Fetal dependency on maternal fatty acids: a pilot study in human pregnancies using the natural abundance variation of ^{13}C

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Abstract

The extent of *de-novo* biosynthesis of non-essential fatty acids (FAs) and the endogenous biosynthesis of long chain polyunsaturated FAs in human fetuses remains largely unknown. We used natural variations in the ^{13}C:^{12}C ($\delta\ ^{13}C$) of plasma phospholipids of the woman at delivery and of cord blood to infer fetal biosynthesis of FAs. We studied 39 mother-fetus pairs with uncomplicated pregnancies and term delivery. Eighteen women were supplemented with docosahexaenoic acid (DHA), from pregnancy week 20 until delivery, sourced from an algae (n=13) or fish oil (n=5), each with slightly different ^{13}C content. Twenty-one women did not receive DHA supplementation. We measured the $\delta\ ^{13}C$ value of selected phospholipid FAs (C16:0, C18:0, C18:1n-9, C18:2n-6, C20:4n-6, and C22:6n-3), in maternal and cord plasma samples at delivery using isotope ratio mass spectrometry.

We found significant linear correlations for $\delta\ ^{13}C$ values of FAs between mothers and their fetuses (C16:0, r=0.8535; C18:0, r=0.9099; C18:1n-9, r=0.8079; C18:2n-6, r=0.9466; C20:4n-6, r=0.9257; and C22:6n-3, r=0.9706). Women supplemented with algal DHA had significantly lower DHA $\delta\ ^{13}C$ values in their plasma phospholipids than those supplemented with fish DHA or those who did not receive DHA supplementation ($p<0.001$).

There was no significant difference in $\delta\ ^{13}C$ values of FAs between women at delivery and their fetuses.

These findings strongly suggest that the human fetus is highly dependent on the placental transport of maternal plasma FAs, particularly DHA. The limited fetal biosynthesis of major FAs emphasizes the crucial role of maternal nutrition and placental well-being in fetal development.

Keywords: Carbon 13, natural abundance, placenta, fetal biosynthesis, fatty acids, DHA

Abbreviations used in this paper: fatty acid (FA), long chain polyunsaturated (LCP), arachidonic acid (AA), docosahexaenoic acid (DHA), gas chromatography (GC), combustion (C), isotope ratio mass spectrometry (IRMS), linolenic acid (ALA), linoleic acid (LA), palmitic acid (PA), oleic acid (OA).

Introduction

Fatty acids (FAs) are required by the developing fetus to support rapid cellular growth and function. Deposition of lipids increases exponentially during gestational age, reaching around 7 g/day just before birth [1]. Among these, docosahexaenoic (C22:6n-3; DHA) and arachidonic (20:4n-6; AA) acids are crucial for optimal fetal growth and the development of important organs such as the retina and brain [2, 3].

From the beginning of pregnancy, the woman adjusts her metabolism to support the nutritional needs of the fetal-placental unit by increasing levels of nearly all plasma lipid classes [4]. As the fetus develops, the placenta actively transports substantial amounts of FAs from the mother to the fetus [5], particularly during the third trimester when brain tissue rapidly increases in cell size and number [6]. Higher amounts of DHA and AA acids, and lower amounts of their precursors, linolenic (C18:3n-3, ALA) and linoleic (C18:2n-6, LA) acids, are found in fetal plasma compared to maternal plasma [7]. It has been estimated that the rate of fetal accretion for AA and DHA between 35 and 40 weeks of gestational age is 92 and 42 mg/day, respectively [8].

While the mechanism of FAs transfer across the placenta is well established [9, 10], our understanding of the contribution of maternal FAs supply to the fetus, and the extent of the fetal *de novo* FA biosynthesis (lipogenesis) remains rather limited.

Some information on the placental transfer of FAs comes from an *in vivo* short study using palmitic acid (C16:0, PA), oleic acid (C 18:0, OA), LA and DHA, uniformly labeled with ^{13}C in both control and gestational diabetes mellitus pregnant women. These FAs were administered orally 12 hours before elective cesarean section. The ^{13}C enrichment was then measured in maternal plasma, cord blood, and the placenta. The authors found that 0.5% of administered FAs were detectable in cord blood, except for DHA which was enriched by 3.5% [11]. The preferential placental transfer of DHA over AA, LA, and ALA possibly reflects the high demand for DHA in the growing fetus [12]. Moreover, although the fetal ability to desaturate and elongate LA to AA was demonstrated in 9-term infants [13], endogenous biosynthesis appears less efficient in the case of DHA chain elongation from ALA [14].

Information on the biosynthesis of saturated and unsaturated non-essential FAs by the human fetus is limited to older *in vitro* studies of human subcutaneous fetal tissue and rat fetuses [15]. It is known that FA synthesis in adipose tissue begins to increase continuously from as early as 10 weeks of gestation [16] and that rat fetuses synthesize FAs *de novo* using maternal glucose as a primary precursor [17]. Given the undisputed importance of the diet of the

pregnant woman and of DHA supplementation during pregnancy [18, 19], further understanding of fetal lipid synthesis with respect to maternal DHA and FA placental transfer remains of importance.

