Insulin Response to Fructose and Glucose Infusions into the Sheep Fetus[1] (35408)

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There is still no general agreement whether the fetal pancreas releases insulin in response to hyperglycemia. Insulin secretion by the fetal sheep pancreas has been observed by Alexander *et al.* (1) following a glucose infusion into the fetal circulation. Conversely, Willes *et al.* (2) have reported that injections of fructose or glucose to sheep fetuses did not stimulate insulin release. The present study was carried out to determine whether the rapid infusion of glucose or fructose into the fetal circulation provokes a rise in fetal plasma insulin levels in animals not subjected to operative or anesthetic stress.

Materials and Methods. Eight Dorset or Western ewes (fasted 3 days prior to surgery and bearing singlets of known gestational age) were prepared for surgery as described by Meschia *et al.* (3). Two catheters were placed in the fetal circulation, one for the injection of solutions and the other for withdrawal of blood samples. The injection catheters were never used to obtain blood samples for analysis. Catheter sites in the fetus were either the umbilical vein and umbilical artery or the femoral vein and artery. Maternal catheter sites were the uterine vein and/or the femoral artery. The ewes were permitted a minimum of 15 hr recovery after surgery prior to experimentation.

Catheters were kept patent by daily irrigation with dilute heparin in saline (1000 units/ml). During the first hour prior to hexose injection, up to five control samples (1 to 1.5 ml) were taken not less than 5 min apart. Injections of either fructose or glucose were given as 20 g/100 ml solutions (2 g/kg estimated fetal body wt). Fetal weight was estimated by palpation of the uterus at the time of surgery, as well as from the postconceptual age. The injections were made at a rate of 5 ml/min through a Millipore filter (HA 0.45 μ). Total volumes injected ranged from 20 to 50 ml as a function of estimated fetal weight. The injection catheter was flushed with not more than 1 ml of isotonic saline solution following hexose injection. After injection, 1- to 1.5-ml blood samples were collected in heparinized syringes, every 5 to 10 min for the first 30 min and then hourly from the end of injection time. A postinjection sample was obtained the following day.

Whole blood samples were immediately prepared and analyzed by the methods of Saifer and Gerstenfeld (4) for glucose, and Yaphe and Arsenault (5) for fructose. The glucose-oxidase reagent (Glucostat) was obtained from the Worthington Biochemical Corporation (Freehold, N.J.). Blood for plasma insulin analysis was kept in a 5° ice bath until all samples were obtained. Plasma samples were then stored at —15° until the time for analysis.

Fetal and maternal sheep plasma insulin concentrations were determined by a modification of the method of Morgan and Lazarow (6), using a sheep insulin standard supplied by Eli Lilly and Co. Known insulin concentrations in human sheep plasmas were measured in the assays as a check on repeatability.

Results. A total of eight fetuses ranging in gestational age from 110 days to term (147 days) were studied. Fourteen fructose injec-

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TABLE I. Summary of Estimated Gestational Age at Time of Study and Carbohydrate Infused.

Fetus no.	Estimated gestational age (days)	Carbohydrate infused
1	122	Fructose
	134	Fructose
	143	Fructose
2	120	Fructose
	122	Fructose
	126	Fructose
3	120	Fructose
	127	Fructose
	134	Fructose
	136	Glucose
	143	Glucose
	145	Fructose
4	123	Glucose
	126	Glucose
	133	Fructose
5	110	Fructose
	121	Glucose
6	120	Glucose
	122	Fructose
7	119	Glucose
	121	Fructose
8	137	Glucose

tions were done in seven of the fetuses and eight glucose injections in six of the fetuses. The time from fetal surgery to the experimental period varied from the first to the twenty-ninth postoperative days. Table I lists the estimated gestational ages at which the fetuses were studied for each of the two carbohydrates.

After a fructose infusion into the fetal circulation, there was an increase in fetal plasma insulin concentration and a small but consistent decrease in fetal plasma glucose concentration. The data obtained in fetus No. 4 are presented in Fig. 1. In animal No. 2, which was the only preparation in which both the mother and fetus became progressively more hypoglycemic, the fetal plasma insulin response occurred in only the first of three fructose infusions. In all of the fetuses studied, there were no significant changes in maternal blood fructose, glucose or plasma insulin concentrations as a result of fetal fructose infusions.

