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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 this potential 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. **Diabetes** 40 (Suppl. 2):83–88, 1991

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

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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.

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 peritumoral 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 vitro (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 peritumoral 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 uterus 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, 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 placentially 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
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...
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