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Intermediary Metabolism in Pregnancy

First Theme of the Freinkel Era

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During the first half of gestation in the rat, maternal net body weight increases rapidly, whereas in the second half of gestation, the mass of maternal structures declines, coincident with the rate of maternal fat accumulation. Enhanced maternal food intake, extrahepatic tissue lipoprotein lipase (LPL) activity, and adipose tissue lipogenesis are responsible for the progressive accumulation of maternal fat. However, during late gestation, decreased fat synthesis in maternal adipose tissue, enhanced lipolytic activity, and decreased LPL activity deplete maternal fat depots. These changes, plus enhanced endogenous production of triglyceride-rich lipoproteins, are also responsible for maternal hypertriglyceridemia. This condition benefits the offspring in two ways: *1*) enhanced LPL activity in maternal liver when fasting increases triglyceride consumption for ketone body synthesis, giving the basis for accelerated starvation; and *2*) induction of LPL activity in the mammary gland before parturition diverts maternal circulating triglycerides to milk synthesis in preparation for lactation. The magnitude of the maternal-fetal glucose transfer was higher than that of any of the other substrates studied, including alanine, and despite actions to spare glucose, this transfer causes maternal hypoglycemia, which is especially intense in the fasting condition. This increases sympathoadrenal activity in the mother, which may contribute to her active gluconeogenesis. Glycerol was a more efficient glucose precursor than alanine and pyruvate, and whereas glycerol placental transfer is very small, it is proposed that the fetus benefits from this product of adipose tissue lipolysis when it is previously converted into glucose. In thyroidectomized pregnant rats treated with thyroxine for different periods, restraining maternal accumulation of fat depots during the early part of gestation compromises the normal metabolic adaptations during late gestation, including the capacity for accelerated starvation, which negatively affects fetal development.
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My first steps in the field of intermediary metabolism in pregnancy were made during the years I spent with Professor Norbert Freinkel (1965–1968). His teachings, positive criticism, and enthusiasm have driven my scientific career from that time on, and all my later contributions are built on the foundation I established under his supervision. For this and for his sincere friendship, I dedicate this paper in appreciation of his undying memory.

E.H.

CHANGES IN MATERNAL STRUCTURES

In his earliest contributions related to intermediary metabolism in pregnancy, Freinkel (1) pointed out that the mother should be geared to conserve more exogenous nutrients whenever she eats, in anticipation of the more severe demands caused by the needs of sustaining a continuous flux of nutrients to the fetus even during periods in which food may be unavailable.

Enhanced maternal anabolism in the fed state should be associated with the heightened capacity for catabolism in the fasted state so that both fetal development and maternal survival can be preserved under all conditions (1,2). Although these two manifestations may coexist during late gestation, their onset occurs at different times. During the first half of gestation in the rat, maternal net body weight increases rapidly, coincident with a minimal weight accretion by the conceptus (Fig. 1). Later in gestation, the mass of maternal structures declines while the conceptus weight increases progressively (Fig. 1). It is known that increments in maternal weight coincide with increases in the size of certain structures, e.g., the liver (3–6), and augmented net

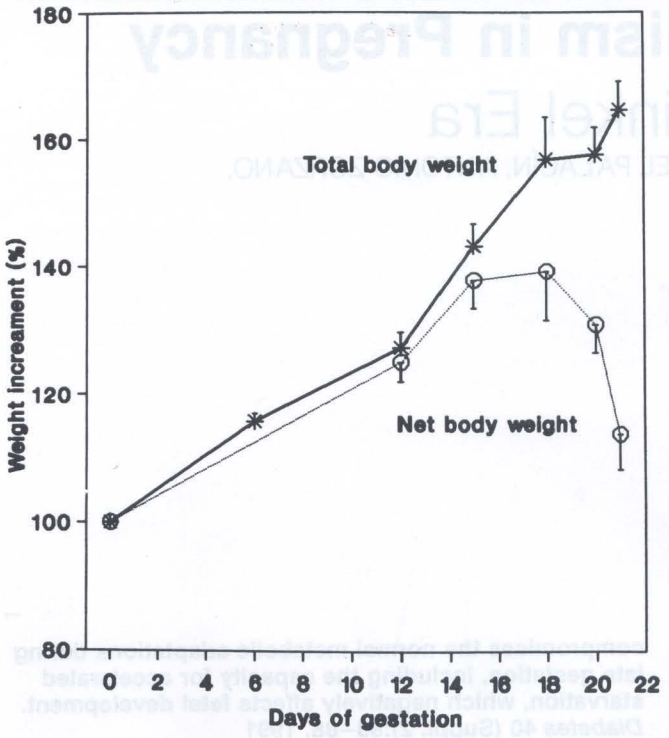

FIG. 1. Increments of total and net maternal (conceptus-free) body weight in rat at different lengths of gestation. Means ± SE of 6 rats/group.

