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Glucose and Insulin Tolerance Tests in the Rat on Different Days of Gestation

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Abstract
To study insulin/glucose relationship during gestation, rats were studied on days 6, 12, 15, 18, 20 or 21 of pregnancy and the results were compared to values in sex-matched virgin control rats. Blood glucose levels were decreased on days 20 and 21 of gestation whereas plasma insulin levels appeared decreased on days 6 and 12, unchanged on day 15 and enhanced on days 18, 20 and 21 of gestation. Total pancreas insulin content was already augmented on day 6 of gestation and continued to increase with gestational time. With the exception of an increase in the 6-day-pregnant rats 22.5 min after an oral glucose load, blood glucose levels did not differ between 6- or 12-day-pregnant rats and virgin controls although plasma insulin levels reached higher values on these days. However, in the 15-day-pregnant rats, glucose tolerance after the glucose load was enhanced while plasma insulin levels did not differ from those in virgin rats during the first 30 min. In the 18-day-pregnant rat blood glucose was more increased but plasma insulin did not differ after the glucose load when compared to virgin rats, whereas 20- or 21-day-pregnant rats showed a glucose tolerance similar to that of virgin rats but their insulin levels shortly after the glucose load were higher. The hypoglycemic response to a high intravenous dose of insulin was decreased in 12-, 18-, 20- and 21-day-pregnant rats. Therefore, whereas in both the 6- and 12-day-pregnant rats there is an enhanced β-cell response to the glucose insulinotropic effect and insulin responsiveness is reduced in 12-day-pregnant rats, the 15-day pregnant rat is in a transitory stage where both insulin sensitivity and the β-cell response return to nonpregnant values. However, from 18 days of gestation on, there is an intense insulin-resistant condition which is only partially compensated by an enormous accumulation of insulin in the pancreas followed by a faster and larger insulin release after a glucose load.

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Introduction

During late pregnancy, the pancreatic β-cell response to different insulinotropic stimuli, especially glucose [1–4], is enhanced resulting in a sustained hyperinsulinemia in spite of a tendency toward hypoglycemia [5–7]. Glucose tolerance at late pregnancy remains within normal nonpregnant limits [7–9] despite the increased insulin secretion rate in response to glucose [1–3]; this indicates a state of insulin resistance. Decreased insulin sensitivity has been well documented in late pregnancy in both women [10–12] and rats [1, 5, 13]. The stage of pregnancy at which these changes appear has only been investigated partially. Although some authors have shown that insulin resistance develops after 16 days of pregnancy in the rat [14] others have not found an alteration in the insulin-stimulated glucose oxidation in adipocytes from 16-day-pregnant rats [15]. We have recently seen that on day 15 of gestation in the conscious rat glucose tolerance is enhanced whereas plasma insulin levels are similar to those in nonpregnant animals [4] suggesting a phase of enhanced insulin sensitivity. Before insulin resistance develops during late pregnancy in humans, there also seems to be a phase of increased insulin sensitivity as shown by the enhanced glucose tolerance found during weeks 11–15 in healthy pregnant women [16, 17] and by the decreased insulin dosage required by insulin-dependent diabetes mellitus patients during early pregnancy [for a review, see ref. 18]. Since insulin/glucose interactions seem to play an important role in the overall metabolic adaptations taking place during pregnancy, the present experimental study in rats was addressed to determining the circulating glucose and insulin levels, the pancreatic insulin content and the short-time hypoglycemic response to a high dose of insulin as index of insulin responsiveness at different days of gestation. Since anesthesia is known to exaggerate the insulin response to glucose in nonpregnant rats [19] and even to affect the glucose metabolism in a different manner in pregnant and nonpregnant animals [20, 21], the animals used for this study were unanesthetized.

Material and Methods

Animals

Female Wistar rats were mated when weighing 160–180 g, and gestation was timed by the appearance of spermatozoa in vaginal smears. Animals were housed in collective cages in a light- and temperature-controlled room (12 h on-off cycle, 22 ± 2 °C) and fed Purina chow pellets (Panlab, Barcelona, Spain) ad libitum. They were always studied under fed conditions on days 6, 12, 15, 18, 20 or 21 of pregnancy, and age- and sex-matched virgin rats were always studied in parallel.

