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Glucose Tolerance Tests During Gestation in the Unanesthetized Rat

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To establish the temporal stages at which changes in insulin/glucose interactions may appear during gestation in the rat, unanesthetized animals were subjected to oral glucose tolerance tests (2 g glucose/kg) at days 15 and 21 of gestation and were compared to virgin female controls. On day 15 glucose tolerance is enhanced in the pregnant rat whereas plasma insulin levels are like those in control animals. On day 21 glucose tolerance does not differ between the two groups although insulin is higher in the pregnant animals. Results show 2 differenciated stages of insulin/glucose relationships throughout gestation in the rat with enhanced insulin sensitivity on day 15 and enhanced insulin resistance during late gestation. It is suggested that these changes contribute to the anabolic tendencies of the mother during mid gestation and her catabolic condition during late gestation.

Key words: Glucose tolerance, Pregnancy, Insulin, Endocrine pancreas, Rat, Insulin resistance.

It is well known that during late gestation in both human and experimental animals there is a decline in the blood glucose concentration in the post-absorptive period or after short periods of starvation (4, 10, 20, 31) although blood glucose concentrations rapidly return to normal values after parturition. In spite of this tendency toward hypoglycemia, plasma insulin levels are elevated in late pregnancy (5, 10, 27). This is the result of increased insulin release (4, 5, 10, 20, 25, 27, 28, 31, 33) which seems to be caused by the dietary (1, 8) and hormonal factors (24) which modify the adenylate cyclase-cAMP system in the pancreatic islets (23). In spite of a rise in insulin secretion in response to glucose during pregnancy, glucose tolerance remains within normal non-pregnant limits for both humans (4, 31) and rats (12, 25). This indicates that a state of insulin resistance develops in late pregnancy, as has also been well documented in women (3, 30) and rats (18, 21, 25). However, the stage of pregnancy at

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which these changes appear and whether they occur simultaneously still need to be established. Although attempts to study this point in the rat have been carried out in anesthetized animals (21), our experience indicates that anesthesia strongly affects the glucose metabolism in the pregnant rat (35). The situation in humans is not clear, since some authors have found an increase in glucose tolerance in weeks 11 to 15 (6, 14) whereas others were unable to demonstrate altered glucose tolerance in early pregnancy (19, 22). Moreover, in patients with insulin-dependent diabetes mellitus, the insulin dosage generally has to be reduced during early pregnancy, indicating an increase in insulin sensitivity (26). To determine whether in the pregnant rat a phase also exists in which insulin sensitivity is enhanced in contrast to the well known decreased insulin sensitivity during late gestation, we studied the response to an oral glucose load in 15- and 21-day unanesthetized pregnant rats and their virgin controls.

Materials and Methods

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

Animals were given 2 g of glucose/kg dissolved in 10 ml of distilled water by stomach tube. Blood samples were collected from the tip of the tail into heparinized tubes 5 min prior to treatment (time 0) or 7.5, 15, 22.5, 30, 60 and 120 min after the glucose load. Plasma aliquots were kept at -30 °C until analyzed for either glucose (15), after deproteinization (32), and RIA-insulin (9) with a rat-specific radioimmunoassay kit generously provided by Novo Industri A/S (Copenhagen, Denmark).

Statistical comparison between groups was made following the student's «t» test.

Results

Oral glucose tolerance tests (OGTT) were carried out in all pregnant and control groups. As shown in figure 1A, in 15 day pregnant rats, plasma glucose was lower and remained significantly below the level in virgin animals at 15, 22.5, 30, 60 and 120 min after the glucose load. When these values are expressed as a percentage of those before the glucose load (time 0), plasma glucose levels in 15 day pregnant rats remained below the level in virgin animals at 30, 60 and 120 min after the glucose load (fig 1B). However with the only exception of a slight but significant increase at 15 min, plasma insulin levels did not differ between the two groups throughout the whole OGTT (fig. 2), indicating an augmented insulin sensitivity in the 15 day pregnant animals. In the 21 day pregnant rats absolute plasma glucose levels were below the level in virgin animals throughout the whole OGTT (fig. 3A), the difference being statistically significant at 0, 15, 22.5 and 120 min. Differently to the 15 day pregnant rats, values of plasma glucose expressed as a percentage of time 0 in 21 day pregnant rats did not differ from those of virgin animals (fig. 3B). However, plasma insulin levels in 21 day pregnant rats expressed as absolute values were significantly higher than in virgins at all the time points studied (fig. 4A) and when expressed as percentage values of those at time 0, they remained significantly higher in pregnant than in virgin rats at 7.5, 15 and 60 min after the glucose load (fig. 4B), indicating a condition of insulin resistance.

