

GLUCOSE TOLERANCE TESTS AND "IN VIVO" RESPONSE TO INTRAVENOUS INSULIN IN THE UNANAESTHESIZED LATE PREGNANT RAT AND THEIR CONSEQUENCES TO THE FETUS

Hyperglycémie provoquée et réponse *in vivo* à l'insuline intraveineuse
chez la rate gravide avancée non anesthésiée
et autres conséquences pour le fœtus

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RÉSUMÉ

Chez la rate gravide au stade avancé on a constaté que la glycémie était plus basse et les taux plasmatiques d'insuline RIA légèrement plus élevés que chez la rate vierge. Les épreuves d'hyperglycémie provoquée par voie orale et intraveineuse ont provoqué des variations de glycémie parallèles dans les deux groupes, alors que les taux d'insuline RIA étaient plus élevés chez les rates gravides. Les taux sanguins de glucose mesurés après des doses faibles (0,1 à 1 UI/Kg) ou fortes (10 UI/Kg) d'insuline par voie intraveineuse ont diminué plus lentement et dans une plus faible mesure chez les rates gravides que chez les rates vierges. Les glycémies fœtales n'étaient pas influencées par le traitement des mères à l'insuline. Ces résultats montrent que la sensibilité et la réponse à l'insuline sont diminuées chez les rates gravides avancées non anesthésiées. Il est suggéré que cette résistance à l'insuline traduit peut-être un mécanisme visant à retarder l'élimination par les tissus maternels des aliments ingérés, assurant ainsi leur mise à la disposition du fœtus.

Mots clés : Grossesse. Résistance à l'insuline. Rat. Glucose.

SUMMARY

In the late pregnant rat, blood glucose levels were lower and plasma RIA-insulin levels were slightly higher than in virgin animals. Oral and intravenous glucose tolerance tests produced parallel changes in blood glucose in both groups whereas plasma RIA-insulin increased more in the pregnant animals. Blood glucose levels after either low (0.1-1 IU/Kg) or high (10 IU/Kg) doses of intravenous insulin decreased more slowly and less in pregnant than in virgin rats. Fetal blood glucose levels were not affected by maternal insulin treatment. Results show that in the unanaesthetized late pregnant rat both insulin sensitivity and responsiveness decreased and it is proposed that this insulin resistance may represent a mechanism to delay disposal of ingested nutrients by maternal tissues, ensuring their availability to the fetus.

Key words : Pregnancy. Insulin resistance. Rat. Glucose.

In late pregnancy the insulin response to glucose is enhanced whereas glucose tolerance is either reduced or within the normal nonpregnant range both in humans (1-3) and rats (4-6), indicating insulin resistance, which has been directly demonstrated by the administration of exogenous insulin in the

Abbreviations used : RIA-insulin : radioimmunoassayed insulin ; Id : insulin dose.

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Received on 24-04-1985 ; revised on 09-04-1986.

pregnant rat (4, 7, 8). By using "in vivo" or "in situ" perfused organ designs, it has been shown that liver, skeletal muscle and adipose tissue from pregnant rats display insulin resistance (4, 8, 9, 10). In "in vitro" systems most authors have shown that the insulin response in tissues from late pregnant rats is unmodified (5, 6, 11, 12, 13) although there are others showing decreased insulin sensitivity (14) which contrast with reported increase in insulin receptor number in the same preparations (14, 15). Although the different response "in vivo" versus "in vitro" may result from the absence of anti-insulin factors in the "in vitro" preparations which are present when pregnant animals are studied "in vivo", the problem has not yet been clarified. Another

unresolved aspect is whether insulin resistance in late gestation is the result of decreased sensitivity, diminished responsiveness, or both. In this regard, we initially reported an impaired insulin response to supramaximal insulin doses in the pregnant rat, suggesting decreased responsiveness (7), whereas Leturque et al. (4), using pentobarbitone anaesthetized animals, showed decreased sensitivity but not responsiveness to large insulin doses. We have recently shown that pentobarbitone affects glucose metabolism differently in pregnant and virgin rats (16), indicating that anaesthesia alone could affect insulin responsiveness in the pregnant rat, as has already been demonstrated in nonpregnant animals (17, 18, 19).

In the present study we tested *in vivo* insulin sensitivity and responsiveness in unanaesthetized late pregnant and virgin rats by determining the circulating glucose and RIA-insulin changes after oral and intravenous glucose load, and also the hypoglycemic response to different intravenous insulin doses. Potential consequences in fetal glycemia produced by maternal insulin treatment were also studied.

