Long Chain PUFA Transport in Human Term Placenta

Phil Cunningham and Lindsay McDermott

Introduction

Docosahexanoic (DHA) and arachidonic (ARA) acids are important for proper fetal development and accumulate in the fetus during gestation. They cannot be synthesized by the fetus and must be gained instead from the maternal blood supply via the syncytiotrophoblast, the transporting epithelium of the placenta. Cell biological experiments suggest that both membrane-associated placental plasma membrane fatty acid-binding protein (pFABPpm) and fatty acid transport protein (FATP) are involved in DHA uptake while fatty acid-binding proteins (FABP) are thought to be involved in intracellular trafficking of long-chain fatty acids. FABP1, 3, and 4 have been detected in the syncytiotrophoblast and there is experimental and theoretical evidence to suggest that these three FABP are under the control of hypoxia inducible factor (HIF), perhaps playing a role in fetal protection from hypoxia. Biophysical measurements reveal these FABP have a higher affinity for DHA over ARA but that affinities for both are lower than those of nonessential fatty acids. Recent research is beginning to uncover the mechanisms of DHA and ARA transmembrane and intracellular transport in the placenta, and it is suggested that maternal health and nutrition during pregnancy could be important in determining fatty acid transport and binding protein expression and, thereby, essential fatty acid delivery to the fetus.

Long-chain PUFA ARA [20:4(n-6)] and DHA [22:6(n-3)] cannot be synthesized by the developing fetus and instead must be gained in adequate supply from the maternal diet for proper development. Prostaglandin E2, important in the normal development of many organs and cells (particularly the central nervous system), is synthesized from ARA as indeed are many eicosanoids (1-5). DHA, meanwhile, is essential for development of the fetal brain and retina and recent reviews, assessing the impact of dietary fatty acids on fetal development (6,7), have led to recommendations of DHA supplementation for pregnant mothers (8). DHA and ARA are metabolites of the essential dietary fatty acids α-linolenic and linoleic, respectively; generation of both metabolites involves elongases and desaturases (9). Because the ability of the fetus and human placenta to desaturate and elongate fatty acids is limited (10), the fetal demand for DHA and ARA has to be satisfied by the mother and their transport to the fetus provided by the placenta. Within the human placenta, the syncytiotrophoblast keeps maternal and fetal circulation separate while allowing nutrient exchange (Fig. 1A). This multinuclear transporting epithelium derives from mononuclear cytotrophoblast cells that in turn derive from the fertilized egg. The syncytiotrophoblast is polarized and consists of a microvillous membrane (MVM) facing the maternal blood and a basal membrane facing the fetal blood (Fig. 1B) (11).

DHA and ARA accumulate in the fetus during pregnancy by a process described as biomagnification. More recently, a human in vivo study using oral doses of 13C-labeled fatty acids showed placenta:maternal DHA concentrations were significantly higher than those of palmitic, oleic, and linoleic acids (12). The question remains, therefore: how does the placenta achieve this selective uptake of essential PUFA? Unesterified fatty acid cellular entry and exit has long been debated (13). Although it is argued that fatty acids can flip flop across membranes (14), a membrane-associated protein has also been directly implicated in this process (15). Indeed, linoleic acid uptake by cultured cytotrophoblast cells is a saturable mechanism indicating the involvement of facilitating proteins, not solely simple diffusion (16). This short review article assesses recent studies of membrane-associated and -intracellular proteins expressed in the placenta and capable of binding and transporting fatty acids, focusing on the essential fatty acids DHA and ARA.

Syncytiotrophoblast fatty acid uptake and efflux

There are a number of membrane-associated fatty acid transport proteins expressed in the placenta and of these FATP4 and pFABPpm are implicated in the selective uptake of DHA.

