Maternal and fetal lipid metabolism under normal and gestational diabetic conditions

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Abstract: Maternal lipids are strong determinants of fetal fat mass. Here we review the overall lipid metabolism in normal and gestational diabetes mellitus (GDM) pregnancies. During early pregnancy, the increase in maternal fat depots is facilitated by insulin, followed by increased adipose tissue breakdown and subsequent hypertriglyceridemia, mainly as a result of insulin resistance (IR) and estrogen effects. The response to diabetes is variable as a result of greater IR but decreased estrogen levels. The vast majority of fatty acids (FAs) in the maternal circulation are esterified and associated with lipoproteins. These are taken up by the placenta and hydrolyzed by lipases. The released FAs enter various metabolic routes and are released into fetal circulation. Although these determinants are modified in maternal GDM, the fetus does not seem to receive more FAs than in non-GDM pregnancies. Long-chain polyunsaturated FAs are essential for fetal development and are obtained from the mother. Mitochondrial FA oxidation occurs in fetal tissue and in placenta and contributes to energy production. Fetal fat accretion during the last weeks of gestation occurs very rapidly and is sustained not only by FAs crossing the placenta, but also by fetal lipogenesis. Fetal hyperinsulinemia in GDM mothers promotes excess accretion of adipose tissue, which gives rise to altered adipocyte profiles. Fetal lipoproteins are low at birth, but the GDM effects are unclear. The increase in body fat in neonates of GDM women is a risk factor for obesity in early childhood and later life.

Keywords: fatty acids; fetal lipids; gestational diabetes; human pregnancy; maternal lipids.

Introduction

Maternal diabetes is still associated with excess maternal and fetal morbidity. Compared with normal pregnancy, women with poorly controlled diabetes have increased risk of preeclampsia, early delivery, cesarean section, and unfavorable intrauterine environment for fetus development, with increased risk of miscarriage, stillbirth, congenital malformations, placental dysfunction, fetus morbidity and mortality, and intrauterine malprogramming [1, 2]. Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy [3–5] and still is a major risk factor for fetal macrosomia or obesity [6, 7]. It was initially proposed that overgrowth of the fetus in maternal diabetes is the result of increased delivery of glucose to the fetus, which consequently develops premature maturation of pancreatic insulin secretion and subsequent hyperinsulinemia, which together with the excess availability of glucose results in overgrowth of the fetus [8]. In fact, the association of high maternal glucose levels and fetus macrosomia has been well documented [9, 10]. However, the increased incidence of macrosomia in newborns is also observed in diabetic pregnancy with satisfactory glycemic control and in GDM mothers who have similar glycated hemoglobin (HbA1c) levels as non-diabetic women [11–13]. In diabetic pregnancy, a wide range of disturbances in lipid metabolism have been reported [14] and maternal lipids are thought to be strong determinants of fetal growth in GDM pregnancies [15].

Both maternal triacylglycerols (TAGs) and non-esterified fatty acids (NEFAs), rather than glucose, are positively correlated with neonatal weight and fat mass in well-controlled GDM women but not in normal pregnancies [15, 16]. This indicates that maternal dyslipemia in GDM actively enhances the availability of lipids to the fetus, contributing to fat depot accumulation and consequent risk of development of macrosomia.

Although lipids cross the placenta with difficulty, changes in placental function must contribute to the increased transfer of maternal lipids to the fetus under conditions of GDM hyperlipidemia. Moreover, it has been also shown that handling or metabolism of long-chain
polyunsaturated fatty acids (LCPUFAs) is altered in the fetus of GDM women [17], suggesting that such a disturbance could also contribute to fat depot accumulation.

The purpose of this chapter is to review the overall changes in lipid metabolism that take place in pregnancy under normal and GDM conditions, analyzing both maternal and fetal size, as well as lipid placental transfer and its consequences for fetal growth.

**Maternal lipid metabolism**

Throughout pregnancy there are major changes in lipid metabolism. In the mother, there is an increase in adipose tissue mass and hyperlipidemia, whereas in the fetus there is a need for essential fatty acids (EFAs) and LCPUFAs, which are necessary for fetal growth and development.

**Adipose tissue metabolism**

During the first two thirds of gestation there is an increase in adipose tissue mass [18, 19], which represents most of the increase in maternal structures that takes place during pregnancy. This is the result of both hyperphagia [20] and increased lipid synthesis [21], which in rats is driven by the enhanced adipose tissue insulin responsiveness that occurs during early pregnancy [22]. During this early stage of pregnancy, there is unchanged [23] or even increased adipose tissue lipoprotein lipase (LPL) activity [24]. This enzyme is extrahepatic, being especially active in adipose tissue, and catalyzes the hydrolysis of circulating TAGs in TAG-rich lipoproteins (chylomicrons and very low density lipoproteins, VLDLs) [25]. The hydrolytic products, NEFAs and 2-monoacylglycerol or glycerol, are taken up by the subjacent tissue for re-synthesis of TAGs [26, 27] and these changes facilitate the accumulation of circulating lipids in maternal fat depots.

