Docosahexaenoic acid supply in pregnancy affects placental expression of fatty acid transport proteins[1–3]

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ABSTRACT

Background: Better understanding of the mechanisms involved in docosahexaenoic acid (DHA) transfer to the neonate may contribute to improve dietary support for infants born prematurely to mothers with placental lipid transport disorders.

Objective: We studied whether DHA supplements modify the messenger RNA (mRNA) expression of placental lipid transport proteins to allow a selective transfer of DHA to the fetus.

Design: Healthy pregnant women ($n = 136$) received, in a double-blind randomized trial, 500 mg DHA + 150 mg eicosapentaenoic acid, 400 μg 5-methyl-tetrahydrofolic acid, 500 mg DHA + 400 μg 5-methyl-tetrahydrofolic acid, or placebo during the second half of gestation. We analyzed the fatty acid composition of maternal and cord blood phospholipids and of placenta; we quantified placental mRNA expression of fatty acid–transport protein 1 (FATP-1), FATP-4, FATP-6, fatty acid translocase, fatty acid–binding protein (FABP) plasma membrane, heart-FABP, adipocyte-FABP, and brain-FABP.

Results: The mRNA expression of the lipid carriers assayed did not differ significantly between the 4 groups. However, the mRNA expression of FATP-1 and FATP-4 in placenta was correlated with DHA in both maternal plasma and placental phospholipids, although only FATP-4 expression was significantly correlated with DHA in cord blood phospholipids. Additionally, the mRNA expression of several membrane lipid carriers was correlated with EPA and DHA in placental triacylglycerols and with EPA in placental free fatty acids.

Conclusions: Correlation of the mRNA expression of the membrane placental proteins FATP-1 and especially of FATP-4 with maternal and cord DHA leads us to conclude that these lipid carriers are involved in placental transfer of long-chain polyunsaturated fatty acids. *Am J Clin Nutr* 2006;84:853–61.

KEY WORDS Cord blood, docosahexaenoic acid, fatty acid transport, long-chain polyunsaturated fatty acids, placenta, pregnancy

INTRODUCTION

Docosahexaenoic acid (DHA) transfer across the placenta is of major importance because its synthesis by the fetus is limited. Prenatal DHA accretion determines myelination during a brain growth spurt, and DHA concentrations in the erythrocyte phospholipids of infants have been associated with electroretinogram responses after birth (1) and with response to visual stimulation until the age of 2 y (2). DHA concentrations in maternal circulation are influenced by its dietary supply (3, 4). A better understanding of the mechanisms involved in its transfer to the neonate is important to improving fetal DHA status, not only in uncomplicated pregnancies but also in disorders associated with poor DHA status. Despite normal plasma arachidonic acid (AA) and DHA concentrations in women with gestational diabetes (5, 6), low erythrocyte phospholipid long-chain polyunsaturated fatty acids (LC-PUFA) were found in their infants (7).

Unesterified fatty acid input into placental and mammalian cells occurs through both a passive flip-flop as well as a saturable protein-mediated mechanism by plasma membrane fatty acid–binding protein (FABPpm/GOT2), fatty acid translocase (FAT/CD36), and a family of 6 fatty acid–transport proteins (FATPs) (8). Although the roles of these proteins in placental fatty acid uptake and metabolism are yet to be fully understood, their complex interaction has been suggested to be involved in the enrichment of LC-PUFAs that occurs in the fetal circulation compared with the maternal side. In the cytosol, FABPs enhance the uptake of fatty acids into the cell by increasing the concentration gradient of unbound fatty acids (9). Two forms of FABPs have been identified in placenta: heart-FABP (H-FABP) and liver-FABP (L-FABP). The mRNA expression of other cytosolic FABPs reported in other tissues might occur in placental tissue, which

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TABLE 1
Characteristics of the 4 randomized study groups[1]

	Placebo	MTHF	DHA	DHA + MTHF	P^2
Maternal age (y)	29.8 ± 5.7 [35]	29.6 ± 5.1 [33]	29 ± 4.7 [35]	29.3 ± 4.6 [33]	0.884
BMI at study entry (kg/m^2)	25.1 ± 2.8 [35]	26 ± 3.6 [33]	26 ± 3.8 [35]	26.2 ± 2.8 [33]	0.324
BMI at delivery (kg/m^2)	29.2 ± 3.6 [34]	28.5 ± 3.8 [29]	31 ± 4.4 [34]	29.4 ± 2.9 [32]	0.179
Placental weight (g)	548 ± 109 [35]	519 ± 148 [33]	542 ± 128 [35]	545 ± 126 [33]	0.784
Infants' birth weight (g)	3349 ± 419 [35]	3254 ± 481 [33]	3283 ± 435 [35]	3220 ± 502 [33]	0.683
Infants' birth length (cm)[2]	50.9 ± 1.9 [26]	51.6 ± 2.1 [25]	50 ± 1.9 [30]	50.5 ± 2 [24]	0.820

[1] All values are $\bar{x}$ ± SD. *n* in brackets. MTHF, 5-methyl-tetrahydrofolic acid; DHA, docosahexaenoic acid.
[2] ANOVA.

should be confirmed in trophoblast cells because placenta samples contain many cell types, including a large number of blood cells (10). Brain-FABP (B-FABP) is strongly expressed in radial glial cells of the developing brain, especially in the perinatal period, and in mammary tissue (11). Because B-FABP is distinguished from other FABPs by its strong affinity to DHA (12), it is of interest to determine its implication in placental LC-PUFA transfer during pregnancy.

In vitro assays have shown that DHA is a potent activator of retinoid X receptors (RXRs) in human embryonic kidney 293 T cells (13). Nuclear RXRs are essential in embryonal development and in the regulation of lipid homeostasis. Because RXR agonists have been reported to increase FATP-1 mRNA expression in hepatoma cell lines and in diabetic Zucker rats (14), DHA supplementation could also modify its mRNA expression. The objective of the present study was to evaluate whether DHA supplementation during the last trimester of pregnancy might selectively modify in vivo the mRNA expression of fatty acid transporters in human placenta at the time of delivery. In a second step, the relation between the postulated fatty acid transporter and LC-PUFA contents in maternal and cord blood was analyzed.

