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# Comparative responsiveness to prolonged hyperinsulinemia between adipose-tissue and mammary-gland lipoprotein lipase activities in pregnant rats

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## ABSTRACT

*The present study was addressed to determining the comparative responsiveness of lipoprotein lipase (LPL) activity, in white adipose tissue and mammary gland, to a prolonged hyperinsulinemic stimulus, in pregnant and virgin rats. Pregnant rats at the 17th day of gestation and virgin animals were subjected, under conscious and unrestrained conditions, to a continuous infusion with either 50% glucose or double-distilled water (controls) (35 ml/day) for 72 h through a catheter in the jugular vein. The basal plasma-glucose levels were lower in pregnant than in virgin rats. After the glucose infusion plasma-glucose levels remained unchanged but plasma-insulin levels were much higher, and this effect was greater in pregnant than in virgin rats.*

*Whereas LPL activity in white adipose tissue in the controls was lower in pregnant than in virgin rats, in rats receiving the glucose infusion it increased more in pregnant than in virgin rats. However, LPL activity in the mammary gland was already higher in control pregnant rats than in virgin controls and the glucose infusion caused a similar increase in both groups. Although there was a linear correlation when individual values, from all the studied rats, for LPL activity in both tissues were plotted against plasma insulin levels, the correlation coefficient was much higher for mammary-gland LPL activity than for adipose-tissue LPL activity.*

*Plasma-triglyceride levels were higher in pregnant than in virgin rats. The glucose infusion did not modify this parameter, probably because of the changes in LPL activity in other tissues which are known to occur in the opposite direction to those observed in this study for adipose tissue and mammary gland. The present results support the notion that the insulin resistant condition which normally occurs during late gestation is responsible for the decreased LPL activity in adipose tissue, but that the mammary gland remains sensitive to insulin and so maternal hyperinsulinemia would contribute to the induction of LPL activity in this organ prior to parturition.*

## INTRODUCTION

Maternal hypertriglyceridemia is a characteristic feature during late pregnancy in both humans (Russ *et al.*, 1954; Konttinen *et al.*, 1964; Knopp *et al.*, 1978) and rats (Scow *et al.*, 1964; Herrera *et al.*, 1969; Argiles and Herrera, 1981), and corresponds to an increase in triglycerides in all plasma lipoprotein fractions (Montelongo *et al.*, 1992). The increment of triglyceride-rich lipoproteins (chylomicrons and very low density lipoproteins) is, however, the most pronounced of these changes (Montelongo *et al.*, 1992; Herrera *et al.*,

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1988; Argiles and Herrera, 1981; Knopp *et al.*, 1978) and is caused by several factors, including an enhanced production of endogenous triglycerides (Wasfi *et al.*, 1980; Hunphrey *et al.*, 1980) and an improved intestinal absorption of dietary triglycerides (Argiles and Herrera, 1989).

Decreased adipose-tissue lipoprotein lipase activity (LPL) (Otway and Robinson, 1968; Herrera *et al.*, 1988; Hamosh *et al.*, 1970; Ramirez *et al.*, 1983) may delay the removal from circulation of triglyceride-rich lipoproteins and this could be an additional factor contributing to maternal hypertriglyceridemia. However, postheparin plasma-LPL activity in late pregnancy does not decrease as would be expected (Kinnunen *et al.*, 1980) in light of the decreased LPL activity found in adipose tissue, although this is probably a consequence of the contraposed action of the enhanced LPL activity present in other tissues, especially the mammary gland, as shown by studies in pregnant rats (López-Luna *et al.*, 1994; Ramirez *et al.*, 1983).

The opposite nature of the changes in LPL activity found during late gestation in adipose tissue and mammary gland has important physiological implications since it allows circulating triglycerides to be diverted from storage in the former tissue to be taken up by the latter for use in milk synthesis (Ramirez *et al.*, 1983; Argiles and Herrera, 1989; Herrera *et al.*, 1994).

