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Effect of Insulin on Glucose Uptake in Near-Term Fetal Lambs (42042)

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Abstract. Glucose clamp experiments were performed in 27 chronically catheterized, late-gestation fetal lambs in order to measure the effect of fetal insulin concentration on fetal glucose uptake at a constant glucose concentration. Fetal arterial blood glucose concentration was measured over a 30-min control period and then maintained at the control value by a variable glucose infusion into the fetus while insulin was infused at a constant rate into the fetus. Plasma insulin concentration increased from 21 ± 10 (SD) to 294 ± 179 (SD) $\mu U \cdot ml^{-1}$. The exogenous glucose infusion rate necessary to maintain constant glycemia during the plateau hyperinsulinemia averaged 4.3 ± 1.6 (SD) $mg \cdot min^{-1} \cdot kg^{-1}$. In a subset of 13 animals, total fetal exogenous glucose uptake (FGU; sum of glucose uptake from the placenta via the umbilical circulation plus the steady-state exogenous glucose infusion rate) was measured during the control and hyperinsulinemia period. FGU was directly related to insulin concentration ($y = 4.24 + 0.07x$) at insulin levels < 100 μU/ml and increased 132% above control at insulin levels above 100 μU/ml. Hyperinsulinemia did not affect fetal glucose uptake from the placenta via the umbilical circulation. These studies demonstrate that insulin concentration is a major factor controlling glucose uptake in the near-term fetal lamb, and that an increase of fetal insulin does not affect the transport of glucose to the fetus from the placenta.

Previous investigations of the effect of insulin in the ovine fetus relied on methodology that did not allow exact quantification of the effect of insulin independent of glucose concentration. For example, insulin has been infused into the fetal lamb producing fetal hypoglycemia (1), and an increased net glucose uptake by the fetus from the placenta (2). However, in these studies fetal insulin concentrations were not measured. Furthermore, since the rate of glucose entry into cells is partly dependent on plasma glucose concentration, the fetal hypoglycemia that developed during hyperinsulinemia may have limited the magnitude of the insulin-induced cellular glucose uptake, leading to an underestimation of the effect of insulin upon glucose utilization.

Given these limitations in previous fetal studies, the present experiments were performed utilizing glucose clamp techniques (3) to measure the specific effect of physiologic and pharmacologic levels of insulin on fetal glucose uptake independent of fetal glucose concentration.

Methods. *Animal preparation.* Pregnant, Columbia-Rambouillet ewes were studied between 125 and 145 days of gestation. The ewes were fasted for 2 days prior to surgery. Surgery was performed under intravenous pentobarbital sedation (5 mg/kg) and tetracaine hydrochloride spinal anesthesia (6 mg in hypertonic glucose). Polyvinyl catheters for infusion of antipyrine, glucose, and insulin were placed in the fetal femoral veins via pedal veins. Catheters for blood sampling were placed in the abdominal aorta via a pedal artery (advanced to the level of the umbilical arteries) and the common umbilical vein. All catheters were tunneled subcutaneously through a flank incision on the ewe and kept within a plastic pouch attached to the ewe's skin. The catheters were flushed every other day with heparinized saline (30 Units heparin per milliliter of saline). The ewes were allowed to recover after surgery for at least 5 days before study. They were kept in carts and allowed an *ad libitum* diet of alfalfa pellets, water, and mineral supplement. At the end of the studies, each animal was sacrificed with a rapid intravenous infusion of Buthanasia. The fetus was autopsied for measurement of weight and for confirmation of catheter location.

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Experimental design. Two sets of experiments were performed. In both sets fetal arterial blood glucose was determined in four blood samples drawn over a 30-min control period. The mean value calculated from these measurements was then maintained with a variable glucose infusion while insulin was infused at a constant rate into the fetus (Fig. 1). Plasma insulin was measured four times in the control period and four times between 90 and 150 min after starting the insulin infusion. During the insulin infusion period, a steady state was observed for glucose and insulin concentrations and for exogenous glucose infusion rate.

