Experimental Chemical Diabetes and Pregnancy in the Rat

Evolution of Glucose Tolerance and Insulin Response

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SUMMARY

The effect of pregnancy on the course of experimental chemical diabetes (CD) has been studied in the rat. Glucose tolerance tests (0.5 g/kg i.v.) have been performed serially in the virgin state (2 mo), late pregnancy (20.5 day of gestation), and 1 and 2 mo after delivery, in control and in CD female rats. During gestation in the controls basal plasma glucose is decreased, and plasma glucose levels after glucose load are also lower than levels found in the virgin state. Glucose tolerance is not significantly affected. Nevertheless, glucose-induced insulin secretion in pregnant animals is increased compared with the virgin state. Glucose tolerance remains unchanged 1 and 2 mo postpartum, but insulin response to glucose becomes significantly lower than in the virgin state. In the pregnant CD rats basal plasma glucose is decreased, but plasma glucose levels after glucose load are similar to values found in the virgin state, thus suggesting decreased glucose tolerance. Glucose-induced insulin secretion is increased compared with the virgin state. Glucose tolerance remains deteriorated 1 and 2 mo postpartum, but insulin secretion is no longer significantly different.

These findings indicate that in CD female rats glucose tolerance is and remains deteriorated by pregnancy, while in normal female rats it is and remains unchanged. Thus, despite increased insulin response to glucose during late gestation in the CD rats, the diabetogenicity of pregnancy is confirmed with this experimental model. DIABETES *31*:75–79, January 1982.

Previous clinical studies in humans have shown that subclinical diabetic women, when pregnant, develop a decreased tolerance to a glucose challenge. Insulin response to glucose was increased (as compared with the postpartum response) but was clearly defective in timing and quantity compared with the insulin response in normal pregnant women.[1] A reversal of glucose disposal to the normal range was observed 6–8 wk after delivery, but the abnormal insulin response persisted.[1]

No experimental studies in animals concerning this phenomena are presently available. Pancreatectomy, alloxan, and streptozotocin have been used in efforts to develop an animal model of human diabetic pregnancy, but generally such maternal diabetes was induced during pregnancy rather than before and hyperglycemia tended to be severe. The insulin response to glucose was not investigated in these mothers.

We have recently developed an experimental model of chemical diabetes (CD) in the adult rat, characterized by a chronic and stable low insulin response to glucose, a slight but consistent elevation of basal plasma glucose values, and slightly impaired glucose tolerance.[2] It remained to be determined whether glucose tolerance and the β-cell function were further impaired by pregnancy in our CD model. Alternatives in glucose and insulin metabolism are known to occur in normal gestation. We therefore studied in vivo glucose tolerance and insulin secretion in CD rats in the virgin state, during late pregnancy, and 1 and 2 mo postpartum.

MATERIAL AND METHODS

Wistar rats were fed ad libitum with pelleted chow (U.A.R. Villemoisson s/orge, France, carbohydrate 47%, protein 20%, and fat 8%). Females were caged with a male for one night (5 p.m. to 9 a.m.) and pregnancy was detected by abdominal palpation 14 days later as previously described.[3] Natural birth occurred 22 days after mating. On

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the day of birth, the newborn rats received streptozotocin (Upjohn, Co., Kalamazoo, Michigan) (100 μg/g) in 25 μl of citrate buffer (0.05 mol/L), pH 4.5, through the saphenous vein. They were left with their own mothers, the number of animals per litter being kept at 8. On day 4 after birth, neonates were tested for glycosuria with Clinistix (Ames Co, Division Miles Labs, Paris, France) and only animals with 3+ values were kept. They were weaned on day 21 after birth.

Spontaneous evolution of neonatal diabetes led to a chemical diabetic state in the adult that was stable and chronic, as previously described.[2] In other litters all the newborns received only citrate buffer and were used as controls.

