Biochemical markers of n-3 long chain polyunsaturated fatty acid intake during pregnancy(*)

R. Matorras[1], L. Perteagudo[2], P. Sanjurjo[2]

Departments of Obstetrics and Gynecology[1] and of Pediatrics[2]. Hospital from Cruces Pais Vasco University, Baracaldo, Vizcaya (Spain)

Summary

Background: To assess the relationship between the mothers' intake of n-3 long chain polyunsaturated fatty acids (LC PUFA) during pregnancy and their levels in plasma and tissue.

Methods: 162 mothers were studied during labor. Three groups were differentiated according to the n3 LC PUFA intake assessed by means of a dietetic interview: superior intake (SIG) (>0.721 g/day), medium intake (MIG) (from 0.382 to 0.721 g/day) and inferior intake (IIG) (<0.381 g/day). Fatty acids (FA) were studied by capillary chromatography in plasma and in erythrocyte phospholipids.

Results: The fatty acids (FA), expressed in absolute values, did not show any significant differences among the aforementioned groups. However, three were some trends which were confirmed when the FA were expressed in percentages. Thus, higher levels of docosahexaenoic acid (DHA) were found in SIG both in plasma and in the erythrocyte membrane, when expressed in percentages. Eicosapentaenoic acid (EPA) was also higher in the SIG in the erythrocyte membrane, whereas in plasma the differences were of marginal significance. On the other hand, arachidonic and linoleic acids had lower values in the SIG in erythrocytes. The theoretical optimal intake of n-3 LC PUFA corresponded to a plasma concentration of 117.9 ± 45.9 mcg/ml n-3 LC PUFA or 2.54% of the total fatty content (2.29% of DHA). The corresponding cut-offs in erythrocyte membranes were 7.54% of total lipids (5.59% of DHA).

Conclusion: The best markers of n-3 LC PUFA intake were DHA for plasma and DHA and EPA for erythroctye phospholipids, all of them expressed in proportions of total FA. The arachidonic and linoleic acids (in percentages) in erythrocyte phospholipids were also good markers of n-3 intake. This probably reflects the metabolic competition between both PUFA families.

Key words: Polyunsaturated fatty acids; Pregnancy; Intake; Recommendations.

Abbreviations: Long chain polyunsaturated fatty acids (LC PUFA); Superior intake groups (SIG); Medium intake group (MIG); Inferior intake group (IIG); Docosahexaenoic acid (DHA); Eicosapentaenoic acid (EPA); Arachidonic acid (AA).

Introduction

The precursors of n-3 and n-6 polyunsaturated fatty acids (PUFA), linolenic and linoleic acids, are essential nutrients. However, their relative intake must be balanced, since they share the enzymes for the synthesis of their long chain (LC) metabolites [1]. These LC derivatives can also come from the diet, but their sources are not widely available. Meat represents the main source of arachidonic acid (AA) (n-6 PUFA), whereas fatty fish and shellfish those of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (both n-3 PUFA) [2]. There are also small amounts of n-3 PUFA in animal offal and eggs.

LC PUFA play an important role in the membrane structure and in the synthesis of the ecoisanoids [3]. During pregnancy they play an important role in fetal development (especially brain development) as well as in a number of conditoins [4].

Although some specific dietetic recommendations regarding the intake of n-3 and n-6 PUFA families during pregnancy have been made [5], there are a number of unknown aspects regarding PUFA in pregnancy. Most recommendations have been based on animal experimentation or on non-pregnant women.

The purpose of the study was to assess the relationship between the mother's intake of PUFA during pregnancy and the levels of PUFA in plasma and tissue, and especially between the theoretical dietetic requirements and PUFA levels.

Materials and Methods

The population under study consisted of 162 women in labor with an at-term vaginal delivery of a live newborn infant. Cases with remarkable pathologies in the mother or the newborn infant were excluded. Among other maternal conditions, hypertension, diabetes, obesity and undernourishement were exclusion criteria. Written informed consent was obtained. A nutritional inquiry was carried out. The first part of the inquiry consisted of the determination of the monthly amount of fish and shellfish consumption, the main sources of n-3 LC PUFA. The frequency and the amount of each of them were investigated in order to established the mean daily edible portion. In each patient the mean total amount of fish (g/day) as well as the mean amount of EPA and DHA were determined by means of Moreiras *et al.* food composition tables [6] for the fish. In the second part of the inquiry the women were asked to remember the whole intake of 3 typical days in each pregnancy trimester. The intake of other sources of PUFA besides fish was thoroughly investigated, representing < 30% of the total amount in our population [7]. Fish intake proved to have a normal distribution.