SUBJECTS AND METHODS

Subjects

One hundred thirty-six healthy women aged 18–40 y with an uncomplicated singleton pregnancy were recruited before week 20 of gestation at the pregnancy clinic of the University Hospital of Granada, Spain. Inclusion criteria were no use of fish-oil supplements since the beginning of pregnancy and no regular use of folate and vitamin B-12 supplements after week 16 of gestation. All women habitually consumed an omnivorous diet. The study protocol was approved by the local Ethical Committee. After a careful explanation of the study details, written informed consent was obtained from all participating women.

The participants were randomly assigned in a double-blind fashion to 1 of 4 groups. From week 22 of gestation, the mothers received daily a fish-oil supplement containing 500 mg DHA + 150 mg eicosapentaenoic acid (EPA) (DHA group), 400 μg 5-methyl-tetrahydrofolic acid (MTHF group), both (DHA + MTHF group), or placebo. In most European countries, including Spain, there is no general folic acid fortification of foods. Detailed information on the participants is given in **Table 1**. The DHA supplement was based on refined interesterified fish oil (>90% of the fat from triacylglycerol) with an increased DHA content (Pronova Biocare, Lysacker, Norway). The components were supplied as 15 g milk-based portions, which contained vitamins and minerals in amounts meeting the estimated additional requirements during the second half of pregnancy (Blemil Plus; Laboratorios Ordesa, Barcelona, Spain) (15). The fatty acid compositions of the placebo and the fish-oil supplements are shown in **Table 2**.

Sampling

Placental tissue was obtained within 10 min after delivery from the center of the parenchyma. Attached decidua was removed. Samples were washed several times in a cold sodium chloride solution (0.9%; 4 °C) to eliminate blood residues. Clean tissue samples were divided, and a 100–200-mg piece was submerged into 2 mL RNAlater solution (Qiagen, Hilden, Germany) for RNA stabilization for up to 4 wk at −20 °C; a second piece of placenta (0.3–0.5 g) was immediately frozen in liquid nitrogen and stored at −80 °C for fatty acid analysis. Because of technical problems in the beginning of the study, 136 placenta samples were available for RNA extraction, but only 99 placenta samples were available for fatty acid analyses (placebo group: $n = 26$;

TABLE 2
Fatty acid composition of the dietary supplements used[1]

Fatty acid	Placebo	Fish oil
	% by wt	
4:0	0.92 ± 0.02	0.67 ± 0.04
6:0	1.41 ± 0.0	0.87 ± 0.05
8:0	1.16 ± 0.01	0.65 ± 0.02
10:0	3.07 ± 0.02	1.66 ± 0.03
12:0	3.94 ± 0.03	2.16 ± 0.01
14:0	12.88 ± 0.04	7.33 ± 0.04
15:0	1.32 ± 0.02	0.85 ± 0.00
16:0	36.45 ± 0.08	22.03 ± 0.16
17:0	0.85 ± 0.00	0.88 ± 0.01
18:0	10.37 ± 0.04	8.82 ± 0.01
20:0	0.21 ± 0.00	0.36 ± 0.01
16:1n−7	2.13 ± 0.00	1.64 ± 0.01
18:1n−9 + n−7	22.48 ± 0.05	20.76 ± 0.13
20:1n−9	ND	1.35 ± 0.02
18:2n−6	1.74 ± 0.04	2.02 ± 0.01
20:4n−6	0.14 ± 0.00	1.16 ± 0.01
22:4n−6	ND	1.02 ± 0.01
18:3n−3	0.53 ± 0.02	0.69 ± 0.02
20:5n−3	ND	5.58 ± 0.04
22:5n−3	0.11 ± 0.00	1.66 ± 0.02
22:6n−3	ND	17.84 ± 0.13

[1] All values are $\bar{x}$ ± SD of 3 samples per group. Data were according to the manufacturer's analyses. ND, not detected.

MTHF group: $n = 27$; DHA group: $n = 25$; and DHA + MTHF group: $n = 21$).

Maternal and cord blood samples (5 and 2 mL, respectively) were collected at the time of delivery into EDTA-containing tubes; plasma was separated within 1 h by centrifugation at 1500 × *g* for 5 min and frozen immediately at −80 °C for later fatty acid analysis.

Extraction of total RNA and quantification by real-time polymerase chain reaction

Total RNA was extracted from placental tissue by using the RNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA concentrations measured with an ultraviolet light (UV) photometer ranged between 150 and 500 μg/mL with an A260/A280 ratio of 1.6 to 1.9.

To eliminate DNA contamination, we digested 100 μL of extracted RNA with 5 μL DNase I (Amplification Grade 1U/μL, Gibco; Life Technologies, Karlsruhe, Germany) using the 10X buffer included in the kit and adding UV-water up to a final volume in the tube of 130 μL. Tubes were incubated for 15 min at room temperature, and the digestion was stopped at 65 °C for 10 min. After digestion, we precipitated RNA with 1.2 (by vol) of isopropanol at −20 °C for ≥30 min. We centrifuged the tubes at 12 000 × *g* for 10 min and discarded the supernatant fluid. The pellet was washed once with 50 μL of 100% ethanol and centrifuged under the same conditions. We allowed the pellet to air dry and finally dissolved it into UV-water to reach a final concentration of ≈1 μg RNA/12.5 μL UV-water. The tubes were kept at −80 °C until analyzed further.

Complementary DNA (cDNA) synthesis was performed with the use of the Advantage RT for PCR Kit from Clontech (BD Biosciences, Palo Alto, USA) by using 1 μg digested RNA and oligo (dT)18 primers, according to the manufacturer's instructions.