MATERIALS AND METHODS

Animals. Wistar femal rats were mated when weighing 150-180 g, and gestation was timed by the appearance of spermatozooids in vaginal smears. Sex- and age-matched virgin rats were studied in parallel. Animals were housed in collective cages in a light-cycle and temperature controlled room (12 h on-off; 23 ± 1 °C), and fed purina chow pellets *ad libitum*.

Glucose tolerance tests. Virgin and 19 day pregnant rats received a solution of glucose in distilled water orally, through a polyethylene cannula connected to a syringe, or injected into a tail vein. The amount of administered glucose was always 2 g/Kg body weight, and other methodological details were as previously described (20). Those doses of glucose were selected under the base of previous experiences with the same (20) or ten times lower doses (21) to compare maximal responses to the insulinotropic stimulus between pregnant and virgin rats. Blood samples were collected from the tip of the tail and placed in heparinized receptacles at 7.5, 15, 22.5, 30, 60 and 120 min after the glucose load. In animals receiving glucose orally, blood was also collected 5 min prior to treatment, in order to obtain 0 time values. An aliquot of whole blood was immediately deproteinized (22) for glucose determination (23). Plasma was separated from another aliquot for the analysis of RIA-insulin (24) by using a radioimmunoassay kit specific for rat generously provided by Novo Industri A/S (Copenhagen, Denmark).

Insulin administration studies. Blood was collected from the tip of the tail in other virgin and 20 day pregnant rats to obtain samples for 0 time values. One hour later, these animals were injected through a tail vein with 1 ml solution/Kg body weight of either saline (0 insulin) or 0.1, 1 or 10 IU Actrapid Monocomponent insulin (from Novo Industri A/S, Copenhagen, Denmark) dissolved in saline. Blood samples were collected from the tip of the tail at 4, 8 and 12 min thereafter, and animals were killed by decapitation at the 16 th min for the collection of blood from the neck wound. Blood and plasma samples were processed as indicated above for the estimation of blood glucose at all time points and for that of plasma RIA-insulin at the 16 th min after the insulin treatment. Values of blood glucose at each time point after the insulin injections were corrected by values found at the same time in animals receiving saline (0 insulin dose), and insulin effect was expressed as :

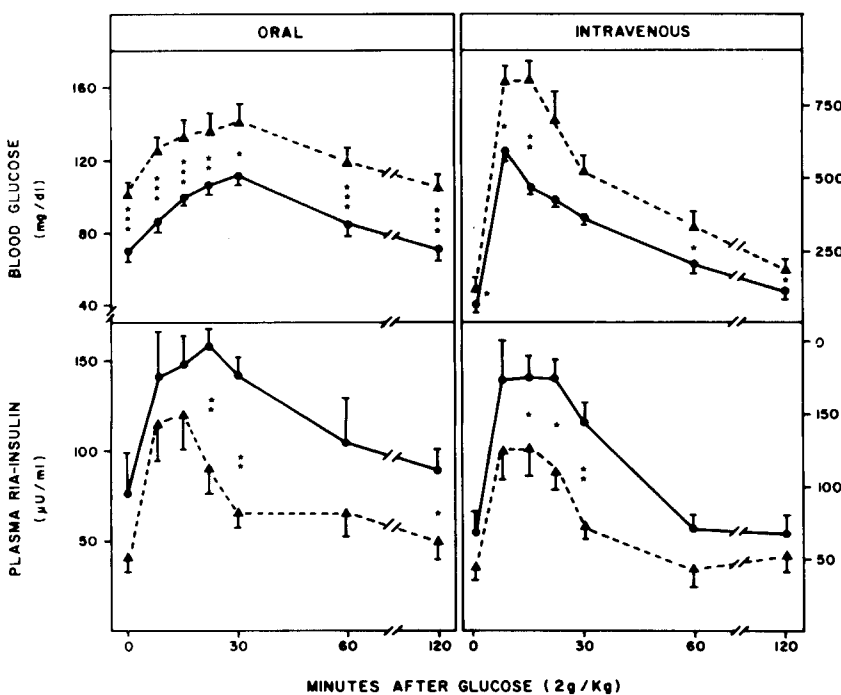


FIG. 1. — Blood glucose and plasma RIA-insulin concentrations in virgin (▲ - - -▲) and 19 day pregnant rats (● - - -●) after oral or intravenous glucose administration (2 g/Kg). Asterisks correspond to the statistical significance between both groups : * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, estimated by the Student's "t" test. Means \pm SEM of 5-8 rats/group.

