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MODULATION OF LIPOPROTEIN LIPASE ACTIVITY IN ADIPOSE TISSUE DURING LATE PREGNANCY

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INTRODUCTION

Maternal hypertriglyceridemia is a common feature during late gestation both in humans[1] and in rats[2]. Two major factors contribute to such hypertriglyceridemia: *1/* an increased hepatic production of endogenous triglycerides (TG) which is supported by the augmented transport of FFA to the liver from adipose tissue[3], and *2/* a delayed removal from circulation of TG-rich lipoproteins caused by reduced lipoprotein lipase (LPL) activity[4]. Since the liver is the major receptor organ for free fatty acids (FFA) and glycerol derived from adipose tissue lipolysis, the enhanced maternal liver TG production is facilitated by the enhanced adipose tissue lipolytic activity that occurs during late gestation[5]. Although it is not known what hormonal changes may be responsible for the decrease in LPL activity in adipose tissue at the last stage of gestation, it is well known that insulin stimulates adipose tissue LPL activity and expression under nonpregnant conditions. Since both insulin sensitivity and responsiveness are decreased during late gestation[6], it may be hypothesized, that insulin resistance during late gestation is responsible for the decreased LPL activity normally seen in maternal adipose tissue. To test this hypothesis, in the present work we have studied whether conditions of prolonged hyperinsulinemia or hypoinsulinemia affect LPL activity in the pregnant rat.

METHODS

Female Wistar rats weighing 160-180 g were fed Purine chow diet (Panlab, Barcelona, Spain). After an overnight fast, one group of rats were made diabetic by a single intravenous injection of 45 mg of streptozotocin (STZ) per Kg body weight dissolved in 50 mM citrate buffer pH 4.5. They were re-fed and 24 h. later an urinary glucose test was made using commercial strips (Dextrostise). Only rats showing positive glucosuria were included in the study. Starting 24 h. after the STZ injection the rats were subjected to a daily subcutaneous (sc) treatment with 1.5 I.U./100 g of ultralente bovine insulin (MC, Novo, Denmark). After 7 days of treatment,

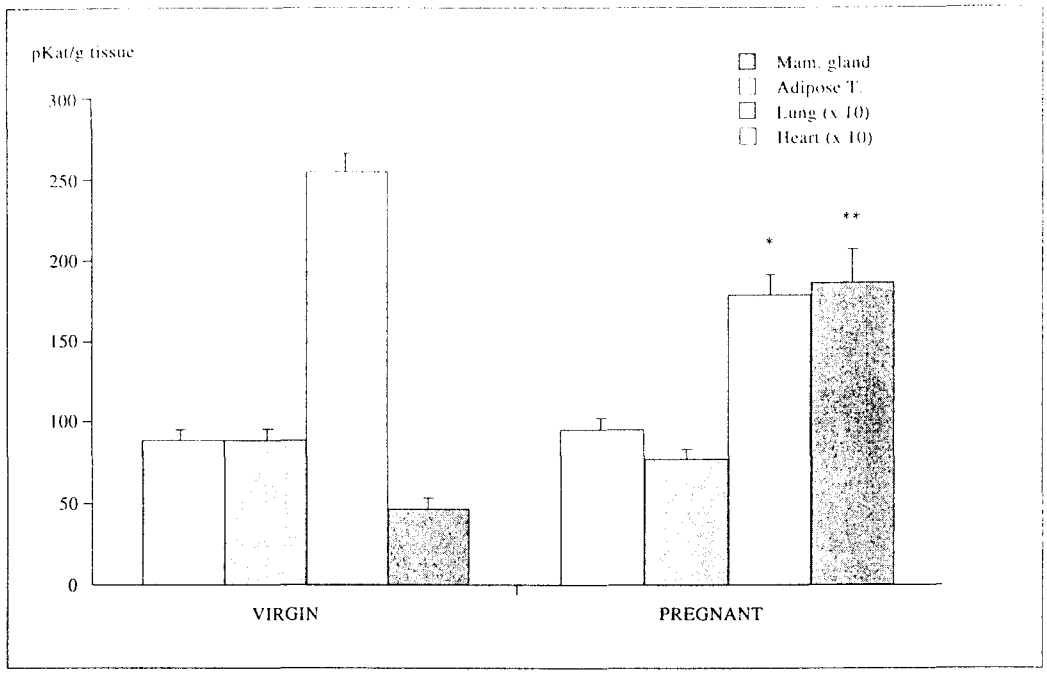

Figure 1. *Lipoprotein lipase activity in different tissues of 20 day pregnant and virgin rats. *Pregnant vs virgin rats (*P< 0.05,**P< 0.01). Differences in heart and lung between pregnant and virgin rats were not significant (P<0.05).*