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From the sum of both interviews we obtained the mean daily intake of n-3 LC PUFA. Mean daily intake of saturated, monounsaturated and n-3 and n-6 PUFA was also calculated. In cases with different dietary habits during pregnancy, each trimester of pregnancy was specifically investigated, averaging the results.

The n-3 LC PUFA intake (EPA and DHA) constituted the criteria in order to arbitrarily divide the women into three groups: the inferior intake group (IIG) (≤ 33 percentile, corresponding to an intake of ≥ 0.721 g/day of n-3 LC PUFA). The theoretical optimal n-3 LC PUFA intake was defined following RDA dietetic recommendations, corresponding to 300-400 mg/day [8].

Maternal fasting blood samples were obtained by venipuncture (5 cc) during the second stage of labor. Women whose last intake was <6 hours were excluded from the study. Samples were studied by means of capillary chromatography following the Lepage and Roy method [9] for plasma transesterification as well as for the phospholipidic fraction of the blood red cells, which was previously separated from the lipidic extract by thin-layer chromatography. Methyl esters were separated in a gas chromatograph Perkin Elmer 8500 (Buckinghamshire, England), by means of capillary columns using a 30 m SP 2330 fused silica capillary column, 0.25 mm ID, 0.20 microm film thickness (Supelco Inc, Bellefonte, PA) and equipped with a flame ionization detector.

The following 11 plasma FA were systematically detected: myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1 n -9), linoleic (18:2 n-6), linolenic (18:3 n-3), dihommogammalinolenic (20:3 n-6), arachidonic (AA) (20:4 n-6), EPA (20:5 n-3) and DHA (22:6 n-3). In the phospholipids of the blood red cells we also detected docosatetraenoic (22:4 n-6) and docosapentaenoic acids (22:5 n-6 and 20:5 n-3). Identification was performed by comparison to commercial standards (Supelco Inc and Laroden Fine Chemicals, Malmö, Sweden). As internal standard C: 17:0 was used. The values for each fatty acid were obtained by electronic integration. Results were expressed as the percentual value of the total fatty acids and as a concentration (mcg/ml). The statistical analysis was performed by means of oneway analysis of variance (ANOVA) with the Scheffé test. The results concerning the fatty acid status of the newborn infants from the aforementioned mothers and the lipid pattern of these mothers and their infants will be reported in two forthcoming articles [10, 11].

Results

Characteristics of the three populations

Mothers from the 3 populations were similar regarding demographic data (Table 1), management of labor and socioeconomic status.

Table 1. — *Demographic data of the patients.*

	Superior intake	Medium intake	Inferior intake
Mother age (years)	29.0±4.4	28.4±4.4	27.7±4.5
Primiparity (%)	49	40	46
Smoking (%)	35.6	25.8	31.6
Gestational age (weeks)	39.4±1.1	39.2±1.76	39.3±1.82
Newborn weight (g)	3426±396	3311±475	3347±413
Body weight (Kg)	60.7±8	58±8.9	58.7±9.5
Weight gain during pregnancy (Kg)	11.3±3.7	10.6±3.6	11.4±4
Energy intake (Kcal/day)	2259±475	2306±403	2287±489
n-6 PUFA intake (g/day)	12.65±7.57	16.77±8.88	15.51±7.55

No significant differences

Plasma fatty acids

Absolute values (Table 2):

Table 2. — *Absolute concentrations of plasma fatty acids in mothers regarding long chain n-3 PUFA intake.*

