Chronic Hyperinsulinemia in the Fetal Rhesus Monkey

Effects of Physiologic Hyperinsulinemia on Fetal Growth and Composition

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SUMMARY

One of the hallmarks of the hyperglycemic-hyperinsulinemic infant of the diabetic mother (IDM) is macrosomia and selective organomegaly. Primary hyperinsulinemia, with insulin levels similar to those observed in human IDMs at delivery, was produced in the fetal rhesus monkey during the last third of gestation. The effects of this physiologically relevant hyperinsulinemia, in the absence of hyperglycemia, on fetal growth were studied. Fetal macrosomia, with a 23% increase in total body weight, was observed in physiologically hyperinsulinemic fetuses. A similar 27% increase in weight was produced by fetal insulin levels that were 10 times higher. A logarithmic correlation was observed between fetal birth weight ratio and fetal plasma insulin concentration. In contrast to this increase in weight, skeletal growth, as measured by crown-heel length and head circumference, was not affected by hyperinsulinemia. Only cardiomegaly was found in the low-dose hyperinsulinemic fetuses, whereas cardiomegaly, hepatomegaly, and splenomegaly were produced by hyperinsulinemia in which insulin levels were in the highest range. Compositional analysis of heart and skeletal muscle indicated no differences in the protein, RNA and DNA concentration, or in the protein-to-DNA ratio in hyperinsulinemic fetuses. We interpret these data as indicating that fetal insulin plays the predominant role in controlling the normal, as well as the augmented, fetal weight characteristic of the human infant of the diabetic mother. DIABETES *33*:656–660, July 1984.

The Pedersen hyperglycemia-hyperinsulinemia hypothesis states that maternal hyperglycemia leads to fetal hyperglycemia and, as a result, in hypertrophy of fetal islet tissue with insulin hypersecretion. The latter leads to greater fetal utilization of glucose and amino acids and enhanced fetal growth.[1] The clinical evidence confirming this hypothesis in the diabetic pregnancy is now extensive.[2] Other clinical examples of pathology associated with excessive fetal growth and fetal hyperinsulinism are the Beckwith-Wiedeman syndrome, infant giants or fetopathia diabetica, B-cell hyperplasia or adenomatosis, and nesidioblastosis.[3] In the case of the human IDM, however, the fetal plasma insulin concentrations are not as high as those produced in animal studies in which primary fetal hyperinsulinemia is produced. In one study of 20 infants of diabetic mothers (IDMs), umbilical plasma insulin concentrations were found to range from 5 to 330 μU/ml.[4] In the case of IDMs who were hypoglycemic 2 h after delivery, the umbilical plasma insulin concentration was approximately 10 times higher than that found in control infants.[5]

The stimulation of fetal overgrowth by the administration of insulin to the mammalian fetus has been described in both the rat[6–8] and rhesus monkey.[9] In all cases, large doses of insulin were given either by single or multiple injections or by chronic subcutaneous infusion. The chronic infusion approach in the rhesus monkey produced fetal insulin concentrations ranging up to 5300 μU/ml. In the hyperinsulinemic rhesus fetus, macrosomia is accompanied by increased placental, liver, heart, and carcass weight. The macrosomic fetal hyperinsulinemic rat also has increased liver and spleen weights. Morphologic study of human IDMs reveals a similar pattern of fetal macrosomia with selective organomegaly, with the fetal heart[10] and liver[10,11] significantly increased in weight.

The purpose of the present studies was to test the hypothesis that even in the absence of fetal hyperglycemia, primary fetal hyperinsulinemia, comparable to that which may be attained in the human IDM, results in fetal macrosomia in the rhesus monkey.

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TABLE 1
The effects of chronic hyperinsulinemia on fetal rhesus monkey size

Insulin dose (U/day)	Day	Gestational age (day)	Fetal plasma insulin (μU/ml)	Fetal weight (mg)	Placental weight (g)	Crown-heel length (cm)	Head circumference (cm)
Control 0 *(N = 9)*	21 ± 0	142 ± 5	28 ± 12	372 ± 54	92.4 ± 12.0	29 ± 2	19 ± 1
Low dose 4.75 (N = 10)	19 ± 1	145 ± 3	340 ± 208*	459 ± 53*	124.6 ± 39.5	*28 ± 2*	*18 ± 1*
High dose 19.0 (N = 10)	*20 ± 1*	141 ± 4	3625 ± 1700†,§	474 ± 48†	141.6 ± 50.8‡	28 ± 2	18 ± 1

*Low dose versus control, $P < 0.005$; †high dose versus control, $P < 0.001$; ‡high dose versus control, $P < 0.01$; §high dose versus low dose, $P < 0.005$.