Corrected insulin effect = $(GoId - GtId) - (GoIo - GtIo)$, where $GoId$ and $GtId$ correspond to glucose values at 0 and t min after a given insulin dose (Id), and $GoIo$ and $GtIo$ correspond to values at 0 and t min after saline (0 dose). **Statistics.** Comparisons between groups were made following a two-way analysis of variance with Newman-Keuls test in the maternal insulin administration experiments whereas the Student's "t" test was followed in the others.

RESULTS

As shown in *Figure 1*, blood glucose levels in the late pregnant rats were significantly lower than in virgin animals, and although in both groups oral and intravenous glucose administration produced a rise and a subsequent decrease in blood glucose values, the difference between the two groups was maintained until the 120 min time studied. Plasma RIA-insulin values before the glucose load were slightly but not significantly higher in pregnant than in virgin rats (*figure 1*). After either oral or intravenous glucose administration there was a greater rise in plasma RIA-insulin levels in pregnant than in virgin animals, this difference becoming significant at 22.5, 30, and 120 min after oral glucose, and at 15, 22.5, and 30 min after intravenous glucose (*Figure 1*).

When exogenous insulin was given intravenously to virgin rats, its corrected effect on blood glucose was significant at 4 min and increased progressively through time and with increasing doses (*Figure 2*). This was not, however, the case in pregnant rats (*Figure 2*) in which the insulin effect was delayed, and was not significant until the 8 th min. This effect

was less pronounced in pregnant than in virgin rats with all insulin doses studied, and it did not increase progressively by increasing the insulin dose from 0.1 to 10 IU/Kg body weight. To obtain a more comprehensive understanding of the hypoglycemic effect of insulin, the area under the curve of the results presented in *Figure 2* was calculated (16 min values were not taken into consideration since blood samples come from the neck instead from the tail; see Methods). As shown in *Figure 3*, the integrated hypoglycemic effect of insulin was less in pregnant than in virgin rats, the difference between the two groups being highly significant for the 1 and 10 IU/Kg body weight doses.

The smaller response to insulin in pregnant rats was not due to their reduced plasma insulin levels. On the contrary, as shown in *Table I*, plasma RIA-insulin levels 16 min after intravenous insulin injections were significantly higher in pregnant than in virgin animals when given 0, 0.1 or 1 IU/Kg body weight, whereas differences were not significant after the 10 IU dose.

To determine whether maternal insulin administration affected fetal blood glucose levels, this parameter was measured in pregnant rats and their fetuses 16 min after insulin treatment. As shown in *Table II*, reductions in blood glucose levels in maternal blood after treatment with increasing insulin doses were not paralleled by a change in fetal glycemia, which caused an increase in the fetal/maternal blood glucose ratio. Plasma RIA-insulin levels in the fetus were not modified by maternal insulin treatment (data not shown).