Glucose tolerance was determined in control and in chemical diabetic (CD) female rats at 2 mo in the virgin state, on day 20.5 of gestation, and 1 and 2 mo after delivery (lactation being terminated 3 wk after delivery). Gestation was obtained in the control and in the CD female rats at 2.5–3 and 3–4 mo, respectively. Each female was serially tested. Intravenous glucose tolerance tests (IVGTT) (0.5 g glucose/kg body wt) were performed immediately after induction of pentobarbital anesthesia (i.p.) (4 mg/100 g body wt). The necessity of frequent and sequential blood sampling after i.v. injection of glucose precludes the use of unanesthetized animals. However, it has been demonstrated that pentobarbital anesthesia slightly modifies both the secretion and physiologic action of insulin. Glucose tolerance is impaired and the plasma insulin response to glucose enhanced.[4] On the other hand, conventional techniques used to collect blood in unanesthetized animals necessitate restraining devices and cause a mild stress with modifying effects on glucose tolerance and insulin secretion.[5] In our experiments control and CD female rats were treated with the same anesthesia protocol to control for metabolic variations related to use of the anesthesia. All tests were performed at 10 a.m., with rats in the nonfasted state. Body temperature was maintained at 37°C using heating lamps. Blood samples (300 μl) withdrawn from the tail vein were immediately centrifuged at 4°C; plasma was stored at −20°C until assayed.

Plasma glucose was measured using a glucose analyzer (Beckman Instruments, Inc., Irvine, California). Plasma immunoreactive insulin (IRI) was estimated using purified rat insulin as standard (R 171, Novo, Copenhagen, Denmark), antibody to mixed porcine + bovine insulin, and porcine monoiodinated ^{125}I-insulin.[6] The lower limit of detection for insulin was 6 μU/ml (0.25 ng/ml), with a within- and between-assay coefficient of variation of 10%. Silicate was used to separate free from bound hormone.[7]

The insulin response during the glucose tolerance tests was calculated as the insulinogenic index[8] (ΔIRI/ΔG), which is the ratio of incremental plasma insulin values integrated over the period (90 min) following the injection of glucose (ΔIRI) to the corresponding incremental integrated plasma glucose values integrated over the same period (ΔG). A 90-min period for the integration of glucose and insulin changes was chosen because glucose and insulin values in the CD females did not return to baseline by 60 min, in contrast to control rats.

RESULTS

Basal glucose and insulin levels. Pregnancy in CD rats is associated with a significantly lower weight gain: 75 ± 8 g compared with 110 ± 6 g in normal rats ($P < 0.01$). This difference is probably related to the difference in number of fetuses per litter, which ranges from 2 to 10 (5 ± 2) in CD and from 9 to 13 (11 ± 1) in normal rats.

As shown in Table 1, late pregnancy in normal rats is associated with a significant decline (50%) of plasma glucose levels in the fed state ($P < 0.001$) without a significant change in insulin levels. Consequently, the plasma insulin/glucose ratio is significantly increased ($P < 0.02$). Plasma glucose and the plasma insulin/glucose ratio returned to initial values 1 and 2 mo postpartum.

Pregnant CD rats show a similar decline (40%) in basal plasma glucose compared with levels in the virgin state ($P < 0.001$). However, the plasma glucose levels in the pregnant CD rats are significantly greater ($P < 0.01$) than the corresponding values obtained in normal pregnant rats. Also, in the pregnant CD rats the plasma insulin/glucose ratio is significantly increased ($P < 0.001$) compared with the value before gestation. Plasma glucose returned to the initial value by 1 mo postpartum but plasma insulin and the plasma insulin/glucose ratio were significantly higher ($P < 0.05$ and $P < 0.01$, respectively) than in the virgin state.