For the real-time polymerase chain reaction (PCR) quantification, a set of primers for FATP-1 (sense: 5'-AGGTGGTTCAGTACATCGGG–3'; antisense: 5'-AGAACTCCCCGATTTGGC–3'), FATP-4 (sense: 5'-ATACCCACTGGACCTTCCG–3'; antisense: 5'-GGTGTTGATGAGGGCTGC–3'), H-FABP (sense: 5'-TTTTGCTACCAGGCAGGTG–3'; antisense: 5'-TCATCTGCTGTTGTCTCATCG–3'), and β_2-microglobulin (housekeeping gene; sense: 5'-AGGTTTACTCACGTCATCCAG–3'; antisense: 5'- TTGGGCTGTGACAAAGTCAC–3') were designed by using the HUSAR Primer Analysis Software (EMBL, Heidelberg, Germany). Primers for FAT/CD36 (sense: 5'-GGAAAGTCACTGCGACATGA-3'; antisense: 5'-CCTTGGATGGAAGAACG AATC-3'), FABPpm/GOT2 (sense: 5'-GGAAGGAAATAGCAACAGTGG-3'; antisense: 5'-TCCTACACGCTCACCATATAAGC-3'), FATP-6 (sense: 5'-GAAATGAGCAGGGTTGGTGTAT-3'; antisense: 5'-AGTGTCTCCAGTACGG TCCC-3'), adipocyte-FABP [A-FABP (sense: 5'-AGATGACAGGAAAGTCAAGAGCA-3'; antisense: 5'- CCTTTCATGACGCATTCCAC-3', and B-FABP (sense: 5'-GCTCTAGGCGTG GGCTTT-3'; antisense: 5'-TCCAGGCTAACAACAGAGTTACAG-3') were designed by Net Primer software (Premier Biosoft International, Palo Alto, CA).

Real-time PCR for FATP-4, H-FABP, and β_2-microglobulin contained 5 μL cDNA, 0.5 μmol/L of each primer, 200 μmol dNTP/L (Amersham Pharmacia Biotech, Freiburg, Germany), 1X SYBR Green (SYBR Green, PCR Core Reagents Kit; Applied Biosystems, Darmstadt, Germany), 2.5 mmol $MgCl_2$/L (SYBR Green, PCR Core Reagents Kit; Applied Biosystems), and 1.25 U AmpliTaq Gold polymerase (Applied Biosystems), which made a total of 25 μL reaction volume. For FATP-1 quantification we used 0.8 μmol/L of each primer and 3 mmol $MgCl_2$/L instead of the concentrations indicated above. The mRNA quantitation of these genes was performed in a Rotorgene 2000 (Corbett Research, Sydney, Australia). The cycling conditions for the real-time PCR were as follows: *1*) initial denaturation at 95 °C for 10 min; *2*) 35 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s for FATP-4, H-FABP, and β_2-microglobulin and at 62 °C for 30s for FATP-1, and synthesis at 72 °C for 30 s; and *3*) final elongation at 72 °C for 10 min. To minimize changes in cycle threshold reproducibility detected from run to run, the samples were quantified in every plate together with a standard curve with the use of serial dilutions of the purified specific PCR product.

Real-time PCR analyses for FAT/CD36, FABPpm/GOT2, FATP-6, A-FABP, and B-FABP were performed in an I-cycler (Bio-Rad, Munich, Germany). With this thermal cycler, cycle threshold values were fairly reproducible (CV = 2.04% from run to run) and the PCR reactions for FAT/CD36, FABPpm/GOT2, FATP-6, A-FABP, B-FABP, and β_2-microglobulin were performed together in the same plate. Thus, standard curves were not required. For each PCR reaction, 1 μL cDNA, 2 μL of each primer (which were previously prepared in a concentration of 5 μmol/L), and 10 μL SYBR Green (SYBR Green, PCR Core Reagents Kit; Applied Biosystems) were used, which made a total of 20 μL reaction volume with water. The cycling conditions for the real-time PCR were as follows: *1*) initial denaturation at 95 °C for 5 min; *2*) 39 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, and synthesis at 72 °C for 45 s; and *3*) final elongation at 72 °C for 1 min.

We further checked the amplification product in a 2% agarose gel in tris-acetate-EDTA buffer, using ethidium bromide staining and the Eagle-eye II System (Stratagene, Amsterdam, Netherlands).

Fatty acid analyses

Fatty acids were extracted from placental tissue with the use of chloroform:methanol (2:1 by vol), and lipid fractions were obtained by thin-layer chromatography as previously described (16). Fatty acids from maternal and cord blood were extracted according to the method of Lepage and Roy (17); only the phospholipid fraction from blood samples was isolated by liquid chromatography with the use of aminopropyl columns (Sep Pak Cartridges; Waters, Milford, MA) and analyzed for fatty acid composition.

Fatty acid composition (% by wt) was determined by capillary gas-liquid chromatography on a Hewlett-Packard 5890 series II gas chromatograph (Hewlett-Packard, Waldbronn, Germany) that was equipped with a BPX70 column (SGE, Weiterstadt, Germany) 60 m in length and with an inner diameter of 0.32 mm (16).

Statistical analysis

All data are presented as means ± SDs. Results of the mRNA quantification of FATP-1, FATP-4, and H-FABP were expressed in arbitrary units, eg, number of copies of the gene/10 000 copies of β_2-microglobulin. Results of the mRNA quantification of FAT/CD36, FABPpm/GOT2, FATP-6, A-FABP,