These changes in maternal-tissue LPL activity are regulated by hormonal variations occurring during late gestation. The decline in adipose-tissue LPL seems to be related to the insulin resistant condition present at this stage of gestation (Herrera *et al.*, 1990; Martin *et al.*, 1993; Knopp *et al.*, 1970). The increase occurring in mammary-gland LPL activity is probably triggered by a decline in the plasma-progesterone concentration and the subsequent release of prolactin that occurs during the last days of pregnancy (Ramirez *et al.*, 1983; Spooner *et al.*, 1977). However, and although not yet confirmed, insulin may also play a role in the changes taking place in LPL activity in this organ during gestation, but in an opposite way to its role in adipose tissue. The mammary gland is highly sensitive to insulin during lactation (Burnol *et al.*, 1987; Da Costa and Williamson, 1993), and in both pregnant and lactating rats the mammary gland expresses insulin receptors which display insulin-stimulated tyrosine kinase activity (Burnol *et al.*, 1990).

By using our recently developed method for prolonged glucose infusion in the unrestrained rat (Ramos and Herrera, 1995b), this study determines the comparative responsiveness of adipose-tissue and mammary-gland LPL activity to continuous hyperinsulinemia during late gestation in the normoglycemic rat.

## MATERIALS AND METHODS

Adult female Wistar rats weighing 170–180 g, from our own colony, housed at 22–24 °C with controlled lighting (lights from 8.00 to 20.00 h), were mated and the beginning of pregnancy was determined by the presence of spermatozoids in vaginal smears. Pregnant rats at day 17 of gestation and age-matched virgin rats were subjected to a continuous intravenous infusion with either double-distilled water or 50% glucose through a catheter placed into the jugular vein by means of an infusion pump (Minipuls II, Gilson, Villiers Le Bel, France) at the rate of 35 ml/day. The surgical procedure was as previously described (Ramos and Herrera, 1995b). In short, a Silastic catheter (Dow Corning Midland, MI, USA; 0.02 inch inside diameter, 0.037 inch outside diameter) was placed into the right jugular vein, under ketamine cocktail anesthesia. After recovery from anesthesia, animals were housed in individual cages and the respective infusions were continued for 72 h. At the time of ending the infusion period, which in the case of pregnant rats corresponded to day 20 of gestation, the animals were killed by decapitation, blood was collected from the neck wound and their mammary glands and lumbar adipose tissue rapidly dissected and placed into liquid nitrogen to be stored at −80 °C until processing.

Plasma samples were used to measure insulin by radioimmunoassay (RIA) (Heding, 1972) using a specific kit for rats (Novo, Denmark), glucose (Huggett and Nixon, 1957), and triglycerides were determined using a commercial enzymatic kit (Menarini). Lipoprotein lipase activity was determined in acetone-ether extracts as described (Llobera *et al.*, 1979), and values were expressed as pkatals (pmol of substrate transformed per second) per amount of fresh tissue weight.

Results were expressed as mean ± SE, and statistical comparison between groups was done using Student's *t*-test. Simple regressions were analyzed by means of Pearson's *r* correlation coefficient for linear variations.

## RESULTS

As shown in Figure 1a, after 3 days of the intravenous infusion with 50% glucose at the rate of 35 ml/day plasma, corresponding to a total amount of 17.5 g of glucose/day, neither virgin nor pregnant rats showed

any change in plasma-glucose levels when compared with rats receiving the water infusion (controls). Plasma-glucose levels remained significantly lower in pregnant than in virgin rats independent of whether they received the aqueous medium or the glucose infusion (Figure 1a). As shown in Figure 1b, the plasma-insulin level was higher in control pregnant rats on the water infusion than in control virgin animals. The glucose infusion raised plasma-insulin levels higher in pregnant rats (3.34 times control rat levels) than in virgin rats (2.11 times control rat level), and although plasma insulin levels were 2.31 times higher in control pregnant rats than in control virgin animals, this difference was 3.66 times in the pregnant rats than in the virgin animals after the glucose infusion (Figure 1b).

