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surface active material flux
tracheal fluid

Primary Hyperinsulinemia Reduces Surface Active Material Flux in Tracheal Fluid of Fetal Lambs

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Summary

We sought to test the hypothesis that hyperinsulinemia *per se* alters the flux of surface active material (SAM) into tracheal fluid by continuously infusing insulin (0.24 ± 0.04 units/kg/hr, mean ± S.E.) from 112 through 135 days gestation into five chronically catheterised fetal lambs, from which tracheal fluid could be collected.

Serum insulin levels in these fetuses (95 ± 10 μU/ml) were greater than in five chronically catheterised control fetuses of the same gestational age (10 ± 1 μU/ml, $P < 0.001$) and in the mothers (38 ± 6 μU/ml, $P < 0.001$). Serum glucose levels in the insulin-treated fetuses (10 ± 1 mg/dl) were lower than in the control fetuses (19 ± 1 mg/dl, $P < 0.001$) and in the mothers (60 ± 3 mg/dl, $P < 0.001$). Arterial blood gases (pH 7.37 ± 0.01, P_{O_2} 23.3 ± 0.05 mm Hg, P_{CO_2} 41.5 ± 0.9 mm Hg) and hematocrit (33 + 1% at 127 days gestation and 31 ± 1% at 135 days gestation) in the insulin treated fetuses were not different from the controls.

SAM flux into the tracheal fluid of the insulin-treated fetuses was 1 μg/kg/hr, coefficient of variation 373%. This was lower than SAM flux in the control fetuses (26 μg/kg/hr, coefficient of variation 28%, $P < 0.01$). Moreover, among the control fetuses, SAM began to appear in tracheal fluid at 119 days gestation and was present in all five fetuses by 125 days gestation, whereas SAM did not begin to appear in the insulin-treated fetuses until 127 days gestation and did not appear at all in three of them.

Speculation

Chronic hyperinsulinemia reduces surface active material flux into tracheal fluid of fetal lambs. This effect may be partially mediated by reduced substrate (glucose) availability for surface active material phospholipid synthesis, storage, and/or secretion.

The incidence of respiratory distress syndrome is reported by Robert *et al.* (21) to be increased almost 6-fold in infants of diabetic mothers, even when suitable corrections are made for gestational age. However, RDS may be less prevalent when maternal diabetes is mild and well controlled (7, 8). The infants of diabetic mothers experiences hyperinsulinemia both *in utero* and in the postnatal period (2, 10, 12, 16, 22), particularly when glucose homeostasis is poor (24).

Stubbs and Stubbs (28) have hypothesised that fetal hyperinsulinemia may be the common link between maternal diabetes mellitus and respiratory distress syndrome. Stubbs *et al.* (27) reported that the glucose uptake of isolated perfused rat lung increased by 30% in the presence of physiologic concentrations of insulin. Sosenko *et al.* (26) and Rhoades *et al.* (20) found evidence of delayed lung maturation in fetuses of glucose intolerant rabbits and rats respectively. Also, Smith *et al.* (23) found that, in the presence of glucocorticoids, lecithin synthesis by cultured fetal lung cells decreased in response to higher concentrations of insulin. In addition, Gross *et al.* (9) have indicated that insulin delays the morphologic maturation of fetal rat lung cultures, causing a decrease in the number of lamellar bodies and alveolar type II cells. However, Epstein *et al.* (6) found no decrease in the rate of incorporation of choline into phosphatidylcholine in lung slices obtained from the fetuses of glucose intolerant monkeys.

We sought to test the hypothesis that hyperinsulinemia *per se* alters the flux of surface active materials (SAM) into tracheal fluid by infusing insulin into chronically catheterised fetal lambs from which tracheal fluid could be collected.

MATERIALS AND METHODS

Gestational age of fetal lambs was determined from the time of mating (Nebeker Farms, Santa Monica, CA). In addition, we estimated fetal age from ossification centers *in utero*, and by extrapolation of fetal weight and crown-rump measurements at delivery; dating by these methods agreed within 3 days.

