0031-3998/89/2605-0467$02.00/0
PEDIATRIC RESEARCH

Vol. 26, No. 5, 1989
Printed in U.S.A.

The Effect of Prolonged Intrauterine Hyperinsulinemia on Iron Utilization in Fetal Sheep

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ABSTRACT. Newborn infants of poorly controlled insulin-dependent diabetic mothers demonstrate a redistribution of iron from serum and tissue stores into red blood cells. These changes may be due to increases in iron utilization during augmented Hb synthesis, which compensates for chronic intrauterine hypoxemia induced by prolonged fetal hyperinsulinemia. We tested this hypothesis by measuring plasma iron, total iron-binding capacity, percent iron-binding capacity saturation (total iron-binding capacity saturation), Hb concentration, total red cell Hb, and total red cell iron in the arterial blood of 11 chronically instrumented fetal sheep after 7–12 d of infusion with 15 U/day of insulin ($n = 5$) or placebo ($n = 6$). The insulin-infused fetal sheep had higher mean ± SD plasma insulin concentrations (448 ± 507 *versus* 11 ± 8 mU/L; $p < 0.001$) and lower arterial oxygen saturations (38 ± 7 *versus* 54 ± 9%; $p < 0.02$). The insulin-infused group had a lower mean plasma iron concentration (20.8 ± 10.9 *versus* 42.1 ± 14.7 μM/L; $p < 0.02$) and total iron-binding capacity saturation (36 ± 20 *versus* 64 ± 22%; $p < 0.02$) and a higher total red cell Hb (45.4 ± 8.7 *versus* 32.6 ± 8.8 g; $p < 0.02$) and total red cell iron content (154 ± 29 *versus* 111 ± 29 mg; $p < 0.02$) when compared with the placebo group. Seven to 12 d of intrauterine hyperinsulinemia decreases serum iron and increases total red cell iron, most likely by stimulating increased Hb synthesis in response to low arterial oxygen saturation. Hyperinsulemia may play a major role in the altered iron metabolism in newborn infants of diabetic mothers. (*Pediatr Res* 26:467–469, 1989)

Abbreviations

TIBC, total iron-binding capacity
TIBC saturation, TIBC saturation
FEP, free erythrocyte protoporphyrin
CaO_2, calculated arterial oxygen content

Infants of insulin-dependent diabetic mothers who are large for dates and hypoglycemic at birth rate are at high risk for the hematologic manifestations of iron deficiency, including abnormally low cord blood serum ferritin and iron concentrations and abnormally high cord blood serum transferrin, total iron-binding capacity and FEP concentrations (1–3). These alterations imply aberrations in fetal iron metabolism ranging from depletion of iron stores (decreased ferritin) to ineffective erythropoiesis (increased FEP) (4). Many infants of diabetic mothers are polycythemic at birth (5), which means that fetal iron delivery to red blood cells must increase to support this augmented Hb synthesis.

The mechanism of altered iron metabolism in these infants has not been studied. We have hypothesized that the changes most likely represent redistribution of iron from tissue stores into red cells for the compensatory increase in Hb synthesis associated with chronic intrauterine hypoxemia (2). The latter is thought to be due to increased fetal oxygen consumption induced by both chronic intrauterine hyperinsulinemia and hyperglycemia (6–8), but may also be due to the effect of significant vascular disease on oxygen or nutrient transfer.

This study tests the hypothesis that prolonged hyperinsulinemia in fetal sheep without hyperglycemia or underlying placental vascular disease results in sufficient arterial blood oxygen desaturation to cause a shift of iron from the plasma into the red cells.

MATERIALS AND METHODS

Animal preparation. Eleven mixed-breed Eastern ewes with time-dated pregnancies between 122 and 126 d gestation were anesthetized with intravenous ketamine and local lidocaine, and had catheters placed into the inferior vena cavae and abdominal aortae of their fetuses via the pedal arteries and veins as previously described (9). Plasma samples for this study were obtained from animals enrolled in a larger series of studies assessing the sequential effects of hyperinsulinemia on fetal blood volume and fatty acid metabolism, and in which plasma was available at the time of death for iron analysis (9–11).

