Effects of Pregnancy and Sex Steroid Administration on Skeletal Muscle Metabolism in the Rat

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SUMMARY

To determine whether the insulin resistance, exaggerated fasting hypoglycemia, and hypoalaninemia of late human and rat gestation may reflect altered skeletal muscle metabolism, hindlimbs of nonpregnant (NPG) and 3-wk-pregnant (PG) rats were noncyclically perfused in situ. In 12- and 24-h-fasted NPG rats, 50–500 μU/ml of insulin significantly augmented glucose uptake 3–9-fold. In PG rats uptake was suppressed 40–50% at comparable hormone concentrations. Release of alanine and phenylalanine was suppressed 20–35% by 250 μU/ml or more of insulin in NPG rats. However, comparable insulin concentrations failed to suppress their release in PG rats. No differences between groups existed with respect to oxygen uptake, glycerol, lactate, or pyruvate release. Suppression of insulin sensitivity in 12-day-pregnant rats was not observed.

Since plasma estradiol (E) and progesterone (P) are increased in pregnancy, E-benzoate (5 μg/day) and/or P (5 mg/day) were injected s.c. into female rats for 21 days. In 12-h-fasted animals, E treatment potentiated whereas P treatment mildly antagonized insulin-induced glucose uptake relative to control values. Separate effects of E and P were offset when administered in combination. Individual or combined regimens did not alter oxygen uptake, or release of alanine, phenylalanine, lactate, or pyruvate.

In late gestation, insulin action on skeletal muscle is resisted with respect to glucose uptake, alanine release, and proteolysis. Fasting hypoalaninemia is not due to impaired alanine release from muscle, but may reflect increased fetal extraction. Fasting hypoglycemia may represent, in part, maternal gluconeogenic precursor deficiency due to fetal alanine removal. E and/or P treatment do not duplicate the observed metabolic changes. DIABETES *30*:545–550, July 1981.

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In late human and rat gestation, endogenous insulin resistance is manifested by elevated basal and postprandial insulin concentrations,[1–3] and reduced sensitivity to the hypoglycemic effects of exogenous insulin and tolbutamide.[4–6] Despite the presence of this diabetogenic stress, there is a paradoxic depression of fasting plasma glucose relative to the nonpregnant state.[1–3] This latter phenomenon may relate to decreased availability of gluconeogenic amino acids for hepatic glucose production and/or increased extraction of both glucose and amino acids by the rapidly growing fetus. Fasting hypoalaninemia that is observed during late gestation is consistent with both of these ideas.[7–8]

Skeletal muscle represents a large tissue mass that is an important site for insulin action, glucose utilization, and alanine production, and alanine is known to be a major gluconeogenic precursor. In the present study, the impact of gestation on these three parameters was examined by employing the isolated, perfused rat hindlimb, a preparation comprised mainly of skeletal muscle.[9] The results suggest that during late pregnancy there is reduced sensitivity to insulin in skeletal muscle. Although administration of estradiol or progesterone to nonpregnant rats also had significant effects on skeletal muscle metabolism, the two steroids administered separately or in combination could not reproduce the results obtained in pregnant animals.

MATERIALS AND METHODS

Animals. Age-matched female Sprague-Dawley rats (Madison, Wisconsin) weighing 250–300 g were subsequently maintained in the virgin state or rendered pregnant. All were housed in air conditioned quarters with a light source from 6 a.m. to 6 p.m. Two studies were performed.

Study I. One group of pregnant rats was studied on days 12 or 13 of a 21-day gestation; a second group was used on days 19 or 20. Control, virgin rats were matched to the age

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and pregestational weight of pregnant animals. Gravid animals were excluded from the study if the number of fetuses was less than six. All rats were allowed free access to water and laboratory chow until fasting was begun for 12 or 24 h before time of investigation.

Study II. Four groups of animals were investigated. Control rats received s.c. injections of sesame oil twice daily for 21 days. A second group was administered estradiol benzoate, 2.5 μg b.i.d.; the third group received progesterone, 2.5 mg b.i.d.; and the final group was injected with the same dosage of both hormones in a combined regimen twice daily. Dissolution of the sex steroids in sesame oil has been described previously.[3] Volumes of sesame oil used for each injection regimen were the same.