Between 108 and 110 days of pregnancy, ewes were operated upon under 0.5% xylocaine epidural anesthesia, as described by Platzker *et al.* (17). Polyvinyl chloride catheters were placed in the fetal carotid artery and jugular vein. A stiff polyethylene catheter was inserted into the fetal trachea. This catheter led to a 600-ml latex bag which was left in the amniotic sac. A separate catheter leading from the latex bag, together with the fetal artery and vein catheters, was brought out through the ewe's flank. This arrangement allowed us to remove accumulated tracheal fluid without applying negative pressure to the lungs, to obtain intermittent arterial blood gas samples from the fetus, and to give intravenous infusions to the fetus. A polyvinyl chloride catheter was also placed in a maternal vein. After operation, the ewes were given 150 mg medroxy progesterone acetate aqueous suspension intramuscularly. The ewes also received 1.2 million units procaine penicillin and 1 g kanamycin intramuscularly for 5 days. The animals were allowed to recover for up to 3 days after the operation. The fetuses received 200,000 units penicillin G and 10 mg kanamycin intravenously every day.

We induced primary hyperinsulinemia by continuous intravenous infusion of insulin (Iletin, Eli Lilly and Company, Indianapolis, IN) using a syringe infusion pump (Harvard Apparatus Company, Millis, MA). Insulin infusions were given to three singletons and to one twin fetus from each of two twin pregnancies. Three additional singletons and the two untreated twins served as controls.

Tracheal fluid was collected daily and stored at −40°C for subsequent analysis. Arterial blood gases were measured on alternate days using a Corning 175 blood gas analyzer (Corning Medical, Medfield, MA). Serum samples were also collected on alternate days and stored at −40°C for subsequent analysis.

The SAM in each tracheal fluid sample was measured on a surface balance (5). SAM flux, in μg/kg/hr, was calculated by multiplying the tracheal fluid SAM concentration in μg/ml by tracheal flow in ml/kg/hr. Fetal weight was extrapolated from the fetal weight data of Barcroft (1), using the actual fetal weight measured at necropsy after fetal death.

Serum glucose concentration in mg/dl was measured using a YSI-23A glucose analyzer (Yellow Springs Instruments, Yellow

Springs, OH). Serum insulin concentration in μU/ml was measured using a competitive binding radioimmunoassay (Beckton Dickson Immunodiagnostics, Orangeburg, NY). Serum cortisol concentration in μg/dl was measured using a competitive binding radioimmunoassay (Clinical Assays, Cambridge, MA). Hematocrit of fetal blood was determined using a mini-centrifuge (International Equipment Company, Needham Heights, MA).

SAM flux was compared using Wilcoxon's Rank sum test for unpaired data (31). The serum insulin and serum glucose levels in the insulin-treated fetuses *versus* the control fetuses and in the insulin-treated fetuses *versus* the mothers were compared using the Student's *t* test for unpaired data (29).

RESULTS

Insulin infusions were given to three singleton and two twin fetuses at a rate of 0.24 ± 0.04 U/kg/hr, mean ± S.E. from 112 through 135 days of gestation. The serum insulin levels in these fetuses during the infusion were 95 ± 10 μU/ml. The serum insulin levels in the three singleton and two twin untreated control fetuses were 10 ± 1 μU/ml. The serum insulin levels in the mothers were 38 ± 6 μU/ml. The differences between the insulin-treated group and the controls and between the insulin-treated group and the mothers were statistically significant, $P < 0.001$, $P < 0.001$, respectively.

The serum glucose levels in the insulin treated fetuses were 10 ± 1 mg/dl. The serum glucose levels in the control fetuses were 19 ± 1 mg/dl. The serum glucose levels in the mothers were 60 ± 3 mg/dl. The differences between the insulin-treated group and the controls and between the insulin-treated group and the mothers were statistically significant, $P < 0.001$, $P < 0.001$, respectively.

Arterial blood gases in the insulin-treated fetuses were pH 7.37 ± 0.01, P_{O_2} 23.3 ± 0.5 mm Hg, P_{CO_2} 41.5 ± 0.9 mm Hg. These values were not different from the controls. Hematocrit of the insulin treated fetuses were 33 ± 1% at the beginning of the experiment and 31 ± 1% at the end. These values were not different from the controls.