Our clinical research group has recently utilized the natural variations in the $^{13}C/^{12}C$ ($\delta\ ^{13}C$) value to measure the contribution of DHA from an algal source to the plasma DHA pool in pregnant women [20]. With this approach, under the premise that the ^{13}C content of the nutrient of interest must be different from the isotopic background, it was possible to trace the metabolism of a given metabolite and separate the dietary from the endogenous component in biological samples [21-23].

In this study, we applied the ^{13}C natural abundance approach to compare the $\delta\ ^{13}C$ values of selected maternal and fetal FAs, to infer the placenta transfer from the mother to the fetus. We aimed to determine whether *de-novo* biosynthesis of non-essential FAs and the biosynthesis of long chain polyunsaturated (LCP) FAs *via* chain elongation and desaturation of essential FAs occur in the human fetus and whether these potential processes contribute to the overall fetal FA turnover.

Methods

The study group consisted of 39 mother-fetus pairs. Ten were part of an old study conducted in Holland [21] and 29 were studied in Italy. Twenty-nine out of the 39 mother-fetus pairs were part of previous studies by our group [20, 21].

Eighteen Italian pregnant women were supplemented with 200 mg/day of DHA from pregnancy week 20 until delivery (DHA^+ group), while 21 women did not take any DHA supplementation (DHA^- group).

In the supplement, DHA was in the form of triglyceride oil from algal (N=13) or fish (N=5) origin. Participation in the study was voluntary, and informed consent was obtained from all subjects. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the local ethical committee of Padova Hospital (protocol number 1333P) and of Sophia Children's Hospital, Erasmus University, Rotterdam.

The inclusion criteria were: single pregnancy, aged 18–40 years, uncomplicated pregnancies, and term deliveries.

Blood samples

Cord blood samples were obtained at delivery whereas, maternal venous blood samples were obtained from the antecubital vein within 2 hours from delivery. Blood collected in EDTA tubes was centrifuged within 2 hours of collection and the plasma aliquots were stored, for a maximum of ten years, at − 80°C in tubes containing pyrogallol as an antioxidant.

Sample preparation

Lipids were extracted from 100 µl of plasma samples using a chloroform-methanol mixture with butylated hydroxytoluene as an antioxidant by the Folch method [24]. Lipid class separation and phospholipid isolation were reported previously [25].

Briefly, the lipid extract was resolved in classes by thin-layer chromatography and the phospholipid fraction was hydrolyzed with HCl-methanol. The resulting methyl ester fatty acids were extracted with hexane. The hexane layer was collected in vials for gas chromatographic analysis.

Gas chromatography analysis

Gas chromatography analysis was performed as previously described [20] and FA composition was reported as the percentage of each FA relative to the total phospholipid FAs (mol %).

δ^{13}C of plasma phospholipids FAs

The δ^{13}C value of FA methyl esters from plasma phospholipids was analyzed by using a gas chromatography-combustion interface isotope ratio mass spectrometer (GC-C-IRMS, Delta V Thermo Fisher Scientific, Bremen, Germany) as previously reported [20]. The system was externally calibrated with certified standard mixtures F8–2 for fatty acid methyl ester (even chain fatty acid methyl and ethyl esters from n-C14:0 to n-C20:0), obtained from Arndt Schimmelmann.

Carbon isotopic analysis was performed in triplicate. The values of δ^{13}C were expressed in milliUrey (mUr) [26]. Each mUr is representative of a one per mill (1 in 1000, ‰) change in the δ^{13}C with respect to the Vienna Pee Dee Belemnite (V-PDB) international reference standard:

$$\delta\,13\mathrm{C}\,(\mathrm{mUr}) = \left[\frac{\mathrm{R\ sample}}{\mathrm{R\ standard}} - 1\right]$$

Where "R" is the ratio of the heavy to light isotope in the sample or standard.

Statistical analysis

Data were presented as mean ± standard deviation (SD) or as median and interquartile range (25°th-75°th percentile). Pearson correlation was used to assess the association between maternal and fetal $\delta^{13}C$ of FAs.

Inter-group comparisons were performed using the Mann-Whitney test, while intra-group comparisons were conducted with the Wilcoxon test.

All tests were two-sided, and a p-value <0.05 was considered statistically significant. Statistical analysis was performed using PASW Statistics 18.0 (IBM Corp, Armonk, NY).

Results

The 28 Italian women were recruited in Padua, Italy between 2015 and 2017, and the 11 Dutch women were recruited in Rotterdam, The Netherlands.

Participants' characteristics were: body weight of 63±9 Kg, weight gain during pregnancy of 11±3 Kg, length of gestation 40±1 weeks, and newborn birth weight of 3453±368 g.

The DHA^+ group was composed of 18 Italian women, whereas there were 10 Italian and 11 Dutch women in the DHA^- group.

FA composition and $\delta^{13}C$ value of DHA supplements

Detailed composition of DHA supplement (from algae or fish) has been determined and recently published [20] and is reported in Table 1. The percentage of DHA of the two preparations was not different being 45.7 and 45.8 mol% in fish and algae supplements, respectively (p = 0.80) whereas it was significantly different in the $\delta^{13}C$ DHA value (-25.3±0.2 vs -15.8±0.2, p<0.001).