Content of Insulin in the Pancreas

One set of animals was decapitated for dissection of the pancreas, which was rapidly placed in ice-cold Hanks' solution; any fat tissue was eliminated under a magnifier. After weighing, the insulin was extracted in acid ethanol [22]. The extract was neutralized with saturated sodium bicarbonate for insulin assay [23].

Glucose Tolerance Tests

Another set of rats was subjected to an oral glucose tolerance test as previously described [1]. Rats were given 2 g glucose/kg dissolved in distilled water by stomach tube. Blood samples were collected from the tip of the tail into heparinized tubes 5 min before treatment (time 0) or 7.5, 15, 22.5, 30, 60 or 120 min after the glucose load. Plasma aliquots were kept at −30 °C until analyzed for glucose [24] after deproteinization [25], and for RIA insulin [23] with a rat-specific radioimmunoassay kit from Novo Industri A/S (Copenhagen, Denmark).

Intravenous Insulin Tolerance Test

In another set of rats blood was collected from the tip of the tail to obtain the 0 time values. One hour later, the rats were injected through a tail vein with either 1 ml of saline/kg or 10 IU of Actrapid monocomponent porcine insulin (from Novo Industri A/S) dissolved in 1 ml saline/kg. Blood samples were col-

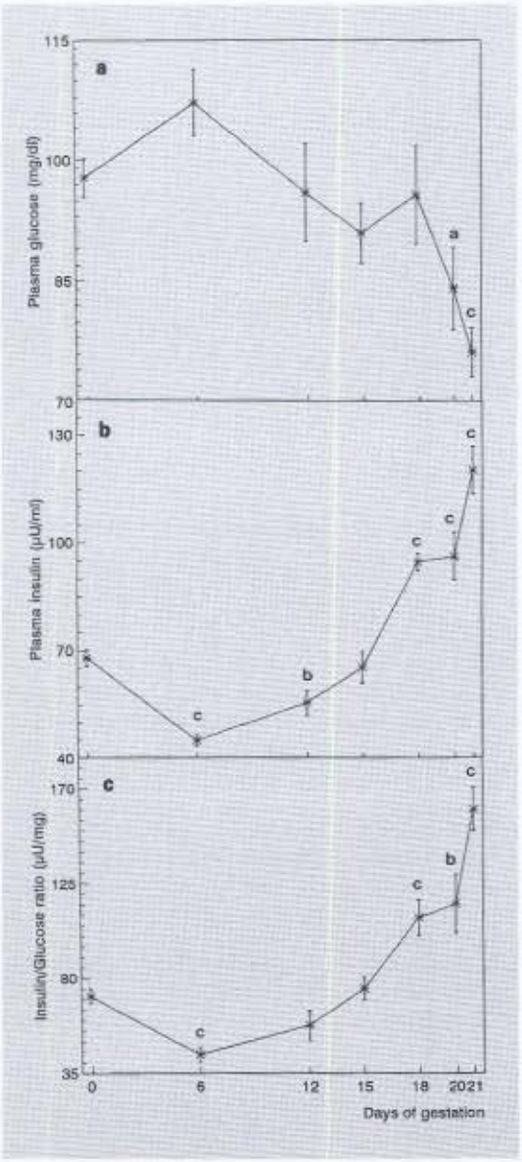


Fig. 1. Plasma levels of glucose (**a**) and insulin (**b**) and insulin/glucose ratio (**c**) at different times of gestation in the rat. Data are means ± SEM of 10–29 rats/group. [a] $p < 0.05$, [b] $p < 0.01$ and [c] $p < 0.001$, vs. values in nonpregnant rats (day 0).

lected from the tip of the tail and placed in heparinized receptacles 4, 8 and 12 min after the injection. Plasma aliquots were used for glucose analysis as above and, at each time point after the insulin injections, were corrected by values found at the same time in animals receiving saline. The insulin effect was expressed as the area under the corrected insulin effect curve, which was calculated as: corrected insulin effect = $(G_0–GI_t) – (G_0–GS_t)$, where G_0 corresponds to glucose values at time 0, and GI_t and GS_t correspond to values t min after insulin or saline, respectively.

