Chapter 1

CARBOHYDRATE-LIPID INTERACTIONS IN PREGNANCY

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I. INTRODUCTION

During the first two thirds of gestation, coinciding with a minimal weight accretion by the conceptus, the mother prepares herself to maintain the rapid fetal growth that takes place during the last trimester of pregnancy. In fact, during early gestation maternal weight gain is greater than the simple intrauterine growth and weight gain. In this phase, the mother conserves more exogenous nutrients whenever she eats, and this anabolic condition is especially manifested in her accumulation of fat deposits. In the later part of gestation, the rapid fetal growth is sustained by the placental transfer from the mother of a variety of substrates; of these glucose is the most abundant. This intense loss of nutrients from maternal circulation is not compensated by her hyperphagia and seems to contribute to a switch to a net catabolic state which is especially evident in adipose tissue and becomes accelerated when food is withheld. Under this condition, exaggerated ketogenesis and gluconeogenesis actively contribute to the availability of fuels to the fetus.

The present chapter reviews these carbohydrate-lipid interactions and analyzes the role of maternal body fat accumulation during the early part of pregnancy in the metabolic adaptations during late gestation. The consequences and intimately related aspects of these interactions are several of the other changes that occur in the maternal/fetal relationship such as the development of maternal hypertriglyceridemia, changes in the maternal amino acid metabolism, placental metabolite transfer, etc. These topics are reviewed in the other chapters of this book.

II. CARBOHYDRATE METABOLISM DURING LATE PREGNANCY

Under basal conditions, maternal hypoglycemia develops after short or prolonged fasting periods during late gestation in both women[1,2] and rats.[3] Since the steady state of a circulating metabolite is the result of the balance between production and utilization, and because glycogen stores are depleted under fasting conditions, this hypoglycemic tendency could be the result of decreased gluconeogenesis, or an enhanced rate of glucose utilization, or both.

Maternal gluconeogenesis depends on both the absolute availability of precursors and on the activity of gluconeogenic enzymes. The circulating level of gluconeogenic substrates fluctuates during fasting in late pregnancy. In the pregnant fasted rat plasma levels of lactate or pyruvate do not fall below levels observed in virgin animals[4–6] and, while profound reductions in gluconeogenic amino acids have been reported,[4,5] other substrates can even increase, as is the case with glycerol.[4–6] The activity of key gluconeogenic enzymes from both the liver and the kidney is enhanced in the pregnant rat,[7]

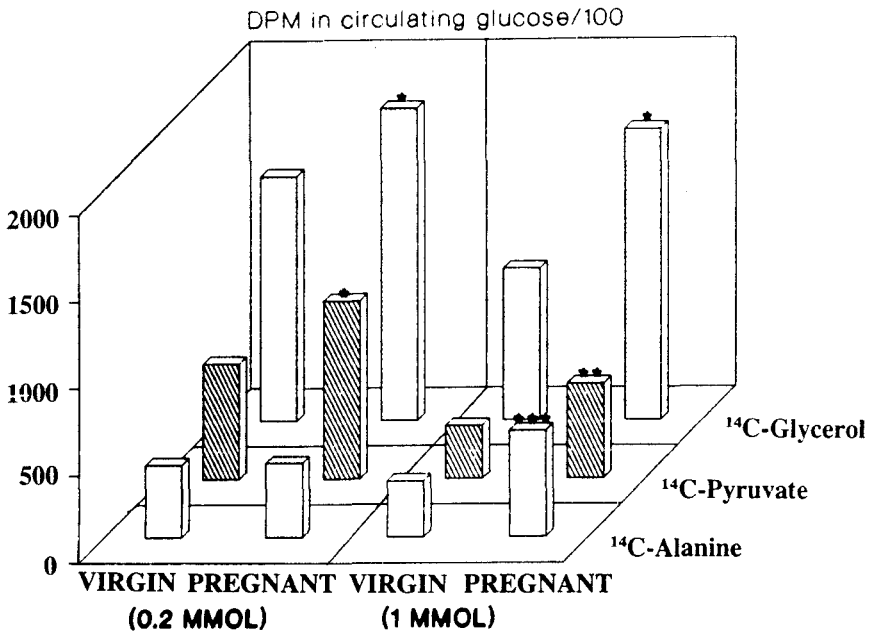

FIGURE 1. Appearance of ^{14}C-glucose in blood 5 min after the intravenous administration of 0.2 or 1 mmol of the correspondent uniformally labeled ^{14}C-tracer to 24-h fasted, 21-d pregnant rats or virgin controls. Other methodological details are described elsewhere.[5] Statistical significance between pregnant and virgin rats is shown by asterisks (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

and we have found that after the *in vivo* administration of either tracer or substrate amounts of labeled precursors, their conversion into glucose in the fasted pregnant rat is always greater than in virgin controls.[3,5,8,9]