FA composition of plasma phospholipids

The quantitative data of plasma phospholipids are available for 28 out of 39 mother-fetal dyads. Unfortunately, due to a computer system crash, we lost the chromatogram of the phospholipids FA belonging to the Dutch group. It was not possible to provide information on these samples, as there was not enough plasma left to repeat the analysis.

The phospholipid FAs composition data are reported in Table 2. There was no significant difference in total phospholipid content between the DHA^+ and DHA^- groups in the mother and fetus.

At delivery, DHA content in maternal plasma phospholipids was significantly higher in the DHA^+ group than in the DHA^- group, 4.32 (3.17-5.07) vs 2.29 (2.12-3.11) mol %, p=0.005.

DHA content in cord plasma phospholipids was higher in the DHA^+ group than in the DHA^- group (6.44 (3.60-7.53) vs 3.97 (2.94-4.93); p=0.003). The content of C22:0 and C24:0 was significantly lower in cord plasma phospholipids of the DHA^+ group compared to the DHA^- group.

Significant differences in FA mol% between mother and fetus can be viewed in Table 2. There were significant differences in the following long chain polyunsaturated FA (LC-PUFA): C18:2n-6, C18:3n-3, C20:3n-6, C20:4n-6, C20:5n-3, C22:4n-6, C22:6n-3. We also found significant differences for C12:0, C18:0, C18:1n-9, C18:1n-7, C20:0 and C24:0.

We calculated the difference of the mol% values for each woman-fetal dyad for all the major FAs and compared them to evaluate if the differences were influenced by DHA supplementation. We did not find any significant difference (Table 2). Figure 1 shows a significant correlation of DHA percentage in plasma phospholipids between mothers and their fetuses

Maternal and cord blood plasma phospholipid FAs $\delta^{13}C$

The $\delta^{13}C$ values of PA, OA, C18:1n-9; LA, AA, and DHA isolated from maternal and cord plasma are illustrated in Figure 2. Significant correlations were observed between the $\delta^{13}C$ values of these FAs in the plasma phospholipid fractions of mothers and their fetuses.

The $\delta^{13}C$ value of plasma phospholipid DHA was significantly higher ($p<0.001$) in women supplemented with DHA from algae (-21.9±2.1 mUr) compared to those who did not receive DHA supplementation (-27.6±2.1 mUr), or who were supplemented with DHA from fish oil (-27.1±0.9 mUr). Moreover, the Dutch women had the lowest ^{13}C content (-29.0±1.3 mUr), possibly reflecting their diet.

The differences in FA $\delta^{13}C$ between mothers and fetuses are reported in Figure 3.

The maternal-fetal difference of $\delta^{13}C$ DHA is similar between DHA^+ and DHA^- groups (p=0.254).

DISCUSSION

In this study, we compared the $\delta^{13}C$ values of selected FAs between maternal and cord/fetal plasma phospholipids in uncomplicated pregnancies. We chose to analyze plasma phospholipids because they are rich in DHA. When designing this study, we hoped to demonstrate some differences between the $\delta^{13}C$ values of selected FAs of the woman and her fetus. Contrary to our expectations, we found: (1) a very close match between the $\delta^{13}C$ values of all the major FAs studied in the mother and their fetus and (2) no effect of the DHA

supplementation on the difference in mol% values between maternal and fetal FAs. These are important information that suggests a very efficient equilibration between the maternal and fetal FAs, and a low or undetectable fetal biosynthesis of the major FAs.

In line with other studies [27, 28] we found that DHA^+ women had higher DHA mol% values in their plasma phospholipids than DHA^- women and that the plasma DHA mol% values of the supplemented fetuses had significantly higher DHA mol% values than those belonging to DHA^- group. In line with this notion, Figure 1 shows the correlation between maternal and fetal DHA mol% values.

The $\delta^{13}C$ values of LA and AA were -29.4 ±1.3 mUr and -28.0±1.8 mUr in the mother and -29.2±1.4 mUr and -28.0±1.8 mUr in the fetuses. We can observe that the $\delta^{13}C$ value of LA was nearly identical in the mother and her newborn. This result was expected and was reassuring as LA is an essential FA and no endogenous biosynthesis is expected neither in the mother nor in her fetus. The $\delta^{13}C$ value found in plasma phospholipids likely reflects the ^{13}C of LA content of the maternal diet (not measured in this pilot study), and likely indicates a condition of steady state, as we asked the pregnant women to maintain their diet constant in terms of the sources of fats and oils. In our study, the mean difference in $\delta^{13}C$ between LA from pregnant women (DHA^- group) and their fetuses was -0.2 mUr with maximum and minimum values of 0.8 and -0.8 mUr respectively. From these data, we extrapolated the error of our method (analytical plus biological variance) to be 0.8 mUr based on the maximum difference between maternal and fetal plasma $\delta^{13}C$ LA.

