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Serum lipid profile in diabetic pregnancy

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Summary

Hyperlipidemia is a common feature in normal pregnancy and consists primarily of triglycerides whose increament during late gestation is distributed into all the lipoprotein fractions, whereas changes in their cholesterol content are more moderate. Diabetes is also generally associated to disturbances in lipoprotein metabolism and has a tendency of developing hypertriglyceridemia rather than hypercholesterolemia. Additive effects would be expected when both diabetes and pregnancy are associated, but the effect varies depending on the lipoprotein fraction that is considered and the type of diabetes. We have found that during pregnancy, insulin treated pregestational or gestational diabetic women have augmented plasma FFA and ß-Hydroxybutyrate levels, but normal lipoprotein profile when compared to healthy controls. Pregnant diabetic women have decreased plasma ß-estradiol levels. Since it is known that estrogens actively contribute to maternal hypertriglyceridemia under normal conditions, it is proposed that the decreased estradiol levels in diabetic pregnant patients impede an exaggerated rise of circulating lipoproteins over the normal range. Overt diabetes in the streptozotocin treated pregnant rat impaires the normal increase in maternal accumulation of fat deposits as a consequence of decreases in both lipogenesis and adipose tissue lipoprotein lipase activity. This condition seems to be responsible for the lower increase in plasma triglycerides during late gestation in the overt diabetic animals as compared to others whose diabetes was circumscribed to the second half of gestation and whose fat deposit accumulation was well preserved. These findings in diabetic pregnant women and in streptozotocin treated rats allow the proposal that a balance between the degree of metabolic control and hormonal dysfuntion (including sex hormones) may determine the development or not of dyslipidemia in diabetic pregnancy.
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Keywords: diabetes, pregnancy, free fatty acids, ß-OHbutirate, estradiol, lipoprotein lipase human, rat.

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Perfil lipídico en la gestación diabética

Resumen

En el embarazo normal se produce una hiperlipidemia que consiste principalmente en un incremento de los triglicéridos al final de la gestación, los cuales aumentan en todas las fracciones lipoproteicas. Sin embargo, los cambios en los niveles de colesterol son más moderados. En la diabetes se produce también frecuentemente una alteración en el metabolismo lipoproteico, con tendencia al desarrollo de hipertrigliceridemia más que hipercolesterolemia. Así pues, cabe esperar que cuando coinciden la diabetes con la gestación se produzcan efectos sumatorios, aunque ello varie en función de la fracción lipoproteica que se considere y del tipo de diabetes. Nosotros hemos observado que durante la gestación, en mujeres con diabetes pregestacional o gestacional, sometidas a tratamiento insulínico, se produce un aumento de los niveles plasmáticos de ácidos grasos libres y de ß-hidroxibutirato, pero un perfil lipoproteico normal, en comparación con lo observado en mujeres gestantes sanas. Las mujeres gestantes diabéticas presentaron unos niveles plasmáticos de ß-estradiol más bajos que los controles. Puesto que los estrógenos se sabe que contribuyen a la hipertrigliceridemia en la gestación normal, se propone que esos niveles bajos de ß-estradiol en las pacientes diabéticas gestantes estarían impidiendo un aumento exagerado de las lipoproteinas circulantes, sobre los niveles normales. Una diabetes intensa en ratas preñadas tratadas con estreptozotocina disminuye el acúmulo de grasas corporales que normalmente se produce en la gestación debido a una disminución de lipogénesis y de actividad de lipoproteina lipasa en el tejido adiposo. Esto parece ser responsable del menor incremento de los niveles de triglicéridos plasmáticos al final de la gestación en estos animales con diabetes circunscrita a la segunda mitad de la gestación, en los que las reservas de grasas corporales habían sido preservadas. Estos hallazgos en mujeres gestantes diabéticas y en ratas tratadas con estreptozotocina permiten proponer que el desarrollo o no de una situación dislipémica en la gestante diabética depende del balance entre el grado de control metabólico y la disfunción hormonal, incluyendo a las hormonas sexuales.
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Palabras clave: diabetes, gestación, ácidos grasos libres, betahidroxibutirato, estradiol, lipoproteinlipasa, humano, rata.

Introduction

During normal pregnancy in women there is an exaggerated increment of circulating lipids. The main component of this manifestation corresponds to triglycerides that, as shown in Figure 1, acquire their highest values during late gestation and return to basal levels shortly after parturition. Similar findings have been previously reported by other investigators (1-6).

The plasma levels of total cholesterol also increases during gestation in women, although the change is less striking than that found in triglycerides Figure (1), and it has been even reported that cholesterol levels in women with heterozygous familial hypercholesterolemia decreased to normal levels during pregnancy (7). Plasma cholesterol levels in the rat increase much less than triglycerides durig pregnancy (8). A decline in plasma cholesterol levels during gestation has been found in monkeys (9) and rabbits (10). The reason for this interspecies difference is not completely understood, although the hypocholesterolemic effect of pregnancy under certain conditions seems to be related with the estrogen induced increase in LDL degradation through LDL receptors. In the rat it has been shown that estrogen treatments reduce serum LDL and this change has been associated with enhanced uptake of LDL by the liver (11, 12).

