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Effect of High Levels of Insulin on Glucose Utilization and Glucose Production in Pregnant and Nonpregnant Sheep (42807)

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Abstract. The present study was conducted to test the hypothesis that pregnancy in sheep alters the effects of insulin on glucose utilization and glucose production. Euglycemic, hyperinsulinemic glucose clamp experiments were performed in chronically catheterized, unstressed, fed or 24-hr fasted, nonpregnant sheep and fed, pregnant sheep. Endogenous glucose production rate for the whole sheep and glucose utilization rate of the uterine and nonuterine maternal tissues were measured in control and high-insulin periods by tracer technique using [6-^{3}H]glucose. Control glucose utilization rate in the fed, nonpregnant sheep was significantly ($P < 0.05$) greater than that in the fasted, nonpregnant sheep, 2.29 ± 0.17 and 1.86 ± 0.11 mg/min/kg, respectively, and also in the nonuterine maternal tissues of the pregnant sheep (1.71 ± 0.18 mg/min/kg). Insulin stimulated glucose utilization 116.4 ± 14.8% in the fed, nonpregnant sheep but only 82.8 ± 11.0% in the fasted, nonpregnant sheep and 94.2 ± 14.3% in the nonuterine tissues of the fed, pregnant sheep. Also, insulin suppressed endogenous glucose production to 53.2 ± 5.6% in the fed, nonpregnant sheep, to 3.9 ± 3.1% in the fasted, nonpregnant sheep, and to 9.0 ± 3.7% in the fed, pregnant sheep. In the pregnant animals, uterine glucose uptake and uterine glucose utilization were not different and were not altered by changes in maternal insulin concentration. The results indicate that during late pregnancy glucose utilization is reduced and resistance to the effect of insulin to enhance glucose utilization is present in the nonuterine maternal tissues compared with nonpregnant, fed sheep. In contrast, the effectiveness of insulin to suppress glucose production in the pregnant sheep is greater than that in nonpregnant, fed sheep. These results also demonstrate that differential changes in the effect of insulin can exist simultaneously between peripheral (glucose consuming) and central (glucose producing) tissues. The changes in glucose utilization and in insulin effect in the pregnant sheep are both qualitatively and quantitatively similar to those of the nonpregnant sheep when fasted, suggesting that similar substrate and/or hormonal factors may be involved.

Glucose metabolism during pregnancy is determined primarily by the rates of glucose utilization in nonuterine maternal and uterine tissues, the rate of maternal endogenous glucose production, and the maternal diet which provides glucose or glucogenic substrates and contributes to establishing the hormonal milieu. The major hormone affecting glucose utilization and production is insulin. Previous studies of the effect of insulin on maternal glucose metabolism during pregnancy have documented a resistance to the effect of insulin on systemic glucose utilization (1–6) and glucose utilization by adipocytes (7–12). The mechanisms responsible for this insulin resistance have not been defined but have been attributed to increased circulating concentrations of various hormones such as placental lactogen (1, 2), prolactin (10), and growth hormone (10). These studies were deficient, however, in not discriminating between the effect of insulin on the nonuterine maternal tissues and those of the uterus. This deficiency in study design is particularly important because there does not appear to be a significant effect of insulin on uterine glucose uptake (13). Thus, it remains to be determined not ony how circulating hormones might alter insulin action in only some tissues but also which tissues are affected, and to what extent such insulin resistance contributes to overall glucose metabolism.

Insulin also acts to inhibit glucose production. This aspect of insulin action during pregnancy has not been addressed. Also, *in*

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vivo studies of insulin action during pregnancy, in general, have not controlled for differences in the nutritional states of pregnant animals and controls. Among nonpregnant animals, nutritional state can have strong influences on insulin function (14), and nutritional state certainly can affect maternal glucose concentration and the rate of maternal glucose production (15).

