Fetal Lipid Requirements: Implications in Fetal Growth Retardation

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The importance of lipid metabolism in intrauterine growth retardation (IUGR) has not been extensively studied. Although little is known of the effect of marginal dietary intakes of essential fatty acids on fetal lipid metabolism, there is evidence that placental lipids of infants that are small for gestational age (SGA) are low in 20:4 n-6 and 22:6 n-3. There also is evidence that the placenta transports long-chain polyunsaturated essential fatty acids from the maternal to the fetal circulation. The degree to which the placenta may modify the essential fatty acid precursors, 18:2 n-6 and 18:3 n-3, to longer-chain homologues utilized in fetal tissue synthesis is unknown and is central to understanding whether or not reduced placental transfer of 20:4 n-6 and 22:6 n-3 limits fetal growth. It is also not clear whether limited transfer of 20:4 n-6 and 22:6 n-3 by the placenta may arise from limitations inherent in the metabolic capability of the placenta, or whether it is caused by marginal or low maternal intake of essential fatty acid precursors. Nutritional status of the mother during gestation has been related to fetal growth. In general, reduced nutritional status with respect to n-6 and n-3 essential fatty acids has been correlated with reduced neonatal growth and head circumference. These observations also associate lower nutritional status with lower scores for some behavioral assessments in infants. During the last trimester of gestation, large amounts of essential and nonessential fatty acids are utilized in the synthesis of fetal tissues. The degree to which reduced accretion of lipid is caused by reduced placental transfer of lipid is not clear, nor are the mechanisms that limit fat accretion in the SGA infant apparent.

INTRAUTERINE/FETAL GROWTH RETARDATION

Intrauterine growth retardation is a common generic clinical term used to describe the perinate with a birth weight at or below a specific weight percentile for sex and gestational age. In North America, the 10th centile for birth weight is used. In Britain and Europe, an infant is considered growth-retarded if the birth weight is at or below
the third percentile, or is two standard deviations below normal. As part of the clinical definition, IUGR is a result of pathologic processes inhibiting the normal intrinsic growth potential of the fetus. Comparatively, SGA infants are clinically distinct from intrauterine growth-retarded infants by the absence of pathologic processes. SGA infants are considered normal small fetuses, reflecting the normal but lower distribution of perinatal weight. Much of the current literature, however, does not make this distinction, and in most discussions the two terms appear synonymous. Further research is needed to develop more appropriate definitions of IUGR, focusing more on qualitative aspects of growth than on size (1). Quality or "composition" of growth must also be distinguished from quantity of growth. Determining which tissues grow or fail to grow if nutrition or placental function is compromised may be important to developmental progress of the fetus/infant.

The causes associated with growth retardation are a result of fetal, maternal, and placental disorders working alone or in combination. Nutritional deficiencies affecting fetal growth may result from any of these disorders. In some fetal disorders (e.g., inborn errors of metabolism), nutritional status and growth of the fetus may be affected by the inability to metabolize or utilize certain nutrients. Placental function and nutritional status of the mother during gestation are also related to fetal growth. Inadequate placental vascular development, site of placental development in the uterus, placental function, or maternal nutritional status have been related to the development of intrauterine or fetal growth retardation (1,2). These inadequacies may affect the transport of nutrients from the mother to the fetus. Although the potential impact of disturbed fatty acid transport on the development of fetal growth retardation is unknown, a study comparing the fatty acid composition of placentae from term and preterm human pregnancies has been reported (3). Fatty acids in total triacylglycerol and in phosphatidylcholine and phosphatidylethanolamine phosphoglycerides of placental membrane phospholipids from appropriate-for-gestational-age (AGA) and SGA infants were studied. No differences in the content of triacylglycerol between AGA and SGA placentae was observed. However, a reduction of n-6 fatty acids, particularly 20:3 n-6 and 20:4 n-6, in SGA placental triacylglycerol fatty acids was noted. A reduction in n-3 fatty acids, especially 22:6 n-3 in phosphatidylcholine, was also reported. The investigators suggested that these changes in fatty acid composition in placental membrane phospholipids can affect the transport of important nutrients to the fetus as well as alter the formation of eicosanoids. Although it is not certain how these changes in the placenta affect the fetus, a study by Al et al. (4) noted that the placenta is, for the most part, a fetal organ. Thus, it is possible that significant correlations between placental weight and some fetal fatty acid values may be explained by the closer similarity of placental to fetal plasma fatty acid composition than to maternal plasma fatty acid composition (4).