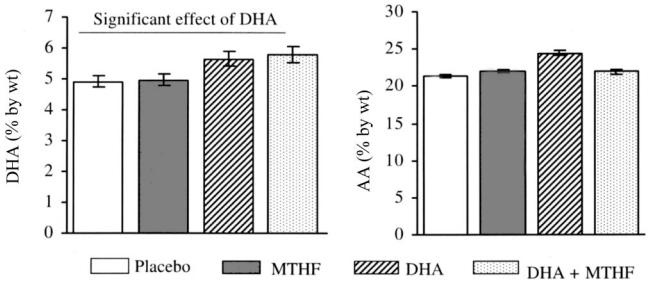

FIGURE 1. Mean (±SD) percentages of docosahexaenoic acid (DHA) and arachidonic acid (AA) in placental phospholipids from subjects who consumed different nutritional supplements during the second half of pregnancy: placebo ($n = 26$), 5-methyl-tetrahydrofolic acid (MTHF; $n = 27$), DHA ($n = 25$), or DHA + MTHF ($n = 21$). Two-factor ANOVA (followed by a post hoc Bonferroni test) was used to evaluate the 2 sources of variation in the study (DHA and MTHF supplementation). DHA supplementation resulted in a significantly higher percentage of DHA in placental phospholipids in both the DHA and DHA + MTHF groups ($P = 0.008$), whereas no significant changes were observed after MTHF supplementation ($P = 0.177$). Percentages of placental AA were not significantly different between the groups ($P = 0.395$ for DHA supplementation and $P = 0.097$ for MTHF supplementation). No significant interactions between DHA and MTHF supplementations were reported for AA ($P = 0.459$) or DHA ($P = 0.991$) in placental phospholipids.

and B-FABP were calculated as cycle threshold ratios of each gene with respect to β_2-microglobulin. Differences in mRNA expression and fatty acid composition were evaluated by using 2-factor analysis of variance (ANOVA) followed by multiple comparisons with Bonferroni correction. Two-factor ANOVA was used to evaluate the 2 sources of variations in the study (DHA and MTHF supplements). Correlations between mRNA expression and fatty acids were assessed by using Pearson's correlation coefficients. We used SPSS for WINDOWS (release 10.0; SPSS Inc, Chicago, IL) for the statistical analysis. P values <0.05 were considered statistically significant.

RESULTS

DHA supplementation during the second half of pregnancy (DHA and DHA + MTHF groups) resulted in significant DHA incorporation into placental phospholipids at the time of delivery, whereas the proportion of AA remained unchanged (**Figure 1**); MTHF supplementation did not affect the placental fatty acid profile significantly. However, the mRNA expression of FATP-1, FATP-4, FATP-6, FAT/CD36, FABPpm/GOT2, H-FABP, A-FABP, and B-FABP in placental tissue did not differ significantly between the groups after dietary supplementation (**Figure 2**). Nevertheless, we detected a significant positive correlation between the proportion of DHA in placental phospholipids and mRNA expression in the membrane proteins FATP-1 and FATP-4 in the placenta (**Figure 3**). The percentage of EPA in placental phospholipids was also positively correlated with FATP-1 ($r = 0.32$, $P = 0.002$) and FATP-4 ($r = 0.48$, $P = 0.0001$) expression. Furthermore, the ratio of AA to DHA in placental phospholipids was inversely correlated with mRNA expression in both proteins (FATP-1: $r = -0.29$, $P = 0.007$; FATP-4: $r = -0.33$, $P = 0.002$) and in triacylglycerols (**Table 3**) and free fatty acids (FFAs) (**Table 4**) but not in cholesteryl esters (data not shown). These results indicate that FATP-1 and FATP-4 are mainly involved in dietary n−3 LC-PUFA esterification in placental phospholipids.

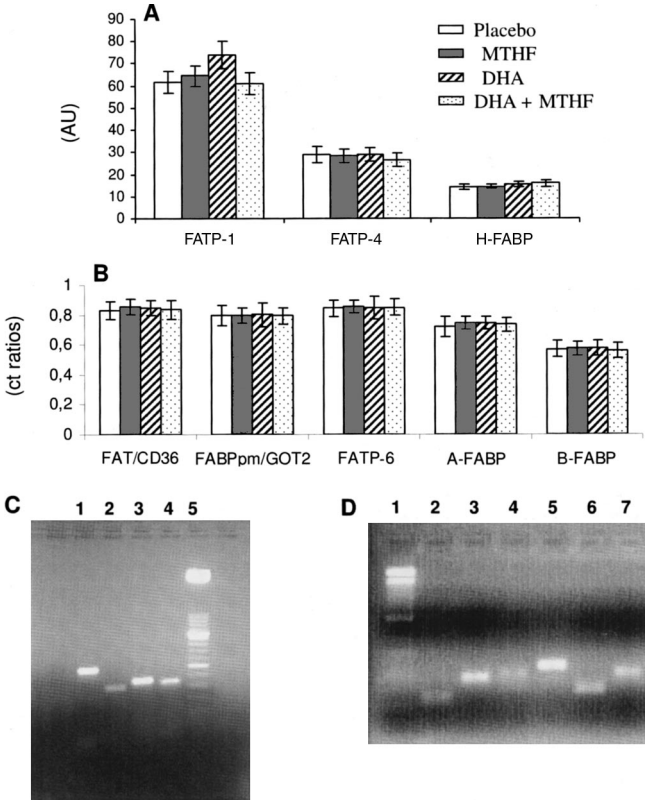

FIGURE 2. Messenger RNA (mRNA) expression of fatty acid transport proteins (FATPs) in placenta from subjects who consumed different nutritional supplements during the last trimester of pregnancy: placebo ($n = 35$), 5-methyl-tetrahydrofolic acid (MTHF; $n = 33$), docosahexaenoic acid (DHA; $n = 35$), or DHA + MTHF ($n = 33$). A: mRNA expression of FATP-1, FATP-4, and heart fatty acid–binding protein (H-FABP) were not significantly different between the 4 supplementation groups (2-factor ANOVA). Arbitrary units (AUs) represent the number of copies/10 000 copies of β_2-microglobulin. B: mRNA expression of fatty acid translocase (FAT/CD36), plasma membrane FABP (FABPpm/GOT2), FATP-6, adipocyte-FABP (A-FABP), and brain-FABP (B-FABP) in placenta also was not significantly different between the 4 groups (ANOVA). Cycle threshold (ct) ratios were calculated by dividing the ct value of the selected gene by the ct value of the housekeeping gene β_2-microglobulin. C and D: Polymerase chain reaction products from villi central human placental tissue were resolved on a 2% agarose gel and viewed by staining with ethidium bromide. C: Lane 1: β_2-microglobulin; lane 2: H-FABP; lane 3: FATP-4; lane 4: FATP-1; lane 5: marker XIII 50 base pairs/band. D: Lane 1: marker XIV 100 base pairs/band; lane 2: β_2-microglobulin; lane 3: FAT/CD36; lane 4: FABPpm/GOT2; lane 5: FATP-6; lane 6: A-FABP; and lane 7: B-FABP.