Increments in plasma triglycerides during late gestation are distributed among all the lipoprotein fractions whereas changes in their cholesterol content are more moderate (3-5, 13).

Diabetes is also generally associated with hypertriglyceridemia rather than hypercholesterolemia (14-16), and therefore summatory effects appear when both pregnancy and diabetes are associated. Although with wide variations, due to the type of lipoprotein fraction that is considered and the type of diabetes one consider, exaggerated increments in plasma tiglycerides rather than choles-

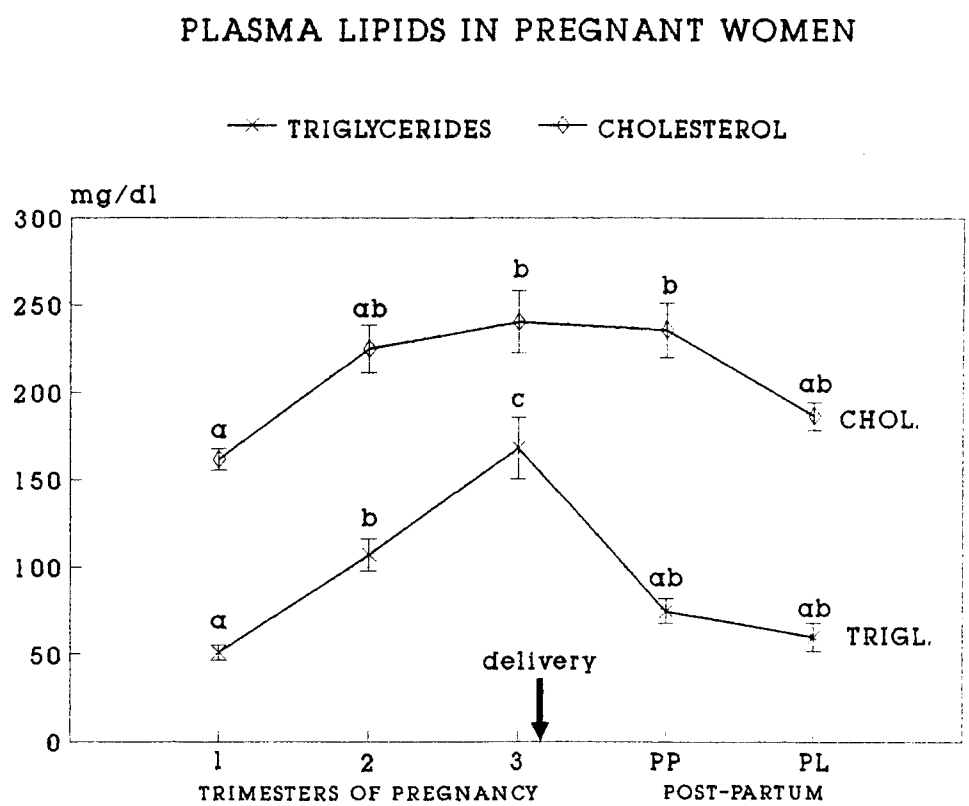

Figure 1 Plasma cholesterol and triglyceride concentration dirung gestation and 2-4 weeks after parturition (PP) and at the end of lactation (PL) in women. Means ± SEM. Statistical comparison between the groups is shown by the letters: for the same parameter, different letters correspond to a significant difference between two time points.