On the basis of such deficiences in study design and knowledge regarding the possible changes in insulin action during pregnancy, we conducted the present study in nonpregnant, fed and fasted sheep and in pregnant sheep in late gestation. Glucose utilization and production were quantified with tracer techniques; the effect of insulin was assessed using glucose–insulin clamp procedures; and the differential effect of insulin on uterine and nonuterine maternal tissues was determined by comparing uterine glucose uptake (measured by the Fick principle) with whole-body glucose utilization (measured with tracer technique) at different plasma insulin concentrations.

Materials and Methods. Experiments were performed on Columbia–Rambouillet ewes. The pregnant ewes each carried one fetus and were studied between 130 and 145 days gestation (term = 150 days). Each animal was fasted for 2 days prior to surgery. Surgery was performed under intravenous pentobarbital sedation (5 mg/kg) and pontocaine spinal anesthesia (6 mg in hypertonic glucose). Polyvinyl catheters were inserted according to the following plan. One catheter for both insulin and tracer glucose infusions and one catheter for glucose infusion were placed into a femoral vein in each ewe. In the pregnant ewes a third catheter for antipyrine infusion (used to measure uterine blood flow) was placed into a fetal femoral vein via a pedal vein after exposing a fetal hindlimb through a standard hysterotomy procedure (15). Catheters for blood sampling were placed into the maternal femoral artery and in the pregnant ewes into the uterine vein draining the pregnant uterine horn. All catheters were tunneled subcutaneously through a flank incision and kept within a plastic pouch attached to the ewe's skin. The catheters were flushed every other day with heparinized (0.9%, w/v) NaCl in water (30 units heparin per milliliter of NaCl solution). The ewes were allowed to recover 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 least two sheep were always kept together. At the end of the studies, each animal was killed with a rapid intravenous infusion of T[61] euthanasia solution (Taylor Pharmaceutical Co., Decatur, IL). Autopsy of the pregnant ewes was performed in order to examine and weigh the uterus and its contents.

Infusates. Antipyrine (10% in saline) was used for the estimation of uterine blood flow. Glucose (50% in water) (Travenol Laboratories, Inc., Deerfield, IL) was used for infusion. Insulin (pure pork insulin, Eli Lilly Corp., Indianapolis, IN) was made up to a final concentration of 600 milliunits/ml in 42 ml of a mixture containing 7 ml sheep plasma, 30 ml 0.9% NaCl, and 5 ml of 45 m*M* potassium phosphate. Tracer glucose infusate contained 1 mCi of [6-^{3}H]glucose (ICN Radiochemicals, Inc., Costa Mesa, CA) in 30 ml of 0.9% NaCl. Each infusate was counted before and after the experiment; there was no significant difference between the two counts for all experiments. All infusions were performed with Sage Model 355 syringe infusion pumps.

Study design. Thirteen fed, pregnant ewes were each studied on one occasion. Eleven nonpregnant ewes were studied once when fed and once following a 24-hr fast during which time the ewes had access only to a mineral supplement and water.

Tritiated glucose was given to the ewe as a primed, constant infusion and was continued for the duration of the study (prime dose $\cong$ 50 μCi/kg, constant rate $\cong$ 0.05 μCi/min/kg). In the pregnant ewes antipyrine was infused into the fetus (0.1 ml/min) for the duration of the study and was used to measure uterine blood flow (16). Blood samples were drawn at 60, 90, 100, 110, and 120 min after starting the infusion, from the femoral artery in the nonpregnant ewes and from the femoral artery and the uterine vein simultaneously in the pregnant ewes.

Following the control period samples, each ewe received a primed, constant infusion of insulin according to the protocol reported by DeFronzo *et al.* (17). The prime

dose was estimated to raise the plasma insulin level to a desired level assuming an insulin space in the ewe of 15% body wt. Insulin infusion rates (1.0 to 4.0 munits/min/kg) were constant for each experiment.

During the insulin infusion, glucose concentration was maintained at the average of the glucose concentration for the control period by variable glucose infusion rate (GIR), using our modification (13) of the euglycemic glucose clamp procedure of DeFronzo *et al.* (17). The initial value for GIR was arbitrarily chosen as 2.5 mg/min/kg based on our preliminary measurements of glucose turnover rates in sheep (15).