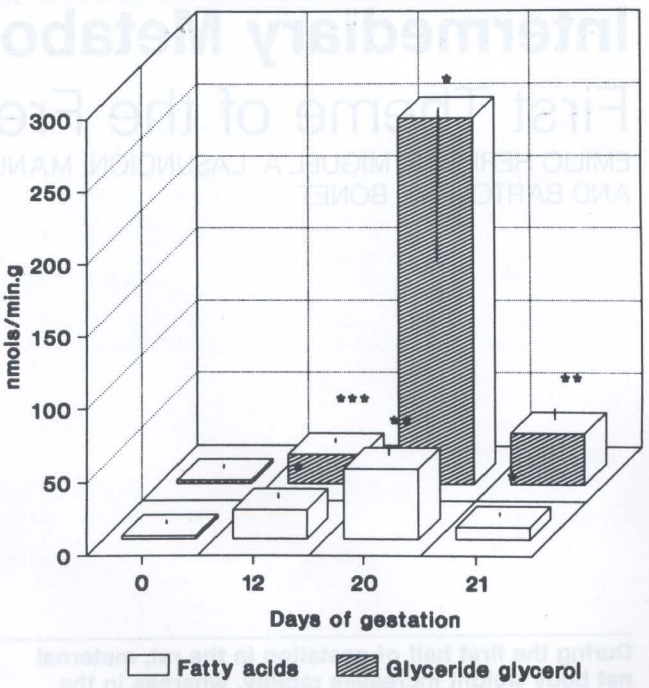

FIG. 2. Utilization of glucose for fatty acids and glyceride glycerol by periuterine adipose tissue in situ in virgin and pregnant rats at different lengths of gestation (4–6 rats/group). Animals received 20-min infusion with D-[U-^{14}C]glucose through left uterine artery. Estimation of lipid synthesis was done by taking into account differences between left and right tissue ^{14}C-labeled lipid values, arterial plasma glucose concentration, and blood flow through left uterine artery, as previously described (23).

nitrogen retention (4,7), but quantitatively, the most important factor to contribute to these increments corresponds to fat depots, which has been shown in both pregnant women (8) and rats (9,10). Recent carcass analysis studies in the rat have shown that although this maternal body fat accumulation lasts until day 19 of gestation, it declines from this time until labor (11).

ADIPOSE TISSUE METABOLISM

Enhanced maternal food intake and extrahepatic tissue lipoprotein lipase (LPL) activity (12) and adipose tissue lipogenesis (13) are responsible for the progressive accumulation of maternal fat depots during the first half of gestation. During late gestation, however, a maternal tendency to favor the depletion rather than the accumulation of fat is the result of several juxtaposed factors, including a critical switch in adipose tissue lipid synthesis. This particular point has not been clarified, because maternal adipose tissue lipid synthesis has been reported to be either reduced (14,15), unaffected (16), or even slightly enhanced (17,18). In addition, redistribution of blood flow to organs, especially the uterus (19,20), and the changes in plasma metabolite concentration that take place during gestation could affect substrate availability to adipose tissue. To avoid these problems, we studied the direct incorporation of certain metabolites into lipids by periuterine adipose tissue in situ after different lengths of gestation in the rat. With this purpose, we followed an adaptation of the technique we previously used to study the placental transfer of metabolites in the rat (21,22). Tracer was infused for 20 min throughout the left uterine artery of a pentobarbital sodium–anesthetized rat, and the amount of label in lipids present in the periuterine fat pad from the left uterine horn was compared with that found in the fat pad from the right horn. The amount of substrate taken up by the tissue was calculated as a function of the metabolite concentration in the maternal arterial blood, the difference between radioactivity levels in the fat pads from the left and the right uterine sides, and the left uterine blood flow, as already described (23,24). We checked glucose, alanine, lactate, glycerol, and palmitate as substrates and found that glucose was quantitatively the most important to be incorporated into adipose tissue triglycerides. The conversion of glucose into fatty acids increased progressively with gestation until day 20 and then declined (Fig. 2). The conversion of glucose to glyceride glycerol rather than fatty acids was much higher on day 20 of gestation and declined sharply at day 21. Because this picture coincides with that seen for maternal body weight and composition, it indicates that these changes in adipose tissue lipid synthesis may play an important role in maternal fat accumulation during the first two-thirds of pregnancy.

