

Lipid metabolism in the fetus and the newborn

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Summary

During late gestation, although maternal adipose tissue lipolytic activity becomes enhanced, lipolytic products cross the placenta with difficulty. Under fasting conditions, free fatty acids (FFA) are used for ketogenesis by the mother, and ketone bodies are used as fuels and lipogenic substrates by the fetus. Maternal glycerol is preferentially used for glucose synthesis, saving other gluconeogenic substrates, like amino acids, for fetal growth. Placental transfer of triglycerides is null, but essential fatty acids derived from maternal diet, which are transported as triglycerides in lipoproteins, become available to the fetus owing to the presence of both lipoprotein receptors and lipase activities in the placenta. Diabetes in pregnancy promotes lipid transfer to the fetus by increasing the maternal–fetal gradient, which may contribute to an increase in body fat mass in newborns of diabetic women. Deposition of fat stores in the fetus is very low in the rat but high in humans, where body fat accretion occurs essentially during the last trimester of intra-uterine life. This is sustained by the intense placental transfer of glucose and by its use as a lipogenic substrate, as well as by the placental transfer of fatty acids and to their low oxidation activity. During the perinatal period an active ketonemia develops, which is maintained in the suckling newborn by several factors: (i) the high-fat and low-carbohydrate content in milk, (ii) the enhanced lipolytic activity occurring during the first few hours of life, and (iii) both the uptake of circulating triglycerides by the liver due to the induction of lipoprotein lipase (LPL) activity in this organ, and the presence of ketogenic activity in the intestinal mucosa. Changes in LPL activity, lipogenesis and lipolysis contribute to the sequential steps of adipocyte hyperplasia and hypertrophy occurring during the extra-uterine white adipose tissue development in rat, and this may be used as a model to extrapolate the intra-uterine adipose tissue development in other species, including humans. Copyright © 2000 John Wiley & Sons, Ltd.

Keywords fetal metabolism; adipose tissue; essential fatty acids; ketone bodies; suckling; diabetes

Metabolic changes occurring in the mother sustaining fetal lipidic metabolism

Fetal metabolism, and consequently fetal growth, directly depend on the nutrients crossing the placenta, and therefore the mother adapts her metabolism in order to support this continuous draining of substrates. The mother develops hyperphagia from early gestation which, together with endocrine changes, allow her net body weight to be increased (free of the conceptus), and such change mainly corresponds to the accumulation of fat depots which occurs during the first two-thirds of gestation both in women [1–3] and in rat [4–6]. During the last trimester of gestation, maternal lipid metabolism switches to a catabolic condition, as shown by an accelerated breakdown of fat depots. Adipose tissue lipolytic activity becomes enhanced

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both in women [7,8] and in rat [9,10]] as a consequence of an increase in both mRNA expression and activity of the hormone sensitive lipase [11], the key enzyme for the lipolytic cascade.

In light of the above, one would expect an intense transfer of maternal adipose tissue lipolytic products to the fetus but this is not the case. As shown in Figure 1, the substrate crossing the placenta in the largest quantities is glucose, followed by amino acids [12–14], whereas both FFA and glycerol cross the placental barrier in smaller proportions [15]. In fact, when interspecies comparisons of fetal accretion are made, it emerges that in humans, which at birth have a high body fat content, the placenta is relatively permeable to free fatty acids. It has been suggested that during early gestation, embryonic and fetal lipids are derived from maternal FFA crossing the placenta, whereas in advanced gestation there is a gradual shift to *de novo* synthesis in fetal tissue [16]. As shown in Figure 2, plasma FFA level is higher in 24 h-fasted 20-day pregnant rats than in virgin rats, which agrees with the active adipose tissue lipolytic activity in the former and the limited capability of the placenta for FFA transfer, which may also be responsible for the low plasma level of FFA found in fetal plasma. Plasma FFA are mainly directed to the liver, where they can be used for either esterification in the synthesis of glycerides or oxidation and ketone body synthesis. Both of these pathways are enhanced in the fasted mother during late gestation as shown in the rat [17–19], and plasma ketone body level in 24 h-fasted 20-day pregnant rats is much higher than in virgin rats (Figure 2). Despite ketogenesis not being active in the fetus [20,21], ketone bodies in fetal plasma reach the same level as in the mother (Figure 2) since they are easily transferred through the placenta. The fetus therefore benefits from this product of maternal fatty acid metabolism since ketone bodies may be used not only as fuels [21] but also as lipogenic substrates [22,23].