Maternal arterial blood samples were drawn at 15 min intervals to measure plasma insulin concentration. Additional blood samples for glucose and tritiated glucose concentrations in both nonpregnant and pregnant ewes and antipyrine concentrations in the pregnant ewes were drawn at 60, 100, 110, 120, and 130 min after starting the insulin infusion (200, 240, 250, 260, and 270 min of the study).

Chemistry. Whole blood glucose concentrations were measured using a Yellow Springs Model 23A glucose analyzer. Plasma insulins were determined by the double-antibody method (Serono). Antipyrine was determined in whole blood using a Technicon autoanalyzer according to the methods of Meschia *et al.* (16) and Wilkening *et al.* (18). Tritiated glucose was analyzed as previously described (19).

Calculations. Glucose flux rates were calculated as previously described (21). The model and symbols appropriate for the present investigations are presented in Fig. 1. All calculations (Table I) are made assuming steady-state conditions.

The model represents the distribution and kinetics of glucose in a single-compartment system. Within this compartment, two glucose pools are analyzed, that of the nonuterine maternal tissues and that of the uterine tissues. At steady state and tracer glucose equilibrium, irreversible glucose disposal by the uterus ($R_{o,u}$) is assumed equal to the net rate of glucose transfer to the uterus from the mother ($R_{u,m}$), since we have shown that over the normal glucose concentration range for pregnant sheep (including the range of glucose concentrations observed in the present study), glucogenesis by the uterine contents (uteroplacenta and fetus) is negligible (15, 19–21). For the same reason the glucose specific radioactivity (SA) which is sampled in the maternal femoral artery (SA_m) is considered representative of that in both the maternal and the uterine (including fetal) glucose pools (15, 19–21). In nonpregnant sheep, $R_{o,u}$, $R_{u,m}$, and the weight of the uterus (approximately 100 g or about 0.2% of the pregnant animal's weight) are very small in contrast to $R_{o,m}$ and the weight of the ewe and have been considered equal to zero for calculation of glucose fluxes in the nonpregnant sheep. Total glucose entry or its steady-state equivalent, total glucose disposal, is assumed equal to endogenous glucose production because in the sheep consuming a standard alfalfa pellet diet, it is unlikely that there will be net absorption of glucose from the gastrointestinal tract (22). During the insulin infusion period $R_{m,o}$ is equal to the rate of endogenous glucose production (EGPR) plus the rate of exogenous glucose infusion. Since glucose disposal by nonmetabolic pathways (e.g., in the urine) was negligible, glucose disposal was considered equal to glucose utilization.

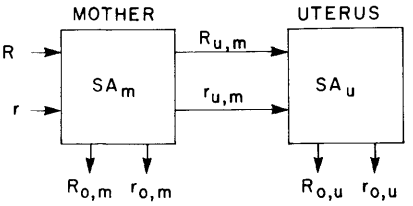

FIG. 1. Model for the calculation of net fluxes of glucose and tracer glucose into and out of the mother and the uterus. The symbols and notation are explained in Table I.

Statistical methods. Each nonpregnant ewe was studied once when fed and once when fasted. These studies were conducted at least 2 days apart. Each pregnant ewe was studied once. Differences between the control and insulin periods were compared with the paired student t test and by analysis of variance. Differences among groups were compared with the nonpaired student t test and by analysis of variance. Differences were considered significant at $P < 0.05$. Results

TABLE I. SYMBOLS AND EQUATIONS (See Fig. 1)

R	Net tracer glucose flux
r	Net tracer glucose flux (including tracer glucose infusion rate)
SA	Glucose specific activity
	Subscripts[a]
m	Maternal glucose pool
u	Uterine glucose pool
o	Outside of the glucose pool
$R_{m,o}$	r/SA_m
$R_{o,m}$	r/SA_m in nonpregnant sheep
$R_{o,m}$	$(r - r_{u,m}/SA_m)$ in pregnant sheep
$r_{u,m}$	Uterine blood flow × uterine arteriovenous blood tracer glucose concentration difference
$R_{u,m}$	Uterine blood flow × uterine arteriovenous blood glucose concentration difference
$R_{o,u}$	$r_{u,m}/SA_m$

[a] The order of subscripts represents to pool x from pool y.

are expressed by means and standard errors of the means.

