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Control by insulin of adipose tissue lipoprotein lipase activity during late pregnancy in the rat

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During late gestation there is a marked increase in maternal plasma lipids both in humans[1-4] and in rats[5-9]. The increments of triglyceride(TG)-rich lipoproteins (chylomicrons and VLDL) are among the most pronounced of these changes[4,6,9,10]. They are partially caused by an enhanced production of endogenous TG[11,12] which, among other factors, is supported by the augmented transport of FFA to the liver from adipose tissue[13,14] and by improved intestinal absorption of dietary TG[15]. Delayed removal from circulation of TG-rich lipoproteins due to decreased adipose tissue lipoprotein lipase (LPL)[6,8,10,16-18] cannot be ruled out as an additional factor that may contribute to maternal hypertriglyceridemia. Besides this role, reduced adipose tissue LPL activity during late gestation allows blood TG to be diverted from storage in adipose tissue to other tissues where they are required to fulfill pregnancy-related metabolic needs. The prominent example are mammary glands where there is an increased uptake of circulating TG prior to parturition for use in milk synthesis[15,17]. Even in the liver under fasting conditions[10,19] the circulating TG are increasingly taken up to be used as substrates for maternal ketogenesis.

These changes in maternal tissue LPL activity are regulated by hormonal variations occurring during late gestation. The changes occurring in LPL activity in the mammary gland during that phase are probably triggered by the well known decline in plasma progesterone concentration and the subsequent release of prolactin that occurs during the last days of pregnancy[17,20]. However, it is not known what hormonal changes may be responsible for the decrease in LPL activity in adipose tissue. It is well known that insulin stimulates adipose tissue LPL expression under nonpregnant conditions[21,22], but both insulin sensitivity and responsiveness are decreased during late gestation[23-25]. It may be hypothesized, therefore, that insulin resistance is responsible for decreased maternal adipose tissue LPL activity. We have studied this possibility by determining how conditions of prolonged hyperinsulinemia and hypoinsulinemia in the pregnant rat affect LPL activity.

METHODS

Two experimental conditions were used. In one of them adult female Wistar rats were made diabetic by iv injection of a freshly prepared solution of streptozotocin (STZ, 45 mg/kg body wt). Starting 24 h after the STZ injection the rats were subjected to a daily sc treatment with 1.5 u/100 g of ultralente bovine insulin (MC, Novo, Denmark). On the 7th day the animals were mated with nondiabetic males and divided into two groups: one kept on insulin until sacrificed ('diabetics + insulin', D + Ins); the other group had the treatment discontinued from the mating time ('diabetics', D). Normal untreated sex and age matched virgin and pregnant controls were studied in parallel. All animals were killed by decapitation on the 20th day of gestation. The animals of the D + Ins group were killed 24 h after the last insulin treatment. Blood was collected and lumbar fat pads were rapidly excised and kept at − 80 °C until processed.

In another series of experiments, pregnant rats on the 17th day of gestation and age matched virgin controls had a permanent cannula surgically implanted in the external jugular vein under ketamine anesthesia. After recovering from the anesthesia the cannula was connected to an infusion pump and under unrestrained conditions animals received a continuous infusion (35 ml/day) through the cannula of either a 50% glucose solution or double distilled water. They were decapitated on the 20th day of gestation and blood and lumbar fat pads collected as above.

Plasma samples were used to measure insulin by RIA[26] using a kit specific for rat (Novo, Denmark)

and glucose[27]. LPL activity was determined in acetone-ether extracts as described[28].

RESULTS AND DISCUSSION

To establish whether major changes in circulating insulin levels in the pregnant rat would modify their adipose tissue LPL activity STZ-diabetic rats with or without treatment with high doses of exogenous insulin were compared with nondiabetic pregnant and virgin rats. As shown in Fig. 1a, plasma insulin levels were higher in nondiabetic pregnant than in virgin animals. These findings were expected since it is well known that under normal conditions there is hyperinsulinemia during late gestation[7,29]. Diabetic rats show significant reductions in plasma insulin levels, whereas insulin treatment of diabetic rats caused a significant increase compared not only with virgin, but also with pregnant nondiabetic controls (Fig. 1a). Plasma glucose levels showed an opposite trend to that of insulin. As may be seen in Fig. 1b, pregnant controls had significantly reduced plasma glucose levels, compared with virgin rats, whereas the pregnant diabetic rats (D) were markedly hyperglycemic. Insulin treatment (D + Ins) reduced the glycemia to levels slightly, but not significantly lower than in pregnant controls.

