Impaired Insulin Secretion In the Neonatal Rhesus Monkey after Chronic Hyperinsulinemia *In Utero* (43080)

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Abstract. **The secretion of insulin by the pancreas of the newborn rhesus monkey that had been made experimentally hyperinsulinemic *in utero* was studied in 18 animals. Chronic *in utero* hyperinsulinemia was produced by the continuous subcutaneous delivery of 4.75 units of insulin per day for 18 ± 1 days. After delivery, the insulin-containing pump was removed to allow neonatal insulin levels to drop to normal levels. By 6.5 ± 1.0 hr after pump removal, plasma glucose, insulin, and C-peptide immunoreactivity (CPIR) were comparable in the control and experimental animals. At that point 300 μg of glucagon/kg body weight was given iv to stimulate insulin secretion. After 30 min a significant elevation (expressed as the percentage of basal levels) in plasma glucose by 250%, insulin by 200%, and CPIR by 200% was observed in the control animals. In contrast, no changes in plasma insulin or CPIR concentrations occurred, with an attenuated glucose response that was only one-fifth of the control response, in the experimental animals. These results along with the observed lowered concentrations of CPIR in the plasma and insulin in the pancreas at birth can be interpreted as evidence that insulin is an inhibitor of its synthesis and secretion *in utero* and that this abnormal intrauterine environment causes changes that persist into extrauterine life.**

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The pancreas of the fetus of the diabetic mother, because of her disturbed metabolism, is exposed to concentrations of metabolic growth substrates that are different than in normal pregnancy. Glucose and the amino acids arginine, leucine, and lysine are potent stimulators of insulin synthesis and secretion. The stimulatory effect of elevated concentrations of these substrates is to produce fetal hyperinsulinemia (1). The existence of chronic fetal hyperinsulinemia leads to the fetopathy of the infant of the diabetic mother, most notably fetal macrosomia and neonatal hypoglycemia (2). The synthesis and secretion of insulin is influenced by substrate, hormone, and neural factors (3). Some, like glucose, glucagon, or acetyl choline, stimulate insulin secretion. Insulin itself has been shown to be an inhibitor of its own secretion both *in vivo* (4) and *in vitro* (5). The functional development of the fetal pancreas has been shown to be influenced by substrate concentration (6). Because hyperinsulinemia is also present during periods of substrate excess, it has not been possible to dissociate the competing and opposite effects of hyperinsulinemia and substrate excess in the developing pancreas of the infant of the diabetic mother. The hyperinsulinemic fetal rhesus monkey model, however, can be used to separate these two effects (7) and to study the consequences of *in utero* hyperinsulinemia on pancreatic development, when substrate concentrations are not elevated. We report here the use of this model to determine if insulin is an inhibitor of its synthesis and secretion *in utero* and if this inhibition persists into extrauterine life.

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Materials and Methods

Eighteen time-dated pregnant rhesus monkeys were used in this study. These animals were housed and cared for in the AAALAC accredited New England Regional Primate Research Center in Southboro, MA. The studies described were reviewed and approved by the IACUC of the Primate Center. All surgical procedures were performed aseptically in an approved sterile operating room of the Primate Center. At approximately 130 days gestation a laparotomy was performed

under ketamine anesthesia on pregnant animals. A fetal limb was exteriorized and an Alzet minipump implanted subcutaneously in that limb. After the limb was returned into the uterus, the laparotomy was closed. In 11 fetuses the minipump was loaded to deliver 4.75 units of sodium pork insulin/day in a solution of 1.6% glycerol. The other seven fetuses were controls in which the diluent, 1.6% glycerol, but not insulin, was infused.

After 18 ± 1 (mean ± SD) days, the fetuses were delivered by Cesarean section while the mothers were under ketamine anesthesia. A peripheral maternal blood sample was taken for the measurement of plasma substrates and hormones. Fetal umbilical artery (UA) and vein (UV) samples were taken before the clamping of the cord. Neonates were immediately transferred to an infant incubator set at 37°C. Spontaneous respiration occurred while these neonates were being vigorously towel dried.