The rats were fed a controlled diet of rat chow to maintain equal weights among the groups throughout the 21 days of steroid administration. This was accomplished by feeding the steroid-treated rats the same volume of food that the sesame oil control rats consumed the previous day. Animals were fasted 12 h before experimentation.

Perfusion technique. After fasting and induction of anesthesia (pentobarbital, 50 mg/kg i.p.), the abdomen was opened and the uterine, ovarian, iliolumbar, and inferior mesenteric vessels were ligated. In pregnant animals, the uterus (with fetuses) and ovaries were removed and weighed. After ligating the aorta just distal to the renal vessels, an 18-gauge polyethylene cannula was inserted and tied into the lumen of the abdominal aorta proximal to the aortic bifurcation. The perfusion pump was started. The inferior vena cava was ligated and punctured with a 16-gauge polyethylene catheter. This catheter was tied into place and the entire flow was collected. The surgical preparation required less than 5 min.

The perfusion medium consisted of Krebs-Henseleit bicarbonate buffer[10] containing bovine serum albumin, fresh beef erythrocytes, and glucose. Erythrocytes obtained from the jugular vein of a stunned steer were collected into a solution combining sodium citrate, citric acid, and glucose[11] and was used within 72 h. The cells were washed by suspension three times in two volumes of saline (156 meq NaCl/L) and twice in two volumes of Krebs-Henseleit buffer. On the morning of experimentation the cells were washed again in buffer and resuspended in buffer containing 3% bovine serum albumin. The albumin was Cohn fraction V with a free fatty acid content of 2.342 μmol/100 mg (Sigma Chemical Co., St. Louis, Missouri). The volume was adjusted to yield a hematocrit of 33% and a hemoglobin level of 12 g/dl. Glucose was added to achieve a concentration of 5.5 mM. The pH was brought to 7.85 with 10 M NaOH. When the medium was gassed with 95% O_2-5% CO_2 and warmed to 37°C, the pH fell to 7.4. Crystalline pork insulin (Eli Lilly and Co., Indianapolis, Indiana) previously diluted in physiologic saline containing 1% bovine albumin, was added to the perfusion medium to give concentrations of 50, 100, 250, 500, and 1000 μU/ml.

The perfusion apparatus represents a modification of that described by Exton and Park.[11] The perfusion medium was placed into a 2-L flask attached to a rotor that was modified to allow gas delivery and medium withdrawal during rotation. A constant flow of 95% O_2-5% CO_2 maintained oxygen saturation at > 99%. This saturation was possible due to the thin layer of perfusate always lining the rotating flask. The flask rested in a waterbath maintained at 37°C. A Harvard peristaltic pump (Millis, Massachusetts) delivered perfusate from the flask through a blood filter and a bubble trap to the arterial cannula at a constant flow rate of 6 ml/min. At this flow rate the arterial perfusion pressure was approximately 61 mm Hg, as monitored by an aneroid manometer attached to the bubble trap. With the exception of the rotor, all components of the apparatus were maintained at 37°C in a plexiglass chamber designed for two concurrent perfusions to take place. In all instances perfusions were performed in an open-loop system in which media were not recycled.

Sampling and chemical determinations. After cannulation and a 5-min washout period, the venous effluent was collected at 5-min intervals for a total of 30 min. Arterial samples were obtained at the beginning of the first and end of the last collection periods. The samples were centrifuged, and the supernatant was stored at −20°C and then analyzed for glucose using a YSI glucose analyzer (Yellow Springs Instrument Corp., Yellow Springs, Ohio). The supernatant was also deproteinized with perchloric acid, final concentration 0.3 M, and analyzed fluorometrically for lactate,[12] pyruvate,[13] alanine,[14] phenylalanine,[15] and glycerol.[16] Arterial and venous samples also were drawn during the final period for pO_2 determinations. Steady-state conditions for glucose uptake and alanine release were established after 20 min. Data presented in all figures represent flux rates measured at 30 min.