Decreased fat synthesis in maternal adipose tissue during late gestation is accompanied by two other changes that also justify the decreased body fat content occurring at this stage. One of them is enhanced lipolytic activity, which was found in the 19-day pregnant rat when I was working under Freinkel in collaboration with Knopp (17). This active lipolysis is further accelerated in the late pregnant rat under fasting conditions (17). The other change is a reduction in adipose tissue uptake of fatty acids from plasma triglycer-

ides (25) caused by a decrease in LPL activity (10,26–29). Because this enzyme catalyzes the hydrolysis of circulating triglycerides associated with triglyceride-rich lipoproteins (very-low-density lipoprotein and chylomicrons), it facilitates tissue uptake of hydrolytic products.

Decreased LPL activity in adipose tissue during late gestation would also reduce the fractional catabolic rate of triglyceride-rich lipoproteins. This effect and the enhanced production of triglycerides, by both the liver (30,31) from endogenous substrates and the intestinal mucosa from dietary fat (32), are responsible for the development of the well-known hypertriglyceridemia of late gestation (10,26,32,33).

CONSEQUENCES OF MATERNAL HYPERTRIGLYCERIDEMIA

Maternal triglycerides are practically unavailable to the fetus because of placental impermeability to triglycerides. However, maternal triglycerides may benefit offspring in one of two ways.

On the one hand, and contrary to what happens in the nonpregnant rat, in which liver LPL activity is very low and does not change with starvation, we have shown that 24 h starvation in the 20-day pregnant rat causes a marked increase in the activity of this enzyme in liver (10). Because a parallel picture is seen in both liver triglyceride concentration and plasma ketone body concentrations (10), through this mechanism, the liver, which under normal conditions is a triglyceride-exporting organ, becomes a receptor of circulating triglyceride, thus allowing increased triglyceride consumption for ketogenic substrates. This and the enhanced ketone body production from the free fatty acids reaching the liver as a consequence of the augmented adipose tissue lipolysis (10,17) underlie the concept of maternal "accelerated starvation" already proposed by Freinkel (1) in 1965. Through this mechanism, during periods of dietary deprivation, the late pregnant mother can rapidly and persistently use her fat depots accumulated during previous gestational stages for the synthesis of ketone bodies as alternative fuels for many tissues, thereby allowing the preservation of other more essential metabolites such as amino acids and glucose (34). The fetus also benefits from maternal ketonemia because ketones cross the placental barrier freely and may be used by the fetus not only as fuels but as lipogenic substrates (35,36).

On the other hand, maternal hypertriglyceridemia constitutes a floating store easily used for milk synthesis before parturition. In the rat, we found that at this gestational time, maternal hypertriglyceridemia declined (29), and a similar change has been described in pregnant women after delivery (37,38). This effect in the rat coincided with an additional increase in mammary gland LPL activity (29), and these two effects are interconnected because neither one occurred when induction of the enzyme was blocked with progesterone (29). Therefore, the induction of LPL activity in the mammary gland before parturition diverts maternal circulating triglycerides to milk synthesis in preparation for lactation.

PLACENTAL TRANSFER OF METABOLITES

The anabolic condition of the mother during early gestation therefore switches to a catabolic one during the later stages,

