

Reversion of insulin resistance in the rat during late pregnancy by 72-h glucose infusion

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Ramos, Pilar, and Emilio Herrera. Reversion of insulin resistance in the rat during late pregnancy by 72-h glucose infusion. *Am. J. Physiol.* 269 (*Endocrinol. Metab.* 32): E858–E863, 1995.—To determine whether sustained exaggerated hyperinsulinemia in normoglycemic rats modifies insulin responsiveness during pregnancy, 17-day-pregnant and virgin rats were studied after receiving a continuous intravenous infusion (35 ml/day) of either 50% glucose or bidistilled water (controls) for 72 h. Plasma glucose was unchanged, whereas insulin was highly increased, and the effect was more marked in pregnant than in virgin rats. Insulin responsiveness, estimated under the hyperinsulinemic euglycemic clamp with 0.8 IU insulin \cdot h⁻¹ \cdot kg⁻¹, was lower in control pregnant than in virgin rats but higher in pregnant than in virgin rats in those that had received the glucose infusion. The tissue glucose utilization metabolic index (GUI) was estimated with 2-deoxy-D-[1-³H]glucose in the clamped rats. The GUI was lower in heart, white- and red-fiber skeletal muscle, and adipose tissue in control pregnant rats than in control virgin rats, and, although the glucose infusion decreased that index in both red-fiber muscle and adipose tissue in virgin rats, glucose increased the index in red-fiber muscle in pregnant rats to the level found in virgin controls. Results therefore show that, when unaccompanied by hypoglycemia, sustained exaggerated hyperinsulinemia decreases insulin responsiveness in virgin rats but reverts insulin resistance in late-pregnant rats.

hyperinsulinemic-euglycemic clamp; tissue glucose utilization; gestation; insulin responsiveness

THE HYPERINSULINEMIA and insulin resistance that have been observed during late pregnancy in both humans (25) and rats (16, 18) are not completely understood at present. Although the existence of insulin resistance has been substantiated in most insulin target tissues when the hyperinsulinemic-euglycemic clamp technique has been used (18, 19), it has not been clearly found when studied in isolated preparations (3, 20), and maternal hyperlipidemia and/or the increase in circulating concentrations of counterregulatory hormones have been suggested as being responsible for postreceptor defects. Maternal hyperinsulinemia, which develops progressively during the course of gestation, may also contribute to the reduced responsiveness of maternal tissues, because prolonged hyperinsulinemia is associated with decreased tissue responsiveness (23). There are reports (17, 32) showing that under nonpregnant conditions chronic hyperinsulinemia, induced by daily injections of insulin, is associated with decreased cellular insulin sensitivity. However, it has also been reported that induced hyperinsulinemia in the rat may increase insulin responsiveness both in vivo and in vitro (17, 30) or even induce insulin hypersensitivity in adipose tissue and insulin resistance in skeletal muscle (4, 28). These

findings suggest that hyperinsulinemia has different long-term effects on insulin responsiveness.

The present study was designed to investigate the effect on insulin responsiveness of the sustained exaggerated hyperinsulinemia that is unaccompanied by hypoglycemia, caused by a 72-h intravenous glucose infusion in late-pregnant and virgin control rats, and to determine the tissues that are involved. Because anesthesia can cause different metabolic perturbations in pregnant and nonpregnant rats (33), we have adapted the euglycemic clamp technique and the method using labeled 2-deoxyglucose to quantify glucose utilization in various tissues for use in conscious animals. Our results demonstrate that, under these conditions, insulin action is significantly impaired in the virgin rat but is significantly enhanced in the pregnant rat.