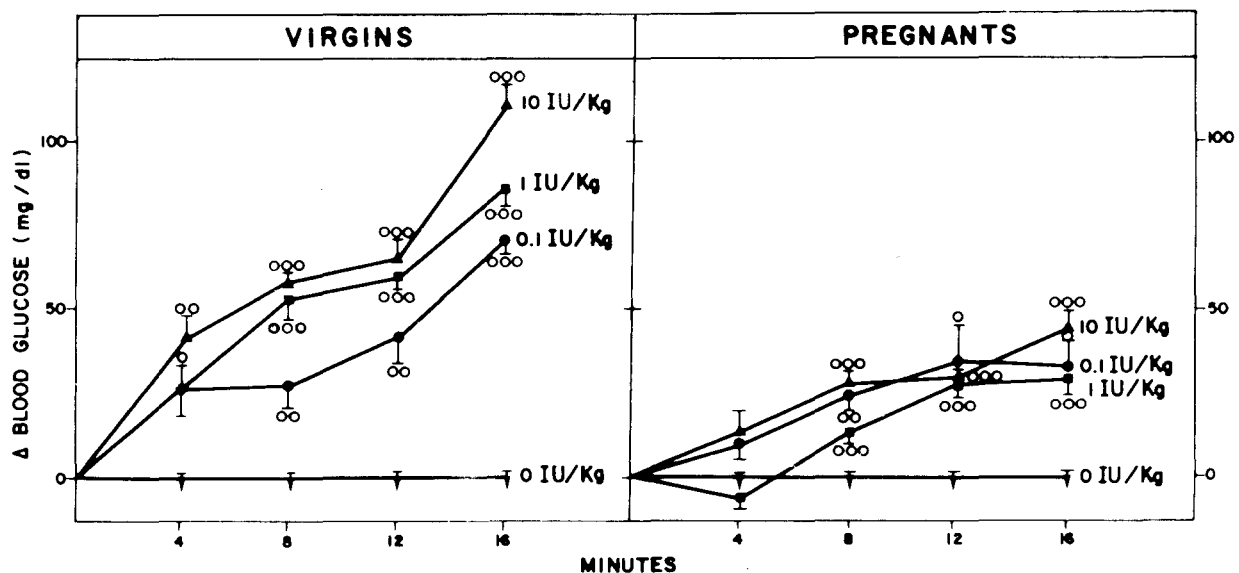


FIG. 2. — Hypoglycemic effects of intravenous insulin administration to virgin and 20 day pregnant rats. Values are expressed as the blood glucose change after the different insulin doses. Statistical significance versus values at 0 minutes is shown as : o = $P < 0.05$, oo = $P < 0.01$, ooo = $P < 0.001$. Means \pm SEM of 7-9 rats/group. Comparison between groups was done by the Newman-Keuls test.

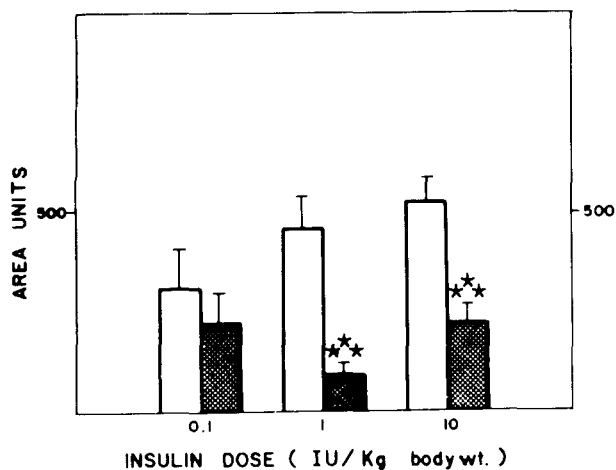


FIG. 3. — Integrated hypoglycemic effects of intravenous insulin administration to virgin (open bars) and 20 day pregnant rats (shaded bars). Values correspond to the area under the curve of the results in Figure 2 for the first 12 min after treatments. Means \pm SEM of 7-9 rats/group. Comparison between groups was done by the Newman-Keuls test.

DISCUSSION

These results show that at late gestation in the unanaesthetized rat there is reduced glycemia and enhanced insulinemia, in agreement with previous findings (25, 26). They also show that after both oral and intravenous glucose load there is a parallel increase in blood glucose levels in pregnant and in virgin animals whereas in the pregnant group there was a greater increase in plasma RIA-insulin levels as well as decreased sensitivity (shift to the right in the dose-response curve) and responsiveness (reduced response to supra-maximal doses) to exogenous insulin. Both glucose and insulin were administered to all the animals at the same concentration per unit of body weight. In this way the greater dilution of absorbed glucose in the pregnant rat due to its transfer to the conceptus structures and the greater degradation of insulin produced by the insulinase activity of the placenta (27) were properly compensated by the higher body weight of the pregnant rats. This conclusion is supported by the parallel change in circulating glucose (Figure 1) and RIA-insulin levels (Table I) found in pregnant and virgin rats after their respective glucose or insulin treatments. These findings indicate that the late pregnant rat is hypersensitive to the insulinotropic stimulus of glucose and insulin resistant. These conclusions coincide with most previous reports in both the rat (4-7) and in man (1-3, 28) although discrepancies exist between some investigators. For example, Leturque et al. (4, 8) found insulin resistance in the pregnant rat given small insulin doses but not with maximal doses. This result may differ from ours with large doses of insulin because in the other study pentobarbitone anaesthesia was used, which is

TABLE I. — Plasma RIA-insulin levels 16 min after intravenous insulin injections in virgin and 20 day pregnant rats

Insulin dose	RIA-insulin (μ U/ml)		P
	Virgin rats	Pregnant rats	
0	36 \pm 5	80 \pm 17	< 0.05
0.1	29 \pm 5	81 \pm 13	< 0.01
1	640 \pm 141	1092 \pm 22	< 0.05
10	7177 \pm 126	6889 \pm 286	N.S.

