DIFFERENT RESPONSES TO MATERNAL DIABETES DURING THE FIRST AND SECOND HALF OF GESTATION IN THE STREPTOZOTOCIN-TREATED RAT

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ABSTRACT. To understand the mechanism of exaggerated hypertriglyceridemia in diabetic pregnancy, streptozotocin-treated rats receiving a daily insulin supplement were mated with normal males and divided into four groups: i) kept under this regime until the 20th day of gestation (DI+II), ii) the same regime until the 12th day of gestation (DI), iii) the insulin treatment was suspended during the first half of gestation (days 0–12) and then restored on a daily basis until the 20th day (DII), and iv) no insulin treatment was given after mating (D). All animals were studied on day 20. Despite increased food intake, maternal conceptus-free body weight was greatly reduced in the D animals as compared with the other groups whose values did not differ. Both the plasma glucose and β-hydroxybutyrate levels were increased more in D than in DI rats and values in both groups were greater than in the others. Insulin levels showed an opposite trend to that of glucose, but the values in DI+II rats were higher than in untreated intact control rats (C). The plasma triglyceride concentration was highest in the DI rats, followed by the D group whose values were still significantly higher than in either C or DI+II rats. Plasma free fatty acid levels were lower in D than in any of the other groups, although they were also lower in DI+II and DI than in C animals. Adipose tissue lipoprotein lipase activity was highest in DI+II animals and their values were very similar to those found in DII, whereas the values in the C, D and DI animals were all similar and much lower. Results indicate that reductions in fat accumulation during the first half of gestation impair the activation of lipolytic activity in the severe diabetic mother during late gestation. During this period lipolysis helps sustain maximal hypertriglyceridemia, which develops in animals whose diabetes was circumscribed to the second half of gestation. In general, our findings show that anabolic changes during the first half of gestation affect metabolic events during late gestation.

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During late gestation in normal pregnancy there is an increase in maternal circulating triglycerides (TG) both in humans (1–3) and in rats (4–7). In addition to hyperphagia (6) and enhanced absorption of dietary lipids (8), hypertriglyceridemia is the result of an increase in the hepatic TG production (9–11) and a reduction of circulating TG uptake by adipose tissue (12) caused by reduced lipoprotein lipase (LPL) activity (13–15). The liver, which is the major destination for products of adipose tissue lipolysis (16), partially converts them into TG which return to circulation in very low density lipoprotein (VLDL) particles (17). The enhanced maternal liver TG production (9–11) is facilitated by the enhanced adipose tissue lipolytic activity that occurs during late gestation (18,19). When a human or rat mother is diabetic the hypertriglyceridemia is exaggerated (20,21). Neither the factors that cause this manifestation nor the specific effects it may have on the perinatal complications known to occur in diabetic pregnancy are yet well established (22), but it is well known that supraphysiologic increases in plasma

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lipoproteins may cause permanent derangement in lipid lipoprotein metabolism in the mother (23). Using thyroidectomized pregnant rats we previously found that when maternal hypothyroidism is present during the first half of gestation but not during the second half, normal maternal metabolic changes during the last phase of gestation are impaired, including the normal reduced net increase in the conceptus-free maternal body weight (24). This finding indicates that fat deposited during the first half of gestation may be responsible for several of the metabolic adaptations that normally occur during late gestation. To determine whether maternal diabetes in the first or second half of gestation also alters normal metabolic response during late gestation, and to attain a better understanding of the factors responsible for exaggerated maternal hypertriglyceridemia in pregnancies complicated with diabetes, streptozotocin (STZ)-diabetic rats were mated and treated with substitution doses of insulin for different periods before being studied on day 20 of pregnancy.

MATERIALS AND METHODS

Animals and Experimental Design

Female Wistar rats from our own colony, weighing 160–180 g, were housed in a temperature-controlled room (21–23°C) with 12-h light-dark cycles and fed a Purina Chow diet (Pamlab, Spain). After an overnight fast some animals were treated i.v. with 45 mg/kg STZ (Sigma, USA) dissolved in 50 mM citrate buffer pH 4.5. They were refed and 24 h later a urinary glucose test was performed. Only rats showing glucosuria were included in the study, and these were treated s.c. with insulin bovine ultralente monocomponent insulin, (Novo-Nordisk, Denmark) 1.5 IU/day per 100 g body weight. After 7 days of treatment these rats were mated with normal males, and positive pregnancy was estimated by the appearance of sperm in vaginal smears. From this time on insulin treatment was maintained in some pregnant rats for either the whole study (DI+II), until the 12th day of gestation (DI), or else the treatment was interrupted on day 0 of pregnancy in other animals that were kept without treatment either for the whole study (D) or up to the 12th gestational day at which time daily insulin treatment was restored until sacrifice (DII). Untreated age-matched nondiabetic pregnant rats were studied in parallel as controls (C). All animals were killed by decapitation; the blood was collected into heparinized tubes and the plasma was kept at –20°C until processed.