Results. Figure 2 presents data in the nonpregnant animals, and Fig. 3 presents data in the pregnant animals. These results demonstrate that steady-state conditions were present during the sampling periods of the control and the insulin infusion studies. Mean values during each sampling period are shown for glucose and insulin concentrations in Table II and for glucose fluxes in Table III.

Nonpregnant fed studies. Control period values for glucose and insulin concentrations and glucose flux rates were normal for Columbia–Rambouillet sheep in our laboratory. During the insulin infusion glucose concentration was not changed, while insulin concentration increased 10.4-fold (Table II, Fig. 2). Also during the insulin infusion, total glucose entry ($R_{m,o}$) (or disposal $R_{o,m}$) increased by 116.4 ± 14.8% above control (Table III). During the insulin infusion the difference between the total glucose entry rate ($R_{m,o}$) and the exogenous glucose infusion rate, considered to represent the nonsuppressed endogenous glucose production rate (GPR), was significantly less (53.2 ± 5.6%, $P < 0.001$) than that of the control.

Nonpregnant fasted studies. The fasting glucose concentrations were not different from the fed state ($P > 0.5$), but the fasting arterial plasma insulin concentration was significantly lower than that in the fed state ($P < 0.05$) (Table II). The mean total glucose entry ($R_{m,o}$) rate was significantly less (18.8%, $P < 0.05$) in the fasted versus fed states.

During the hyperinsulinemic period in the fasted ewes, glucose concentrations were not different from those of the control, while insulin increased to an average value which was comparable to that of the fed state (Table II). Total glucose entry ($R_{m,o}$) increased by 82.8 ± 11.0% above the control ($P < 0.001$) (Table III). In the fasted state, the glucose entry ($R_{m,o}$) during hyperinsulinemia was significantly lower (35.1%, $P < 0.05$) than that of the fed state.

During hyperinsulinemia in the fasted

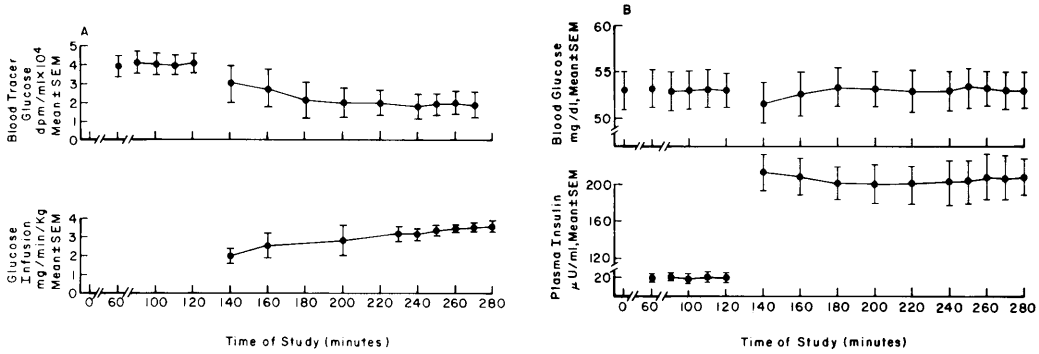

FIG. 2. Combined values (mean ± SEM) for arterial blood tracer glucose concentrations and glucose infusion rates (A) and arterial blood glucose and plasma insulin concentrations (B) in the nonpregnant, fed and the nonpregnant, fasted ewes. Values from 9 to 120 min were averaged for the control sampling period, and values from 240 to 270 min were averaged for the insulin infusion sampling period.