Determination of weight of perfused muscle. After 30-min perfusions were completed in 43 selected experiments, Niles Blue dye was injected into the arterial circulation. Delineation and weight of stained muscle was determined by the method of Ruderman.[9] The ratio of perfused muscle weight to total body weight (in absence of fetuses in pregnant animals) was recorded.

In seven groups of animals (various control, pregnant, and sex steroid-treated rats) the mean ratio was 0.228 ± 0.0003. Each subgroup was within 1.7% or less of that mean (range 0.226 to 0.234). Accordingly, the overall ratio of 0.228 was used to calculate the weight of perfused muscle in each animal by multiplying this number by the total body weight. This value was inserted into the equation described below.

Calculations. Substrate uptake or release was calculated using the following equation: $R = (A - V)(1 - h/100)(F)(1/G)$, with R, release (or uptake) in nmol/min/g muscle perfused; A, arterial concentration in nmol/ml; V, venous concentration in nmol/ml; h, hematocrit; F, flow in ml/min; and G, grams of muscle perfused.

Student's *t* tests for paired and unpaired data were used to assess the significance of changes within a group and differences in mean values between groups, respectively.

RESULTS

Effects of pregnancy on skeletal muscle metabolism. The effects of insulin on glucose uptake and on lactate and pyruvate release by the perfused hindlimbs of pregnant and nonpregnant rats fasted for 12 h are shown in Figure 1. In nonpregnant and 2-wk-pregnant rats, glucose uptake was progressively and significantly increased above control perfusions devoid of insulin when concentrations of the hormone were 50–500 μU/ml. At concentrations of 500 μU or more, a maximum ninefold increase occurred. The glucose

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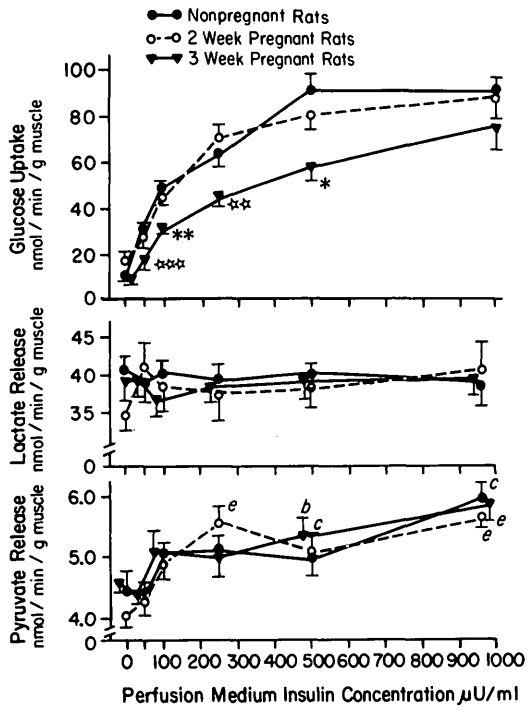


FIGURE 1. Effects of insulin on glucose uptake, and lactate and pyruvate release in noncyclically perfused hindlimbs of nonpregnant and 2- and 3-wk-pregnant rats. Animals were fasted 12 h before perfusion. Each point represents the mean ± SEM of at least six experiments. The following symbols indicate significance of the difference between means of pregnant animals and the corresponding means of nonpregnant animals perfused with equal concentrations of insulin: ☆ ($P < 0.05$); ☆☆ ($P < 0.025$); ☆☆☆ ($P < 0.01$); ** ($P < 0.005$); and * ($P < 0.001$). The following letters indicate significance of the difference between the means of animals perfused with insulin and mean values of animals in the same group perfused without insulin: *a* ($P < 0.05$); *b* ($P < 0.025$); *c* ($P < 0.01$); *d* ($P < 0.005$); and *e* ($P < 0.001$).