The mean difference of $\delta^{13}C$ values of AA between pregnant women and their fetuses was -0.1 mUr with a range from a maximum of 1.3 mUr to a minimum of -1.6 mUr. The mean differences between $\delta^{13}C$ values of phospholipid LA (precursor) and AA (product) were 1.2 mUr (max 4.2, min -1.1 mUr) in fetuses and 1.3 mUr (maximum 4.9, minimum -1.3 mUr) in mothers. The difference between these values was close to zero. If there were significant endogenous biosynthesis of AA from LA, we would have expected some, consistent $\delta^{13}C$ difference between the precursor and product, which was not observed. Only 10 out of 39 mother-fetus pairs had an AA $\delta^{13}C$ difference greater than the maximum error of our method. These findings suggest that the AA supply to the fetus was far larger than any endogenous biosynthesis if it occurs at all [29]. We cannot exclude, however, that the acetyl molecules incorporated during chain elongation had the same $\delta^{13}C$ of LA. In both fetuses and neonates, the main precursor of lipogenesis is glucose [30]. In Europe, carbohydrates like potatoes and bread/wheat are the main sources of glucose. Both have C3 signature, with low ^{13}C content (-

24<δ^{13}C<-30 mUr). Unfortunately, we did not measure plasma glucose δ^{13}C nor δ^{13}C of the carbohydrates of women's diet. This made it difficult to calculate the contribution, in terms of ^{13}C of the acetyl molecules, derived from glucose, incorporated during chain elongation. It is also possible that our method was not sensitive enough to detect a trivial endogenous biosynthesis of AA as the variation in enrichment was determined by the addition of 2 carbon atoms out of 20, and only in the case that the added carbon atoms were different in C^{13} from those of the parent FA, namely LA. In this scenario, it would be impossible to calculate AA fetal biosynthesis in the absence of an AA supplement with a different δ^{13}C than that of LA. Such a study would be feasible with the use of an AA supplement with a high content of ^{13}C. Obtaining information on AA is important, as AA is the second most represented LCPUFA in the brain [31] and data on its perinatal metabolism are scanty, especially in humans.

The mean difference of δ^{13}C values of DHA between pregnant women and their fetuses was +0.2 mUr in the DHA^+ group, and -0.2 mUr in the DHA^- group. Notably, in the women receiving algal DHA (usually with higher ^{13}C content) the maternal-fetal DHA δ^{13}C difference was below our methodological error for all study dyads, but one pair (-2.9 mUr). These findings strongly suggest a marked dependency of the human fetus from the maternal LCP supply, with no significant fetal biosynthesis as reported in animal studies [29].

Stable isotope studies in preterm infants have demonstrated that premature infants can synthesize both AA and DHA [21, 32, 33] but the synthesis is not sufficient to meet their high nutritional requirements [34]. Information on endogenous biosynthesis in the human fetus, especially in the presence of a likely "abundant" maternal supply, is not available so far.

We were surprised to find very low δ^{13}C differences for PA and OA between mothers and their fetuses. Given that fetal lipogenesis is known to occur, with some lipid synthesis detected in adipose tissue from 10 weeks onwards [16], we anticipated more pronounced differences.

The mean δ^{13}C difference for PA between pregnant women and their fetuses at delivery was -0.1 mUr with a maximum of 2.1 mUr and a minimum of -2.2 mUr. For OA the mean difference was -0.3 mUr with a maximum of 1.6 mU and a minimum of -3.2 mUr. We expected some consistent differences between the mean of the maternal-fetal differences of PA vs OA or at least some random differences in individual patients. We found no statistically significant differences between the maternal-fetal differences of PA and OA compared with LA (p=0.818 and p=0.544, respectively by Wilcoxon test).

Our pilot study could not demonstrate the endogenous fetal biosynthesis of PA and OA so, we conclude that in our subjects this was very low or absent. An alternative hypothesis could be that lipogenesis does occur at the cellular level (mainly in adipose tissue which most studies focused on) with limited exchange with plasma lipids.

This point requires a few additional considerations. If, from one side, it could be hypothesized that the same glucose was used for lipogenesis in the mother and the fetus (well-known dependency of fetal glucose from maternal supply [35] it is also true that lipogenesis in the last trimester of pregnancy is low [4] and most likely the ^{13}C enrichment of plasma phospholipid PA in maternal plasma is from dietary origin. We thought that if fetal PA biosynthesis from glucose were to be more active than in pregnant women, this would have resulted in a more evident maternal-fetal difference.

In addition, in agreement with previous studies, [36, 37] our findings indicate that a daily supplementation of 200 mg of DHA in the second half of pregnancy significantly increases the DHA percentage in maternal plasma phospholipids. Our stable isotope study demonstrated that maternal DHA supplementation (richer in ^{13}C) increased the DHA enrichment of the fetus indicating the transfer and incorporation of DHA from dietary supplement into the fetal plasma phospholipids.

Despite the limited sample size our data shows that 200 mg/day of DHA from algae did not reduce AA levels in both mothers and fetuses.

In conclusion, this pilot study did not show clear evidence of fetal synthesis of major FAs including saturated, unsaturated, the essential LA and LCP FAs AA and DHA.

Our study has some limitations. First, we did not measure plasma palmitate and glucose levels, the main sources of acetyl CoA for maternal and fetal lipogenesis. Second, we asked the pregnant women to maintain a constant diet but we did not ask them to compile a food diary. Third, the limited number of patients makes it impossible to look at differences linked to the type of delivery and fetus gender.

Future studies using our non-invasive method with a larger number of patients may confirm these preliminary findings or provide a deeper understanding of the fetal dependency on placenta FA transport in healthy pregnant women on a normal diet, as well as in cases of nutrition disturbances or maternal diseases.