Expression of the Results

Results are expressed as means ± SEm. Statistical comparisons were made by a multiple linear regression analysis with a 95% confidence interval, by using the PRESTA statistical program [26]. Comparison between two groups was done by the Student's t test.

Results

As shown in figure 1a, on days 6, 12, 15 and 18 of gestation blood glucose levels did not differ from those in nonpregnant control rats (0 days) whereas in both 20- and 21-day-pregnant rats values were significantly lower. When compared to values in virgin rats (0 days), plasma insulin levels appeared decreased in pregnant rats on days 6 and 12 of gestation, and were unchanged on day 15 whereas they were significantly higher on days 18, 20 and 21 of gestation (fig. 1b). The insulin/glucose ratio was significantly lower in 6-day-pregnant rats than in virgins, lower still – although not significantly so – in 12-day-pregnant rats, similar in 15-day-pregnant rats and significantly higher in pregnant rats on days 18, 20 and 21 of gestation as compared to virgin controls (fig. 1c).

As shown in figure 2a, the weight of the pancreas did not differ between 6-day-pregnant rats and virgin rats but it was significantly heavier in 12-day-pregnant rats and continued to increase progressively as gestational time advanced. Both the pancreatic insulin concentration (fig. 2b) and the total pancreas

insulin content (fig. 2c) already appeared augmented on day 6 of gestation and then increased progressively with gestational time; total pancreatic insulin on day 21 of gestation was ten times greater than in virgin animals (fig. 2c).

An oral glucose tolerance test was carried out in another set of rats to obtain an index of the sensitivity of insulin release as a result of the glucose stimulus and an indirect index of insulin responsiveness. Due to the differences in basal glucose and insulin levels among the groups (fig. 1), values were expressed as a percentage of those at time 0. As shown in figure 3, in 6-day-pregnant rats plasma glucose reached a significantly higher level 22.5 min after glucose load than virgin controls although values did not differ between the two groups at any of the other time points. In these 6-day-pregnant rats, plasma insulin levels reached higher values than in virgin rats at 15, 22.5, 60 and 120 min after glucose. In 12-day-pregnant rats, the changes in plasma glucose values were very similar to those in virgin controls, whereas plasma insulin levels appeared significantly higher in the former at 15 and 22.5 min and lower at 120 min. In the 15-day-pregnant rats, blood glucose levels reached the same value as in virgin controls up to 15 min after the glucose load and, after this time point, the decline in plasma glucose levels was faster in the pregnant rats. However, whereas plasma insulin levels did not differ between 15-day-pregnant rats and virgin controls during the first 30 min after the glucose load, they were higher in the former group at 60 and 120 min (fig. 3). In the 18-day-pregnant rat plasma glucose values 15 and 22.5 min after the glucose load appeared higher than in virgin rats, whereas the increase in plasma insulin did not differ between the two groups until 30 and 120 min when the values were lower in the 18-day pregnant rats than in virgin animals (fig. 3). A