The FATP family contains 6 members, each with a distinct expression pattern (17). The localization of FATP4 expression in trophoblasts and the yolk sac of the developing wild-type mouse chorioallantoic placenta suggests a role for FATP4 in fat absorption in early embryogenesis (17). Indeed, homozygous deletion of the FATP4 gene in mice results in early embryonic lethality (17). In cytotrophoblast cells isolated from human term placentas and cultured in a mix of oleic and linoleic acids, expression of FATP2, 3, 4, and 6 remained unchanged and was not correlated with oleic acid uptake (18). Placental mRNA expression of FATP1 and FATP4, on the other hand, were directly correlated with DHA in both maternal plasma and placental phospholipids and FATP4 expression was significantly correlated with DHA in cord blood phospholipids, suggesting that this protein is specifically involved in (n-3) long-chain PUFA placental transfer (19). Interestingly, a G/A polymorphism in exon 3 of the FATP4 gene, giving rise to the Gly209Ser substitution, is significantly associated with several features of the insulin resistance syndrome (20). Modeling of FATP4 against acetyl-CoA synthetase, a protein possessing >95%
sequence similarity with and structural conservation to FATP4, suggests FATP4 consists of a large N-terminal domain and a smaller C-terminal domain interfaced with an AMP binding motif. Asp488 and Arg503, residues critical for fatty acid transport activity, are located within the N-terminal domain of the protein in close proximity to the AMP binding motif. The variable Gly209 residue was located far from the fatty acid-binding region in a domain implicated in protein-protein interactions (20). It is known that polymorphisms in Δ5 and Δ6 desaturase enzymes are related to maternal (n-6) and (n-3) fatty acid status (21) and it will be interesting to determine whether FATP4’s G/A polymorphism is similarly related to maternal long-chain PUFA status.

Plasma membrane FABP (FABPpm/mAspAt) gene expression was detected in villous tissue placenta extracts, but its expression was not modified by fish oil supplementation during pregnancy (19). Although FABPpm is similar in size (40 kDa) to pFABPpm, pFABPpm has a distinct amino acid composition and isoelectric point from FABPpm and also lacks mitochondrial aspartate aminotransferase activity (22). pFABPpm is found solely on MVM purified from term placentas (23). BeWo cells (an experimental model of the trophoblast) incubated with pFABPpm antiserum had 64 and 68% reductions in DHA and (an experimental model of the trophoblast) incubated with oleic acid uptake, respectively, and oleic acid uptake was reduced by pFABPpm antiserum had 64 and 68% reductions in DHA and (an experimental model of the trophoblast) incubated with oleic acid uptake, respectively, and oleic acid uptake was reduced by pFABPpm antiserum had 64 and 68% reductions in DHA and (an experimental model of the trophoblast) incubated with oleic acid uptake, respectively, and oleic acid uptake was reduced by pFABPpm antiserum had 64 and 68% reductions in DHA and (an experimental model of the trophoblast) incubated with oleic acid uptake, respectively, and oleic acid uptake was reduced by pFABPpm antiserum had 64 and 68% reductions in DHA and (an experimental model of the trophoblast) incubated with oleic acid uptake, respectively, and oleic acid uptake was reduced by pFABPpm antiserum had 64 and 68% reductions in DHA and (an experimental model of the trophoblast) incubated with oleic acid uptake, respectively, and oleic acid uptake was reduced by pFABPpm antiserum had 64 and 68% reductions in DHA and (an experimental model of the trophoblast) incubated with oleic acid uptake, respectively, and oleic acid uptake was reduced by pFABPpm antiserum had 64 and 68% reductions in DHA and (an experimental model of the trophoblast) incubated with oleic acid uptake, respectively, and oleic acid uptake was reduced by pFABPpm antiserum had 64 and 68% reductions in DHA and (an experimental model of the trophoblast) incubated with oleic acid uptake, respectively, and oleic acid uptake was reduced by pFABPpm antiserum had 64 and 68% reductions in DHA and.

FIGURE 2  Analysis of human FABP1, 3, 4, and 5 for upstream HIF regulatory elements as identified within TRANSFAC (2008) patterns (41). The dashed vertical line indicates the ENSEMBLE gene start site and for each genomic sequence, 2000 bp upstream and 3000 bp downstream are depicted. Exons are marked for the message immediately above the genomic line and coding sections are indicated above that. FABP1 and 5 have 2 transcripts each; FABP1a differs from FABP1b by a 16-bp displacement in the start of the coding region in the first exon and FABP5a’s first exon is entirely untranscribed. All genes show putative HIF binding sites upstream of the protein coding region, as indicated by ticks below the genomic sequence. The above image was created using FIGURE for GCG (version 11.1, Accelrys).