Figure 1 shows the comparative *in vivo* conversion of equimolecular amounts of either ^{14}C-pyruvate, alanine, or of glycerol into circulating glucose when given to 24-h fasted,21-d pregnant rats or virgin controls. Glycerol is seen to be more efficiently converted into glucose than any of the other two substrates and gluconeogenetic activity from either pyruvate or glycerol always appears higher in pregnant than in nonpregnant animals. However, when chemically small amounts (0.2 mmol) of the different tracers are given to the rats the synthesis of glucose from alanine is the same in pregnant as in virgin animals, whereas it is enhanced when either pyruvate or glycerol are examined. This result agrees with the low concentration of circulating amino acids but not of pyruvate or glycerol commented on above found at late pregnancy, since a reduced availability of a specific substrate may restrain its actual entrance into a pathway even though the activity of its key enzyme might be enhanced. In the case of pregnancy this hypothesis is supported by the results found when larger amounts of each tracer are given, 1 mmol (Figure 1).

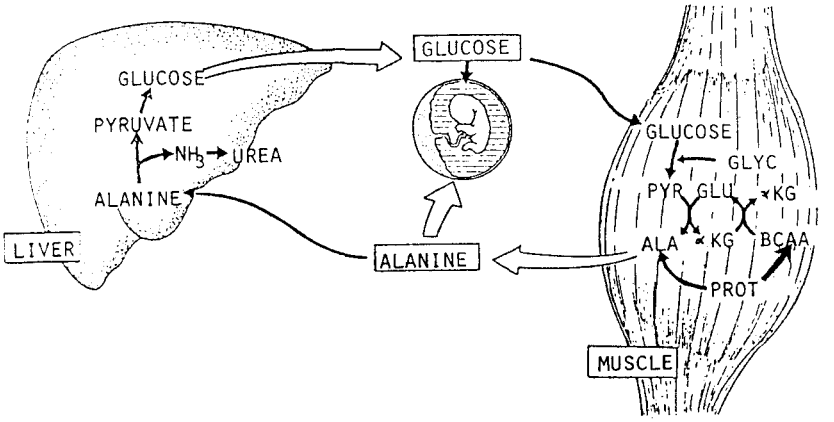

FIGURE 2. Schematic representation of the glucose-alanine cycle in the fasting condition modified in pregnancy by the presence of the conceptus. The size of the arrow corresponding to the flux of alanine to the fetus has been proportionally magnified to indicate that the transfer of this and other amino acids occurs against gradient, whereas that of glucose is carried out by facilitated diffusion.

Under this condition the conversion of each administered substrate, including alanine, into glucose is greater in pregnant than in virgin animals. This greater amount of tracer may have compensated for the differences of endogenous alanine levels in overcoming their potentially limited use as an efficient gluconeogenic substrate in the pregnant rat.

Two clear conclusions may then be reached from these findings. The first is that although gluconeogenesis is enhanced in the late-pregnant fasted rat, alanine (and probably other gluconeogenic amino acids as well) is not used as a preferential substrate in this pathway because of its reduced availability. The amino acids can transfer across the placental barrier, even against the gradient,[10] and this is what makes their concentrations in fetal plasma higher and more stable than in maternal plasma (References 11 and 13; Chapter 3 of this book). As shown in Figure 2, the resulting glucose-alanine cycle between liver and skeletal muscle in the mother is interfered with the presence of the fetus. The maternal capacity to convert circulating alanine into glucose is limited by the active transfer of alanine through the placenta, whereas at the same time, a considerable proportion of the newformed glucose is also transferred to the fetus and becomes less available for maternal tissues. It is then obvious that maintenance of enhanced maternal gluconeogenesis requires an important source of alternative substrates.

The second conclusion that may be reached from our experiment on *in vivo* gluconeogenesis in late-pregnant rats is that the most efficient in its conversion into glucose of the three substrates studied is glycerol. This conclusion is not surprising since, as shown in Figure 3, unlike the pathways for the other substrates, this pathway from glycerol up to glucose does not require