Placental transfer of glycerol is also very limited (Figure 2), which together with the active adipose tissue lipolytic activity during late gestation described above, justifies the increase in plasma glycerol level seen

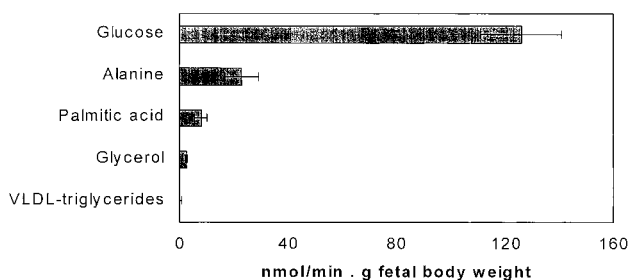


Figure 1. Transfer of D-glucose, L-alanine, palmitic acid, glycerol and very low density (VLDL) triglycerides in the 20-day pregnant rat placenta *in situ*. Placental transfer was measured as previously described [120], based on the infusion of ^{14}C -labeled substrates through the left uterine artery and making proper correction of data for specific activity dilution and uterine artery blood flow

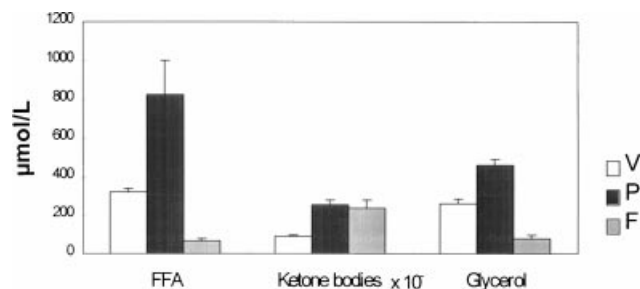


Figure 2. Plasma level of FFA, ketone bodies (β -hydroxybutyrate plus acetoacetate) and glycerol in 24 h-fasted virgin (V), 20-day pregnant rats (P) and their fetuses (F) measured by enzymatic procedures. Differences between values of pregnant vs virgin rats were always statistically significant ($p < 0.001$) whereas those between fetuses and their mothers were significant for both FFA and glycerol ($p < 0.001$) but not for ketone bodies

in the 24 h-fasted 20-day pregnant rat, and its low concentration in fetal plasma. Maternal glycerol is, however, being used as a preferential substrate for glucose synthesis, as reported in the rat [24–26], and this mechanism not only warrants the availability of glucose for placental transfer but saves the use of other gluconeogenic substrates like amino acids, which are less available in maternal circulation [27,28] but are essential for fetal growth.

Another metabolic adaptation normally occurring during late gestation is the development of maternal hypertriglyceridemia. Such hypertriglyceridemia occurs in the mother resulting from an enhanced liver production of VLDL-triglycerides [29,30], together with an increase in the transfer of triglycerides among the different lipoprotein fractions [31,32], an increase in the intestinal absorption of dietary lipids [33] and a reduced clearance of triglyceride-rich lipoproteins due to decreased extrahepatic lipoprotein lipase (LPL) activity which occurs both in women [32] and in rats [11].