Thirty minutes after delivery, a 22-gauge vialon catheter was inserted into a femoral vein which was aseptically exposed after local anesthesia with lidocaine without epinephrine. The catheter was advanced into the inferior vena cava and kept patent by being filled with normal saline with 3 units of heparin/ml. This procedure lasted less than 30 min so that by 1 hr of age each neonate was catheterized. The Alzet minipump was removed under local analgesia with lidocaine from 1 to 4 hr after delivery in the experimental group and 1 hr after delivery in the control group. These neonates were kept in the incubator and fasted during the study period. At hourly intervals blood samples were taken for measurement of plasma substrates and hormones. The samples were handled aseptically so that red blood cells could be returned to the neonates. The red blood cells were suspended in normal saline to the volume of the original sample and returned within 15 min of that sample.

Sampling in the control group lasted up to 8 hr, with a mean of 7 ± 1 hr ($n = 6$), at which time an intravenous glucagon tolerance test was carried out on the neonates. The glucagon tolerance test was performed at 6.5 ± 1.0 hr ($n = 7$) after pump removal in the experimental group. Because the insulin delivering pump was removed at various times between 1 and 4 hr after delivery, the mean age of the experimentals at the time of testing was 9 ± 2 hr compared with 7 ± 1 hr for the controls. The glucagon tolerance test consisted of giving 300 μg of glucagon/kg of body weight followed by a blood sample taken at 5 and 30 min later, for plasma glucose and immunoreactive insulin and C-peptide immunoreactivity (CPIR) (8). Because of technical difficulties, complete data were not collected on five of the original 18 animals; thus, these were excluded from analysis.

In a separate series of studies in which either 19.0 or 4.75 units of insulin were given to the fetus *in utero* for 18 to 21 days, the pancreatic insulin content was determined. The pancreases were removed in eight control and eight experimental animals of which two received 19.0 units/day and six received 4.75 units/day. The pancreases were quick frozen in liquid nitrogen and stored at −70°C until insulin content was determined. The frozen pancreases were weighed and then homogenized in an acid-ethanol solution to extract insulin, the concentration of which was then determined by radioimmunoassay.

Data analysis were carried out using the StatView program on a Macintosh SE computer. The effect of glucagon on control and experimental animal glucose, insulin or CPIR was assessed by the one factor ANOVA-repeated measures test. Significance was ascribed to P values of <0.05. Differences between values at the three time points were determined by the Fisher PLSD at the significance of $P < 0.05$. The unpaired t test was used to compare differences in relative responses after glucagon and data collected at delivery with significance being attributed to $P < 0.05$. All data are presented as mean ± SD.

Results

Table I summarizes the data collected at delivery in both mothers and neonates. As expected, plasma insulin concentration in the experimental animals is significantly elevated ($P < 0.001$) by approximately one order of magnitude above the control level. This degree of chronic fetal hyperinsulinemia produced a significant ($P < 0.005$) increase in body weight of 25% above the controls. The UA plasma glucose concentration is also significantly ($P < 0.025$) reduced in the experimental group. In contrast, the UV plasma glucose concentration is not affected. The UV-UA plasma glucose concentration gradient in all fetuses was positive with a mean difference of 0.28 ± 0.11 mM in the controls compared with a significantly ($P < 0.025$) greater difference of 0.56 ± 0.22 mM in the experimentals. Fetal CPIR, which reflects fetal insulin production and release, is significantly ($P < 0.05$) reduced by approximately 50%. However, no correlation exists between plasma CPIR and plasma UA ($r = 0.14$; $n = 17$) or UV ($r = 0.04$; $n = 17$) glucose concentrations. No differences in the plasma concentrations of glucagon, cortisol, epinephrine, norepinephrine, total amino acids, or total branched chain amino acids were found between the two groups. Also as expected, maternal glucose and insulin levels were not affected by fetal hyperinsulinemia.

Figure 1 summarizes the insulin content of the group of animals that was used for tissue composition studies including pancreatic insulin content. A significant ($P < 0.05$) 50% reduction in extractable insulin was found in the insulin-treated group, 16.3 ± 10.7 nmol/g of pancreas versus 32.2 ± 14.8 nmol/g in the