As shown in Figure 2a, white adipose-tissue LPL activity appeared lower in control pregnant rats than in virgin animals. The glucose infusion enhanced LPL activity in the adipose tissue of both groups although

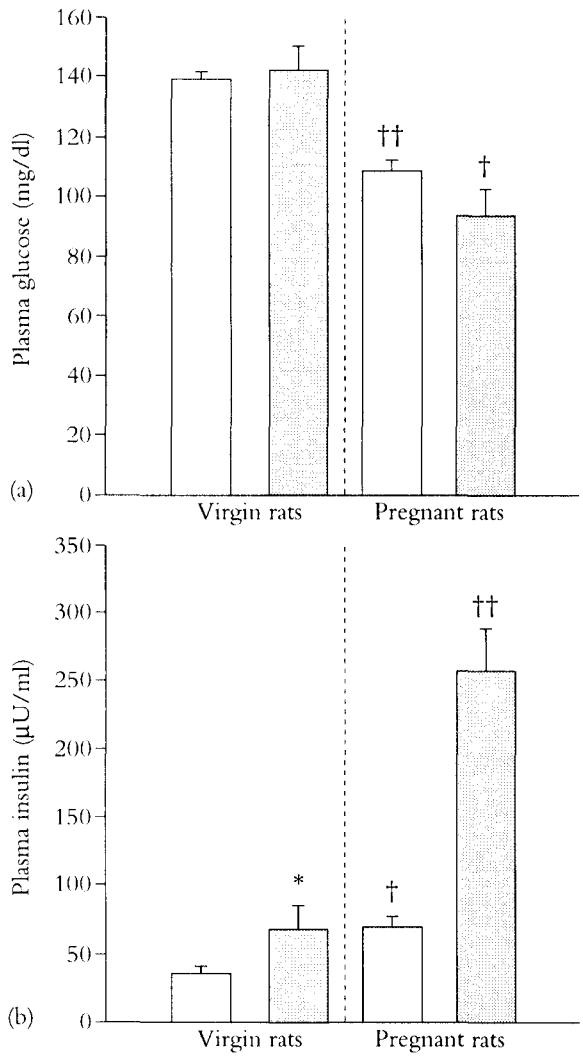


**Figure 1** Plasma glucose (a) and insulin levels (b) in virgin and 20-day pregnant rats at the end of 72 h of continuous intravenous infusion with double-distilled water (hollow bars) or 50% glucose (shaded bars) at the rate of 35 ml/day. Statistical comparison between pregnant and virgin rats, $^{\dagger}p < 0.01$; $^{\dagger\dagger}p < 0.001$, and between glucose and glucose infusions, $^{*}p < 0.05$

