging 2.4% of the values in the six normal fetuses (3.77 ± 0.75 $U \cdot g^{-1}$).

DISCUSSION

In our study, streptozotocin injections into near term fetal lambs resulted in a 50% decrease in fetal plasma insulin concentration that was associated with a 97.6% reduction in pancreatic insulin content and a failure of insulin secretion to occur in response to hyperglycemia produced by fetal glucose infusion. In addition to the hypoinsulinemia, fetal hyperglycemia developed together with reduced umbilical glucose uptake by the fetus. Because fetal glucose concentration and umbilical glucose uptake could be normalized by an insulin infusion, it is unlikely that direct or toxic effects of streptozotocin on the fetus or the uteroplacenta were primarily responsible for the hyperglycemia and the reduced rate of umbilical glucose uptake. These results indicate that changes in fetal glucose concentration primarily affected fetal glucose uptake from the umbilical circulation. Whether or not the hyperglycemia resulting from the hypoinsulinemia was produced by a reduction of insulin suppression of

endogenous glucose production or by a decrease of insulin-mediated glucose utilization cannot be determined by this study.

In other animal studies, streptozotocin has reduced plasma and pancreatic insulin concentration by both an acute (1–3 days) direct cytotoxic action on the pancreatic β cells (11) and over a longer period (> 1 wk) by an apparent immune injury to the β cells (12). Streptozotocin also causes injury of a nonspecific nature to liver and kidney cells (11). The mechanism of this cytotoxicity is not known nor is the reason(s) for the selectivity of the tissue and cell injury.

Use of streptozotocin in fetal lambs has received only limited study. In an abstract report Shelley (13) described results of administration of streptozotocin at quite high doses (200–400 $mg \cdot kg^{-1}$ fetal weight) to late gestation fetal lambs documenting subsequent fetal hyperglycemia and an impaired insulin secretion in response to acute hyperglycemia. Similar observations were made by Brinsmead and Thorburn (7) who gave comparable high doses of streptozotocin to midgestation (70–85 days) fetal lambs. The few (four of 11) fetuses that survived until near term also were hyperglycemic relative to a group of normal fetuses (18 $mg \cdot dl^{-1}$ for streptozotocin-injected fetuses *versus* 9.4 $mg \cdot dl^{-1}$ for control) and did not show a marked increase in plasma insulin concentration in response to acute hyperglycemic challenge. Recently, Philipps *et al.* (8) reported that injection of one dose of 100 $mg \cdot kg^{-1}$ fetal weight of streptozotocin into fetal lambs resulted in progressive fetal hyperglycemia and reduced umbilical glucose uptake although they did not observe a change in fetal plasma insulin concentration. In these studies, however, experiments were conducted only 1 and 2 days after the streptozotocin injection.

The present study adds to these previous reports in several respects. First, plasma glucagon levels increased after streptozotocin suggesting an intact capacity for glucagon secretion. Second, oxygen levels and umbilical oxygen uptake were normal in the streptozotocin-treated fetuses, both in relation to their own values during a control study and when compared to other normal animals studied in our laboratory at the same time (4). Thus, streptozotocin injections and the resulting metabolic changes did not affect the magnitude of fetal oxygen consumption. Given the markedly reduced umbilical glucose uptake by the streptozotocin-treated fetuses, their oxygen consumption must have been fueled by other substrates (*i.e.* endogenously produced glucose, amino acids, or lactate).

Third, umbilical glucose uptake was significantly and markedly reduced after streptozotocin injections. We have shown previously that high levels of insulin in fetal lambs have no effect on uteroplacental transfer of glucose to the fetus (3). In the present study there was no evidence that the reduced umbilical uptake of glucose by the fetus was due to a selective effect of hypoinsulinemia on uteroplacental glucose transfer. Thus, the fetal hyperglycemia in the present study resulted from the hypoinsulinemia and secondarily reduced the maternal-fetal arterial plasma glucose concentration gradient thereby reducing uteroplacental transfer of glucose to the fetus. This hypothesis is corroborated by two further observations: *1*) the glucose infusion into the streptozotocin-treated fetuses produced an increase in glucose concentration and a further reduction in umbilical glucose uptake but no change in fetal insulin concentration; *2*) insulin infusion into the streptozotocin-treated fetuses reestablished the maternal-fetal arterial plasma glucose concentration gradient and the rate of umbilical glucose uptake that were present during the normal basal period of the control study. Thus, change in uteroplacental to fetal glucose transfer was consistently dependent on changes in fetal glucose concentration but not dependent at all on changes in fetal insulin concentration. These observations demonstrate that at least at the levels of maternal and fetal glucose concentration in these studies the net rate of uteroplacental glucose uptake is not regulated by fetal insulin concentration. These observations corroborate a similar demonstration in normal fetal sheep reported previously (3). Furthermore, because there was not a significant reduction in net uteroplacental glucose uptake with streptozotocin-induced hypoinsulinemia, these observations also imply that uteroplacental glucose transfer and net uptake were not altered by direct effects of streptozotocin.

Mechanisms responsible for the fetal hyperglycemia after streptozotocin injection were not addressed by this study. Several possible mechanisms seem reasonable, acting alone or together. First, the hypoinsulinemia may have reduced fetal cellular glucose uptake. This reduced uptake may have been due to a direct reduction of insulin stimulation of insulin receptors and/or to the secondary development of insulin resistance that appears to be a part of experimental, streptozotocin-induced diabetes mellitus (14). Second, the hyperglycemia may have been due to enhanced fetal endogenous glucose production due either to a release of insulin suppression of glucose production or to the increased activity of other stimulants of glucose production such as hypoxia (15), glucagon (16), and adrenalin (17). Glucagon levels increased approximately 2-fold in these fetuses, although much greater increases have been found necessary to produce fetal hyperglycemia and endogenous glucose production in normal glycemic, normal insulinemic fetal sheep (18). Other counterregulatory hormone levels were not assayed in the present study. Finally, it is possible that streptozotocin may have produced fetal hyperglycemia by directly limiting fetal cellular glucose uptake, independent of the reduction of insulin effect. In the present study this possibility seems unlikely given the data presented in Table 3 and Figure 3 that show return to control values of umbilical glucose uptake with restoration of control values for fetal glucose concentration and maternal-fetal arterial plasma glucose gradient using an insulin infusion into the fetus.

In conclusion, the present study provides evidence that streptozotocin induces fetal insulin deficiency by reducing pancreatic insulin content, insulin secretory capacity, and hypoinsulinemia. In response to the fetal hypoinsulinemia, fetal hyperglycemia develops, reducing the maternal-fetal glucose concentration gradient and umbilical glucose uptake. These data indicate that the normal concentration of insulin in the fetus determines uteroplacental-fetal glucose exchange indirectly by regulating fetal glucose concentration.

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