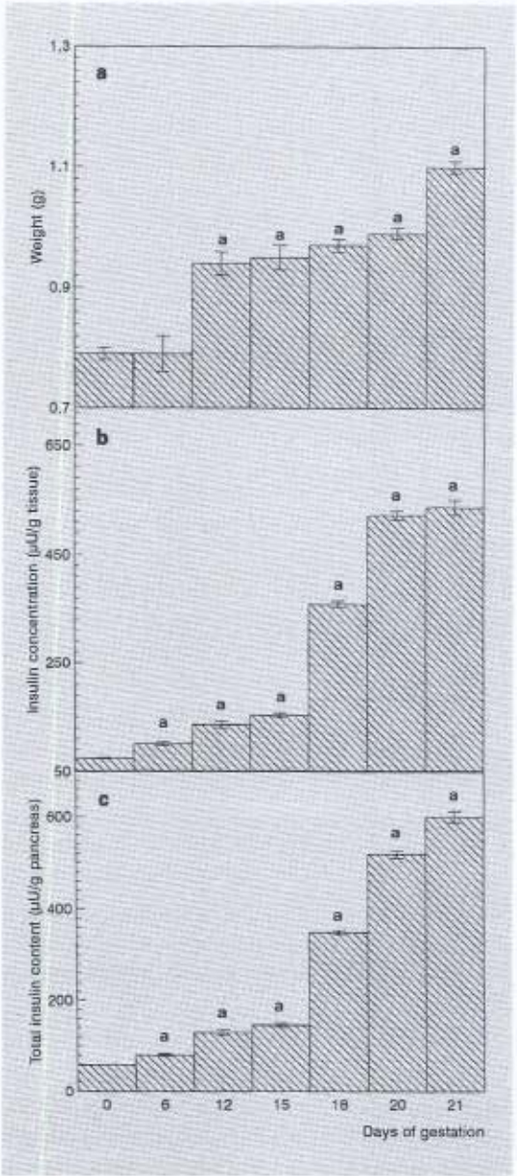
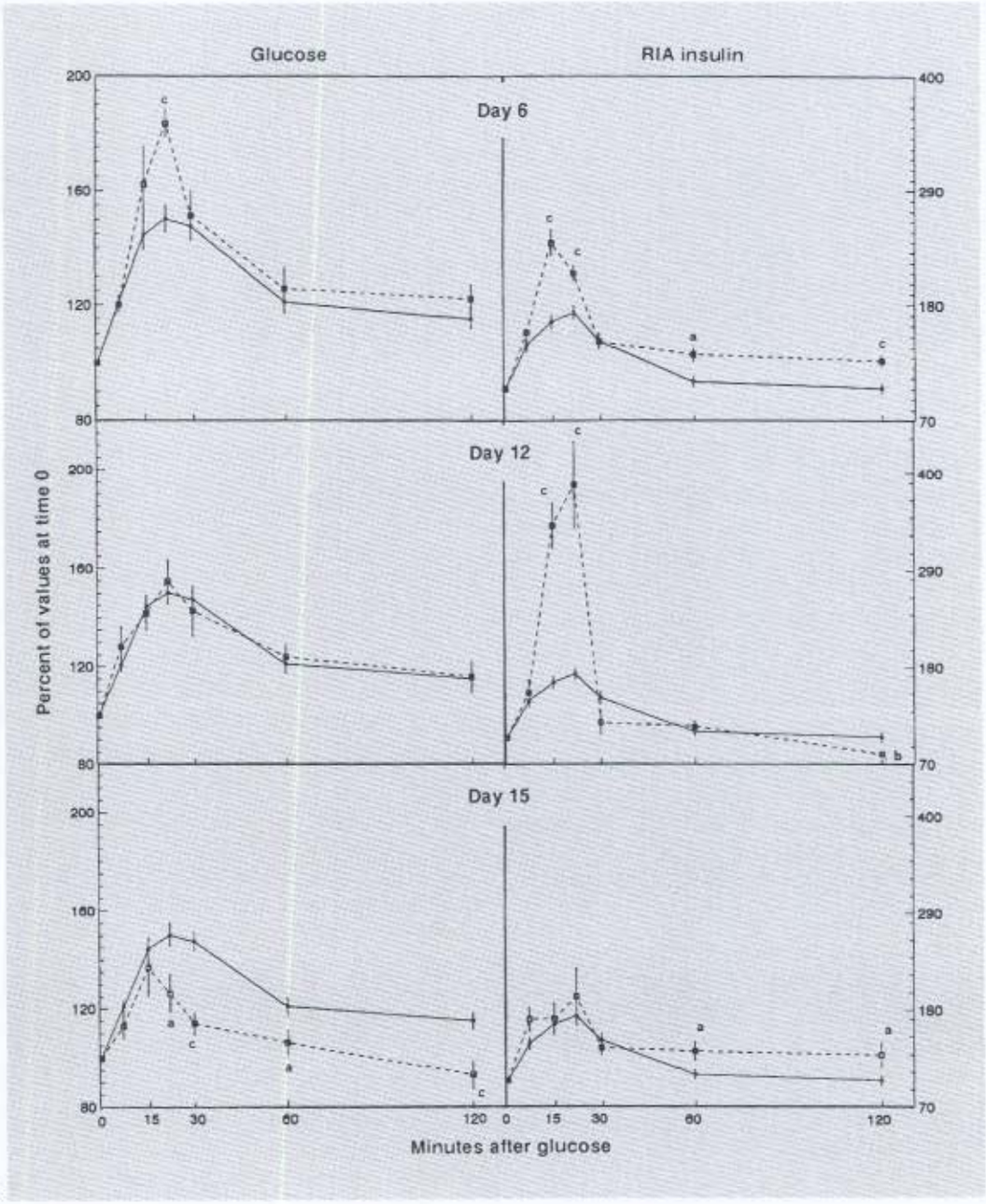