TABLE 1
Effect of pregnancy on body weight and basal plasma glucose and insulin levels of normal and CD rats

	Body weight (g)	Plasma glucose (mg/dl)	Plasma insulin (μU/ml)	Plasma insulin/glucose ratio
Normal rats				
Virgin (2 mo)	174 ± 3 (9)	156 ± 6 (9)	51 ± 7 (9)	0.33 ± 0.04 (9)
Late pregnancy (20.5 day)	330 ± 13 (7)	83 ± 3 (7)‡	45 ± 7 (8)	0.55 ± 0.08 (7)*
Postpartum (1 mo pp)	252 ± 14 (7)	159 ± 7 (6)	60 ± 7 (6)	0.38 ± 0.04 (6)
Postpartum (2 mo pp)	272 ± 5 (8)	145 ± 3 (8)	54 ± 5 (8)	0.40 ± 0.05 (8)
CD rats				
Virgin (2 mo)	153 ± 3 (9)	203 ± 5 (9)¶	30 ± 3 (9)‖	0.15 ± 0.01 (9)¶
Late pregnancy (20.5 day)	281 ± 11 (8)	128 ± 15 (7)‡‖	52 ± 11 (6)	0.41 ± 0.05 (7)‡
Postpartum (1 mo pp)	258 ± 3 (6)	180 ± 11 (5)	45 ± 4 (5)*	0.25 ± 0.02 (5)†‖
Postpartum (2 mo pp)	265 ± 9 (8)	173 ± 5 (8)‡¶	47 ± 4 (8)†	0.27 ± 0.03 (8)†§

Values are mean ± SEM. The number of observations is shown in parentheses.
When values of pregnant or postpartum rats are different from those of the corresponding virgin rats, significance is given by * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$.
When values of chemical diabetic rats are different from those of the corresponding normal rats, significance is given by § $P < 0.05$, ‖ $P < 0.01$, ¶ $P < 0.001$.

The latter changes were accentuated 2 mo postpartum ($P < 0.01$).

Response to IVGTT. Plasma glucose and insulin levels following intravenous glucose are shown in Figure 1. In the normal female rats plasma glucose levels after glucose load during pregnancy were lower ($P < 0.001$) than the values found in the virgin state. No significant change of mean incremental glucose area calculated for the 90-min test occurred (Figure 2). The increment of plasma insulin level after the glucose load and the mean incremental insulin area (Figure 2) were not significantly greater during late pregnancy than in the virgin state (Figure 1). However, calculation of the insulin/glucose ratio before and during pregnancy indicates that it is significantly increased ($P < 0.05$) during late pregnancy (Figure 2). Glucose values 1 and 2 mo postpartum were very close to those found in the virgin state, resulting in no significantly different incremental glucose areas. The insulin levels reached during IVGTT are lower postpartum than during pregnancy and even lower than in the virgin state (Figure 1). The mean incremental insulin area also is lower 1 and 2 mo postpartum ($P < 0.01$), and the insulinogenic index returns to a value lower than in the virgin state ($P < 0.05$) (Figure 2). Results obtained in virgin CD rats in response to IVGTT confirm our previous results.[2] In the pregnant CD rats, plasma glucose levels after glucose load are similar to values found in the virgin state (Figure 1). This pattern contrasts with that observed in normal rats. In this group the plasma glucose levels obtained after i.v. glucose during pregnancy are always lower than those obtained in the virgin state. Moreover, the incremental glucose area is significantly ($P < 0.05$) increased in the pregnant CD rats (Figure 2) because basal plasma glucose values during pregnancy