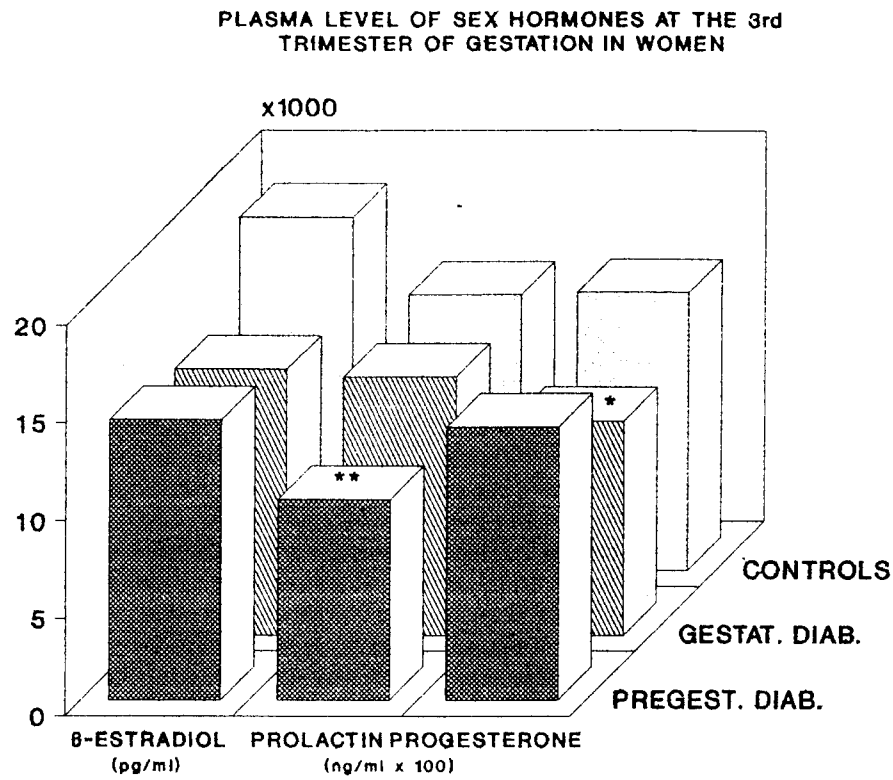

Figure 2 Plasma level of sex hormones at the 3rd trimester of gestation in pregestational diabetic, gestational diabetic and control women. Statistical comparison versus controls is shown by asterisk: *= $p<0.05$, **= $p<0.01$. Other details in ref. 23.

terol have been found in diabetic pregnancy (17-19). There are even conditions where diabetic pregnancy appears associated with normal triglyceride levels (20-22) and even decreased cholesterol levels (18). Although the reason for this variation is not know, there are specific factors which may be responsible, and this paper is addressed to analyzing these possibilities.

Lipoprotein profile in human diabetic pregnancy

To determine how diabetes affects plasma lipoprotein levels in human pregnancy, we have recently carried out a longitudinal study with pregestational diabetic (PDG) women who where under insulin therapy at least during late pregnancy, and who were compared to healthy pregnant women. The women were studied after an overnight fast at the 1st, 2nd and 3rd trimester of gestation an also between 2 and 4 weeks after delivery and after the end of lactation (23). The observed results may be summarized as follows: free fatty acids and ß-Hydroxybutyrate levels were higher in PGD and GD patients than in controls during gestation but this difference disappeared after parturition. Total triglycerides and VLDL-, LDL-, and HDL- triglycerides increased with gestational time in the 3 groups and declined at postpartum, and although total cholesterol and VLDL-, LDL- and HDL- cholesterol also followed a similar trend their rise was less pronounced and the decline after parturition slower than that of triglycerides in the 3 groups with no difference among them. Measuring the triglyceride/cholesterol ratio in the different lipoproteins it appeared that gestation caused an increase in the ratio in LDL and HDL but not in VLDL in all 3 experimental groups. Plasma progesterone, ß-estradiol and prolactin increased very sharply with gestation and declined at postpartum in the 3 groups but when compared to normal controls absolute values for ß-estradiol were significatively lower in PGD patients at the 1st and 2nd trimester, those of prolactin were lower in this same groups at 1st, 2nd and 3rd trimester, and progesterone was lower in GD patients at the 3rd trimester (23).

Figure 2 summarizes the plasma level of these hormones in the 3 groups at the 3rd trimester of gestation values, and it is seen that values for ß-es-

tradiol were lower, although not significantly, in-both PGD and GD patients whereas prolactin levels were lower in PGD patients and the levels for progesterone were lower in GD women than in controls. Other authors have also reported decreased plasma estradiol and prolactin levels in pregnant diabetic women having wide glycemic excursions (24, 25). Due to the known effects of estrogens, which increase VLDL production (26), decrease hepatic lipase activity (27, 28) and increase HDL-triglyceride levels (29), it is proposed that the hyperlipidemia which occurs under normal conditions during gestatiton seems to be primarily driven by the increase in circulating steroid hormones. The decreased level of these hormones in the diabetic women during gestation found in this study may have restrained the development of an overt hyperlipidemic condition in our diabetic pregnant patients. It is therfore proposed that the development or not of a dyslipidemic condition in diabetic pregnancy depends on the balance between the metabolic control and the level of sex hormones.

Post-heparin lypases in pregnancy

In the search for the causes of the changes observed in plasma lipoprotein-triglyceride levels at different stages of gestation, we measured the lipolytic activities in plasma 10 min after the i.v. administration of 50 IU heparin/kg. As shown in Figure 3, it was found that post-heparin lipoprotein lipase (LPL) activity was decreased in the third trimester in all the groups and returned to basal levels after parturition, when plasma lipoprotein-triglycerides wer also normalized. In fact, either VLDL-, LDL- or HDL-triglycerides were weakly but significantly correlated in a linear form with postheparin LPL activity (umpublished results), indicating that the decrease in this enzymatic activity may somehow contribute to the observed changes.