MATERIALS AND METHODS

Pregnant rhesus monkeys (*Macaca mulatta*) of known gestation were supplied and maintained by the New England Regional Primate Research Center in Southborough, Massachusetts. As previously described,[9] primary hyperinsulinemia was produced by surgically implanting, under maternal ketamine anesthesia, an Alzet osmotically driven minipump (Alza Corp., Palo Alto, California) subcutaneously in a limb of the fetus at hysterotomy between days 113 and 128 of gestation. The pregnancy was then maintained for approximately 3 wk without any medications to the mothers other than prophylactic antibodies. Ten fetuses received 19.0 U (high-dose) per day of sodium pork insulin in buffered 1.6% glycerol. A second group of 10 fetuses received 4.75 U (low-dose) of insulin per day, one quarter of the original insulin dose. A third group of 9 fetuses served as controls. Of the control group, 4 fetuses were implanted with Alzet minipumps that delivered only 1.6% glycerol. The remaining 5 fetuses did not undergo the original implantation procedure. The control and 19-U/day, insulin-treated fetal groups include data from some previously studied animals (5 controls and 7 insulin-treated) as well as newly studied (4 control and 3 insulin-treated) animals.

Eighteen to 21 days after the start of insulin delivery, a second hysterotomy was performed. At this time, free-flowing maternal peripheral blood samples were taken for hormone and substrate determinations. Free-flowing fetal umbilical artery and vein blood samples were immediately taken, after the umbilical cord was exteriorized, for glucose and insulin measurements. An umbilical catheter was then passed into the fetus, through which it was exsanguinated in utero. The dead fetus was then delivered, weighed, measured, and dissected.

Plasma samples were analyzed for glucose with the Yellow Springs Instruments (Yellow Springs, Ohio) glucose analyzer 23A. Plasma insulin concentration was determined by a modification of the two-antibody radioimmunoassay of Hales and Randle.[12] Muscle, skeletal, and heart composition was determined by analysis of protein by the method of Lowry et al.,[13] RNA by the method of Halliburton and Thomson,[14] and DNA by the method of Dische.[15]

Data analysis was performed by the use of one-way analysis of variance on parametrically distributed data, and the Kruskal-Wallis analysis of variance on nonparametrically distributed data was used to identify differences among the three treatment groups. Regression analysis was performed to identify correlations. When comparisons between groups in the three treatment groups were performed, the Bonferroni-modified *t* test was used. In the case of the muscle composition studies, in which only the control and high-dose groups were studied, the unpaired Student's *t* test was used. Significance was attributed to $P < 0.05$. Data are given as mean ± SD.

RESULTS

Of the 10 fetuses that received the high-dose insulin for at least 18 days, 6 were alive at delivery on day 21 of hyperinsulinemia. The remaining 4 fetuses were delivered as stillbirths after 18 (one), 19 (one), and 21 (two) days of hyperinsulinemia. Of the low-dose–treated fetuses, 2 were delivered as stillbirths on day 21. Anthropomorphic meas-