these rats were mated with normal males and positive pregnancy was estimated by appearence of spermatozoids in vaginal smears. From this time on insulin treatment was mantained in some pregnant rats for the whole study (Diabetic + Ins) or else the treatment was interrupted on day 0 of pregnancy and the rats were kept without treatment for the whole study (diabetic). 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 Diabetic+Ins group were killed 24 h. after the last insulin treatment. Plasma from blood collected from the neck wound into heparinized tubes was kept at -20°C until processing. Lumbar fat pads were rapidly excised and placed into liquid nitrogen, and stored at -80°C until processing for lipoprotein lipase activity.

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 continous infusion (35 ml/day) through the cannula of either a 50% glucose solution, 1 I.U. of insulin/day or double distilled water. They were decapitated on the 20th day of gestation and blood and lumbar fat pads, mammary gland, heart and lung were collected as above.

Plasma aliquots were used to measure glucose and insulin by RIA using a specific kit for rats (Novo, Denmark). LPL activity was assayed in acetone-diethyl ether extracts as described[7].

RESULTS AND DISCUSSION

As shown in figure 1, heart and lung LPL activity did not change between virgin and pregnant rats when studied under basal conditions. However, as also shown in figure 1, the activity of this enzyme in adipose tissue appeared significantly lower in pregnant than in virgin rats, whereas in the mammary gland it was much higher in the former group. On the basis of previous reports[8] these findings were to be expected. Besides contributing to hypertriglyceridemia, the reduced adipose tissue LPL activity during late gestation would allow circulating TG to be diverted from storage in adipose tissue to other tissues. The increase in LPL activity found in the mammary gland is coincident with the previously reported increased uptake of triglycerides by this tissue in rats during late pregnancy[9], indicating that the change in LPL activity contributes to the availability of circulating triglycerides for milk synthesis prior parturition.

To test whether major changes in circulating insulin levels in the pregnant rat would modify adipose tissue LPL activity, STZ-diabetic rats treated with high doses of exogenous insulin and STZ-diabetic rats without insulin treatment were compared with non-diabetic pregnant and virgin rats. As was recently found by us with rats under similar conditions[10], on the 20th day of gestation, normal non-diabetic pregnant rats had lower plasma glucose levels than normal virgin rats, whereas STZ-diabetic pregnant rats were markedly hyperglycemic,

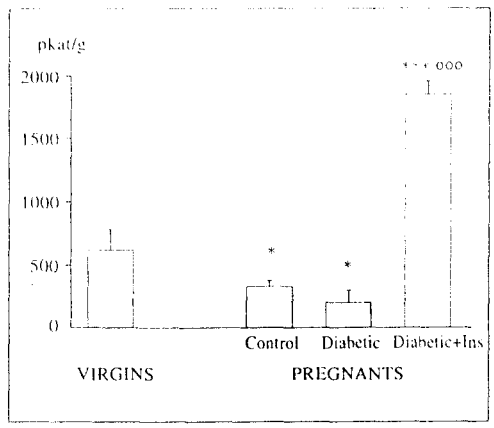

Figure 2. *Lumbar adipose tissue lipoprotein lipase in STZ-diabetic 20 day pregnant rats with or without treatment with exogenous insulin (1.5 units bovine ultralente insulin/100 g. per day) and normal pregnant and virgin controls. *Pregnant rats vs virgins and °vs pregnant controls (*P<0.05, ***or °°°P<0.001). Values are expressed as pKats/g. fresh tissue (1 pKatal= 1 pmole of substrate converted per second).*

and their insulin treatment made these differences disappear. Plasma insulin levels showed an opposite trend of glucose, and were higher in non-diabetic pregnant than in virgin animals, whereas when both groups were diabetic there was a significant reduction in plasma insulin levels. Insulin treatment of the diabetic rats significantly increased plasma insulin levels, in both virgin and pregnant rats, to values that were higher than in their respective non-diabetic controls[10]. This experimental design therefore allowed us to have streptozotocin treated rats with different plasma insulin and glucose levels and to test whether these conditions were also followed by differences in adipose tissue LPL activities.