Processing and Analysis of the Samples

The two uterine horns were immediately excised and weighed with their contents to obtain the whole conceptus weight. This value was subtracted from the rat's total body weight before sacrifice to obtain the net maternal body weight. Lumbar fat pads were rapidly excised and placed into liquid nitrogen, and stored at –80°C until processed for LPL activity, assayed in acetone/diethyl ether extracts as previously described (25). Plasma samples were used for glucose (26) and β-hydroxybutyrate determinations (17) in Somogyi supernatants (28); other plasma samples were used for TG (Menatest, Italy) and free fatty acid (FFA) (29) assays by enzymatic procedures and for insulin radioimmunoassay (30) with a specific rat kit generously provided by Novo (Denmark).

Statistical Analysis

Mean values of five to six rats/group are given. The significance of difference between the means of the two groups was obtained with Student's *t* test.

RESULTS

Our study aimed to determine whether maternal diabetes during specific periods of gestation affects TG elevation during late gestation. Fig. 1 summarizes plasma glucose and insulin levels on the 20th day of gestation in all the animals as an index of their diabetic condition. It can be seen that the STZ-diabetic pregnant rat (D) had remarkably high plasma glucose and reduced plasma insulin levels, compared with either normal untreated pregnant controls (C) or pregnant rats that were treated with STZ but received a daily insulin injection throughout the whole study (DI+II). Fig. 1 also shows that when STZ-diabetic rats were kept on insulin only until the 12th day of gestation [but not later (DI)], plasma glucose levels on the 20th day were significantly higher and plasma insulin significantly lower than in intact controls (C), although the change was milder than that found in the D animals. After insulin treatment, only during the second half of gestation (day 12–day 20, DII) did both the plasma glucose and insulin levels appear similar to those found in the control group (C) (Fig. 1). No difference was found between the plasma glucose levels in the STZ-diabetic rats that received a daily insulin injection throughout the whole study (DI+II) and in the controls (C), but insulin levels were significantly higher in DI+II. This indicated that insulin treatment caused a permanent hyperinsulinemic condition in these animals since samples were collected 24 h after the last insulin injection.

As shown in Fig. 2, the overt diabetic condition of the STZ-treated rats that did not receive insulin (D) caused a marked reduction in maternal weight when