	Superior intake (n=54) mean (mcg/ml)±SD	Medium intake (n=54) mean (mcg/ml)±SD	Inferior intake (n=54) mean (mcg/ml)±SD
Mirystic acid	40.08± 18.94	38.90± 14.66	40.09± 15.26
Palmitic acid	1078.24±242.82	1036.14±226.47	1127.52±274.40
Palmitoleic acid	85.73± 38.01	87.75± 36.96	92.63± 50.35
Stearic acid	274.80± 47.21	267.25± 51.43	269.48± 54.63
Oleic acid	996.87±291.88	928.26±280.60	1030.82±435.32
Linoleic acid	1184.02±328.68	1192.19±247.77	1204.97±279.71
Linolenic acid	12.29± 6.27	14.38± 6.0	13.12± 7.64
Dihommo-gamma-linolenic acid	99.71± 43.23	105.70± 44.15	103.83± 57.62
Arachidonic acid	235.25± 57.38	224.81± 54.79	236.04± 57.59
Eicosapentaenoic acid	17.32± 11.63	17.52± 13.99	13.15± 9.30*
Docosahexaenoic acid	114.42± 29.70	107.49±30.04	102.16± 33.19*

*: p = 0.09.

There was a trend to lower values of DHA and EPA in the IIG, with p values of marginal significance (p=0.09, F=2.47 and 2.40). The levels of the remaining fatty acids were similar in the different groups.

Among the mothers (n=25) who fulfilled the RDA dietetic recommendations (300-400 mg/day of n-3 LC PUFA), the plasma concentration of n-3 LC PUFA was 117.90±45.93 mcg/ml (12.71±8.69 mcg/ml of EPA and 105.19±38.88 mcg/ml of DHA).

The n-6 PUFA intake of mothers with an intake of 300-400 mg/day of n-3 LC PUFA (12.45 g ± 7.59), was similar to those with < 300 mg/day (15.11 g ± 8.25) and to those with > 400 mg/day (12.88 g ± 7.45).

b) Percentual values (Table 3):

Table 3. — *Proportions of plasma fatty acids in mothers regarding long chain n-3 PUFA intake.*

	Superior intake (n=54) mean (%)±SD	Medium intake (n=54) mean (%)±SD	Inferior intake (n=54) mean (%)±SD
Mirystic acid	0.86±0.34	0.84±0.25	0.84±0.24
Palmitic acid	25.25±1.75	24.76±1.82	25.95±2.86 *
Palmitoleic acid	1.99±0.82	2.05±0.68	2.05±0.86
Stearic acid	6.64±1.08	6.58±0.89	6.38±1.03
Oleic acid	24.68±3.78	23.63±3.80	24.65±4.88
Linoleic acid	29.41±4.62	31.06±4.56	29.49±4.73
Linolenic acid	0.31±0.19	0.39±0.20	0.30±0.12 #
Dihommo-gamma-linolenic acid	1.59±0.43	1.64±0.39	1.59±0.45
Arachidonic acid	6.17±1.49	5.97±1.05	5.98±1.12
Eicosapentaenoic acid	0.38±0.28	0.40±0.33	0.28±0.22**
Docosahexaenoic acid	2.59±0.55	2.46±0.59	2.26±0.52 ##

* : p<0.05; F=3.89 (IIG vs MIG, F=3.84, p<0.05)
#: p<0.05; F=3.94 (IIG vs MIG, F=3.13, p<0.05) (MIG vs IIG, F=2.82, p=0.06)
**: p=0.07
##: p<0.01; F=5.27 (SIG vs IIG, F=5.19, p<0.01).

In the MIG the percentual values of palmitic acid were lower than in the IIG. Regarding PUFA, in the MIG the percentual values of linolenic acid were increased in regard to the IIG ($p<0.05$, $F=3.13$), the differences with the SIG being of marginal significance ($p=0.06$, $F=2.82$). There were lower values of DHA in the IIG than in the SIG ($p<0.01$, $F=5.19$). There was a trend to lower values of EPA in the IIG ($p=0.07$, $F=2.67$). Mothers with 300-400 mg/day of n-3 LC PUFA intake, had 2.54±0.69% of those fatty acids in plasma (0.25±0.15% of EPA and 2.29±0.59% of DHA).