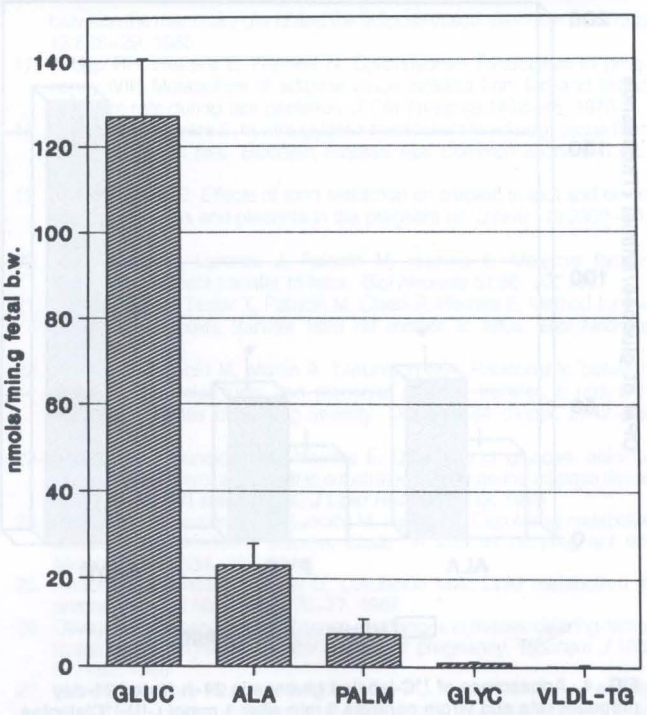

FIG. 3. Placental transfer of D-glucose (Gluc), L-alanine (Ala), palmitic acid (Palm), glycerol (Glyc), and very-low-density lipoprotein triglycerides (VLDL-TG) in 20-day pregnant rat (4–8 rats/group). Placental transfer to fetus was determined by measuring radioactivity in fetuses after infusing ^{14}C-labeled substrates through left uterine artery and making proper correction of data for specific activity dilution of tracer and blood flow through left uterine artery, as previously described (21,22). b.w., Body weight.

and this change must play an important role in fetal development and maternal preparation for lactation.

The importance of the fetal need for maternal metabolites may be inferred from the quality and quantity of the placental transfer. Placental transfer of various substrates to the fetus has been measured by infusing the left uterine artery in the late pregnant rat with different tracers to monitor the appearance of the label in fetal structures (21,22), following a method like the one mentioned to estimate lipid synthesis in lumbar fat pads in situ. Despite maternal hyperlipidemia, the transfer of free fatty acids is quite low and that of either glycerol or very-low-density lipoprotein triglycerides is practically negligible (Fig. 3). However, the importance of the maternal-fetal glucose transfer was much higher than for any of the other substrates studied, including alanine (Fig. 3), which is known to cross the placental barrier by an active mechanism (39). Glucose is a major substrate for the fetus economy (20,40), and at least in the rat, we have shown that it cannot be synthesized in the fetus from placentally transferred substrates (41).

MATERNAL GLUCOSE METABOLISM AND CONSEQUENCES OF MATERNAL FASTING HYPOGLYCEMIA ON SYMPATHOADRENAL ACTIVITY

The intense flux of maternal glucose to the fetus is partially compensated by maternal hyperphagia (12), but the mother is also forced to develop mechanisms that reduce glucose consumption by her own tissues (1,42). Again, this process

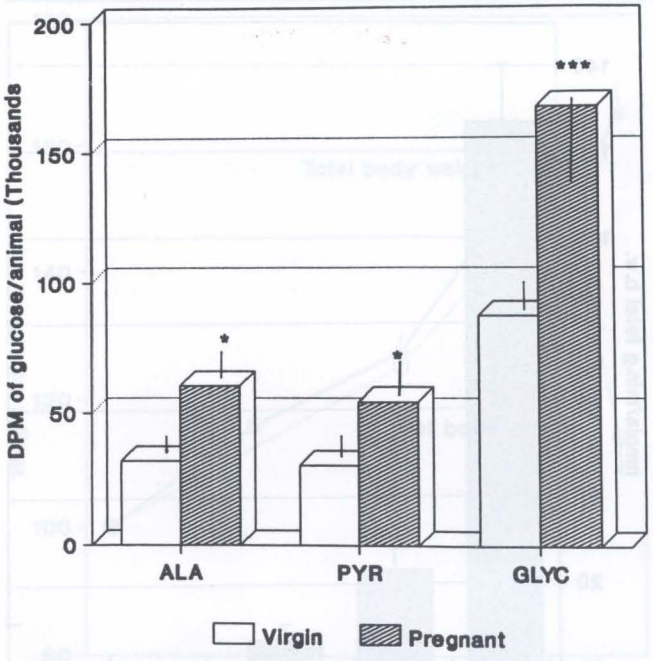

FIG. 4. Appearance of ^{14}C-labeled glucose in 24-h fasted 21-day pregnant rats and virgin controls 5 min after 1 mmol L-[U-^{14}C]alanine (Ala), [3-^{14}C]pyruvate (Pyr), or [U-^{14}C]glycerol (Glyc) i.v. Values correspond to means ± SE of 9–10 rats/group. Animals were studied unanesthetized. Other methodological details were described previously (51). * $P < 0.05$. *** $P < 0.001$.