In the course of gestation very intense changes were found in the postheparin hepatic lipase activity, the values of which decreased sharply at the 2nd and 3rd trimester of gestation to increase after parturition. At postpartum and postlactation, postheparin hepatic lipase reached values that were even higher that either one at the 1st trimester in the same woman or in nonpregnant controls. Figure 4 shows the decrease in postheparin hepatic lipase activity found in the 3 groups studied at the 3rd trimester of gestation as compared to the values found at post-lactation. When plotting any of the lipoprotein-triglyceride values from all of the subjects studied over the post-triglyceride values from all of the subjects studies over the post-heparin hepatic lipase activity, a significant multiplicative regression appeared in each case, although the HDL-triglycerides showed the highest correlation coeficient value (umpublished results). These findings suggest a cause-effect relationship between these parameters and although we do not yet know the mechanism which may be involved, the increase in circulating estrogens occurring during late gestattion could be involved, since under nonpregnant conditions these hormones are known to suppress hepatic triglyceride lipase synthesis (27, 28) and consequently its activity and, in addition, an inverse correlation has been reported between this enzyme and plasma HDL-triglycerides after estrogen treatment in postmenopausal women (29, 30). Although more experimental support is required, it could be proposed that during late gestation the HDL catabolism is reduced, which would cause the elevation observed in HDL despite augmented VLDL-triglycerides and result in a picture which differs from that of other hypertriglyceridemic states.

Effects of overt diabetes on lipid metabolism in pregnancy

A similar difficulty to the one found here in observing any change in the plasma lipoprotein profile in diabetic pregnant women has also been found by other authors. Besides the reasons commented above, the metabolic control of the patients may also influence their potential response in whatever lipoprotein metabolism is concerned. Actually, the non-insulin-dependent diabetics (type II) who had elevated plasma glucose and HbA1c values were found by Hollingsworth and Grundy (19) to have higher total triglycerides and VLDL-triglycerides during late gestattion than insulin-dependent diabetic patients or normal women.

Because of this difficulty and in order to understand the mechanism which produces the high triglycerid levels in certain diabetic pregnant women we were interested in determining how overt diabetes might affect lipid metabolism in pregnancy, and this forced us to find and adequate experimental model (31). Female Wistar rats were i.v. treated with 45 mg of streptozotocin per kg and 24 h later they began a daily treatment of a s.c. injection of 1.5 IU insulin/100 g body wt. They were mated 8 days after the streptozotocin treatment and

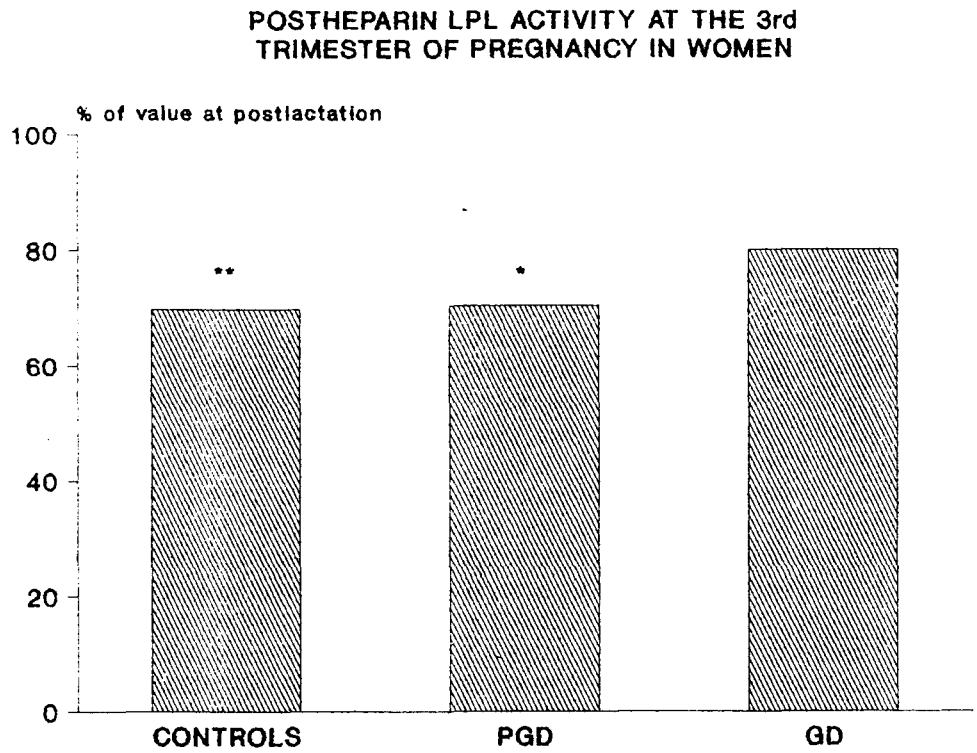

Figure 3 Lipoprotein lipase activity 10 min after i.v. heparin administration (50 IU/kg) in pregestational diabetic (PGD), gestational diabetic (GD) and control women at the 3rd trimester of gestation versus post-lactation. Statistical significance versus values at post-lactation in the same women is shown by asterisk (**= $p<0.01$, *= $p<0.05$). Methodological details as previously reported (refs. 6 and 23).