The increase in maternal fat depot accumulation stops during the third trimester of gestation as result of three main changes: (a) decreased adipose tissue fatty acid synthesis [22]; (b) decreased LPL activity [23], which causes a decline in the hydrolysis and tissue uptake of TAGs in TAG-rich lipoproteins and contributes to the development of maternal hypertriglyceridemia; and (c) increased adipose tissue lipolytic activity [28, 29]. Under normal conditions, the first two pathways are known to be stimulated by insulin whereas the third is inhibited. It is therefore proposed that the insulin-resistant condition present in the mother in the last trimester of pregnancy [30, 31] is responsible or actively contributes to these three changes. In fact, it has been shown that during late pregnancy there is resistance to the action of insulin in adipose tissue metabolism [32]. However, the regulation of adipose tissue lipolytic activity during late pregnancy deserves special attention. Lipolysis is under tight regulation by hormones, catecholamines and insulin being considered their major regulators in humans [33–35]. However, in addition to the insulin-resistant condition, other pregnancy hormones could contribute to such change. In fact, pregnancy-specific hormones have insulin-antagonistic and lipolytic effects [36].

In spite of the important role that increased adipose tissue lipolytic activity might play in maternal–fetal metabolic interactions during pregnancy, there is practically no data available regarding lipolysis during late pregnancy in normal women. There is, however, one report showing increased lipolysis in non-obese pregnant women in the third trimester and a significant negative correlation between insulin and the rate of glycerol production. However, no correlations were found between levels of glucagon, human placental GH (hPGH), estrogens, progesterone, or chromogranin A and the rate of glycerol production [37]. The reason for the lack of such correlation is not known although the mechanisms underlying insulin resistance in late pregnancy are well known. Placental hormones such as human placental lactogen and hPGH, as well as cortisol and estrogens, have been extensively studied as underlying the insulin resistance in late pregnancy. Recent studies have also implicated several factors derived from adipose tissue (collectively known as adipokines or adipocytokines) that mediate insulin resistance in pregnancy [38, 39]. A few of these adipocytokines are known to have increased plasma concentrations during late pregnancy and to decrease insulin sensitivity. Such factors include leptin [40], tumor necrosis factor-α (TNF-α) [41], interleukin-6 [42], visfatin [43], chemerin [44], retinol binding protein-4 (RBP4) [45], and resistin [46]. Other adipokines, such as adiponectin [47, 48] and apelin [49], are known to decrease during late pregnancy and to increase insulin sensitivity. These changes actively contribute to the maternal insulin-resistant condition during late pregnancy. Although these and other adipocytokines are derived from adipose tissue as well as other tissues such as liver, during pregnancy most of them are also produced by the placenta [50] and, therefore, actively contribute to insulin resistance and its effects, enhancing adipose tissue lipolytic activity during late pregnancy.

Increased levels of NEFAs as result of active adipose tissue lipolytic activity during late pregnancy can also contribute to insulin resistance [51]. In addition, the
transcription factor peroxisome proliferator-activated receptor gamma (PPAR-γ), which is normally highly expressed in adipose tissue, is a regulator of adipogenesis and lipogenesis through binding to several genes [52, 53] and inducing synthesis of proteins such as adiponectin [54] and LPL [55]. It has been proposed that PPAR-γ coordinates the balance between fat deposition and mobilization through its effects on lipolysis [56]; it has also been implicated in the regulation of insulin sensitivity [57]. During late pregnancy, the levels of PPAR-γ mRNA and protein are lower in adipose tissue in both control and GDM women than in non-pregnant women [58].

The underlying pathophysiology of GDM is a function of two major metabolic disorders, pronounced peripheral resistance to insulin and inadequate β-cell function [31]. Accumulating evidence indicates that adipose tissue plays a key role in the development of GDM, not only because it is the main site of lipid storage but also because it is responsible for the synthesis and secretion of adipocytokines. Thus, those changes in maternal adipose tissue metabolism during late pregnancy known to be controlled by decreased insulin sensitivity become further affected in this condition, resulting in the increase in plasma NEFAs and ketone bodies seen in GDM pregnant women [59, 60]. Under these conditions, maternal adipokines associated with insulin resistance, such as adipocyte fatty acid-binding protein [AFABP] [61, 62], leptin [63], resistin [64, 65], and RBP4 [66] (among others), are higher whereas those associated with insulin sensitivity, such as adiponectin [47, 48], are lower in GDM than in control pregnant women [39, 67], contributing to the chronic insulin resistance seen in GDM. Although the ability of insulin to inhibit lipolysis is reduced during late pregnancy [68], this is further reduced in GDM subjects [31, 58]. Furthermore, although the cellular mechanisms involved in its development are not yet completely understood, the pronounced resistance to insulin seems to actively contribute to the reduced insulin suppression of lipolysis present in GDM women with advancing gestation [58].

**Maternal dyslipidemia**

During late pregnancy, the net catabolic condition of maternal adipose tissue is associated with hyperlipidemia, which mainly corresponds to increases in TAG levels with smaller rises in phospholipids and cholesterol [23, 69]. The greatest increase in plasma TAGs corresponds to VLDLs as result of their increased production by the liver [70] and decreased removal from the circulation as a result of decreased LPL activity in adipose tissue [23]. Plasma lipoproteins of higher density than VLDLs, such as LDLs and HDLs that normally do not carry TAGs, also become enriched in TAGs during late pregnancy [23] as result of an increase in cholesteryl ester transfer protein (CETP) activity [71, 72] and a decrease in hepatic lipase activity [23]. This latter change also seems to be responsible for the proportional accumulation of TAGs in buoyant TAG-rich HDL₃ subfractions at the expense of cholesterol-rich and TAG-poor HDL [23]. The increase in plasma estrogen levels during pregnancy probably contributes to these changes, because estrogens are known to stimulate hepatic VLDL production in pregnancy [69] and to decrease hepatic lipase activity [73, 74].