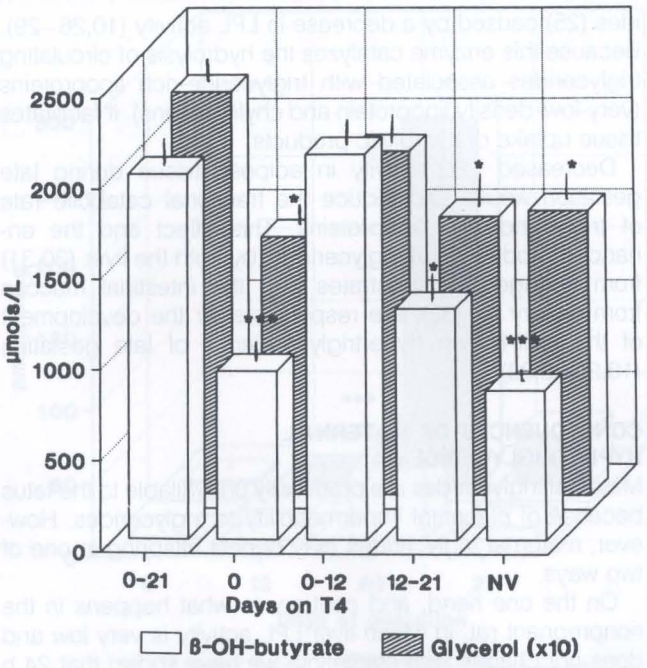

FIG. 5. Plasma β-hydroxybutyrate and glycerol levels in 24-h fasted 21-day pregnant rats thyroidectomized at day 0 of gestation and treated with 1.8 µg · 100 g^{-1} body wt · day^{-1} L-thyroxine (L-T4) for different lengths of gestation. Other methodological details for treating animals were described previously (53). Nonthyroidectomized virgin (NV) animals were studied in parallel. * $P < 0.05$, *** $P < 0.001$, vs. thyroidectomized rats treated for whole period (0–21).

is most evident during periods of food deprivation and is driven by the maternal insulin resistance developed during late gestation, as pointed out by Freinkel and collaborators in 1964 (43) and later supported by more direct experiments in both pregnant women (44,45) and rats (46,47).

These actions for sparing glucose are not sufficient to maintain maternal normoglycemia during late gestation, especially when food is withheld. After an overnight fast in the third trimester of human pregnancy, plasma glucose is significantly lower than in postpartum or nongravid women (43), and when more stringent fasting is instituted by withholding food from pregnant rats (5,6) for ≥1 day, frank hypoglycemia supervenes near term. This action seems to be responsible for the heightened catecholamine excretion found during late gestation in the fasting rat (48), because the adrenal medulla is selectively activated by reductions in blood glucose (49,50).

As we suggested when working under Freinkel in 1969, increased sympathoadrenal activity causes certain metabolic benefits to the fasted pregnant mother (48). It could abet the metabolism of fat and the resistance to insulin, sparing maternal glucose.

The increased sympathoadrenal activity may have an additional benefit to the fasting mother. Because catecholamines have enhancing effects on gluconeogenesis, they may contribute to the augmented gluconeogenic activity also found in the fasted late pregnant rat (6,51). By studying the metabolic effect of anesthetics in the pregnant rat, we have substantiated the connection between the enhanced gluconeogenic activity and the augmented catecholamine release in these animals (52).

With different three-carbon substrates, the production of glucose is greater in fasted pregnant rats than in virgin controls, and glycerol is a more efficient glucose precursor than other more classic ones such as alanine and pyruvate (Fig. 4). Because the plasma concentration of glycerol but not pyruvate, lactate, or gluconeogenic amino acids is augmented in the fasted late pregnant rat (51), these findings indicate that glycerol is a major substrate for the synthesis of glucose in this condition.