Additionally, we observed significant positive correlations between the mRNA expression of several membrane proteins and EPA and DHA in placental triacylglycerol (Table 3) and with EPA in FFAs (Table 4). Furthermore, in the FFA fraction, the percentage of AA was inversely correlated not only with mRNA expression of membrane proteins but also with the cytosolic proteins A-FABP and B-FABP (Table 4). The fatty acid composition of the cholesteryl ester fraction was fairly constant in placenta, and fatty acids in this lipid fraction were not correlated with the mRNA expression of any of the genes analyzed in the present study. These results point to a nonselective mechanism by membrane transport proteins for DHA and EPA storage in placental triacylglycerol, whereas EPA competes with AA by cytosolic FABP for its movement within the placenta.

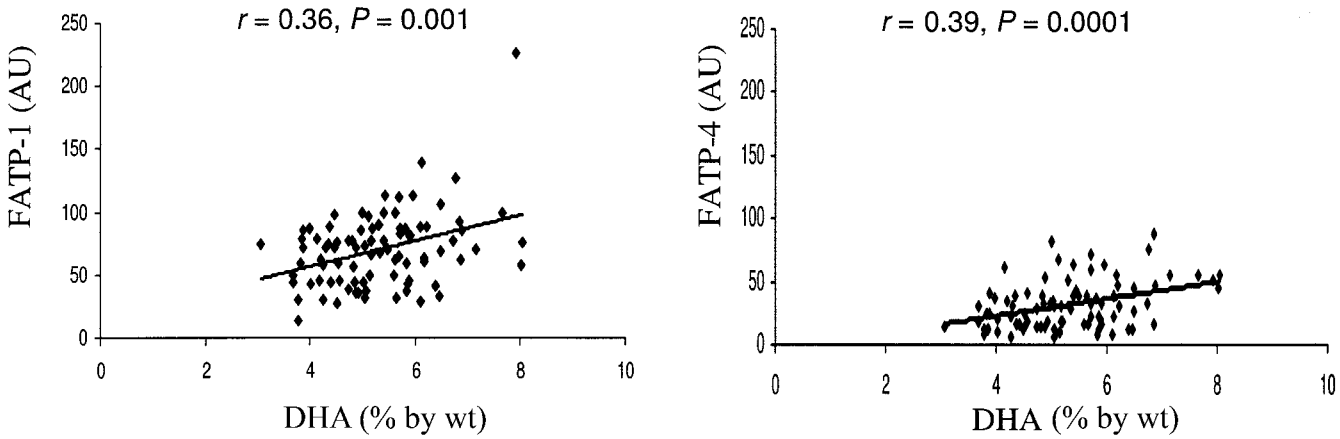

FIGURE 3. Pearson's correlation coefficients between percentages of docosahexaenoic acid (DHA) in placental phospholipids and messenger RNA expression of fatty acid–transport protein 1 (FATP-1) and FATP-4 in the placenta from the subjects ($n = 99$). AU, arbitrary units.

At delivery, the percentage of DHA in maternal plasma phospholipids was also significantly greater in the DHA-supplemented groups than in the non DHA-supplemented groups (placebo group: 5.89 ± 1.61; MTHF group: 6.12 ± 1.52; DHA group: 7.77 ± 1.72; and DHA + MTHF group: 7.59 ± 1.68). In addition, we detected positive correlations between maternal plasma phospholipid EPA and DHA concentrations and the gene expression of the placental membrane transporters FATP-1 and FATP-4 (**Table 5**). In contrast, the percentage of AA in maternal plasma phospholipids was negatively correlated with all of the membrane proteins in the placenta, although the correlations with FATP-1 and FABPpm/GOT were not significant (Table 5). Thus, these results in maternal plasma phospholipids support those previously observed in placenta, ie, that high percentages of DHA and EPA in maternal plasma phospholipids lead to up-regulation of mRNA expression of FATP-1 and FATP-4 by the placenta.

Placental FATP-1 and FATP-4 mRNA expression were inversely correlated with AA and the ratio of AA to DHA in cord plasma phospholipids (**Figure 4** and **Figure 5**). Because there was one outgoing data point in both plots for FATP-1 that might influence the results, we also evaluated correlations without this outlier. FATP-1 expression remained significantly negatively correlated with the percentage of AA in cord plasma phospholipids ($r = -0.26$, $P = 0.005$), whereas only the ratio of AA to DHA showed a nonsignificant trend ($r = -0.18$, $P = 0.053$). Of all the genes analyzed, only the FATP-4 mRNA expression in the placenta was significantly correlated with DHA in cord plasma phospholipids (Figure 5). These results show that mRNA expression of FATP-4 in placental tissue was positively correlated with the uptake of maternal DHA into placental and cord blood phospholipids, which appears to indicate a mechanism for selective materno-fetal DHA transfer in humans.

DISCUSSION

This is the first human in vivo study to analyze mRNA concentrations of selected cellular lipid-binding proteins in placental tissue and the effect of fish-oil supplementation. At the time of delivery, mRNA expression of FATP-1 and FATP-4 in placental tissue was positively correlated with maternal DHA incorporation into placental and cord blood phospholipids, although the only significant finding was for FATP-4 expression in cord blood. In human trophoblast cells, treatment with both

