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Limited Maternal Fuel Availability due to Hyperinsulinemia Retards Fetal Growth and Development in the Rat

EDWARD S. OGATA, RONALD I. PAUL, AND SANDRA L. FINLEY

Departments of Pediatrics, Obstetrics and Gynecology, Northwestern University Medical School, Northwestern Memorial Hospital, and Children's Memorial Hospital, Chicago, Illinois

ABSTRACT. We rendered pregnant rats chronically hyperinsulinemic to determine the effect of reduced maternal metabolic fuel availability on fetal growth and development. We implanted osmotically driven insulin loaded minipumps on day 14 (term 21.5 days) in pregnant rats. This significantly increased maternal plasma concentrations of insulin and reduced glucose from day 15 until term. From day 17 until birth, fetal growth was significantly less for hyperinsulinemic mothers (term birth weight 4.53 ± 0.07 *versus* 5.64 ± 0.06 g, $p < 0.001$). In fetuses of hyperinsulinemic mothers plasma glucose and insulin concentrations were significantly reduced while glucagon concentrations were increased. Total plasma amino acids were significantly reduced in maternal rats and their fetuses from days 17 to 19 while arteriovenous blood gas tensions and pH did not differ between fetuses of hyperinsulinemic and control mothers. Small for gestational age newborn pups of hyperinsulinemic mothers were hypoglycemic for the first 240 min of life as a result of limited hepatic glycogen stores and a delay in the normally expected induction of hepatic cytosolic phosphoenolpyruvate carboxykinase. This occurred despite significant increases in neonatal plasma glucagon concentrations. These data indicate that limitation of maternal glucose and amino acids with normal placental gaseous exchange retards fetal growth, limits hepatic glycogen deposition, and delays neonatal phosphoenolpyruvate carboxykinase induction. Limited fetal insulin secretion resulting from diminished maternal fuel availability may have also been a factor in retarding growth. The delay in phosphoenolpyruvate carboxykinase induction despite enhanced glucagon secretion during fetal and neonatal life suggests a specific "resistance" to this hormone in the rat growth retarded by limited metabolic fuel availability. (*Pediatr Res* 22: 432–437, 1987)

Abbreviations

PEPCK, phosphoenolpyruvate carboxykinase
BOHB, betahydroxybutyrate
SGA_I, small for gestational age related to maternal hyperinsulinemia

Intrauterine growth retardation is often ascribed to "uteroplacental insufficiency," (1) a poorly defined condition. In previous studies, we mimicked "uteroplacental insufficiency" in the rat by maternal uterine artery ligation, a well-established method for retarding fetal growth (2, 3). This reduces fetal arteriovenous oxygen tension and pH, increases carbon dioxide tension, and reduces glucose and branched-chain amino acid concentrations in the fetus (4). The relative importance of each alteration in limiting fetal growth is unknown.

A method (5) by which the maternal rat is rendered chronically hyperinsulinemic during late gestation offered a potential means to determine the extent to which limiting metabolic fuels without altering blood gas tensions or pH affects fetal growth and development. Using this, we found that maternal hyperinsulinemia decreases concentrations of glucose and amino acids in the mother and fetus. This limits fetal growth although not to the same degree as with maternal uterine artery ligation in which both fuel availability and gaseous exchange are impaired. This restriction of maternally derived metabolic fuels also affects fetal biochemical development since growth retarded newborn pups of hyperinsulinemic mothers become hypoglycemic as a result of limited glycogen stores and delayed induction of hepatic PEPCK.

