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Docosahexaenoic Acid in Breast Milk Reflects Maternal Fish Intake in Iranian Mothers

Beheshteh Olang[1,2,3,4]*, Majid Hajifaraji[2], Mohamed Atiya Ali[1,3], Sophie Hellstrand[1], Mohammad Palesh[1,4], Ebrahim Azadnyia[2], Zinat Kamali[2], Birgitta Strandvik[1], Agneta Yngve[1,3]

[1]Unit for Public Health Nutrition, Department of Biosciences and Nutrition, Karolinska Institutet, Stockholm, Sweden; [2]National Nutrition and Food Technology Research Institute, Faculty of Nutrition & Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [3]Department of Health, Nutrition and Management, Oslo and Akershus University College of Applied Sciences, Oslo, Norway; [4]Kermanshah University of Medical Sciences, Kermanshah, Iran.
Email: *Beheshteh.olang@ki.se

ABSTRACT

To estimate essential fatty acid (FA) and long-chain polyunsaturated fatty acid (LCPUFA) concentrations in early breast milk (BM) in relation to habitual fish intake. BM was collected within 72-hours after delivery from consecutively included mothers, 60 in Guilan (coastal) and 60 in Kermanshah (inland) provinces. Mothers were interviewed to complete a food frequency questionnaire. The FA composition was measured with gas chromatography. Mothers in the coastal area had higher intake of fish/seafood. Consumption of saturated fat was higher in Kermanshah and olive intake was higher in Guilan. High fish/seafood intake was associated with higher docosahexaenoic acid (DHA) and lower arachidonic acid (AA)/DHA ratio in BM. There were no differences in linoleic and α-linolenic acid concentrations in BM between the provinces. N-3 FA and DHA concentration were significantly higher in Guilan than Kermanshah, but total n − 6 FAs and AA did not differ and were high in both provinces. The ratios of total (n − 6)/(n − 3) and AA/DHA in BM of mothers from Guilan were significantly lower than those in Kermanshah. The LCPUFA status in BM in two Iranian provinces was generally good and DHA was higher and the AA/DHA was significantly lower in mothers with high fish intake.

Keywords: Essential Fatty Acids; Docosahexaenoic Acid; Arachidonic Acid; Linoleic Acid; Alpha-Linolenic Acid

1. Introduction

In the last 20 years, several studies have examined the maternal intake of fish, seafood and omega (n)-3 long-chain polyunsaturated fatty acids (LCPUFA) during pregnancy and lactation in relation to the impact on infant's health outcome [1,2]. Maternal health during pregnancy as well as fetal and child health have the potential to be improved by LCPUFA [3,4]. The requirement of LCPUFA for normal development is especially high during this period of rapid growth of the foetus and infant and also for long term health of the child.

Maternal stores of LCPUFA contribute to supply the foetus in the last trimester in order to meet higher requirements of the foetus during this stage [5,6]. The efficient placental transfer is reflected in lower concentrations of DHA and AA in the mother's plasma as pregnancy progresses [5,7].

The average concentration of arachidonic acid (AA; $20:4n − 6$) in breast milk (BM) is around 0.45% and that

of docosahexaenoic acid (DHA; $22:6n − 3$) around 0.32% of total fatty acids (FA) [8]. The concentrations are modulated by the diet and especially DHA in BM reflects the intake of fish, seafood and fish oils, because the marine food chain is based on ocean phytoplankton, which synthesize DHA [9]. Eicosapentaenoic acid ($20:5n − 3$, EPA) is also obtained from fish but can be synthesized from $α$-linolenic acid (ALA; $C18:3n − 3$) and thereby reflect also the intake of vegetables [10]. The conversion of linoleic acid (LA; $C18:2n − 6$) to AA is more efficient than the transformation to LCPUFA of the n − 3 series [11]. The lower conversion of ALA to LCPUFA might reflect the high levels of n − 6 FA in modern diet since the transformations of LA and ALA are dependent on competing enzymes. The recommended $(n − 6)/(n − 3)$ ratio in the diet is 4:1 but is in many countries today it is much more than 10:1 [12].