The rate of tracheal fluid production in the insulin treated fetuses was 3.0 ± 0.1 ml/kg/hr. The rate of fluid production in the controls was 2.8 ± 0.1 ml/kg/hr (not significantly different).

SAM flux into the tracheal fluid of the fetal lambs is shown in Figure 1. In the insulin-treated fetuses, SAM flux was 1 μg/kg/hr, coefficient of variation 373%. This was statistically significantly lower than SAM flux in the control fetuses, 26 μg/kg/hr, coefficient of variation 28%, $P < 0.01$.

Among the control fetuses, SAM began to appear in tracheal fluid at 119 days gestation and was present in all five fetuses by 125 days gestation. In contrast, SAM did not begin to appear in the tracheal fluid of the insulin treated fetuses until 127 days gestation and did not appear at all in three of them.

In two of the five insulin treated fetuses, SAM appeared in the tracheal fluid on day 129 (flux 14 μg/kg/hr) and day 130 (flux 13 μg/kg/hr) of gestation in one fetus and on days 127 (flux 3 μg/kg/hr) and 129 (flux 4 μg/kg/hr) of gestation in the other. These were days on which the insulin infusion pump malfunctioned, resulting in low serum insulin levels in the fetuses (1 and 4 μU/ml; 1 and 3 μU/ml, respectively). On the following days, when the insulin infusion pumps were functioning properly, SAM flux into the tracheal fluid returned to 0 μg/kg/hr for a duration of 2 or more days.

Serum cortisol levels in both the insulin treated and the control fetuses were < 1.5 μg/dl throughout the gestational period of this experiment.

We were unable to maintain the insulin-treated fetuses in a physiologically stable state past 135 days gestation. For this reason we have not reported data past 135 days gestation. Eventual intrauterine death of the insulin-treated fetuses was associated with progressively severe hypoglycemia. Hypoxemia seemed to be an agonal event in these fetuses.

DISCUSSION

Continuous intravenous infusion of insulin produced significant elevation of serum insulin levels in five chronically catheterised lamb fetuses. However, we also found hypoglycemia relative to controls in these fetuses. Carson *et al.* (4) have also reported hypoglycemia, together with decreased arterial oxygen content, during sustained insulin infusion in the ovine fetus. In the current study, arterial oxygen content was not measured. However, arterial blood gases, specifically P_{O_2}, and hematocrit were not affected by chronic insulin infusion before 135 days gestation. Eventual intrauterine death of the insulin-treated fetuses in the current study was associated with progressively severe hypoglycemia. Fetal hypoxemia appeared to be an agonal event.

Primary hyperinsulinemia with secondary hypoglycemia was associated with a significant reduction of SAM flux into tracheal fluid of fetal lambs without affecting the rate of tracheal fluid production. The lungs of the fetal lamb produce fluid that flows from the trachea as early as 94 days gestation (13). Lung fluid production continues until birth. Fluid production rates reported by previous investigators have ranged from 2.2 to 4.5 ml/kg/hr. In the studies of Mescher *et al.* (13), SAM appeared in tracheal fluid between 124 and 133 days gestation, and its flux increased rapidly after 135 days to 125 μg/kg/hr at 148 days. Platzker *et al.*