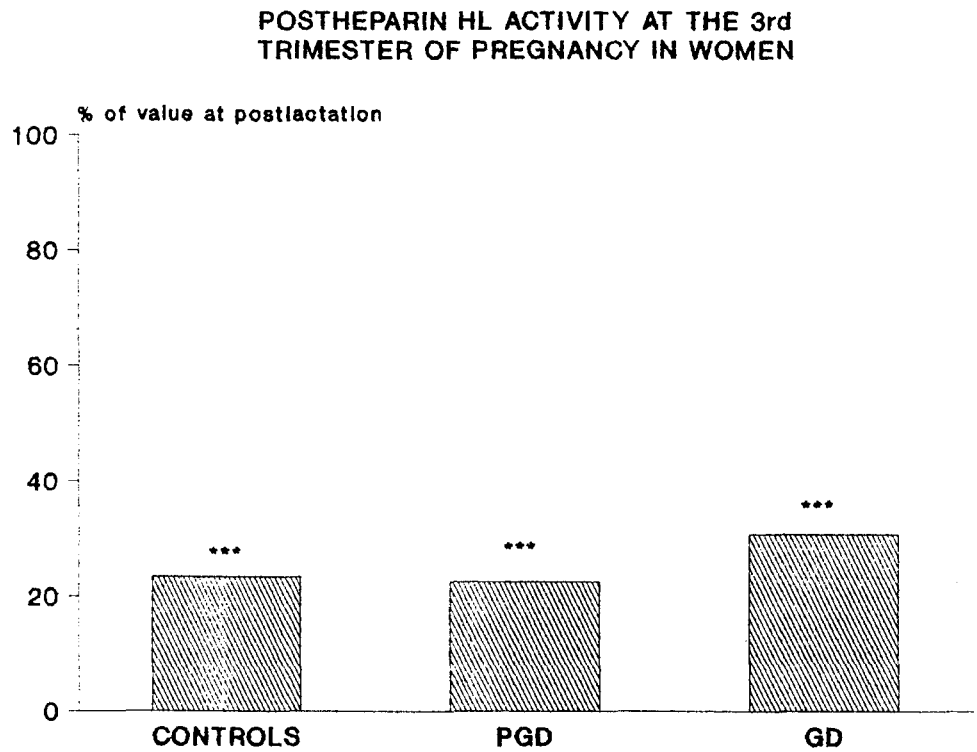

Figure 4 Hepatic lipase activity 10 min after i.v. heparin administration (50 UI/kg) in pregestational diabetic (PGD), gestational diabetic (GD) and control women at the 3rd trimester of gestation versus post-lactation. Statistical significance versus values at post-lactation in the same women is shown by asterisk (***= $p<0.001$). Methodological details as previously reported (refs. 6 and 23).

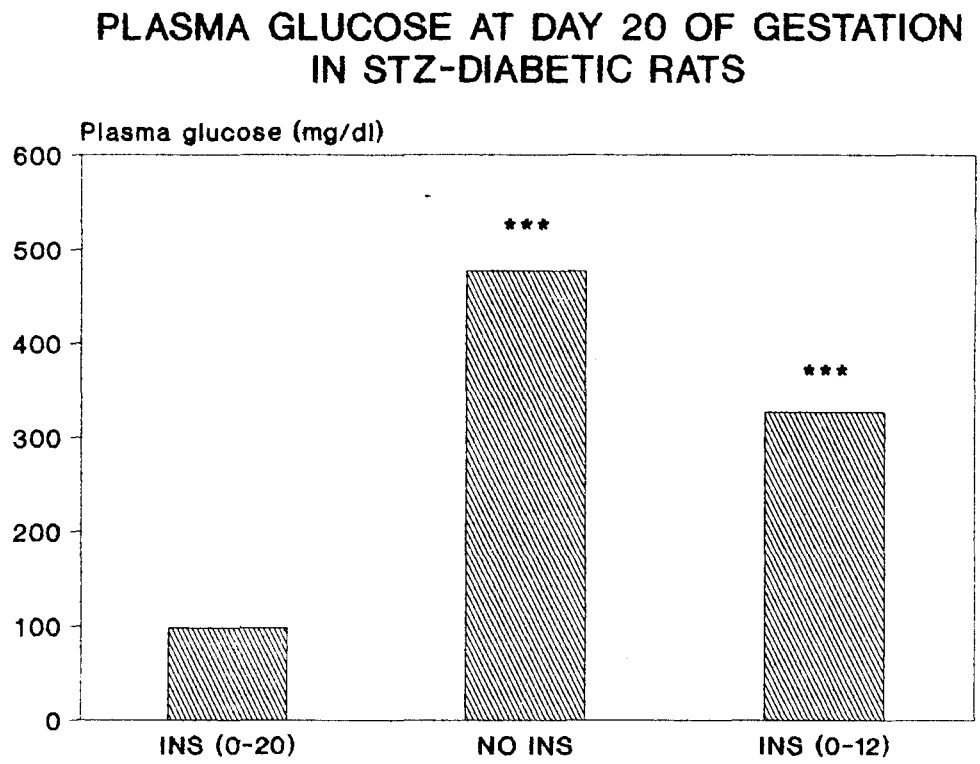

Figure 5 Plasma glucose levels at day 20 of gestation in streptozotocin-diabetic rats receiving or not insulin therapy for various periods of time (see text). Statistical comparisons versus rats receiving insulin treatment for the whole experiment (INS 0-20) in shown by asterisks (***= $p<0.001$).