Table I. Substrate and Hormone Concentrations at Delivery

	Control	Experimental	$P <$
Fetus			
n	7	11	
Gestational age (days)	151 ± 2[a]	151 ± 2	NS[b]
Weight (g)	441 ± 69	554 ± 20	0.005
UA glucose (m*M*)	2.25 ± 0.56	1.40 ± 0.50	0.025
UV glucose (m*M*)	2.52 ± 0.73	1.96 ± 0.50	NS
UA insulin (p*M*)	194 ± 97	2334 ± 589	0.001
UV insulin (p*M*)	187 ± 86	2283 ± 933	0.001
CPIR (p*M*)	280 ± 147	145 ± 87	0.050
Glucagon (ng/liter)	91 ± 21 (6)	90 ± 28 (7)	NS
Cortisol (n*M*)	522 ± 61	585 ± 271 (9)	NS
Epinephrine (p*M*)	1152 ± 1037 (6)	2462 ± 2452 (9)	NS
Norepinephrine (p*M*)	5603 ± 4391 (6)	5538 ± 4557 (9)	NS
Total AA[c] (m*M*)	4.79 ± 0.22 (5)	4.45 ± 0.48 (5)	NS
Total BCAA[c] (m*M*)	0.48 ± 0.16 (5)	0.45 ± 0.15 (5)	NS
Mother			
Glucose (m*M*)	3.36 ± 0.56	3.02 ± 0.62	NS
Insulin (p*M*)	819 ± 259	775 ± 323	NS

[a] Mean ± SD.
[b] NS, not significant.
[c] AA, amino acids; BCAA, branched chain amino acids.

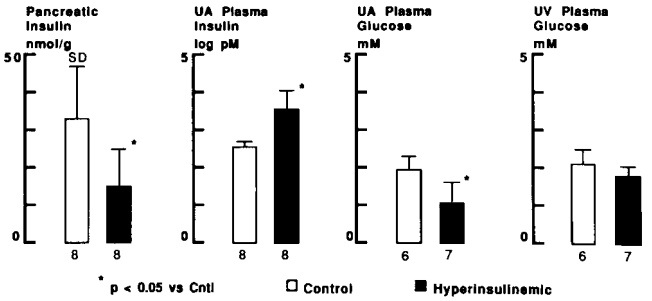

Figure 1. Pancreatic insulin content and plasma insulin and glucose concentration in fetal rhesus monkeys at 144 ± 2 days of gestation.

controls. Like the insulin-treated neonates above, the UA plasma glucose concentration of 1.12 ± 0.51 m*M* in the experimental group is significantly ($P < 0.05$) lower than the 1.96 ± 0.28 m*M* in the controls. However, umbilical vein plasma glucose concentration in the controls of 2.29 ± 0.34 m*M* is not different from the 1.80 ± 0.40 m*M* in the insulin-treated group. No correlation exists between pancreatic insulin content and either UA ($r = 0.41$; $n = 13$) or UV ($r = 0.33$; $n = 13$) plasma glucose concentration. Insulin content, for example, is equally high in fetuses with UA plasma glucose concentrations of 1.1 m*M* and 2.0 m*M* or equally low at 0.5 m*M* and 2.1 m*M*.

Figure 2A shows the change in neonatal venous plasma glucose concentration from delivery until the administration of the glucagon tolerance test. The control animals were able to maintain a mean fasting plasma glucose concentration at above 2.20 m*M* throughout the study period. The experimental animals experienced a significant ($P < 0.05$) drop in plasma glucose reaching its nadir of 0.67 ± 0.44 m*M* at 1 hr postdelivery in six of seven animals and at 2 hr in the remaining animal. The plasma glucose concentration remained significantly ($P < 0.05$) suppressed at 2 hr after pump removal. By 4 hr after pump removal, glucose levels had increased to control levels. When the glucagon tolerance test was performed at 6.5 ± 1.0 hr after pump removal, the control levels of 2.20 ± 0.50 m*M* and the experimental levels of 1.96 ± 1.20 m*M* were comparable.

The venous plasma insulin concentrations during the study period are summarized in Figure 2B. Control insulin levels averaging 139 ± 61 p*M* remained unchanged through the study period. The levels in the experimental animals at delivery of 2283 ± 933 p*M* and at the glucose nadir of 2463 ± 1795 p*M* are significantly ($P < 0.05$) elevated compared with controls. Insulin levels drop progressively after the insulin delivering pump is removed, but remain significantly higher than controls at 2 and 4 hr after pump removal. By the time glucagon is given, 6.5 ± 1.0 hr after pump removal, the plasma insulin concentrations are the same in both groups, 122 ± 65 p*M* in the controls, and 171 ± 59 p*M* in the insulin-treated group.