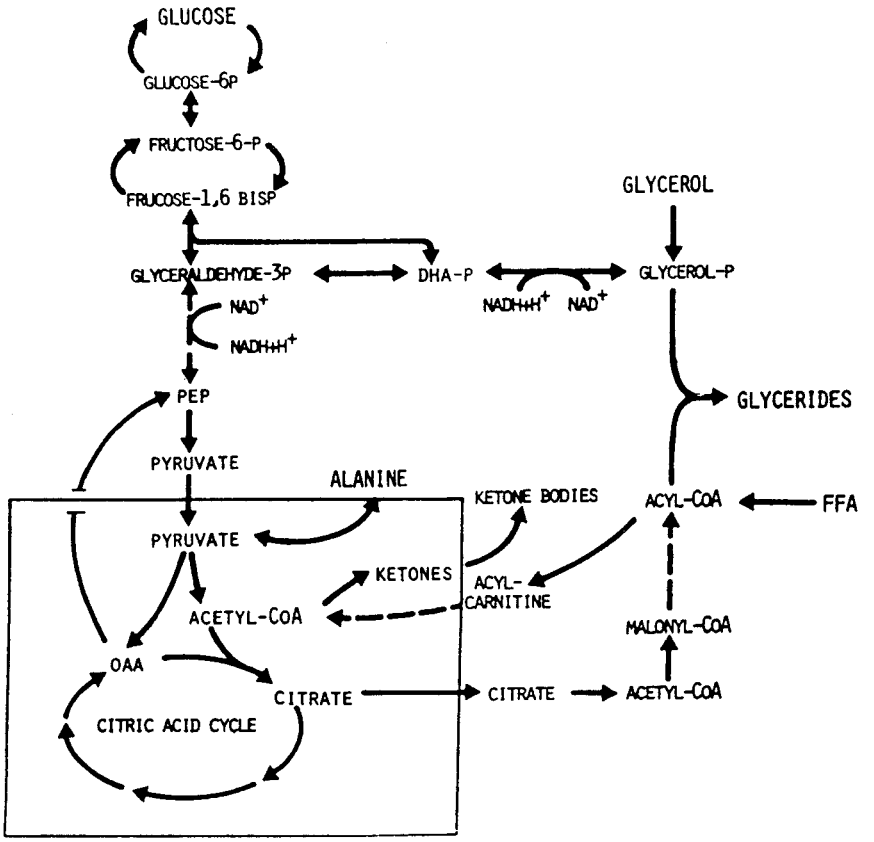

FIGURE 3. Major carbohydrate-lipid interactions in liver. PEP = phosphoenolpyruvate; OAA = oxaloacetate. Dashed lines correspond to several reaction pathways.

intramitochondrial steps. This, together with the high glycerokinase activity in liver and kidney cortex[14] and the physicochemical characteristics of glycerol which may contribute to its short half-life, allow this metabolite to be rapidly converted into glucose,[5] thus becoming an efficient gluconeogenic substrate in pregnancy where its circulating level is enhanced. The enhanced use of glycerol as an efficient alternative gluconeogenic substrate in pregnancy is even seen in the fed condition where the use of other substrates is, however, unchanged.[15,16]

An enhanced glucose utilization seems also to contribute to maternal hypoglycemia. The rates of glucose utilization found in several species, including humans, rats, guinea pigs, rabbits, and sheep, have always been higher in pregnant than in nonpregnant females.[17] This effect specifically corresponds to glucose utilization by the conceptus which represents 30 to 50% of overall maternal glucose utilization,[18-21] even though the rate of

glucose utilization by maternal tissues is lower in late pregnancy than in nonpregnant animals.[17,21] The importance of the fetal consumption of maternal metabolites may be inferred from the quality and quantity of their placental transfer. Our previous studies[13,22,23] on the maternal-fetal transfer of a variety of substrates in the late-pregnant rat placenta *in situ* have shown that the importance of glucose transfer is much higher than for any of the other substrates studied, including alanine, palmitic acid, and glycerol. This preponderance of placental glucose transfer is justified because, as will be commented in Chapter 8, glucose is a major substrate for the fetal economy, one which the fetus cannot synthesize from placentally transferred substrates under normal conditions.[24]

III. CONSEQUENCES OF MATERNAL HYPOGLYCEMIA ON ADIPOSE TISSUE LIPOLYTIC ACTIVITY

Due to the intense transfer of glucose to the fetus, pregnancy therefore constitutes one of the few physiological situations in which mild dietary deprivation elicits meaningful drops in blood glucose. This action seems to be responsible for the heightened catecholamine excretion found during late gestation in the fasting rat,[25,26] since the adrenal medulla is selectively activated by reductions in blood sugar.[27,28]

The increased sympatho-adrenal activity may be responsible for the accelerated mobilization of the fat depot in response to fasting that has long been recognized as one of the metabolic characteristics of late pregnancy.[29] Other additional factors,such as the gestational hormones which are released by the placenta and ovary, must also contribute their share to the enhanced lipolytic activity in maternal adipose tissue since this effect is also present under fed conditions[30,31] whereas catecholamine excretion over 24 h does not vary in pregnant and nonpregnant rats under *ad libitum* feeding.[22]

