Insulin Response to Arginine in Normal Newborn Infants and Infants of Diabetic Mothers

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SUMMARY

The effect of maternal diabetes during pregnancy on insulin release of the newborn infant was examined employing arginine infusion as stimulus. At age two hours, eight normal newborn infants, six of gestationally diabetic mothers (IGDM) and four of insulin-dependent diabetic mothers (IDM) were infused with arginine hydrochloride 0.5 gm. per kilogram body weight for thirty minutes. Eight additional normal newborn infants were infused with isotonic saline. A small but definite rise in plasma IRI was observed in the normal newborns receiving arginine; however, the infants of gestationally diabetic mothers responded with an enhanced blood glucose and plasma insulin rise compared with the normal newborns. It seemed that intermittent or sustained hyperglycemia in utero may have potentiated the neonatal insulin response to arginine. DIABETES *23:* **816-20, October, 1974.**

Infants of diabetic mothers (IDM) often develop hypoglycemia during the early neonatal period. In 1954, Pedersen proposed the hypothesis of maternal hyperglycemia-fetal hyperinsulinism as the mechanism of neonatal hypoglycemia in these infants;[1] however, the effects of maternal diabetes on the control of insulin release in the fetus or newborn infant are not yet clearly understood. When a large intravenous load of glucose is administered rapidly to the infants of gestationally diabetic mothers (IGDM) the initial rise of plasma insulin is greater than that in normal newborn infants.[2] When glucose is administered by prolonged infusion, the sustained insulin response is appropriate and not excessive compared to the rise of blood glucose in IGDM.[3]

Arginine, among other amino acids, has been used as a stimulus to evaluate insulin release in adult man.[4] It has been shown that the arginine-induced insulin release differs from that of glucose in that glucose stimulated insulin release may be blocked by epinephrine, but arginine-initiated insulin release is not blocked.[5] Furthermore, the dependence of arginine-induced insulin secretion on glucose[6] and the synergistic effects of arginine and glucose upon insulin secretion[7] have been demonstrated.

In the present study, arginine was infused to normal newborn infants, infants of gestationally diabetic mothers and infants of insulin-treated diabetic mothers. The purpose of the study was to examine whether diabetes during pregnancy alters the insulin response to arginine in the newborn infant even during the period of relative hypoglycemia in the immediate neonatal period.

SUBJECTS AND METHODS

Sixteen normal newborn infants, six infants of gestationally diabetic mothers, and four infants of insulin-treated diabetic mothers were studied. Informed consent was obtained from the mothers of all infants studied. The mothers of the normal newborns were selected on the basis of (1) no family history of diabetes mellitus, (2) no glycosuria, (3) two-hour postprandial blood glucose concentrations <90 mg. per 100 ml., (4) body weight less than 80 kg., and (5) no known perinatal complications. Infants with an apgar score <6 at one or five minutes were not studied. Gestationally diabetic mothers were selected during the third trimester of pregnancy with glucose disappearance rates kt <1.0 per cent per minute by

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intravenous glucose tolerance tests. They were managed by diet without insulin therapy during pregnancy. Three of the four insulin-treated diabetic mothers had juvenile onset diabetes and had received continuous insulin therapy for more than five years. Patient E.B. had maturity onset diabetes and had been treated intermittently with oral hypoglycemic agents or insulin for the previous ten years. She was treated continuously with insulin for ten months prior to the delivery of the infant studied.

During labor and delivery of the infants, all the women in the study received no glucose intravenously. The normal newborn infants and the infants of gestationally diabetic mothers were delivered spontaneously between thirty-eight and forty-two weeks' gestation. Two infants of insulin-treated mothers were delivered vaginally at thirty-five and thirty-six weeks' gestation after spontaneous labor; the other two infants were delivered by C-section thirty-eight and one-half and thirty-six and one-half weeks' gestation. All studies were conducted in an environmentally controlled room at 50 per cent humidity and an ambient temperature of 34° C in the Perinatal Clinical Research Unit. Maternal venous blood and umbilical arterial and venous blood samples were obtained at birth. The infants were observed from birth to age two hours at which time L-arginine hydrochloride* 0.5 gm. per kilogram body weight was infused for thirty minutes via a peripheral vein. One infant of an insulin-treated mother (L.K.) became symptomatic with tachypnea and tremor by age ninety minutes. Blood glucose concentration was 4 mg./per 100 ml. and arginine infusion was started early at ninety minutes of age. Blood glucose gradually rose during the infusion (table 2) and clinical symptoms subsided. Eight normal newborn infants served as controls and were infused with isotonic saline 10 ml. per kilogram body weight for thirty minutes. Serial venous blood samples were obtained between birth and five hours of age from an indwelling peripheral venous scalp needle. The blood samples were heparinized, kept on ice and centrifuged at 4° C. Whole blood glucose concentrations were determined by the glucose oxidase method. Plasma samples were stored at -20° C until immunoreactive insulin (IRI) and free fatty acid (FFA) concentrations were assayed. Plasma immunoreactive insulin concentrations were determined by the double antibody method of Morgan and Lazarow[8] and plasma FFA by the ultramicro colorimetric method of Novak.[9]

* R-Gene(R) by Cutter Laboratory. (5% Arginine HCl)

The data were analyzed from the group means and standard deviations and evaluated using Students's *t* distribution. In the individual infant at different time intervals, comparison was made by pairing of data analyzed by Student's *t* test.