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Authorship

The authors' contributions were as follows: VP and PC designed research; PS, contributed to study design and to patient recruitment; SV, EC, and GV contributed to patient recruitment; AS, MS, and AC analyzed samples; MS oversaw the statistical analysis. All authors read and approved the final version of the manuscript.

The authors declare no conflict of interest.

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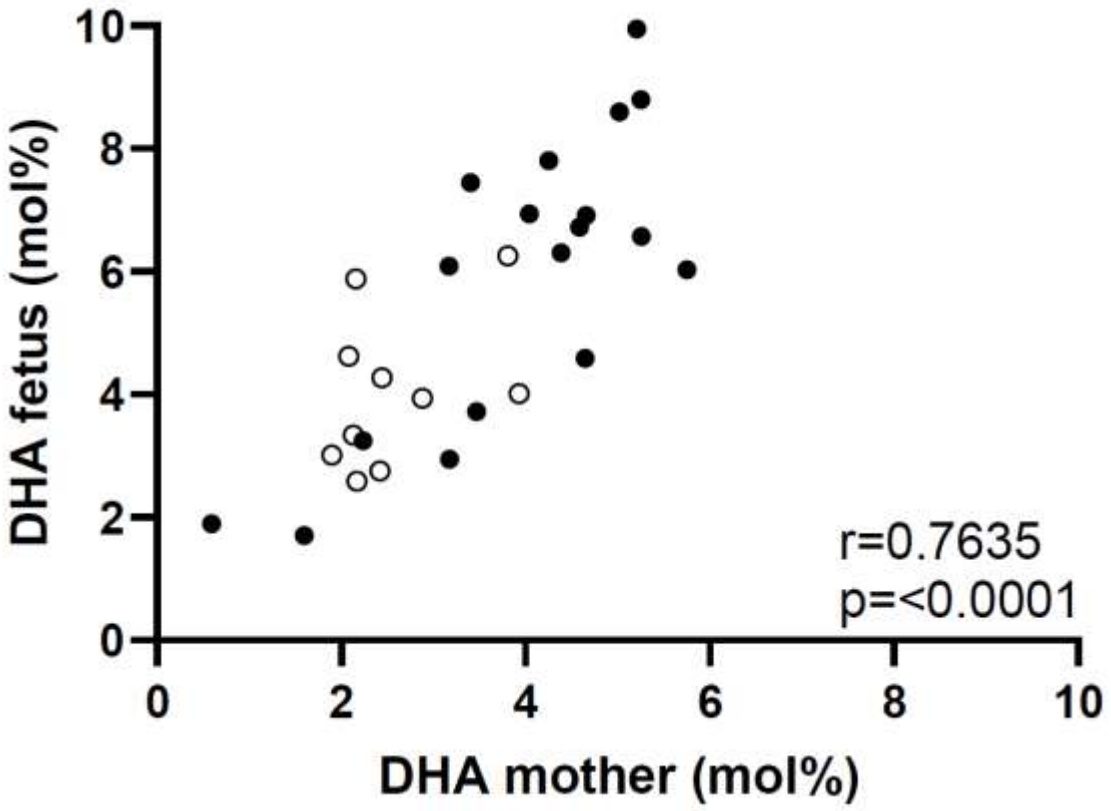

Figure 1. Correlation between maternal and fetal plasma phospholipids' DHA in 28 pregnant women receiving (filled dots) or not receiving (empty dots) DHA supplement