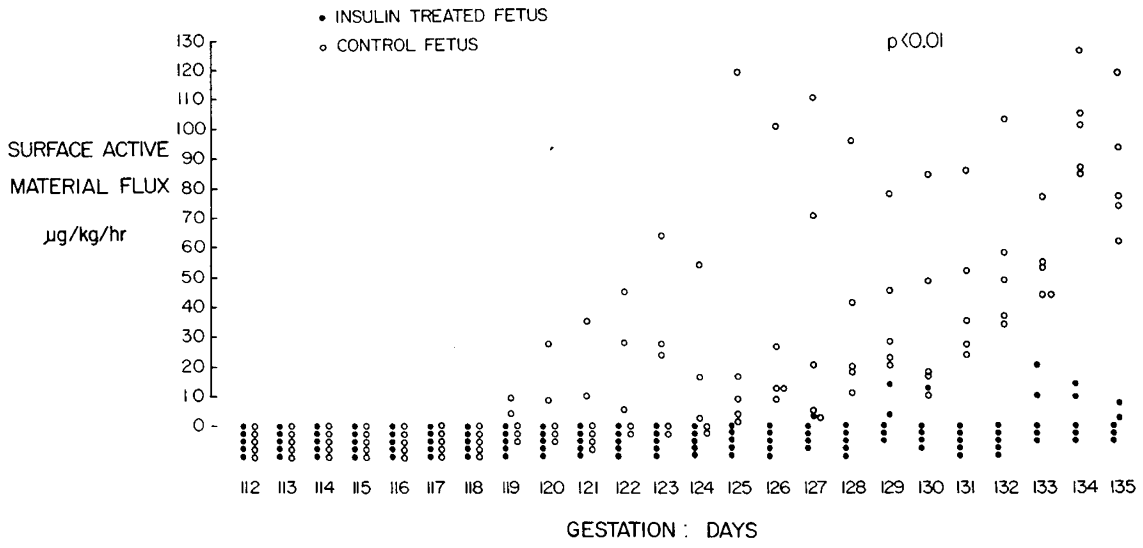

Fig. 1. SAM flux into tracheal fluid of chronically catheterised fetal lambs: comparison of insulin-treated and control fetuses between 112 and 135 days gestation. *P* value refers to significant difference by Wilcoxon's Rank sum test for unpaired data.

(17) isolated small amounts of disaturated lecithin from lung homogenates of fetal lambs at 108 days gestation. However, this method cannot specifically identify disaturated lecithin in SAM. Kikkawa *et al.* (11) found that lamellar inclusion bodies were first detected on electron microscopy in the alveolar type II cells of fetal lamb lungs at 113 days gestation. Lamellar inclusion bodies are the most probable storage form of pulmonary surfactant. Hence, in the current study, primary hyperinsulinemia with secondary hypoglycemia was present from the probable time of inception of SAM synthesis and packaging for secretion. Glucose is probably the major source of glycerol-3-phosphate and dihydroxyacetone phosphate which are necessary for the synthesis of complex lipids (15), although glycerol may be used to some extent (30). Activation of pyruvate dehydrogenase by insulin (18) would increase conversion of glucose to acetyl-coenzyme A, thereby decreasing production of intermediary metabolites such as glycerol-3-phosphate and dihydroxyacetone phosphate. Therefore, in the hyperinsulinemic fetus with decreased substrate (glucose) availability, decreased SAM lipid synthesis might occur. Rhoades (19) has already shown that starvation reduces glucose utilization for lipid synthesis by the lung. However, it should be noted that hyperinsulinemia in the human diabetic pregnancy occurs in the presence of hyperglycemia. The effects of primary hyperglycemia on lung maturation in the ovine fetus remain to be studied. Moreover, the relationship between hyperinsulinemia and end-organ effect is complex. Neufeld *et al.* (14) found increased concentrations of insulin receptors as well as increased affinity of receptors for the hormone in circulating blood monocytes obtained from newborn infants of gestational diabetic mothers.

It is also tempting to speculate on a possible effect of acute fluctuation in insulin levels on the release of SAM into tracheal fluid. SAM appeared transiently in the tracheal fluid of two fetuses when the insulin infusion pumps broke down, and disappeared again when the infusion pumps were repaired. Brown *et al.* (3) have reported that glucocorticoid induction of SAM production in fetal lambs is also reversible.

Sosenko *et al.* (24) reported that maternal administration of cortisol reverses functional delay of lung maturation in fetuses of alloxan diabetic rabbits. In the present study, serum cortisol levels in both the insulin treated and control fetuses were low (<1.5 μg/dl). Mescher *et al.* (13) have shown that SAM release into tracheal fluid of fetal lambs normally precedes the rise in serum cortisol levels which occurs after 140 days gestation in these animals.

CONCLUSION

Primary hyperinsulinemia with secondary hypoglycemia reduces SAM flux into tracheal fluid of chronically catheterised fetal lambs.

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