It is proposed that the two main hormonal factors responsible for the metabolic interactions that result in the development of maternal hypertriglyceridemia during late pregnancy are the high lipolytic activity and low LPL activity in adipose tissue caused by the insulin-resistant condition and the increased liver secretion of VLDLs [69] and decreased hepatic lipase activity caused by estrogen [75]. Figure 1 summarizes these metabolic interactions and the modulating effect of insulin resistance and estrogen on them.

The dyslipidemic condition in diabetic pregnancy has been reported to be very variable. Some studies have found exaggerated hypertriglyceridemia in diabetic pregnancy compared with normal pregnancy in the three trimesters of gestation [76–78], but there are also studies showing no differences [59, 79, 80]. Plasma cholesterol, LDL-cholesterol, and HDL-cholesterol levels were also found to be higher or lower in GDM as compared with normal pregnancy (for a review see [14]). However, small sized and dense LDL particles, which are characteristic of insulin-resistant conditions and have been associated with increased risk of coronary artery disease, have been consistently found in GDM women [80, 81].

The variations in the reported findings related to the dyslipidemic condition of diabetic pregnant women are not completely understood. As commented above, some of the changes occurring in lipid metabolism during late gestation of normal pregnancy are driven by both the insulin-resistant condition and increases in steroid hormones. Plasma levels of these hormones [59, 82] and of sex hormone-binding globulin [83] are decreased in pregnant diabetic women. Furthermore, plasma lipoprotein TAG levels have been shown to correlate with prolactin, estradiol, and progesterone in the course of gestation [59]. Because the degree of metabolic control can affect both the dyslipidemic condition and the level of sex hormones during pregnancy, it is proposed that the development or lack of development of exaggerated hypertriglyceridemia
in diabetic pregnant women compared with the control condition depends on the balance between those two factors.

**Placental lipid transfer in normal and GDM pregnancies**

The placenta separates the maternal and fetal circulations, and any substance destined for fetal supply has to traverse this organ. Because of the rising incidence of newborns with excessive adiposity, lipid transfer across the placenta has received increasing interest in the past decade. Yet, the complexity of the organ and the processes involved, combined with a scarcity of relevant data, have hampered the development of a convincing concept about transfer mechanisms and their regulation. The current concept described below is thus mostly based on extrapolation of results from other tissues [84–88].

**Concept of maternal–fetal lipid transfer and placental metabolism**

Figure 2 depicts current understanding of maternal-to-fetal transfer of fatty acids. About 1%–3% of the fatty acids in the maternal circulation are non-esterified (i.e. the vast majority are in lipoproteins). LDL is the major lipoprotein in the maternal circulation and can be taken up into the syncytiotrophoblast (the placental tissue interfacing with maternal blood) by receptor-mediated endocytosis [89, 90]. After uptake, the LDL-TAG and LDL-cholesterol esters can become hydrolyzed by intracellular lipases and cholesterol-ester hydrolases and contribute to the intracellular fatty acid pool. Other lipoproteins such as VLDLs
and HDLs bind to receptors on the syncytiotrophoblast surface and become hydrolyzed extracellularly. Endothelial lipase has been identified as the major extracellular placental lipase [91, 92], but this is not a uniform finding and there are claims about the presence of LPL [93]. Regardless of the molecular identity of the TAG hydrolase activity [94], the resulting NEFAs must be taken up by the syncytiotrophoblast to eventually contribute to the intracellular fatty acid pool. Uptake is accomplished by fatty acid transporters such as fatty acid transport proteins (FATB)-1, FATB-4, fatty acid translocase (FAT/CD36), and plasma membrane fatty acid binding protein (p-FABPm) [95]. These transporters have overlapping specificities for the various fatty acids, and their relative contribution to the uptake of distinct fatty acids is unknown.

Once inside the syncytiotrophoblast, the fatty acids must be bound to fatty acid binding proteins (FABP), of which the heart-type isoform appears to be the major FABP in the human placenta [96]. From there, fatty acids can enter the various metabolic routes, including β-oxidation, conversion into eicosanoids, and re-esterification to form phospholipids and triglycerides. The latter may then be stored in lipid droplets in the syncytiotrophoblast.

NEFAs can traverse the cytoplasm of the syncytiotrophoblast and are then released into the fetal circulation, where they bind to transport proteins such as α-fetoprotein. They are taken up in the fetal liver, esterified and metabolized or used to form lipoproteins, mostly HDL, which is the main cholesterol-carrying fetal lipoprotein in humans. The various fatty acids contribute differentially to fetal lipid pools (i.e. NEFA, TAG, phospholipids – mainly phosphatidylcholine, and cholesterol-esters) [97].

Interestingly, the human placenta has a preference for the transport of docosahexaenoic acid (DHA) over arachidonic acid (AA), α-linolenic acid (ALA), and linoleic acid (LA) [98]. This could explain the enrichment of LCPUFAs in fetal plasma and in particular of DHA [99, 100] and also reflect the high demand of the growing fetus for DHA, particularly to sustain brain and retinal development.

**Challenges to this concept**

This concept, which has been around for about a decade, suffers from several deficiencies (i.e. it is in contradiction to some important biochemical and biological principles).

Although fatty acid transfer proteins (FATPs) appear to be the main fatty acid transporters involved in the uptake of fatty acids, their conceptual involvement in the overall transfer process poses a problem. This is because FATPs are not only transporters, but also have acyl-CoA-ligase activity [101]. Thus, any fatty acid taken up by an FATP becomes an acyl-CoA immediately after having reached the cytoplasmic side of the FATP. Although this CoA-ligation is necessary to allow fatty acids to enter metabolic pathways, it poses a problem for fatty acid egress from the syncytiotrophoblast. No transporter molecule...
is known to date that accepts acyl-CoA. Thus, fatty acid-CoAs need to be hydrolyzed to release free fatty acids, a reaction catalyzed by acyl-CoA thioesterases. It is unclear whether these enzymes are present in the human placenta, although they have been found in BeWo cells, a choriocarcinoma cell line derived from the human syncytiotrophoblast [102]. This raises the question of whether FATPs account for the uptake of fatty acids destined for transfer to the fetus. Alternatively, simple diffusion could allow the passage of fatty acids across the syncytiotrophoblast to the fetal circulation.