Experimental protocol. Four to 6 d after surgery, the fetal sheep were assigned to an insulin-infused ($n = 5$) or placebo-infused ($n = 6$) group. Insulin-infused fetal animals received 15 U/day of Iletin II (Eli Lilly and Co., Indianapolis, IN) in diluent via a fetal vein (9–11). Placebo-infused animals received the diluent. Fetal arterial samples were obtained for plasma insulin, glucose, iron, and TIBC, oxygen saturation, Hb, and hematocrit after 7 to 12 d of infusion. Maternal arterial plasma was also obtained for analysis of plasma iron and TIBC concentrations.

Biochemical analysis. Plasma insulin concentrations were measured by a double antibody RIA (12), plasma glucose using a Yellow Springs Instrument Glucose Analyzer (model 23A, Yellow Springs, OH), fetal oxygen saturations and Hb concen-

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Supported in part by the National Institute of Health and Human Development Diabetes Center Grant P-50 HD11343 and a grant from Ross Laboratories.

trations with a Radiometer Hemoximeter (model OSM2, Copenhagen, Denmark), and fetal hematocrits by a microhematocrit method. Plasma samples for iron determination were centrifuged immediately and the plasma frozen at −20°C until analysis. Plasma iron concentration was determined by direct iron assay using the chromophore Ferene (Dimension Clinical Chemistry System, E.I. DuPont Co., Wilmington, DE). Plasma TIBC was measured using the same chromophore following elution from an alumina column (Dimension Clinical Chemistry System, E.I. DuPont Co., Wilmington, DE). Interassay variabilites for the iron and iron-binding capacity assays within the range of serum samples tested were <3%. All analyses were done in duplicate.

Determination of red cell volumes was necessary for the calculation of total red cell Hb and iron contents. Red cell volumes were measured using ^{99m}Tc autologous-labelled fetal red cells (13, 14). Briefly, 4 mL of fetal whole blood in acid-citrate dextrose solution was incubated for 15 min each with Sn-pyrophosphate and subsequently ^{99m}Tc. The red cells were washed, resuspended in normal saline and administered in a bolus of 5 mL to the fetus. Fetal arterial blood was sampled at 10 and 20 min, the samples were weighed and radioactivity counted (Traycor analytic sample changer, model 1185, Elk Grove, IL). Fetal red cell volume (mL) was calculated by dividing the total cpm of the standard fetal red cells injected by the cpm/mL of the fetal red cells sampled. Fetal blood volume was calculated by dividing the measured red cell volume by the fetal hematocrit. Values for 10 and 20 min were not different and were averaged. Fetal arterial oxygen contents (CaO_2) were calculated as previously described (15).

Serum and red cell iron calculations. Percent TIBC saturation was calculated by dividing plasma iron by TIBC values and multiplying by 100. Total red cell Hb was calculated by multiplying fetal blood volume by the Hb concentration. The total red cell iron content was calculated by multiplying total red cell Hb by 3.4 mg of elemental iron/g Hb.

Statistical analysis. We compared the mean plasma insulin concentrations, glucose concentrations, oxygen saturations. CaO_2, plasma irons, TIBC, TIBC saturations, Hb concentrations, total red cell Hb, and total red cell iron contents of the insulin-infused group with the values of the placebo group at the end of the infusion period using the two-tailed Student's t test with significance set at a $p < 0.05$. All values are presented as mean ± 1 SD.

RESULTS

The two groups had comparable gestational ages, number of days of infusion, and plasma glucose concentrations (Table 1). After 7–12 d, the insulin-infused group had a significantly higher plasma insulin concentration and significantly lower arterial oxygen saturation but similar CaO_2 when compared with the placebo-infused group.