A. FATE OF THE LIPOLYTIC PRODUCTS

Figure 4 summarizes major pathways present in the adipose tissue metabolism. Enhanced adipose tissue lipolysis increases the release of both free fatty acids (FFA) and glycerol into maternal circulation where they reach high plasmatic values.[5,6,32] The placental transfer of these two lipolytic products is low,[33,34] particularly in the case of glycerol, which results in their respective concentrations in fetal circulation being much lower than those seen in maternal blood.[32] As indicated by their specific rise in plasma after hepatectomy in both the nonpregnant and the late pregnant rat,[35,36] the liver is the main receptor of these two lipolytic products. In the liver, fatty acids and glycerol are converted into their active forms—acyl-CoA and α-glycerol phosphate, respectively. Acyl-CoA derivatives are either used for esterification in the synthesis of glycerides or for degradation to acetyl-CoA and ketone body synthesis through the β-oxidation pathway; α-glycerol-phosphate is used for

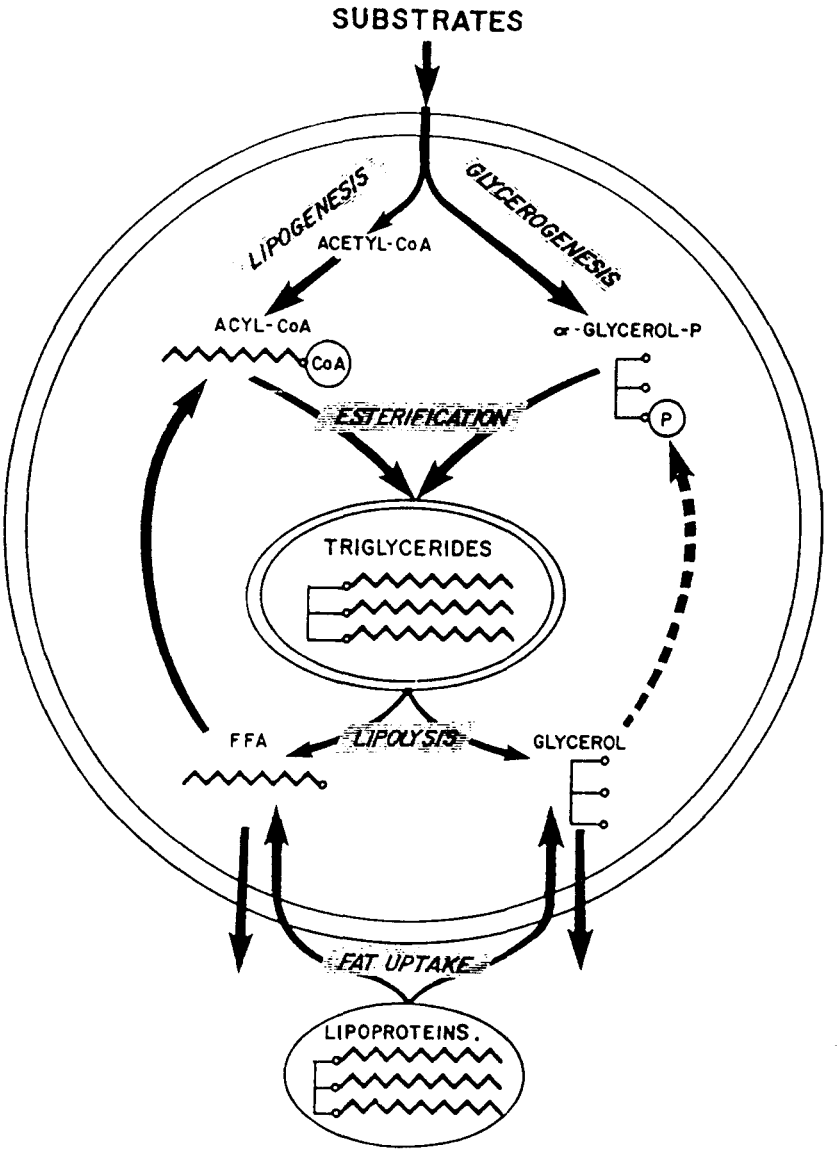

FIGURE 4. Major pathways in adipose tissue. Although adipose tissue is able to convert glycerol in its active form (α-glycerol-P) for reesterification,[66] this is done in a very small proportion[67] as compared to the conversion of FFA into their acyl-CoA derivatives. This differentiation is indicated in the figure by the dotted line between glycerol and α-glycerol-P.

glyceride glycerol synthesis or gluconeogenesis (Figure 3). As we have previously reported, gluconeogenic substrates in the liver, such as alanine and pyruvate, may be channeled at the level of dihydroxyacetone phosphate to

the synthesis of α-glycerol-phosphate, which is used for the esterification of fatty acids.[37,38] As also shown in Figure 3, and differently from the other gluconeogenic substrates, the pathway for glycerol conversion into glyceride glycerol is much shorter. We have also shown that the use of glycerol for glyceride glycerol synthesis is not only more efficient than the use of alanine but that differently from this amino acid, the enhancement of this pathway in the liver of the fed 21-d pregnant rat parallel to the gluconeogenic pathway from the same substrate.[16]

Liver production of triglycerides is enhanced during late pregnancy[39–41] and we have seen here that, among other factors, hepatic glyceride synthesis is supported by the augmented transmission of FFA and glycerol to the liver from adipose tissue lipolysis. Therefore, this effect must actively contribute to the rises in triglyceride-rich lipoproteins found in maternal plasma (see Chapter 2).