The proportion of fatty acids on the maternal side of the syncytiotrophoblast that are transferred to the fetal circulation is entirely unknown; it is not even known whether it is most of the fatty acids or only a small fraction. Some information comes from in vivo studies using palmitic acid (PA, 16:0), oleic acid (OA, 18:1 n-9), LA (18:2 n-6), and DHA (22:6 n-3), all labeled with 13C-stable isotopes [103]. These were administered to the mother 12 h prior to delivery by cesarean section, at which time enrichment of the 13C-labeled fatty acids in cord blood was determined. Interestingly, only about 0.5% of the fatty acids administered to the mother were enriched in the fetal circulation. The exception was DHA, which was enriched by 3.5%. It is pertinent that the absolute transfer rate for PA and LA is in the range of 6–7 mmol/24 h. Extrapolation to the amount of fat stored in the fetus during the period of rapid development (i.e. the third trimester) demonstrated that maternal-to-fetal fatty acid transfer in normal pregnancies contributes to only about 20% of the neonatal fat stores (about 350 g) [104].

A further open question relates to the lipid droplets found in the syncytiotrophoblast. Although there is evidence that the droplets store fatty acids in TAGs, it is unknown whether they can also mobilize the TAGs or whether the released fatty acids contribute to the fatty acid pool destined for release to the fetal circulation or to the metabolic pool, or both.

The effect of GDM

There are multiple determinants of overall metabolite transport across the placenta, including that of fatty acids. At the level of the placenta, key roles are played by its structure, morphology, metabolism, and the number and activity of transporter molecules involved. If VLDL- and HDL-borne fatty acids contribute to overall transfer, then the lipase activity(ies) could be rate limiting. Also, thioesterase activity is relevant if the fatty acid-CoAs must be hydrolyzed to NEFAs and CoA. In addition, the utero-placental and umbilical blood flow, as well as the maternal-to-fetal concentration gradient, can become main determinants in the case of flow-limited nutrients. Each of these determinants could be modified by maternal GDM. Thus, the measurement of any single contributor to overall transfer does not allow conclusions to be made about potential GDM effects. Rather, methods have to be used that integrate each distinct GDM-associated change and provide an overall readout. This is why studies using stable isotopes are so powerful. Using this method it was demonstrated that the fetal enrichment of three maternally administered fatty acids (PA, OA, and LA) was similar in normal and GDM pregnancies, and that only DHA enrichment was reduced by about 50% [103]. Because fetal enrichment was measured in cord blood, this finding does not necessarily reflect the extent of transfer. An unknown proportion of fatty acids could have been incorporated into fatty acid ester pools (i.e. in TAGs, phospholipids and/or cholesterol esters) in fetal tissues and thus escaped measurement in cord blood. In addition, DHA and AA are retained in the placenta in GDM and integrated into phospholipids. This could also explain the lower DHA enrichment in cord blood in GDM [105].

The only study that used the ex vivo placental perfusion system (a method integrating the whole placental tissue and all its changes associated with a given condition) in diabetic pregnancies found increased placental uptake and release of AA into the fetal circulation [106]. However, this was in type 1 diabetes mellitus and, therefore, might not be representative of GDM.

Collectively, with all its limitations, the available evidence does not suggest that the fetus receives more fatty acids in a condition of maternal nutritional oversupply, such as GDM, than in non-GDM pregnancies.

Placental lipid metabolism in GDM

The GDM placenta contains more TAGs and phospholipids, but not cholesterol esters, than the normal placenta [107, 108]. These depots are almost exclusively stored in the syncytiotrophoblast as lipid droplets [109]. This location suggests that the build up of lipid droplets is fuelled mostly by maternally derived lipids.

In fact, in vitro experiments have demonstrated the key role of fatty acids in driving lipid droplet formation. Droplet formation is accompanied by increased expression of adipophilin (a lipid droplet-associated protein) and of fatty acids acting in concert with insulin [110, 111]. This process also requires proper activity of FABP-4 [112]. Because the maternal concentrations of both insulin and
fatty acids are elevated in a medically managed GDM pregnancy, one could envisage their contribution to enhanced placental lipid storage in GDM. However, these results were obtained in trophoblast cells from normal pregnancies and, therefore, extrapolation to GDM has to be made with some caution. Thus, direct evidence is pending.

The energy requirements of the placenta are thought to be covered by maternal glucose sources. However, only about one third of maternal glucose taken up by the placenta is used to sustain placental activities, of which about 80% is channeled into the glycolytic pathway, resulting in lactate production [107]. Elevation of maternal glucose supply by higher circulating concentrations in GDM are unlikely to stimulate glycolysis because this pathway, at least in the normal placenta, is already saturated at low glucose concentrations (about 5–8 mmol/L) [110, 113]. Thus, the placenta probably depends on additional energy sources, and fatty acids are likely candidates. The presence of active enzymes involved in β-oxidation, predominantly in the syncytiotrophoblast (the metabolically most active placental cell type), provides the molecular basis [114, 115]. However, the proportion of fatty acids stored in lipid droplets or oxidized to cover the extra energy requirements of increased placental activity in GDM is unknown.