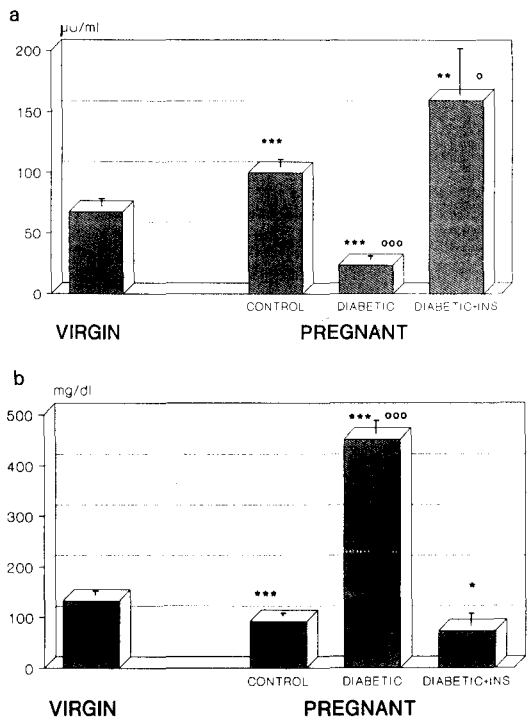

FIG. 1. Plasma insulin (1a) and glucose (1b) levels in STZ-diabetic 20 day pregnant rats with or without treatment with exogenous insulin (1.5 units bovine ultralente insulin/100 g per day) and normal pregnant and virgin controls. *P vs virgins and °vs pregnant controls (* or ° $P<0.05$, ** or °° $P<0.01$ and *** or °°° $P<0.001$).

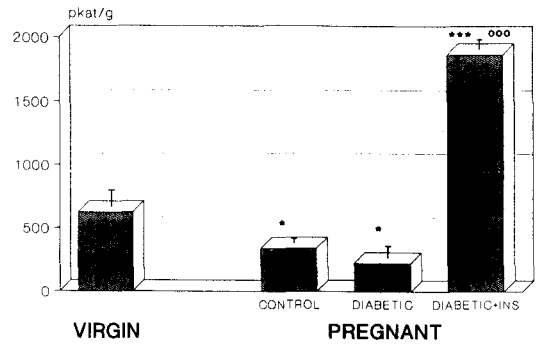

FIG. 2. Lumbar adipose tissue lipoprotein lipase activity in STZ-diabetic 20 day pregnant rats with or without treatment with exogenous insulin (1.5 units bovine ultralente insulin/100 g per day) and normal pregnant and virgin controls. Statistical comparisons as indicated in Fig. 1. Values are expressed as pkatals/g fresh tissue (1 pkatal = 1 pmole of substrate converted per second).

These different insulin and glucose patterns among the 4 groups was accompanied by important changes in adipose tissue LPL activity. As shown in Fig. 2, lumbar fat pad LPL activity in pregnant controls was significantly lower than in virgin rats and this finding agrees with the well known decrease in maternal adipose tissue LPL activity found during late pregnancy[6,8,10,16-18]. In diabetic pregnant animals LPL activity did not differ from that found in nondiabetic pregnant controls (Fig. 2), which indicates that the already low LPL levels, characteristic of pregnancy, cannot be further decreased by diabetes. The values of diabetic animals receiving insulin treatment (D + Ins) are of special interest since their adipose tissue LPL activity was higher than that of any other groups, including not only nondiabetic and diabetic pregnant rats, but also virgin animals ($P<0.001$). This result was unexpected and coincided with the enhanced plasma insulin level of these animals (Fig. 1a). These insulin levels were still high although the blood samples had been collected 24 h after the last insulin treatment, suggesting that earlier plasma insulin levels would be even higher. This hyperinsulinemia in the pregnant rat may have compensated for, or caused a reduction in maternal insulin resistance since it is known that sustained hyperinsulinemia in the nonpregnant rat may enhance insulin sensitivity[30]. Since it is well known that adipose tissue LPL activity is stimulated by insulin[20,21], it is proposed that in our diabetic pregnant rats, receiving high insulin dosage, the exaggerated hyperinsulinemia was effective in overcoming maternal insulin resistance to the extent allowing a major induction of adipose tissue LPL activity.

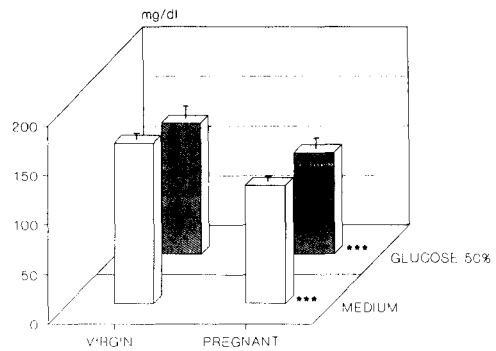

FIG. 3. Effect of iv glucose infusion (50% glucose in distilled water, 35 ml/day) in unrestrained pregnant and virgin rats from days 17 to 20 of gestation, on plasma glucose levels. *pregnant vs virgins (*** $P<0.001$). Differences between glucose and water infused animals were not significant ($P>0.05$).

