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Role of lipoprotein lipase activity on lipoprotein metabolism and the fate of circulating triglycerides in pregnancy

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The mechanism that induces maternal hypertriglyceridemia in late normal pregnancy, and its physiologic significance are reviewed as a model of the effects of sex steroids on lipoprotein metabolism. In the pregnant rat, maternal carcass fat content progressively increases up to day 19 of gestation, then declines at day 21. The decline may be explained by the augmented lipolytic activity in adipose tissue that is seen in late pregnancy in the rat. This change causes maternal circulating free fatty acids and glycerol levels to rise. Although the liver is the main receptor organ for these metabolites, liver triglyceride content is reduced. Circulating triglycerides and very-low-density lipoprotein (VLDL)–triglyceride levels are highly augmented in the pregnant rat, indicating that liver-synthesized triglycerides are rapidly released into the circulation. Similar increments in circulating VLDL-triglycerides are seen in pregnant women during the third trimester of gestation. This increase is coincident with a decrease in plasma postheparin lipoprotein lipase activity, indicating a reduced removal of circulating triglycerides by maternal tissues or a redistribution in their use among the different tissues. During late gestation in the rat, tissue lipoprotein lipase activity varies in different directions; it decreases in adipose tissue, the liver, and to a smaller extent the heart, but increases in placental and mammary gland tissue. These changes play an important role in the fate of circulating triglycerides, which are diverted from uptake by adipose tissue to uptake by the mammary gland for milk synthesis, and probably by the placenta for hydrolysis and transfer of released nonesterified fatty acids to the fetus. After 24 hours of starvation, lipoprotein lipase activity in the liver greatly increases in the rat in late pregnancy; this change is not seen in virgin animals. This alteration is similar to that seen in liver triglyceride content and plasma ketone body concentration in the fasted pregnant rat. In the fasting condition during late gestation, heightened lipoprotein lipase activity is the proposed mechanism through which the liver becomes an acceptor of circulating triglycerides, allowing their use as ketogenic substrates, so that both maternal and fetal tissues may indirectly benefit from maternal hypertriglyceridemia. Changes in the magnitude and direction of lipoprotein lipase activity in different tissues during gestation actively contribute both to the development of hypertriglyceridemia and to the metabolic fate of circulating triglycerides. Any deviation in these metabolic adaptations occurring in the human mother may have consequences that modify her lipoprotein profile, even postpartum. Hormone-induced changes in pregnancy mirror those seen with oral contraceptive steroids and provide a teleologic rationale for the lipoprotein changes induced by sex steroids. (AM J OBSTET GYNECOL 1988;158:1575-83.)

Key words: Hypertriglyceridemia, lipoprotein lipase, lipoproteins, pregnancy

Maternal hypertriglyceridemia is one of the most striking and consistent changes occurring at late gestation in both humans and experimental animals, but its physiologic significance is not yet completely understood. The fetus does not directly benefit from this maternal condition. The passage of intact triacylglycerol across the placenta has not been detected in any study. It is known that the supraphysiologic increase in circulating triglyceride-rich lipoproteins during pregnancy constitutes a risk of permanent hyperlipidemia postpartum. We review major adaptations of triglyceride metabolism occurring during late gestation to attain a better understanding of the mechanism for the induction of maternal hypertriglyceridemia in normal pregnancy and the physiologic significance of this change. The key role of maternal lipoprotein lipase activity in determining the tissular fate of circulating triglycerides is emphasized. For ethical reasons, most data were derived from studies of the pregnant rat, but some data from pregnant women are also presented to document the validity of the extrapolation from the experimental data.

Material and methods

Studies in the rat. Female Wistar rats from our own colony and weighing 160 to 180 gm were mated at 2
Fig. 1. Change of maternal free-conceptus body weight (solid line) and carcass fat content (dashed line) in the pregnant rat. n = Five to seven rats per group.