An important pathway for fatty acids, especially AA, is conversion into eicosanoids such as prostacyclin and thromboxane. In type 1 diabetes mellitus, AA conversion into thromboxane and prostacyclin is enhanced, with preferential formation of thromboxane B2 [116]. This imbalance of vasoactive eicosanoids with preference for the vasoconstricting thromboxane B2 could explain the reduced feto-placental (i.e. umbilical) blood flow in type 1 diabetic pregnancies. Because GDM is only rarely accompanied by a change in umbilical blood flow, this pathway has failed to receive attention in this condition and studies have not been conducted so far.

Fetal lipid metabolism

Role of LCPUFAs in fetal growth

Fatty acids are essentials for fetal development because they are used as structural components, as a source of energy, as precursors of bioactive compounds such as eicosanoids, and as regulators of transcription factors. Practically all fatty acids can provide energy, but structural and metabolic functions mainly require polyunsaturated fatty acids (PUFAs) of the n-3 or n-6 series. The fetus must obtain these PUFAs from the mother, who obtains them either from the diet or by supplementation [117]. Both ALA and LA are so-called essential fatty acids (EFAs) and must be present in the maternal diet, whereas their LCPUFA derivatives can be synthesized from EFAs through the n-3 and n-6 pathways, respectively, by the processes of elongation and desaturation. Metabolically important n-3 LCPUFAs are eicosapentaenoic acid (EPA, 20:5 n-3) and DHA; AA is an important n-6 LCPUFA. Although none of these three fatty acids are absolutely required in the maternal diet because they can be synthesized from EFA precursors, the fetus depends mainly on their exogenous supply as conversion of EFAs to LCPUFAs in term and preterm neonates is very limited. Thus, because production of EPA, DHA, and AA might be inadequate during periods of rapid intrauterine growth, they are considered essential for the fetus [118]. The major proportion of those LCPUFAs are found in the maternal circulation in their esterified form associated with plasma lipoproteins, with only a minor proportion in the form of NEFAs [119, 120]; therefore, the metabolic interactions summarized in Figure 1 are essential to ensure appropriate availability of those LCPUFAs to sustain fetal development.

During the first 8 weeks of intrauterine life, PUFAs are already required by gametes and embryo [121], but their net rate of utilization is small and does not represent an additional demand on the mother or her diet. Until around 25 weeks of gestation in humans the accumulation of lipids and specific fatty acids by the fetus is relatively small, but increases logarithmically with gestational age [86]. This period corresponds to the greatest accretion of individual fatty acids and it is crucial that there is sufficient supply of both AA and DHA for proper functions [122] such as retinal and cerebral function.

Intervention trials have been conducted in different countries to determine the effects of marine oil or DHA and EPA supplementations in different periods of pregnancy. Although there were no clear differences in birth weight it was found that low n-3 LCPUFA levels were associated with dysfunctional brain and retinal development, suggesting that pregnant women should achieve an average intake of at least 200 mg/day of DHA to achieve a desirable outcome [123].

Arachidonic acid has been shown to be of particular importance for growth and development [124] and a linear regression between birth weight and plasma AA (but no other plasma fatty acid) has been found in premature infants [125]. Although not completely established, the growth-promoting effect of AA could be related to its function as a precursor of prostaglandins and other eicosanoids, or to its structural role in membrane phospholipids.
Fatty acids as source of energy for the fetus

The fetus has classically been considered to be primarily dependent on glucose oxidation for energy production [126]. However, several recent reports have re-evaluated the role of mitochondrial fatty acid oxidation (FAO) in human placental and fetal metabolism [114, 115, 127, 128]. The mRNA expression and activity of FAO enzymes in several human fetal tissues and in placenta [129] suggest that FAO actively contributes to fetal and placental energy production. Several recessive inherited disorders in genes of the FAO pathway have been reported to be associated with prematurity, intrauterine growth retardation, and other disorders that may progress to coma and death [130, 131].

Even in animal studies, despite the predominant role of glucose oxidation for energy production, disturbances of enzymes of the FAO pathway are associated with reduced fertility, fetal demise, and fetal growth restriction [132–134]. Furthermore, inactivation of genes encoding the transcription factors that regulate the FAO pathway, such as the peroxisome proliferator-activated receptors (PPARs), cause embryonic lethality and failure of the syncytiotrophoblast to develop and sustain pregnancy [135].

It is therefore suggested that, in contrast to the results obtained in animal studies, FAO plays an important role in the human fetus as a source of metabolic energy for placental function and fetal development. It is to be expected that there are differences in the proportion of each fatty acid being oxidized by the placental–fetal unit, LCPUFAs from maternal origin probably being the preferential substrate.

Lipid synthesis

From weeks 26–40 of gestation, human fetal weight increases more than fourfold, and beyond 30 weeks of gestation fat accumulation exceeds that of non-fat components [136]. In fact, the rate of fat accretion is approximately linear between 36 and 40 weeks of gestation [137]. By the end of pregnancy, fat accretion ranges from 1.6–3.4 g/kg/day [138] giving a total fetal body fat of approximately 14% of mean birth weight [139]. Although about half of that fat is derived from maternal sources passing across the placenta over the whole period of gestation [140], the remainder could be due to lipogenic activity within the fetus. It is known that as early as weeks 12–20 of gestation human fetal tissues are capable of incorporating different labeled substrates into lipids both in vitro [141–143] and in vivo [144]. It has even been shown that fatty acid synthesis from various precursors is higher in human fetuses than in their mothers, and that the rate of this synthesis decreases rapidly after birth, particularly in the liver [145]. Those lipids correspond to fatty acids, TAGs, phospholipids, and cholesterol. When palmitate is used as substrate, it is mainly incorporated into phospholipids and TAGs, whereas the synthesis of sterols is lower [146]. These data indicate that from an early embryonic stage, human tissues contain all the enzymatic machinery to synthesize lipids from carbohydrates. It should also be pointed out that there are differences in the rate of lipogenesis, not only between different species but also between different organs in the same species. An example is fatty acid synthesis in the brain of fetal rats, which is higher than in the liver [147].