METHODS

Animals. Female Wistar rats bred in our laboratory were used. They were housed at 22–24°C with light from 0800 to 2000. They had free access to water and chow pellets (Letica, Barcelona) with a composition of 17% protein, 3% fat, 4.3% fiber, 5% minerals, 58.7% carbohydrates, and 12% humidity (2,900 Kcal/kg diet). The beginning of pregnancy was determined by the presence of spermatozoa in vaginal smears. All the rats were subjected to a long-term infusion in unrestrained conditions, following the previously described method (24). Pregnant rats at day 17 of gestation and age-matched virgin rats were anesthetized with an intraperitoneal injection of 0.3 ml/200 g body weight of a ketamine cocktail [(in mg/ml) 50 ketamine, 5 diazepam, and 1 atropine; 5:4:1, vol/vol/vol]. A Silastic catheter (Dow Corning, 0.02 in. ID, 0.037 in. OD) was tunneled under the back skin and introduced for 3 cm into the right jugular vein. Another catheter was placed in the right femoral vein. This catheter was exteriorized on the back of the rat and was filled with 0.9% NaCl solution until use. The animals were housed individually in cages designed so that the infusion catheter passed through a simple pulley system between the infusion pump (Minipuls II, Gilson) and its attachment to the implanted catheter; thus the part of the infusion catheter that was inside the cage remained taut, but the rat was allowed complete freedom of movement during the entire infusion period. The infusion was started at the rate of 35 ml/day immediately after rats recovered from anesthesia. One-half of the rats were infused with 50% glucose in bidistilled water (0.31–0.33 mmol \cdot kg⁻¹ \cdot min⁻¹ in virgins and 0.23–0.24 mmol \cdot kg⁻¹ \cdot min⁻¹ in pregnant rats), whereas the other one-half were infused with sterile bidistilled water and were considered controls. Distilled water was chosen rather than saline to avoid sodium overload. The respective infusions were kept running for 72 h. Blood samples were obtained daily from tail tips for determination of blood glucose (14) and plasma insulin (10). All the experiments were performed between 1000 and 1200, that is, in the postabsorptive period, because spontaneous food consumption stopped with the onset of light. At the end of the 72-h infusion period, some animals were

killed by decapitation; blood was collected from the neck wound and livers were immediately dissected and placed in liquid nitrogen to be stored at -80°C until processed. Free fatty acids (FFA) were measured in plasma following an enzymatic procedure (26). A frozen liver aliquot was used to purify glycogen (7) before hydrolyzation in acid for glucose determination, as previously described (11).

Euglycemic clamp studies. The amount of infused glucose needed to maintain euglycemia in rats receiving an insulin infusion at a constant rate was determined in the conscious rat by adapting the glucose clamp technique used by Leturque et al. (18) in anesthetized rats. Briefly, at the end of the 72-h infusion, the catheter placed in the jugular vein was connected to a two-way interconnector that received the flux from two different infusion pumps (Precidor infusion pump type 5003, Infors HT). Human insulin (Actrapid monocomponent, Novo) was infused by means of one of the pumps at a constant rate of $16 \mu\text{l}/\text{min}$ ($0.8 \text{ IU} \cdot \text{h}^{-1} \cdot \text{kg}^{-1}$). The blood glucose concentration was maintained constant at basal levels by a variable rate glucose infusion (20%) through the other pump and also via the same catheter. Small amounts of blood ($5 \mu\text{l}$) were collected from the tip of the tail every 5 min, starting just before the beginning of insulin infusion, to measure the glucose concentration with a Reflux II M analyzer (BM-Test-Glycémie 20–800 R, Boehringer Mannheim). The exogenous glucose infusion rate was adjusted to maintain euglycemia by altering the percent dial of the Precidor pump depending on the changes observed in the blood glucose concentrations. A steady-state glucose infusion was normally achieved within 30 min after the clamp was started. Some additional blood samples ($200 \mu\text{l}$) were collected to determine the steady-state insulin concentration (10).