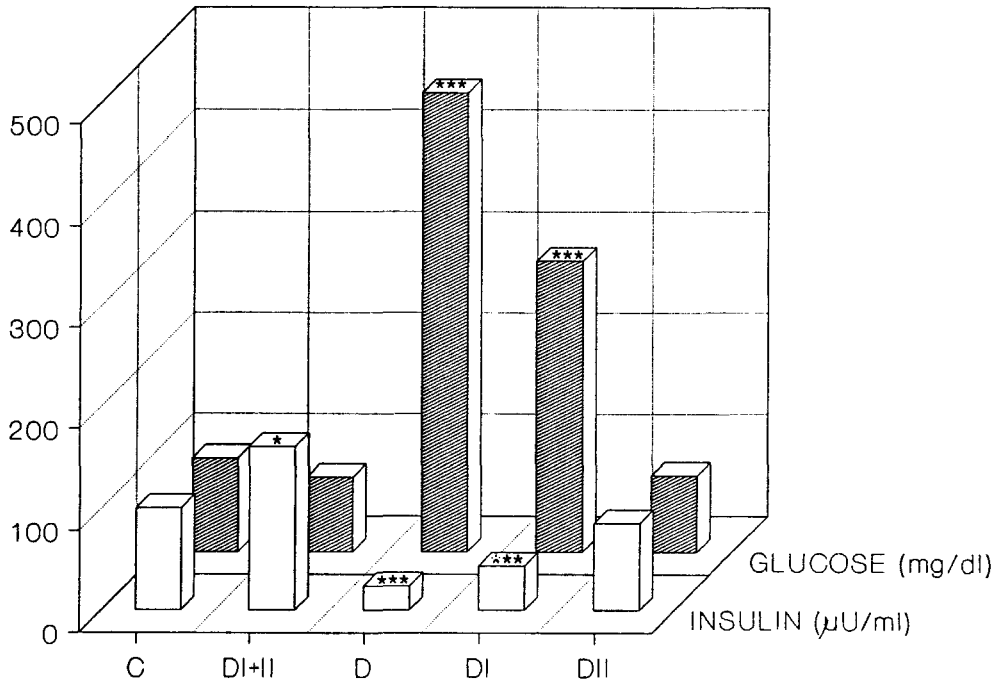

Fig. 1. Plasma glucose and insulin in STZ-diabetic pregnant rats receiving a daily insulin supplement (1.5 IU/100 g) for the entire study (DI+II), until the 12th day of gestation (DI), or when treatment was suspended during the first half of gestation (days 0–12) and then restored on a daily basis until the 20th day (DII) or no insulin was given after mating (D). Non-diabetic pregnant rats were used as controls (C), and all animals were studied on day 20 of gestation. *P <0.05, **P <0.01, *** P <0.001, compared with group C.

compared with the other groups, which corresponds not only to a reduction in the conceptus weight but also in maternal structures (net conceptus-free maternal weight). These changes were not caused by reductions in food intake since we specifically found that total daily food consumption in the D animals was even higher than in controls (data not shown). All the insulin treatment schedules caused a normalization in net maternal weight, whereas conceptus weight was reduced in both D and DI+II animals (Fig. 2).

Fig. 3 summarizes the values of the plasma concentration of lipid components in the different groups. TG levels were significantly higher in STZ-diabetic pregnant rats not receiving insulin (D) than in the C animals (Fig. 3). The values were even higher in rats that received the insulin treatment during the first half of pregnancy (DI) despite their hyperglycemia being milder than in the D group. Plasma TG levels, however, in DII and DI+II animals were similar to those in the C group (Fig. 3). An opposite trend to that seen for TG was found in plasma FFA levels in which the lowest values were found in the D animals; the plasma FFA levels were similar in DI and DI+II animals but still significantly lower than in C, whereas values in DII did not differ from those of normal controls (Fig. 2). The levels of β-hydroxybutyrate were highest in the untreated group of pregnant diabetic rats (D) and relatively similar among the other four groups. There was no statistical difference between the untreated normal group (C) and the diabetic group that received insulin therapy during the second half of pregnancy (DII), whereas in those that received insulin therapy during the first half of pregnancy only (DI), β-hydroxybutyrate levels were higher (P <0.05), and in those receiving the insulin supplement during the whole study (DI+II) they were lower (P <0.05) than in C animals.

To establish whether differences in circulating TG could be related to adipose tissue LPL, the activity of this enzyme was determined in lumbar fat pads. As shown in Fig. 4, LPL activity was much higher in DI+II rats than in normal controls. LPL activity values were slightly but significantly lower in adipose tissue of both D and DI than in C, whereas they were higher in DII (P <0.05).