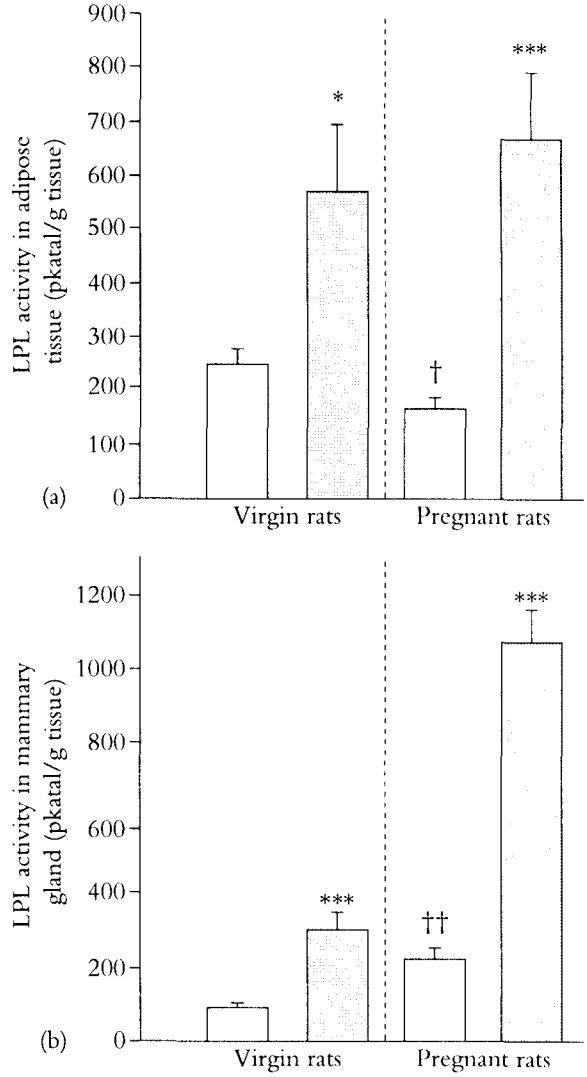


**Figure 2** Lipoprotein lipase activity (LPL) in lumbar adipose tissue (a) and mammary gland (b) in virgin and 20-day pregnant rats at the end of a continuous intravenous infusion with double-distilled water (hollow bars) or 50% glucose (shaded bars) at the rate of 35 ml/day. Statistical comparison between glucose and water infusions, $^{*}p < 0.05$; $^{***}p < 0.001$. Statistical comparison between pregnant and virgin rats, $^{\dagger}p < 0.05$; $^{\dagger\dagger}p < 0.01$; $^{\dagger\dagger\dagger}p < 0.001$

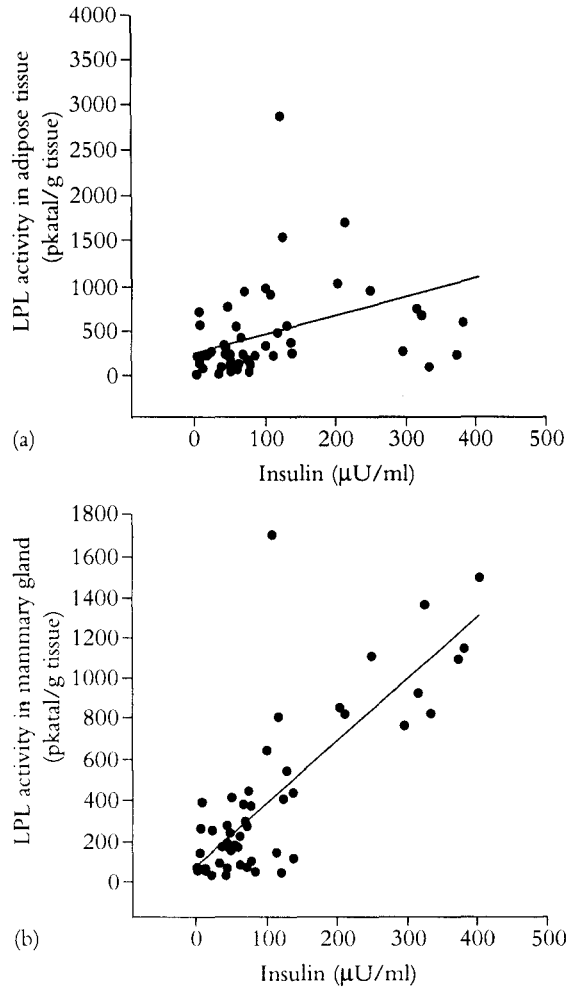


**Figure 3** Linear correlations between lipoprotein lipase (LPL) activity in adipose tissue and plasma insulin (a) ($y = 250.83 + 2.07$ x, $r^2 = 0.1598$, $p = 0.00275$, $n = 54$) and between LPL activity in mammary gland and plasma insulin (b) ($y = 79.04 + 3.05$ x, $r^2 = 0.6387$, $p = 2.1 \times 10^{-12}$, $n = 51$). Constructed with all the individual values used for Figures 1 and 2

the effect was greater in pregnant than in virgin rats, and the differences found in control animals disappeared in animals receiving the glucose infusion. In contrast to the findings in adipose tissue, LPL activity in mammary gland was higher in control pregnant rats than in control virgin animals (Figure 2b). However, the glucose infusion had a similar effect enhancing the LPL activity in mammary gland in both groups, and values attained after the glucose infusions remained significantly higher in pregnant and virgin rats (Figure 2b).