After a glucose infusion into the fetal circulation there was, in every case, a marked increase in fetal plasma insulin concentration and in fetal blood fructose concentration. Figure 2 presents the data obtained in fetus No. 8. There were no significant changes in maternal blood glucose, fructose, or plasma insulin concentrations following glucose injection into the fetuses. Exogenous glucose was a more potent stimulant of fetal insulin release than exogenous fructose, as judged by the maximum insulin reponse for either carbohydrate infusion. The mean $\pm$ SE maximum insulin response to fructose infusion in all fetuses was 21.9 $\pm$ 2.9 μU/ml, and for glucose was 66.9 $\pm$ 9.1 μU/ml. These mean insulin concentrations were significantly different ($p<0.001$). Pooled fetal plasma insulin concentrations before and after glucose injection are shown in Fig. 3. Insulin controls, insulin concentrations after glucose injection, and maximum insulin values are differentiated in Fig. 3. All maximum insulin concentrations after glucose infusion occurred within 50 min after injection. Since all peaks

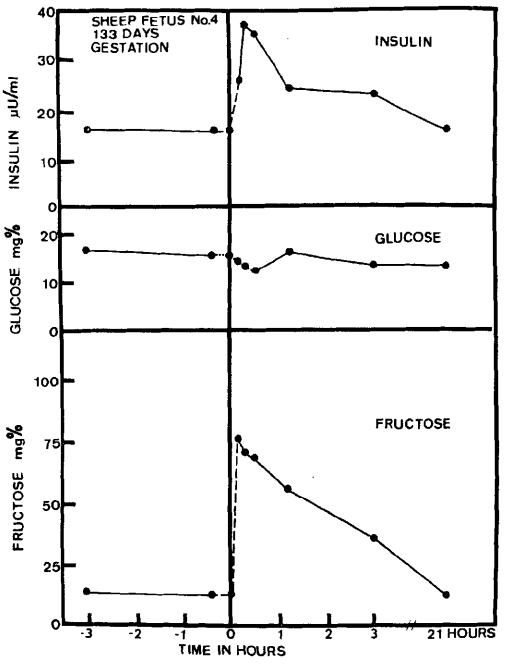

FIG. 1. Fetal arterial blood glucose and fructose concentrations and fetal arterial plasma insulin concentrations are shown before and after the injection of a fructose solution (20 g/100 ml).

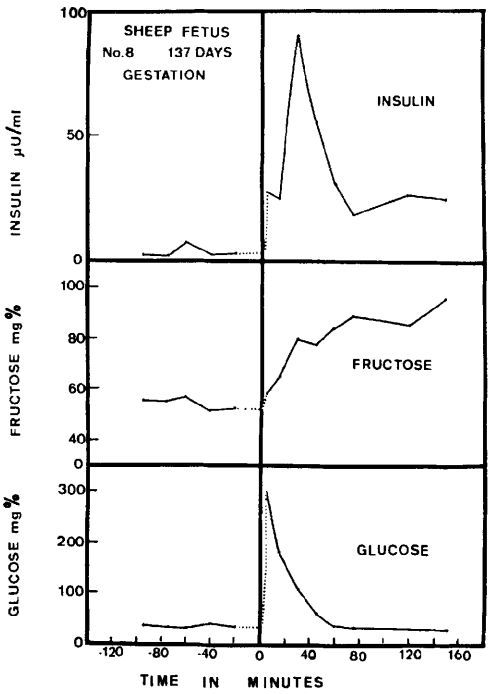

FIG. 2. Fetal arterial blood glucose and fructose concentrations and fetal arterial plasma insulin concentrations are shown before and after the injection of a glucose solution (20 g/100 ml).

occurred above 30 μU/ml and all control values were below 20 μU/ml, the increase of fetal plasma insulin concentrations above control levels following glucose injection is clearly demonstrated.

Fetus No. 3 was most intensively studied, and the data on this animal are presented in Fig. 4. This fetus received four fructose infusions and two glucose infusions during the period from 118 to 145 days of gestational age, following which the fetus was born alive and well. After each carbohydrate infusion, there is a clearly demonstrable insulin peak which is much greater following glucose infusions than fructose infusions. The increase in blood fructose concentration in the fetus following the infusion of glucose is also clearly seen.

Discussion. There are many conflicting reports in the literature concerning the insulin response of the mammalian fetus to an induced hyperglycemia. Some of these differences may be due to the fact that different species have been studied; however, two previous studies of carbohydrate infusions into the fetal circulation of sheep also show dissimilar results. One study by Willes *et al.* (2) utilized a surgical preparation similar to ours; that is, surgery was carried out on the fetus for the implantation of catheters, the animal was allowed to recover from surgery, and on subsequent days the carbohydrate infusions were carried out. These investigators found no insulin response to the infusion of glucose into the fetal circulation at a dosage of 0.5 to 1 g/kg. In addition, they found no elevation of fetal blood fructose concentration following the glucose infusions. In contrast, a recent study by Alexander *et al.* (1) utilizing acute animal preparations (that is, studying the infusion of carbohydrates into the fetal circulation while the pregnant animal was still under surgical stress) showed a clear-cut insulin response to fetal hyperglycemia in two of three animals. Our studies, carried out in chronic preparations over the last 35 days of gestation, confirm that glucose