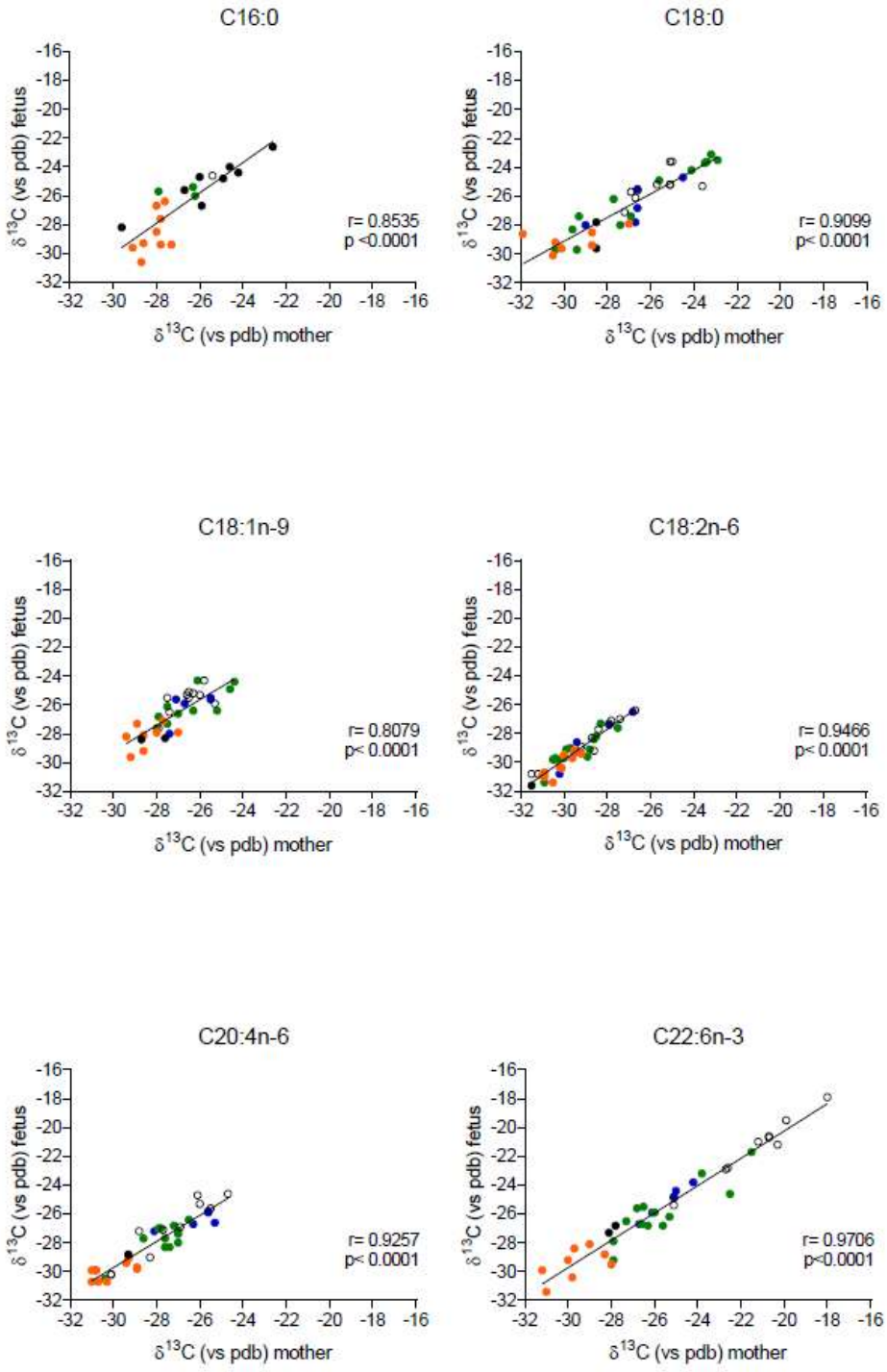

Figure 2. Correlation between the $\delta^{13}C$ of the major plasma phospholipid FAs measured in the mother and the fetus. The orange points are for the Dutch women, the empty points are for the DHA^- Italian Women, and the blue (fish-DHA) and the green (algal-DHA) points are for the DHA^+ Italian women. Correlations were made by the Pearson test.