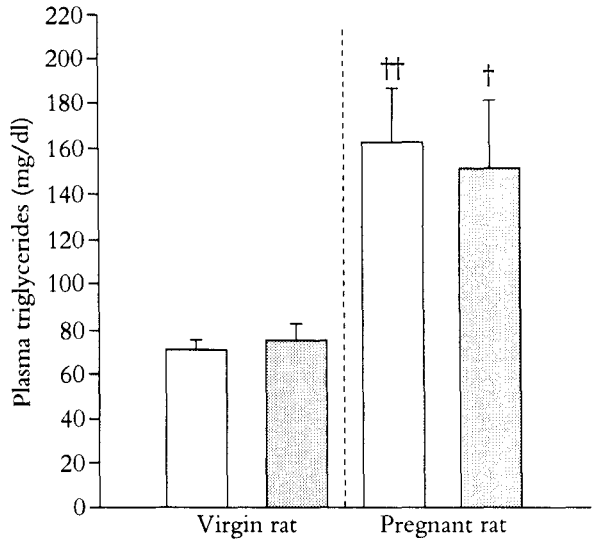


**Figure 4** Plasma triglyceride levels in virgin and 20-day pregnant rats at the end of 72 h of continuous intravenous infusion with double-distilled water (hollow bars) or 50% glucose at the rate of 35 ml/day. Statistical comparison between pregnant and virgin rats $^{\dagger}p < 0.05$; $^{\dagger\dagger}p < 0.001$

Due to the parallel effects of the glucose infusion on LPL activities in both adipose tissue and mammary gland in the two groups, it was considered of interest to calculate the linear correlations between these parameters using all the individual values. As shown in Figure 3a, plotting LPL activity in adipose-tissue values against plasma-insulin levels showed a significant linear correlation with a low correlation coefficient ($r^2 = 0.1598$). A significant linear correlation also appeared when LPL activity values in mammary gland were plotted against the plasma-insulin levels but, in this case (Figure 3b), the correlation coefficient value was high ($r^2 = 0.6387$), indicating a closer relationship between these parameters than the one for adipose tissue.

In spite of the strong enhancing effect of the glucose infusion on LPL activities in both adipose tissue and mammary gland in pregnant and virgin rats, the infusion did not modify the plasma-triglyceride levels. As shown in Figure 4, plasma-triglyceride levels were higher in control pregnant rats than in virgin animals and these values were unaffected by the glucose infusions in either group.

## DISCUSSION

Present findings show that after 3 days of intravenous infusion with high glucose doses in unrestrained rats, both pregnant and virgin rats remain euglycemic and hyperinsulinemic and that LPL activity is greatly en-

hanced after this treatment in both white adipose tissue and mammary gland. From previous reports we know that this experimental protocol causes a sustained hyperinsulinemic condition in the rats during the 3 days of treatment with the glucose infusion, the effect being greater in pregnant than in virgin rats, whereas plasma-glucose levels initially demonstrate a slight rise followed by a decline to basal levels in both groups (Ramos and Herrera, 1995a). The stronger effect of the glucose infusion on plasma-insulin levels in pregnant than in virgin rats concurs with the well-known enhanced sensitivity of β-cells to insulinotropic agents during pregnancy (Martin *et al.*, 1986; Davidson, 1984). We also know that whereas the 3 day glucose infusion reverts the insulin resistant condition in late pregnant rats, it decreases insulin responsiveness in virgin animals (Ramos and Herrera, 1995a). There then exists the possibility that the observed effects are not only a consequence of the changes in circulating insulin levels but also of the changes in tissue responsiveness to insulin. This seems to be specifically true of LPL activity in adipose tissue, where it was found that LPL activity does not correlate well with plasma-insulin levels, as shown by the low although significant correlation coefficient. Under normal conditions, adipose tissue contributes to the insulin resistant condition of late pregnancy in the rat (Leturque *et al.*, 1986) and this idea agrees with the low LPL activity values already seen in the control pregnant rats despite their hyperinsulinemic condition.