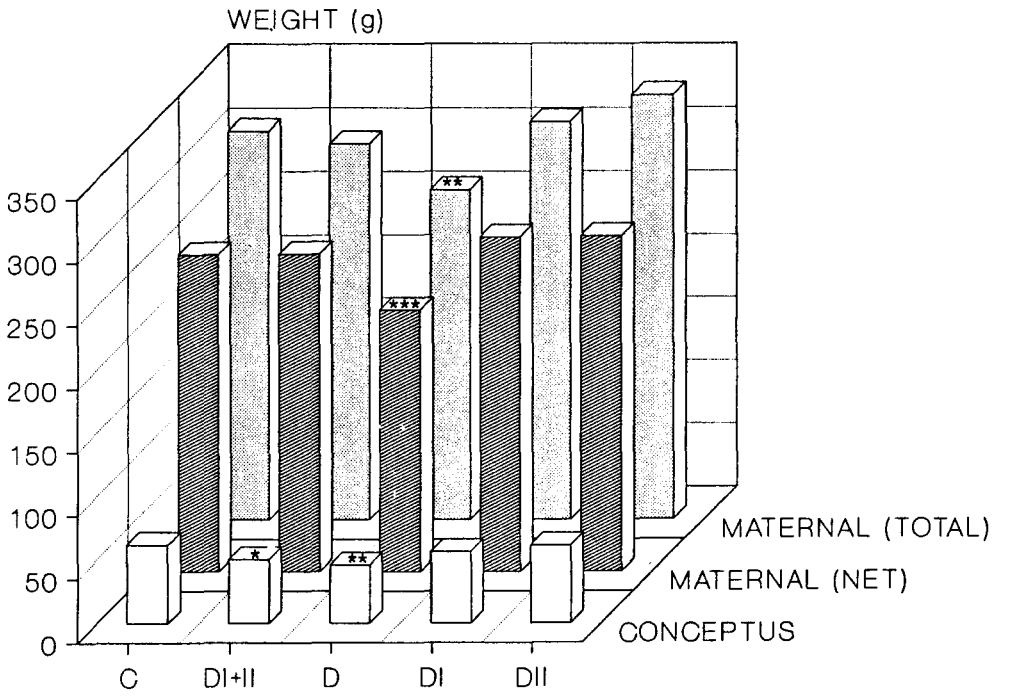

Fig. 2. Total body, net conceptus-free maternal and conceptus weights in STZ-treated 20-day pregnant rats and controls. Group identification and statistical comparisons as in Fig. 1.

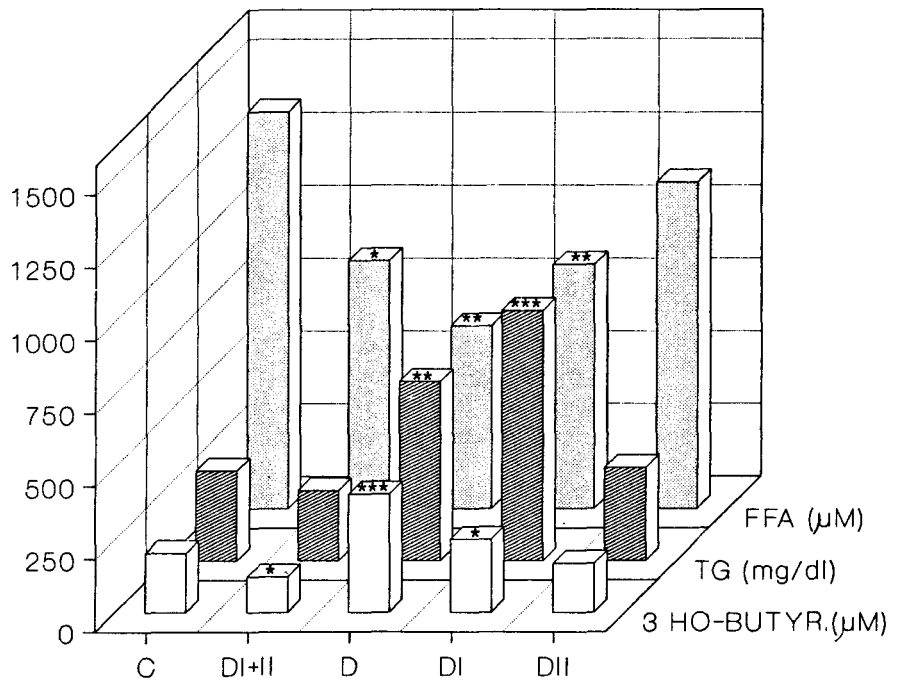

Fig. 3. Plasma levels of FFA, TG and β-hydroxybutyrate (3 HO-butyr.) in STZ-treated 20-day pregnant rats and controls. Group identification and statistical comparisons as in Fig. 1.