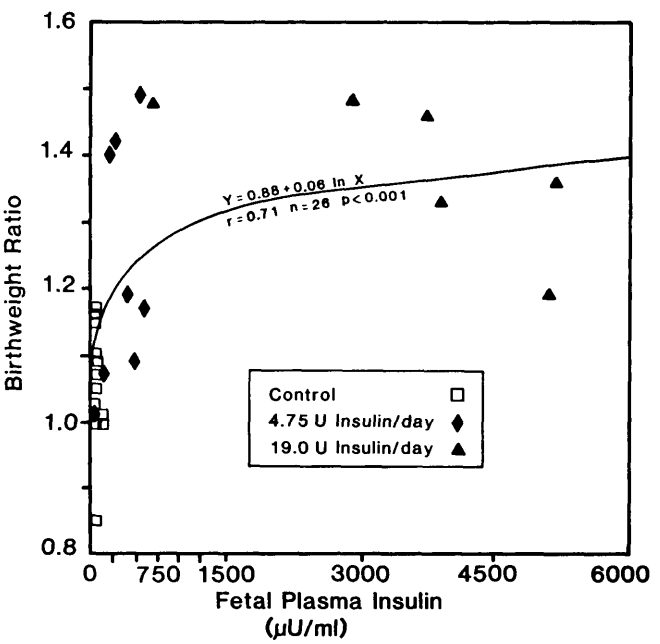


FIGURE 1. Correlation between fetal birth weight ratio and fetal umbilical artery plasma insulin. Controls are represented by open squares (□), fetuses receiving 4.75 U of insulin/day by closed diamonds (♦), and fetuses receiving 19.0 U of insulin/day by closed triangles (▲).

TABLE 2
Organ weights (g) of control and hyperinsulinemic fetal rhesus monkeys

Insulin dose (U/day)	Brain	Liver	Lung (total)	Heart	Thymus	Kidney (total)	Spleen	Adrenal (total)
Control 0 (N = 9)	48.0 ± 5.1	11.1 ± 2.5	7.5 ± 1.7	2.3 ± 0.6	1.3 ± 0.5	2.7 ± 0.5	0.8 ± 0.3	0.2 ± 0.1
Low dose 4.75 (N = 10)	50.7 ± 5.4	14.2 ± 1.6	10.4 ± 3.2	3.0 ± 0.7*	1.6 ± 0.5	3.0 ± 0.8	0.8 ± 0.4	0.2 ± 0.1
High dose 19.0 (N = 10)	47.6 ± 4.9	16.6 ± 4.4†	9.2 ± 2.8	3.7 ± 0.9‡,‖	1.7 ± 0.7	3.4 ± 0.9	1.4 ± 0.4§	0.2 ± 0.1

*Low dose versus control, $P < 0.05$; †high dose versus control, $P < 0.01$; ‡high dose versus control, $P < 0.001$; §high dose versus control, $P < 0.005$; ‖high dose versus low dose, $P < 0.05$.

urements were made, body and organ weights determined, and tissue samples taken for histology, but no chemical determinations were attempted in these stillborn fetuses. Because the duration of hyperinsulinemia was the same as in the fetuses alive at delivery, they are included in the treatment groups for anthropomorphic, body weight, and organ weight analysis.

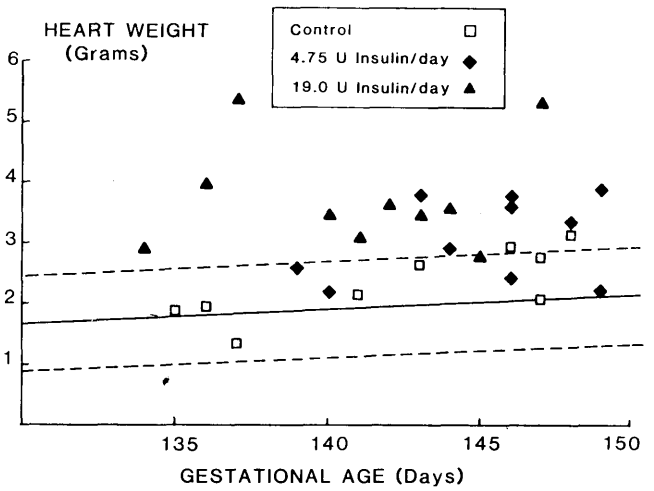
Table 1 summarizes the effects of chronic hyperinsulinemia on the fetal rhesus monkey. The duration of treatment and gestational age at delivery of the three groups of fetuses were the same. Fetal umbilical artery plasma insulin concentrations were significantly elevated in the fetuses receiving 4.75 and 19 U of insulin per day (340 ± 208 [$P < 0.005$] and 3625 ± 1700 μU/ml [$P < 0.001$], respectively) compared with the control group (28 ± 12 μU/ml) as well as to each other ($P < 0.005$). The corresponding arterial plasma glucose concentrations were 21 ± 9 and 22 ± 7 mg/dl for the low- and high-dose–treated groups and 34 ± 9 mg/dl for the controls. Fetal body weight was likewise significantly increased in the low- and high-dose groups (459 ± 53 [$P < 0.005$] and 474 ± 48 g [$P < 0.001$], respectively) compared with controls (372 ± 54 g). The placental weight was significantly ($P < 0.05$) increased compared with the control group only in the high-dose group. In contrast, the crown-heel length and the head circumference were not different among the three groups.