MATERIALS AND METHODS

We adapted the method of Grupposo *et al.* (5) to cause maternal hyperinsulinemia. On day 14 (term 21.5 days) of gestation, we anesthetized 9-wk-old pregnant rats with ketamine (30 mg/kg body weight) and implanted a 2.2 × 0.5 cm osmotically driven minipump in the subcutaneous tissue of the dorsum of the neck after local administration of 1% xylocaine. Animals awakened within 1 h. The minipump (Alzet Corp., Palo Alto, CA) had been filled with porcine insulin in glycerol (Iletin II, U-500, Eli Lilly, Indianapolis, IN) and placed in 154 mM NaCl 24 h before implantation to prepare its cellulose ester membrane. Once implanted, the minipumps continuously deliver 0.42 U of insulin/h for the duration of gestation. Control rats received minipumps loaded with 154 mM NaCl. Rats had *ad libitum* access to standard rat chow (Purina, St. Louis, MO). Rats with insulin pumps were supplied with a glucose solution (10% v/v) rather than water. We measured maternal weight and food intake daily and obtained blood from the cut tail tip of awake mothers.

We performed two series of studies to characterize the effect of maternal hyperinsulinemia in the offspring. In the first series, we sequentially measured growth and metabolic variables of fetuses from days 18 through 21 of gestation, and in the second series we measured metabolic fuels and hormones related to glucoregulation in newborn pups during the first 240 min of life. We used only fetuses and pups from mothers with eight or more in a litter to control for the effect of litter size on fetal growth.

Fetal studies. To estimate maternal-fetal fuel provision and gaseous exchange, we adapted the method of Girard *et al.* (6) in which blood is sampled from the fetus with the fetal-placental

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Correspondence Edward S. Ogata, M.D., Division of Neonatology, Prentice Women's Hospital, 333 East Superior Street, Room 1106, Chicago, IL 60611.
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circulation intact. The mother was anesthetized and the uterus exposed and sequentially incised so that individual fetuses could be removed leaving the placenta attached to the uterine wall. We sampled only two to three fetuses from a litter in order to avoid any potential effect of prolonged maternal anesthesia. Thus eight to 12 litters are represented in each group. The fetuses were dried and maintained at 37° C with heat lamp and warm moist gauze, and care was taken not to apply tension to the umbilical cord. Fetal blood was obtained from cut axillary vessels and maternal blood obtained simultaneously from the cut tail tip. After blood sampling the fetus was killed by decapitation and the liver quickly removed and frozen in liquid nitrogen. In selected litters, we measured mixed arteriovenous gas tensions and pH as previously reported (4). We measured carcass and placental weight, plasma concentrations of glucose, insulin, and glucagon, hepatic cytosolic PEPCK activity, and glycogen concentrations. We could measure only fetal body weight and plasma glucose concentrations on days 14 to 16.

Newborn studies. We have reported our technique for determination of metabolic variables in the newborn (3). On day 21.5, rat pups were delivered by cesarean section after stunning and cervical dislocation of the mother. Pups were quickly dried, separated from their placentas, and placed in chambers which provide humidified room air at 37 ° C. Pups had blood collected from the severed axillary artery and were then decapitated. The liver was quickly frozen. We measured sequentially the changes in glucose, insulin, glucagon, and hepatic glycogen concentrations in littermates of hyperinsulinemic and control mothers at 0, 20, 60, 120, and 240 min and hepatic cytosolic PEPCK activity at 0, 120, and 240 min.

Analyses. Plasma glucose concentrations (Beckman II Glucose Analyzer) were determined for each fetus and pup. Blood for hormone analysis was treated with Aprotinin (Trasylol 25:1 V/V) and the plasma frozen at −20° C until assay. The limited amount of plasma obtained from each fetus or pup sometimes precluded measurement of both insulin and glucagon in each sample. Similarly, both glycogen and PEPCK could not be measured for each fetus or pup because of limited liver mass. A separate series of fetuses and their mothers had plasma amino acid concentrations determined.

Our analytical techniques have been reported (2–4). Both plasma insulin and glucagon concentrations were determined with double antibody radioimmunoassay with rat insulin and porcine glucagon respectively as standards. Hepatic cytosolic PEPCK activity and glycogen concentrations were determined as before. Mixed arteriovenous blood gas tensions and pH were determined with an ILC 1301 automated blood gas analyzer. Plasma BOHB and amino acids were determined as before.