Maternal triglycerides do not directly cross the placenta (see Figure 1) but, besides being a source of essential fatty acids for the fetus (see below), they may be used by the fetus as a source of oxidative substrates, although in an indirect manner and under a metabolic emergency condition, such as starvation. Despite the fact that the adult liver lacks LPL expression, 24 h starvation causes a marked increase in liver LPL activity in pregnant rats, although not in nonpregnant rats [34,35], and such change is paralleled by a similar increase in both liver triglycerides and plasma ketone body concentrations [36]. It is believed that such LPL activity in the liver of the starved pregnant rat has an extrahepatic origin, but it is proposed that through this mechanism, the liver, which under normal conditions is a triglyceride-exporter organ, becomes a heightened acceptor of circulating triglycerides, thus allowing their increased consumption as ketogenic substrates, and therefore contributing to the enhanced maternal ketonemia commented above. This condition not only promotes ketone bodies availability to the fetus but must also contribute to a reduced utilization

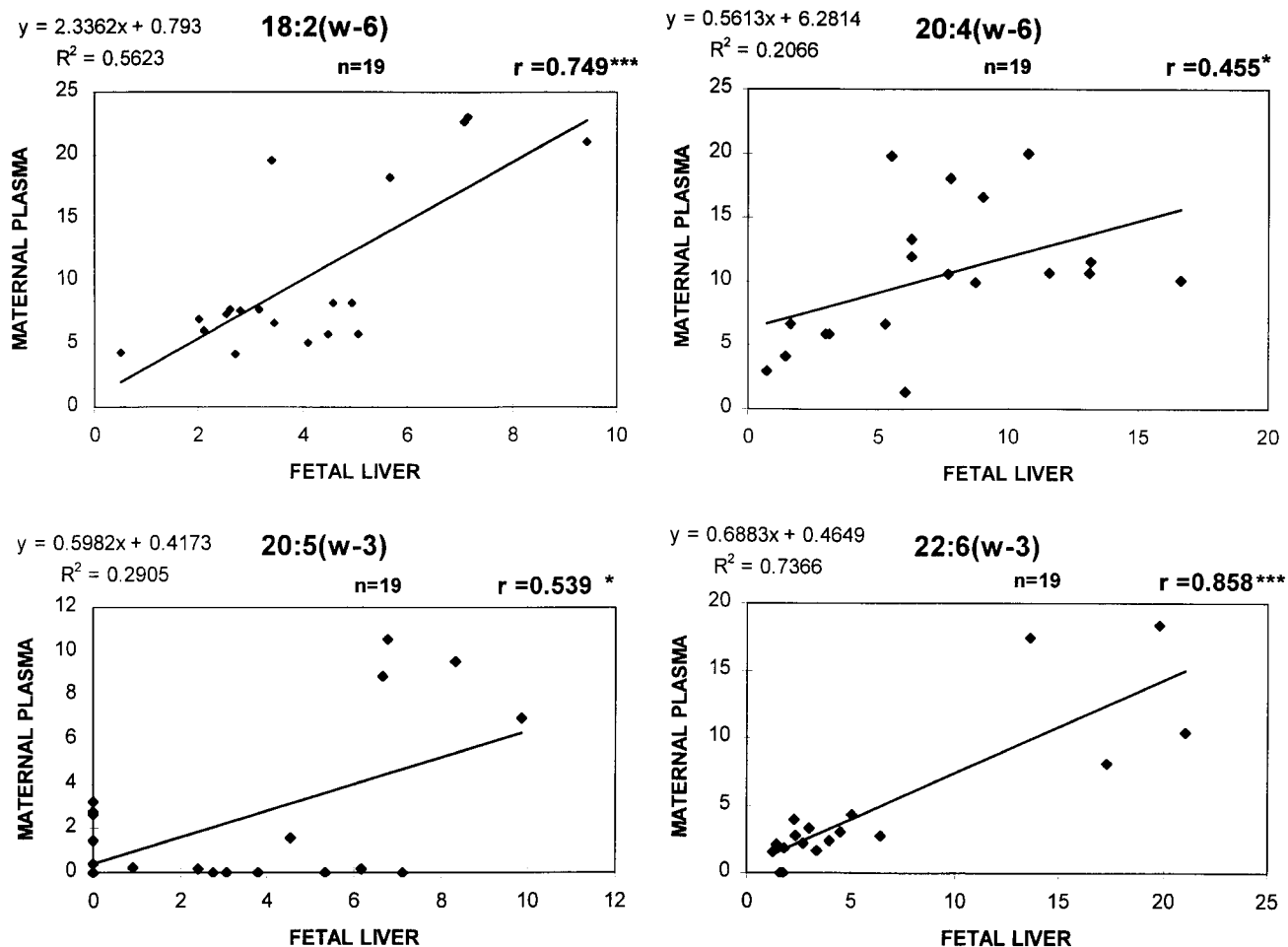


Figure 4. Linear correlation of essential fatty acids between maternal plasma and fetal liver in 20-day pregnant rats fed with a semisynthetic diet [121] containing 5% of either palm oil, sunflower oil, olive oil or fish oil as the only fat source from the day of mating. Asterisks indicate statistically significant correlation ($*=p < 0.05$. $***=p < 0.001$)

and triglycerides [47,69]. It has been shown that the transfer of linoleic acid paralleled the increased transplacental passage of lipids in diabetes, but the uptake of arachidonic acid and its preferential incorporation into triglycerides rather than into phospholipids of the placental tissue and fetal effluent are increased in perfused human term placenta from women with Type 1 diabetes mellitus (DM) [70]. Thus, both the transfer and distribution of this essential LCPUFA is altered in Type 1 DM. An increased transport of linoleic and arachidonic acids was also noted in streptozotocin-induced diabetic rats [71].