Fatty acids in phospholipids of red blood cells

Absolute values (Table 4):

Table 4. — *Absolute concentrations of fatty acids in the erythrocyte membrane in mothers regarding n-3 long chain PUFA intake.*

	Superior intake (n=54) mean (mcg/ml)±SD	Medium intake (n=54) mean (mcg/ml)±SD	Inferior intake (n=54) mean (mcg/ml)±SD
Mirystic acid	3.64± 1.71	4.23± 1.78	3.51± 0.23 *
Palmitic acid	150.62±64.43	159.96±57.47	150.10±67.69
Palmitoleic acid	3.52± 3.80	4.60± 3.83	3.19± 3.30
Stearic acid	60.59±31.00	64.32±30.26	61.32±30.90
Oleic acid	91.49±39.66	101.19±37.14	91.07±47.14
Linoleic acid	59.97±29.06	70.19±25.36	62.25±28.78
Linolenic acid	0.88± 1.22	1.27± 1.25	0.79± 0.91 *
Dihommo-gamma-linolenic acid	15.79± 9.68	18.01±10.27	15.59±11.03
Arachidonic acid	62.47±36.79	67.22±32.44	66.85±38.31
Eicosapentaenoic acid	3.18± 2.20	3.09± 1.8	2.50± 1.99
Docosahexaenoic acid	16.92±11.09	23.08±15.19	18.63±14.37 #
Docosapentaenoic n-6 acid	6.62± 5.62	7.74± 6.19	6.80± 5.11
Docosapentaenoic n-3 acid	7.53± 4.33	8.34± 3.88	7.75± 5.21
Docosahexaenoic acid	35.26±17.70	36.74±17.47	33.50±21.55

a: p = 0.08.
#: p=0.07.

There was a trend of marginal significance to higher levels in the MIG of the following fatty acids: mirystic acid ($p=0.08$, $F=2.51$), linolenic acid ($p=0.08$, $F=2.52$ and docosatetraenoic n-6 ($p=0.07$, $F=2.64$).

Among mothers strictly fulfilling RDA recommendations, n-3 LC PUFA concentration was 40.51±22.72 mcg/ml (2.44±1.71 mcg/ml of EPA, 8.10±6.23 mcg/ml of docosapentaenoic n-3 and 29.97±16.13 mcg/ml of DHA).

Percentual values (Table 5):

Concerning n-6 PUFA, the SIG had lower values of linoleic acid than the MIG ($p<0.05$, $F=3.74$). The SIG also had lower values of AA than IIG ($p<0.05$, $F=0.37$). There was a trend of marginal significance to lower values of docosatetraenoic acid ($p=0.09$, $F=2.47$).

Regarding n-3 PUFA, the levels of EPA and of DHA were significantly increased in the SIG compared with the IIG ($p<0.01$, $F=5.28$ and $p<0.05$, $F=3.88$). There was a trend of marginal significance to lower values of linolenic acid in the IIG ($p=0.07$, $F=2.60$).

Table 5. — *Proportions of fatty acids (% ± SD) in the erythrocyte membranes of mothers regarding long chain n-3 PUFA intake.*

	Superior intake (n=54) mean (%)±SD	Medium intake (n=54) mean (%)±SD	Inferior intake (n=54) mean (%)±SD
Mirystic acid	0.68±0.30	0.65±0.24	0.69±0.35
Palmitic acid	28.93±3.35	28.22±3.50	28.91±3.85 *
Palmitoleic acid	0.68±0.70	0.71±0.49	0.57±0.55
Stearic acid	11.79±2.21	11.20±1.99	12.24±3.03
Oleic acid	18.48±2.51	18.61±2.51	18.00±2.67
Linoleic acid	11.91±2.08	13.07±2.45	12.62±2.13 *
Linolenic acid	0.18±0.24	0.23±0.21	0.14±0.11 #
Dihommo-gamma-linolenic acid	1.93±0.70	1.96±0.59	1.84±0.46
Arachidonic acid	12.14±1.88	12.42±1.64	13.14±2.21 +
Eicosapentaenoic acid	0.55±0.29	0.50±0.28	0.40±0.18 **
Docosatetraenoic acid	3.56±0.76	3.92±0.90	3.84±0.82 ##
Docosatetraenoic n-6 acid	1.29±0.72	1.35±0.72	1.24±0.53
Docosatetraenoic n-3 acid	1.51±0.44	1.56±0.46	1.45±0.45
Docosahexaenoic acid	6.02±1.19	5.61±1.01	5.48±0.97 ++