The amount of DHA in cord blood and breast milk is correlated to the level of DHA in maternal blood [13]. Maternal supplementation of fish or cod liver oil during pregnancy has been shown to affect the cognitive func-

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*Corresponding author.

tion of the infants [14]. A fast increase of DHA in breast milk was seen during supplementation of fish oil as well as a fast decrease after the supplementation period [9]. It has been suggested that pregnant and lactating women should have an average daily intake of at least 200 mg DHA [15], which can be reached by eating fish and seafood, including fatty fish, approximately twice a week. Supplementation of DHA during pregnancy was associated with lower AA concentrations in umbilical arterial walls [16] and several studies has indicated that also AA is important for the infant's development [17]. Low fish consumption is one of the nutritional problems in Iran [18]. Fish, fish oil and caviar from the Caspian Sea are the most important dietary sources of n − 3 PUFA in the north of Iran [19]. The average fish consumption per capita per year is 18 kg in the world; however, it is only 5 to 6.7 kg in Iran.

The aim of this study was to estimate if EFA and LCPUFA concentrations in BM differed in two provinces of Iran, deviating in regard to dietary fish intake.

2. Material and Methods

2.1. Study Design

This cross-sectional study was conducted on the mothers in the delivery ward, who were consecutively asked for participation. The study was performed in two provinces of Iran, the Guilan close to, and the Kermanshah province far from the Caspian Sea, and in two public hospitals, the Alzahra Hospital in Rasht, the capital of Guilan, and the Moutazedi Hospital in Kermanshah, the capital of Kermanshah, during July to September 2008.

The study was approved by the Medical Ethics Committee of the Kermanshah University of Medical Sciences in 2008. Informed consent was obtained from all mothers. Ethics approval for the statistical analyses of the results to be conducted in Sweden was obtained in 2009.

2.2. Subjects

Sixty mothers were selected in each hospital. Two mothers in Kermanshah and one mother in Guilan rejected to participate and then the next one was chosen. Nobody was excluded due to disease.

2.3. Breast Milk Sampling and Fatty Acid Analysis

Two-5 ml BM was collected from mother's breast by hand pumping into tubes 8 to 72 h after delivery.

The tubes were kept in ice box and sent within half an hour to the provincial health centre for freezing at −20°C until analysis, which was performed at the laboratory of National Nutrition and Food Technology Research Institute (NNFTRI) in Tehran. The milk samples were ho-

mogenized by Vortex (Heidolph Vortex Shaker REAX 1. 220 V. 30 W Germany) at 2400 rpm for 30 sec, and 500 μl was mixed with 2 ml KOH in methanol (2 N) and 2 ml hexane, and again mixed by use of a Vortex for 2 min. For esterification the sample was maintained for 30 min in ambient temperature and centrifuged (Rotina 35 R, Hettich, Tuttingen, Germany) for 2 min at 5000 rpm at 4°C. Na_2SO_4 (1 g) was added to the upper phase and the fatty acid methyl esters (FAME) were removed and 2 μl of the esterified sample was injected into the gas chromatograph (GC-CP3800, Varian, USA) equipped with a fused silica capillary column 50 m × 0.25 mm (I.D. CP-SIL 88, for FAME, Varian Chrompack, USA-Netherlands) supplied with 0.2 μm stationary phase (Varian Chrompack, USA-Netherlands) and a flame ionization detector. Oven temperature was maintained at 45°C for 2 min, then increased by 10°C/min to 175°C and held there for 15 minutes, followed by increase at 3°C/min to 220°C and kept there for 25 min (total time: 70 min). The injector and the detector temperatures were set at 260°C and 270°C, respectively. Helium was the carrier gas with a split ratio of 30:1 and a column flow of 0.5 ml/min. FA were identified by comparing the retention times of FAME with a standard FAME mixture (SUPELCO 37 Comp. FAME mix, Pennsylvania, USA). All samples were analysed in duplicate with gas chromatography. The results are expressed as weight/weight percent. The values are given as mean ± SEM if not otherwise indicated.