Triglycerides and phospholipids are accumulated in the placenta in both human and rat diabetes [72], indicating an enhanced uptake, hydrolysis and re-esterification activity. In the diabetic late pregnant rat a correlation in plasma triglycerides and FFA between the mother and the fetus as well as an enhanced placental transfer of maternal fat to the fetus were found [73,74]. Since the higher birth weight in human diabetic pregnancy has been positively correlated with the extent of both maternal hyperlipoproteinemia [75] and maternal FFA levels [76], it is proposed that maternal hyperlipidemia and enhanced maternal-fetal fat transport may contribute

to the fetal macrosomia frequently found in newborns of diabetic women.

Fat depots in the fetus

The continuous and active transfer of nutrients through the placenta fulfills the energetic demands, growth and fat storing deposition of the fetus. The latter is highly variable among mammalian species, lipid storage during fetal life being an exception rather than a rule, and in most species, body fat content at birth is very low and white adipose tissue is barely detectable, including in the rat [77,78]. However, in the human newborn, fat represents around 16% of body weight [79], and most of it is found in the form of white adipose tissue. In humans, body fat accretion occurs essentially during the last trimester of intra-uterine life. In fact, from week 30, fat accumulation exceeds that of nonfat components [80], and at week 36 of gestation, 1.9 g of fat accumulates for each gram of nonfat daily weight gain, and by term gestation, the deposition of fat accounts for more than 90% of the calories accumulated by the fetus [81], permitting the accumulation of 2.4 g of fat/kg/day [80,82].

Two main factors contribute to this rapid accumulation of lipids in the human fetus during late gestation: (i) besides being quantitatively the main substrate crossing the placenta, glucose is the main energy source for the fetus [83], and approximately 70% of fetal glucose uptake is converted to fat [16]. Both fetal liver and adipose tissue have been shown to have the capacity to synthesize fatty acids *de novo* [84,85]. The enlargement of body fat mass in newborns of diabetic women depends on the insulin-induced increase in triglyceride synthesis and storage as a result of the increased insulin production by the fetal pancreas which, in turn, is secondary to a larger glucose availability *in utero* [86]. (ii) As commented above, fetal essential fatty acids reflect those present in the mother's plasma, indicating that maternal fatty acids are available to the fetus throughout their placental transfer. In addition, fetal fatty acid oxidation is low [84] allowing the preferential channeling of fatty acids to adipose tissue for triglyceride synthesis.