Table I. Plasma triglycerides and VLDL lipids in 20-day-pregnant and nonpregnant rats

<table>
<thead>
<tr>
<th></th>
<th>Nonpregnant</th>
<th>Pregnant</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Triglycerides (gm/dl)</td>
<td>76.5 ± 5.6</td>
<td>390.4 ± 61.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VLDL-triglycerides (mg/dl)</td>
<td>31.6 ± 2.5</td>
<td>232.7 ± 25.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VLDL-cholesterol (mg/dl)</td>
<td>2.5 ± 0.1</td>
<td>13.9 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VLDL-triglycerides/VLDL-cholesterol (mg/dl)</td>
<td>12.1 ± 0.4</td>
<td>15.8 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
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</table>

n = Four to seven rats per group. p = Significance of the difference between pregnant and nonpregnant rats.

months of age. Gestational time was estimated by the appearance of spermatozoïds in vaginal smears. Age- and sex-matched virgin animals were always studied in parallel with the experimental animals. All animals were killed by decapitation, and blood collected from the neck wound was processed for plasma metabolite analysis and isolation of lipoproteins by sequential ultracentrifugation. For the analysis of very-low-density lipoprotein (VLDL) subfraction distribution, purified VLDL, corresponding to 1.8 mg triglycerides, was applied to heparin–Sepharose CL 6B columns in 5 mmol/L Tris HCl buffer (pH 7.4) containing 0.05 mol/L NaCl and 0.025 mmol/L MnCl₂, and eluted stepwise with 0.05, 0.12, 0.20, 0.50, and 1.00 mol/L NaCl in 5 mmol/L Tris HCl buffer. Carcass fat content was determined by both body specific gravity and gravimetry of purified lipid extracts. Adipose tissue lipidolysis was determined in lumbar fat pad pieces incubated in vitro in the presence of U-¹³C-glycerol (0.5 μCi/ml), fatty acid–free bovine albumin (10 mg/ml), and glucose (5 mmol/L), as described elsewhere. Liver triglycerides were measured in lipid extracts after removal of phospholipids. For determination of lipoprotein lipase activity, tissues were rapidly excised and placed in liquid nitrogen, and the enzyme was assayed in acetone/diethyl ether extracts as previously described. Values were expressed as a function of protein concentration.

Studies in pregnant women. Twelve pregnant women who registered for obstetric care were studied. Subjects were selected according to the following criteria: (1) values from routine analysis in normal range for gestational period of at least three blood samples obtained during gestation, (2) normal course and outcome of pregnancy, (3) no endocrine abnormalities, (4) single pregnancy, and (5) customary diets and no medication except multivitamin and iron supplementation. All women gave informed consent and were seen during the first and third trimesters of gestation. Blood samples were obtained in tubes containing 1.5 mg/ml
ethylendiaminetetraacetic acid (EDTA) from seated subjects after an overnight fast. After centrifugation, plasma was subjected to ultracentrifugation at \( d = 1.006 \) for VLDL, purification and analysis of VLDL-triaclylglycerol and VLDL-cholesterol (VLDL-C) in a Hitachi 705 automatic analyzer (Hitachi, Tokyo, Japan). Aliquots of VLDL were used for studying heparin-Sepharose profiles, as indicated above. After the blood samples were taken for lipoprotein analysis, heparin was injected intravenously in a dose of 50 IU/kg. Ten minutes after heparin injection a second blood sample was taken into a heparinized tube kept on ice. Plasma was separated and stored at -80°C until assayed. Postheparin lipoprotein lipase was measured by using a stable and specific substrate,\(^{16,17}\) and potential interference of hepatic lipase in the assay was eliminated by assaying parallel samples in the presence of 1.0 mol/L NaCl.

Expression of results. Results are expressed as mean ± SEM; statistical comparison between groups was done by Student t test.