Finally, fatty acid translocase (FAT/CD36) gene expression was also detected in placental villous tissue extracts (19). CD36 is a membrane glycoprotein involved in fatty acid uptake in the stomach, upper intestinal tract, heart, skeletal muscle, and adipose tissues (15,25). Indeed, it was recently shown that FAT/CD36 gene deletion decreases fat consumption in mice (25). Its expression did not differ in placenta tissue from control, preeclamptic and intrauterine growth restricted (IUGR) pregnancies (26). Because levels of ARA and DHA were reduced in placental tissue from normal and preeclamptic pregnancies (27) and the fetal:maternal ratio of DHA and ARA was significantly lower in IUGR cases when compared with normal pregnancies (28), it is not clear that FAT/CD36 is of major importance in placental fatty acid transport.

Intracellular transport
Fatty acids are bound and transported within the cell by FABP. The 9 members of this protein family have distinct tissue expression patterns and possess individual and common functions, some of which have been mapped to specific regions of the proteins’ 3-dimensional structure (29). Despite varying in primary sequence homology from 15 to 70%, all members have a common fold, a β barrel structure capped by 2 α helices, and, with the exception of FABP1, all bind long-chain fatty acids with a 1:1 stoichiometry. Of the 9 family members, FABP1, 3, 4, 5, and 7 have been described in the placental syncytiotrophoblast. Measurement of human FABP1, 3, and 4’s affinity for a range of fatty acids using the acylated intestinal FABP method revealed none that had a specific affinity for particular fatty acids. Affinities generally decreased with decreasing chain length and increasing double bond number (30). It is unlikely then that FABP are responsible for the fetal accumulation of DHA and ARA, although it is probable that they participate in their intracellular trafficking.

FABP1 and FABP3
FABP1 (liver FABP) and FABP3 (heart FABP, mammary derived growth inhibitor) had a greater affinity for DHA than ARA, perhaps suggesting a preferential transportation of DHA (30). Both FABP types were first detected in purified human cytotrophoblast cells by Campbell et al. (31) using immunoblotting. Daoud et al. (16) have since shown that FABP1 and 3 gene expression is upregulated 3- and 5-fold, respectively, upon differentiation of cytotrophoblast cells into syncytiotrophoblast...
tissue. Interestingly, it has also been observed that FABP1 and 3 expression increases in primary cytotrophoblasts cultured under hypoxic conditions (32) and analysis of upstream regulatory elements of both genes revealed potential HIF binding sites (Fig. 2). Hypoxia is implicated as a cause of IUGR in newborns (11). Although FABP1 and 3 expression were reported to be unaltered in trophoblast tissue from IUGR term pregnancies compared with control appropriately grown for gestational age tissue (33), the causes of IUGR in these cases, and whether hypoxia was involved, are not known. Moreover, these findings were obtained by immunoblotting of placental homogenates using commercially available antibodies. The antibodies were not rigorously tested for FABP specificity and, given the 3-dimensional topology common to all 9 FABP family members (29), it is possible these antibodies may have detected several additional FABP types. Thus, it will be important to obtain quantitative PCR data for the FABP to determine the role of hypoxia in their placental expression.

FABP3 expression was unaltered in placental homogenates from diabetic pregnancies while FABP1 expression was upregulated 112% in insulin-dependent diabetes mellitus and 64% in gestational diabetes mellitus compared with control tissue (33). Diabetic pregnancies are associated with elevated levels of DHA, ARA, and γ-linolenic acid in placental phospholipids (34). Interestingly, FABP1 expression is induced by PPARγ/retinoid X receptor (35). Given that PPARγ can bind many unsaturated long-chain fatty acids, it is possible that fatty acid accumulation by the placenta in diabetic pregnancies is causing elevated FABP1 expression.