Lipid metabolism during the perinatal period

Source of lipids in maternal milk around parturition

Fat constitutes about 50% of the total caloric value of human milk [87], triglycerides corresponding to the major lipidic component in both colostrum and mature milk [88–91]. The induction of lipogenic activity in mammary glands does not occur until after parturition as reported in the rat [92] and, therefore, lipids in colostrum must come from maternal circulation. In fact, as shown in Figure 5, around parturition in the rat, LPL activity in mammary glands increases whereas in adipose tissue it decreases. Through this mechanism, plasma triglycerides

are driven to be taken up by mammary glands for milk synthesis instead of being accumulated in adipose tissue [33,93] (see Figure 3). Besides the induction effect of prolactin on mammary glands LPL [94,95], these changes are mediated by the opposite responsiveness to insulin seen around parturition between mammary glands and adipose tissue, which is enhanced in the former [42,96] and decreased in the latter [41,42,97]. These changes allow essential fatty acids from maternal diet circulating as triglyceride-rich lipoproteins in maternal plasma to become available to the suckling newborn.

Ketonemia in the neonatal period

Since nonesterified fatty acids (long and medium chain length) are the major precursors for ketone bodies synthesis, the relative high-fat and low-carbohydrate diet present in milk contributes to the marked hyperketonemia normally present in both humans and rats during the suckling period [98]. In adults, adipose tissue lipolysis determines the main supply of long chain fatty acid to the liver, and immediately after birth [99] and during suckling, although less intensely, the rate of lipolysis has been shown to be enhanced both in humans [100] and in rats [101]. The enhanced lipolysis occurring in the first few hours of life appears to be regulated by catecholamine release, resulting in cAMP production and increased protein kinase C activity [99,102]. During the suckling phase, the enhanced lipolysis seems to be caused by an enhanced sensitivity to lipolytic hormones, like thyrotropin [100], and a decreased plasma insulin/glucagon ratio, which also favors lipolysis [103,104].

Although the liver in the adult is considered to be the sole tissue capable of synthesis and release of ketone bodies to the circulation, intestinal mucosa has been shown to synthesize ketone bodies in neonatal rats due to the expression of the key enzyme hydroxymethylglutaryl-