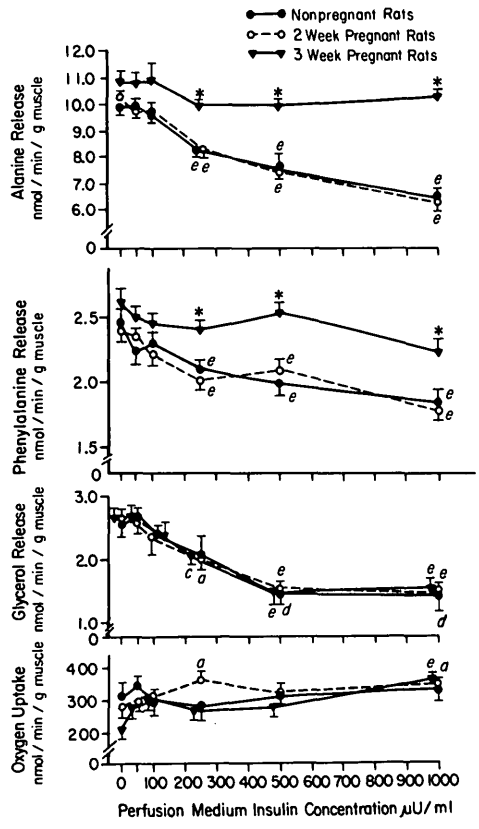
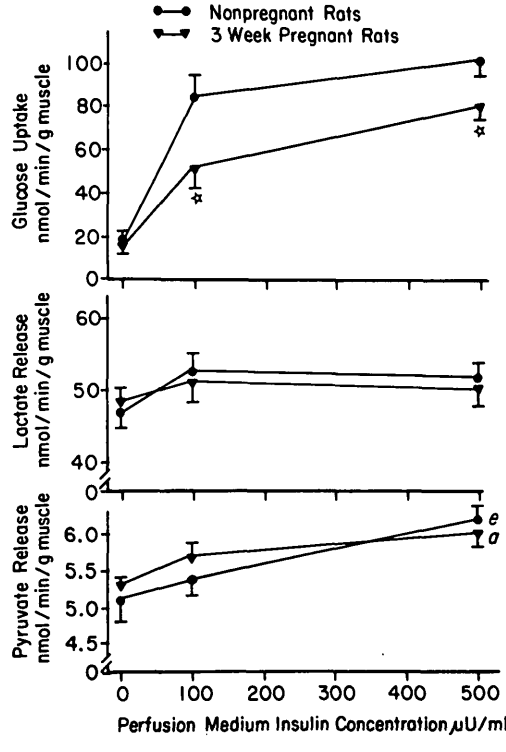


FIGURE 2. Effects of insulin on oxygen uptake and on the release of alanine, phenylalanine, and glycerol in noncyclically perfused hindlimbs of nonpregnant and 2- and 3-wk-pregnant rats. Animals were fasted 12 h before perfusion. Refer to Figure 1 legend for additional details.

FIGURE 3. Effects of insulin on glucose uptake and on the release of lactate and pyruvate in noncyclically perfused hindlimbs of nonpregnant and 3-wk-pregnant rats. Animals were fasted 24 h before perfusion. Refer to Figure 1 legend for additional details.



uptake response to insulin in 2-wk-pregnant rats did not differ significantly from that of nonpregnant rats.

Insulin-stimulated glucose uptake was markedly blunted in 3-wk-pregnant rats and was significantly below values of nonpregnant control and 2-wk-pregnant rats at insulin concentrations of 50–500 μU/ml. At 1000 μU/ml no differences among groups were observed. In nonpregnant and pregnant rats, insulin, up to 1000 μU/ml, did not alter lactate release. At this insulin concentration a small, significant increase was found in pyruvate release. No difference in the lactate and pyruvate fluxes were found between the pregnant and nonpregnant rats.

Figure 2 depicts the effects of insulin on skeletal muscle release of alanine, phenylalanine, and glycerol as well as on oxygen uptake in the animals fasted for 12 h. In nonpregnant rats, a significant decrease ($P < 0.001$) in both alanine and phenylalanine release was found with insulin concentrations of 250 μU/ml or more. These responses were similar in the 2-wk-pregnant rats. Insulin, up to 1000 μU/ml, did not decrease the alanine or phenylalanine released from muscle in 3-wk-pregnant rats. The differences in release between this group and the nonpregnant and 2-wk-pregnant group were significant in perfusions with insulin at or above 250 μU/ml. In nonpregnant rats, glycerol release was reduced 50% with 500 μU/ml insulin. A small increase in oxygen uptake was observed with 1000 μU/ml insulin. The