Results and comment

In normal pregnancy maternal fat storage occurs during the early stages of gestation; in later phases an accelerated transfer of glucose and amino acids to the fetus occurs. As shown in Fig. 1, the maternal, conceptus-free body weight of the pregnant rat progressively increases up to day 19 of gestation and then stops; this change is paralleled by the increase in maternal carcass fat content, which clearly declines at day 21. During late gestation an intense extraction of maternal glucose and amino acids by the placenta occurs to support the rapid fetal accretion,\(^{18,21}\) and these changes are responsible for the maternal tendency toward hypoglycemia.\(^{22}\)

Heightened glucose and amino acid extraction and the endocrine changes occurring in the mother have intense effects on the metabolic activity of adipose tissue. As shown in Fig. 2, in vitro lipolytic activity in pieces of lumbar fat pads from 19-day-pregnant rats is higher than activity in controls. This difference is enhanced under fasting conditions, in which greater maternal hypoglycemia\(^{23}\) and reactive endocrine changes, like enhanced catecholamine production,\(^{24}\) stimulate maximal breakdown of maternal fat depot.

The active adipose tissue lipolytic activity during late gestation is responsible for both the arrest in the progressive increment of fat storage seen during earlier phases (Fig. 1) and, as shown in Fig. 3, for the increase of free fatty acid and glycerol levels found in maternal circulation. The liver is the main receptor organ for these lipolytic products,\(^{25}\) and may either metabolize them through different pathways or reesterify them for triglyceride synthesis and later release to circulation in the form of VLDL.

Fig. 2. Rates of in vitro lipolysis (glycerol released) in pieces of lumbar fat pads from fed and 48-hour fasted 19-day-pregnant rats (P) and their nonpregnant controls (NP). Asterisks correspond to the significance of the difference between pregnant and nonpregnant rats (**p < 0.001). n = Six rats per group.

Triglyceride liver content in the rat mother is decreased during late gestation, but circulating triglycerides are greatly enhanced (Fig. 3). These findings indicate that triglycerides synthesized in liver from circulating free fatty acids and glycerol or from endogenous substrates are rapidly released into the circulation. In agreement with this interpretation, it can be seen in Table I that maternal hypertriglyceridemia in the late pregnant rat specifically corresponds to an increase in circulating VLDL-triglycerides, and although VLDL-cholesterol levels are also enhanced, the triglyceride/cholesterol ratio in VLDLs is significantly greater in pregnant than in nonpregnant rats. An enhanced production of triglyceride-rich lipoproteins (presumably chylomicrons) by the intestine from dietary lipids is also seen in late pregnancy in the rat, as indicated by the quicker and greater appearance of labeled lipids in plasma shortly after oral administration of \(^{14}C\)-tripalmitine in 20- and 21-day-pregnant rats compared with controls.\(^{26}\) These findings are also consistent with the increased production of triglyceride-rich lipoproteins in the late pregnant rat, as previously suggested by Childs et al.\(^{27}\)
Fig. 3. Plasma metabolite levels and liver triglyceride content in 20-day-pregnant rats (P) and their nonpregnant controls (NP). Asterisks correspond to the significance of the difference between pregnant and nonpregnant rats (***p < 0.001). n = Six to eight rats per group.

Table II. Plasma VLDL lipids and postheparin lipoprotein lipase activity in pregnant women

<table>
<thead>
<tr>
<th></th>
<th>First trimester</th>
<th>Third trimester</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL-triglycerides (mg/dl)</td>
<td>51.0 ± 5.1</td>
<td>112.8 ± 16.5</td>
<td>&lt;0.001</td>
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<tr>
<td>VLDL-cholesterol (mg/dl)</td>
<td>5.22 ± 0.85</td>
<td>21.92 ± 3.13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VLDL-triglycerides/VLDL-cholesterol (mg/dl)</td>
<td>5.85 ± 0.23</td>
<td>5.28 ± 0.35</td>
<td>NS</td>
</tr>
<tr>
<td>Postheparin lipoprotein activity (mkat/ml)</td>
<td>524 ± 51</td>
<td>71 ± 16</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

n = 12 women per group. p = Significance of the difference between third and first trimesters of gestation.