In an assessment of 1560 intrauterine growth-retarded fetuses, it was reported that the risk for perinatal mortality and morbidity progressively increases with decreasing birth weight (5). These infants of low birth weight are at highest risk for brain- and nervous system-related handicaps (6). Because the brain is one of the
most lipid-concentrated organs in the body, the lipid requirements and supply during fetal growth are of critical importance.

ROLE OF LIPIDS IN THE DEVELOPING FETUS

Developmental processes in fetal growth occur in a critical sequence over a limited period of time. The significance of lipids during these developmental periods is well established (7–9), and their supply is in high demand. Dietary fat provides the main energy store in pregnancy, is the vehicle for supply of lipid-soluble vitamins, and has functional significance by providing structural components of cell membranes, particularly during the processes of cellular multiplication, differentiation, and cell-to-cell interactions. Furthermore, the 20-carbon polyunsaturated fatty acids are precursors for a group of biologically active compounds. These compounds are the eicosanoids and include thromboxanes, prostaglandins, prostacyclins, leukotrienes, lipoxins, and hydroperoxy and hydroxy fatty acids. The profile of eicosanoids formed can be altered with a change in dietary balance of n-6 and n-3 fatty acids. Eicosanoids are involved in regulation of diverse physiologic processes. This diversity depends on which essential fatty acid precursor is predominant, the site of its release, and the subsequent eicosanoid profile synthesized. The physiologic processes vary from inflammatory and hypersensitivity reactions to control of vasoconstrictive and thrombogenic activities (10). Two such processes relevant to fetal nourishment and parturition are regulation of blood flow and coagulation.

Synthesis of C-20 and C-22 polyunsaturated fatty acids, in particular arachidonic and docosahexaenoic acids, occurs by the desaturation and elongation of the essential fatty acids linoleic (18:2 n-6) and α-linolenic (18:3 n-3) acids, respectively. Both arachidonic acid (20:4 n-6) and docosahexaenoic acid (22:6 n-3) are key components of all membranes but are particularly enriched in neural, mitochondrial, and vascular membranes. These two fatty acids are readily incorporated into the structural lipids of the developing brain (10). As the degree to which the fetus is capable of desaturation and elongation is not clear, the supply of essential fatty acids and long-chain polyunsaturated fatty acids is critical and central to the synthesis of structural lipids and hence to normal development of the fetus (7,11–13). With respect to lipid requirements during development, a large number of changes occur in body composition in the developing fetus during the third trimester of pregnancy. This period encompasses a major change in both body composition of the fetus—involving a rapid increase in adipose tissue development—and growth of the brain, an organ also highly concentrated in complex lipids (14). The rate of incorporation of long-chain polyunsaturated fatty acids is high in growing tissues, particularly in brain structures (15–17). Hence, the third trimester shows the largest quantitative requirement for essential fatty acids and thus for supply of long-chain polyunsaturated fatty acids. It has also been suggested that the last trimester is the time when IUGR is most common and usually most pronounced (18).
Accretion rates for fatty acids obtained from fetal and infant spinal cord were quantitatively assessed by Clandinin et al. (16). Sample spinal cord segments from the level of C-1 and L3-4 vertebrae were obtained during autopsy. Accretion of approximately 5.4 mg of fatty acid per gram of tissue in the cervical region occurred during the last trimester of pregnancy. During this period, saturated and n-9 monounsaturated fatty acids are the primary ones deposited (45% and 18% of total tissue fatty acids, respectively). Accretion of fatty acids from the n-6 family was also apparent, representing 16% of fatty acids. Accretion in the lumbar region during the last trimester was lower, but increased during the first 13 weeks of life. An estimated 0.88 mg of n-6 and 0.13 mg of n-3 fatty acids accrue in the cervical region of the spinal cord during the last trimester of fetal development. This accretion of fatty acids parallels increasing neuromotor spinal functions and postnatal development of coordinated motor activity (16).