Fig. 2. Weight (**a**), insulin concentration (**b**) and total insulin content of the pancreas (**c**) at different times of gestation in the rat. Data are means ± SEM of 5–33 rats/group. [a] $p < 0.001$, vs. values in nonpregnant rats (day 0).


Glucose
RIA insulin
Day 6
Day 12
Day 15
Percent of values at time 0
Minutes after glucose
200
160
120
80
400
290
180
70
0
15
30
60
120

3

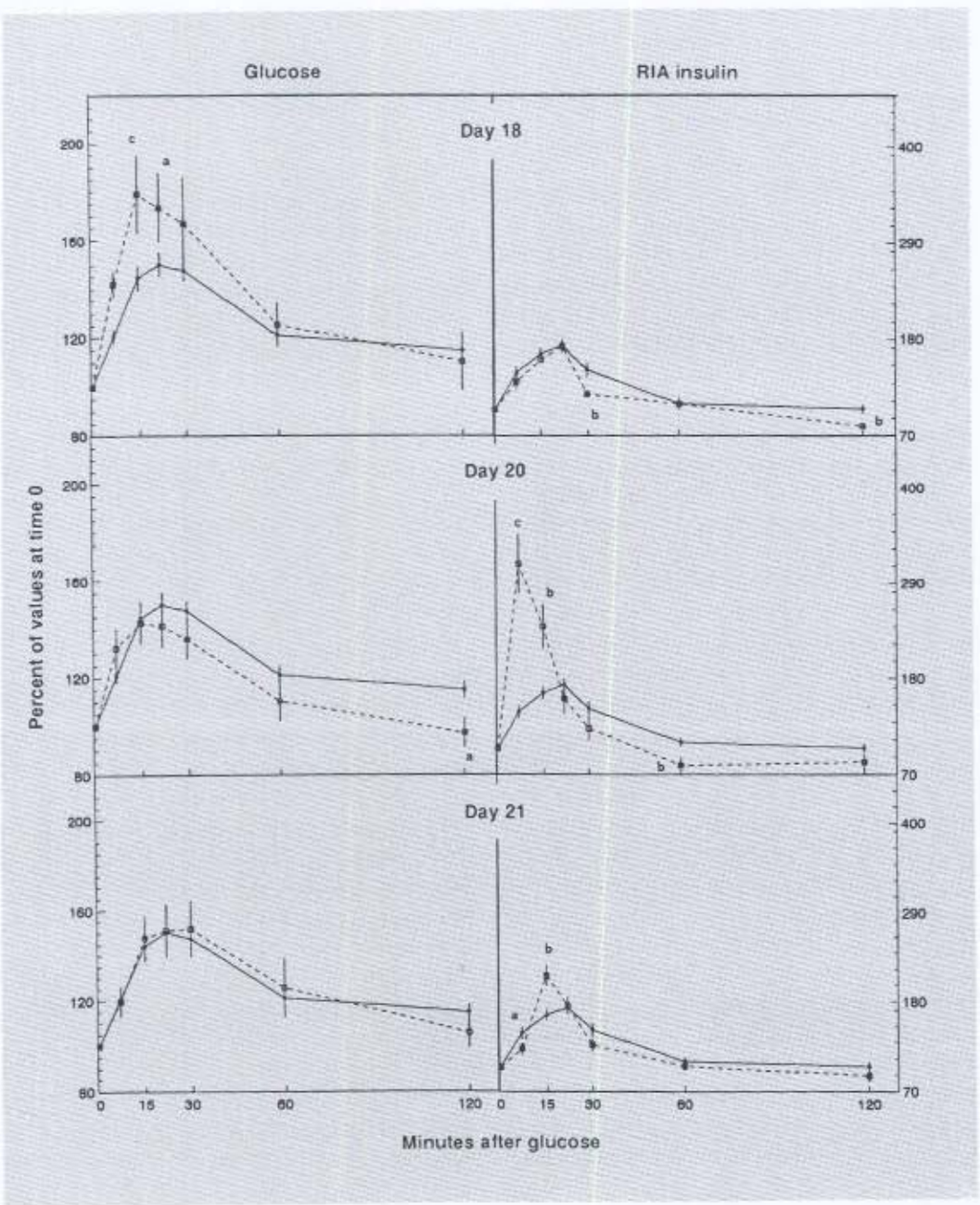


Fig. 3. Percent changes in circulating glucose and insulin levels after 2 g of oral glucose/kg body weight at different times of gestation in the rat. Values of non-pregnant rats are shown by continuous lines and those of pregnant rats by dotted lines. Data are means ± SEM of 8–33 rats/group. [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$, vs. values in nonpregnant rats.

Table 1. Corrected hypoglycemic effect of intravenous insulin (10 IU/kg body weight) at different times of gestation in the rat

Days of gestation	Area units
0	32.6 ± 1.5
6	26.6 ± 3.4
12	23.4 ± 1.2[a]
15	31.8 ± 2.0
18	17.4 ± 2.1[b]
20	17.0 ± 2.2[b]
21	14.0 ± 1.0[b]

Values are expressed as area units of the values obtained 4, 8 and 12 min after the insulin injection. Values are means ± SEM of 8–12 rats/group.

[a] $p < 0.05$, [b] $p < 0.001$, vs. day 0 (virgin animals).