Statistical analysis was performed using the Student's paired *t* test for comparison of values within a series and unpaired *t* test for comparison of values between groups (7). Analysis was performed on a Cyber 170-780 computer at the Vogelback Computer Center of Northwestern University. Data are presented as the mean ± SEM. Values from at least 25 fetuses or pups are represented in each measurement.

RESULTS

Maternal. Maternal rats with insulin loaded pumps had significantly diminished plasma glucose and elevated insulin concentrations compared to controls from day 15 until term (Fig. 1). Plasma BOHB did not differ between hyperinsulinemic and control mothers and ranged from 0.050 to 0.069 μmol/ml on each day of gestation.

While maternal weight gain expressed as body weight or percent increase from baseline did not differ between hyperinsulinemic and control rats, as gestation progressed the weight of the mother excluding uterine contents was significantly greater in hyperinsulinemic than control mothers as a result of the retarded fetal growth of hyperinsulinemic mothers. We compared the

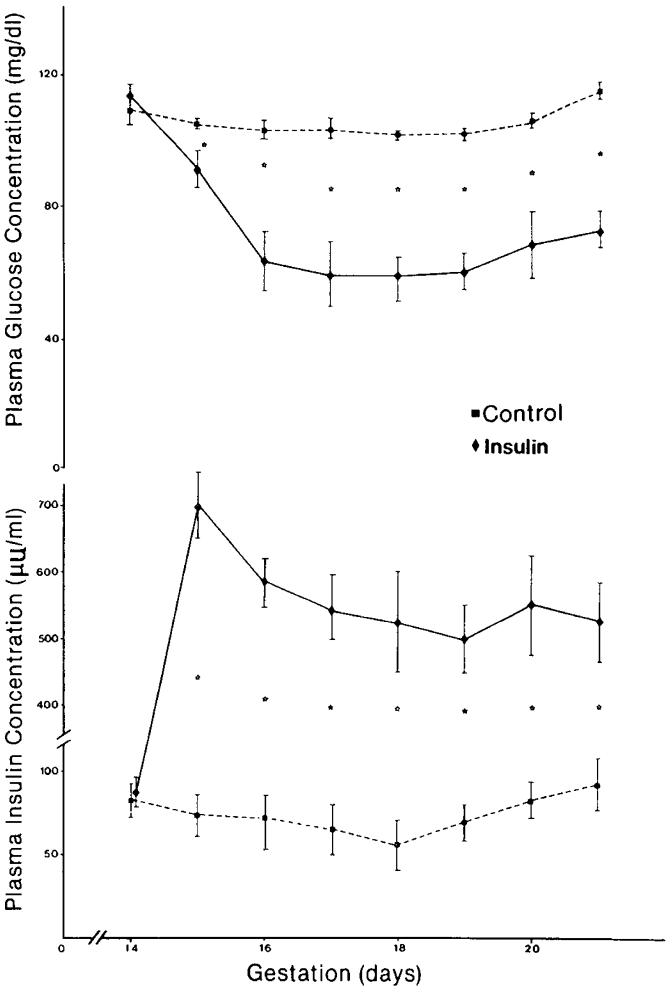

Fig. 1. Maternal plasma glucose and insulin concentrations. By 24 h following implantation, mothers with insulin-loaded pumps have significantly diminished plasma glucose and elevated insulin concentrations (* $p < 0.01$ to 0.001).

weight of the total conceptus relative to that of the mother by the relation: mass of all fetuses and placentas/total maternal weight (8). The total conceptus on day 17 comprised approximately 0.025 of total maternal weight in controls and 0.020 in mothers receiving insulin. By day 21, this was 0.19 of total body weight in controls and 0.16 in mothers receiving insulin. The values in controls reflect the rapid growth of the conceptus relative to maternal weight during late gestation. These values were significantly less for hyperinsulinemic mothers from day 17 onward ($p < 0.01$ to 0.001).