TABLE 3
Pearson's correlation coefficients between messenger RNA (mRNA) expression of lipid transport proteins in placenta and long-chain polyunsaturated fatty acids (% by wt) in placental triacylglycerols ($n = 99$)[1]

mRNA expression	EPA	DHA	AA	AA:DHA
Membrane proteins				
FATP-1	$r = 0.16$, $P = 0.128$	$r = 0.37$, $P = 0.0001$	$r = 0.05$, $P = 0.646$	$r = -0.31$, $P = 0.004$
FATP-4	$r = 0.22$, $P = 0.041$	$r = 0.47$, $P = 0.001$	$r = 0.06$, $P = 0.579$	$r = -0.41$, $P = 0.001$
FATP-6	$r = 0.19$, $P = 0.086$	$r = 0.17$, $P = 0.110$	$r = -0.02$, $P = 0.869$	$r = -0.14$, $P = 0.217$
FABPpm/GOT2	$r = 0.26$, $P = 0.018$	$r = 0.25$, $P = 0.027$	$r = -0.06$, $P = 0.619$	$r = -0.22$, $P = 0.051$
FAT/CD36	$r = 0.23$, $P = 0.030$	$r = 0.25$, $P = 0.016$	$r = 0.01$, $P = 0.913$	$r = -0.32$, $P = 0.002$
Cytosolic proteins				
H-FABP	$r = 0.11$, $P = 0.315$	$r = 0.13$, $P = 0.227$	$r = 0.02$, $P = 0.839$	$r = -0.11$, $P = 0.309$
B-FABP	$r = 0.10$, $P = 0.451$	$r = 0.13$, $P = 0.294$	$r = 0.10$, $P = 0.423$	$r = -0.15$, $P = 0.229$
A-FABP	$r = 0.20$, $P = 0.070$	$r = -0.03$, $P = 0.793$	$r = 0.01$, $P = 0.991$	$r = -0.04$, $P = 0.712$

[1] AA, arachidonic acid; A-FABP, adipocyte fatty acid–binding protein; B-FABP, brain-FABP; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FABPpm/GOT2, plasma membrane fatty acid–binding protein; FAT/CD36, fatty acid translocase; FATP, fatty acid–transport protein; H-FABP, heart-FABP. Pearson's correlation coefficients were derived from simple correlation analysis of lipid transport proteins in placenta and long-chain polyunsaturated fatty acids in placental triacylglycerols with the use of data from all 4 groups.

TABLE 4
Pearson's correlation coefficients between messenger RNA (mRNA) expression of lipid transport proteins in placenta and long-chain polyunsaturated fatty acids (% by wt) in placental free fatty acids ($n = 99$)[1]

mRNA expression	EPA	DHA	AA	AA:DHA
Membrane proteins				
FATP-1	$r = 0.27$, $P = 0.013$	$r = 0.10$, $P = 0.353$	$r = -0.26$, $P = 0.015$	$r = -0.21$, $P = 0.049$
FATP-4	$r = 0.37$, $P = 0.001$	$r = 0.04$, $P = 0.713$	$r = -0.38$, $P = 0.001$	$r = -0.23$, $P = 0.032$
FATP-6	$r = 0.21$, $P = 0.054$	$r = -0.08$, $P = 0.454$	$r = -0.26$, $P = 0.016$	$r = -0.02$, $P = 0.882$
FABPpm/GOT2	$r = 0.23$, $P = 0.041$	$r = -0.03$, $P = 0.781$	$r = -0.29$, $P = 0.009$	$r = -0.09$, $P = 0.452$
FAT/CD36	$r = 0.24$, $P = 0.021$	$r = -0.19$, $P = 0.077$	$r = -0.47$, $P = 0.001$	$r = -0.07$, $P = 0.537$
Cytosolic proteins				
H-FABP	$r = 0.10$, $P = 0.347$	$r = -0.03$, $P = 0.766$	$r = -0.09$, $P = 0.420$	$r = -0.05$, $P = 0.673$
B-FABP	$r = 0.09$, $P = 0.471$	$r = -0.29$, $P = 0.017$	$r = -0.41$, $P = 0.001$	$r = 0.09$, $P = 0.456$
A-FABP	$r = 0.10$, $P = 0.383$	$r = -0.33$, $P = 0.003$	$r = -0.32$, $P = 0.003$	$r = 0.18$, $P = 0.117$

[1] AA, arachidonic acid; A-FABP, adipocyte fatty acid–binding protein; B-FABP, brain-FABP; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FABPpm/GOT2, plasma membrane fatty acid–binding protein; FAT/CD36, fatty acid translocase; FATP, fatty acid–transport protein; H-FABP, heart-FABP. Pearson's correlation coefficients were derived from simple correlation analysis of lipid transport proteins in placenta and long-chain polyunsaturated fatty acids in placental free fatty acids with the use of data from all 4 groups.

peroxisome-proliferator activated receptor γ and RXR agonists was reported to increase the mRNA expression of FATP-1 and FATP-4 but not of FATP-2, FATP-3, and FATP-6; only FATP-4 mRNA expression was related to fatty acid accumulation in these cells (18). Both studies suggest an important role of FATP-4 in the fatty acid transfer by the placenta, whereas the contribution of FATP-1 seems to be less than that of FATP-4. FATP-1 and FATP-4 are the most closely related members of the family (19), and they exhibit both fatty acid transport and acyl-CoA synthetase activities (20). It is known that acyl-CoA fatty acids serve as substrate for reacylation of phospholipids, the formation of intracellular lipid stores, or mitochondrial oxidation; moreover, the amino acid residues 258–475 of FATP-1, which are peripherally associated with the inner leaflet of the plasma membrane, may facilitate direct contact of acyl-CoA fatty acids with placental membrane phospholipids (21). Considering that phospholipid is the most abundant lipid fraction in the placenta (16) and that the percentages of EPA and DHA in maternal plasma in the present study were significantly greater in subjects who received fish-oil supplements (results not shown), FATP-1 and FATP-4 might preferentially incorporate DHA and EPA in placental phospholipids via esterification coupled influx. Moreover, molecules could be specialized in the uptake rate of different fatty acids, as suggested by experiments showing that, unlike FATP-4, FATP-1 can mediate the uptake of arachidonate into transfected cells (22, 23). The fact that FATP-6 was not significantly correlated with DHA in placental phospholipids could also be due to a different mechanism for fatty acid transfer compared with FATP-1 and FATP-4; FATP-6 and also FABPpm/GOT2 have been localized in close physical proximity to FAT/CD36 on the plasma membrane (24, 25), which supports the concept that both proteins could mainly facilitate fatty acid flux through the membrane by a translocation mechanism that uses FAT/CD36 instead of CoA activation of long-chain fatty acids.