SOURCES OF LIPIDS IN THE DEVELOPING FETUS

Fetal demands for lipids are met by both placental transfer and endogenous synthesis (14,19). Similar to the adult, the human fetus cannot synthesize the essential fatty acids 18:2 n-6 and 18:3 n-3. Thus, the fetus relies on deriving these fatty acids from the maternal blood across the placenta. Robertson and Sprecher (20) noted that the free fatty acid content of the placenta is different from that of maternal plasma, suggesting that sources other than maternal circulation exist for the derivation of some components of the placental free fatty acid pool. The amount transported and the mechanism by which these fatty acids reach the fetus from the maternal circulation remain to be clarified. Placental transfer of long-chain polyunsaturated fatty acids in mammals is well established. Arachidonic acid and docosahexaenoic acid concentrations increase in the fetus as gestational age increases (20-22). Although the mechanism responsible for this transfer is not clear, it has been suggested that either the fetal or placental capacity to form the longer-chain fatty acids from parent fatty acids is increased, or that a preferential transfer of the longer-chain fatty acids across the placenta from the maternal to fetal circulation occurs (23,24). Both fetal plasma (25) and fetal erythrocytes (26) have been suggested to play a major role in the transport of essential fatty acids into the fetus. Carrier proteins have also been implicated in the uptake of polyunsaturated fatty acids in fetal rat hepatocytes (27). Previous observations suggested that sufficient amounts of long-chain n-6 and n-3 fatty acids for deposition in growing tissues could easily be synthesized from precursor fatty acids in full-term and preterm infants. However, Clandinin et al. (15,16) showed a lag in accretion of brain and liver long-chain polyenoic fatty acids during fetal development. A lag in the mobilization of essential fatty acid deposits in the liver has been demonstrated for a varying period of time following birth (17).
Thus, it is likely that synthesis of chain-elongated and desaturated fatty acids limits maximum postnatal accretion of these essential fatty acids. In this regard, the concept that intrauterine accretion of long-chain polyenoic fatty acids occurs primarily as a function of mechanisms involving placental transfer is consistent with observations reported by other investigators (4,28).

**FETAL LIPID REQUIREMENTS**

Although the requirement for lipids in the developing fetus persists throughout gestation, the supply of lipid becomes critically important in the third trimester. This trimester is a period of rapid brain growth and rapid accretion of body fat depots, subject to adequate supply of energy to the fetus. At 28 weeks of gestation, accretion of fat in the normal fetus ranges between 1.2 and 1.8 g/kg/d. By 36 to 40 weeks of gestation, fat accretion is approximately linear, ranging between 1.6 and 3.4 g/kg/d (29). Fatty acid analysis of brain tissue from various species indicates that this complex organ contains large amounts of long-chain polyunsaturated fatty acids, predominantly 20:4 n-6 and 22:6 n-3. These long-chain fatty acids accrue substantially during the last trimester of development in brain (15). Accretion of n-9 and saturated fatty acids also occurs at this time. No significant accretion of 18:2 n-6 and 18:3 n-3 was noted during the last trimester (7). An increase in all long-chain polyunsaturated fatty acids has also been reported in cord blood plasma phospholipids in infants between 24 and 44 weeks of gestation (30).

Analysis of fatty acid accretion in fetal liver enabled assessment of minimal fatty acid requirements for tissue synthesis. The liver contains a significant amount of 20n-6 and 22n-3 fatty acids at the end of the second trimester and during the early period of the third trimester of fetal development. During the progression of the third trimester, liver weight, when expressed in grams per kilogram of body weight, decreases as total body weight increases; thus, the liver represents a declining potential reserve of essential fatty acids for the developing fetus (17).

Beyond 30 weeks of gestation, accumulation of fat considerably exceeds that of nonfat components (29). Fetal requirements (mean ± 2 SD) for essential fatty acids and long-chain fatty acids are estimated to be 1100 mg of n-6 fatty acids per day (400 mg/kg of body weight) and 140 mg of n-3 fatty acids per day (50 mg/kg of body weight) (14), based on analysis of fatty acid accretion and estimates for tissue synthesis in fetal brain, liver, and adipose tissue. Rapid accretion of n-6 and n-3 long-chain polyenoic fatty acids in the fetal brain has been indicated by examination of intrauterine fatty acid accretion (15). During the last trimester, the major fatty acids to accrue in the brain are the chain elongation-desaturation products. It is estimated that the developing brain accumulates approximately 43 mg of n-6 polyenoic and 22 mg of n-3 polyenoic fatty acids per week (14). Examination of accretion of fatty acids in the fetal liver from 22 weeks of gestation to term indicates that 13.5 mg of n-6 and 3.8 mg of n-3 fatty acids accrue per week (17).
RELATIONSHIP BETWEEN FATTY ACIDS AND FETAL GROWTH