2.4. Dietary Measurements

The dietary intake of fish and seafood and other fats were assessed by structural interview using a food frequency questionnaire (FFQ) containing 17 items. These included fish, fish oil, sea food, olive, olive oil and dietary fat consumed during pregnancy. The dietary assessments were completed by first author at an interview with the participants, usually at the same time the BM was collected. The response options for the dietary intake were arranged in five categories from "never", "not regularly", "once a week", "twice a week" or "more than twice a week". In the analysis these five categories were reduced to three, named "never/not regularly", "once a week" and "≥2 times a week".

2.5. Statistical Analysis

Data analysis was performed with Excel and Social Sciences software version 17.0 (SPSS Inc., Chicago, IL, USA). Standard t-test was used to compare the mean differences of total FAs in BM and weight gain during pregnancy between the mothers in the two provinces. The fish or seafood intake was categorized. The DHA concentration of total FA in BM and the AA/DHA ratio were compared with the frequency of eating fish or sea-

food during pregnancy by using Kruskal-Wallis test and Mann Whitney's U-test. Chi-square test was used to evaluate the difference in dietary fat consumption including fish or seafood intake between the groups. Differences were considered statistically significant at $p < 0.05$. Mean (SD) is given if not otherwise indicated.

3. Results

3.1. Characteristics of the Two Study Populations

The mean age of the mothers was 26.6 (6.7) years in Kermanshah and 26.2 (6.4) in Guilan, ranging 14 - 40 years and 15 - 43 years, respectively. The weight before pregnancy was not available in 12 cases (4 in Kermanshah and 8 in Guilan). The mean weight did not differ between the groups, being 59.6 (11.6) kg in Kermanshah and 63.7 (13.9) in Guilan. The weight gain during pregnancy was significantly higher in Guilan than Kermanshah ($p = 0.025$). The gestational age was less than 37 weeks in 9 cases (3 in Kermanshah and 6 in Guilan) and in 4 cases data were missing about gestational age.

There were no differences in birth weight, length and head circumference between infants in Kermanshah (n = 58) and infants in Guilan (n = 62). In Guilan was included two pair of twins and anthropometric measurements in two cases were missing in Kermanshah.

3.2. Dietary Data

The FFQ was incomplete in two cases, which were excluded. The result showed that the subjects in Guilan had a significantly higher intake of fish or seafood compared to the mothers in Kermanshah ($p < 0.001$) (**Table 1**). Consumption of saturated fat from other animal sources was higher in Kermanshah ($p = 0.001$) and that of olives and olive oil was higher in Guilan ($p < 0.001$). There were no differences in the intake of other vegetable oils

Table 1. The percentage of mothers eating fish, sea food and other fat products more than once a week during pregnancy in inland (Kermanshah) and coastal (Guilan) provinces of Iran, as reported in the food frequency questionnaire (n = 58 and n = 60, respectively).

Mothers' intake	Kermanshah (%)	Guilan (%)	p-value
Fish and sea food	21	60	0.001
Fish oil supplements	10	8	NS
Olives and olive oil	17	55	0.001
Vegetable oils	76	78	NS
Fat from other animal sources	84	51	0.001

NS = Non significant.

(such as sunflower and corn oil) between the two provinces.

3.3. Fatty Acid Composition of Early BM

There was no difference in the FA pattern between milk sampled at different times after delivery. **Table 2** shows the concentrations of the major FAs in the BM. The FA concentrations were similar regarding the EFAs, LA and ALA.

The total n − 3 PUFA and DHA in BM were higher in the Guilan compared to the Kermanshah district. Total n − 6 PUFA and AA concentrations showed no differences between the two groups. The ratios of total (n − 6)/(n − 3) FA as well as AA/DHA in BM of the mothers from Guilan were lower than those in Kermanshah. Mead acid (eicosatrienoic acid, ETA; 20:3n − 9) was not identified.