Plasma CPIR is significantly ($P < 0.05$) lower in the experimental animals at delivery, 145 ± 87 p*M*, vs 280 ± 147 p*M* in the controls, as is illustrated in Figure 2C. The difference between the two groups remains significant up to 4 hr after pump removal. Because of a gradual decline in the control levels and a concomitant rise in the experimental levels from 2 hr after pump removal, the C-peptide levels in both groups are

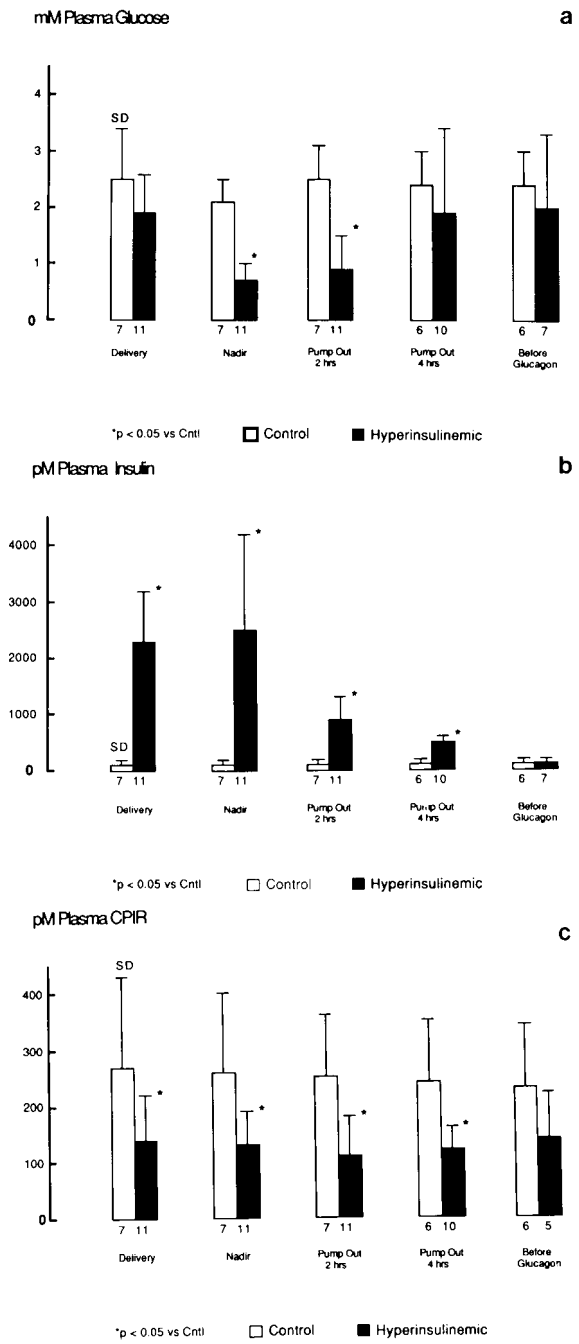

Figure 2. Plasma concentration of glucose (A), insulin (B), and CPIR (C) during the immediate neonatal period in control and hyperinsulinemic (*in utero*) rhesus monkeys.

not different when glucagon is given, 155 ± 59 p*M* in the experimentals and 224 ± 130 p*M* in the controls.

The plasma concentrations of glucose, insulin, and CPIR had been comparable in both groups for at least 1 hr before iv glucagon. By this point, basal insulin secretion, as assessed by the CPIR to glucose molar ratio, was the same in both groups at $10.8 \pm 6.1 \times 10^{-8}$ for controls and $10.4 \pm 4.7 \times 10^{-8}$ for the experimentals. Only when the stimulation of insulin secretion was attempted with glucagon were differences observed.

The response of plasma glucose, insulin, and CPIR to iv glucagon is shown in Table II. This table summarizes the response of all of the control ($n = 6$) and experimental ($n = 7$) animals for which a complete data set was collected. The mean plasma UA glucose concentration at delivery was 2.21 ± 0.67 m*M* for the controls compared with 1.55 ± 0.57 m*M* for the seven experimental animals, which is significantly different from the controls ($P < 0.05$). Of the seven experimental animals, two had glucose levels of 0.73 and 0.89 m*M*, which are two or more standard deviations below the mean control levels. If these two animals are excluded, a subgroup of five experimental animals results with a mean UA plasma glucose concentration of 1.84 ± 0.32 m*M* (range, 1.51–2.35 m*M*) which is not different from the control group (range, 1.46–3.24 m*M*). The data from this subgroup of experimental (euglycemic at delivery) animals is summarized in the right-hand column next to the data for all experimentals in the center column. Table 2 summarizes both the concentrations immediately before iv glucagon and 5 and 30 min postglucagon, and the relative responses as a percent of the preglucagon values at 5 and 30 min.