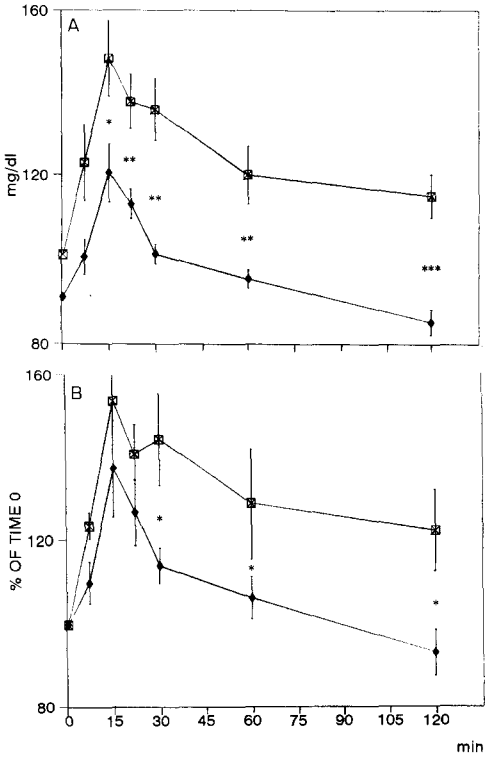

Fig. 1. *Plasma glucose concentration expressed as either absolute values (A) or as percentage of time 0 (B) at different times after oral glucose administration (2 g/kg b. w.) to virgin (□) and 15 day pregnant rats (◆).*
Asterisks correspond to the statistical comparisons between the two groups at each time point (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$; no asterisk = not significant, $p > 0.05$). n = 6-8 rats/group.

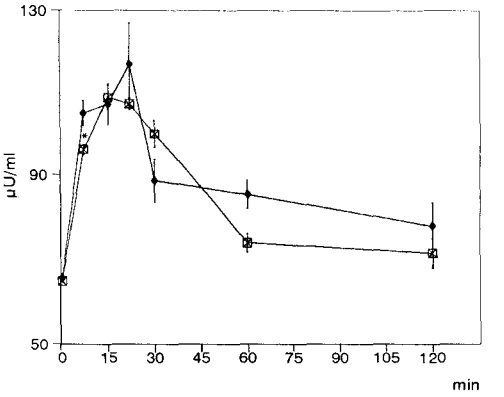

Fig. 2. *Plasma RIA-insulin concentration at different times after oral glucose administration (2 g/kg b. w.) to virgin (□) and 15 day pregnant rats (◆).*
Statistical comparisons between the groups as in figure 1.

Discussion

Using OGTT in unanesthetized animals, the present results indicate that during gestation in the rat there are at least 2 clearly differentiated stages of glucose/insulin relationship. In mid gestation (day 15) an increased glucose tolerance in the presence of unchanged insulin levels reflects enhanced insulin responsiveness. During late gestation, at day 21, glucose tolerance normalizes as a result of the cost of enhanced insulinotropic response to glucose stimulus, indicating a condition of reduced insulin sensitivity. These changes are in some way similar to what has been described in man. Studies in normal pregnancy have shown unaltered glucose tolerance in early gestation (week 10) (2) followed at 11-15 weeks by increased insulin response to glucose ingestion when glucose tolerance is improved (6, 14) whereas during late gestation glucose tolerance decreases gradually and insulin resistance is clearly established (6, 7, 14, 19, 22).

The cause by which at day 15 of gestation there is an increase in the glucose tolerance in spite of unchanged plasma insulin levels after the same glucose load is not known, but as a recent review has stated (13), increased insulin responsiveness seems to be multifactorial and the different pregnancy-related changes in the secretion of progesterone, estradiol, placental lactogen and prolactin may act eith-

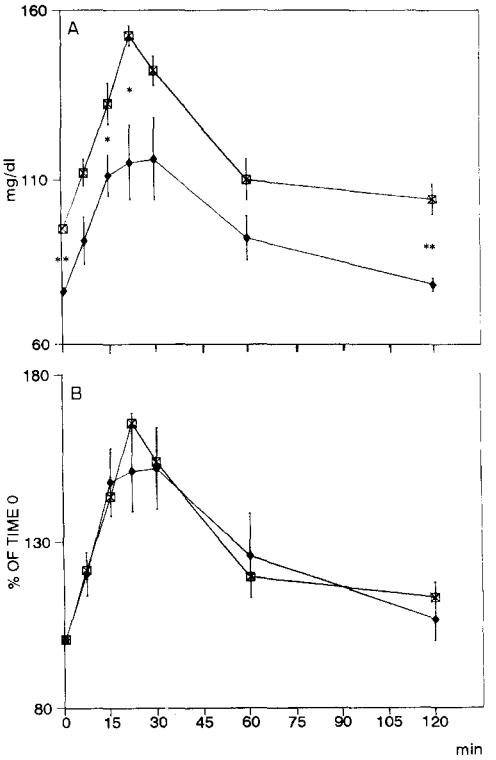

Fig. 3. *Plasma glucose concentration expressed as either absolute values (A) or as percentage of time 0 (B) at different times after oral glucose administration 2 g/kg b. w.) in virgin (□) and 21 day pregnant rats (◆).*
Statistical comparisons as in figure 1. n = 6-8 rats/group.