In the fasting condition the use of both FFA for ketogenesis[3,42] and glycerol for gluconeogenesis[5] is greatly enhanced in the liver of the pregnant mother, and these changes may benefit the fetus which, at this late phase of the gestational period, is at its maximum accretion rate so that requirements for substrates, metabolic fuels, and essential components are greatly enhanced. Ketone bodies freely cross the placenta[34] and may be used as fetal fuels[43–45] or even as substrates for brain lipid synthesis.[46] Increased glycerol levels in maternal circulation together with both the preferential use of this metabolite as a gluconeogenic substrate and the efficient transfer of glucose to the fetus commented on above also benefit the fetus under conditions of reduced availability of other substrates such as amino acids.[4,5] These metabolic interactions occurring in the starved mother are summarized in Figure 5, where the critical role played by enhanced maternal adipose tissue lipolytic activity in both the fetus and her own tissues is indicated.

IV. MATERNAL BODY FAT ACCUMULATION

Sustained accelerated lipolytic activity in adipose tissue requires the availability of sufficient fat depots. The importance of body fat accumulation to key metabolic interactions for both the mother and the fetus during late gestation is consistent with the fact that this accumulation of body fat is one of the most striking features of gestation in both women[47,48] and experimental animals.[49–52] Body fat accumulation increases progressively with gestation but stops or even declines during the last trimester.[47,48,52,53] This change in body fat content seems to account for most of conceptus-free maternal body weight increase during gestation which has been found to coincide very closely with the increase in carcass fat content in the pregnant rat at different days of gestation.[53]

From studies in the pregnant rat model, fat accumulation during the two first trimesters of gestation has been associated to three major changes: (1) maternal hyperphagia, (2) an increase in *de novo* fatty acid synthesis and

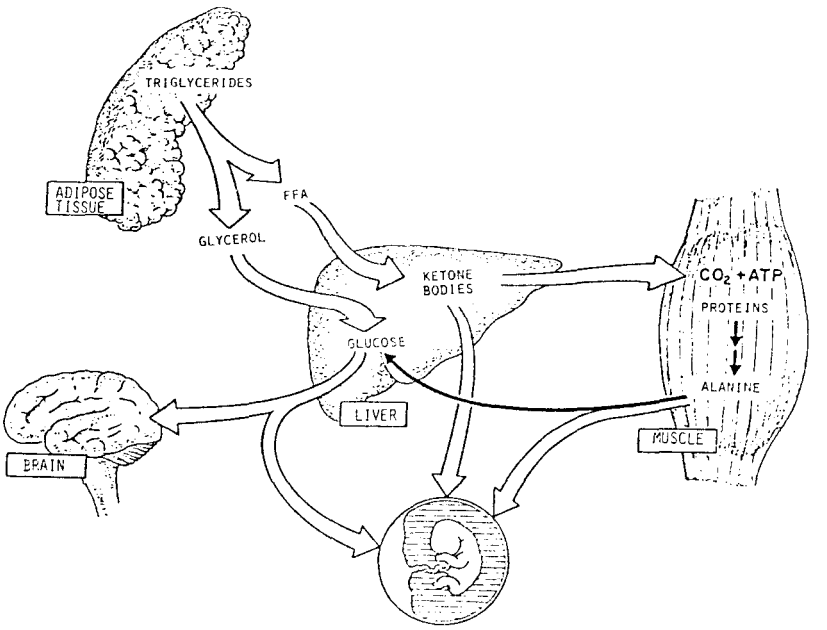

FIGURE 5. Metabolic response to starvation at late pregnancy showing the preponderant role of adipose-tissue lipolysis as a source of substrates for both ketogenesis and gluconeogenesis. (From Herrera E. et al., *Biol. Neonate*, 51, 70, 1987. With permission of S. Karger AG, Basel.)

heightened glucose incorporation into glyceride glycerol, and (3) an increase in tissue-lipoprotein lipase activity.

From studies in the rat it is known that hyperphagia supervenes shortly after mating and increases as gestation time advances becoming especially intense from midgestation until close to parturition.[54,55] This change increases the availability of exogenous substrates and must contribute actively to the maternal accumulation of fat depots which is not found when the animals' food intake is restricted or suppressed.[49,51,56,57]

A technique based on the infusion of ^{14}C-glucose through the left uterine artery to measure the appearance of ^{14}C-lipids in periuterine adipose tissue *in situ* and take into account the periuterine blood flow as well as the specific activity of the tracer in maternal plasma allowed the glucose utilization for fatty acid and glyceride glycerol synthesis in pregnant rats to be estimated at different days of gestation.[58] It was found that both lipogenesis (fatty acids synthesis) and glycerolgenesis (glyceride glycerol synthesis) (see Figure 4) from glucose progressively increased until day 20 and then decreased sharply on day 21. This active lipid synthesis must therefore also contribute to the fat accumulation occurring during the two first trimesters of gestation.