from that time on they were either untreated or kept under daily insulin treatment for different periods of time.

As shown in Figure 5, streptozotocin treated animal studied at day 20 of gestation develop intense hyperglycemia (NO INS) when compared to rats receiving insulin therapy for the whole experiment (INS (0-20)). Figure 5 also shows that when streptozotocin diabetic ras were kept on insulin only until the 12th day were significantly higher than in rats receiving insulin treatment for the whole time (INS (0-20)) but lower than those not receiving insulin treatment ($p<0.05$).

Plasma triglycerides at day 20 of gestation in the streptozotocin diabetic rats not treated with insulin for the whole gestational period were higher than in those receiving insulin therapy for the whole time. However, as shown in figure 6, the increase in plasma triglycerides occurring during the last half of gestation (from day 12 to 20) was greater when the diabetes was circumscribed to the second half of gestation (INS 0-12d) than in rats not treated with insulin (NO INS) for the whole gestational period (Figure 6), who had higher plasma glucose levels (Figure 5). Because of its intrinsic basic interest and because this finding may allow some of the controversial results reported at times in diabetic pregnancy in women to be understood, it deserves to be analyzed.

A certain proportion of plasma triglycerides are derived from those free fatty acids that, after being released by adipose tissue, reach the liver where they are reesterified and return to circulation in the form of VLDL (32). We know that under normal conditions all these metabolic stages are accelerated during late gestation (6, 33-35) although some of the changes are affected by (or depend on) events that have taken place at earlier gestational stages. Adipose tissue lipolytic activity appears enhanced from mid gestation up to parturition, as shown by both the progressive increase in plasma FFA and glycerol levels and the increase in hormone sensitive lyp ıse activity already found

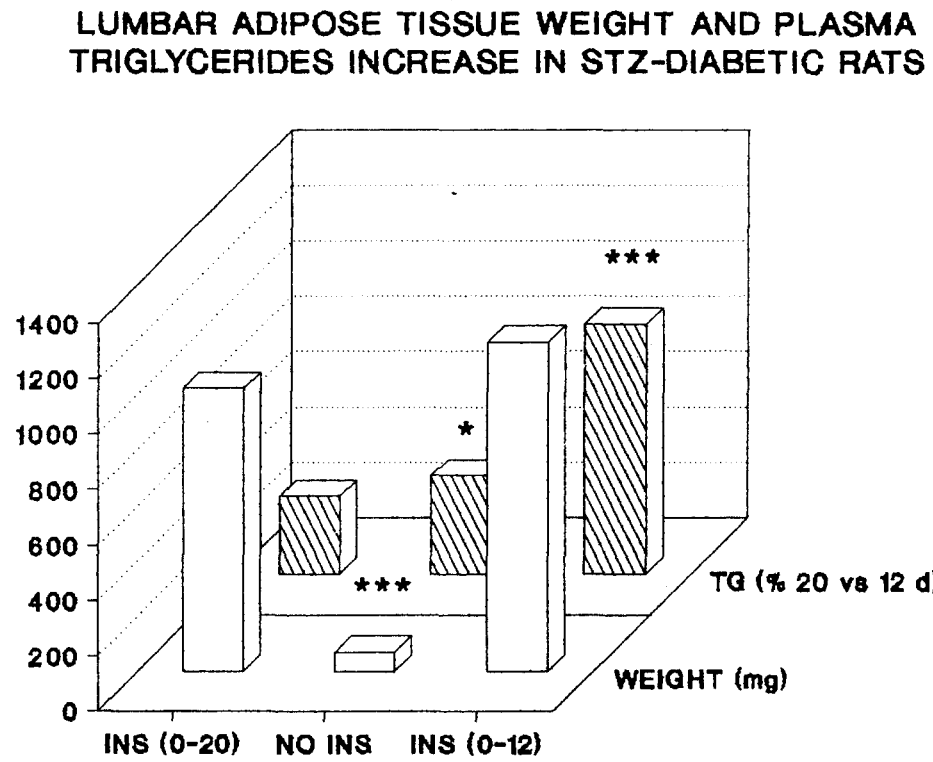

Figure 6 Lumbar adipose tissue fresh weight at day 20 of gestation and plasma triglycerides increase between days 12 and 20 of gestation in streptozotocin-diabetic pregnant rats receiving or not insulin therapy for various periods of time (see text). Statistical comparison between the groups as in figure 5.