Fetal. From days 14 to 16, fetal body and placental weight did not differ between hyperinsulinemic and control mothers, while from day 17 onward, fetuses and placentas of hyperinsulinemic mothers were significantly lighter. We therefore termed the fetuses of hyperinsulinemic mothers as SGA_I (Fig. 2). On days 15 through 21, SGA_I fetuses had significantly lower plasma glucose concentrations than controls. This probably resulted from diminished maternal glucose concentrations since fetal/maternal glucose ratios were equivalent between SGA_I and control mothers on all days (Table 1). Hepatic concentrations of glycogen were significantly lower in fetuses of hyperinsulinemic mothers from days 17 to 21. From days 19 to 21, hepatic glycogen concentrations in SGA_I fetuses were 10.0 ± 0.3, 18.1 ± 3.2, and 34.1 ± 5.0 mg/g liver and in control fetuses, 29.0 ± 6.3, 38.9 ± 5.8, and 49.0 ± 4.6 mg/g liver ($p < 01$ to .001 for all values).

On days 17, 18, and 19, total amino acids were significantly

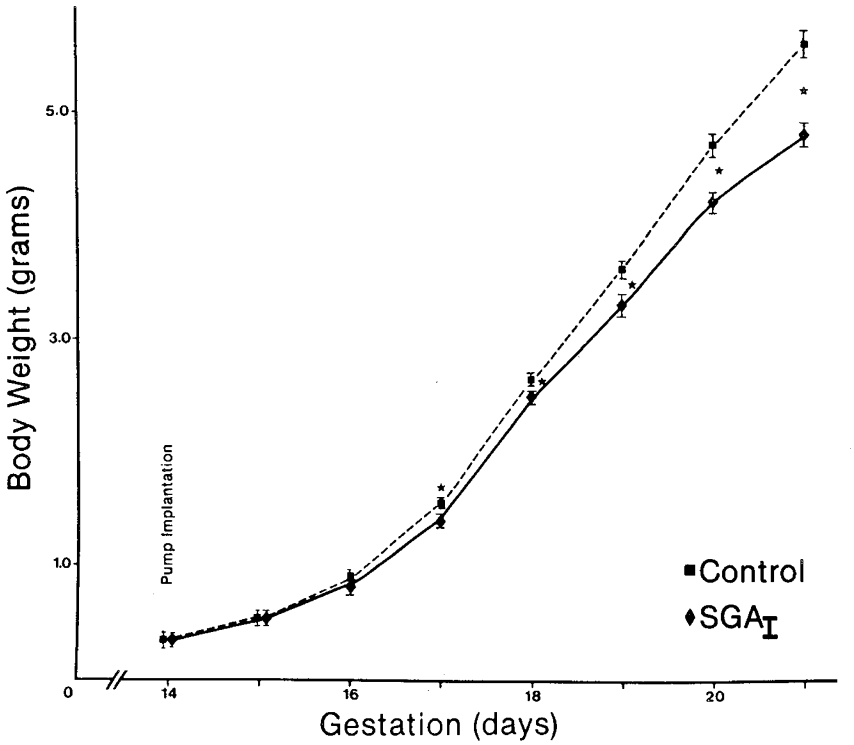

Fig. 2. Intrauterine growth of pups hyperinsulinemic (SGA_I) and control mothers. From day 17 onward, SGA_I fetuses weighed significantly less than controls (* $p < 0.01$ to 0.001).

Table 1. *Fetal plasma glucose (mg/dl) and fetal/maternal glucose ratios*

Gestation (days)	15	16	17	18	19	20	21
Glucose							
SGA_I *	20.8 ± 1.0 *	21.7 ± 1.2 *	20.6 ± 0.8 *	25.1 ± 2.7 *	34.6 ± 2.9 *	47.3 ± 3.7 *	56.7 ± 2.7 *
Control	49.0 ± 6.0	51.3 ± 6.6	53.4 ± 5.7	49.1 ± 2.3	54.8 ± 5.3	70.1 ± 5.3	68.7 ± 4.5
Fetal/maternal glucose							
SGA_I	0.44 ± 0.04	0.49 ± 0.06	0.47 ± 0.04	0.48 ± 0.06	0.48 ± 0.04	0.62 ± 0.03	0.58 ± 0.03
Control	0.40 ± 0.05	0.50 ± 0.10	0.57 ± 0.06	0.51 ± 0.08	0.55 ± 0.07	0.61 ± 0.03	0.62 ± 0.06