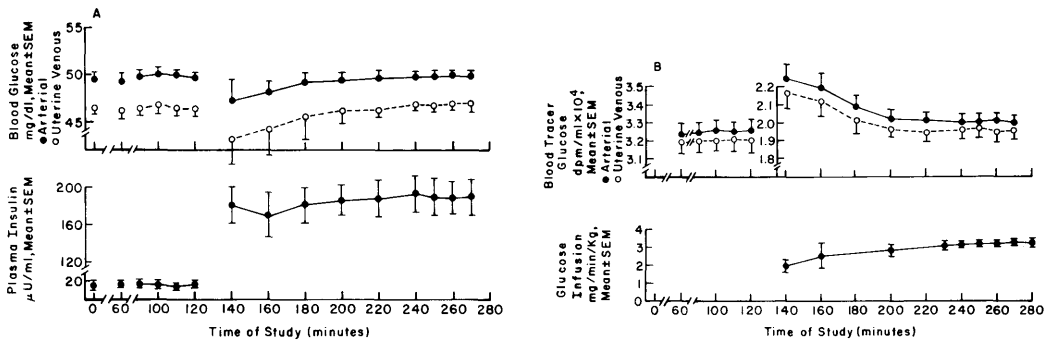

FIG. 3. Values (mean ± SEM) for arterial (●) and uterine venous (○) blood glucose and arterial plasma insulin concentrations (A) and for arterial (●) and uterine venous (○) blood tracer glucose concentrations and glucose infusion rate (B) in the fed, pregnant ewes. The control sampling period was from 90 to 120 min, and the insulin infusion sampling period was from 240 to 270 min.

state, total glucose entry ($R_{m,o}$) was not different from the exogenous glucose infusion rate required to maintain constant glycemia ($P > 0.5$) (Table III), demonstrating virtually complete suppression of endogenous glucose production.

Pregnant animals. Uterine blood flow was not different between control (1263 ± 142 ml/min) and insulin infusion (1231 ± 163 ml/min) periods in the pregnant ewes. Glucose concentrations during the control period were not different between the fed and the fasted, nonpregnant animals (Table II). The insulin concentration was intermediate to the values for the fed and fasted states of the nonpregnant animals (Table II). The total glucose entry rate for the pregnant animals was not significantly different from that for the fed and fasted nonpregnant animals (Table III). However, the glucose utilization rate by the nonuterine maternal tissues was significantly lower than the glucose utiliza-

TABLE II. WEIGHTS AND GLUCOSE AND INSULIN CONCENTRATIONS IN NONPREGNANT AND PREGNANT SHEEP DURING CONTROL STUDY AND FOLLOWING INSULIN INFUSION [MEAN (SEM)]

	Weight (kg)		Arterial blood glucose (mg/dl)	Arterial plasma insulin (μU/ml)
	Total ewe	Nonuterine		
I. Nonpregnant, fed[a]				
Control	48		54.2	20
	(2)		(2.1)	(2)
High insulin	48		54.2	208[c]
	(2)		(2.1)	(20)
II. Nonpregnant, fasted				
Control	48		51.8	11[c]
	(2)		(2.1)	(2)
High insulin	48		52.0	215[c]
	(2)		(1.8)	(34)
III. Pregnant[b]				
Control	50.2	44.6	49.9	17
	(2.7)	(2.9)	(1.9)	(2)
High insulin	50.2	44.6	49.9	188[c]
	(2.7)	(2.9)	(1.9)	(20)

[a] Nonpregnant sheep, $n = 11$ (same sheep studied fed and fasted).
[b] Pregnant sheep, $n = 13$.
[c] Different from nonpregnant control, $P < 0.05$.

TABLE III. GLUCOSE FLUXES [MEAN (SEM)]

	$R_{m,o}$[a] (mg/min/kg)	$R_{o,m}$[b] (mg/min/kg)	$R_{o,u}$ mg/min	$R_{u,m}$ (mg/min)	GIR[a] (mg/min/kg)	EGPR[a] (mg/min/kg)
I. Nonpregnant, fed[c]						
Control	2.29	2.29				
	(0.17)	(0.17)				
High insulin	4.84	4.84			3.66	1.19
	(0.36)	(0.36)			(0.31)	(0.13)
II. Nonpregnant, fasted						
Control	1.86	1.86				
	(0.11)	(0.11)				
High insulin	3.16	3.16			3.08	0.08
	(0.16)	(0.16)			(0.17)	(0.06)
III. Pregnant[d]						
Control	2.22	1.71	35.5	35.6		
	(0.18)	(0.18)	(3.1)	(3.1)		
High insulin	3.52	3.20	34.9	36.9	3.24	0.27
	(0.32)	(0.34)	(3.8)	(4.2)	(0.24)	(0.11)
					3.65[b]	
					(0.27)	