Figure 1 shows the birth weight ratio (the actual birth weight/expected birth weight for gestational age based on the data of Mellits et al.[16]) plotted against the fetal plasma insulin concentration. Regression analysis revealed a highly significant logarithmic correlation, with $r = 0.71$, described by the equation: birth weight ratio = 0.88 + 0.06 ln fetal plasma insulin concentration.

The organ weights of the fetuses in the three treatment groups are given in Table 2. Except for the heart, which was significantly heavier at 3.0 ± 0.7 g ($P < 0.05$) compared with 2.3 ± 0.6 g for the controls, the organ weights of the fetuses receiving low-dose insulin were not different from the controls. The weights of the liver, heart, and spleen were, however, increased compared with the controls in the high-dose insulin group. Brain, lung, kidney, thymus, and adrenal weights were not affected by hyperinsulinemia.

Figure 2 shows the distribution of individual weights of the heart and brain in the three groups superimposed on normal data for weight versus gestational age from Mellits et al.[16]

Figure 2A shows that there is only a minimal overlap in the heart weights between the control group and the insulin-treated groups, illustrating the cardiomegaly found in these



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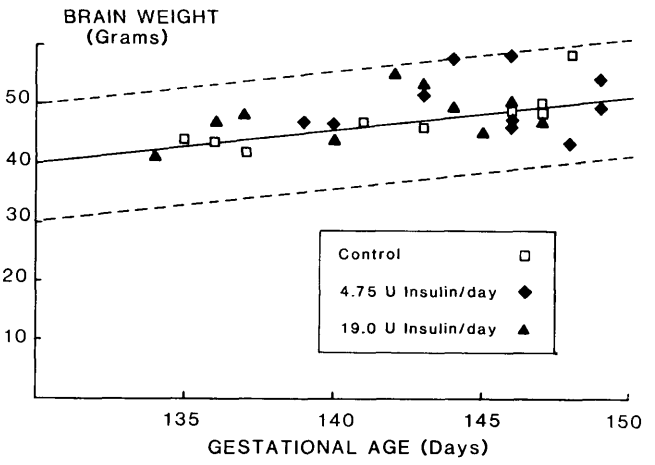

B

FIGURE 2. (A) Heart weight at delivery of fetal rhesus at various gestational ages compared with a larger control population represented by the solid line (——); regression ± 2 SD by broken lines (---), as reported by Mellits et al.[16] (B) Brain weight at delivery of fetal rhesus at various gestational ages compared with the data of Mellits et al.[16]

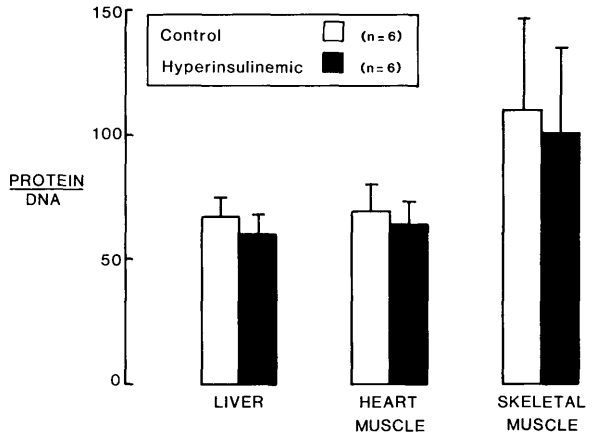

FIGURE 3. The protein:DNA ratio in liver, heart muscle, and skeletal muscle of control and high-dose–treated, hyperinsulinemic rhesus fetuses.

fetuses. Figure 2B, in contrast, shows that fetal brain weight is unaffected by hyperinsulinemia.