RESULTS

I. *Preinfusion glucose and insulin concentrations (table 1)*

At birth, the normal newborn infants and infants of gestationally diabetic mothers had similar glucose concentrations (76.1±3.9 vs. 78.8±7.0 mg. per 100 ml., Mean ± S.E.M.). The mean plasma insulin concentration of the sixteen normal newborns at birth was 10.3±1.5 μU/ml., a value similar to the insulin concentration of the fasting adult. However, the insulin concentration of the IGDM at birth varied greatly, ranging from 9 to 51 μU/ml. (mean 22.6±7.5 μU/ml.). At two hours of age, prior to the infusion of arginine or saline, the mean blood glucose concentration of the IGDMs (36.3±5.4 mg. per 100 ml.) also was not significantly different from that of the normal newborns (44.9±3.3 mg./per 100 ml.) ($p>0.1$); the plasma insulin concentration of the IGDMs, however, was slightly higher than that of the normal newborns (10.8±2.3 vs. 6.5±1.1 μU/ml., $0.02 < p < 0.05$).

II. *Blood glucose response to arginine (figure 1, top panels)*

After arginine infusion was initiated, the blood glucose levels of the normal newborns rose slightly; however, a similar trend was seen in the normal newborns who received saline. The mean blood glucose concentrations of all the normal newborn infants at 30, 60, 120, and 180 minutes postinfusion, whether they received arginine or saline, were not significantly

TABLE 1

Preinfusion blood glucose and plasma insulin concentrations in normal newborns and infants of gestationally diabetic mothers

Time of sampling		Normal (16)*	IGDM (6)	P‡
Birth	Glucose (mg./100 ml.)	76.1±3.9†	78.8±7.9 (5)	N.S.
(Umbilical vein)	Insulin (μU./ml.)	10.3±1.5	22.6±7.5 (5)	<0.01
Age 2 hours	Glucose	45.1±3.3	36.0±5.4	N.S.
(peripheral vein)	(mg./100 ml.)			
	Insulin (μU./ml.)	6.5±1.1	10.8±2.3	<0.05

*Number of infants studied
†Mean ± S.E.M.
‡Normal vs. IGDM

different from their preinfusion levels, i.e. at age two hours ($p > 0.1$).

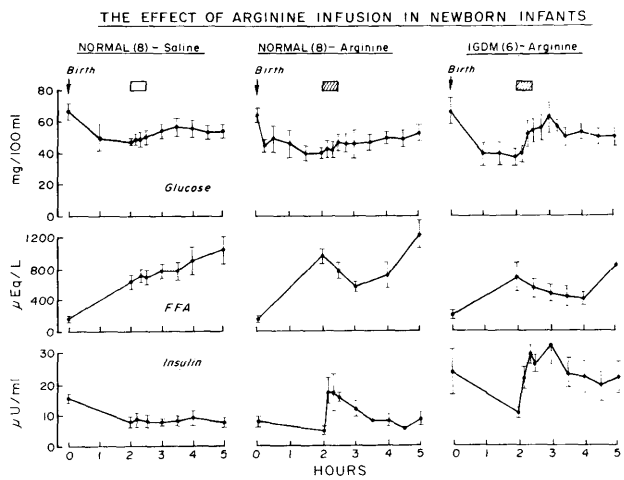

FIG. 1. Blood glucose, plasma FFA and insulin concentrations of normal newborn infants and infants of gestationally diabetic mothers (IGDM) from birth to age five hours. Isotonic saline solution 10 ml./kg. (□) or arginine hydrochloride solution 0.5 gm./kg. (▨) was infused intravenously over a thirty-minute period starting at age two hours.

The infants of gestationally diabetic mothers behaved differently in that mean blood glucose rose 22 mg./per 100 ml. above preinfusion levels one hour after arginine infusion was started ($.02 < p < 0.05$). In individual infants, blood glucose concentration remained higher than the preinfusion level ($.02 < p < 0.05$) until the end of the study period (age five hours). However, the mean blood glucose concentrations of IGDM at 30, 60, 120 and 180 minutes after arginine infusion were not significantly different from those of the normal newborn ($p > 0.1$).

Three of the four infants of insulin-treated mothers were hypoglycemic when arginine infusions were started (table 2). Blood glucose concentrations were 4, 16 and 12 mg./per 100 ml., respectively. In each case blood glucose concentration rose during infusion and was maintained after the arginine infusion was stopped. The fourth infant, E.B., had a blood glucose concentration of 65 mg./100 ml. at age two hours and no further rise of blood glucose was observed when arginine infusion was given.