In the first set of experiments umbilical blood flow and the fetal uptake of glucose from the placenta were not measured and assumed to remain constant in the control and the insulin infusion periods. Thus, in these experiments the average steady-state glucose infusion rate necessary to maintain control glycemia was considered a measure of the response of fetal tissues to elevated insulin concentrations.

In the second set of experiments net fetal glucose uptake from the placenta was measured during the control period and the insulin infusion period. Net fetal glucose uptake from the placenta was calculated by the Fick principle as the product of umbilical blood flow and the umbilical venous–arterial blood glucose concentration difference. Glucose uptake by the fetus was considered equal to the measured net fetal glucose entry rate from the placenta during the control period, and equal to the sum of the net glucose entry rate from the placenta plus the steady-state exogenous glucose infusion rate during the insulin infusion period. These experiments allowed a comparison of basal fetal glucose uptake with fetal glucose uptake at elevated insulin concentrations.

Fetal glucose clamp/methodology. During the insulin infusion, fetal arterial glucose was maintained at the control level using a euglycemic glucose clamp technique. As introduced by DeFronzo *et al.*, this technique permits an elevation of plasma insulin concentration while maintaining plasma glucose at a euglycemic or control glucose concentration (3). In the present experiments, euglycemia was defined for each animal as the average glucose concentration measured during the 30-min control period preceding the insulin infusion.

The insulin infusion was started with a priming dose given as a logarithmically decreasing rate of infusion over 10 min (the total priming dose over 10 min averaging twice the final infusion rate) and ending with a desired constant rate of infusion. The priming dose was estimated after selecting a desired plasma insulin level and assuming an insulin

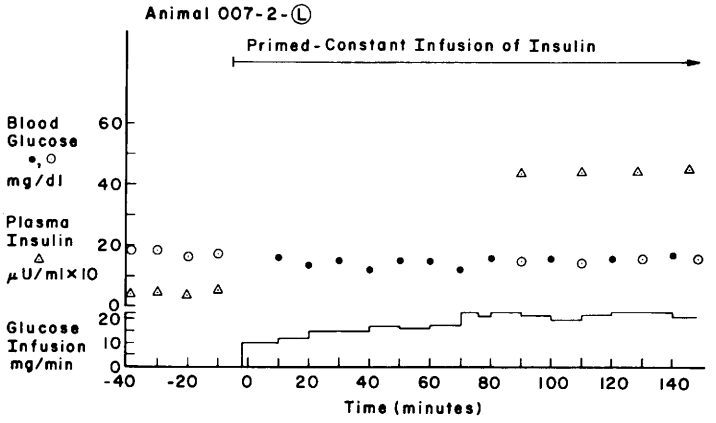

FIG. 1. Representative glucose clamp experiment in one fetus. The circled glucose concentration values (⊙) represent the times at which samples were taken from the umbilical vein and the fetal artery for measurement of glucose and antipyrine concentrations across the umbilical circulation. The uncircled glucose values (·) represent the glucose concentrations used to calculate the rate of the exogenous glucose infusion needed to keep the glucose concentration constant.

space in the fetus of 15% body weight. This approach resulted in constant insulin concentrations by 30 min of infusion. The 15% insulin space was estimated from data in newborn lambs by Cowett *et al.* (4) and was tested to be reasonably accurate by preliminary experiments in our lab.

A variable rate of glucose infusion into the fetus was started during the first 5 min of the insulin infusion. The glucose infusion rate (GIR) was subsequently increased or decreased every 5 to 10 min according to fetal arterial blood glucose concentrations, using the formula

$$\text{new GIR} = \text{previous GIR} \times \frac{G_d}{G_i} + [(G_d - G_i) \times 0.4 \times \text{fetal weight}] \div 10 \text{ min}$$

where G_d is the desired glucose concentration ("clamp" value in $mg \cdot dl^{-1}$), G_i is the glucose concentration ($mg \cdot dl^{-1}$) measured at any time "i," 0.4 is the estimated fetal glucose space expressed as a fraction of fetal body weight. The initial glucose infusion rate was chosen arbitrarily as 2.0 $mg \cdot min^{-1} \cdot kg^{-1}$ based on data from preliminary experiments. Fetal weight was estimated by gestational age norms using the equation: log weight $= -0.951 + 0.0543(X) - 0.000155(X^2)$, where X = gestational age in days. This equation was derived empirically in our laboratory.