The mRNA expression of practically all of the membrane proteins has been correlated with EPA and DHA accumulation in placental triacylglycerol, which indicates nonselective mechanisms of membrane transport proteins for lipid storage. However, fatty acids in cholesteryl esters were not associated with the expression of fatty acid–binding proteins.

FFAs are bound to cytosolic fatty acid–binding proteins for their transfer to cord plasma. Percentages of EPA, but not of DHA, in placental FFAs were positively correlated with the mRNA expression of all membrane proteins, which may have been due to the low incorporation of DHA into the FFA fraction. In placental choriocarcinoma (BeWo) cells, almost 45% of the total amount of [^{3}H]DHA taken up by the cells was esterified into triacylglycerols, whereas 35% was incorporated into phospholipids fractions and <7% in FFAs. In contrast, AA, oleic acid, and linoleic acid accumulation as FFAs in the Bewo cells were

TABLE 5
Pearson's correlation coefficients between messenger RNA (mRNA) expression of membrane lipid transport proteins in placenta and long-chain polyunsaturated fatty acids (% by wt) in maternal plasma phospholipids at the time of term delivery ($n = 136$)[1]

mRNA expression	EPA	DHA	AA	AA:DHA
FATP-1	$r = 0.30$, $P = 0.002$	$r = 0.32$, $P = 0.001$	$r = -0.17$, $P = 0.072$	$r = -0.28$, $P = 0.003$
FATP-4	$r = 0.27$, $P = 0.004$	$r = 0.23$, $P = 0.012$	$r = -0.26$, $P = 0.005$	$r = -0.21$, $P = 0.027$
FATP-6	$r = 0.05$, $P = 0.613$	$r = 0.01$, $P = 0.987$	$r = -0.25$, $P = 0.007$	$r = -0.16$, $P = 0.088$
FABPpm/GOT2	$r = 0.08$, $P = 0.4$	$r = 0.01$, $P = 0.928$	$r = -0.17$, $P = 0.063$	$r = -0.10$, $P = 0.320$
FAT/CD36	$r = 0.03$, $P = 0.728$	$r = -0.01$, $P = 0.985$	$r = -0.19$, $P = 0.035$	$r = -0.11$, $P = 0.221$

[1] AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FABPpm/GOT2, plasma membrane fatty acid–binding protein; FAT/CD36, fatty acid translocase; FATP, fatty acid–transport protein. Pearson's correlation coefficients were derived from simple correlation analysis of long-chain polyunsaturated fatty acids in maternal phospholipids and mRNA expression of membrane lipid transport proteins in placenta with the use of data from all 4 groups.

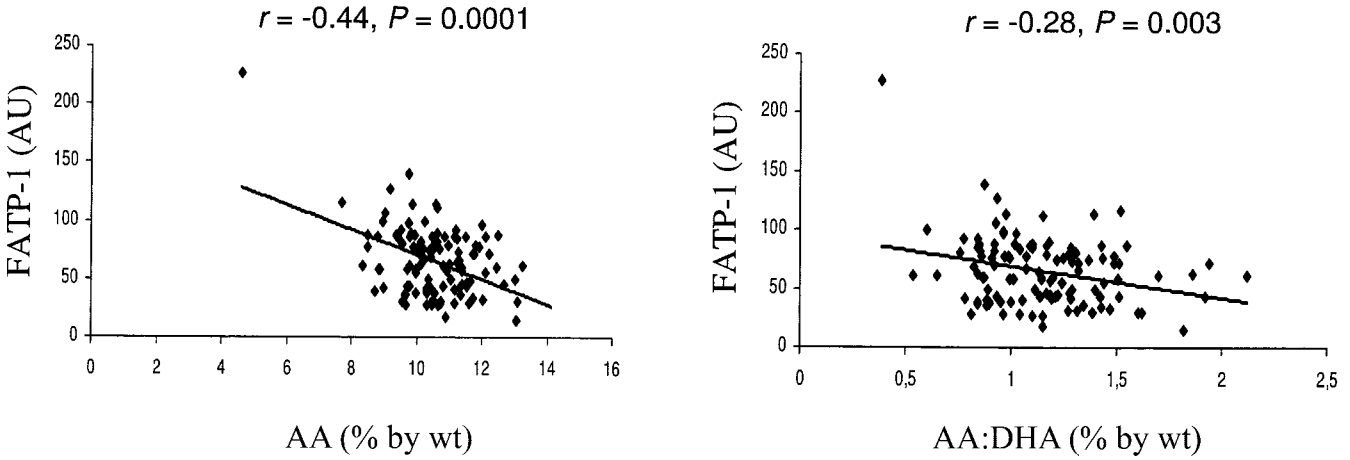

FIGURE 4. Pearson's correlation coefficients between percentages of arachidonic acid (AA) and the ratio of AA to docosahexaenoic acid (DHA) in cord blood phospholipids and messenger RNA expression of fatty acid–transport protein 1 (FATP-1) in the placenta from the subjects (n = 136). AU, arbitrary units.

≈20%, respectively, whereas no information was reported for EPA (26). It is important to note that the percentage of AA in FFAs was inversely correlated with the expression of all membrane proteins and also with the cytosolic proteins A-FABP and B-FABP. Several studies (27, 28) in animals suggest that EPA and AA are competitors for the desaturation and elongation pathway, and this competition might explain the negative correlations with FABP and AA in the placenta, because the percentage of AA was not different in any of the placental lipid fractions among the supplemented groups (results not shown).