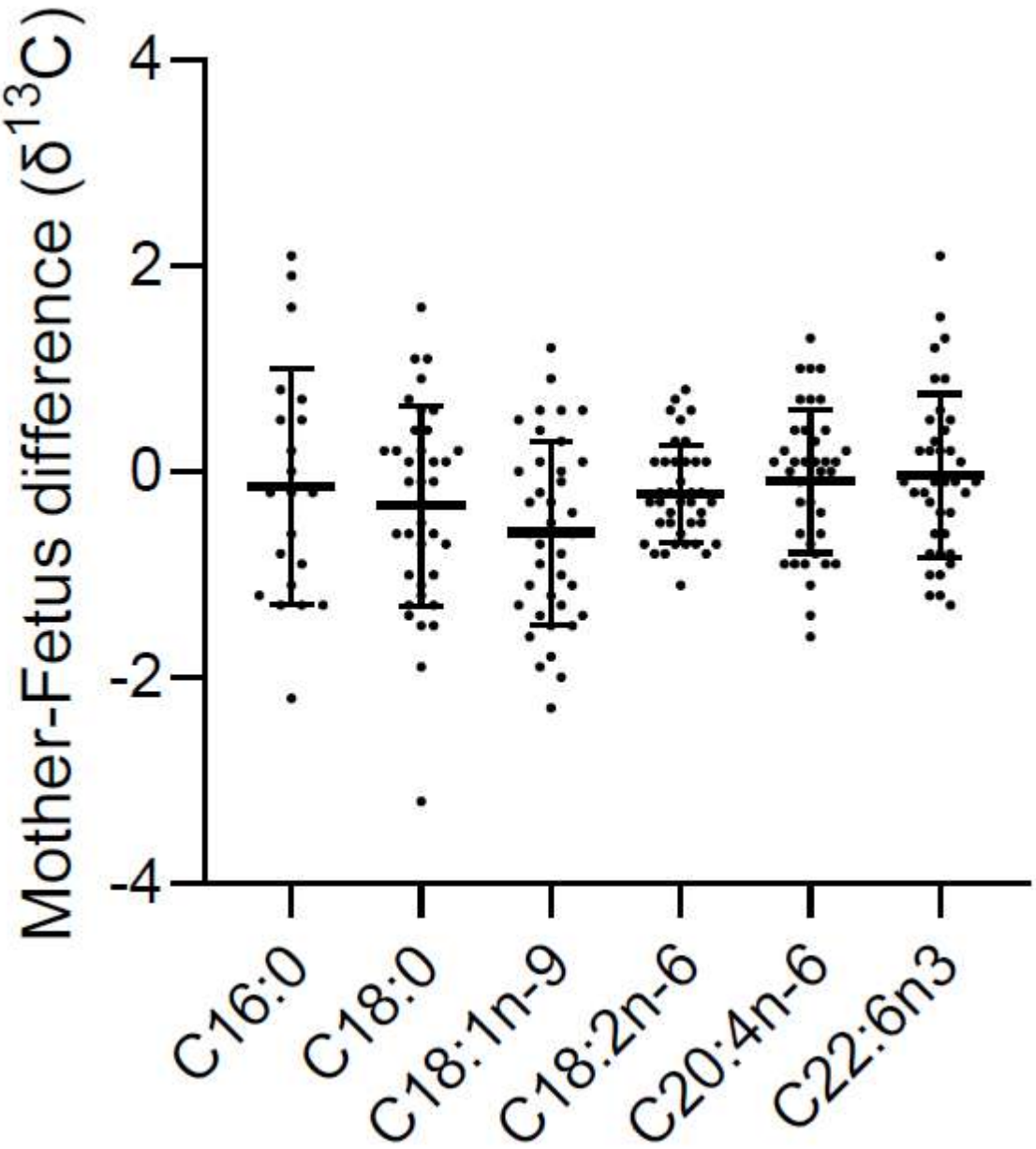

Figure 3. Differences between fetal and maternal $\delta^{13}C$ of major FAs

Table **1** Fatty acid composition and $\delta^{13}C$ of DHA supplements

FA	Fish Group (mol%)		Algae Group (mol%)			Fish Group $\delta^{13}C$ (mUr)		Algae Group $\delta^{13}C$ (mUr)		
	Mean	SD	Mean	SD	p	Mean	SD	Mean	SD	p
8:0	0.94	0.10	0.83	0.12	0.37	-	-	-	-	-
10:0	0.56	0.09	0.45	0.11	0.27	-	-	-	-	-
12:0	-	-	-	-	-	-	-	-	-	-
14:0	3.64	0.04	0.98	0.08	<0.001	-25.0	0.2	-	-	-
16:0	15.56	0.15	22.34	0.05	0.007	-24.0	0.2	-9.3	0.2	<0.001
18:0	4.50	0.09	2.13	0.10	<0.001	-23.7	0.2	-	-	-
20:0	0.59	0.13	0.29	0.02	0.007	-	-	-	-	-
16:1n-7	3.44	0.05	0.43	0.04	<0.001	-24.0	0.2	-	-	-
18:1n-9	9.76	0.09	21.64	0.21	<0.001	-24.0	0.3	-27.3	0.1	<0.001
18:2n-6	1.46	0.08	2.70	0.09	<0.001	-	-	-27.9	0.1	-
18:3n-6	0.65	0.09	-	-	-	-	-	-	-	-
18:3n-3	-	-	-	-	-	-	-	-	-	-
20:4n-6	3.01	0.13	0.65	0.03	<0.001	-26.3	0.2	-	-	-
20:5n-3	7.53	0.21	-	-	-	-25.1	0.3	-	-	-
22:5n-3	1.97	0.01	-	-	-	-	-	-	-	-
22:6n-3	45.75	0.07	45.82	0.32	0.80	-25.3	0.2	-15.8	0.2	<0.001

Values are means ±SD, N= six different batches

Fatty acid composition is expressed as the percentage of fatty acid in total fatty acids. $\delta^{13}C$, carbon-13 isotopic abundance

p determined by independent t-test

Table from Simonato M, Visentin S, Verlato G, et al. DHA turnover in pregnant women using the natural abundance variation of ^{13}C: a pilot study. British Journal of Nutrition. 2023;129(2):240-246. doi:10.1017/S000711452200108

Table 2

FA (mol %)	**W. DHA$^+$**	**W. DHA$^-$**	P[a]	**F. DHA$^+$**	**F. DHA$^-$**	P[a]	P[b] DHA+	P[b] DHA-	**(F.-M.) DHA$^+$**	**(F.-M.) DHA$^-$**	P[a]
12:0	0.09 (0.04;0.12)	0.09 (0.05;0.13)	0.832	0.27 (0.10;0.44)	0.25 (0.15;0.33)	0.555	0.001	0.007	016 (0.01;0.34)	0.15 (0.09;0.19)	0.654
14:0	0.68 (0.49;0.75)	0.62 (0.55;0.74)	0.689	0.69 (0.60;0.90)	0.67 (0.49;0.79)	0.494	0.145	0.386	0.13 (-0.16;0.31)	0.08 (-0.12;0.18)	0.621
16:0	37.56 (35.41;38.30)	38.27 (36.10;39.47)	0.654	37.05 (35.00;38.78)	36.99 (34.96;37.79)	0.689	0.420	0.508	-0.97 (-3.13;2.28)	-2.04 (-2.80;2.35)	0.869
16:1n-7	0.98 (0.74;1.29)	0.86 (0.52;1.27)	0.555	1.06 (0.78;1.65)	0.96 (0.75;1.19)	0.308	0.122	0.646	0.23 (-0.23;0.76)	0.16 (-0.34;0.37)	0.524
18:0	8.51 (7.70;9.56)	8.94 (7.59;10.47)	0.494	12.15 (10.91;15.40)	14.26 (11.64;15.98)	0.577	0.000	0.013	4.49 (1.99;7.30)	5.51 (3.45;6.15)	0.981
18:1-n9	9.90 (8.24;10.50)	9.49 (7.55;11.48)	0.944	6.53 (5.79;7.34)	6.34 (5.89;7.50)	1.000	0.000	0.003	-2.84 (-4.31;-2.03)	-2.98 (-4.46;-1.10)	0.981
18:1-n7	1.38 (1.24;1.65)	1.36 (1.14;1.48)	0.322	2.33 (1.97;2.62)	2.34 (2.06;2.62)	0.654	0.002	0.005	0.78 (0.59;1.31)	1.07 (0.91;1.35)	0.121
18:2-n6	19.19 (16.64;20.66)	20.99 (19.02;22.62)	0.057	6.36 (5.87;7.83)	7.76 (6.50;9.22)	0.045	0.000	0.005	-12.43 (-13.46;-	-13.23 (-15.13;-	0.226