* $p < .01$ to .001.

diminished in SGA_I fetuses compared to controls (day 17, 6.03 ± 0.40 *versus* 9.03 ± 0.50 mM, $p < 0.01$). This resulted from the significant diminution of plasma amino acids in the hyperinsulinemic mothers (day 17, 2.51 ± 0.39 *versus* 3.88 ± 0.39 mM, $p < 0.01$). While total values differed, no consistent differences in specific amino acids could be discerned. On days 20 and 21, there were no differences between SGA_I and control fetuses nor between their mothers.

Plasma insulin concentrations were significantly diminished and glucagon concentrations increased (Fig. 3) in SGA_I fetuses on days 17 through 21. SGA_I and control fetuses had similar blood gas tensions and pH from days 18 to 21. On day 19, SGA_I fetuses had values of pH 7.20 ± 0.008; PCO_2 61 ± 5 torr; PaO_2 24 ± 3 torr; while controls had pH 7.20 ± 0.008; PCO_2 60 ± 5 torr; PO_2 25 ± 3 torr. These values did not differ. The hypercarbia reflects the mild maternal hypoventilation resulting from anesthesia in both groups which we have previously observed.

Fetal weight correlated with fetal plasma glucose for SGA_I and control fetuses on days 20 and 21 (day 20: $r = 0.3256$, $n = 55$, $p < 0.01$; day 21: $r = 0.5730$, $n = 56$, $p < 0.001$), but not with fetal insulin concentrations.

Neonatal. Newborn SGA_I pups were significantly lighter than controls (4.53 ± 0.07 *versus* 5.64 ± 0.06g, $p < 0.001$) and had significantly diminished plasma glucose concentrations during the first 240 min of life (Fig. 4) and decreased plasma insulin concentrations at birth (Fig. 5). Plasma glucagon concentrations were significantly elevated at birth and 20 min. (Fig. 5).

SGA_I pups had significantly diminished hepatic glycogen values from birth to 120 min. Both SGA_I and control pups decreased hepatic glycogen during the first 240 min (Table 2).

Hepatic PEPCK activity. Fetuses of hyperinsulinemic and control mothers had equally low hepatic cytosolic PEPCK activity which did not change during fetal life. Control pups significantly increased PEPCK activity between birth and 240 min while SGA_I pups did not (Table 2).

DISCUSSION

Maternal hyperinsulinemia from day 14 until term reduces maternal and fetal plasma concentrations of glucose and amino acids and is associated with retarded fetal growth and delayed biochemical maturation. The effects of fuel limitation extend into the neonatal period since SGA_I pups developed hypoglycemia because of limited hepatic glycogen stores and PEPCK activity. Fetal and neonatal glucagon secretion was appropriate under these circumstances but did not induce PEPCK or correct neonatal hypoglycemia.

The limitation of concentrations of maternal glucose from

days 15 to 21 and amino acids from days 17 to 19 along with unaltered maternal/fetal ratios of these fuels indicates that maternal hyperinsulinemia reduced fetal fuel availability to different degrees. These were associated with limited fetal growth. The extent of limitation of glucose and amino acids could not be quantitated since we did not measure uteroplacental and umbilical blood flow; however, SGA_I and control fetuses had similar arteriovenous blood gas tensions and pH suggesting that blood flow was normal in hyperinsulinemic mothers. Other factors such as acidosis and ketosis can also delay fetal growth. The normal plasma BOHB concentrations and pH in hyperinsulinemic mothers indicate that these did not contribute to growth retardation.

Insulin is a critical growth stimulating hormone during the perinatal period (10). Plasma insulin concentrations were significantly limited in SGA_I fetuses by day 17 presumably as a result of diminished glucose and possibly amino acids. Because of limited sampling, we could not determine whether fetal insulin and glucagon secretion were altered before this time although glucose was limited earlier. These observations suggest that prolonged fetal hyperinsulinemia retarded fetal growth; however, numerous other factors as fuel availability were probably as important in causing growth retardation. For example, while fetal body weight correlated with fetal plasma glucose, it did not correlate with fetal plasma insulin.