As shown in figure 2, lumbar fat pad LPL activity in normal 20 day pregnant

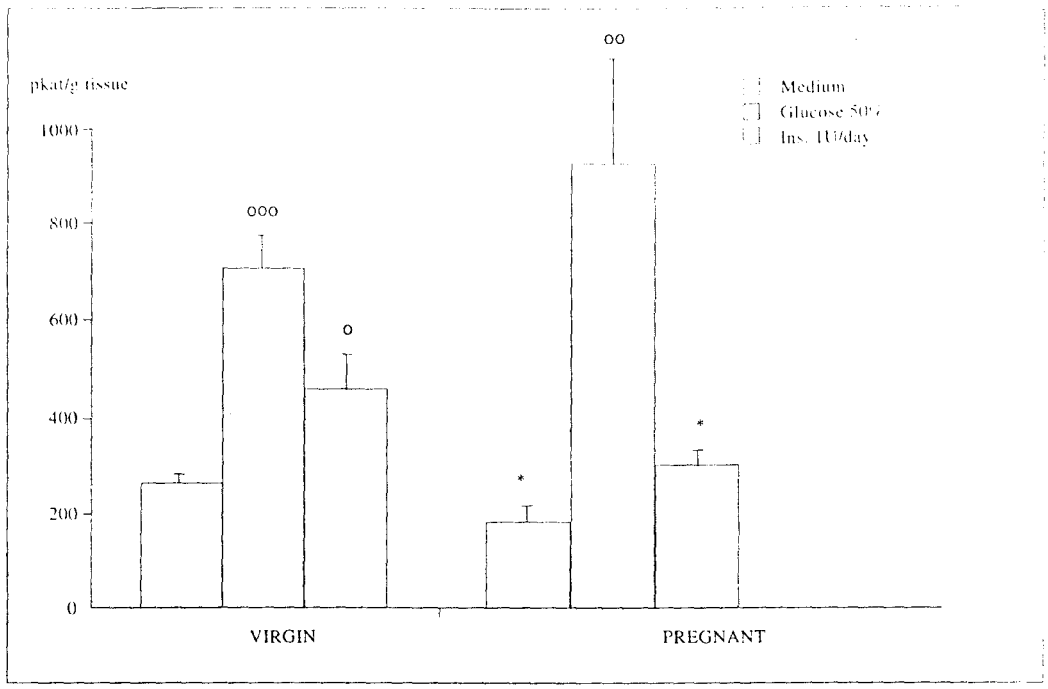

Figure 3. *Lumbar adipose tissue lipoprotein lipase in i.v. infused with glucose (50% glucose in distilled water, 35 ml/day) or insulin (1.5 I.U./day) and unrestrained pregnant and virgin rats from days 17 to 20 of gestation. *Pregnant vs virgin rats, °glucose or insulin vs water infused animal. *or °P<0.05, °°P<0.01, °°°P<0.001. Values of enzyme activity are expressed as in figure 2 for mammary gland and adipose tissue, and as pKats/mg. of protein for lung and heart.*

untreated rats (controls) was significantly lower than in virgin rats and these findings agree with the well-known decrease in maternal adipose tissue LPL activity seen during late pregnancy. In STZ-diabetic pregnant animals not receiving insulin therapy, LPL activity did not change from that found in non-diabetic pregnant controls, which indicates that the already low LPL levels characteristic of pregnancy, cannot be further decreased by diabetes. On the contrary, when the STZ-diabetic pregnant rats received the insulin treatment, adipose tissue LPL activity appeared much higher than in either non-diabetic or diabetic pregnant rats or than in virgin animals. This result was unexpected and has special interest since it coincided with the enhanced plasma insulin level of these animals. This hyperinsulinemia in the treated diabetic pregnant rat may have compensated for - or decreased- the maternal insulin resistance since it is known that sustained hyperinsulinemia in the nonpregnant rat enhances insulin sensitivity[11]. Since it is well-known that adipose tissue LPL activity is stimulated by insulin[12], these findings suggest that prolonged hyperinsulinemia in pregnant diabetic rats, which are receiving a high insulin dosage, is effective in overcoming maternal insulin resistance to the extent of allowing a major induction of adipose tissue LPL activity. These findings there-

fore support the hypothesis that under normal conditions, insulin resistance during late pregnancy is responsible for the decreased LPL activity normally seen in maternal adipose tissue.

The above hypothesis was also tested by another experimental strategy. Continous endogenous hyperinsulinemia in normal pregnant rats was produced by infusing *i.v.* either 50% glucose (35 ml/day), 1 I.U. insulin/day, or distilled water to unrestrained pregnant rats from days 17 to 20 of gestation. Virgin rats were studied in parallel to test whether the observed changes were specific for the pregnant condition. 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 (data not shown). Insulin infusion produced hypoglycemia in pregnant rats as compared with those receiving the water infusion. Plasma glucose levels were always slightly lower in pregnant than in virgin animals, and this difference was true in all the groups studied. Plasma insulin levels were elevated in animals receiving glucose treatment and the effect was significantly higher in pregnant than in virgin rats in agreement with the well-known enhanced sensitivity of the ß-cell to insulinotropic agents during pregnancy[13].