The glucose response in the controls exhibited the expected normal delayed increase observed in human neonates reaching significance ($P < 0.05$) only after 30 min. The glucose response of the experimental animals was similar in that glucose concentrations were only significantly ($P < 0.05$) elevated after 30 min. However, the glucose response is significantly ($P < 0.05$) attenuated compared with the controls, both in absolute and relative terms, at 30 min. The insulin response to glucagon in the controls is also similar to that of human neonates, with a significant ($P < 0.05$) increase in concentration peaking at 5 min and remaining elevated at 30 min. The experimental animals, in contrast, exhibited no significant increase in plasma insulin levels resulting, therefore, in a significant ($P < 0.05$) difference in the relative response between them and the controls. The CPIR response of both the control and experimental animals mirrors the insulin response. There is both an absolute and relative lack of CPIR response to an iv glucagon challenge after *in utero* hyperinsulinemia in all experimental animals regardless of whether they were euglycemic.

Discussion

The absence of an increase in the plasma insulin and C-peptide immunoreactivity after intravenous glucagon in neonatal monkeys that had been hyperinsulinemic *in utero* documents a functional pancreatic abnormality caused by experimentally produced chronic *in utero* hyperinsulinemia. Our data offer support to the hypothesis that the abnormal intrauterine environment of the IDM causes pathophysiologic changes that may persist into extrauterine life. Fetal hyperinsuline-

Table II. Neonatal Glucose, Insulin, and CPIR Response to Intravenous Glucagon, 6 Hr after Alzet Minipump Removal

	Control			Experimental[a]			Experimental[b] (euglycemic *in utero*)		
n		6			7			5	
Time (min)	0	5	30	0	5	30	0	5	30
Glucose (m*M*)	2.20 ± 0.50	3.55 ± 1.32	5.14 ± 2.60[c]	1.96 ± 1.20	2.41 ± 1.33	2.51 ± 1.55[c,d]	1.83 ± 0.70	2.30 ± 1.13	2.42 ± 1.46[c,d]
% of 0 min		154 ± 36	236 ± 78		118 ± 16	124 ± 32[d]		123 ± 16	125 ± 37[d]
Insulin (p*M*)	122 ± 65	262 ± 152[c]	249 ± 156[c]	171 ± 59	215 ± 94	202 ± 75	154 ± 52	202 ± 101	205 ± 92
% of 0 min		230 ± 74	209 ± 80		123 ± 28[d]	117 ± 26[d]		127 ± 34[d]	124 ± 26[d]
CPIR (p*M*)	224 ± 130	301 ± 223	436 ± 403[c]	155 ± 59	158 ± 54	155 ± 62[d]	178 ± 74	171 ± 22	162 ± 25[d]
% of 0 min		133 ± 17	187 ± 75		101 ± 15	100 ± 21[d]		96 ± 12	92 ± 14[d]

[a] All experimental animals that completed the study, mean plasma UA glucose of 1.55 ± 0.57 m*M*.
[b] Experimental animals that at delivery had a mean plasma UA glucose of 1.84 ± 0.32 m*M*, which is not different from the control group of 2.21 ± 0.67 m*M*.
[c] $P < 0.05$ vs 0 min.
[d] $P < 0.05$ vs control.

mia may be a determinant in causing these changes by altering the development of the intra-islet control processes as well as affecting the receptor mediated endocrine functions of those islet produced hormones.

The synthesis and secretion of hormones by the endocrine pancreas is under the control of a variety of integrated mechanisms. Insulin, for example, is an inhibitor of glucagon secretion, whereas glucagon is a stimulator of insulin secretion. The pancreatic hormone, somatostatin suppresses the release of all the other peptides, insulin, glucagon, and pancreatic polypeptide (9). The important role of intra-islet control of hormone release is illustrated by studies in which insulin release from dissociated β cells is higher than from intact islets. Reaggregation of these cells again reduces the amount of insulin that they secrete (10). *In vivo* (4) and *in vitro* (5) studies have demonstrated that exogenous insulin can inhibit pancreatic insulin secretion. There have also been reports which have not been able to demonstrate the feedback inhibition of insulin secretion (11). It is possible that species and experimental design differences are responsible for the contradictory literature in this field although the preponderance of data supports the existence of an insulin feedback loop.