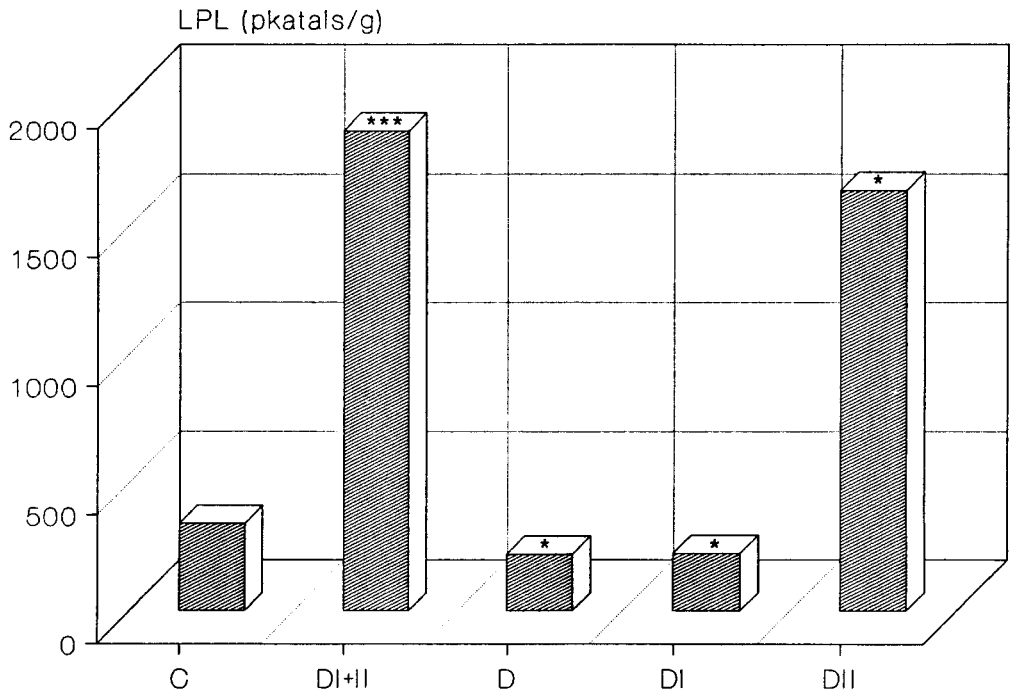

Fig. 4. Lumbar adipose tissue LPL in STZ-treated 20-day pregnant rats and controls. Group identification and statistical comparisons as in Fig. 1.

DISCUSSION

Besides confirming maternal diabetes as a cause of exaggerated hypertriglyceridemia during late gestation, our study with STZ-diabetic pregnant rats shows that this effect is further enhanced when the mother receives insulin supplementation treatment during the first half of pregnancy (DI), but not when this treatment is circumscribed to the second half of pregnancy (DII). Only a few reports of changes in circulating lipids during diabetic pregnancy have been published and, therefore, the mechanism whereby maternal hyperlipidemia is developed in this situation is not well established. Since most of the maternal body mass increment during gestation corresponds to fat deposition (31), the intense reduction in this parameter found here in the overtly diabetic mother indicates that lipolytic activity is exaggerated. However, this condition produces a decrease rather than an increase in plasma FFA levels, probably due to their accelerated removal by the liver. This interpretation is sustained by the elevation in plasma β-hydroxybutyrate and TG that was found. Under normal conditions, as shown in Fig. 5, the liver is the major acceptor of the products of adipose tissue lipolysis, FFA and glycerol (16), and subsequent to their conversion to acyl-CoA and α-glycerol phosphate, they are used for TG synthesis and returned to circulation as VLDL. FFA may be used for β-oxidation, and plasma ketone body levels are an index of this. Plasma levels of both TG and β-hydroxybutyrate are known to rise in nonpregnant diabetics (32,33), indicating an augmented flow through these two pathways. Present findings in the diabetic pregnant rat would therefore suggest that these pathways are also enhanced in the late pregnant diabetic rat. Reduced extrahepatic use of circulating TG-rich lipoproteins is known to play a role in maternal hypertriglyceridemia under normal conditions due to retarded LPL activity in adipose tissue (12–15). However, LPL activity was only slightly modified in the untreated diabetic pregnant rat, indicating that this parameter does not contribute to the specific changes caused in plasma TG under this condition.

Our results show that these metabolic changes in the diabetic pregnant rat are all completely abolished when the animals received insulin treatment during either the entire gestation or during the second half only, but not when the treatment was circumscribed to the first half of gestation. This indicates that insulin deficiency during late gestation is the major inductor of the observed changes. Despite their milder diabetic condition the diabetic rats treated with insulin during the first half of gestation had a greater degree of hypertriglyceridemia than animals that received no