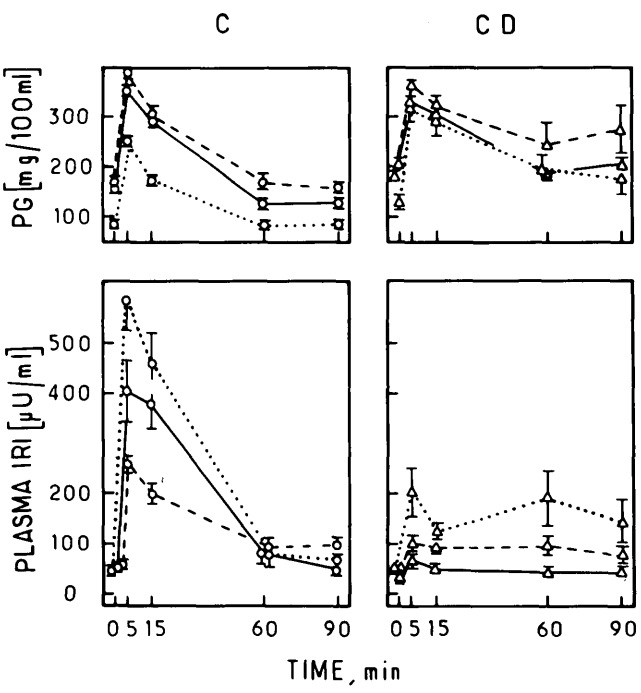

FIGURE 1. Effect of pregnancy on glucose tolerance and plasma insulin response to glucose (0.5 g/kg i.v.) in control (○—○) and CD rats (△—△). The two groups have been tested in the virgin state (2 mo) (—), during late gestation (20.5 days) (·····), and 1 mo postpartum (- - -). Each point is the mean ± SEM of 6 to 9 animals in each group.

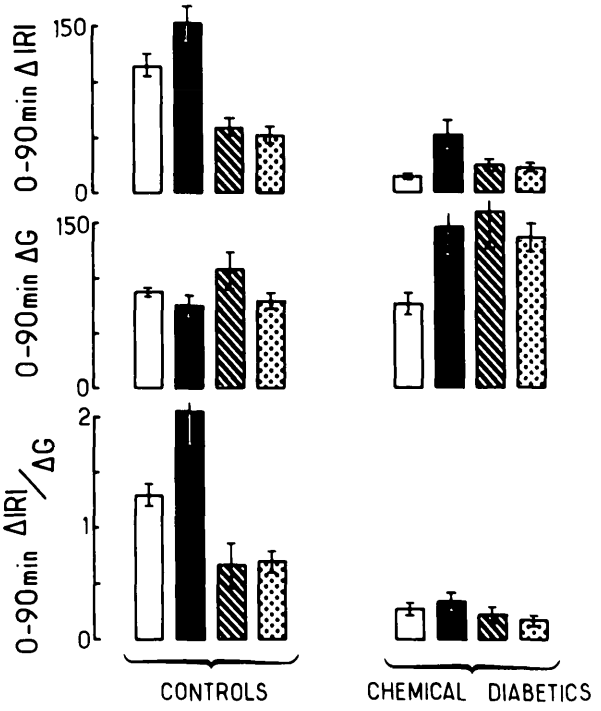

FIGURE 2. Effect of pregnancy on mean incremental glucose area (ΔG), mean incremental insulin area (ΔIRI), and insulinogenic index (ΔIRI/ΔG) in control and CD rats. Parameters presented here have been calculated from values obtained during the 90-min intravenous glucose tolerance test (see Figure 1). In the two groups, the test was performed in the virgin state (2 mo) (□), during late gestation (20.5 days) (■), 1 mo postpartum (▨), and 2 mo postpartum (▩). Each bar is the mean ± SEM of 6 to 9 observations in each group.

are lower than the values in the virgin state. The increment in insulin after glucose load, as shown by calculating the incremental insulin area (Figure 2), is significantly enhanced during pregnancy ($P < 0.05$). Comparison of the insulinogenic index before and during CD pregnancy (Figure 2) indicates that this index is not significantly increased during pregnancy, in contrast with normal female rats.

The incremental glucose area in CD rats remains significantly elevated 1 or 2 mo postpartum compared with the value obtained in the virgin state ($P < 0.05$). The insulin response to glucose returns to the virgin pattern and the insulinogenic index is not significantly different from the index calculated in the virgin state (Figure 2).

These data provide evidence that 2 mo after pregnancy glucose tolerance remains deteriorated in previously CD rats, while in normal rats glucose tolerance is unchanged.