The reversion of insulin resistance caused by the prolonged glucose infusion, and not the resulting absolute plasma-insulin values may therefore be the factor responsible for the increased LPL activity observed in adipose tissue in late pregnancy in rats after the glucose infusion; this effect was even greater than in virgin animals. These findings support our earlier suggestion (Herrera *et al.*, 1990) that under normal conditions, insulin resistance during late gestation is responsible for the decreased LPL activity normally seen in maternal white adipose tissue.

In contrast to white adipose tissue, mammary-gland LPL activity is higher in control pregnant than in virgin animals, it increases similarly in both groups to the hyperinsulinemic condition caused by the prolonged glucose infusion, and it correlates very closely with plasma-insulin levels when individual values from all the studied rats are plotted together. All of this indicates that, besides the role of insulin-enhancing LPL activity in mammary gland, which agrees with findings from other groups in non-pregnant conditions (Da Costa and Williamson, 1994; Oller do Nascimento *et al.*, 1989), LPL activity in late pregnancy in the rat is not influenced by the insulin resistant condition normally seen in other tissues during this stage of gestation. This conclusion agrees with the fact that the mammary gland expresses insulin receptors in pregnant rats (Camps *et al.*, 1994), and with our recent finding that the intrinsic autophosphorylation activity of insulin receptors is augmented in mammary gland from pregnant rats (Carrascosa, Ramos, Molero and Herrera, unpublished data). Such normal responsiveness to insulin in mammary gland at late pregnancy occurs even though the insulin-sensitive glucose transporter GLUT4 disappears when GLUT1 becomes the most abundant glucose carrier (Camps *et al.*, 1994). This is probably a consequence of the disappearance of adipocytes in this organ, when epithelial cells become the most abundant cells in it (Camps *et al.*, 1994; Jensen *et al.*, 1991), and may contribute to the normal responsiveness to insulin of the mammary gland during late gestation.

Maternal hyperinsulinemia during late gestation therefore seems to contribute, together with the changes in the sex hormones (mainly, the increase in plasma-prolactin levels), to the induction of LPL activity in mammary gland prior to parturition. This mechanism, together with the decrease in LPL activity in certain maternal tissues, mainly adipose tissue, drives the triglyceride-rich lipoproteins to the mammary gland (Herrera *et al.*, 1994), and allows circulating triglycerides to be hydrolyzed and taken up, thus contributing very actively to the synthesis of milk in preparation for lactation.

On the basis of the increased LPL activity in both white adipose tissue and mammary gland seen here after the prolonged glucose infusion, one would expect enhanced uptake of circulating triglycerides by these tissues and therefore a consequent reduction in plasma-triglyceride levels. This was not, however, the case in either pregnant or virgin rats receiving the glucose infusion (Figure 4). Since LPL activity is modulated in an organ- and tissue-specific manner (for review see Braun and Severson, 1992) it would be expected that the same hormonal stimulus would cause an opposing effect in other tissues. In accordance with this view, we have recently shown that the same prolonged hyperinsulinemic condition caused by a glucose infusion similar to the one used in this study causes significant reductions of LPL activity in the heart and lungs in virgin and pregnant rats and in red fiber skeletal muscle in pregnant rats (Ramos, 1993). These reductions in LPL activity in certain tissues therefore counteract the increases seen here in white

adipose tissue and mammary gland, and, although they would channel circulating triglycerides towards the latter tissues, they do allow plasma triglycerides to be kept within a normal range.

In conclusion, present findings support the notion that during late gestation decreased white adipose-tissue LPL activity is a consequence of the insulin resistant condition that normally occurs at this time, whereas the mammary gland remains acutely sensitive to insulin and maternal hyperinsulinemia which contributes to the induction of LPL activity in this organ. The tissue-specific responsiveness of LPL allows the circulating triglycerides to be driven towards one organ or another even though the plasma triglycerides remain stable.

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