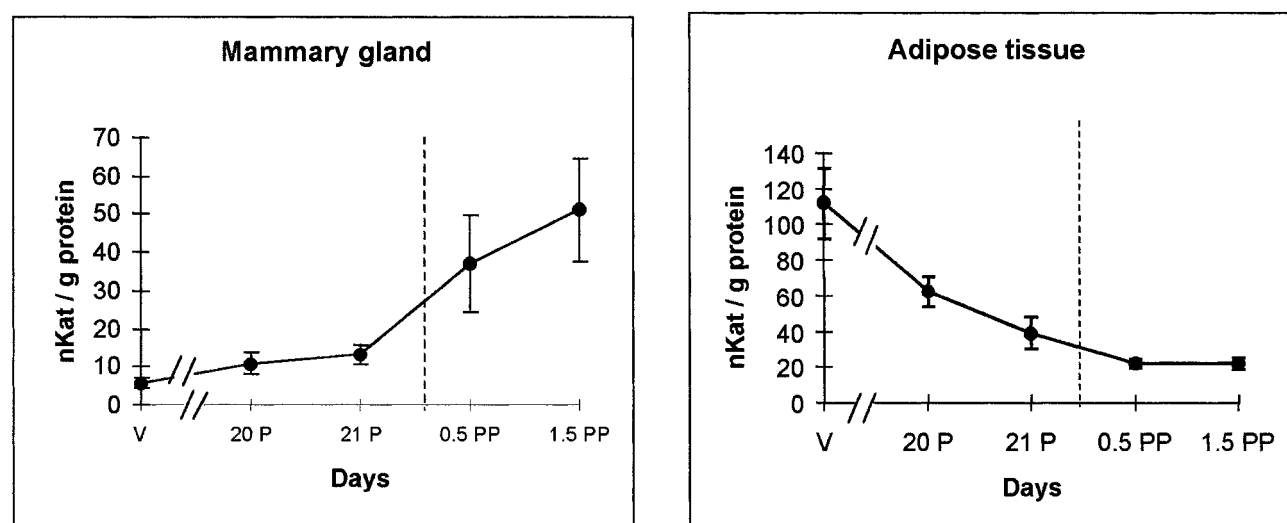


Figure 5. Changes in mammary gland and adipose tissue lipoprotein lipase activity in virgin (V), pregnant (P) and postpartum (PP) rats. Values of LPL activity in both P and PP rats vs virgin rats were statistically significant ($p < 0.05$) at all the time points studied. Experimental details are as previous described [93]

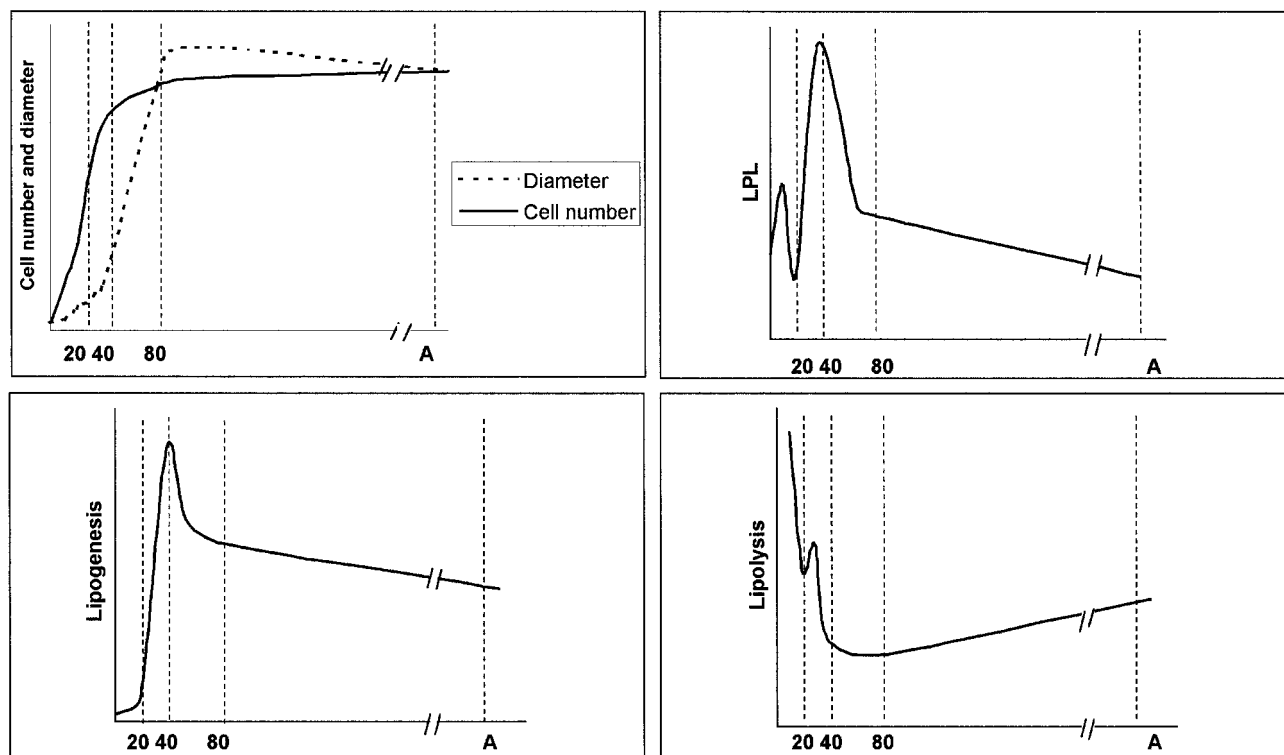


Figure 6. Qualitative morphologic and biochemical changes taking place during the development of white adipose tissue in the rat, based on the references mentioned in the text. The numbers in abscissae correspond to days after birth whereas A represents adults

CoA synthase [105], which is suppressed on weaning suckling rats [105,106]. Although the rate of intestinal ketogenesis does not exceed 10% of that in the suckling liver, it represents an additional strategy to provide ketone bodies to developing tissues [107].