Fetal adipose tissue development

White adipose tissue (WAT) appears in the second trimester of intrauterine life, and adipogenesis in different fat deposit sites begins at 14–15 weeks [148–150]. Adipogenesis begins with the accumulation of mesenchymal stem cells, which develop into adipocytes near the networks of capillaries [148, 149]. Then, early fat cell clusters develop into WAT, consisting of vascular structures and densely packed white adipocytes [151]. Thereafter, fat lobules slowly develop with predominating multilocular fat cells. After the 23rd week, the total number of fat lobules remains approximately constant and the growth of adipose tissue is determined mainly by an increase in size of the fat lobules. At the beginning of the third trimester, adipocytes are found in the characteristic fat depot areas but are still rather small [150]. After birth, the number of fat lobules remains constant, whereas their size continuously grows. This increase in fat mass is mainly the result of an enlargement in existing fat cell size [152, 153].

The process of adipogenesis implies the determination of preadipocytes from stem cells and the terminal differentiation of preadipocytes into mature adipocytes [149, 154]. Adipogenesis is a complex process that is not completely characterized and involves a cascade of gene activation processes, of which PPAR-γ is the master regulator [53, 155, 156], although its endogenous ligand is still unknown.

Several perinatal factors, including elevated insulin levels in amniotic fluid [157], have been associated with subsequent obesity development in offspring. Some studies suggest a direct relation between maternal prepregnancy body mass index (BMI) and/or maternal gestational weight gain and the newborn’s body fat mass [158–161].
Lifestyle intervention studies that comprise physical activity and dietary counseling seem to be effective strategies for reducing excessive gestational weight gain and improving maternal and neonatal outcomes [162–164]. Natural long-chain fatty acids act in preadipocytes as adipogenic hormones through their participation as transcriptional regulators of the expression of lipid-related genes such as those encoding PPARs, and thus promote adipogenesis [165, 166]. Evidence from animal and human studies suggests that changing the n-6:n-3 fatty acid ratio in the diet favors the possibility of altering the early stages of adipose tissue development during fetal life [167]. It was shown that the n-6 LCPUFA AA inhibits cell proliferation and promotes the differentiation of preadipocytes to adipocytes, whereas the n-3 LCPUFAs DHA and EPA seem to counteract this process [167, 168]. The effect of n-3 supplementation during pregnancy and lactation on infant body weight composition has only recently received attention. To date, results are inconclusive although there is no evidence that supplementation with n-3 fatty acids and reduction in dietary AA intake during pregnancy affects offspring fat mass [169, 170].

Newborns from well-controlled GDM mothers can have normal body weight but higher fat mass than those from healthy mothers [16, 171]. Enlargement of body fat mass in newborns of GDM mothers seems to be exclusively the result of an increase in the TAG content of single adipocytes rather than a change in the fat cell number [172]. Serum glucose levels and consequent insulin levels in cord blood of GDM women are augmented in comparison with control pregnancies [16]. Because neonatal plasma insulin levels have been shown to correlate with body fat mass and fat cell weight [173], such hyperinsulinemia in the fetus of GDM mothers would facilitate TAG synthesis, being responsible for increased storage in single adipocytes.

Adipose tissue is considered an endocrine organ, secreting adipocytokines. Given their importance in fetal growth and maturation for both survival at birth and overall health, adipocytokines have been studied in the human newborn of normal and complicated pregnancies [174–179]. There is a wealth of information on factors contributing to fetal growth, but we focus here on adipocyte fatty-acid binding protein (AFABP), leptin, adiponectin, retinol binding protein 4 (RBP4), and angiopoietin-like protein 4 (ANGPTL4). These factors have been studied in control and GDM conditions [67].

AFABP is responsible for intracellular fatty acid trafficking, which contributes to the regulation of hormone-sensitive lipase activity [180], one of the enzymes controlling adipose tissue lipolysis. In pregnant women it was found that AFABP serum concentration was higher in GDM than in control subjects, whereas in cord blood serum it was lower in GDM than in control subjects. AFABP concentration in cord serum of control subjects was higher than in the corresponding maternal serum; this was not the case in GDM, even though AFABP correlated with neonatal fat mass in these GDM fetuses [67], indicating a direct interaction between the two.

Leptin concentration in cord serum has a strong positive correlation with neonatal fat mass [181]. Higher leptin concentrations have been found in fetal plasma from women with either type I diabetes or GDM compared with non-diabetic controls [182, 183]. The relationship between fetal leptin and birth weight has not been clearly established and, although higher concentrations of leptin in cord plasma have been reported in diabetic pregnancies associated with fetal macrosomia [184–186], lower concentrations have also been reported [187].

RBP4 is another adipocytokine that, in addition to its role in the transport of retinol from liver to target tissues, regulates glucose metabolism and reduces insulin sensitivity in humans [183, 188]. Its concentration in GDM pregnant women and cord blood serum is higher than in controls [67, 189], which could contribute to the exaggerated insulin resistance in women with GDM and their newborns [16]. Maternal levels of RBP4 show a positive correlation with maternal fat mass [189], although the implications for fetal growth and development remain unknown.