Results are expressed as means \pm S.E.M. of 5 - 9 rats per group. P values correspond to the statistical difference between pregnant and virgin animals (N.S. = not significant, $p < 0.05$), estimated by the Student's «t» test.

TABLE II. — Plasma glucose levels 16 min after intravenous insulin injections in 20 day pregnant rats and their respective fetuses

Insulin dose (IU/Kg body weight)	Plasma glucose (mg/dl)		
	Mothers	Fetuses	Fetal/maternal ratio
0	98 \pm 5	29 \pm 3***	0.29 \pm 0.03
0.1	74 \pm 8	27 \pm 5***	0.33 \pm 0.08
P	N.S.	N.S.	N.S.
1	52 \pm 5	25 \pm 7**	0.53 \pm 0.10
P	<0.001	N.S.	N.S.
10	54 \pm 5	24 \pm 3***	0.46 \pm 0.06
P	<0.001	N.S.	< 0.01

Results are expressed as means \pm S.E.M. of 5 - 9 rats per group. P values correspond to the statistical difference between insulin treated and untreated (0, insulin dose) group whereas asterisks correspond to the significance between fetuses and their respective mothers (* = $P < 0.01$; *** = $P < 0.001$), estimated by the Student's «t» test.

known to affect insulin tolerance and insulin responsiveness in nonpregnant rodents (17-19), and we have recently shown that anaesthesia affects carbohydrate metabolism differently in virgin and pregnant rats (16). The response to supra-maximal doses of insulin in the pregnant rat may be augmented to the level of nonpregnant animals when studied under pentobarbitone anaesthesia. The present results do not indicate the tissues involved in the insulin resistance of the pregnant animals, but some conclusions may be drawn from previous findings. In adipose tissue from pregnant rats insulin response has been found decreased (14), normal (11) or even augmented (12, 29, 30) but the number of insulin receptors was consistently found increased (14, 15, 30). In the liver of pregnant rats the insulin response was normal (5, 6) and insulin binding to liver receptors was unmodified (31, 32). Skeletal muscle could, however, be the main site involved in insulin resistance in the

pregnant rat. This tissue is responsible for 35 % of glucose uptake in response to an intravenous glucose injection (33), and Rushakoff and Kalkhoff (10, 34) have reported that the insulin effect on glucose metabolism is reduced in skeletal muscle preparations from the late pregnant rat. Maternal endocrine changes (35) and the neuroendocrine counterregulatory response to hypoglycemia (36) interact with skeletal muscle insensitivity to produce the insulin-resistance at late gestation. Urinary excretion of catecholamines and adrenal medulla activity are greater in response to fasting hypoglycemia in pregnant than in virgin rats (37, 38). Thus the possibility that insulin resistance in pregnant rats may be influenced by hypoglycemic compensatory hypersecretion of catecholamines should not be dismissed.

The observed stability of fetal glycemia is remarkable particularly under maternal treatment with insulin doses causing intense hypoglycemia in the mother. This finding contrasts with the correlation which exists between plasma maternal glucose and placental glucose transfer when the rat mother is hyperglycemic (39). It is however consistent with findings in the pregnant rat when the mother is hypoglycemic, such as is the fasted condition (40) or after an intravenous infusion with insulin (41), where fetal glycemia was found unmodified. As maternal hypoglycemia must necessarily produce reduced placental glucose transfer, stable fetal glycemia in this condition indicate that the fetus is protected against maternal hypoglycemia by an unknown mechanism which may imply a reduction in the use of transferred glucose at the expense of enhanced utilization of alternative fuels. Insulin resistance in the late pregnant rat may also represent a fuel preserving mechanism for the fetus. Since maternal insulin does not cross the placenta (27) or affect placental metabolite transfer (41), the amount of nutrients crossing the placenta is directly dependent on their concentration in the maternal circulation. Consequently, maternal beta-cell hypersensitivity to insulinotropic agents, which produces amplified oscillations in plasma insulin levels during daily starved-fed transitions (28), is partially compensated for by reduced insulin responsiveness causing delayed disposition of the ingested nutrients by maternal tissues and thus ensuring their availability to the fetus.

Acknowledgments — The present study was performed with grants from the Fondo de Investigaciones Sanitarias de la Seguridad Social and the Comisión Asesora de Investigación Científica y Técnica, of Spain. The authors thank Caroline S. Delgado for her editorial help.

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