Although the direct passage of glycerol through the placenta is rather limited (Fig. 3), the fetus may benefit from this product of adipose tissue lipolysis when it is previously converted into glucose. In more-direct experiments, we have shown that this system of glucose arrival in the fetus from maternal glycerol works actively in vivo in the late pregnant fasted rat (41), and this synthesis of glucose from products of fat breakdown may constitute an additional mechanism for ensuring the continuous availability of glucose to the fetus.

ROLE OF MATERNAL ANABOLIC CHANGES DURING EARLY GESTATION IN ADAPTATIONS DURING LATE PREGNANCY

The accumulation of fat depots during the first two-thirds of gestation plays an important role in the enhanced maternal catabolism that takes place during late pregnancy. This condition has pivotal importance in sustaining proper availability of substrates for the rapid and continuous development of the fetus.

This argument is supported by the reduced growth found

in 21-day fetuses of thyroidectomized rats that had received substitutive treatment with L-thyroxine only from day 12 of gestation and whose maternal net body weight increase was impaired, as also occurred in rats kept hypothyroid for the whole period (53). The increase in blood β-hydroxybutyrate and glycerol levels after 24 h starvation at day 21 of gestation was smaller in thyroidectomized rats kept without L-thyroxine treatment for either the first 12 days of gestation (group 12–21) or the whole time (group 0) than in those in which the building up of maternal fat depots was preserved by the substitutive L-thyroxine treatment during the first half of gestation (groups 0–12 and 0–21) (Fig. 5). However, values found in the former groups were very similar to those seen in nonthyroidectomized virgin rats starved for 24 h, thus indicating that their capacity for accelerated starvation was impaired. These findings indicate that restraining the accumulation of metabolic depots during the early part of gestation compromises the catabolic adaptations normally occurring at late gestation and that this limitation has a negative effect on fetal development.