Newborn SGA_I pups had significantly diminished plasma glucose concentrations from birth to 240 min (Fig. 4). Limited metabolic fuels during intrauterine life was associated with appropriately increased glucagon secretion in SGA_I fetuses. In addition, SGA_I pups demonstrated a greater then expected cose concentrations from birth to 240 min as a result of limited neonatal glucagon surge compared to controls. These changes should not only have maintained plasma glucose concentrations but also facilitated hepatic PEPCK induction. Hepatic glycogen concentrations decreased in SGA_I fetuses indicating an appropriate response to glucagon. On the other hand, the failure of the SGA_I pups to increase hepatic PEPCK as control pups suggests a "resistance" to glucagon with respect to its role as a gluconeogenic enzyme precursor. Ninety percent of PEPCK activity is present in the cytosol (11, 12), and while the three other critical gluconeogenic enzymes appear during intrauterine life, PEPCK activity increases only after delivery (13, 14). During normal perinatal development, glucagon stimulates PEPCK induction by increasing intracellular cyclic AMP (15, 16) while excessive insulin limits induction by inhibiting mRNA transcription (17, 18). We (3) and others (13, 19, 20) have observed this relative "resistance" in rat pups rendered growth retarded by maternal uterine artery ligation.

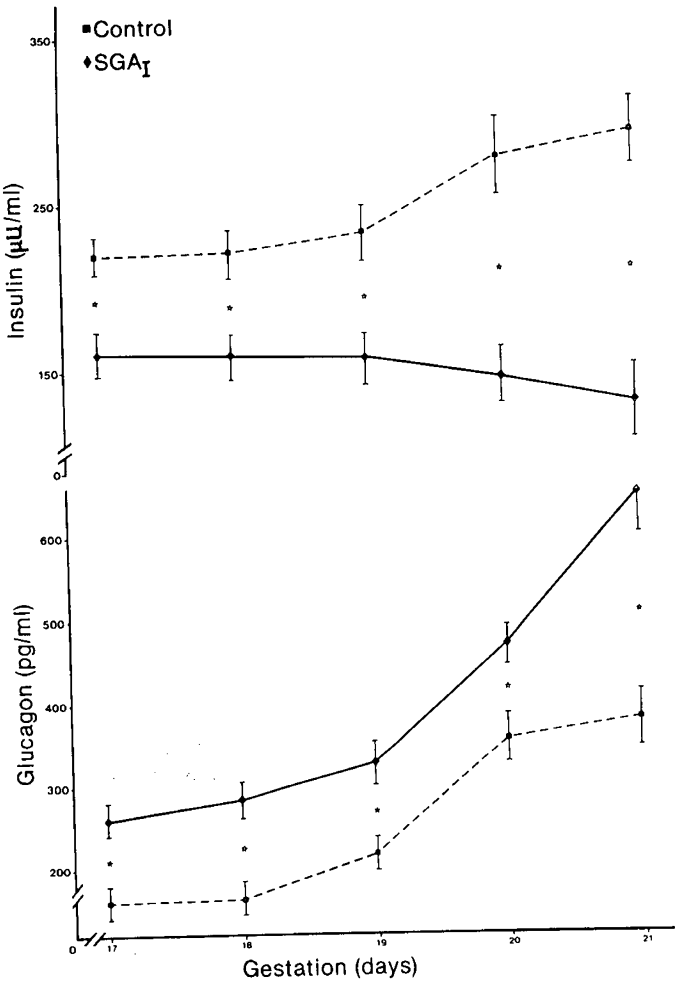

Fig. 3. Plasma concentrations of insulin and glucagon in pups of hyperinsulinemic (SGA_I) and control mothers. From day 17 until term, SGA_I fetuses had significantly diminished insulin and elevated glucagon concentrations (* $p < 0.01$ to 0.001).