similar response to glucose load was found in rats at 20 or 21 days of pregnancy, when the change in plasma glucose was similar to that in virgin controls. In spite of this similarity in plasma glucose values, the insulin levels reached in the 20-day-pregnant rats were significantly higher than in virgin controls shortly after the glucose load (7.5 and 15 min) whereas at later test times they declined more rapidly (fig. 3). In the 21-day-pregnant rats the insulin levels appeared lower than in virgin controls at 7.5 min whereas at 15 min they reached significantly higher values, with no difference between the two groups at later time points.

Since the above data indicate not only a different sensitivity by the pancreatic β-cell to the insulinotropic stimulus of glucose in the different groups but also a difference in insulin responsiveness, this point warranted further study. To obtain an index of insulin responsiveness at different stages of pregnancy, another set of rats received a high intravenous dose of insulin (10 IU/kg) and its corrected effect on blood glucose was measured during the following 12 min. As shown in table 1, when compared to the respective virgin control rats, the corrected insulin effect in 6-day-pregnant rats was slightly, but not significantly, decreased whereas in the 12-day-pregnant rats the effect was significantly lower than in virgin animals. However, in the rats at 15 days of gestation the corrected insulin effect increased to the level of the virgin rats and from day 18 of gestation on, the corrected insulin effect was intensely and significantly decreased in the pregnant rats as compared to virgin rats, the largest difference appearing in the 21-day-pregnant rats (table 1).