*: $p<0.05$; $F=3.74$ (MIG vs SIG, $p<0.05$, $F=3.65$)
#: $p<0.07$
+: $p<0.05$, $F=3.76$ (IIG vs SIG, $p<0.05$, $F=3.54$)
**: $p<0.01$, $F=3.76$ (IIG vs SIG, $p<0.01$, $F=5.18$)
##: $p=0.09$
++: $p<0.05$, $F=3.88$ (IIG vs SIG, $p<0.05$, $F=3.54$)

Among mothers with an intake of 300-400 mg/day of n-3 LC PUFA, those FA represented the 7.54±1.29% of the total fatty content of the erythrocyte membrane (0.42±0.17% of EPA, 1.53±0.44% of docosapentaenoic acid and 5.59±1.02% of DHA).

Discussion

PUFA are receiving greater attention during pregnancy because of their importance in a number of physiological functions, especially brain and vessel development [12]. They have also been linked with a number of pathological condition [13, 14].

It should be remembered that some of them, such as linoleic and linolenic acids, are essential nutrients[15] and that their LC derivates have a relatively limited source [16]. Recently some recommendations for n-3 and n-6 fatty acid intake during pregnancy have been given [5]. However, the recommendation for each single fatty acid has not been specified. The optimal intake of linolenic acid has been estimated at 800-1100 mg/day [8], but without taking into account age group, sex or pregnancy. The difficulty in accurately assessing the intake of a number of nutrients is well-known, especially from a practical point of view. For this reason there is much interest in objective biochemical parameters that reflect

dietary intake. From our combined biochemical/dietetic study some approximate biochemical references can be obtained. The fatty acid content of plasma reflects the diet of the preceding weeks, and that of erythrocyte membranes reflects that of the preceding 2-3 months [17]. Regarding the methodology for expressing FA values, there is some discrepancy. While concentrations reflect the intake with more accuracy, presumably percentage values reflect FA metabolism with more accuracy. Thus our results are expressed in both forms. We calculated the mean levels of n-3 LC PUFA of the group which had the optimal intake of n-3 LC PUFA following RDA [8]. By subtracting two standard deviations from this mean value, we obtained a preliminary cut-off, under which one could expect a deficient n-3 LC PUFA intake. This corresponds to a plasma concentration of n-3 LC PUFA of 26.04 mcg/ml or 1.16% of total fatty content (1.11% of DHA). The corresponding cut-offs in erythrocyte membrane were of 4.96% of total lipids (3.55% of DHA).

Our study is limited to some extent by being based on the RDA dietetic recommendations, which do not differentiate regarding age, sex or pregnancy [8]. Consequently a certain margin of inaccuracy cannot be discounted and which should be rectified in the future if the aforementioned dietetic recommendations are redefined.

When our results were expressed in absolute values, both in plasma and in erythrocyte phospholipids, there were no significant differences among the three intake groups. However, there were some trends which were confirmed when the FA were expressed in percentages. Thus, the FA expressed in percentages of total FA are more accurate markers of n-3 LC PUFA intake.

We found higher levels of DHA in SIG both in plasma and in the erythrocyte membrane, when FA were expressed in percentages. EPA was also higher in the SIG in the erythrocyte phospholipids, whereas in plasma the differences were of marginal significance. These results are consistent with the increased intake of those FA in the SIG. This is in agreement with the increased levels of DHA and EPA among the pregnant women with high intake of fatty fish – other n-3 PUFA sources not being analysed – reported in plasma [16] as well as in erythrocyte phospholipids [17].

On the other hand, AA and linoleic acids (both n-6 PUFA) had lower values in the SIG in erythrocytes, whereas docosatetraenoic acid (also n-6 PUFA) showed a trend to lower values. This probably reflects a metabolic competition between both PUFA families [1].

It is concluded that the best marker of n-3 LC PUFA intake, is the percentage of DHA, both in plasma and in erythrocyte phospholipds, and the next best marker is the percentage of EPA, especially in erythrocyte phospholipids.

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Address reprint requests to:
Dr. ROBERTO MATORRAS
Maria Diaz De Haro, 7
48013 Bilbao (Spain)