PPARα, β, and γ play essential roles in regulating lipid metabolism, energy homeostasis, trophoblast invasion, early placenta development, and in the physiology of clinical pregnancy and its complications (36). PPARγ agonist GW1929 did not enhance FABP3 expression in primary cultured trophoblasts (32) and, similarly, FABP3 gene expression was reported to be not significantly modified by fish oil supplementation during the 2nd half of pregnancy (19). Both these findings agree with previous experiments by Schachtrup et al. (35), whereby it was shown that FABP3’s PPAR regulatory element was not activated by any PPAR/RXR combination with or without the PPAR pan-agonist bezafibrate.

Overall, it appears that in the placenta FABP3 expression is controlled by HIF, whereas FABP1 expression can be controlled by either PPARγ or HIF.

**FABP4 and FABP5**

FABP5 (epidermal FABP, keratinocyte FABP, mal1) protein expression has not been detected in the trophoblast, although FABP5 gene expression has. Levels of FABP5 gene expression were not elevated upon exposure to hypoxic conditions in cultured human cytotrophoblast cells (32) despite its two gene transcripts containing putative HIF responsive elements (Fig. 2). Campbell et al. (31,37), however, were unable to detect FABP5 in purified primary human cytotrophoblasts using immunoblotting, and immunohistochemistry of paraffin embedded human placenta sections revealed trophoblast cells to be immunonegative for FABP5 expression. Instead, FABP5 was localized in the endothelium of fetal venules, capillaries, and some veins (37).

Biophysical measurements show FABP4 (adipocyte FABP, adipocyte P2) binds DHA more tightly than ARA, perhaps indicating favored intracellular DHA transportation (30). Recently, hypoxia was shown to cause an increase in lipid droplet accumulation in cultured human cytotrophoblasts and immunoblotting of whole-cell lysates revealed a concomitant increase in FABP4 expression (32). Indeed, analysis of upstream HIF regulatory elements in the FABP4 gene reveals one potential binding site (Fig. 2). FABP4 is expressed in adipocytes and macrophages, as is FABP5 (38). FABP4 gene ablation experiments in mice revealed a modest adipose tissue phenotype; some studies, but not all, showed small reductions in β-adrenergic-stimulated lipolysis in adipocyte FABP null mice adipose tissue, possibly due to a compensatory increase in FABP5 expression (38). Macrophage FABP4, on the other hand, plays a role in the development of dietary atherosclerosis (38). It will be informative to localize FABP4 within the placenta and determine whether it is expressed in the syncytiotrophoblast, lipid droplet, or macrophage. Macrophages represent up to 40% of the cell population in the placenta; placental macrophages, known as Hofbauer cells, are located close to trophoblasts and fetal capillaries (39) and are perhaps involved in paracrine signaling (40).

**FABP7**

Two recent studies investigating FABP7 (brain FABP) expression in the trophoblast have yielded conflicting results. The first detected FABP7 using RT-PCR with mRNA of villous sections of placenta trophoblast (19). A separate study, which again used RT-PCR but this time with mRNA of primary cultured cytotrophoblasts, reported that no FABP7 was detected (32). Different FABP7 oligonucleotides were used in both studies and this, along with the different mRNA preparations, perhaps offers an explanation for this discrepancy. Examination of fatty acid binding to FABP7 has also produced inconsistent results. Unlike other FABP, FABP7 was shown to have a 40-fold higher affinity for DHA than ARA (38). Measurements using the acrylodated intestinal FABP method, on the other hand, showed FABP7 binding DHA and ARA with dissociation constant values of 13 nm and 18 nm, respectively (30). FABP7 knockout mice showed decreased concentrations of DHA in the neonatal brain (38) and, given that the fetus accumulates DHA during gestation (12), we speculate that there is a specific role for this protein in the placenta.

Figure 1B summarizes current knowledge of the location of fatty acid transporting proteins within the placenta. It is likely that pFABPpm and FATP4 are specifically involved in the preferential uptake of DHA by the placenta and that intracellular transport is achieved by FABP1, 3 and 4. FABP1 and 4 are preferential uptake of DHA by the placenta and that intracellular transport is achieved by FABP1, 3 and 4. FABP1 and 4 are possibly under the control of HIF, whereas FABP3 expression was unaltered in placental homogenates from diabetic pregnancies while FABP1 expression was upregulated 112% in insulin-dependent diabetes mellitus and 64% in gestational diabetes mellitus compared with control tissue (33).

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**Literature Cited**