As the fetus grows and develops, body mass increases between the end of the first trimester and term. During this period there is less reliance on hyperplasia and more on cellular hypertrophy accompanying tissue maturation. In the third trimester, this increase in body mass represents in part preparation for extrauterine life, as adiposity and glycogen storage increase. An increase in the number of cells and tissue formation require formation of new membranes, most of which are high in arachidonic and docosahexaenoic acids (31). Thus, it is logical that these fatty acids must be related to tissue growth. Dynamic markers of circulating pools providing these essential fatty acids are not clear postnatally, but lipoprotein phospholipid and cholesterol ester fractions are likely candidates (32).

Research by Leaf et al. (12) examined the relation between plasma choline phosphoglyceride long-chain polyunsaturated fatty acid composition and measurements of fetal growth and maturity. Analysis indicated a strong correlation of 22:6 n-3 content in plasma phosphatidylcholine with gestational age as well as with fetal head circumference and birth weight. Arachidonic acid correlated most strongly with weight and head circumference. In a study of infants of low birth weight, a relation was found between the long-chain n-6 and n-3 essential fatty acids, birth weight, and head circumference (28). The authors suggest that this does not necessarily imply that low birth weight is a result of essential fatty acid deficiency, but suggests that the relation of lipids to vascular nutrition may be important in placental and fetal development. Research by Koletzko and Braun (33) also provides evidence for a positive correlation between arachidonic acid, as measured in plasma triglyceride content, and body weight. As plasma triglyceride primarily reflects dietary intake, and protein and energy intakes were not controlled in this study, it is not clear how 20:4 n-6 levels in plasma triglycerides might be related to growth. A recent study (11) examined the relation of long-chain polyunsaturated fatty acid supply with prenatal growth. This study analyzed long-chain polyenoic fatty acids in phospholipids from plasma and erythrocytes from the umbilical artery wall. Fifty-two preterm infants ranging in weight from 650 to 1860 g and with gestational ages between 26 and 36 weeks were investigated. Because plasma phospholipids are affected by relatively short-term dietary influences, measures from umbilical artery walls were used as longer-term indicators of long-chain polyenoic status. Results indicate that the relative amounts of n-6 and n-3 long-chain polyenoic fatty acids in umbilical artery walls and relative amounts of n-3 long-chain polyenes in cord plasma were positively correlated with gestational age. Levels of docosahexaenoic acid measured in the arterial cord vessel walls were significantly correlated with weight, head circumference, and length at birth, independently of gestational age at birth. This study suggests that a striking relation exists between docosahexaenoic acid status and prenatal growth. An earlier experiment (34) also examined the relation of fatty acid composition of umbilical artery and vein wall to normal or retarded fetal growth. SGA infants in this study were identified by an abdominal circumference of less than the 10th centile for gestational age. Levels of docosahexaenoic acid measured
in the umbilical venous and arterial wall were positively associated with birth weight and head circumference. Carlson et al. (35) have related plasma phosphatidylcholine 20:4 n-6 concentrations in preterm infants to growth, and 20:5 n-3 levels to reduction in growth.

Ontogeny of the human fetal gastrointestinal tract occurs between the 14th day of gestation and the 12th week of embryonic life, by which time glucose and amino acids are actively transported (36). By 26 weeks of intrauterine life, the fetus has the capacity for limited digestion and absorption. Before this, functional development is limited. The development may be influenced by the intrauterine nutritional state of the fetus. In rats, malnourishment at this stage or postnatally alters normal morphologic and functional maturation of the intestinal tract (A.B.R. Thomson and M.T. Clandinin, unpublished observations). In suckling rats, the brush border membrane lipid composition can also be influenced by dietary composition, which can alter enterocyte enzyme activity and transport properties. The effect of maternal diet on the developing intestinal tract in utero is unknown, as are changes that may be occurring in fatty acid-containing components of the amniotic fluid. However, it appears that early nutritional uptake has effects on intestinal function in later life.