[a] Rate expressed per kilogram of total weight of ewe.
[b] Rate expressed per kilogram of nonuterine weight of ewe.
[c] Nonpregnant sheep, $n = 11$ (same sheep studied fed and fasted).
[d] Pregnant sheep, $n = 13$.

tion rate for the fed ($P < 0.05$) but not the fasted nonpregnant animals. Glucose utilization rate and net glucose uptake rate by the uterus were not different (Table III); thus, there was no apparent significant endogenous glucose production by the uterus or its contents.

During hyperinsulinemia in the pregnant animals, insulin concentration was not significantly different from that in the fed and fasted states in the nonpregnant animals (Table II). Glucose concentration did not change significantly relative to the control period. The glucose utilization rate of the nonuterine maternal tissues ($R_{o,m}$) increased by 1.49 ± 0.11 mg/min/kg (Table III). This value was a significantly ($P < 0.05$) smaller increment than occurred in the nonpregnant fed ewes but was not different from that of the nonpregnant ewes following fasting (Fig. 4). This increment for glucose utilization in the pregnant ewes during hyperinsulinemia, when expressed as a percentage change from the control rate of GUR, was 94.2 ± 14.3%, a value less than but not different from the percentage change for the nonpregnant fed ewes and greater than but not different from the percentage change for the nonpregnant ewes following fasting (Fig. 5). These differences were not changed when glucose concentrations were normalized to the glucose concentration of the nonpregnant, fed ewes, assuming proportionality between GUR and glucose concentration over this limited range of glucose concentration (Fig. 4).

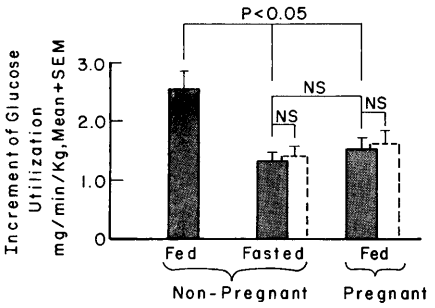

FIG. 4. Increment of glucose utilization rate (GUR) during the hyperinsulinemic glucose clamps. Values for nonpregnant ewes are expressed per kilogram of total weight of ewe; values for the pregnant ewes are expressed per kilogram of the nonuterine weight of the ewes. The open bars, outlined by broken lines, represent the same values normalized to the arterial blood glucose concentration (G) of the nonpregnant, fed ewes, assuming proportionality between GUR and G.

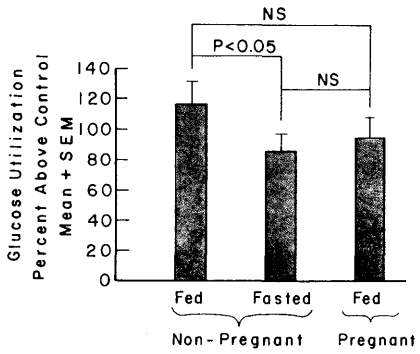

FIG. 5. Increment of GUR during the hyperinsulinemic glucose clamp, expressed as a percentage change above control values for each group.

Both $R_{o,u}$ and $R_{u,m}$ were not different from each other and from the same values during the control period; thus, there was no apparent significant effect of insulin on uterine glucose uptake or utilization.

During hyperinsulinemia in the pregnant sheep the exogenous glucose infusion rate required to maintain constant glycemia was (Table III) significantly ($P < 0.05$) less than the total glucose utilization rate for the whole ewe, demonstrating a nearly complete suppression of endogenous glucose production similar to the effect of hyperinsulinemia in the nonpregnant, fasted animals (Fig. 6). This GIR was not different from the GIR during hyperinsulinemia in the fed, nonpregnant ewes but was significantly greater ($P < 0.05$) than the GIR during hyperinsulinemia in the nonpregnant ewes following fasting.