III. *Plasma insulin response to arginine (figure 1, lower panels)*

During the thirty minutes of arginine infusion, both the normal newborn infants and the infants of gestationally diabetic mothers had small but significant rises in plasma immunoreactive insulin concentrations. When arginine infusion was stopped, the plasma IRI levels gradually declined. However, in both the normal newborn infants and the infants of gestationally diabetic mothers, plasma IRI levels remained higher than their preinfusion levels ($0.02 < p < 0.05$) until the end of the study period at five hours of age. No significant fluctuation of IRI concentrations was seen in normal infants who received saline.

In comparing the insulin responses of both groups of infants who received arginine, i.e. normal newborns and IGDMs, the insulin concentrations of the IGDMs at the end of the arginine infusion were slightly higher than those of the normal newborns ($0.02 < p < 0.05$). After the infusion, however, the infants of gestationally diabetic mothers maintained their IRI concentrations at a significantly higher level than those of the normal newborns throughout the remaining study period (21.0 ± 4.8 vs. 8.9 ± 1.9 μU/ml. at age five hours, $p < 0.01$) while their blood glucose concentrations were not significantly different (48.3 ± 4.9 vs. 51.1 ± 5.1 mg./per 100 ml., $p > 0.1$).

IV. *Plasma FFA (figure 1, mid-panels)*

Plasma free fatty acid concentrations were low at birth in all infants. After birth, the plasma FFA rose in the normal newborn infants who received saline. Arginine infusions to normal newborns and IGDMs resulted in a transient decline of plasma FFA levels

TABLE 2

Blood glucose concentrations (mg./100 ml.) in four infants of insulin-treated diabetic mothers before, during and after the arginine infusion

	Age of infant			Minutes after starting arginine infusion*								
	At birth (UV)	60′	120′	10	20	30	45	60	90	120	150	180
L.K.	107	4	4 (90′)	9	20	25	30	51	46	58	59	68
L.H.	150	35	16	26	21	47	39	46	52	36		
N.L.	152	19	12	21	25	30	33	35	42	43	44	42
E.B.	87	73	65	73	60	52	42	37	42	57	62	67

* Arginine HCl 0.5 gm./kg. body weight was infused intravenously for thirty minutes

which coincided with the rise in plasma insulin concentrations. The magnitude and time of FFA suppression, however, varied greatly in both groups of infants. By the end of the study period, although plasma IRI levels remained elevated in IGDMs, plasma FFA levels were rising in all infants studied.

DISCUSSION

The purpose of the present study was to examine the effect of an altered intrauterine environment caused by maternal diabetes on the pancreatic insulin response of the infant during the neonatal period. Arginine was used as a stimulus to evaluate insulin release.

Early in gestation the human fetal pancreas synthesizes and secretes insulin,[10,11] but it is insensitive to a glucose stimulus for release of insulin.[12,13] By term gestation, the fetus in utero[14,15] and the newborn infant during the neonatal period respond to glucose with rises in plasma insulin, though the responses are attenuated compared with those of adult man.[3] In contrast, the human fetal pancreas may respond to amino acids with insulin release earlier in gestation. Milner et al. and Shaeffer et al. have shown that isolated pancreatic tissue from the human fetus at fourteen to twenty weeks' gestation releases insulin when incubated in vitro with amino acids but not with glucose.[13,16] Grasso, in studies of premature infants, showed a greater insulin rise in infants receiving intravenous infusion of amino acids than in those receiving glucose.[17] In the present study, the normal newborn infants responded to infusion of arginine with a small but significant rise of plasma insulin concentration compared with infants receiving saline. However, the magnitude of the insulin response was smaller than that observed in the group of infants studied by Reitano et al.[18] This difference may be attributed to the larger dose of arginine administered by them (1.25 gm. to 2.5 gm. per kilogram body weight[18] contrasted with 0.5 gm. per kilogram body weight in the present study).

Furthermore, the relatively low plasma insulin response to arginine may have occurred in the normal newborn infants because their blood glucose concentrations were lower than in adults. In adult man, the insulinogenic effect of arginine depends upon a normal blood glucose concentration[6] and is enhanced by hyperglycemia.[7] In premature infants the synergistic effect of glucose and arginine upon insulin release also has been demonstrated.[18] Even though the blood glucose concentrations of the IGDM at age two hours were slightly lower than those in normal newborns, the infants of gestationally diabetic mothers infused with arginine responded with a greater and more sustained rise of blood glucose and plasma insulin.

Ghadimi et al.[19] reported that the plasma free amino acid concentrations of three pregnant diabetic women and their newborns at birth were in the range of those of normal mothers and their newborn infants. Thus, the exaggerated insulin response to arginine in the infants of gestationally diabetic mothers apparently is not explained by prior stimulation with excessive plasma amino acid concentrations in utero. Nevertheless, intermittent or sustained hyperglycemia in utero may have potentiated the neonatal insulin response to arginine.

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