In contrast to the above-mentioned adipocytokines, adiponectin increases insulin sensitivity and its levels in human pregnancies are lower in GDM than in controls [187]. Adiponectin is synthesized in several fetal tissues, which justifies its high concentrations in umbilical plasma [190]. Under normal conditions, cord blood adiponectin concentration increases with gestational age and correlates positively with birth weight [191], indicating that it contributes to fetal adiposity and development. However, in the cord blood of GDM pregnant women adiponectin levels are lower than in controls and no correlation was found with neonatal fat mass or body weight [67].

ANGPTL4 is secreted into serum from adipose tissue, liver, and placenta [192] and its overexpression in mice or treatment with it increase TAG levels as result of irreversible inhibition of LPL activity [193]. This protein has been recently studied in human pregnancy and newborns. It was found that GDM pregnant women who delivered newborns with high fat mass had high concentrations of both TAGs and NEFAs and low concentrations of ANGPTL4 [194]. Their neonates had lower concentrations of TAGs and no differences in NEFA and ANGPTL4 levels, but high
insulin concentrations, which would contribute to their increased fat depots.

In addition to the adipocytokines mentioned above, there are several other factors that affect fat depot and lipid metabolism directly or indirectly in the fetus, therefore contributing to development. Potentially, they could affect fetal body composition and growth in both normal and diabetic pregnancies but their nature and involvement remain to be investigated.

**Fetal lipoproteins**

During early gestation [10–16 weeks post-conception] the cholesterol concentration in umbilical cord plasma is around 80 mg/dL and cyclically decreases to around 50 mg/dL at term [195, 196]. The cord serum TAG concentration averages 30–50 mg/dL [197–199]. LDL-cholesterol also declines during gestation in the human fetus, attaining concentration values of 25–30 mg/dL at term [196, 200, 201]. However, HDL-cholesterol concentration is stable throughout gestation and averages 30–50 mg/dL [197–199]. The cord serum TAG concentration remains around 80 mg/dL and cyclically decreases to around 50 mg/dL at term [196, 200, 201]. All these values for plasma lipoproteins are lower than in adults. In the case of HDL, which represents the main lipoprotein class in cord blood, plasma contains higher levels of large HDL, and a broader range of particle sizes [202, 203]. The plasma VLDL concentration of cord blood at term is also lower than in adults [204, 205], although it increases and the VLDLs become enriched in apoprotein E (apo E), apo CIII, and TAGs during the first week of life [205].

In agreement with the low levels of lipoproteins in the human fetus at birth, apolipoprotein B (apo B) and apolipoprotein A-I (apo A-I) concentrations are also low [205–207]. There are also some changes in the apoprotein content of certain particles. This is the case for HDL, which, despite maintaining a relatively stable concentration in the human fetus, shows an increase in its apo A-I concentration between weeks 20 and 40 of gestation, causing an increase in the ratio of apo A-I to HDL-cholesterol [208]. The main feature of fetal HDL is the high proportion of apo E [209], which is mainly associated with lower-density HDL classes. The concentration of apo E in cord plasma is close to or even higher than in adult plasma. Because fetal HDL and apo E have the ability to modulate placental gene expression, it has been proposed that the function of apo E-rich HDL in the fetal circulation is different to that of adult HDL with its higher proportion of apo A-I [210].

During gestation there is a progressive increase in hepatic LDL receptors in the human fetus, attaining higher activity at mid-gestation than in adult liver [200]. This change has an important role in lowering the concentration of LDL cholesterol, as commented above. After birth, cholesterol feeding decreases LDL receptor activity, as occurs in adults [211], therefore it is suggested that the absence of this regulatory process in the fetus contributes to the elevated LDL receptor activity and low LDL-cholesterol levels during late intrauterine life.

The human fetus has low activity of lecithin-cholesterol acyl-transferase activity [212, 213], causing a lower level of plasma cholesterol esterification [214] that could also contribute to the differences in lipoprotein composition compared with the adult.

Little is known about the effects of diabetes on lipoproteins in the fetus, although marked differences have been reported. An increase in LDL-cholesterol and decrease in HDL-cholesterol [215] and increases in NEFAs, total cholesterol, free cholesterol, esterified cholesterol, phospholipids, TAGs, apo A-I, and apo B in newborns of well-controlled type I diabetic mellitus mothers have been found [216]. Recently, HDL-proteome remodeling resulting in altered functionality of HDL was found to be associated with impaired cholesterol efflux capability and diminished anti-oxidative particle properties in newborns of GDM mothers [217].

**Effects of altered maternal lipids in GDM women on fetal growth**

It is well known that diabetic pregnancies are associated with a high incidence of fetal growth disorders and, although it was previously proposed to be the result of maternal hyperglycemia, it is now evident that control of fetal growth is far more complex than previously thought [218, 219]. Besides obvious disruptions to the control of glucose concentration and metabolism in diabetes, there is evidence that disturbances in maternal lipid metabolism could also contribute to these disorders [14, 220]. As expected, an altered maternal lipid profile affects the quantity and quality of lipids being transferred to the fetus. Increases in maternal plasma TAG levels in GDM women have been related to neonates that are either large or small for their gestational age [220, 221]. Circulating concentrations of TAGs in the third trimester of pregnancy in diabetic women have been considered a stronger predictor of birth weight than glucose concentrations [15, 221].

