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The influence of sardine consumption on the omega-3 fatty acid content of mature human milk

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Abstract

Objectives: The purpose of this study was to investigate what effect the ingestion of sardines, rich in omega-3 series polyunsaturated fatty acids, has on the composition of breastmilk.

Methods: This was a prospective study of 31 nursing mothers under observation at the *Hospital Guilherme Álvaro*. Each was given 2 kg of fresh sardines twice with a 15-day interval. Milk was sampled and a 24-hour dietary recall questionnaire was applied on days 0, 15 and 30. Milk was assayed for fatty acid content by gas chromatography. Statistical analysis of the results was performed using nonparametric tests with significance set at $p < 0.05$.

Results: The results demonstrate that the nutritional intake of the nursing mothers was adequate at all three sample points. With regard to the omega-3 series fatty acid content of the breastmilk, it was observed that regular consumption and shorter intervals between ingestion and milk collection resulted in higher concentrations of docosapentaenoic acid and docosahexaenoic acid at 15 and 30 days into the study. Fatty acids from the omega-3 and omega-6 series exhibited a significant correlation, r^2 was 0.58 and 0.59 at 15 and 30 days, respectively.

Conclusion: These results suggest that incorporating fish into the diets of nursing mother during lactation, in the form of 100 g of sardines two or three times a week, contributes to an increase in omega-3 series fatty acids.

J Pediatr (Rio J). 2006;82(1):63-9: Breastmilk, human milk, polyunsaturated fatty acids, fish intake, fish consumption, docosahexaenoic acid (DHA), omega-3 fatty acids, lactation, breastfeeding.

Introduction

The central nervous system begins to mature during intrauterine life and continues to do so until seven years, exhibiting greatest intensity during the first two years of life.[1] The morphogenic process, which is directly related to cerebral function, requires a supply of specific fatty acids, in particular arachidonic and docosahexaenoic acids (DHA). This means that appropriate maternal nutrition is essential to the fetus during pregnancy and lactation, since there is increased functional and biochemical demand on maternal long chain polyunsaturated fatty acids (PFA).[2-4]

Omega-3 long-chain fatty acids can be found in the brain and retina and are active in growth processes, contributing to myelination and visual function development, to psychomotor development and to several behavior-related features of neural function.[1-5]

After birth these essential fatty acids are transferred to infants in sufficient quantities via the breastmilk of mothers whose nutritional status is adequate. In developing countries, where health and nutrition conditions can be adverse, it is possible for nutritional deficiencies to result in compromise to the processes of elongation and desaturation, i.e. the processes by which arachidonic and docosahexaenóico acids are formed from linoleic and linolenic acids, respectively.[1,2,4]

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Studies comparing breastfed and formula-fed children demonstrated that the first group had both higher concentrations of long-chain PFA and better visual acuity.[6] Preterm children (less than 32 weeks' gestation) had lower concentrations of DHA in the brain, liver and blood cells and also inferior visual acuity, when compared with fullterm children.[1,6]

Populations whose habitual diets include saltwater fish produce breastmilk with higher proportions of omega-3 fatty acids, when compared with populations that rarely consume sea fish.[7-9] Studies of fish oil supplementation with nursing mothers have demonstrated that their breastmilk contains satisfactory levels of fatty acids from the omega-3 series.[8,10,11]

There is evidence that demonstrates the influence of diet on breastmilk. The purpose of this study, therefore, was to verify whether quantities of sardines easily incorporated into the habitual diet of nursing mothers could successfully be used as a source of omega-3 series fatty acids, modulating the levels of these nutrients in mature breastmilk. This guideline could then be used with the general population and in particular with vulnerable groups, such as preterm and low birth weight children.

Methods

Thirty-one nursing mothers (sample of convenience) were followed at the Childcare Clinic of the *Hospital Guilherme Álvaro* Lactation Center, Santos (SP). All were interviewed, and, after enrollment on the study, on days 0, 15 and 30, their milk was sampled and a 24-hr dietary recall was filled out. Sardines were provided on days 0 and 15.