Selectivity of the human placenta for the transport of individual fatty acids has been suggested as one mechanism that might explain greater concentrations of some LC-PUFAs, such as DHA and AA, in the fetal than in the maternal circulation. Kuhn and Crawford (29) suggested that the selective esterification of fatty acids within the placenta (especially as phospholipids) may allow their selective sequestering into the fetus, because these esterified fatty acids do not recross the placental barrier, whereas FFAs cross the placenta in either direction. A different compartmentalization of individual fatty acids in various lipid fractions within the placenta might be a powerful mechanism for selective transfer of fatty acids. In this study we showed FATP-1 and FATP-4 expression to be correlated with the preferential incorporation of EPA and DHA in placental phospholipids, which could be the mechanism for a selective and preferential DHA transfer to the neonate. Given that only FATP-4 expression was significantly correlated with cord blood phospholipid DHA, we propose that FATP-4 plays a critical role in the materno-fetal transport of LC-PUFAs. Indeed, homozygous deletion of the FATP-4 gene in mice resulted in early embryonic lethality (30). Moreover, in FATP-1– and FATP-4–deficient mice, there was no compensatory up-regulation of other family members (19), which highlights the importance of these 2 fatty acid transporters. In vitro studies identified a placental plasma membrane FABP (p-FABPpm), located exclusively in the placenta, that showed a higher affinity and binding capacity for DHA and AA than for linoleic and oleic acids (31). p-FABPpm and classic FABPpm/GOT2 are both membrane proteins of similar size (≈40 kDa), but they differ in amino acid composition, isoelectric point, and aspartate aminotransferase activity. Because the complete amino acid or cDNA sequence of p-FABPpm has not been published, we could not quantify the expression of p-FABPpm in the present

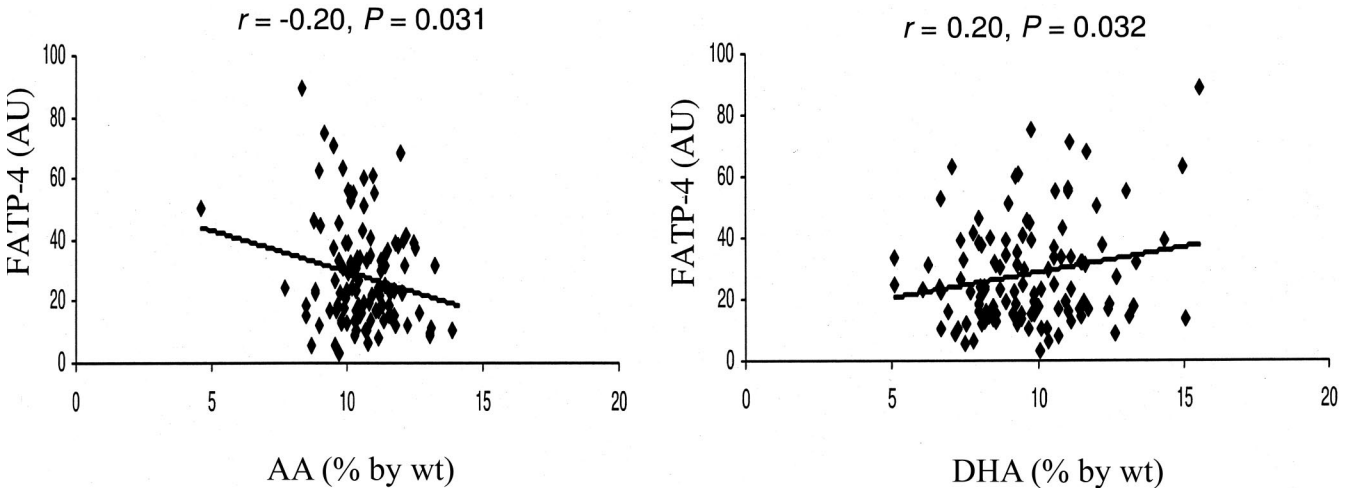

FIGURE 5. Pearson's correlation coefficients between percentages of arachidonic acid (AA) and docosahexaenoic acid (DHA) in cord blood phospholipids and messenger RNA expression of fatty acid–transport protein 4 (FATP-4) in the placenta from the subjects (n = 136). AU, arbitrary units.

study. In addition, in liver it was initially disputed whether classic FABPpm had aspartate aminotransferase activity (32), although further studies on cDNA transfection established definitively that both proteins were identical (33). We must also wait for definitive evidence on the structure and function of this specific placental protein, ie, p-FABPpm.

At the outset of this study, we hypothesized that DHA supplementation during pregnancy could modify the expression of FATP-1 in human placenta or the expression of other lipid transport proteins because DHA was reported to act as a potent agonist of RXR in vitro. In the present study, the level of DHA supplementation was higher than habitual intakes at week 30, and at the time of delivery, and overrode differences in national fish consumption in European countries such as Spain, Germany, and Hungary (data not shown). Daily maternal supplementation with 500 mg DHA during the last trimester of pregnancy resulted in significant concentrations of DHA in maternal plasma phospholipids at the time of delivery. However, we detected no significant differences in the placental mRNA expression of FATP-1, FATP-4, FATP-6, FAT/CD36, FABPpm/GOT2, H-FABP, A-FABP, and B-FABP in the experimental groups. Lengqvist et al (34) reported that DHA and other LC-PUFAs induced RXR activation in vitro, even at low micromolar concentrations. Mean DHA concentrations in placental tissue in this study (considering the sum of DHA in all placental lipid fractions) were 1.4 μmol/g in the subjects who consumed fish-oil supplements and 1.2 μmol/g in the subjects who did not ingest fish-oil supplements; thus, the mean difference between the DHA-supplemented and -nonsupplemented groups was only 0.2μmol/g, which might be too small to induce significant group differences in the mRNA expression of the lipid transport proteins studied.

We conclude that fish-oil supplementation during the second half of pregnancy did not significantly modify the gene expression of FATP-1, FATP-4, FATP-6, FAT/CD36, FABPpm/GOT2, H-FABP, A-FABP, or B-FABP in human placenta at the time of delivery. However, the mRNA expression of FATP-1, and particularly of FATP-4, in placental tissue was positively correlated with the uptake of maternal DHA into placental and cord blood phospholipids, which appears to indicate a mechanism for selective materno-fetal DHA transfer in humans.

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