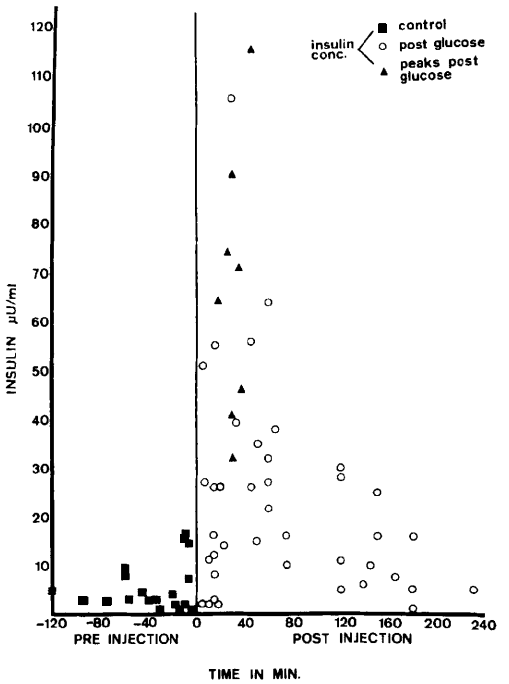

FIG. 3. Fetal arterial plasma insulin concentrations before and after glucose injections into the fetus are shown. The data were obtained during 8 glucose injections into 6 fetuses.

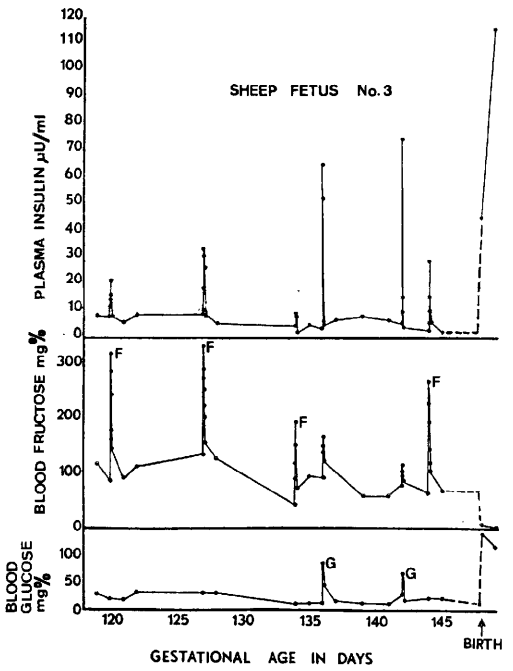

FIG. 4. Fetal arterial blood glucose and fructose concentrations and fetal arterial plasma insulin concentrations are shown. The gestational age at which the 4 fructose injections (F) and 2 glucose injections (G) were done in this fetus is shown.

or fructose infusions into the fetal circulation cause a rise in plasma insulin concentration in the fetus. Furthermore, we have confirmed the findings of Huggett *et al.* (7), in acute animal preparations; *i.e.*, that the infusion of glucose into the sheep fetus leads to a significant elevation of fetal blood fructose concentration. Previously we had shown, in the same type of preparation used in this study, that the injection of exogenous insulin into the fetal circulation increases fetal glucose utilization (8). Thus, according to our data, the sheep fetus is able to respond to an increased insulin concentration by increasing glucose utilization and responds to hyperglycemia by an increase in plasma insulin concentration.

There is no ready explanation for the dissimilarity of results reported in the literature. Immaturity of the fetal pancreas and of the fetal endocrine system has been invoked to explain either the lack or the delay of insulin reponse. In order to be valid, this explanation must assume large differences in the rate of maturation of sheep fetuses. For example, in our experiments we have observed an insulin response to glucose infusion at 119 days of gestational age, whereas in the experiments of Willes *et al.* (2) and in one experiment reported by Alexander *et al.* (1), no response was observed in fetuses older than 140 days. An alternative hypothesis might be that the stress associated with surgery, anesthesia, and injection of a large volume of fluids at room temperature into the fetal circulation was sufficiently severe in certain experiments to block the insulin response.

Summary. Twenty g/100 ml of glucose or fructose solutions at a dose of 2 g/kg were injected into the fetal circulation of pregnant sheep over a gestational age range from 110 days to term. Fourteen fructose injections and 8 glucose injections were carried out in a total of eight fetuses. Fetal plasma insulin concentrations increased significantly after either fructose or glucose injections. After glucose injection, the rise in fetal plasma insulin concentration was most marked and fructose concentrations in the fetal circulation increased.

1. Alexander, D. P., Britton, H. G., Cohen, N. M., and Nixon, D. A., Biol. Neonatorum **14**, 178 (1969).

2. Willes, R. F., Boda, J. M., and Manns, J. G., Endocrinology **84**, 520 (1969).

3. Meschia, G., Makowski, E. L., and Battaglia, F. C., Yale J. Biol. Med. **42**, 154 (1970).

4. Saifer, A., and Gerstenfeld, S., J. Lab. Clin. Med. **51**, 448 (1958).

5. Yaphe, W., and Arsenault, G. P., Anal. Biochem. **13**, 143 (1965).

6. Morgan, C. R., and Lazarow, A., Diabetes **12**, 115 (1963).

7. Huggett, A. St. G., Warren, F. L., and Warren, N. V., J. Physiol. (London) **113**, 258 (1951).

8. Colwill, J. R., Davis, J. R., Meschia, G., Makowski, E. L., Beck, P., and Battaglia, F. C., Endocrinology **87**, 710 (1970).

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