Calculations. Once the steady state of the infused glucose was attained during the clamp, the rate of glucose infusion (R) normalized to the body weight was used to determine the glucose disposal rate (M) as an index of insulin responsiveness. Because exogenous insulin does not cross the placenta (29), the increase in glucose utilization in the pregnant rat corresponds only to the effects of insulin on maternal tissues. It was therefore considered convenient to use a new index, M_2 , that corresponded to the R value normalized to the conceptus-free body weight. Because basal glucose levels differed between pregnant and nonpregnant rats, the rate of glucose clearance was also measured as an index of insulin responsiveness, as proposed by Doberne et al. (5). This index is defined as the ratio between the glucose utilization rate (R), which under the studied conditions equals the rate of glucose infusion at steady state, and the glucose level. Finally, because the different experimental groups not only had different basal glucose and insulin values but also different body weights, the insulin sensitivity index (S_{ip}) proposed by Ader and Bergman (2) was also measured. This index is calculated as the ratio of the increase in glucose utilization measured during the clamp (ΔR) and the product of glucose concentration during the same period (G) \times the increase in plasma insulin (ΔI) and body weight (body wt): $S_{ip(\text{clamp})} = \Delta R/G \times \Delta I \times \text{body wt}$.

Estimate of glucose utilization metabolic index in different tissues. The method used is derived from the technique adapted by Ferré et al. (6) to quantify glucose utilization in different tissues of the anesthetized rat. We further adapted the method for use in the unrestrained rat. Briefly, the nonmetabolizable glucose analogue 2-deoxy-D-[1- ^3H]glucose (18.3 Ci/mmol, from Radiochemical Center, Amersham, UK) was administered as a bolus ($30 \mu\text{Ci}$) 60 min after commencement of the euglycemic-hyperinsulinemic clamp experiment through the catheter placed in the jugular vein. The clamp was maintained while blood samples ($50 \mu\text{l}$) were collected from

the catheter placed in the femoral vein at 1, 2, 5, 7, 10, 20, 30, 45, and 60 min after the 2-deoxy-D-[1- ^3H]glucose. Blood was immediately deproteinized in $\text{Ba}(\text{OH})_2/\text{ZnSO}_4$ (27), and the supernatant was used to determine glucose concentration (14) and 2-deoxy-D-[1- ^3H]glucose radioactivity. At the end of the experiment, rats were killed by decapitation, and a larger blood sample was collected from the neck wound for plasma insulin determination (10). Heart, lumbar adipose, and hindlimb muscles were removed. The muscles were dissected and divided into those corresponding to red fiber (soleus, and red portions of the gastronemius and quadriceps) and those corresponding to white fiber (white portions of the gastronemius and quadriceps). All tissues were immediately immersed in 0.5 ml of 1 M NaOH to determine their 2-deoxy-D-[1- ^3H]glucose 6-phosphate content, as previously described (6). This parameter was corrected by the change in the blood 2-deoxyglucose-specific activity to estimate the glucose utilization index (GUI) with the equation

$$\text{GUI} = \frac{[2\text{-deoxy-D-[1-}^3\text{H]glucose 6-phosphate}]_t}{\int_0^t \text{SA} \cdot dt}$$

where 2-deoxy-D-[1- ^3H]glucose 6-phosphate concentration is expressed as disintegrations $\cdot \text{min}^{-1}$ (dpm) $\cdot \text{kg}$ of tissue $^{-1}$, t is 60 min, and SA is the blood specific activity expressed as dpm of 2-deoxy-D-[1- ^3H]glucose per milligram of glucose.

Expression of the results. Results are expressed as means \pm SE. Statistical comparisons were made by a multiple lineal regression analysis, with a 95% confidence interval, by using the Presta statistical program (1). Comparison between two groups was made with Student's t -test.