In GDM pregnant women having normal levels of serum glucose, insulin, and glycerol as well as normal insulin resistance values (as assessed by the homeostasis
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model, HOMA), the neonatal fat mass was found to be higher than in controls [15, 16]. In these same GDM women, but not in controls, at a time close to delivery, maternal plasma TAG and NEFA concentrations correlated with all neonatal anthropometric measures (i.e. birth weight, BMI, and fat mass). These findings indicate that in GDM, but not in controls, maternal lipids are strong predictors of fetal growth. Comparing maternal lipids (i.e. TAGs, NEFAs, and cholesterol) in GDM and control pregnant women, similar concentrations were found but there were higher NEFA concentrations in the cord blood serum of GDM women [16].

An altered intrauterine fatty acid metabolism could also contribute to the elevated fat depots in neonates of GDM women. Such a possibility is suggested by the finding of a lower proportion of DHA and lower total n-6 and n-3 PUFAs in umbilical arterial plasma, but not in umbilical vein plasma, in GDM subjects compared with controls [17]. Whereas umbilical vein blood comes from the placental capillaries, umbilical artery blood arrives from fetal tissues. Therefore, the decreased proportion of those PUFAs in the umbilical arteries of GDM fetuses indicates their enhanced utilization by fetal tissues, which would also contribute to the tendency for increased fat depot accumulation.

In a literature review related to the fetal origins of obesity, it was reported that most studies showed a positive correlation between birth weight and childhood obesity (for a review see [222]). Recently, a significant correlation between the percentage of body fat at birth and child body fat at follow-up was found in offspring of lean and obese women with normal glucose tolerance and GDM [223]. Therefore, the increase in body fat in neonates of GDM women seems to be a significant risk factor for obesity in early childhood and possibly in later life.

Expert opinion

Although human maternal and fetal lipid metabolism under normal conditions is well understood, there are still several open questions regarding the process of placental fatty acid transfer. It has been studied by several authors and using different methodological systems, but as most PUFAs in maternal plasma are esterified and associated with lipoproteins, the system is quite complex and not completely known. Most functional studies on human placentas have been carried out at the end of pregnancy using ex-vivo perfusion systems or by analyzing components contributing to transfer. However, full elucidation of the order and interaction of intracellular events, their control, and how these events are modified at different stages of pregnancy still needs additional information. Maternal dyslipidemia is quite variable under gestational diabetic conditions and depends on the degree of maternal insulin resistance and steroid hormone levels. Concerning fetal lipid metabolism, the role of maternally derived LCPUFAs on fetal development and specific functions is now well known. Although glucose oxidation is regarded as the primary route for energy production, the important role of mitochondrial fatty acid oxidation for placental and fetal metabolism has recently been recognized. Early studies demonstrate an active contribution of human fetal lipogenesis to fetal adipose tissue development. The rapid accumulation of fetal fat depots during the last weeks of intrauterine life call for additional studies to determine the factors controlling fetal lipogenesis under normal and pathological conditions such as GDM. These studies seem imperative, especially in view of newborn fat rather than birth weight being an important risk factor for later obesity.

Outlook

The important role of maternal nutrition on fetal epigenetic characteristics has now been established. It is expected that in the next 5–10 years additional efforts will be made to identify the most appropriate nutritional conditions during pregnancy and lactation to prevent the risk of long-term pathologies, with a focus on those associated with the highest mortality risk such as obesity and diabetes and their subsequent increased incidence of cardiovascular diseases. Additional research on the dynamics of placental function and the molecular biology of placental cells would provide a better understanding of placental lipid transfer under normal and diabetic conditions.

Highlights

- Glucose plays a key role in the development of neonatal fat mass and maternal dyslipidemia in GDM, enhances the availability of lipids to the fetus, and thereby actively contributes to increased fetal adipose tissue mass.
- During early pregnancy, maternal insulin resistance is not yet developed, but the maternal pancreas is more responsive to feeding. The resulting hyperinsulinemia stimulates adipose tissue LPL activity and
lipogenesis, causing an accumulation of maternal fat deposits.

- The third trimester of pregnancy is characterized by increased catabolism in maternal adipose tissue, which contributes to the development of hypertriglyceridemia. Insulin resistance, estrogens, and adipocytokines are responsible for these changes. They are further altered in diabetic pregnancy, although maternal dyslipidemia appears quite variable.

- The vast majority of fatty acids in the maternal circulation are esterified and associated with lipoproteins. Their placental transfer requires the associated lipoproteins to be recognized by specific receptors and the TAG and cholesterol esters to be hydrolyzed after uptake. Released NEFAs participate in placental intrinsic metabolism and a proportion is finally released to fetal circulation. These interactions are modified in GDM.

- The fetus needs PUFAs from the maternal circulation, especially DHA and AA, which are crucial for intrauterine development and specific functions such as retinal and cerebral development. The fetus also uses fatty acids for energy production through the mitochondrial fatty acid oxidation pathway.

- During the last weeks of intrauterine life there is a rapid increase in fetal fat accumulation. Although around half of the fat is derived from maternal sources, fetal lipogenesis from different substrates is quite active and greatly contributes to adipose tissue development. This process seems to be further enhanced under GDM conditions, contributing to the risk of excessive fetal fat accumulation.

- The fetal lipoprotein profile is different to that of adults, and there are also changes in the apolipoprotein content in specific particles (e.g. enrichment of apo E in HDL).

- In GDM women close to delivery, but not in controls, maternal lipids are strong predictors of fetal late with neonatal anthropometric measures, indicating that maternal lipids are strong predictors of fetal growth in GDM.

- The increase in body fat in neonates of GDM women is a significant risk factor for later development of obesity.

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