The glucose clamp equation was programmed into a Texas Instruments TI-59 calculator which was used for calculating each glucose infusion rate change.

Infusates. Glucose for infusion consisted of dextrose 10% w/v in water (Travenol). The glucose concentration in this solution was measured at 80 mg/ml by a Beckman Astra glucose analyzer. Antipyrine for infusion was prepared as a 10% solution in water and was infused into the fetus at about 10.0 $mg \cdot min^{-1}$. Insulin (porcine insulin, Eli Lilly Co.) was diluted in a mixture of normal saline (30 ml) and maternal sheep plasma (7 ml) to a concentration of about 75 mU/ml and infused into the fetus at rates varying from about 0.45 to about 3.0 mU/min/kg fetal weight. Glucose, antipyrine, and insulin solutions were infused with Sage Model 355 syringe pumps. The total volume of all infusates given during the study was about twice the volume of blood taken for samples.

Sampling and chemical methods. Blood for determination of glucose, insulin, and antipyrine concentrations was drawn into plastic syringes lined with a dried mixture of EDTA and sodium fluoride. Plasma for insulin concentration was immediately separated in a refrigerated centrifuge and frozen at −70°C. Umbilical venous and arterial blood samples were drawn simultaneously. Whole blood glucose concentration for the glucose clamp was measured with a Yellow Springs Glucose Analyzer (Model 23A) which was calibrated to an accuracy of ±1.0 $mg \cdot dl^{-1}$. Samples were analyzed in duplicate and the average value was used for entry into the calculator. A standard of about the same concentration as the samples was run before each blood sample for calibration.

Whole blood glucose concentration for calculation of fetal glucose uptake was measured with glucose oxidase (Sigma) using a protein-free filtrate of whole blood after hemolysis with five parts distilled H_2O and protein precipitation with equal parts of 0.3 N zinc sulfate and 0.3 N barium hydroxide. This method has an accuracy of ±0.1 $mg \cdot dl^{-1}$ in our laboratory. Antipyrine concentrations were measured using a Technicon Autoanalyzer. These values were used to calculate umbilical blood flows according to the method of Meschia *et al.* (6). Antipyrine blood flows have an accuracy of ±10% when compared with umbilical blood flows determined simultaneously with microspheres and electromagnetic flow probes. Insulin was measured in plasma using a double antibody method (Serono) that had a within-assay variance of ±6% and a between-assay variance of ±10%. Samples from one study were always batched to avoid the higher interassay variance.

Statistics. In the first group of animals, only one study was conducted in each animal. In the second group, two complete studies at high insulin concentrations were performed in each animal. The results of these two separate studies did not differ by more than 10% in each animal. Thus, the results of the two separate studies were averaged and the mean value for each animal was used for comparisons among animals. In three animals

of the second group, 13 separate studies were performed, each at a different insulin concentration covering the physiologic range of insulin from about 15 to 90 $\mu U \cdot ml^{-1}$. Differences between groups were tested by the paired student t test. Linear regression analyses were performed using the standard least-squares method.

Results. In the first set of experiments, 39 insulin infusions were performed in 27 animals. The mean control period fetal arterial blood glucose and arterial plasma insulin concentrations for all 27 animals were 17 ± 7 (SD) $mg \cdot dl^{-1}$ and 21 ± 10 (SD) $\mu U \cdot ml^{-1}$, respectively. During the insulin infusion period, blood glucose concentration averaged 17 ± 6 (SD) $mg \cdot dl^{-1}$ (not different from control, $P > 0.8$), and plasma insulin concentration averaged 294 ± 178 (SD) $\mu U \cdot ml^{-1}$. The average steady-state exogenous glucose infusion rate required to maintain the control period glycemia during the plateau insulin infusion period was 4.3 ± 1.6 (SD) $mg \cdot min^{-1} \cdot kg^{-1}$. The magnitude of this steady-state exogenous glucose infusion rate was not significantly related to the glucose concentration ($R = 0.34$, $P > 0.1$).