RESULTS

Body weight, food intake and circulating components during the infusion. As shown in Table 1, the continuous infusion with 50% glucose for 72 h affected neither body weight nor conceptus weight in 17-day-pregnant rats nor body weight in nonpregnant rats compared with control animals receiving bidistilled water. Food intake was higher in control pregnant than in control nonpregnant rats, and the glucose infusion decreased the daily food intake similarly in both groups (Table 1). Plasma glucose concentrations in the basal state were lower in pregnant than in virgin rats, and whereas the glycemia increased on the 1st day of glucose infusion in both groups, values returned to basal levels at the 2nd and 3rd days of infusion and were lower in pregnant than in virgin rats (Table 1). Basal plasma insulin levels were higher in pregnant than in virgin rats, and this was also true in control animals receiving the infusion with bidistilled water. The infusion with 50% glucose significantly increased plasma insulin concentrations in both groups, although the values attained by the pregnant rats were almost three times higher than those in nonpregnant rats (Table 1). In contrast to plasma glucose levels, the insulin levels remained enhanced in both groups receiving the glucose infusion until the end of the treatment, and they always remained higher in pregnant than in virgin rats (Table 1). At the 3rd day of the infusion, plasma FFA levels were higher in the pregnant rats than in the virgin rats receiving the bidistilled water infusion; however, because they had decreased more in the pregnant than in the virgin rats

Table 3. Effect of 50% glucose infusion for 72 h on insulin sensitivity indexes during hyperinsulinemic-euglycemic clamp in pregnant and virgin rats

	R, μmol/min	M, μmol·min ⁻¹ ·kg ⁻¹	M ₂ , μmol·min ⁻¹ ·kg ⁻¹	Glucose Clearance	
				ml/min	ml·min ⁻¹ ·kg ⁻¹
Virgin					
Control (n = 19)	23.3 ± 1.1	111.7 ± 6.1	111.7 ± 6.1	4.6 ± 0.2	22.8 ± 1.1
50% Glucose (n = 9)	18.3 ± 1.7	79.4 ± 4.4	79.4 ± 4.4	3.4 ± 0.3	13.4 ± 0.7
P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Pregnant					
Control (n = 10)	15.5 ± 1.1*	52.8 ± 3.9*	62.78 ± 7.8*	4.0 ± 0.2	15.8 ± 1.3*
50% Glucose (n = 8)	25.0 ± 1.7*	83.9 ± 5.0	96.7 ± 5.0	6.9 ± 0.5*	23.0 ± 1.7*
P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

All values are means ± SE. R, glucose utilization rate in the steady state; M, (R/body weight) or glucose disposal rate in the steady state, absolute and normalized by body weight; M₂, (R/conceptus-free body weight). Glucose clearance is expressed as rate in the steady state, absolute and normalized by body weight. P, significant difference between rats receiving bidistilled water (control) and 50% glucose; *P < 0.05 between pregnant and virgin rats.

ever, in pregnant rats that had received the 50% glucose infusion, all of the indexes for insulin responsiveness were higher than in their respective controls and were similar to those found in virgin controls (Table 3). When all these indexes are compiled, an insulin sensitivity index (S_{ip}) can be seen (Fig. 1) that corresponds to the increase in glucose clearance caused at steady state by the increment in plasma insulin. The S_{ip} value was lower in pregnant than in virgin control rats. Infusion with the 50% glucose solution decreased that index in virgin rats and increased it in pregnant rats to the level found in virgin controls.

GUI in various tissues. The GUI in various tissues was estimated in rats kept under the hyperinsulinemic-euglycemic clamp conditions for a total period of 120 min. As shown in Table 2, both blood glucose and plasma insulin levels remained stable in all the groups from the 60-min time to the completion of the study. As shown in Table 4, the GUI in both heart and white fiber skeletal muscle was lower in pregnant control rats than in virgin controls, but this difference disappeared in the rats that had previously received the 50% glucose infusion be-

cause of a nonsignificant reduction in the virgin rats and a slight increase in the pregnant animals. However, the GUI in red fiber skeletal muscle was lower in pregnant control rats than in virgin controls, and, although the glucose infusion significantly decreased that index in virgin rats, it increased it in pregnant rats to the level found in virgin controls (Table 4). As also shown in Table 4, the GUI in adipose tissue of the control rats appeared lower in pregnant than in virgin animals. However, although the infusion with 50% glucose decreased this parameter in virgin rats, it had no effect in pregnant animals, so the difference between the two groups disappeared.