A 50% reduction in plasma C-peptide concentration and pancreatic insulin content at delivery by exogenously given insulin supports the hypothesis that insulin inhibits its own synthesis and secretion *in utero*. Although we have previously reported the placenta is capable of metabolizing circulating C-peptide (8), the reduced levels in the insulin treated fetuses are not likely to be due to a different rate of metabolism of C-peptide in the two groups. Because fetal plasma glucose concentrations are reduced in the umbilical arteries, the possible reduction of insulin secretion by reduced plasma glucose concentrations must be considered. However, no relationship exists between umbilical artery or vein plasma glucose concentration and plasma C-peptide concentration.

Human and nonhuman primate transplacental arterial plasma glucose gradient is smaller than other mammals (12). This is because the primate placenta is highly permeable to glucose. This facilitates the transfer of glucose to the fetus even when fetal glucose utilization is increased. In fetal sheep, increasing fetal plasma insulin by an average of 16 times results in a corresponding fetal glucose utilization rate increased by 132% of control (13). The hyperinsulinemic fetal rhesus has insulin levels that are about 12 times higher than basal. We expect that fetal glucose utilization would be affected to about the same extent as in the sheep, that is, about 100% above basal. Given the 20-fold difference in the maternal glucose pool and the ability to maintain plasma glucose concentration even if fetal utilization were doubled, it would only represent an additional increase in utilization of 5% by maternal tissues. Because the fetal glucose concentration is a function of maternal glucose concentration, the mother should protect the fetus from hypoglycemia as long as the mother is well fed and in good health.

Because it is not possible to measure substrate or hormone levels of the fetus at other times during the experiment, we can only assume that values obtained at delivery, especially plasma glucose, are reflective of values throughout the *in utero* period. At delivery, fetal plasma cortisol and catecholamine levels are not different in the two groups. If chronic hypoglycemia were produced in the hyperinsulinemic fetuses, the levels of these hypoglycemic counter-regulatory hormones would have been elevated. This data supports the argument that fetal euglycemic hyperinsulinemia is produced using this animal model. We can also assume that because CPIR levels for up to 8 hr after delivery remain similar to delivery levels, these levels are also reflective of predelivery levels.

Further support for ruling out fetal glucose levels being responsible for the suppressed insulin comes from the separate studies of pancreatic insulin content. In

these studies also, no relationship exists between plasma glucose concentrations at delivery and pancreatic insulin content. Insulin content is equally high or low in different animals with either high or low glucose concentrations. We conclude that *in utero* hypoglycemia, therefore, is not responsible for the reduction of insulin content by 50%. The reduced content may be the result of reduced synthesis, reduced β cell mass, or an altered rate of pancreatic development. Histological examination of pancreases from other hyperinsulinemic fetuses has failed to identify any morphological differences between them and controls (unpublished observations). Feedback inhibition of insulin synthesis is the most likely explanation for the reduced insulin content.

After the delivery of the neonates, and after plasma glucose, insulin, and CPIR levels had normalized, an *in vivo* assessment of neonatal endocrine pancreatic function was carried out by the intravenous administration of glucagon. Glucagon was chosen because it is a potent insulin secretagouge whose mechanism of insulin secretion functions independently of glucose (14). Because glucagon is also a mobilizer of hepatic glucose stores (15), it was expected that the stimulatory effect of glucose on insulin secretion could also be assessed.

Intravenous glucagon in the control animals produced the expected rise in plasma insulin concentration within 5 min of administration even before plasma glucose concentration was significantly affected. At 30 min, insulin and CPIR levels were both elevated. This is the same response that is observed in adult man and demonstrates that glucagon receptors are present in the neonate and that they are functional in the rhesus neonate. The equally normal increase in plasma glucose in the control neonates also confirms the presence of and the intactness of the glucagon receptor.

The C-peptide, insulin, and glucose responses elicited by the iv administration of 300 μg of glucagon/kg to the controls were the expected normal responses previously reported in fetal sheep (16), human neonates (17), and adults (14), although delayed in the neonate compared with the adult. The number of glucagon receptors in the fetus is greatly reduced compared with the adult. A progressive increase in the number of high affinity sites is observed in the transition from fetus to adult without a change in the number of low affinity sites or binding constants (18). The dose of glucagon required to elicit the maximal plasma glucose response is greater than in the adult, presumably because of the reduced number of glucagon receptors compared with the adult.