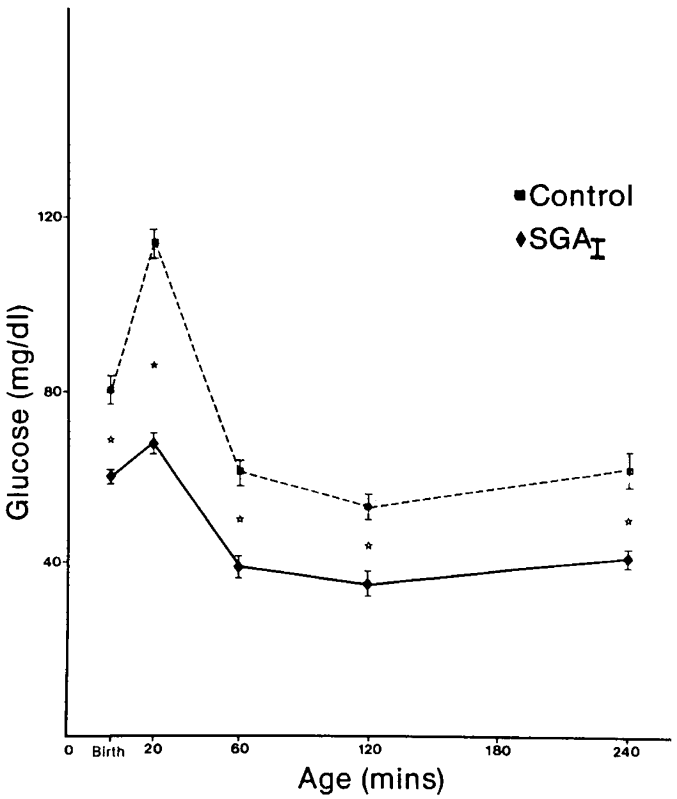

Fig. 4. Plasma glucose concentrations of newborn SGA_I and control pups. SGA_I pups had significantly diminished values from birth to 240 min (* $p < 0.01$ to 0.001).

Another cause of the neonatal hypoglycemia may be inappropriate modulation of insulin. While SGA_I pups had significantly diminished insulin concentrations at birth, subsequent values did not differ from controls. Since SGA_I pups were hypoglycemic compared to controls, their elevated insulin values relative to glucose suggest a possible inability to limit insulin secretion.

Our findings extend the initial observations of Grupposo *et al.* (5) who reported that term pups and day 20 fetuses of hyperinsulinemic mothers were significantly smaller and had lower plasma glucose and insulin concentrations than controls. In