In the second set of experiments net fetal glucose uptake from the placenta was measured during the control period and during the steady-state insulin infusion period in 13 animals. The results are presented in Table I. Umbilical blood flow averaged 196 ± 48 (SD) $ml \cdot min^{-1} \cdot kg^{-1}$ during the control period and 199 ± 51 (SD) $ml \cdot min^{-1} \cdot kg^{-1}$ during the steady-state insulin infusion period. These values were not significantly different ($P > 0.5$). Mean umbilical venous and arterial blood glucose concentrations did not change significantly with insulin infusion ($P > 0.5$). Mean fetal glucose uptake from the placenta was 4.0 $mg \cdot min^{-1} \cdot kg^{-1}$ during the control period and 4.8 $mg \cdot min^{-1} \cdot kg^{-1}$ during the steady-state insulin infusion period. This increase in glucose uptake by the fetus from the placenta was not significant ($P > 0.2$).

The mean total fetal glucose uptake during the steady-state insulin infusion period was 9.3 ± 1.3 (SD) $mg \cdot min^{-1} \cdot kg^{-1}$, compared with the average control period glucose uptake of 4.0 ± 1.1 (SD) $mg \cdot min^{-1} \cdot kg^{-1}$, representing a 132.2% increase of fetal glucose uptake above control. As shown in Fig. 2, there was no significant relationship between insulin concentration and total glucose uptake above insulin concentrations of 100 $\mu U/ml$, suggesting a maximal effect of insulin above this value.

The total exogenous glucose uptake rate in 13 studies done in three animals during steady-state hyperinsulinemia was measured at plasma insulin concentrations less than 100 $\mu U/ml$ (Fig. 3). Below this insulin level, total steady-state glucose uptake and insulin concentration were correlated (mean $R = 0.86$, $P < 0.001$) according to the average linear regression equation $y = 4.69 + 0.07x$.

Discussion. These studies represent, to our knowledge, the first application of glucose clamp methodology to the evaluation of the effect of insulin on the rate of glucose uptake by the fetus. With this methodology the effect of an increase of fetal plasma insulin concentration on fetal glucose uptake is measured independently of changes in glucose concentration.

In the fetus, glucose clamp methodology differs from its application in postnatal life in that basal glucose entry into the fetus (placental to fetal glucose transfer) is relatively constant from the control period to the hyperinsulinemia period whereas in postnatal life, basal glucose entry (endogenous glucose production) is a decreasing function of insulin concentration, at least over the physiologic range of insulin concentration (7). In extrauterine life total glucose utilization rate (measured, for example, by tracer dilution) may be the only reliable measure of insulin effect, while in fetal life either the glucose utilization rate or the glucose uptake rate determined by the sum of the steady-state exogenous glucose infusion rate and the placental to fetal glucose transfer rate may serve as measures of insulin effect. In the present studies, the assumption of equality between exogenous glucose uptake by the fetus and fetal glucose utilization was considered valid as we have demonstrated previously that, in the range of glucose concentrations encountered in these studies, fetal endogenous glucose production is negligible (8).

The major requirements for the application of glucose clamp methodology to the fetus include the precise maintenance of a constant fetal arterial plasma glucose concentration