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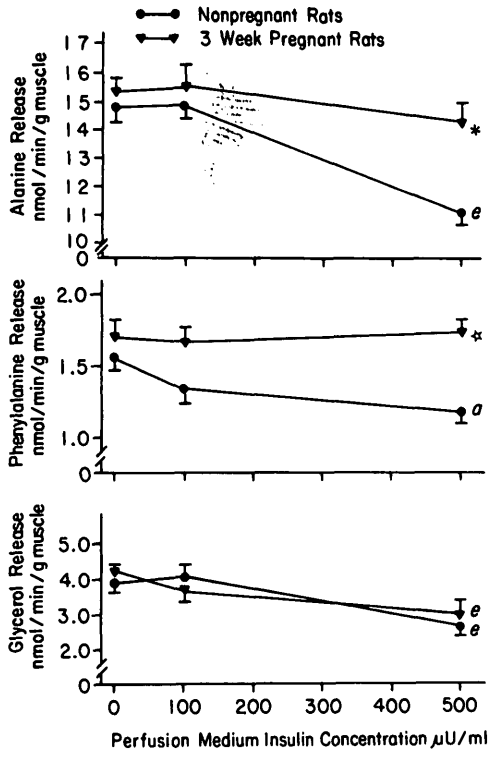

FIGURE 4. Effects of insulin on alanine, phenylalanine, and glycerol release in noncyclically perfused hindlimbs of nonpregnant and 3-wk-pregnant rats. Animals were fasted 24 h before perfusion. Refer to Figure 1 legend for additional details.

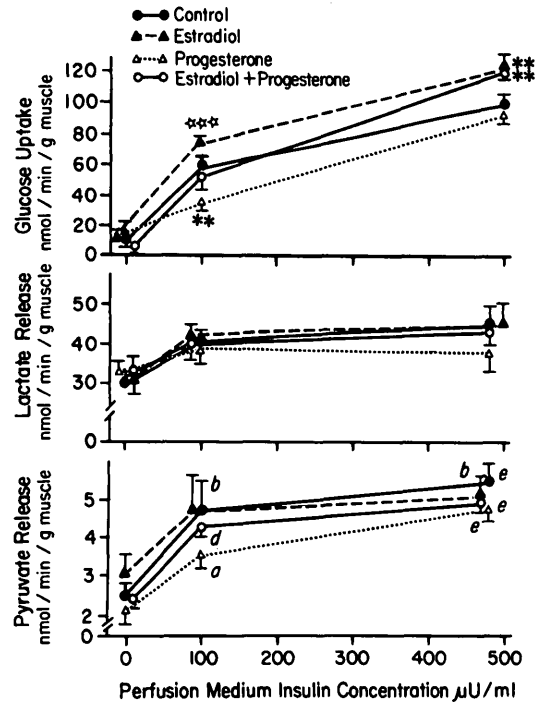

FIGURE 5. Effects of insulin on glucose uptake and on the release of lactate and pyruvate in noncyclically perfused hindlimbs of female rats administered estradiol benzoate (5 μg) and/or progesterone (5 mg) daily for 21 days. Control animals received daily injections of sesame oil, the vehicle in which the sex steroids was dissolved. Animals were fasted 12 h before perfusion. Refer to Figure 1 legend for additional details.

glycerol and oxygen fluxes of the pregnant rats did not differ from those found in the nonpregnant rats.

The flux rates of glucose, lactate, pyruvate, alanine, phenylalanine, and glycerol in nonpregnant and 3-wk-pregnant rats fasted for 24 h are shown in Figures 3 and 4. These results are similar to those described for rats fasted for 12 h (Figures 1 and 2). Again, rats in late gestation demonstrated significant impairment of insulin-mediated glucose uptake and insulin-induced suppression of alanine and phenylalanine efflux.