As shown in Table II, the increase in circulating VLDL-triglycerides and VLDL-cholesterol also occurs in women during late gestation. However, lipoprotein metabolism differs from that in the rat, because the VLDL-triglycerides/VLDL-cholesterol ratio remains unchanged. Although the reason for this species difference is not yet known, it may be caused by the lack of neutral lipid transfer protein in the rat, that is, however, present in humans. This lipid transfer protein is known to mediate the exchange of esterified cholesterol and triglycerides between VLDL and higher density lipoproteins. The absence of this protein in the rat may result in the specific accumulation of triglycerides in the VLDL particles, whereas in women they are more easily transferred to lipoproteins of higher density. This interpretation agrees with our unpublished findings of an enhanced triglyceride/cholesterol ratio in both low-density lipoprotein (LDL) and high-density lipoprotein (HDL) in women in the third trimester of pregnancy. The unchanged VLDL-triglycerides/VLDL-cholesterol ratio in pregnant women also agrees with the fact that the proportional distribution of VLDL subfractions does not change in human pregnancy, as indicated by the heparin-Sepharose elution profile in Fig. 4. We had previously found that this profile was greatly modified in late pregnancy in the rat. The benefits to the mother from the increase in circulating triglycerides is not fully understood. Circulating maternal triglycerides cannot be directly used to
Fig. 4. Heparin-Sepharose column elution profile in 5 mmol/L Tris buffer, pH 7.4, supplemented with 0.05 (A), 0.12 (B), 0.20 (C), 0.50 (D), or 1.00 (E) mol/L NaCl of VLDL (d < 1.006) purified by ultracentrifugation from plasma of pregnant women at different months of gestation. Values of absorbance at 280 nm are indicated by solid lines, and those of triglyceride content per fraction (µg) by dashed lines.

Support fetal metabolic needs, because they cannot cross the placental barrier. We have focused our attention on maternal lipoprotein lipase activity, because this enzyme catalyzes triglyceride hydrolysis in circulating triglyceride-rich lipoproteins and facilitates hydrolytic products uptake by the subjacent tissue. Because this enzyme is normally bound to capillary endothelium and is released by heparin, we measured its activity in plasma from pregnant women 10 minutes after administration of 50 IU/kg intravenous heparin. As shown in Table II, postheparin lipoprotein lipase activity decreases at late gestation. This change exceeds the extent to which lipoprotein lipase would be diluted in a larger plasma volume and occurs when maternal
circulating triglycerides are highest. Our findings indicate either that the overall plasma triglyceride removal capacity by maternal tissues decreases as gestation advances or that the different tissues redistribute their use of circulating triglycerides. To determine how specific tissues participate in this reduced postheparin lipoprotein lipase activity, lipoprotein lipase activity was measured in different tissues in the pregnant rat. As shown in Fig. 5, and as was expected, the highest activity in nonpregnant rats was found in adipose tissue, and the lowest in the liver. During late gestation a significant decline in lipoprotein lipase activity was observed in liver, heart, and adipose tissue, whereas an increase was seen in mammary gland and placenta between days 20 and 21 of gestation. In keeping with the decline in fat stores in late gestation (Fig. 1) and with the reductions in liver triglycerides (Fig. 3), these findings suggest that circulating triglycerides, whether alimentary or endogenous, are diverted from those tissues where lipoprotein lipase activity decreases (liver, heart, adipose tissue) and are taken up by the mammary gland, placenta, and possibly other maternal tissues in which enzyme activity increases.

Changes in lipoprotein lipase activity in specific tissues may have important metabolic implications for both the mother and the fetus. The intense reduction of lipoprotein lipase activity found in maternal adipose tissue during late gestation confirms previous reports from this and other laboratories. Because of the high level of lipoprotein lipase activity present in this tissue as compared with others under nonpregnant conditions (Fig. 5), and because of its high proportional corporeal mass, the reduction in adipose tissue lipoprotein lipase activity must produce a reduction in the clearance of circulating triglyceride-rich lipoproteins. This hypothesis is supported by the diminished uptake of VLDL-triglycerides in isolated adipocytes taken from rats during late pregnancy; this effect and the previously described increase in circulating triacylglycerol production constitute the major factors responsible for the intense maternal hypertriglyceridemia observed during late gestation.