DISCUSSION

Our study in the normal rat confirms that the β-cell is able to adapt to the metabolic and endocrine changes of pregnancy by enhancement of insulin response to glucose. This fact is well documented in both the normal woman[9–12] and the normal rat.[13–16] However, the precise mechanism of this change is still a matter of controversy. Although islet hyperplasia may be partly responsible,[13,17] it has been proposed that in pregnancy the pancreatic β-cell may develop an unusually sensitive mechanism for responding to glucose.[14] It has been suggested that these changes may be mediated by hormones such as human placental lactogen,[18] estrogens [13,19] and progesterone.[14,19,20,21] In our experiments, despite the increased insulin response to glucose, in the nor-

mal rat glucose tolerance (as tested by i.v. glucose) is unaffected by pregnancy. This result is in accord with previous data in the rat and the human[1,16,22] suggesting that insulin action is less efficient upon the maternal target tissues. Such a conclusion has been drawn by Fisher et al.,[23] who estimated in vivo insulin sensitivity in pregnant women by the glucose clamp technique. In the pregnant rat the decreased insulin sensitivity has been attributed to impaired insulin binding,[24] but contradictory results have been published.[25,26]

In the CD rats our data indicate that pregnancy causes a decline of basal plasma glucose level and an increased basal plasma insulin level compared with those in virgin rats. Following i.v. glucose administration, glucose tolerance, as estimated by the increment of plasma glucose above the basal value (Figure 2), is decreased and the insulin response is increased in the pregnant CD rats. The fact that the insulin response to glucose is increased during diabetic pregnancy shows that the endocrine pancreas of pregnant rats is still able to meet, but only partially, the increased insulin requirements of pregnancy. This is in contrast to the conclusions of Van Assche et al.,[27] who demonstrated that, unlike the nondiabetic rat, the amount of β-cells does not increase in the pancreas of the pregnant diabetic rat. However, these authors used pregnant rats with severe streptozotocin diabetes. Nevertheless, during pregnancy our experimental model of chemical diabetes shows that normal carbohydrate tolerance is not maintained. In that sense our experimental design mimics the development of gestational diabetes in women; i.e., in gestational diabetic patients the development of hyperglycemia appears related to the defect in timing and quantity of insulin release in response to glucose.[1] Postpartum glucose tolerance remains deteriorated in the CD rats compared with the virgin state, thus indicating aggravation of the diabetic state. Such a conclusion is supported by (1) plasma glucose values 90 min after i.v. glucose (272 ± 22 mg/dl) higher than preinjection values (173 ± 5 mg/dl) in the CD group 2 mo postpartum, while in the virgin CD group plasma glucose values 90 min after i.v. glucose returned to preinjection levels and (2) a significantly increased incremental glucose area in the CD group 2 mo postpartum compared with the value obtained in the virgin state. Note that the deteriorated glucose tolerance is still present in the stable nonpregnant state 5 wk after lactation ended (2 mo postpartum). Moreover, this deterioration is probably not related to the effect of age, since the incremental glucose area measured in 7-mo-old virgin CD rats is not significantly different from that previously measured at 3 mo in the same CD rats (unpublished data). This is accompanied by a return of insulin response to the range found in the virgin state, which may be related to a change in peripheral sensitivity to insulin. As far as insulin sensitivity is reflected by the basal value of the plasma insulin/glucose ratio, it appears that insulin sensitivity is abnormally high in the CD virgin rats compared with normal virgin rats (Table 1). During gestation this hypersensitivity no longer exists in the CD rats and does not return after delivery. The decrease of peripheral insulin sensitivity triggered by gestation persists after termination of pregnancy in the CD rats, in contrast to normal females. This probably accounts for the persistence of deteriorated glucose tolerance.

Finally, since emphasis has been placed on maternal hyperglycemia during gestational diabetes as being the major determinant of fetal obesity and β-cell hyperactivity,[28] studies of the effects of maternal chemical diabetes on the rat fetus should be performed.

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