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REFERENCES

1. Freinkel N: Effects of the conceptus on maternal metabolism during pregnancy. In *On the Nature and Treatment of Diabetes.* Leibel BS, Wrenshall GA, Ed. Amsterdam, Excerpta Med., 1965, p. 679–91
2. Freinkel N: Banting Lecture 1980: of pregnancy and progeny. *Diabetes* 29:1023–35, 1980
3. Poo LJ, Lew W, Addis T: Protein anabolism of organs and tissues during pregnancy and lactation. *J Biol Chem* 128:69–77, 1939
4. Beaton GH, Beare J, Ryu MH, McHenry EW: Protein metabolism in the pregnant rat. *J Nutr* 54:291–304, 1954
5. Scow RO, Chernick SS, Brinley MS: Hyperlipemia and ketosis in the pregnant rat. *Am J Physiol* 206:798–804, 1964
6. Herrera E, Knopp RH, Freinkel N: Carbohydrate metabolism in pregnancy. VI. Plasma fuels, insulin, liver composition, gluconeogenesis, and nitrogen metabolism during late gestation in the fed and fasted rat. *J Clin Invest* 48:2260–72, 1969
7. Zuspan F, Goodrich S: Metabolic studies in normal pregnancy. I. Nitrogen metabolism. *Am J Obstet Gynecol* 100:7–13, 1968
8. Hytten FE, Leitch I: *The Physiology of Human Pregnancy.* 2nd ed. Oxford, UK, Blackwell, 1971, p. 359–64
9. López-Luna P, Muñoz T, Herrera E: Body fat in pregnant rats at mid- and late gestation. *Life Sci* 39:1389–93, 1986
10. Herrera E, Lasunción MA, Gomez-Coronado D, Aranda P, López-Luna P, Maier I: Role of lipoprotein lipase activity on lipoprotein metabolism and the fate of circulating triglycerides in pregnancy. *Am J Obstet Gynecol* 158:1575–83, 1988
11. López-Luna P, Maier I, Herrera E: Carcass and tissue fat content in the pregnant rat. *Biol Neonate* 60:29–38, 1991
12. Knopp RH, Boroush MA, O'Sullivan JB: Lipid metabolism in pregnancy. II. Postheparin lipolytic activity and hypertriglyceridemia in the pregnant rat. *Metabolism* 24:481–93, 1975
13. Fain JN, Scow RO: Fatty acid synthesis in vivo in maternal and fetal tissues in the rat. *Am J Physiol* 210:19–25, 1966
14. Flint DJ, Sinnett-Smith PA, Clegg RA, Vernon RG: Role of insulin receptors in the changing metabolism of adipose tissue during pregnancy and lactation in the rat. *Biochem J* 182:421–27, 1979
15. Knopp RH, Sauder CD, Arky RA, O'Sullivan JB: Two phases of adipose tissue metabolism in pregnancy: maternal adaptations for fetal growth. *Endocrinology* 92:984–88, 1973
16. Flint DJ: Role of insulin and the insulin receptor in nutrient partitioning between the mammary gland and the adipose tissue. *Biochem Soc Trans* 13:828–29, 1985
17. Knopp RH, Herrera E, Freinkel N: Carbohydrate metabolism in pregnancy. VIII. Metabolism of adipose tissue isolated from fed and fasted pregnant rats during late gestation. *J Clin Invest* 49:1438–46, 1970
18. Chaves JM, Herrera E: In vitro glycerol metabolism in adipose tissue from fasted pregnant rats. *Biochem Biophys Res Commun* 85:1299–1306, 1978
19. Rosso P, Kava R: Effects of food restriction on cardiac output and blood flow to the uterus and placenta in the pregnant rat. *J Nutr* 110:2350–54, 1980
20. Lasunción MA, Lorenzo J, Palacín M, Herrera E: Maternal factors modulating nutrient transfer to fetus. *Biol Neonate* 51:86–93, 1987
21. Lasunción MA, Testar X, Palacín M, Chieri R, Herrera E: Method for the study of metabolite transfer from rat mother to fetus. *Biol Neonate* 44:83–92, 1983
22. Herrera E, Palacín M, Martin A, Lasunción MA: Relationship between maternal and fetal fuels and placental glucose transfer in rats with maternal diabetes of varying severity. *Diabetes* 34 (Suppl. 2):42–46, 1985
23. Palacín M, Lasunción MA, Herrera E: Utilization of glucose, alanine, lactate and glycerol as lipogenic substrates by periuterine adipose tissue in situ in fed and starved rats. *J Lipid Res* 29:26–32, 1988
24. Palacín M, Lasunción MA, Asunción M, Herrera E: Circulating metabolite utilization by periuterine adipose tissue "in situ" in the pregnant rat. *Metabolism* 40:534–39, 1991
25. Herrera E, Gómez-Coronado D, Lasunción MA: Lipid metabolism in pregnancy. *Biol Neonate* 51:70–77, 1987
26. Otway S, Robinson DS: Significance of changes in tissues clearing-factor lipase activity in relation to the lipemia of pregnancy. *Biochem J* 106: 677–82, 1968
27. Hamosh M, Clary TR, Chernick SS, Scow PO: Lipoprotein lipase activity in adipose tissue and mammary tissue and plasma triglyceride in pregnant and lactating rats. *Biochim Biophys Acta* 210:473–82, 1970
28. Llobera M, Montes A, Herrera E: Lipoprotein lipase activity in liver of the rat fetus. *Biochem Biophys Res Commun* 91:272–77, 1979
29. Ramirez I, Llobera M, Herrera E: Circulating triacylglycerols, lipoproteins and tissue lipoprotein lipase activities in rat mothers and offspring during the perinatal period: effect of postmaturity. *Metabolism* 32:333–41, 1983
30. Kalkhoff RK, Bhatia SK, Matute ML: Influence of pregnancy and sex steroids on hepatic triglyceride biosynthesis. *Diabetes* 21 (Suppl. 1): 365–69, 1972
31. Wasfi I, Weinstein I, Heimberg M: Hepatic metabolism of (1^{-14}C) oleate in pregnancy. *Biochim Biophys Acta* 619:471–81, 1980
32. Argiles J, Herrera E: Appearance of circulating and tissue ^{14}C-lipids after oral ^{14}C-tripalmitate administration in the late pregnant rat. *Metabolism* 38:104–108, 1989
33. Konttinen AT, Pyorala T, Carpen E: Serum lipid patterns in normal pregnancy and preeclampsia. *J Obstet Gynaecol Br Commonw* 71:453–58, 1964
34. Freinkel N, Metzger BE, Nitzan M, Hare JW, Shambaugh GE, Marshall RT, Surmaczynska BZ, Nagel TC: "Accelerated starvation" and mechanisms for the conservation of maternal nitrogen during pregnancy. *Isr J Med Sci* 8:426–39, 1972
35. Shambaugh GE, Morozak SC, Freinkel N: Fetal fuels. I. Utilization of ketones by isolated tissues at various stages of maturation and maternal nutrition during late gestation. *Metabolism* 26:623–35, 1977
36. Patel MS, Johnson CA, Ratan R, Owen DE: The metabolism of ketone bodies in developing human brain: development of ketone body utilizing enzymes and ketone bodies as precursors for lipid synthesis. *J Neurochem* 25:905–908, 1975
37. Knopp RH, Warth MR, Charles D, Childs M, Li RJ, Mabuchi H, Van Allen M: Lipoprotein metabolism in pregnancy, fat transport to the fetus, and the effects of diabetes. *Biol Neonate* 50:297–317, 1986
38. Desoye G, Schweditsch MO, Pfeiffer KP, Zechner R, Kostner GH: Correlation of hormones with lipid and lipoprotein levels during normal pregnancy and postpartum. *J Clin Endocrinol Metab* 64:704–12, 1987
39. Palacín M, Lasunción MA, Martín del Río R, Herrera E: Placental formation of lactate from transferred L-alanine and its impairment by aminooxyacetate in the late-pregnant rat. *Biochim Biophys Acta* 841:90–96, 1985
40. Battaglia FC, Meschia G: Principal substrates of fetal metabolism. *Physiol Rev* 58:499–527, 1978
41. Palacín M, Lasunción MA, Herrera E: Lactate production and absence of gluconeogenesis from placental transferred substrates in fetuses from fed and 48-h starved rats. *Pediatr Res* 22:6–10, 1987
42. Leturque A, Ferré P, Burnol A-F, Kande J, Maulard P, Girard J: Glucose utilization rates and insulin sensitivity in vivo in tissues of virgin and pregnant rats. *Diabetes* 35:172–77, 1986
43. Bleicher SJ, O'Sullivan JB, Freinkel N: Carbohydrate metabolism in pregnancy. V. The interrelations of glucose, insulin and free fatty acids in late pregnancy and postpartum. *N Engl J Med* 271:866–72, 1964
44. Freinkel N, Metzger BE, Nitzan M, Daniel R, Surmaczynska B, Nagel T:

Facilitated anabolism in late pregnancy: some novel maternal compensations for accelerated starvation. In *Proceedings of the VIIIth Congress of the International Diabetes Federation.* Malaise WJ, Pirart J, Eds. Amsterdam, Excerpta Med., 1974, p. 474–88 (Int. Congr. Ser., no. 312)
45. Daniel RR, Metzger BE, Freinkel N, Faloona GR, Unger RH, Nitzam M: Carbohydrate metabolism in pregnancy. XI. Response of plasma glucagon to overnight fast and oral glucose during normal pregnancy and in gestational diabetes. *Diabetes* 23:771–76, 1974
46. Knopp RH, Ruder HJ, Herrera E, Freinkel N: Carbohydrate metabolism in pregnancy. VII. Insulin tolerance during late pregnancy in the fed and fasted rat. *Acta Endocrinol* 65:352–60, 1970
47. Martín A, Zorzano A, Caruncho I, Herrera E: Glucose tolerance tests and "in vivo" response to intravenous insulin in the unanaesthesized late pregnant rat and their consequences to the fetus. *Diabete Metab* 12:302–307, 1986
48. Herrera E, Knopp RH, Freinkel N: Urinary excretion of epinephrine and norepinephrine during fasting in late pregnancy in the rat. *Endocrinology* 84:447–50, 1969
49. Goldfien A, Zieleli S, Despointes RH, Bethune JE: The effect of hypoglycemia on the adrenal secretion of epinephrine and norepinephrine in the dog. *Endocrinology* 62:749–57, 1958
50. Young JB, Landsberg L: Sympathoadrenal activity in fasting pregnant rats: dissociation of adrenal medullary and sympathetic nervous system responses. *J Clin Invest* 64:109–16, 1979
51. Zorzano A, Lasunción MA, Herrera E: Role of the availability of substrates on hepatic and renal gluconeogenesis in the fasted late pregnant rat. *Metabolism* 35:297–303, 1986
52. Zorzano A, Herrera E: Effects of anesthetics and starvation on in vivo gluconeogenesis in virgin and pregnant rats. *Metabolism* 33:553–58, 1984
53. Bonet B, Herrera E: Different response to maternal hypothyroidism during the first and second half of gestation in the rat. *Endocrinology* 122:450–55, 1988