In contrast, intravenous glucagon had little or no effect on the experimental neonates. Plasma levels of CPIR and insulin were unchanged indicating that the β cell's ability to either recognize or respond to the glucagon signal had been functionally altered by the exogenously produced fetal hyperinsulinemia. The lack of effect was not likely due to neonatal hypoglycemia because plasma glucose levels in experimental animals were similar to controls 4 hr after pump removal, 2.5 hr before glucagon was given. Fetal hypoglycemia was likewise ruled out because the insulin and CPIR response was also absent in the subgroup of experimental animals that were euglycemic at delivery and presumably not hypoglycemic *in utero.*

Both glucose and glucagon are insulin secretagogues. The possibility that they are acting synergistically in stimulating insulin release must be considered. Table II shows that the control animals exhibited a significant insulin response at 5 min after glucagon, even though the glucose response was not significant. The experimental animals likewise had no glucose response at 5 min, but in their case no insulin response was observed. The fact that at 30 min the control animals did not have further elevated insulin, even though glucose levels are double basal levels, suggests that synergistic effects play an important role. It is obvious that there is variability in the responses of both groups. Two control animals had a minimal glucose response of +4% and +24% whereas their insulin responses were +73% and +124%. Conversely, the experimental animal with the highest glucose response of +37% at 5 min had only a +5% increase in insulin. These data argue against the possibility that the absence of an insulin and CPIR response is due to the fact that glucose levels did not increase.

The hydrolysis of hepatic glycogen stores to increase plasma glucose concentration in response to iv glucagon is a receptor mediated event that is mediated by a sequence of protein phosphorylation steps that generate active phosphorylase a. The fact that plasma glucose levels were increased by only 20% of the control increase (0.55 mM vs 2.94 mM) in the experimental animals could mean that the glucagon receptor mediated signaling mechanism has been altered in the experimental animals by the abnormal intrauterine environment of the chronically hyperinsulinemic fetal rhesus monkey. The poor glucose response might be due to reduced hepatic glycogen content. This animal model, however, is characterized by elevated hepatic content during *in utero* chronic hyperinsulinemia (19). Another explanation for this response could be that chronic *in utero* hyperinsulinemia causes hyperglucagonemia followed by glucagon receptor down regulation. We have previously reported that no hyperglucagonemia exists in this model (7, 19). Plasma glucagon levels in the animals used in these current studies confirm that at delivery plasma glucagon levels are the same in both groups of animals.

A defect in hepatic glucagon receptor or postreceptor action has been identified. Whether this same defect is also present in the pancreas cannot be determined at

this time. Whether the pancreas of the *in utero* hyperinsulinemic fetus as a neonate is appropriately responsive to glucose or amino acid stimulation has not been established as of yet. Additional *in vivo* and *in vitro* studies will be required. Nevertheless, this current study demonstrates that pancreatic insulin content is reduced *in utero* and that postnatally glucagon does not stimulate insulin secretion and attenuated hepatic glucose production. The absence of an insulin or CPIR response to glucagon could also reflect a defect in the stimulus-secretion coupling of the β cell or a more generalized derangement of the mechanisms which control endocrine pancreas functioning via intra-islet regulatory processes.

The persistence of aberrant metabolism in the offspring of the diabetic mother has been reported in both humans and rats. Several longitudinal studies have documented higher incidences of subsequent obesity and diabetes in the human infant of the diabetic mother (20). Reduced insulin secretion and abnormal glucose tolerance has been observed in the female offspring of rats with mild streptozotocin induced diabetes. This abnormality was also passed to their offspring, thus demonstrating a nongenetic transgenerational transmission of a defect in insulin secretion (21, 22).

In the studies reported here, the fetal rhesus pancreas is not exposed to high concentrations of secretogogues and is not hypersecreting insulin. The exogenously produced fetal hyperinsulinemia causes feedback inhibition of insulin synthesis and secretion *in utero*. After delivery when neonatal insulin and glucose concentrations are normalized, basal insulin secretion, based on the CPIR to glucose ratio at the time that glucagon is given, is the same in the experimental and control animals. The absence of an insulin and CPIR response to glucagon during the neonatal period even after insulin and glucose concentrations have returned to normal levels indicates that abnormal pancreatic function persists into extrauterine life.

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