Effects of female sex steroid administration on skeletal muscle metabolism. Figure 5 shows the effects of insulin on glucose uptake, lactate release, and pyruvate release in control and steroid-treated rats fasted for 12 h. In control rats, 500 μU/ml insulin stimulated glucose uptake ninefold over uptake in perfusions without insulin. Glucose uptake in rats treated with estradiol benzoate was elevated above uptake in control rats during perfusions with insulin at concentrations of 100 μU/ml ($P < 0.01$) and 500 μU/ml ($P < 0.05$). Glucose uptake was blunted in progesterone-treated rats ($P < 0.005$) as compared with the control group when perfusion insulin was 100 μU/ml. This difference was not observed when the concentration was raised to 500 μU/ml. With combined administration of the two hormones, the opposing effects of estradiol and progesterone on insulin-stimulated glucose uptake appeared to be offset at insulin concentrations of 100 μU/ml, since no differences from control responses were found. However, at 500 μU/ml of insulin, the combined regimen enhanced glucose uptake above that of the control group ($P < 0.025$). In control and steroid-treated rats, insulin up to 500 μU/ml did not alter lactate release. At 500 μU/ml, a significant increase was found in pyruvate release. No differences in lactate and pyruvate release were observed between the control and sex steroid-treated animals.

Figure 6 demonstrates the effects of insulin on alanine, phenylalanine, and glycerol release and oxygen uptake in control rats and animals receiving sex steroids after 12 h of fasting. In control and steroid-treated rats, a significant decrease in alanine and phenylalanine release was found with 500 μU/ml insulin, as compared with perfusions without insulin. At this concentration, glycerol release was decreased 50%. Insulin did not significantly alter oxygen uptake. The alanine, phenylalanine, glycerol, and oxygen fluxes of the sex steroid-treated rats did not differ significantly from the corresponding fluxes in control rats.

DISCUSSION

Skeletal muscle responsiveness to insulin action on glucose uptake was markedly reduced in late rat pregnancy, suggesting that this tissue is a major site of insulin resistance. Failure to observe a similar phenomenon in 2-wk as opposed to 3-wk-pregnant animals indicates that this blunted hormone action is a relatively late event during the course of normal gestation. This is consistent with in vivo findings previously reported in both human and animal investigations.[3,17,18]

Results of our studies also suggest that the insulin resistance of late pregnancy involves amino acid as well as glucose metabolism in muscle. Prepartum rats fasted for either 12 or 24 h failed to decrease muscle alanine or phenylalanine efflux throughout a wide range of insulin concentrations. However, in control and 2-wk-pregnant rats release of both amino acids was suppressed when concentrations of

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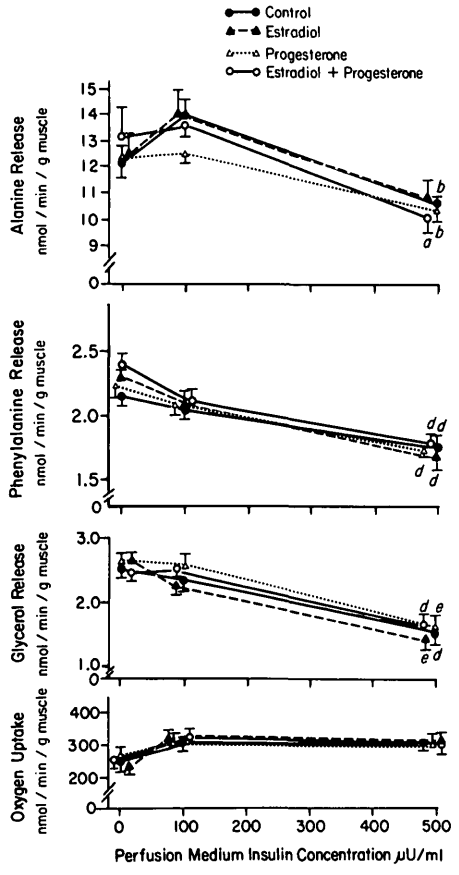

FIGURE 6. Effects of insulin on oxygen uptake and on the release of alanine, phenylalanine, and glycerol in noncyclically perfused hindlimbs of female rats administered estradiol benzoate (5 μg) and/or progesterone (5 mg) daily for 21 days. Control animals received daily injections of sesame oil alone. Animals were fasted 12 h before perfusion. Refer to Figure 1 legend for additional details.

insulin were increased, results that are similar to previous studies of normal rat muscle using the same animal model.[19,20]