As shown in figure 3, both glucose and insulin insfusion were effective in significantly augmenting adipose tissue LPL activity in virgin rats, which agrees with previous findings[12]. Insulin infusion in 20 day pregnant rats caused a milder increase in adipose tissue LPL activity than in virgin rats (figure 3), which may be a consequence of either the reduced insulin sensitivity known to be present during late gestation[6], of the hypoglycemic condition of the former animals that would impede a normal response, or both, hypoglucemia and reduced insulin sensitivity. As also shown in figure 3, the effect of the glucose infusion was, however, more pronunced in pregnant than in virgin rats. It is seen that after 3 days of glucose infusion lumbar adipose tissue LPL activity attained even higher values in pregnant than in virgin rats in spite of basal activities being lower in the former. Since glucose infusion produced much plasma insuline levels[14], it may be proposed that this exaggerated and prolonged hyperinsulinemia can compensate for maternal insulin resistance by allowing adipose tissue LPL activity to be restored.

SUMMARY AND CONCLUSIONS

Prolonged hyperinsulinemia in the pregnant rat, caused either by daily treatment with exogenous insulin of the STZ-diabetic rat or a continous infusion of glucose, produces an increase of adipose tissue LPL activity. These findings support the hypothesis that under normal conditions, insulin resistance during late pregnancy is responsible for the decreased LPL activity normally seen in maternal adipose tissue.

Key words: Insulin, LPL, Pregnancy.

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REFERENCES

1 Knopp RH, Montes A and Warth MR. Carbohydrate and lipid metabolism in normal pregnancy. Food and nutrition board. Laboratory indices of nutritional status pregnancy. *Natl Acad Sci Washington* 1978, pp 35-88.

2 Scow RO, Chernick SS and Brinley MS. Hyperlipemia and ketosis in the pregnant rat. *Am J Physiol* 1964;**206**:796-804.

3 Carmaniu S and Herrera E. Conversion of (U-^{14}C)-glycerol, (2-^{3}H)-glycerol and (1-^{14}C)-palmitate into circulating lipoproteins in the rat. *Rev Esp Fisiol* 1979;**35**: 461-466.

4 Hamosh M, Clary TR, Chernick SS et al. Lipoprotein lipase activity in adipose tissue and mammary tissue and plasma triglycerides in pregnant and lactating rats. *Biochem Biophys Acta* 1970;**210**:473-482.

5 Knopp RH, Herrera E and Freinkel N. Carbohydrate metabolism in pregnancy. VIII Metabolism of adipose tissue isolated from fed and fasted pregnant rats during late gestation. *J Clin Invest* 1970;**49**:1438-1446.

6 Knopp RH, Ruder HJ, Herrera E et al. Carbohydrate metabolism in pregnancy VII. Insulin tolerance during late pregnancy in the fed and fasted rat. *Acta Endocrinol* 1970;**65**:352-360.

7 Llobera M, Montes A and Herrera E. Lipoprotein-lipase activity in liver of rat fetus. *Biochem Biophys Res Comm* 1979; **91**:272-277.

8 Ramírez I, Llobera M and Herrera E. Circulating triacylglycerols, lipoproteins and tissue lipopprotein lipase activities in rat mothers and offspring during the perinatal period: effect of postmaturity. *Metabolism* 1983;**32**:333-341.

9 Argiles J and Herrera E. Lipids and lipoproteins in maternal and fetus plasma in the rat. *Biol Neonate* 1981;**39**:37-44.

10 Martín A and Herrera E. Different responses to maternal diabetes during the first and second half of gestation in the streptozotocin-treated rat. *Israel J of Medical Sciences* 1991;**27**:442-448.

11 Trimble ER, Weir GC and Gjinovci A. Increased insulin responsiveness in vivo and in vitro consequent to induced hyperinsulinemia in the rat. *Diabetes* 1984;**33**:444-449.

12 Robinson D and Speake B. Role of insulin and other hormones in the control of lipoprotein lipase activities. *Biochem Soc Trans* 1989;**17**:40-42.

13 Freinkel N. Of pregnancy and progeny. *Diabetes* 1980;**29**:1023-1035.

14 Herrera E, Ramos P and Martín A. Control by insulin of the adipose tissue lipoprotein lipase activity during late pregnancy in the rat. Frontiers in diabetes research. Lessons from animal diabetes III. Ed. E. Shafrir. 1991, XI. 6 pp 551-554.