The concentration of DHA was higher in BM after premature than after term delivery, being 0.76 (0.26) vs. 0.56 (0.27) ($p = 0.03$), but the AA concentration showed no significant differences after term and premature delivery. The AA/DHA ratio was lower after premature delivery 1.91 (0.44) vs. 2.94 (2.10) than after term delivery ($p = 0.016$). There was no difference in the total concentrations of n − 6 and n − 3 fatty acids. In **Table 3** are only compared data after the term deliveries in the two provinces in relation to fish and seafood intake, but similar significant differences were obtained when all milk samples were included.

BM from the youngest tertile (14 - 23 years) of mothers showed the highest concentration of PUFA (Kruskall-Wallis, $p = 0.01$), which was mainly related to the n-6 fatty acids ($p = 0.03$). There was no significant correlation between age groups regarding fish or seafood intake.

4. Discussion

This study demonstrates that regional differences in Iran regarding fish and seafood intake were reflected in the FA pattern of the early BM on a group level, confirming that dietary fish and seafood intake of the mother influences the DHA content of BM [9]. The results also corroborate the very high concentration of n − 6 FA earlier reported in BM from Iran [20].

The mean concentrations of DHA and AA in BM in our study were higher than those reported in a meta-analysis of 16 different studies on colostrum [21] and DHA showed about 50% higher concentration than the meta-analysis comparing 106 studies from mature milk [8]. We do not have a clear explanation to this, but walnuts are frequently used in Iran, which might be a contributing factor [22]. The total n − 6 PUFA did not show any differences between mother's milk between the two districts and one of the explanations to this might be the high consumption of vegetable oils generally in Iran [19,

Table 2. The concentration of major fatty acids in breast milk (weight%) of mothers in inland (Kermanshah) and coastalnd (Guilan) provinces of Iran.

Fatty acids	Kermanshah (n = 60)	Guilan (n = 60)	p-value
C12:0	1.62 ± 0.10	1.67 ± 0.12	NS
C14:0	4.12 ± 0.14	4.10 ± 0.13	NS
C16:0	24.9 ± 0.32	26.1 ± 0.24	0.001
C18:0	6.50 ± 0.14	6.53 ± 0.17	NS
C20:0	0.25 ± 0.00	0.23 ± 0.01	NS
C22:0	1.09 ± 0.04	1.10 ± 0.04	NS
C24:0	0.92 ± 0.05	0.91 ± 0.06	NS
∑ SFA	39.4 ± 0.8	40.7 ± 0.77	NS
C14:1ω7	0.24 ± 0.00	0.20 ± 0.00	0.001
C16:1ω7	1.77 ± 0.09	1.81 ± 0.08	NS
C18:1ω9	30.08 ± 0.30	28.85 ± 0.3	0.001
C20:1ω9	0.83 ± 0.02	0.76 ± .021	0.04
∑ MUFA	32.9 ± 0.41	31.6 ± 0.4	NS
C18:2ω6 (LA)	16.30 ± 0.33	16.03 ± 0.4	NS
C18:3ω3 (ALA)	0.81 ± 0.03	0.81 ± 0.03	NS
C20:4ω6 (AA)	1.40 ± 0.04	1.32 ± 0.04	NS
C20:5ω3 (EPA)	1.31 ± 0.06	1.35 ± 0.06	NS
C22:5ω3 (DPA)	0.35 ± 0.01	0.40 ± 0.02	NS
C22:6ω3 (DHA)	0.50 ± 0.02	0.66 ± 0.03	0.001
∑ω3FA	3.04 ± 0.10	3.35 ± 0.11	0.04
∑ ω6FA	17.7 ± 0.36	17.4 ± 0.41	NS
∑ PUFA	20.8 ± 0.41	20.8 ± 0.42	NS
LA/ALA	21.2 ± 0.55	20.8 ± 0.54	NS
AA/DHA	3.40 ± 0.31	2.28 ± 0.14	0.001
AA/(EPA+DHA)	0.82 ± 0.03	0.70 ± 0.03	0.01
∑ ω6/∑ω3	6.10 ± 0.20	5.44 ± 0.17	0.01

Mean ± SEM. SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, LA = linoleic acid, ALA = α-linolenic acid, AA = arachidonic acid, EPA = eicosapentaenoic acid, DPA = docosapentaenoic acid, DHA = docosahexaenoic acid, PUFA = polyunsaturated fatty acids.

