Effects of Insulin on the Biochemical and Morphologic Maturation of the Fetus

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Fetal hyperinsulinemia has been implicated in producing fetal macrosomia and other changes characteristic of the fetus of mothers with diabetes mellitus. Direct experimental evidence in support of the hypothesis that insulin alone can produce fetal macrosomia has been lacking in view of the fact that all previous studies have resorted to induction of maternal and hence fetal hyperglycemia to elicit an increased secretion of insulin by the fetus. The principal objective of the investigation was to study the growth and biochemical composition of the primate fetus exposed to high concentrations of insulin in the presence of normal glucose concentration in fetal and maternal compartments.

Twenty-five pregnant rhesus monkeys (Macaca mulatta) of known gestational age were used in the study. Of these, 15 were included in the experimental group, 5 in the sham operated control group, and 3 in the non-operated control group. Under ketamine anesthesia, the uterus was exposed and the hind limb of the fetus was delivered through a small incision. After obtaining aliquots of amniotic fluid and blood samples from the uterine vein, 500 µl. of fetal blood was obtained from the popliteal vein of the fetus. The samples were analyzed for glucose, insulin, glucagon, amino acids, free fatty acids, and lipids. A subcutaneous tunnel was then formed over the extensor surface of the thigh, and an osmotically driven mini-pump (measuring 6.5 x 25.0 mm.) filled with 170 µl. of aqueous solution of porcine insulin containing 3,200 units/ml. was inserted. The rate of infusion was 6 µl. per day corresponding to 18 units of insulin. The incisions in the fetal thigh, in the amniotic membrane, the uterine wall, and the abdominal wall were closed. Twenty-one days after implantation, at which time the fetuses ranged between 134-147 days (term being 170 days), the animals were re-anesthetized and the fetuses were removed by cesarean section. Maternal blood samples and amniotic fluid samples were obtained as previously. Fetal organs were removed for gross, microscopic, and chemical analysis (glycogen, lipids, protein, RNA, and DNA). Tissue samples for biochemical analyses were frozen in liquid nitrogen and stored at -70° C. until time of processing.

There was a 40% fetal loss within 48 hours after initial surgery in both insulin implanted or sham operated groups. Three of the insulin implanted fetuses were delivered as stillbirths after 19, 20, and 21 days exposure respectively. Because the duration of hyperinsulinemia of these fetuses was the same as those who were delivered alive, they were included in the full treatment group for the purposes of anthropomorphic and organ weight analysis. Of the seven fetuses implanted with insulin mini-pumps and delivered alive, only four could be classified as completely meeting the experimental conditions, since one mini-pump was found to be plugged at delivery, and in two cases, the pump had been extruded into the amniotic space.

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The mean glucose concentration of mother and fetus being 47 mg/dl and 27 mg/dl respectively, was unaffected by administration of insulin into the fetus. The same also pertained to the concentration of insulin in maternal plasma the mean of which was 63 μU/ml. before, and 68 μU/ml. after the implant. There was, however, more than 100-fold increase in the concentration of insulin in fetal plasma (from 30 μU./ml. to 3,525 μU/ml. and a more than 20-fold increase in that of amniotic fluid from 21 μU/ml. to 460 μU/ml.

There was a slight reduction in the 9 amino acids tested for in the hyperinsulinemic fetuses, although there was no change in their ratios. The lowering of plasma amino acids is ascribed to an increased protein synthesis by the macrosomic, hyperinsulinemic fetus. The concentrations of plasma lipids, free fatty acids (FFA), and 3-hydroxybutyrate (3-HB) were unaffected. The very low concentration of 3-HB (less than 1/20 of that of fasting adults) suggests that the fetus does not utilize FFA to an appreciable extent. The concentration of glucagon was lower in the hyperinsulinemic fetuses. Because of the small numbers, the difference, however, was not statistically significant. In adult man insulin is known to inhibit glucagon secretion.

The relative increases of total body and organ weights for the hyperinsulinemic fetuses in comparison to controls were as follows: body, 34%; placenta, 67%; liver, 61%; heart, 106%; spleen, 179%; lung, 31%; kidney, 11%; brain, 4%. There was no increase in the crown-heel length. Analysis of liver tissue revealed a 33% ($p < 0.05$) increase in glycogen concentration and a 50% increase in lipids ($p > 0.05$); DNA and RNA, and protein-DNA ratios remained unaffected indicating that hyperinsulinemia resulted in an increase in cell numbers rather than in cell size. There was extensive erythropoesis in the liver of the hyperinsulinemic fetuses; erythropoetic tissue was essentially absent in controls. It remains to be elucidated whether the increase in erythropoesis in the hyperinsulinemic fetuses is due to a direct effect of insulin or to a lower fetal pO_2. Examination of the fetal lung by light and electronmicroscopy revealed no differences between the two populations. The same is also true regarding the concentration of phospholipids.

We conclude that fetal hyperinsulinemia unaccompanied by hyperglycemia or other changes in the composition of the fetal plasma does lead to fetal macrosomia of a type characteristic of infants of diabetic mothers. We infer that the oxygen consumption of such fetuses is increased, and that the disproportionately small hyperplasia of the lung, in combination with hyperviscosity and hepatosplenomegaly contributes to the respiratory distress often seen in infants of diabetic mothers.

The elimination of maternal hyperglycemia and thus fetal hyperinsulinemia should lead to a totally normal development of fetuses of diabetic mothers without vascular disease.

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