Lipoprotein lipase is an enzyme which is normally bound in its active form to the capillary endothelium of extrahepatic tissues via noncovalent

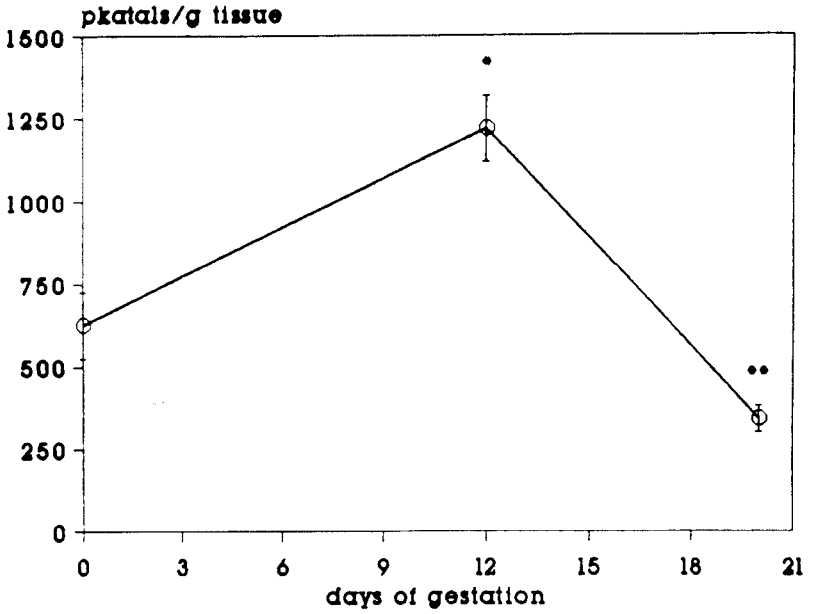

FIGURE 6. Lipoprotein lipase activity (LPL) in lumbar fat pads of pregnant and virgin rats (0 days of gestation). The enzyme was assayed in acetone-ether delipated extracts by a radiochemical method as described elsewhere.[68] Mean ± SEM. Asterisks correspond to the statistical comparisons vs. values at day 0 or gestation (* = $p < 0.05$, ** = $p < 0.01$).

interaction with heparan sulfate molecules. It hydrolyzes the triglycerides circulating in plasma in the form of triglyceride-rich lipoproteins into FFA and glycerol. The products of this triglyceride hydrolysis are partially taken up by the subjacent tissue;[59] thus, this enzyme controls the so-called "fat uptake" in adipose tissue (see Figure 4). The enzyme is released to circulation after heparin administration because the heteropolysaccharide competes with the glycosamine glycan molecules that bind it to the capillary endothelium. Some time ago Knopp et al. showed that after heparin administration, plasma lipoprotein lipase activity increased at day 12 of gestation in the rat,[54] indicating that at this stage of gestation the capacity for the hydrolysis of circulating triglycerides and the tissue uptake of the hydrolytic products is enhanced. Figure 6 shows the lipoprotein lipase activity that was found at 0, 12, and 20 d of gestation in the lumbar fat pads of pregnant rats. It is seen that this activity increases at day 12 of gestation as compared to nonpregnant animals (day 0). It may be then suggested that at this time maternal adipose tissue actively hydrolyzes and takes up circulating triglycerides and that this action may also contribute to the accumulation of body fat occurring during the first part of gestation.

As also seen in Figure 6 lipoprotein lipase activity in adipose tissue decreases at day 20 of gestation in the rat, which confirms previous findings.[60–62] This effect together with the reduction in fatty acids and in glyceride

glycerol synthesis and the increased lipolytic activity commented on above, results in the net increase in fat-depot breakdown that occurs before parturition producing the decrease in body fat content that follows the previous rise during gestation in the rat that was recently reported by us.[52] This transition from an anabolic to a catabolic condition in the maternal lipid metabolism specifically occurs in the phase of maximal fetal growth.[52] It is proposed that although lipids can only cross the placental barrier with difficulty,[34,63] maternal accumulation of fat stores could spare glucose for fetal growth being therefore of essential importance not only to the maternal metabolism but also to fetal development.