Mean plasma iron concentration and percent iron-binding saturation were significantly lower in the insulin-infused group than in the placebo group and were accompanied by a significantly higher total red cell iron content (Fig. 1). TIBC was similar in both groups. The blood volume of the insulin-infused group was 0.447 ± 0.073 L and was significantly more than the 0.345 ± 0.062 L value of the control group. The total red cell Hb content was significantly greater in the insulin-infused group, although the mean Hb and hematocrit concentrations of the two groups were not different (Table 2).

The iron status of the ewes was measured in the arterial plasma of four animals in which the fetus had been infused with insulin and five in which the fetus had been infused with placebo. The mean maternal plasma iron of the insulin-infused fetal animals was 45.6 ± 8.2 μM/L, with a TIBC saturation of 73 ± 20%. These values were not different from the maternal plasma iron (46.7 ± 6.6 μm/L) and TIBC saturation (71 ± 17%) of the placebo-infused fetuses. There also were no differences between these maternal values and those of the placebo-infused fetal sheep.

Table 1. *Gestational age, infusion time, plasma glucose and insulin concentrations, and fetal oxygen characteristics of insulin- and placebo-infused sheep at time of assessment of iron status (mean ± SD)*

	Insulin ($n = 5$)	Placebo ($n = 6$)	p
Gestational age (d)	135 ± 1	133 ± 2	NS
Time of infusion (d)	11 ± 1	9 ± 2	NS
Plasma insulin (mU/L)	448 ± 507	11 ± 8	<0.001
Plasma glucose (mmol/L)	0.50 ± 0.17	0.74 ± 0.28	NS
Arterial oxygen saturation (%)	38 ± 7	54 ± 9	<0.02
C_aO_2 (ml/L)	64 ± 16	76 ± 21	NS

DISCUSSION

Our study demonstrates that 7 to 12 d of hyperinsulinemia significantly alters the iron status of late gestation fetal sheep. Prolonged fetal insulin infusion is associated with a decreased plasma iron concentration and iron-binding saturation, increased total red cell Hb and iron content, and lower fetal arterial oxygen saturation. These results suggest that iron is transferred from plasma into red cells to support accelerated Hb synthesis in response to prolonged fetal hypoxemia. The study supports our hypothesis that chronic fetal hypoxemia associated with prolonged fetal hyperinsulinemia is a likely etiology of the altered iron status described in newborn infants of diabetic mothers (1–3).

We have previously proposed two potential mechanisms to account for the serum iron changes seen in infants of diabetic mothers. These include increased iron utilization during hypoxemia-augmented erythropoiesis and impaired placental iron transfer secondary to diabetic placental vascular disease (1, 3). The hyperinsulinemia sheep model allowed us to eliminate placental vascular disease as a variable and to demonstrate that

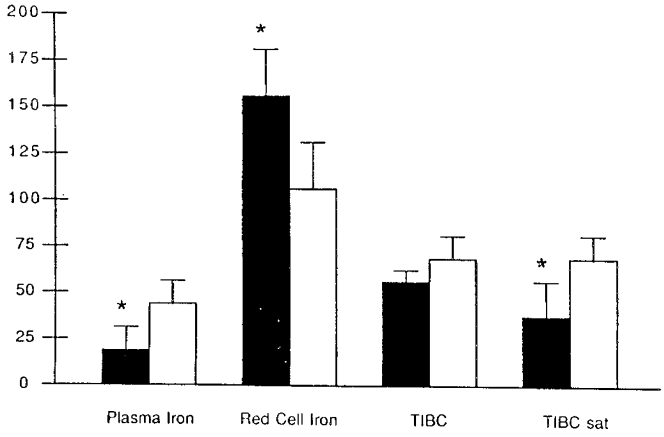

Fig. 1. Iron status of insulin- (■) and placebo- (□) infused fetal sheep. Plasma iron and TIBC are expressed as μM/L, red cell iron as mg, and TIBC saturation as a percentage. Values are mean ± SD. * $P < 0.02$ versus placebo-infused group.