To lend further support for the above hypothesis, we produced continuous endogenous hyperinsulinemia in normal pregnant rats. Unrestrained animals were iv infused with 50% glucose (35 ml/day) or distilled water from days 17 to 20 of gestation. To test whether the observed changes were specific for the pregnant condition, virgin rats were infused in parallel.

As shown in Fig. 3, in spite of receiving a total amount of 17.5 g/day glucose, neither virgin nor pregnant rats showed hyperglycemia as compared with those receiving the water infusion. Plasma glucose levels were slightly lower in pregnant than in virgin animals, and this difference was true whether animals received the aqueous medium or the glucose infusion (Fig. 3). As shown in Fig. 4, plasma insulin was elevated in animals receiving glucose treatment and the effect was significantly

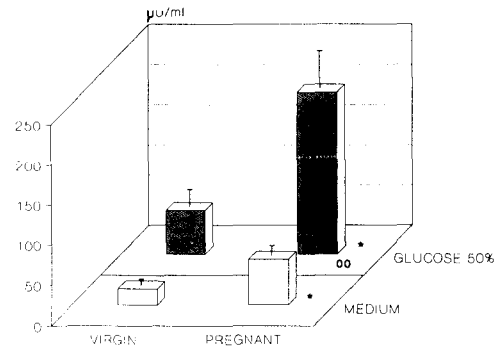

FIG. 4. Effect of iv glucose infusion (50% glucose in distilled water, 35 ml/day) in unrestrained pregnant and virgin rats from days 17 to 20 of gestation, on plasma insulin. *pregnant vs virgins, °glucose vs medium (*$P<0.05$, °°$P<0.01$).

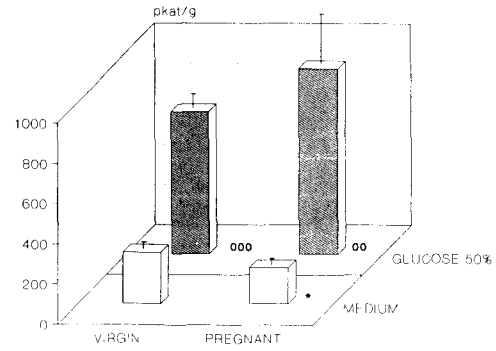

FIG. 5. Effect of iv glucose infusion (50% glucose in distilled water, 35 ml/day) in unrestrained pregnant and virgin rats from days 17 to 20 of gestation, on lumbar adipose tissue lipoprotein lipase activity. *pregnant vs virgin animals, °glucose vs water infused animals *$P<0.05$, °°$P<0.01$, °°°$P<0.001$). Values of enzyme activity are expressed as in Fig. 2.

higher in pregnant as compared with virgin rats. On the one hand this finding agrees with the well known enhanced sensitivity of the B-cell to the insulinotropic agents during pregnancy[25,31,32]. On the other hand, it shows that the hyperinsulinemia attained in the glucose-infused pregnant rats was able to counteract their reduced insulin sensitivity. When plasma insulin levels attained in these animals (Fig. 4) are compared to those of glucose (Fig. 3) it becomes clear that the lower insulin sensitivity of the pregnant vs the virgin animals is the result of higher insulin levels in the former, causing a similar glucose change in both groups. In any event, in spite of the higher insulin requirement, the so-called 'net insulin effect' appears to be similar in both groups, as shown by their similar glucose values (Fig. 3). As shown in Fig. 5, the glucose infusion was effective in augmenting the reduced basal adipose tissue LPL activity in the pregnant rats to values even higher than those in virgin rats. These results demonstrate that a compensation of maternal insulin resistance allows adipose tissue LPL activity levels to be restored.

CONCLUSIONS AND COMMENTS

By following two different experimental strategies, one based on daily treatment with exogenous insulin of the STZ-diabetic pregnant rat and the other based on continuous infusion of glucose from 17th to 20th day of gestation, we have been able to cause prolonged hyperinsulinemia in the pregnant animals. Under this condition, plasma glucose levels were very similar to those seen in untreated control animals, and in both experimental models with hyperinsulinemia adipose tissue LPL activity was significantly enhanced. These findings support the

notion ' that under normal conditions, insulin resistance during late gestation is responsible for the decreased LPL activity normally seen in maternal adipose tissue. Besides its basic interest, our findings may provide better understanding of lipid metabolism during pregnancy. Since reduced adipose tissue LPL activity has been attributed a role in the delayed removal of plasma TG-rich lipoproteins, which has not been documented directly, these experimental models demonstrate that this delay may be corrected without causing important changes in plasma glucose levels. Work is presently in progress to assess this problem. It is known that exaggerated hyperlipidemia during gestation may cause permanent dyslipidemia[33] and modifying maternal LPL activity may be a useful therapeutic approach to this problem.

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