The effects of lipids on growth of the fetus may also occur indirectly, through hormonal control of various systems. Hormonal effects on development have been shown to exist at the level of the central nervous system. Thyroid hormone in particular has been shown to control at least two very important steps of brain maturation: neurite outgrowth and myelination (37). Deficiency or excess of thyroid hormone can alter the distribution of catecholamine receptors or acquisition of muscarinic receptors. In a recent study (M.T. Clandinin et al., unpublished observations), raised levels of thyroid stimulating hormone occurred in developing rat pups fed diets reflecting the composition of a current infant formula containing physiologic amounts of 22:6 n-3. These results suggest that changes in the balance of 20:4 n-6 to 22:6 n-3 during the development of neural-endocrine tissues can affect metabolic controls by endocrine mechanisms.

Hormones also control the regulation of fetal lung surfactant glycerophospholipid. Pulmonary surfactant consists of 90% lipid and is synthesized and assembled by alveolar epithelial cells. The main function of surfactant is to decrease surface tension, protecting the alveoli against collapse. This property is primarily conferred by dipalmitoyl phosphatidylcholine. The other major lipid components include unsaturated phosphatidylcholine and phosphatidylglycerol. Among the hormonal regulators of lung surfactant are glucocorticoids, prolactin, thyroid hormones, estrogens, androgens, growth factors, insulin, catecholamines, and cyclic AMP (38). Prostaglandins have also been identified as mediators of lung growth as well as surfactant secretion (38). The impact of fetal lipid metabolism on development and maturation of this critical functional constituent in the lung is not well known.

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**DISCUSSION**

*Dr. Battaglia*: During the last day and half, we have talked about the asymmetric growth-retarded baby. It is certainly true that statistically the brain is closer to normal weight than is body weight or other anthropomorphic measurements, but the question of whether it has developed normally in IUGR fetuses is a big one for me. Looking at your data on the distribution of the many long-chain polyunsaturated fatty acids present in the brain, it almost looks like a way in which you can “fingerprint” a tissue. I wonder whether you have looked at normal infants and IUGR infants with supposed brain sparing who have died for other reasons in terms of brain composition?

*Dr. Clandinin*: No, but we have perhaps done one half of it. We have characterized the compositional changes in neuronal and glial cells in the rat during development and in response to different diet treatments, and it would be fairly easy for us to begin to compare those data with some of the rat models. It would be a very good thing to do.

*Dr. Haschke*: Data on DHA (docosahexaenoic acid) and arachidonic acid in infant brains indicate that arachidonic acid is not influenced by diet, whereas the DHA concentration in the brain is much influenced by the diet. It is highest in fish eaters and lowest in vegetarians.

*Dr. Clandinin*: I have seen those data, and one of the difficulties is the way the analyses are expressed as a relative % fatty acid analysis as opposed to a microgram per gram of total tissue quantitative analysis. So it is an interesting observation, but it’s difficult to compare it to this kind of data. I guess what you are alluding to is that if the DHA content or the EPA content of the diet varies a lot, the omega-3 would vary, whereas the arachidonic is relatively constant. My interpretation of that would be that arachidonate is one of the really major components in brain, and what I feared about some of the earlier studies in which infants were fed fish oil was that you would compromise arachidonate status unless you fed arachidonate. I guess what one would need to look at in the Gibson data is whether in specific cases in which the dietary DHA was high, was the arachidonate lower? I don’t know the answer to that.
Dr. Haschke: The data for preterm infants who were fed fish oil-supplemented formula (containing 0.2% DHA, 0.3% EPA) without arachidonic acid indicated that arachidonic acid in plasma and red blood cells was suppressed (1). Moreover, in these infants, it was shown that linear growth was also suppressed (2). However, it seems that if formulas with lower levels of DHA are fed, arachidonic acid in plasma and red blood cells is no more suppressed. Gibson has unpublished data for infants who were given DHA and GLA (\(\gamma\)-linolenic acid), who therefore didn't receive arachidonic acid but received a precursor. In those infants, arachidonic acid and DHA in erythrocytes and plasma were very close to the values in breast-fed infants. So it seems to be a matter of balance how much we give to infants. However, it is likely that premature infants deviate from term infants as far as requirements are concerned.