A. ROLE OF MATERNAL LIPIDIC ACCUMULATION DURING THE FIRST HALF OF PREGNANCY ON CATABOLIC ADAPTATIONS DURING LATE GESTATION

Changes in lipid metabolism and maternal capacity for an enhanced response to starvation and even the consequences in fetal growth during late gestation are very much influenced by the maternal capacity to accumulate lipid stores during earlier stages of pregnancy. It has previously been shown that at term, thyroidectomized pregnant rats treated with replacement doses of thyroxine (T_4) from midgestation (day 12) but not earlier, have fetuses with a lower pituitary growth hormone content and body weight than those receiving the T_4 treatment between days 0 to 12 of gestation.[64] Conceptus-free maternal body weight was also substantially decreased until late gestation in those animals that were kept hypothyroid during the first half of gestation but not in those in which hypothyroidism was circumscribed to the second half of gestation.[64] These findings may be interpreted as implying that maternal hypothyroidism during the first half of gestation impairs the anabolic changes in the mother addressed to building up her fat stores and limits the proper availability of substrates to sustain the normal catabolic events that support fetal development during late gestation. Since this alteration may be especially manifest during the periods of food restriction when the mother must guarantee the proper availability of substrates to maintain the continuous and rapid fetal growth during the last trimester of gestation, the hypothesis was tested more directly. It was found that besides impairing maternal body weight, 24-h starvation causes a lower increase in plasma levels of β-hydroxybutyrate and glycerol in the 21-d pregnant rats that were kept hypothyroid during the first half of gestation as compared to any of the other groups studied, including those animals that were kept under T_4 treatment during the first half of gestation only, and were therefore hypothyroid at the time of the study.[65] These results indicate that by impairing the maternal capacity to build up fat stores, hypothyroidism that occurs during the first 12 d of gestation, but not during the second half of gestation, compromises both the normal catabolic response during late gestation and the adequate metabolic realignments necessary for accelerated starvation, thereby resulting in possible damage to the fetus.

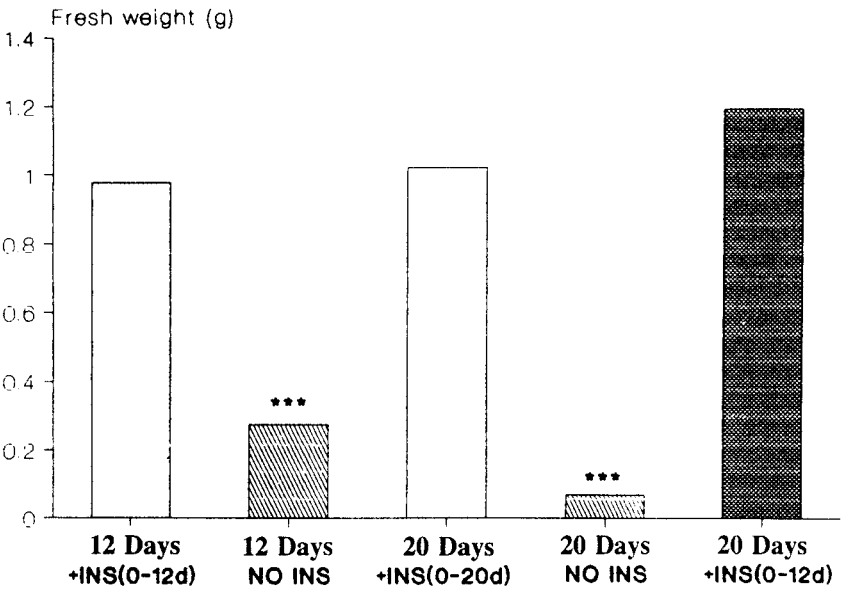

FIGURE 7. Lumbar adipose tissue mass in 12- or 20-d pregnant streptozotocin diabetic rats. Animals were intravenously treated with 45 mg/kg body weight of streptozotocin prior to mating. Some rats were treated daily with subcutaneous replacement insulin therapy (1.5 IU/100 body weight) from the time of receiving the streptozotocin until sacrifice, which was done either at day 12 (+INS(0 to 12 d)) or 20 of gestation (+INS(0 to 20 d)). Another group received the replacement insulin therapy only up to the 12th day of gestation, and thereafter were maintained diabetic until the 20th day in which animals were sacrificed (+INS(0 to 12 d)). A final group of animals did not receive any therapy and were sacrificed either at day 12 or 20 of gestation (NO INS). Statistical comparisons vs. animals receiving insulin therapy for the whole time are shown by asterisks: *** = $p < 0.001$.