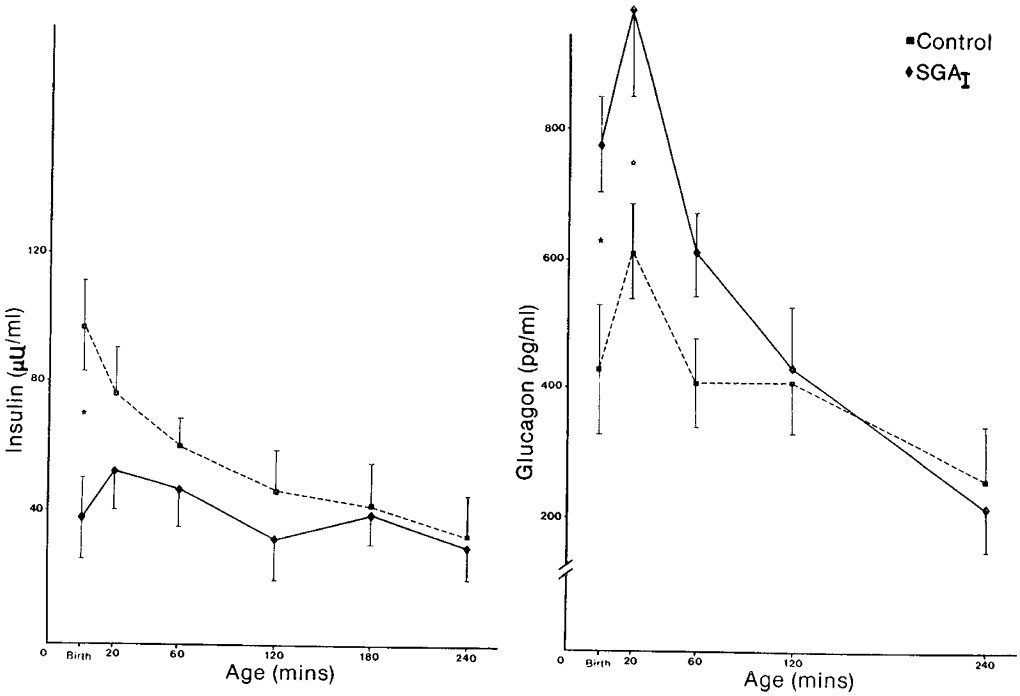

Fig. 5. Plasma insulin and glucagon concentrations of SGA_I and control pups. SGA_I pups had significantly diminished insulin concentrations at birth (* $p < 0.01$) and significantly elevated glucagon concentrations at birth and 20 minutes (* $p < 0.01$).

Table 2. *Hepatic glycogen concentrations (mg/g liver) and cytosolic PEPCK activity (μmol/PEP/g liver/min)*

Age (min)	0	20	60	120	240
Glycogen					
SGA_I	36.1 ± 6.0	20.1 ± 2.1	20.0 ± 3.1	17.0 ± 5.4	13.0 ± 5.3
*	*	*	*	*	*
Control	50.3 ± 7.3	43.2 ± 6.4	38.3 ± 5.1	23.4 ± 4.1	12.0 ± 5.2
PEPCK					
SGA_I	0.092 ± 0.014			0.134 ± 0.012	0.135 ± 0.009
					*
Control	0.100 ± 0.015			0.127 ± 0.013	0.227 ± 0.009

* $p < .01$ to .001

contrast to our observations, they reported a significantly diminished fetal/maternal glucose relationship on day 20 in hyperinsulinemic mothers suggesting impaired glucose transport. In addition, while we found significant limitations of maternal and fetal total amino acids associated with hyperinsulinemia from days 17 to 19 only, Grupposo *et al.* (5) reported diminished total maternal and fetal amino acid concentrations on day 21.5. These may represent differences in anesthesia or handling of the fetus.

While our model of maternal hyperinsulinemia resulted in sustained fetal hypoglycemia, the significant reductions in fetal total amino acids from days 17 to 19 were followed by normal values on days 20 and 21. The mechanism for this is unclear. We can only speculate that the "antiinsulin factors" which normally develop during late gestation were sufficient in the hyperinsulinemic mother to correct plasma amino acid but not glucose concentrations.

In previous studies, we used maternal uterine artery ligation on day 18 in the rat as a model of "uteroplacental insufficiency." This retarded fetal growth (3) and was associated with limited fetal glucose and amino acids (4). While this was for a shorter period (days 18–21) than with maternal hyperinsulinemia, the offspring of mothers with uterine artery ligation were significantly smaller. The factor responsible for this difference was the alteration of gas exchange and pH. Unlike SGA_I fetuses, the fetuses of mothers with uterine artery ligation were hypercarbic, hypoxic, and acidotic for at least 48 h following ligation suggesting that maintenance of adequate blood gas tensions and pH may be an extremely important factor in determining fetal growth. This is consistent with models of chronic maternal hypoxia which profoundly retard fetal growth (21).

Recently a syndrome (22, 23) has been described of women who fail to develop the normally expected "diabetogenic state" (24) of late pregnancy. Such women have normal rather than attenuated glucose clearance during the third trimester and are at a 20 to 30% risk of bearing growth-retarded infants. It has been suggested that such women fail to develop one or all of the antiinsulin factors of late pregnancy and thereby limit fuel availability to the fetus. To the extent that maternal hyperinsulinemia limits fuel availability, this model in the rat mimics the "failed diabetogenic state" and may be useful for studies concerning this syndrome.

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