Dr. Clandinin: Your point is a good one in that it is a matter of balance between the two, because basically both use the same pathways. In terms of the Carlson study, probably the early effect on the arachidonate was a result of that the way those infants were supplemented with fish oil also provided a significant amount of EPA, and that probably wasn't a good thing to do. It is now possible, as you know, to develop a feed without the EPA so you can avoid that problem.

Dr. Williams: Your estimated weekly requirements for long-chain n-3 polyunsaturated fatty acids, based on the tissue accretion, are actually fairly close to the mean adult weekly intakes of those fatty acids in the UK. How would you suggest that these requirements are being met?

Dr. Clandinin: Certainly we would expect that on a per kilogram basis the requirement of the infant would be high compared with an adult because of the intensive tissue growth that is going on. If you do the same kind of calculations from intakes of human milk, you come up with similar numbers.

Dr. Williams: But in utero how are they being met?

Dr. Clandinin: In utero I think we know that the essential fatty acids cross the placenta. There have been early suggestions, from some of the Crawford work that has not been followed up. There may even be selective transfer of some of the chain-elongated forms of the essential fatty acids, but in terms of essential fatty acid transfer and metabolism by placenta to fetus, this is an unstudied area.

Dr. Williams: But would you suggest that there would have to be selective mobilization of these fatty acids from the maternal adipose tissue stores? If the dietary intakes are equivalent only to the fetal requirements, the mother’s requirements and the fetal requirements could not be met.

Dr. Clandinin: There are two aspects in that question. One is that the adipose store of the mother is actually rather large with respect to essential fatty acids, and the other is that what we recommend for an adult as a minimum requirement is vastly below what they normally eat—what they normally eat is an order of magnitude greater than what we recommend as a minimum intake.

Dr. Page: It might be pertinent just to comment on the recent study we have been completing in my laboratory in Aberdeen. We did a perfused placental study in which we were able to demonstrate that there was indeed an enhanced transfer of linoleic and \(\alpha\)-linolenic acids compared with oleic, and also of DHA, which was even greater. We were unable to find any evidence of chain elongation in our study, and we could not actually demonstrate any enhanced transfer of arachidonic acid. We are following that up, but we think it is metabolized very quickly in the perfused placenta.

Dr. Godfrey: It is important that we do not overinterpret the Leaf data (3) on head circumference and concentrations. Firstly, it is not adjusted for gestational age, and secondly, head
circumference at birth is very strongly tied in with placental size, and reduced placental transfer of fatty acids may underlie the lower concentrations. So I think it is very dangerous to infer cause and effect there. Secondly, in relation to the socioeconomic differences in birth weight, differences in maternal size account for a part of that, and differences in maternal smoking for another part of that gradient. Having said that, the WIC (women-infants-children) Nutritional Supplementation Program in the United States was not associated with consistent increases in birth weight, but it was associated with consistent increases in head circumference at birth. So there is a very complex set of areas to address there. In our data from Southampton, we find relations between the mothers’ diet and fetal growth that occur at all levels of maternal social class (4). So I think it is dangerous to point specifically towards essential fatty acids; there is a whole series of issues that needs to be addressed.

Dr. Clandinin: I would agree with you. To come back to the serine and glycine story, I actually don’t think that serine in liver goes to glycine; I think its purpose is to make choline.

Dr. Battaglia: I guess what we mean by essential is that all those nutrients need to be there in adequate amounts, and you have called attention to one set of structural compounds that are needed. I think it is interesting to start working on the placental delivery of these compounds—we need to know a lot more about them. Every time I hear a discussion of diet and fetal growth, I keep thinking of the marvelous biologic example that I know most of you are aware of—hibernation. Bears that hibernate don’t eat and they don’t drink water, but they produce very nice baby bears that are not growth-retarded. So you can build a perfectly normal baby without any intake in the mother provided there is a mechanism that stops all nitrogen loss. I guess that what we are trying to find out for human is that when you have a restriction, what are the adaptive mechanisms that are made successfully in one pregnancy and not so successfully in another?

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