									10.65)	11.05)	
18:3-n6	0.11 (0.06;0.13)	0.10 (0.08;0.13)	1.000	0.00 (0.00;0.15)	0.11 (0.09;0.14)	0.226	0.278	0.575	-0.04 (-0.10;0.05)	0.02 (-0.03;0.04)	0.245
18:3-n3	0.14 (0.12;0.18)	0.13 (0.09;0.16)	0.621	0.00 (0.00;0.00)	0.00 (0.00;0.00)	0.524	0.000	0.005	-0.14 (-0.18;-0.11)	-0.14 (-0.17;-0.08)	0.654
20:0	0.37 (0.34;0.51)	0.38 (0.31;0.43)	0.832	0.59 (0.53;0.70)	0.75 (0.54;0.94)	0.160	0.000	0.005	0.23 (0.06;0.33)	0.42 (0.16;0.49)	0.089
20:2-n6	0.28 (0.23;0.35)	0.31 (0.23;0.39)	0.524	0.25 (0.20;0.40)	0.32 (0.24;0.36)	0.464	0.678	0.646	0.01 (-0.11;0.18)	-0.02 (-0.07;0.05)	0.832
20:3-n6	3.12 (2.74;3.58)	2.90 (2.42;3.74)	0.689	4.02 (3.49;5.21)	4.19 (4.01;4.56)	0.944	0.010	0.074	1.23 (0.02;2.03)	1.17 (0.50;1.97)	0.944
20:4n-6	8.53 (7.13;10.35)	8.53 (7.73;9.33)	1.000	16.07 (13.88;17.62)	15.20 (14.42;17.59)	0.832	0.000	0.005	7.20 (5.73;9.25)	7.36 (6.65;8.93)	0.796
20:5n-3	0.31 (0.19;0.50)	0.27 (0.16;0.38)	0.621	0.20 (0.00;0.26)	0.17 (0.12;0.26)	1.000	0.000	0.005	-0.16 (-0.27;-0.08)	-0.09 (-0.15;-0.05)	0.245
22:0	0.78 (0.59;0.90)	0.84 (0.68;1.15)	0.332	0.69 (0.60;1.00)	1.12 (0.75;1.34)	0.016	0.868	0.258	0.03 (-0.24;0.25)	0.25 (-0.19;0.60)	0.265
22:4n-6	0.29 (0.16;0.33)	0.28 (0.25;0.42)	0.226	0.63 (0.46;0.94)	0.54 (0.50;0.62)	0.408	0.000	0.007	0.37 (0.23;0.62)	0.28 (0.18;0.31)	0.109

22:5n-3	0.34 (0.27;0.42)	0.30 (0.26;0.41)	0.724	0.37 (0.15;0.52)	0.36 (0.23;0.42)	0.724	0.983	0.721	0.05 (-0.17;0.10)	0.01 (-0.06;0.08)	0.906
22:6n-3	4.32 (3.17;5.07)	2.29 (2.12;3.11)	0.005	6.44 (3.60;7.53)	3.97 (2.94;4.93)	0.003	0.001	0.005	2.03 (0.27;3.55)	1.16 (0.40;2.47)	0.524
24:0	0.43 (0.26;0.56)	0.52 (0.47;0.60)	0.097	0.56 (0.40;0.76)	0.81 (0.71;0.88)	0.040	0.030	0.022	0.23 (-0.06;0.75)	0.30 (0.05;0.39)	0.981
24:1n-9	1.62 (1.17;1.86)	1.22 (1.03;1.29)	0.121	1.11 (0.81;1.74)	1.23 (1.09;1.84)	0.332	0.088	0.386	-0.31 (-0.63;0.44)	0.12 (-0.15;0.33)	0.208
PL (mg/dL)	208.1 (166.3;208.1)	185.5 (152.3;242.5)	0.906	54.2 (45.7;60.5)	62.2 (50.8;73.6)	0.121	0.000	0.005	-53.88 (-60.38;-45.50)	-62.05 (-73.48;-50.62)	0.133

Plasma phospholipid fatty acid composition (mol %) in DHA^+ and DHA^- pregnant women and their fetuses

W. = Women

F. = Fetus

(F.-M-) = Fetus-Mother difference in mol%

[a] Inter group comparison made by Mann Whitney test

b Intra group comparison made by Wilcoxon test

FA=fatty acid

DHA^+: group with 200 mg/day of DHA supplementation

DHA^-: group without DHA supplementation