Progressive increments in placental lipoprotein lipase activity both facilitate the hydrolysis of maternal triglycerides and direct the transfer of the released nonesterified fatty acids across the tissue to the fetus, as has already been proposed to occur in different species, including humans.

The low lipoprotein lipase activity in maternal liver may contribute to the maintenance of a low concentration of triglycerides in that organ in the fed state. Inasmuch as we had previously found that lipoprotein lipase activity increased in the liver of newborn rats under fasting conditions and that this change was related to high ketone body production, lipoprotein lipase activity was measured also in the fasted pregnant
As shown in Fig. 6, although 24-hour starvation does not affect liver lipoprotein lipase activity in nonpregnant rats, it causes a marked increase in lipoprotein lipase activity in pregnant rats. A parallel picture is seen in both liver triglyceride and plasma ketone body concentrations. In the pregnant rat, after 24-hour starvation, concentration of these substances increases greatly, whereas a much smaller increment is observed in the nonpregnant rat (Fig. 6). This finding points to the interconnection of these three parameters. Data in the literature show that in the nonpregnant, adult, fasted rat liver, lipoprotein lipase activity may be enhanced by the administration of intralipid, suggesting a substrate inductor mechanism.55

It is not yet known whether the observed liver lipoprotein lipase increment in the fasted pregnant rat has an intrahepatic or extrahepatic origin, but the exaggerated hypertriglyceridemia observed during pregnancy may be proposed as the inductor process. Through this mechanism the liver, which under normal conditions is a triglyceride-exporter organ, becomes a heightened acceptor of circulating triglycerides, thus allowing their increased consumption as ketogenic substrates. Enhanced production of ketone bodies in the fasted state benefits both fetal and maternal metabolism, because the availability of other substrates is limited. Ketone bodies easily cross the placental barrier, and may be used as energy sources by the fetus60; in the mother, ketone body metabolism may compensate for hypoglycemia, particularly in maintaining cerebral oxidative metabolism, as well as for the reduced utilization of circulating triglycerides caused by low lipoprotein lipase activity.

The other maternal tissue in which lipoprotein lipase activity is greatly modified during late gestation is the mammary gland (Fig. 5). In the rat model it was previously found that just prior to parturition maternal hypertriglyceridemia declined and approached pregestation levels.61 Although not tested around parturition, a similar change has been described in pregnant women after delivery.1,4,50 This effect occurred in the rat with no change in adipose tissue lipoprotein lipase activity, the values of which remained very low at this
time, but it did coincide with a further increase in mammary gland lipoprotein lipase activity. The direct relationship between these two changes is supported by our previously reported findings. In the pregnant rat that reduction in circulating triglycerides did not occur when the induction of the enzyme in the mammary gland was blocked by progesterone treatment, which avoids the prolactin peak normally occurring before parturition. We therefore conclude that, around parturition, the increase in circulating triglyceride-rich lipoproteins is not as high as would be expected, or a decline may even occur, because of augmented removal through mammary gland lipoprotein lipase activity. In this way maternal circulating triglycerides are diverted to milk synthesis in preparation for lactation.

We conclude that changes in the magnitude and even the direction of lipoprotein lipase activity in different tissues during gestation actively contribute not only to the development of hypertriglyceridemia but also to the metabolic fate of circulating triglycerides. Through this mechanism several needs of maternal and fetal metabolism are fulfilled. However, any change in these dynamic and intricate metabolic adaptations seen in the mother throughout gestation may directly modify her lipoprotein profile. Under pathologic conditions the alteration may be permanently maintained, thereby increasing the risk for the development of cardiovascular disease. Hormone-induced changes in pregnancy mirror those seen with oral contraceptive steroids and provide a teleologic rationale for the lipoprotein changes induced by sex steroids.

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