DISCUSSION

In the present study, the glucose infusion previously used to develop insulin resistance in the nonpregnant rat (8) was investigated for its potential effect on the pattern of insulin responsiveness in the unanesthetized rat during late pregnancy. It was found here that continuous glucose infusion caused an initial rise in plasma glucose that returned to basal levels after 42 h, whereas plasma insulin remained markedly increased after 72 h, and this effect was more marked in pregnant than in nonpregnant rats. Whereas the treatment caused insulin resistance in the nonpregnant rat, it reversed the insulin resistance found in pregnant animals. A decrease in the food intake of the animals during the glucose infusion might have influenced the different response to insulin, but the reduction was similar for both groups, and their daily caloric intake remained relatively constant during the infusion period, due primarily to calories from the infused glucose. Previous reports by Hager et al. (8) also noted that a 72-h glucose infusion reduced food intake and provoked hyperinsulinemia and insulin resistance in the nonpregnant rat, although the hyperglycemic condition of their animals was maintained throughout the whole infusion time. This difference with our results may arise from the fact that the glucose infusion rate they used was slightly higher (60–66 mg·kg⁻¹·min⁻¹) than the one used here (55–60 mg·kg⁻¹·min⁻¹).

The cause of the insulin resistance in the nonpregnant animals receiving the glucose infusion is not known, although it must be secondary to the increased

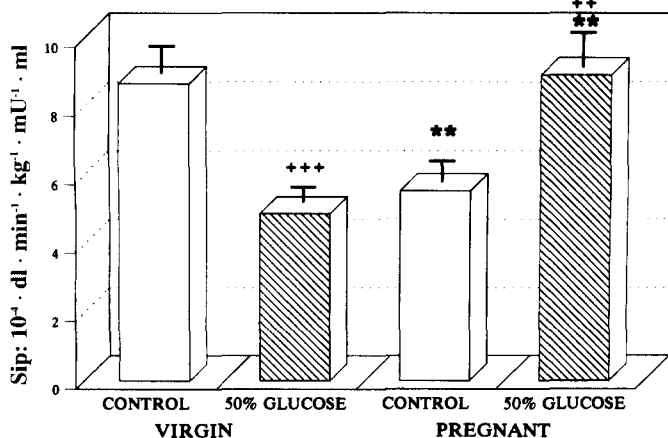


Fig. 1. Insulin sensitivity index (S_{ip}) during hyperinsulinemic-euglycemic clamp in virgin and 20-day-pregnant rats that previously received an infusion of bidistilled water (control) or 50% glucose. S_{ip} corresponds to the increase in glucose clearance caused at steady state by the increment in plasma insulin. Values are means ± SE of 8–18 animals/group. Statistical comparisons between pregnant and virgin rats: **P < 0.01; between control rats and those on 50% glucose: ++P < 0.01; +++P < 0.001.

gether with the present results, allows us to propose that maternal insulin resistance is also responsible for the decreased lipoprotein lipase activity normally seen in adipose tissue during late gestation.

In conclusion, exaggerated hyperinsulinemia produced by 72 h of continuous glucose infusion decreases insulin responsiveness in nonpregnant rats but increases it in late-pregnant animals. These differences will make it possible to determine which of the metabolic adaptations normally occurring during late gestation are caused by maternal insulin resistance. Our current data suggest that enhanced adipose tissue lipolytic activity, decreased glycogen storage capability, and decreased adipose tissue lipoprotein lipase activity should be considered among the maternal metabolic adaptations that are caused by insulin resistance.

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