20]. These vegetable oils, such as sunflower oil, contain very high concentrations of n − 6 fatty acids, which would explain why the total n − 6 FA did not differ between the groups. High AA concentration in BM has previously been reported in colostrum [21] and from Iran and Iraq, and reflects the high consumption of red meat [20,23].

The high concentrations of total n − 3 PUFA in BM in both provinces was mainly due to higher ALA and EPA

Table 3. Mean (SD) molar % concentration of (DHA; C22: 6n − 3) and the ratio of (AA; C20:4n − 6) to DHA in breast milk after term delivery in relation to the intake of fish and/or seafood during pregnancy, as reported in the food frequency questionnaires. P-values refer to Kruskal-Wallis test.

Fatty acid	Fish and seafood intake			p-value
	Non/seldom (n = 64)	1/Week (n = 25)	≥2/Week (n = 17)	
DHA	0.52 (0.23)#	0.62 (0.27)	0.69 (0.34)	0.048
AA/DHA	3.2 (2.4)#*	2.4 (1.1)	2.45 (1.6)	0.022

Mann-Whitney's U-test was used for differences between the groups, #p < 0.05 non/seldom compared to >2 times/week, *p < 0.05 non/seldom compared to once/week.

concentrations than previously reported [20,21,23]. We did not have a full dietary record, but the high EPA concentration and the relatively small differences between the groups compared to other studies, might be related to a high vegetable intake, as this has shown to highly influence the EPA concentration [10]. Such interpretation is supported by the relatively high ALA concentration. However, unusually high levels of LCPUFA might be due to co-elution, which could not be excluded but is less probable. The absence of a commonly found FA in colostrum, the eicoatrienoic acid (20:3n − 9, ETA, mead acid) might be an indicator that the high LCPUFA was valid, since mead acid is suggested to be a marker of essential fatty acid deficiency.

The results indicate that mothers with fish or seafood intake at least once a week had the highest DHA concentration and the lowest AA/DHA ratio. Our milk samples were collected in 8 - 72 hours after delivery and the level of LCPUFA is high in this period although related to mother's diet [24]. Other studies have shown that the increased content of LCPUFA in BM in early lactation was related to higher infant DHA status at 1 year of age [25]; suggesting that the levels of LCPUFA in BM might have a long term effect on the infant's FA status. In pregnant women in the republic of Seychelles, who had high habitual fish consumption, this was not correlated to the DHA concentration in BM 1 month post partum or in maternal serum at 28 weeks of gestation [7]. Despite the high fish intake, the DHA concentration in breast milk varied 5-fold but was similar to the reported mean in the worldwide review [8].

In our study the variation was even larger, ranging 0.11% to 1.58%, which might explain why we could find a correlation to the fish intake.

There was no difference of mean age of the mothers in the two provinces, but the youngest mothers (14 - 23 years) had the highest n − 6 PUFA concentration in BM, not correlated with higher fish or seafood intake. No data were collected about maternal parity but we cannot ex-

clude such influence since it has been shown that DHA decreases with parity [26]. On the other hand, the influence of Western diet and processed food, rich in vegetable oils [27], might be higher in the youngest age group and thereby explain the higher concentrations of n − 6 fatty acids in that group.

Socioeconomic factors, including family size, income and mothers education have, in an urban Iraqian population of mothers, been shown to influence n − 3 FA concentrations in the BM [28]. We did not have such data in this small pilot study and also other confounding factors regarding maternal habits, like smoking was not registered, and are, together with the restricted dietary recording, the limitations of our study. Such influences can therefore not be separated from genetic [29] or ethnic [30] differences, which would also be of importance for the FA pattern in BM. Thus we can summarize that regardless of these limitations, the results indicate an association between fish intake and DHA in BM, while the influence on EPA seems to be more complex.

5. Conclusion

There are accumulating data showing the effect of maternal dietary fatty acid intake on the FA composition of BM. This study shows that Iranian mothers have a good LCPUFA status in BM compared with the literature and that the DHA status was associated with the reported fish and sea food intake.

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