TABLE I. DATA FROM FETUSES IN WHICH UMBILICAL BLOOD FLOW AND FETAL GLUCOSE UPTAKE WERE MEASURED DURING THE GLUCOSE CLAMP

	Arterial plasma insulin (μU/ml)	Arterial blood glucose (mg/ml)	Umbilical venous blood glucose (mg/ml)	Umbilical venous–arterial blood glucose concentration difference (mg/ml)	Umbilical bloodflow ($ml \cdot min^{-1} \cdot kg^{-1}$)	Fetal glucose uptake from the placenta ($mg \cdot min^{-1} \cdot kg^{-1}$)	Exogenous glucose infusion ($mg \cdot min^{-1} \cdot kg^{-1}$)	Total glucose uptake ($mg \cdot min^{-1} \cdot kg^{-1}$)
Control period								
Fetus								
1	18	0.266	0.283	0.017	186	3.1		
2	12	0.194	0.214	0.020	223	4.5		
3	14	0.176	0.210	0.034	163	5.5		
4	8	0.164	0.193	0.029	127	3.7		
5	20	0.189	0.202	0.013	210	2.8		
6	30	0.136	0.158	0.022	186	4.0		
7	24	0.172	0.187	0.014	236	3.4		
8	21	0.108	0.122	0.014	201	2.8		
9	10	0.052	0.058	0.006	331	2.1		
10	33	0.192	0.216	0.024	156	3.7		
11	25	0.192	0.222	0.031	183	5.6		
12	10	0.129	0.174	0.035	162	5.6		
13	15	0.207	0.234	0.028	180	4.0		
$n = 13$ $\bar{X}$	18	0.168	0.190	0.022	196	4.0		
SD	8	0.050	0.053	0.009	48	1.1		
SEM	2	0.014	0.015	0.002	12	0.3		
Hyperinsulinemia period								
Fetus								
1	244	0.291	0.305	0.014	172	2.4	8.0	10.3
2	238	0.171	0.202	0.031	217	6.7	3.4	10.1
3	217	0.183	0.220	0.037	124	4.6	4.7	9.3
4	442	0.167	0.195	0.027	125	3.4	5.3	8.6
5	613	0.178	0.205	0.027	203	5.5	3.8	9.1
6	329	0.121	0.150	0.030	211	6.3	3.2	9.5
7	458	0.174	0.190	0.016	260	4.1	7.8	11.9
8	227	0.084	0.106	0.022	217	4.8	3.1	7.8
9	332	0.055	0.067	0.011	300	3.4	4.1	7.5
10	440	0.185	0.208	0.023	160	3.7	7.8	11.6
11	58	0.181	0.211	0.030	166	5.4	2.5	7.9
12	37	0.150	0.178	0.028	178	4.9	3.5	8.4
13	87	0.207	0.233	0.026	259	6.7	2.0	8.7
$n = 13$ $\bar{X}$	286	0.165	0.190	0.025	199	4.8	4.6	9.3
SD	165	0.055	0.056	0.007	51	1.3	2.0	1.3
SEM	46	0.015	0.016	0.002	14	0.4	0.6	0.4

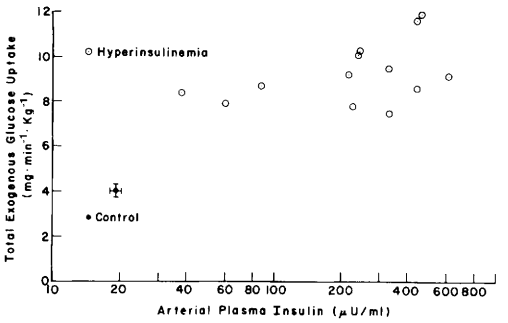

FIG. 2. Total glucose uptake by the 13 fetuses in which both umbilical glucose uptake and exogenous glucose infusion rate were measured. The control values are shown as an average value including the SEMs for both total exogenous glucose uptake and insulin concentration.

from the control to the hyperinsulinemic period and either the maintenance of constant maternal arterial plasma glucose concentration, placental glucose metabolism and umbilical blood flow from the control period to the hyperinsulinemic period, or alternatively measurement of net umbilical glucose uptake during both the control and hyperinsulinemic periods. The advantage of measuring directly net fetal glucose uptake from the placenta is that umbilical blood flow, maternal glycemia, and placental glucose metabolism are not easily controllable variables; thus, net fetal glucose uptake from the placenta may vary independently of fetal glucose concentration.