The protein, RNA, and DNA contents of heart of the insulin-treated fetuses were not different from the controls. Similarly, skeletal muscle composition was also found not to be different. Figure 3 illustrates the lack of effect of high-dose insulin treatment on the protein:DNA ratio in liver, heart, and skeletal muscle.

DISCUSSION

The degree of macrosomia in fetuses receiving low-dose insulin, with a mean insulin concentration of 340 μU/ml, was similar to that produced by the administration of the high-insulin dose, with a mean insulin concentration of 3625 μU/ml. Fetal weight increase in these two groups of insulin-treated fetuses was 23% and 27% compared with 22% for a group of 61 human IDMs.[4] Fetal weight, expressed as birth weight ratio, correlated with fetal umbilical plasma insulin levels, as shown in Figure 1. The logarithmic nature of this correlation confirms that fetal hyperinsulinemia in the physiologic range is capable of producing significant fetal weight gain.

There were, however, some differences in response to the two doses of insulin. Fetuses exposed to the high-insulin dose also had significantly heavier placentas, hearts, livers, and spleens. In contrast, those given the low dose only had significantly heavier hearts. The selective cardiomegaly found in the low-dose–treated rhesus fetuses is similar to that reported in human IDMs from a Scandinavian population.[11]

Skeletal growth stimulation in these fetuses was not observed, since crown-heel length was found to be the same as controls. The increase in weight of the hyperinsulinemic fetuses was most likely attributable to increased lipid, carbohydrate, and protein stores. Although whole body composition analysis was not performed, gross pathologic examination at autopsy identified large deposits of adipose tissue in the thoracic cavity, perirenally and pericardially, in both groups of hyperinsulinemic fetuses. In contrast, gross adipose tissue was not found in the control fetuses.

We have previously reported that hepatic protein, RNA, and DNA concentrations in the hyperinsulinemic rhesus fetus were the same as in controls.[9] In skeletal (psoas) and heart muscle, the concentration of protein, RNA, and DNA was also found to be the same. We interpret these liver, heart, and muscle composition data as evidence that hyperinsulinemia in the presence of normal-to-low substrate concentrations stimulates cell hyperplasia in some tissues. When fetal plasma substrate concentrations are elevated, as in the case of maternal diabetes, both hyperplasia and hypertrophy have been reported.[10] The normal or low fetal plasma substrate concentrations in the presence of hyperinsulinemia, however, do not preclude the enhanced fetal uptake of substrates, as has been shown in the case of glucose in the fetal sheep.[17]

In almost all animal studies in which primary fetal hyperinsulinemia has been produced, fetal macrosomia has resulted. The one exception is the fetal pig.[18] Worthy of note, also, is the lack of fetal macrosomia in piglets delivered of sows made diabetic with alloxan.[19] Even in the absence of hyperinsulinemia, rabbit fetal weight has been found to be correlated with fetal plasma insulin levels.[20] In the human, the relationship between fetal size and placental insulin receptor number supports the conclusion that fetal insulin mediates fetal growth.[21]

Fetal hyperinsulinemia in the absence of fetal hyperglycemia, with insulin concentrations that may be attained in the fetus of the diabetic mother, results in macrosomia and cardiomegaly in the fetal rhesus monkey. The macrosomia is comparable to that observed in the human infant of a diabetic mother whose disease has been inadequately controlled, particularly during the latter third of pregnancy. The fetal birth weight ratio of all the rhesus fetuses studied was found to correlate with the log of the plasma insulin concentration. This correlation is similar to the correlation between newborn birth weight ratio and the log of fetal umbilical plasma C-peptide immunoreactivity found in the human IDM.[22] In the human IDM, additional substrates, such as glucose, amino acids, and free fatty acids from the mother, may add to the excess of fetal fuels for growth. Differences between the animal models and human diabetes must be recognized, since they are not identical pathologic states. Nevertheless, the hyperinsulinemic rhesus fetus and human IDM share many similar characteristics, thus providing experimental insight into the pathophysiology of fetal macrosomia. We suggest, therefore, that fetal insulin plays a predominant role in controlling the normal as well as the augmented fetal growth characteristic of the human infant of the diabetic mother.

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