Increased fat accumulation during the first part of pregnancy also plays an important role in the hypertriglyceridemia normally occurring during late gestation, and this is clearly seen in the streptozotocin diabetic pregnant rat. Figure 7 shows that the lumbar adipose tissue mass at day 12 of gestation is much lower in rats that were made diabetic by the intravenous injection of 45 mg of streptozotocin/kg body weight prior to mating than in animals subjected to the same treatment receiving a daily subcutaneous-replacement insulin therapy (1.5 IU/100 g body weight). At day 20 of gestation the difference between the two groups was even greater than that found at day 12 due to the greater reduction in the adipose tissue mass of those streptozotocin diabetic rats not receiving insulin therapy. However, when streptozotocin-treated pregnant rats receiving insulin therapy up to the 12th day of gestation were made diabetic from this day until the 20th by suppressing the insulin treatment during this period, the lumbar fat pad weight appeared the same as in those that had received insulin for the whole time. These findings

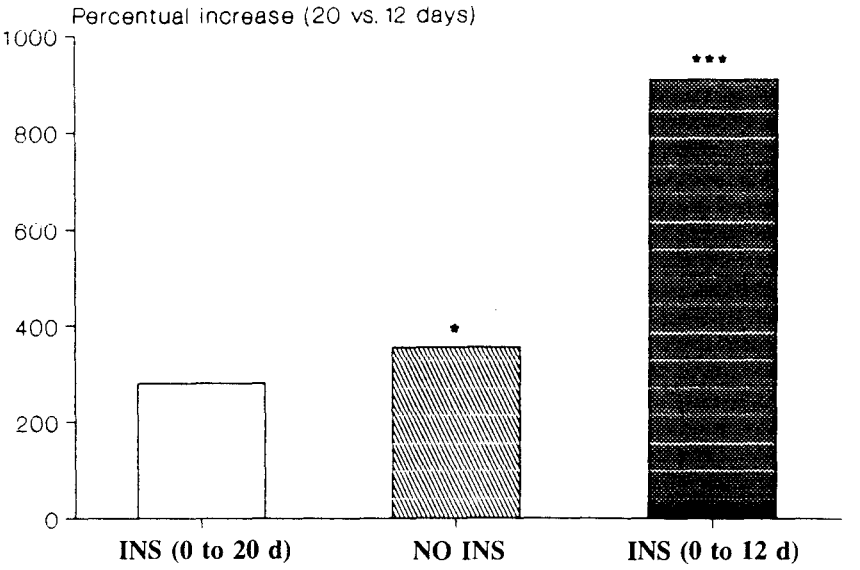

FIGURE 8. Percentual increase in plasma triglycerides from day 12 to 20 of gestation in rats treated with streptozotocin and receiving, or not receiving insulin therapy. Methodological details and identification of the groups are as in Figure 7. Statistical comparisons vs. animals receiving insulin therapy for the whole time (INS(0 to 20 d)) are shown by asterisks: * = $p < 0.05$, *** = $p < 0.001$.

indicate that diabetes during the first half of gestation but not during the second half impairs the normal accumulation of body fat occurring during gestation. As shown in Figure 8, the increase in plasma triglyceride levels occurring from day 12 of gestation until day 20 in streptozotocin pregnant rats that were permanently kept diabetic (NO INS) was only slightly higher than in those kept under insulin therapy for the whole time (INS 0 to 20 d). However, plasma triglyceride levels increased very sharply in those streptozotocin diabetic rats that received the insulin therapy during the first 12 days of gestation (INS 0 to 12, in Figure 8) and had therefore preserved their fat depots during that period (see also group INS 0 to 12 in Figure 7). Two conclusions may be then obtained from these data: (1) Impaired fat accumulation during the first half of gestation in the rats that were diabetic during this period results in the plasma triglycerides rise during the second half of gestation being much lower than in those diabetic animals in which body fat stores were normally accumulated during that first part of gestation, and (2) the intense catabolic response during late gestation in the overtly diabetic pregnant rat is only seen when fat depots are preserved during the first half of gestation since, otherwise, an insufficient amount of substrates are available for mobilization.

The findings therefore show that provoking hypothyroidism or diabetes in pregnant rats only during the first half of gestation, blocks fat accumulation, thereby hindering maternal catabolic responses and hypertriglyceridemia during the second gestational phase and impairing normal fetal growth. Thus it may be concluded that maternal fat accumulation during the first half of gestation is of pivotal importance on the metabolic adaptations normally occurring during late gestation.

V. CONCLUDING REMARKS

During early gestation the mother is in an anabolic state which, among other factors (endocrine?), is supported by increments in both food intake and adipose tissue lipogenesis. At this stage of gestation the small size of the fetus requires minimal maternal expenditure of substrates on sustaining its development, and maternal fat deposits are accumulated. In late gestation, on the contrary, the increased need for substrates and oxidative fuels by the conceptus as the result of the high rate of fetal accretion is supported by an active influx of metabolites from the mother. Glucose is quantitatively the most abundant of the different substrates that cross the placenta, and despite enhanced maternal gluconeogenesis, this transfer is the cause of the maternal tendency to hypoglycemia during short periods of food deprivation. Hypoglycemia forces the mother to switch to a catabolic state which is particularly visible in adipose tissue metabolism when the mother fails to eat; the availability of glycerol, which becomes an important gluconeogenic substrate, increases and exaggerated ketogenesis develops. This mechanism allows the husbanding of other essential compounds such as amino acids, therefore assuring the proper availability of substrates for the fetus.

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