The success of the application of glucose clamp methodology in the present experiments can be assessed by considering the results from the subset of 13 animals studied by both glucose clamp and Fick principle methodologies (Table I). First, from control to hyperinsulinemia, umbilical blood flow did not change significantly, increasing an average of only 1.9%. Second, fetal arterial glucose concentration did not change significantly, decreasing an average of only 0.3 $\pm$ 0.4 SEM mg$\cdot$dl^{-1} or 1.8%. In individual experiments, however, fetal arterial glucose concentration varied over the range $\pm$ 2.5 mg$\cdot$dl^{-1} from control. Umbilical venous glucose concentration decreased only 0.04 $\pm$ 0.30 SEM mg$\cdot$dl^{-1} or 0.2%. Thus, the umbilical venous–arterial blood glucose concentration difference increased an average of 0.3 mg$\cdot$dl^{-1} or 12.7%. Net fetal glucose uptake from the placenta did not vary consistently with insulin infusion. It remained virtually constant in three experiments, decreased in three, and increased in seven, with a mean increase of 19.6% which just failed to achieve statistical significance ($P < 0.10$, >0.05). In contrast, in every fetus, total fetal glucose uptake increased in response to insulin by over twofold.

In the present studies changes in the fetal arterial blood glucose concentration from control to hyperinsulinemia were inversely correlated with changes in the umbilical venous–arterial blood glucose concentration difference ($R = -0.71$, $P < 0.01$) and with changes in umbilical glucose uptake ($R = -0.65$, $P < 0.02$). As shown in Fig. 4, to the extent that fetal arterial blood glucose concentration did not change during hyperinsulinemia, the umbilical venous–arterial blood glucose concentration difference did not change, demonstrating that insulin had no effect on placental to fetal glucose transport. The lack of a sensitivity to fetal insulin of placental to fetal glucose transport shown in the present studies can be added to our previous observations which showed no effect of markedly elevated levels of maternal insulin on placental glucose uptake or placental to fetal glucose transport (9). Thus, we conclude that in sheep, in spite of the presence of insulin receptors on the placenta, transport of glucose by the placenta from mother to

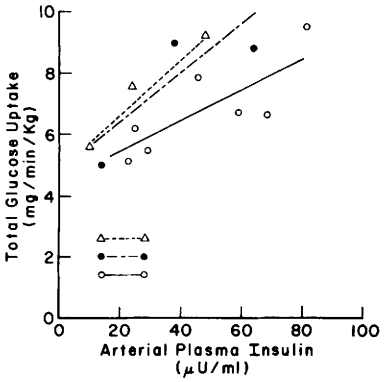

FIG. 3. Individual linear regressions in three fetuses for total glucose uptake versus insulin concentrations less than 100 μU/ml. The average linear regression equation of the three individual regressions is $y = 4.24 + 0.07x$, $R = 0.85$, $P < 0.001$.

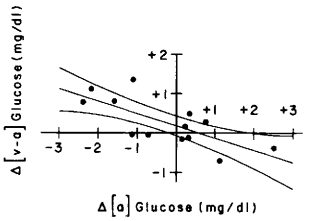

FIG. 4. The change in the umbilical venous–arterial glucose concentration (Δ[v–a] glucose, mg/dl) from the control to the insulin infusion period is plotted versus the change in the arterial glucose concentration (Δ[v–a] glucose, mg/dl). The linear regression relationship ($y = 0.18 - 0.31x$, $R = -0.71$, $P < 0.01$) is shown including the 95% confidence limits.

fetus is independent of acute variations of insulin above the normal level.

On the other hand, to the extent that fetal arterial glucose concentration did change, the resulting changes in umbilical venous–arterial glucose concentration difference and net fetal glucose uptake from the placenta were relatively large, in agreement with a previous study aimed at establishing the relation of placental glucose transfer to maternal and fetal glucose concentrations (10). For example, a 1.0 $mg \cdot dl^{-1}$ decrease (~6%) in fetal arterial glucose concentration at a fixed maternal arterial glucose concentration resulted in an approximate 20% increase in the umbilical venous–arterial glucose concentration difference and a 15% increase in net fetal glucose uptake from the placenta. According to Fig. 3 a 6% increase in fetal insulin concentration at constant fetal arterial glucose concentrations resulted in a 2.4% increase in net fetal glucose uptake from the placenta. Together, these studies emphasize the precision with which fetal glucose metabolism is controlled by maternal and fetal glucose concentrations.