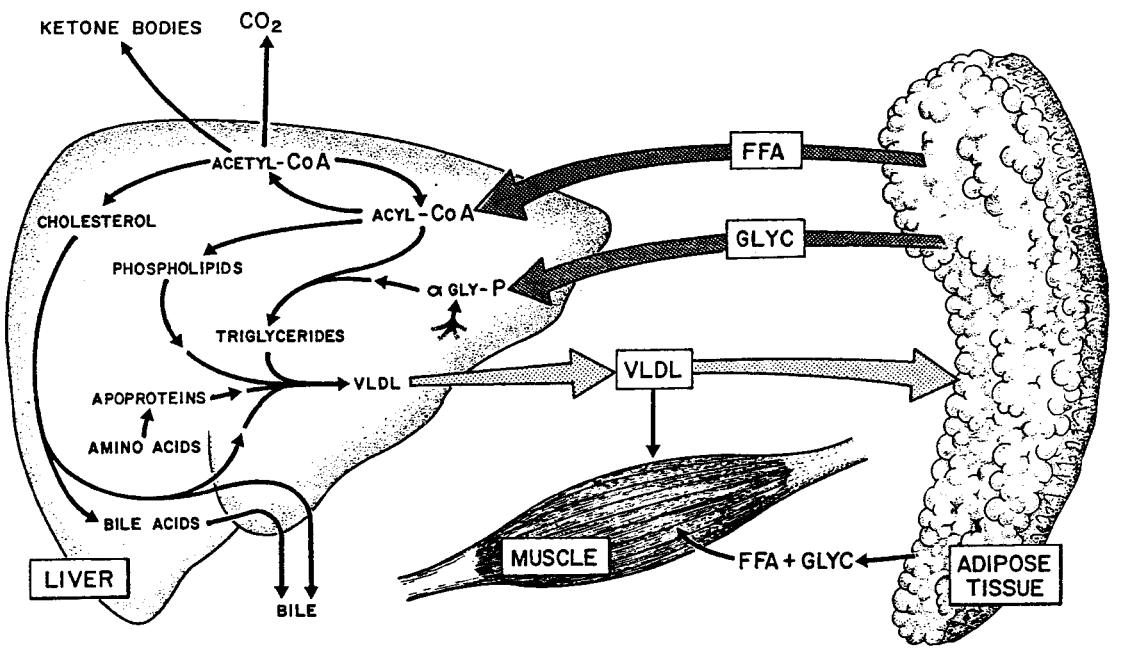

Fig. 5. Major metabolic pathways for the use of adipose tissue lipolytic products, FFA and glycerol, under normal conditions.

treatment. The preservation of adipose tissue mass in these animals during the anabolic phase of gestation compared with diabetic animals may have provided them with sufficient substrates to maintain an exaggerated FFA flow to the liver during the catabolic phase of gestation. Therefore, it may be possible that although lipolytic activity may be enhanced as in the other two groups, the higher availability of substrates in the diabetic animals treated with insulin during the first gestational phase, compared with those that did not receive treatment, allowed the former to release more FFA into circulation. This interpretation agrees with the higher plasma FFA levels found in this same group compared with the overtly diabetic animals. However, on the basis of the reduced circulating β-hydroxybutyrate levels in the former, it may also be argued that FFA removed by the liver when insulin deficiency is not so intense are mainly driven to TG synthesis rather than to β-oxidation. More direct experiments are required to determine which of these possibilities is more correct.

The last aspect that deserves comment in light of the present results is the enhanced LPL activity found in adipose tissue of the STZ-diabetic rats that were treated with insulin during the whole gestational period or during the last half of gestation only. In these animals plasma glucose levels were similar to those in normal pregnant controls, but insulin levels were either increased or normal even though insulin treatment was given 24 h earlier, indicating that during that period animals were subjected to a hyperinsulinemic environment. Since insulin is known to be a major effector of adipose tissue LPL activity, our findings suggest that the hyperinsulinemia in the STZ-treated pregnant rats receiving exogenous insulin may be responsible for augmented adipose tissue LPL activity. This hypothesis is also supported by more direct experiments carried out recently by us (34), and indicates that the decreased insulin sensitivity generally found in normal pregnancy (35–37) is responsible for reduced maternal adipose tissue LPL activity.

In conclusion, besides describing major changes that occur in lipid metabolism during gestation in experimental diabetes, our results show the role of anabolic changes occurring in the mother during the first half of gestation, which permit normal metabolic responses during the last phase of gestation. This conclusion agrees with a similar one obtained in thyroidectomized pregnant rats treated with thyroxine for different periods of gestation (24), indicating that any hormonal disturbance occurring in the mother during the first half of gestation that affects her normal metabolic adaptations may modify the

quantity of maternal substrates available to fetuses during the last phases of gestation and thereby influence the fetal development.

This article is dedicated to the memory of Professor Norbert Freinkel, our dear friend and great teacher.

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