Discussion. These results demonstrate a significantly lower rate of glucose utilization under basal conditions and in response to high levels of plasma insulin in the nonuterine tissues of late gestation pregnant sheep compared with those of fed, nonpregnant ewes. Additionally, insulin suppressed endogenous glucose production in the pregnant sheep more than in fed, nonpregnant ewes. These metabolic conditions in the pregnant sheep were qualitatively and quantitatively similar to those in the nonpregnant ewes that had been fasted overnight. Furthermore, these differences in glucose metabolism were independent of glucose concentration, indicating that dietary and hormonal factors were probably responsible.

In the present study, weight-specific and absolute glucose entry rates for the whole ewe were not different between pregnant and nonpregnant fed ewes. This observation contrasts with observations by Prior and Christenson (23) and by Bergman (24) who showed that glucose entry (weight-specific or absolute) was significantly greater among pregnant sheep. Their studies, however, included a greater proportion of ewes carrying twins and triplets than of ewes carrying singleton fetuses. Thus, our data suggest that endogenous glucose production in late gestation pregnant sheep carrying a single fetus can be comparable to that of nonpregnant sheep. Similar results have been observed for the rabbit, guinea pig, rat, and human (25).

On the other hand, the rate of glucose utilization in nonpregnant, fed sheep is considerably greater than that of the comparable nonuterine, maternal tissues in pregnant sheep. This difference persists even when glucose concentrations are normalized to those of the fed, nonpregnant ewes. This observation indicates that factors other than glucose concentration were responsible for the difference in glucose utilization. One could arrive at a similar conclusion by a recalculation of the data presented by Prior and Christenson (23) to develop an estimate of nonuterine maternal glucose utilization. While a relative insulin resistance of pregnancy has been suggested by a variety of studies in other mammals, we believe this is

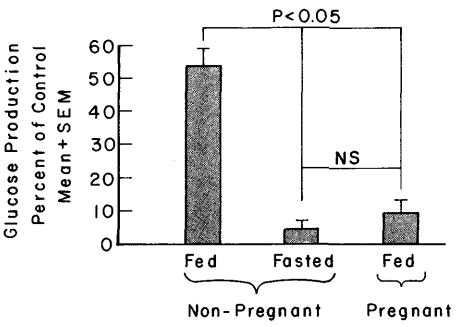

FIG. 6. Endogenous glucose production (EGPR) during the insulin infusion period, expressed as a percentage of the control values.

the first demonstration that the nonuterine tissues of the pregnant sheep have a decreased rate of glucose utilization compared to nonpregnant sheep despite similar insulin and glucose concentrations.

During the glucose clamps, insulin produced two separate effects on glucose turnover: first, endogenous glucose production was suppressed, and second, peripheral tissue glucose utilization was enhanced (17). The enhanced insulin suppression of endogenous glucose production in the fasted, nonpregnant animals may relate to both hormonal and substrate changes. Such changes and their mediating mechanisms have not been investigated. In the pregnant ewe, however, this effect may have a reasonable biochemical basis. Studies in pregnant sheep by Gill and Hart (26) have shown a reduction in hepatocyte glucagon binding and an increase in hepatocyte insulin binding. This increased insulin/glucagon binding ratio may promote insulin effects such as suppression of GPR relative to glucagon effects such as enhancement of GPR. With respect to the effect of insulin on glucose utilization, it is important to emphasize that comparisons of GUR between nonpregnant and pregnant sheep require that in pregnant sheep, the GUR of the nonuterine, maternal tissues is measured separately from that of the uterine tissues. At any time, glucose disposal in the pregnant animal is distributed between the uterine tissues and the nonuterine maternal tissues. In the pregnant sheep this distribution is about 30% uterine/70% nonuterine maternal (15). It is important to quantify this distribution to avoid the false assumption that GIR alone is a measure of insulin response in the pregnant sheep. In fact, only that portion of the GIR that is distributed to the GUR of the nonuterine tissues should be compared to insulin concentration to quantify an insulin–GUR, dose–response relationship that can be compared with the nonpregnant state. This reasoning is supported also by the observation, made in an earlier study in term pregnant sheep (13) and confirmed in the present study, that uterine glucose uptake and utilization are not responsive to changes in maternal insulin concentration.