The present studies can be compared with previous results from our laboratories (2) which demonstrated an approximate 57% increase in total glucose uptake (4.4 ± 0.7 $mg \cdot min^{-1} \cdot kg^{-1}$ to 6.9 ± 0.9 $mg \cdot min^{-1} \cdot kg^{-1}$) during an insulin infusion of about 4 $mU \cdot min^{-1} \cdot kg^{-1}$. In these studies, fetal hypoglycemia was allowed to develop during the fetal insulin infusions (0.22 ± 0.01 SEM $mg \cdot ml^{-1}$ to 0.16 ± 0.01 SEM $mg \cdot ml^{-1}$ of arterial plasma glucose) and the increased glucose uptake resulting from the increased insulin concentrations was supplied by an increased maternal-to-fetal glucose transfer rate. In the present studies, fetal glucose concentration was maintained constant ("clamped") at the control level and the extra glucose needed for the increased glucose uptake resulting from the higher insulin concentration was supplied by the exogenous glucose infusion. The difference in the magnitude of the insulin-stimulated glucose uptake rates between the two studies (Simmons *et al.*, 57% increase; present studies, 133% increase) suggests that either maximal insulin stimulation of glucose uptake was not achieved in the previous studies or that at maximal insulin effect in the fetus, glucose uptake is dependent on glucose concentration.

In the present studies the glucose uptake rate during the hyperinsulinemia period was greater in those animals with the highest fetal arterial glucose concentrations, but the increase in the FGU was not significantly related to glucose concentration (Fig. 5). Thus, the effect of insulin which we have demonstrated appears to be independent of glucose concentration, at least over the range of concentration observed in these animals.

The maximal effect of insulin which these results demonstrate (an approximate doubling of glucose uptake) is similar to results in adult ewes (11) but contrasts sharply with

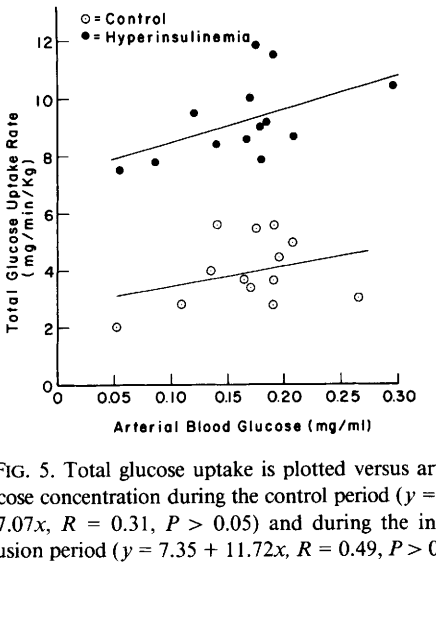

FIG. 5. Total glucose uptake is plotted versus arterial glucose concentration during the control period ($y = 2.79 + 7.07x$, $R = 0.31$, $P > 0.05$) and during the insulin infusion period ($y = 7.35 + 11.72x$, $R = 0.49$, $P > 0.05$).

results in adult humans in whom four- to fivefold increases in glucose utilization rates have been found at maximal insulin effect (1, 7), and with results in adult rats in which threefold increases in glucose utilization rates were observed at maximum insulin effect (12). In neonatal lambs, Susa *et al.* (13) observed an approximate doubling of glucose utilization rate (total of endogenous glucose production plus exogenous intravenous glucose infusion) at constant glycemia during insulin infusions that produced insulin concentrations greater than 60 μU/ml. The studies in the adult sheep and neonatal lambs were conducted at glucose concentrations similar to those for the human and rat studies, but three- to fourfold greater than the glucose concentrations in the present fetal lamb studies. Thus, the similarity of the maximal effect of insulin on glucose uptake among fetal, neonatal, and adult sheep appears to be independent of glucose concentration as well as maturity.

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