at this gestational stage (36). This sustained lipolytic activity in adipose tissue during the second half of gestation is supported by the enhanced fat depots which are accumulated during the earlier stages of pregnancy (6, 37). This accumulation of fat depots during the first half of gestation is greatly impaired in rats which are kept diabetic for that period and in whose fat depots become practically exhausted during the second half of pregnancy (Figure 6). It is therefore proposed that this depletion of fat stores restrains the availability of FFA in the liver for triglyceride synthesis and is responsible for the increase in their plasma levels being lower than that found when animals were not diabetic during the first half of gestation and their fat depots were normal (Figure 6).

Two main factors, which may be impaired in diabetics, contribute to the anabolic state of maternal adipose tissue under normal conditions in early gestation. One is enhanced lipogenesis (38) and the other is increased LPL activity which would facilitate hydrolysis and uptake of plasma triglycerides associated to either chylomicrons or VLDL (39). These two parameters are enhanced at day 12 of gestation in the rat (40) and may be induced by the changes in circulating insulin that occur during this early gestational phase when the sensitivity of the ß-cell is already enhanced but insulin sensitivity has not yet begun the deterioration it will experience during late gestation (41).

In the diabetic condition both lipogenesis and LPL activity in adipose tissue at mid-gestation could be greatly reduced and may be responsible for the impairment in the fat depot accumulation in the mother. This possibility has been directly tested in the case of LPL activity which, as shown in Figure 7, we found to be greatly reduced in lumbar adipose tissue in both 12 and 20 day pregnant streptozotocin diabetic rats (NO INS) as compared to those receiving insulin therapy for the whole experiment (+INS).

We see, therefore, that the metabolic adaptations which occur during the first half of gestation influence those that take place during late gestational phases, when fetal development is accelerated and its needs for metabolic fuels and plastic and essential substrates are greatly enhanced (40). De-

velopment of exaggerated hypertriglyceridemia during late gestation in the diabetic mother causes an accumulation of triglycerides in the placenta. We know that differently from FFA, maternal triglycerides do not directly cross the placental barrier although the presence of LPL in this tissue may hydrolize them and the released FFA may reach the fetus for reconversion into triglycerides (42). This hypothesis is supported both by the fact that, as shown in Figure 8, despite placental impermeability to maternal triglycerides, the pattern of changing triglyceride level is similar in fetal and maternal plasma, and, also, by the linear correlation between maternal triglycerides and newborn body weight that other authors have found in pregnant women (43), which is consistent with the tendency of offspring of diabetic pregnant women to be heavier.

Summary

A characteristic feature of normal gestation is the progressive increase in plasma triglycerides. This change corresponds not only to VLDL-triglycerides but also to LDL-and HDL-triglycerides. Although there are reports which show an increase in all of these lipoprotein fractions in diabetic pregnancy, this is not the case in pregnant women whose glycemic levels are well controlled. Since, on some occasions, pregnant diabetic women have decreased plasma ß-estradiol levels, and it is know that estrogens actively contribute to maternal hypertriglyceridemia under normal conditions, it is proposed that those decreased estradiol levels in pregnant diabetic patients impede an exaggerated rise of circulating lipoproteins over the normal range.

Besides other factors responsible for the development of maternal hypertriglyceridemia during late gestation, postheparin lipoprotein lipase and hepatic lipase activities show both a decrease and a significant inverse correlation with these lipoprotein-triglycerides. This correlation is specially striking in the case of postheparin hepatic lipase versus HDL-triglycerides, suggesting a cause-and-effect relationship.

Overt diabetes in the streptozotocin treated pregnant rat impairs the normal increase in mater-