The adult liver does not express LPL, but the fetal liver contains a high level of LPL activity, as initially shown in the rat [108] and later in other species [109], although in human fetal or neonatal liver it has not been documented. This activity increases after birth and further increases under starving conditions in the newborn [110], and although it declines progressively with age, it remains higher in the liver of the suckling rat than after weaning, when this activity declines to the undetectable level seen in adults [111]. Liver LPL activity in newborn rats parallels changes in liver content of triglycerides as well as those of circulating triglycerides and ketone bodies [110]. It is therefore proposed that in the suckling neonate, long chain fatty acids derived from milk lipids, which are transported as chylomicron triglycerides, are channeled to the liver courtesy of its LPL, and such a change may also contribute to the high ketogenic capacity of that organ during the suckling period.

Changes in the metabolism of adipose tissue during development

As described earlier, great interspecies differences exist in the time course of the development of adipose tissue. In

contrast to the active intra-uterine development of adipose tissue occurring in humans, it occurs after birth in rat and this characteristic provides an appropriate model to study adipose tissue development. Figure 6 summarizes in a qualitative manner the major changes known to take place in rat adipose tissue development along the time, differentiating the end of the suckling period which occurs 20 days after birth, the phase of highest hyperplasia, between 20 and 40 days after birth, and the phase of highest cell hypertrophy occurring between 40 and 80 days after birth. Changes in adipose tissue LPL activity gives an index of tissue capability to take up circulating triglycerides, and as shown in Figure 6, it peaks at mid-suckling to decline around weaning but increasing again at 30–40 days of age to decline thereafter [111–114]. The lipogenic activity is very low through the suckling period [115,116] coinciding with the enhanced availability of fatty acids from milk lipids, whereas it rapidly increases during weaning, when diet composition switches from high fat to high carbohydrate. The rate of lipolysis is high after birth, partially declines as the suckling period advances [101,117], shows a small peak just after weaning, and declines afterwards.

In summary, when adipose tissue hyperplasia predominates, corresponding to the suckling period in the rat, lipogenesis is not very active, but the uptake of fatty acids from circulating triglycerides mainly coming from those in milk, appears enhanced. At the same time, adipose tissue lipolytic activity is enhanced, allowing a

rapid turnover of triglycerides within the adipocytes and therefore contributing to the enhanced rate of cell proliferation. From weaning up to around 40 days after birth, the number of adipocytes is still progressively increasing but they start to be filled up with fat (resulting in a rapid increase in cell size) owing to the intense increase in both LPL activity and lipogenesis, which compensates for the reduction of plasma triglycerides caused by the decreased lipid content of the diet during weaning as compared to suckling. The slight increase in cell size occurring around 80 days after birth (Figure 6) could be explained by the intense decline in adipose tissue lipolysis, and the peak of lipogenic activity at a time when new adipocytes are no longer formed. From then on, until adulthood, which in the rat corresponds to the age of 150–180 days, both adipocyte size and number remain constant and the activities of both LPL and lipogenesis progressively decline, whereas the lipolytic activity remains low with a tendency to recover. A similar metabolic process to that occurring in the development of rat adipose tissue after birth takes place in other species during the intra-uterine life, as shown in sheep [118,119], although it is not yet known whether this is also the case in the human fetus, where adipose tissue is, however, known to have lipolytic activity, but with a low sensitivity to hormones [117].

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