Discussion

Present findings show that as early as day 6 of gestation there is an accumulation of insulin in the pancreas and an enhanced β-cell response to the glucose insulinotropic effect in the rat. Whereas this situation remains on day 12, in the 15-day-pregnant rat there is a transitory stage where both insulin sensitivity and the β-cell response to the glucose load seem to return to the normal value as compared to nonpregnant control rats although the insulin content in the pancreas remains elevated. From 18 days of gestation on, the glucose/insulin relationship becomes highly imbalanced as shown by the intense insulin resistance and the enormous accumulation of insulin in the pancreas which is followed by a faster and greater insulin release into the circulation after a glucose load.

Although the insulin release from isolated in situ pancreas has already been reported to be augmented in the rat on day 10 of gestation [27], to our knowledge this is the first study showing an enhancement in both pancreatic

insulin content and the plasma insulin levels after an oral glucose load as early as the 6th day of pregnancy in the rat. Under basal conditions at this stage of pregnancy, maternal glycemia remains unchanged but plasma insulin levels are reduced, indicating that the enhanced release of insulin is only triggered under an intense insulinotropic stimulus like that caused by a glucose load, and presumably, whenever the rat eats, which would cause exaggerated insulin variations. The state of mild but significant insulin resistance already seen here in conscious rats at 12 days of gestation contrasts with the normal unchanged insulin sensitivity found by Leturque et al. [28] in anesthetized rats at the same gestational time, but anesthesia itself is known to depress glucose utilization [29], and this decrease could cover small changes in insulin sensitivity. Our findings of depressed insulin responsiveness in the 12-day-pregnant rat, however, coincide with the decreased glucose metabolism previously found in hemidiaphragms and adipocytes from 10-day-pregnant rats in the presence of insulin [30]. A role of counterregulatory hormones may be suggested as responsible for such an effect. The plasma levels of estrogens, progesterone, glucocorticoids and placental lactogen gradually increase as pregnancy progresses, and all have been proposed as antagonizing insulin action during pregnancy [for a review, see ref. 31].

The altered insulin/glucose relationship present at early gestation completely switches to a normal condition by the 15th day of gestation. The situation may be comparable to what occurs in humans during the 11th–15th week of gestation where an enhanced insulin sensitivity phase has been found [16, 17]. The present study does not establish the hormonal or environmental factors that may be responsible for such a change, and more experiments are required to investigate them. A normal sensitivity to insulin in the presence of hyperphagia sets the appropriate scene for the active anabolic condition present in the pregnant rat at this stage of gestation [32].

Between the 15th and the 18th day of gestation, the condition appears to be similar in some way to that found in the Zucker fatty rat [33] or in the long-term experimental hyperinsulinemic rat [34, 35] where, under hyperinsulinemic conditions, a phase of normal or even augmented insulin sensitivity occurs before insulin resistance develops. We have seen here that in the 18-day-pregnant rat plasma insulin is already augmented and the accumulation of insulin by the pancreas becomes maximally accelerated. The hyperinsulinemia itself together with the other hormonal factors antagonizing insulin action, which also reach their maximum level during this gestational phase [31, 36], may be responsible for the development of maternal insulin resistance.

The situation of insulin resistance becomes maximally developed at late gestation, as seen here in 20- and 21-day-pregnant rats and concurs with previous findings [1, 5, 13, 14]. At this late phase of gestation we have seen here that the pancreas of the mother rat accumulates an enormous amount of insulin and, besides maintaining elevated basal levels of insulin, whenever she eats she releases a greater amount of insulin into her circulation, as indicated by the exaggerated increase in plasma insulin after oral glucose. Although the contribution to the insulin resistance by other hormonal changes occurring during this late gestational phase cannot be discarded, taken together all of the insulin changes constitute the adequate conditions for the development of a fully insulin-resistant state.

Although most of the observed changes in the insulin/glucose interactions are coincident with the known anabolic condition of the

mother during the first two thirds of gestation and her shift into a catabolic condition during late gestation [37], more specific experiments are required to establish the direct relationships between these parameters.

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