The reduced peripheral (nonuterine) tissue glucose utilization ($R_{o,u}$) also may have a biochemical basis. During late pregnancy, Vernon *et al.* (11), Hove and Blom (27), and Blom *et al.* (28) have shown a trend toward hypoglycemia, hypoinsulinemia, and increased free fatty acid and growth hormone concentrations. These trends are enhanced with fasting and underfeeding. The relative hypoglycemia and hypoinsulinemia may lead to lower rates of glucose utilization while the increased fatty acid and growth hormone levels may add to glucose intolerance. Elevated maternal plasma levels of chorionic gonadotrophin that increase in late pregnancy in sheep also may add to the inhibition of glucose utilization (10, 11). Similar changes in hormonal milieu have been observed in late pregnancy in human (3), rat (7), and rabbit (29) and have been associated with both glucose intolerance and insulin resistance. In the present study, sheep appear to be similar in that high levels of insulin can enhance tissue glucose utilization but to a lesser extent than in nonpregnant fed ewes, indicating that the mechanisms producing the reduced basal glucose utilization rate of the nonuterine tissues in sheep cannot be reversed easily with insulin (at least at the levels and duration achieved in the present study). Thus, the plasma hormonal and substrate milieu and the tissue insulin binding capacity of late pregnancy in sheep are sufficient to reduce both basal glucose uptake and tissue glucose utilization at high levels of insulin. Whether such conditions are operative at lower levels of relative hyperinsulinemia remains to be determined.

The comparison of nonuterine maternal glucose utilization in pregnant sheep with glucose utilization in nonpregnant sheep, for both basal and insulin-stimulated conditions, bears some further qualification. Because of changes in body composition that occur during pregnancy, such as increased blood volume, water content, and adiposity (10, 30), the nonuterine maternal tissue mass in the pregnant animal may have a different weight-specific rate of glucose utilization independent of changes in insulin sensitivity or glucose tolerance. Thus, the differences between basal and insulin-stimulated GUR of nonpregnant and pregnant nonuterine tis-

sues observed in this study may include a component of tissue compositional differences as well as the effect of pregnancy-related glucose intolerance and reduced insulin responsiveness. The partitioning and quantification of these two aspects of glucose metabolism in pregnancy remain to be determined.

The magnitude of the increase in GUR in the nonpregnant fed ewes in the present studies is comparable to that found in nonpregnant sheep by Weekes *et al.* (31). Lower percentage increases in GUR were reported by Weekes *et al.* (31) for insulin levels less than 100 μunits/ml and by Brockman (32). The magnitude of the maximal insulin-induced increase in GUR by the nonpregnant, fed sheep, about twofold in the present studies, is considerably less than that in humans in whom four- to sixfold increases in GUR at maximal insulin effect have been reported (17, 33). Thus, species differences may play a large role in determining the response of glucose utilization to increased concentrations of plasma insulin.

The mechanisms responsible for the change in insulin effect on GPR and GUR with fasting were not studied in the present experiments but are likely to include both an altered hormonal milieu and a substrate mix. The decrease in the effect of insulin on peripheral glucose utilization in the pregnant sheep is consistent with the previously suggested hypothesis that peripheral insulin resitance spares glucose for the conceptus. On the other hand, the comparable effect of insulin on suppression of endogenous glucose production in the pregnant and the nonpregnant fasted ewes is a novel observation which will require further verification. At this time the importance of this phenomenon remains speculative, although it may account for the tendency toward hypoglycemia in late pregnancy, a trend apparently ubiquitous among mammals (24). In nonpregnant sheep, brief fasts of 24 hr do not appear to affect glucose concentration very much (20), consistent with the present observations. Longer fasts result in hypoglycemia, a process that appears to be accelerated in pregnancy. In both cases endogenous glucose production in ruminants appears to be dependent on a normal dietary energy and substrate intake and fails to be sustained by an increase in glucogenesis with fasting.

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