Table 2. *Hematologic status of insulin- and placebo-infused fetal sheep at time of iron analysis (mean ± SD)*

	Insulin ($n = 5$)	Placebo ($n = 6$)	p
Hb (g/L)	101 ± 7	96 ± 15	NS
Hematocrit	0.350 ± 0.023	0.326 ± 0.053	NS
Total red cell Hb (g)	45.4 ± 8.7	32.6 ± 8.8	<0.02

hyperinsulinemia is an independent variable that affects fetal iron utilization.

The specific mechanism by which fetal hyperinsulinemia alters iron utilization is unknown. We speculate that the driving force for the increased red cell iron utilization seen in our fetal sheep was the accelerated Hb synthesis associated with the significant decrease in arterial oxygen saturation. Chronic fetal hyperinsulinemia causes arterial oxygen desaturation (8, 16), most likely by increasing fetal oxygen consumption (8). Hyperinsulinemia increases fetal erythropoiesis in rhesus monkeys, most likely in response to hypoxemia (6), although hyperinsulinemia may also independently stimulate erythropoiesis (17). Expansion of the red cell mass in response to hypoxemia with its attendant increase in Hb synthesis results in increased utilization of available iron (18, 19).

In our study the insulin-infused animals had 40% more total Hb in red cells than the placebo-infused group. This difference in Hb content requires the delivery of an additional 44 mg of elemental iron to the erythrocytes of the insulin-infused fetus. Iron for additional Hb is potentially available from several sources, including fetal plasma, fetal tissue stores, and maternal plasma. Further research is necessary to quantitate the relative contributions of these sources during pathologic states that augment fetal erythropoiesis. Fetal sheep have relatively low tissue iron stores (20), a factor that may potentially limit the amount of elemental iron available from this source during a sudden increase in Hb synthesis and may explain the rapidity with which a significant decrease in plasma iron was observed in our study.

The time course over which fetal iron status changed was consistent with a previous model of hematologic adaptations to chronic arterial oxygen desaturation in neonatal sheep (21), and reflects the amount of time required to incorporate iron into Hb in third trimester fetal sheep (19). As in hypoxemic neonatal sheep (21), arterial oxygen content values at 7 to 12 d were similar in the two groups, implying that the 40% increase in total red cell Hb compensated for the 30% decrease in arterial oxygen saturation. The cost of this compensation was lower serum iron concentrations and iron-binding saturations. An analogous finding in newborn human infants has been described where cord serum ferritin concentrations, representing storage iron, are inversely related to cord Hb concentrations (22).

Although total red cell Hb was significantly higher in the insulin-infused animals, the slightly higher Hb and hematocrit concentrations were not significantly different than the placebo-infused group. The lack of a significant difference between the two groups may have been due to a β error. Alternatively, plasma Hb concentrations may take longer than the duration of our study period to rise, because plasma volume also appears to vary with the duration and severity of hypoxemia (23–25).

Neonatal iron deficiency on the basis of maternal iron deficiency is thought to be relatively rare because fetal iron status remains normal unless the mother is profoundly iron deficient (26). Nevertheless, the long-term consequences of decreased fetal plasma iron concentrations and red cell iron sequestration on nonheme tissues in fetal conditions characterized by accelerated heme synthesis are of potential importance to the developing human during late gestation. Iron deficiency during early postnatal life has been known to affect the development and function of multiple organ systems, including the central nervous system (27, 28). Further studies are necessary to understand the effect of prolonged fetal hyperinsulinemia on iron stores and iron delivery to nonheme tissues in the fetus.

This study in hyperinsulinemic fetal sheep supports the hypothesis that hyperinsulinemia is at least in part responsible for the hematologic manifestations of altered iron status previously described in newborn infants of insulin-dependent diabetic mothers and presents further evidence that strict maternal glycemic control, which may prevent fetal hyperinsulinemia during the third trimester, is critical for fetal and neonatal well being.

Acknowledgments. The authors acknowledge the excellent technical assistance of Ann Beauregard, Dennis Berard, and Edrie Murphy as well as the continued support of Dr. William Oh during these studies. We also thank George L. Ross, M.S., for the development of the mechanized sheep lift, which was indispensable to the surgical preparation of the fetal sheep.

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