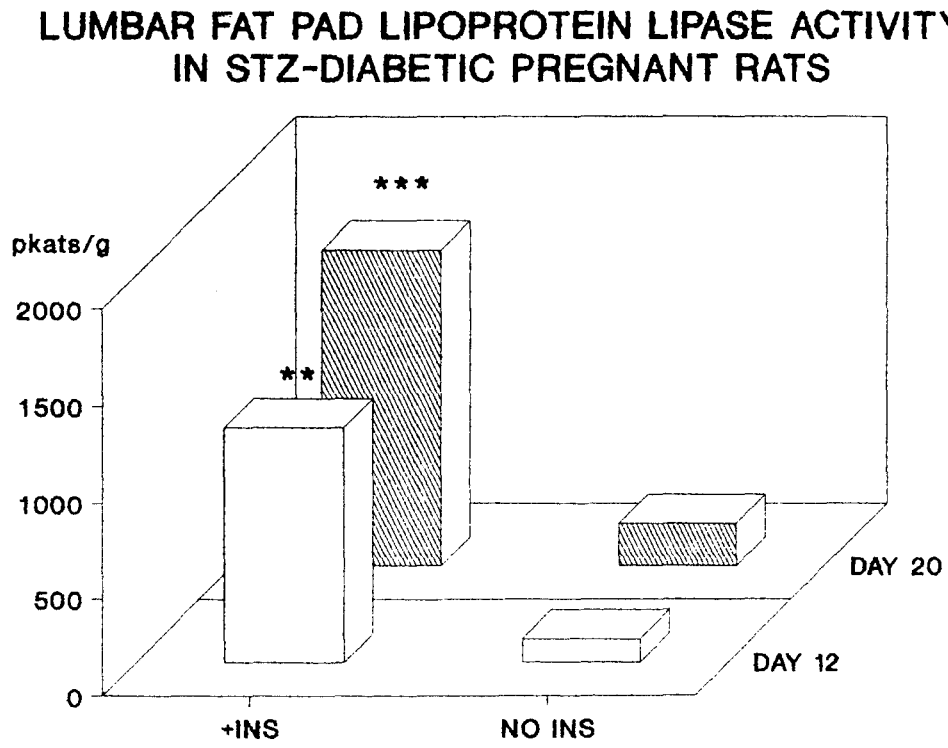

Figure 7 Lumbar fat pad lipoprotein lipase activity in streptozotocin-diabetic pregnant rats receiving (+INS) or not insulin therapy (NO INS). Statistical comparison between the groups for the same gestational day is shown by asterisk (**= p<0.01, ***= p<0.001). Lipoprotein lipase determination as in ref. 6.

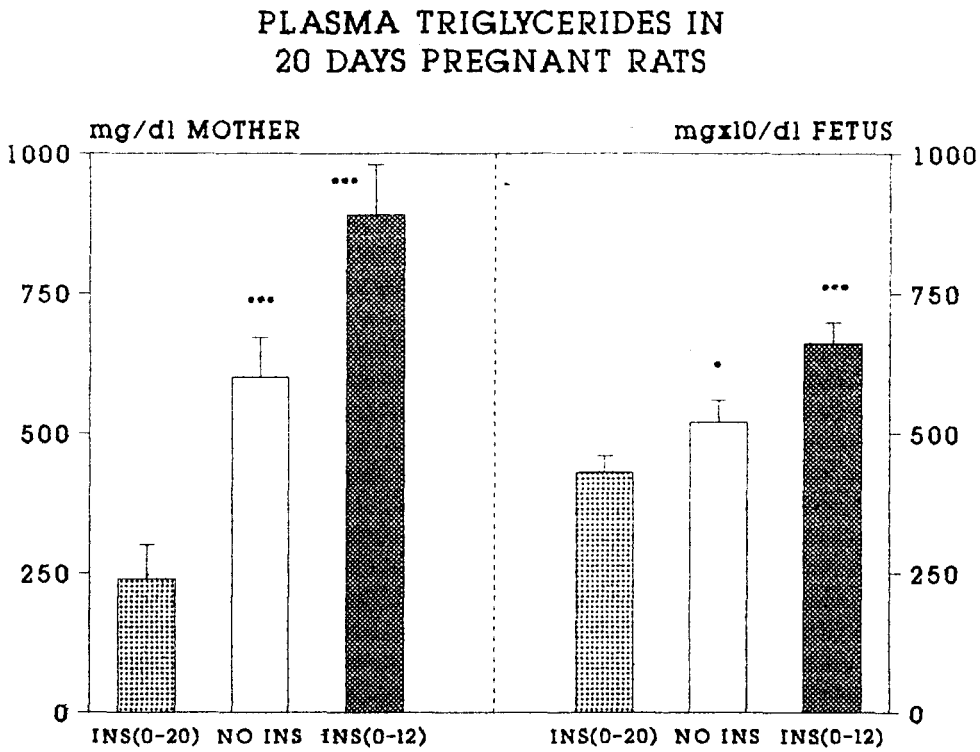

Figure 8 Plasma level of triglycerides in 20 day pregnant streptozotocin-diabetic rats receiving or not insulin therapy for different periods of time and in their fetuses. Means ± SEM. Statistical comparisons versus rats on insulin therapy for the whole time (INS (0-20)) is shown by asteriks (*= p<0.05, **= p<0.01, ***= p<0.001).

nal accumulation of fat depots as a consequence of decreases in both lipogenesis and lipoprotein lipase activity. This condiiton seems to be responsible for the lower increase in plasma triglycerides during late gestation in these animals as compared to those in which diabetes was circumscribed to the second half of gestation and fat depot accumulation was well preserved.

Maternal triglycerides do not cross the placental barrier although the presence of lipoprotein lipase activity in the placenta permits their hydrolysis and releases FFA which become available to the fetus. This hypothesis fits with the linear correlation found between maternal and fetal triglyceride concentrations in the diabetic pregnant rat and the known tendency of offspring of hypertriglyceride pregnant women to be heavier.

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