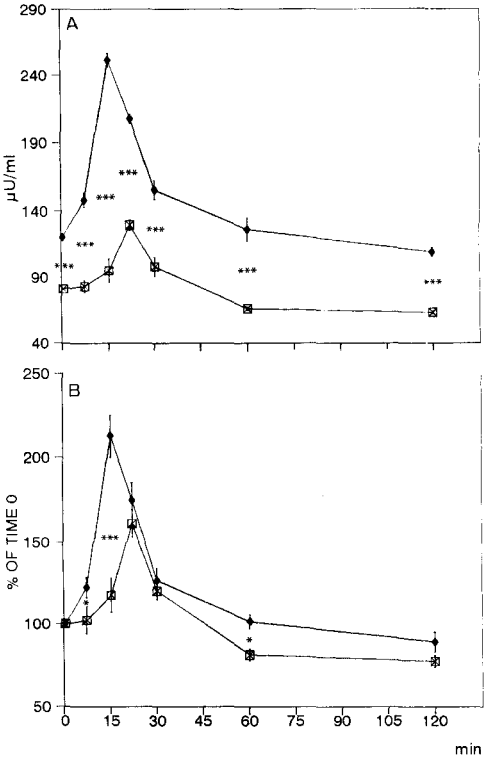

Fig. 4. *Plasma RIA-insulin concentration expressed as either absolute values (A) or as percentage of time 0 (B) at different times after oral glucose administration 2 g/kg b. w.) in virgin (□) and 21 day pregnant rats (◆).*
Statistical comparisons as in figure 1. n = 6-8 rats/group.

er sequentially or simultaneously on it. The hyperphagia (17) which exists at this stage of gestation would cause exaggerated hyperinsulinemic episodes in the mother, and it has been shown in nonpregnant rats that sustained increments in circulating insulin levels may increase insulin sensitivity (34). In any case, this double condition of augmented circulating insulin levels whenever the mother eats together with enhanced insulin sensitivity may be responsible for the increment in maternal structures during the first two thirds of gestation. We have already shown that this increase in maternal structures specifically corresponds to fat storage (11), of which the insulin changes ocurring at this phase of gestation may be the major inducer.

The above argument accords well with the arrest or even the decrease in the maternal net body weight known to occur during late gestation (11), which coincides with the appearance of the decreased insulin sensitivity, the appearance of an ex-

aggerated beta-cell response to the same glycemic stimulus and unmodified changes of plasma glucose levels after glucose load found here in the 21 day pregnant rats. Under this condition, prior anabolic changes ocurring in the mother turns to enhanced catabolism, as indicated by the different metabolic adaptations that occur during the late gestational phase (7, 10, 11).

Here again the juxtaposition of several hormonal factors may cause the increased insulin resistance appearing during late gestation. Positive correlations between increases in cortisol levels and the decreases in glucose tolerance have been established in normal pregnant women (14), and similar effects of pregnancy and corticosterone treatment on glucose uptake and protein degradation of skeletal muscle have also been found in rats (29), suggesting the active implication of glucocorticoids in the induction of insulin resistance during late pregnancy. The contribution of other hormonal changes, including the sustained high plasma insulin levels per se, which might bring about a decrease in insulin sensitivity (16), cannot be discarded and their partial responsability for maternal insulin resistance remains to be established.

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Resumen

Con el propósito de establecer las etapas de la gestación en que aparecen los cambios en las relaciones insulina/glucosa en la rata, se someten los animales sin anestesiar a un test de tolerancia oral de glucosa (2 g/kg) los días 15 y 21 de gestación, y se comparan con ratas hembras controles. El día 15 de la gestación la tolerancia a la glucosa aumenta, mientras que los niveles de insulina permanecen al mismo nivel que las controles. El día 21 la curva de tolerancia a la glucosa es similar en los dos grupos, a pesar de que los niveles de insulina aumentan más en las preñadas. Estos resultados muestran la existencia de dos fases totalmente distintas, en cuanto a las relaciones insulina/glucosa, con un aumento de la sensibilidad a la hormona el día 15 y una resistencia insulínica al final de la gestación. Se sugiere que estos cambios contribuyen a las tendencias anabólicas que se presentan en la madre a mitad de la gestación y su condición catabólica al final de la misma.

Palabras clave: Tolerancia a la glucosa, Gestación, Insulina, Páncreas endocrino, Rata.

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