Phenylalanine release was always in parallel with release of alanine. Since phenylalanine is a marker for net skeletal muscle protein degradation,[21] it would appear that a primary effect of insulin in the fasting state is to restrain release of amino acids by promoting protein synthesis and/or suppressing muscle protein breakdown. This suggests that a major component of insulin resistance in late pregnancy is a blunted effect of insulin on either process, resulting in a net increase in muscle proteolysis. The results also explain, in part, the increased urinary nitrogen excretion observed in late pregnancy[8,22] and the experimental evidence that the prepartum period represents a catabolic phase in which protein reserves are taxed despite increased food intake and elevated plasma concentrations of insulin.[23]

Increased plasma concentrations of estradiol and progesterone are characteristic of late rat and human gestation. Progesterone by itself or in combination with estradiol also has been implicated in the development of hyperinsulinemia in this state.[3,24]

For these reasons a possible relationship between estrogen or progesterone action and the effects of gestation on muscle metabolism was sought.

Administration of estradiol benzoate to nonpregnant rats increased insulin-stimulated glucose uptake in skeletal muscle, corroborating results of investigations that have used the intact, incubated rat diaphragm.[25,26] This effect was opposite to that observed in pregnant animals, but is consistent with findings that treatment with natural estrogens improves carbohydrate tolerance in monkeys, rats, and human subjects.[27–29]

Contra-insulin effects of progesterone on skeletal muscle were not pronounced. A moderate depression of insulin-stimulated glucose uptake was observed with insulin concentrations of 100 μU/ml. This depression was not evident at 500 μU/ml of insulin. Since exogenous progesterone has not been shown to alter glucose tolerance in man[24] or in rats,[3] the mild insulin-antagonism described above may be compensated in vivo by pancreatic hypertrophy and insulin hypersecretion that occur with progesterone administration.[3]

Neither sex steroid separately or in combination altered effects of insulin on release of alanine or phenylalanine from perfused muscle. It is concluded that factors other than these steroids are responsible for the resistance to insulin on muscle proteolysis in pregnancy, and that progesterone by itself contributes to reduced glucose uptake in response to insulin to a lesser extent than does pregnancy per se.

After food deprivation in both human and rat gestation, there is a more rapid fall of plasma glucose, insulin, and amino acids, including alanine, as compared with the nonpregnant state.[8,30,31] Explanations for the exaggerated hypoglycemia include impaired hepatic gluconeogenesis, diversion of glucose and gluconeogenic precursors to the fetal-placental unit, and impaired release of amino acid substrate from muscle to liver for hepatic glucose production.

The first possibility seems unlikely, because the hepatic capacity for glucose production is unimpaired when gluconeogenic precursor availability is not rate-limiting.[8,22,32] The role of the fetal-placental unit, however, is ostensibly important, since fetal growth rates have reached maximal levels and extraction of glucose and amino acids from the maternal circulation is substantial.[33] The observed generalized lowering of amino acid concentrations as well as glucose during a maternal fast supports this view.[7,8]

One must also consider whether the availability of amino acids like alanine and other major gluconeogenic precursors is compromised in late gestation. This does not appear to be the case in perfused muscle of pregnant rats, since alanine release was normal in perfusions devoid of insulin and continued at the same rate despite progressively increased insulin concentrations. These observations suggest that unrestrained muscle alanine efflux in 12- and 24-h-fasted, pregnant rats cannot explain the associated hypoalaninemia observed in these animals during short-term starvation.[7,8]

Our findings suggest that insulin resistance in maternal skeletal muscle reduces glucose uptake. This could explain the greater plasma excursions of this substrate after feeding, and would serve late pregnancy by assuring availability of this critical nutrient for the rapidly growing conceptus. In a similar fashion, resistance to insulin action on net muscle proteolysis guarantees flux of amino acids to the fetal-placental unit for continued tissue growth. Although efflux of gluconeogenic precursors like alanine from muscle is opti-

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mal in late gestation, the magnitude of the release appears to be insufficient to meet maternal needs completely. As a consequence, it would appear that substrate deficiency supervenes, a functional impairment of hepatic gluconeogenesis develops, and hypoglycemia in the mother becomes accentuated during starvation.

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