The mothers were required to fulfill the following criteria for inclusion: be breastfeeding exclusively (no water, teas or cow's milk or bottles), a non-smoker, disease-free during prenatal and postnatal periods, not on medication, not allergic to or intolerant of sardines during the prenatal and postnatal periods and available to attend follow-up appointments. Inclusion criteria for their infants were as follows: birth weight greater than or equal to 2,500 g; gestational age of 37 to 42 weeks; disease and incident free during the perinatal and postnatal periods and aged 15 days or more (postpartum) at the start of the study.

A consent form, approved in advance by the by Ethics Committee at the *Universidade Federal de São Paulo* (UNIFESP-EPM), was obtained from all mothers involved.

The data assessed were number of pregnancies, number of deliveries, gestational weight gain and anthropometric measurements (weight and stature) of the nursing mothers, while body mass index (BMI) was used to verify nutritional status in accordance with WHO criteria,[12] which define normal values as 18.5 to 24.9 kg/m^2, overweight as 25 to 29.9 kg/m^2 and degree I obesity as 30 kg/m^2 to 34.9 kg/m^2, degree II obesity as 35 kg/m^2 to 39.9 kg/m^2, and degree III obesity as $\geq$ 40 kg/m^2.

The dietary investigation used at each sample point (D0, D15 and D30) was a 24-hr dietary recall.[13] The *Programa de Apoio à Nutrição* software package was used to quantify the nursing mothers' dietary intake of calories, macronutrients (proteins, carbohydrates and lipids), saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids and cholesterol (version 2.5, produced by the Healthcare Information Technology Center at the *Universidade Federal de São Paulo*/*Escola Paulista de Medicina*).[14]

Each nursing mother was given 4 kg of fresh sardines (2 kg on day 0 and 2 kg on day 15) and was instructed to eat them at least twice a week, in order to attain a total of 500 g per week. The population's preferred method of preparing sardines, frying them, was respected, guaranteeing a regular supply of long chain polyunsaturated fatty acids. The quantity of sardines provided was estimated with the chance of intrafamily dilution taken into account. After the nursing mothers had consented to take part in the study, milk was collected on three separate occasions: day zero (D0), after 15 days (D15) and after 30 days (D30).

During the D15 and D30 milk collection sessions mothers were asked how frequently they had consumed sardines during the previous two weeks.

The samples of mature breastmilk were collected by manual expression, as described by Marmet.[15] Before collection, breasts were cleaned with sterile gauze dampened with de-ionized water. Collection was standardized to take place immediately after feeding (minimum 40 ml) and from both breasts. Polypropylene flasks that had been treated prior to use were employed for collection. After collection, samples were duly transported and frozen at -20 ºC.

After defrosting the milk, a 5 ml portion was separated so that lipids could be extracted by the Folch et al. method.[16] The total fat content was measured by the gravimetric method. Methyl esters from fatty acid lipid fractions were assayed by the Hartman & Lago[9] method and identified by gas chromatography.[17] The gas chromatograph was a Chrompack CP 9001® with a flame ionization detector (FID) and a CP-sil 88 silicon capillary column measuring 50 by 0.25 mm, and the detector and injector temperatures were set at 270 ºC and 250 ºC, respectively. The gas flow rates employed were 40 ml/minute for H_2, 40 ml/minute with N_2 and 40 ml/minute with synthetic air. The temperature program set was: 180 ºC for 12 minutes, followed by heating of 5 ºC a minute up to 220 ºC, which temperature was maintained for the last 5 minutes, making a total run time of 25 minutes.

Fatty acids were identified in the breastmilk using fatty acid standards by SIGMA® for direct comparison of the fatty acid methyl ester retention times and quantification of their percentage contribution to total fatty acids. The same procedures were used to determine the composition of the sardines.

The following nonparametric tests were employed in the analysis of results: Friedman analysis of variance by ranks;[18] in order to compare diet and breastmilk fatty acid content at the study points. The same analysis was used for total fat content. A multiple comparison test[18] was applied when the analysis of variance revealed significant values; the Wilcoxon test[18] was used to compare % delta values (Δ%) for each fatty acid of interest and for total fat; the Spearman correlation coefficient[18] was used to study the relationship between omega-6 and omega-3 acids at the different sample points, and finally, the Mann-Whitney test[18] was used to study the measurements taken of %Δ between consumption on close/regular days against on widely spaced/irregular days. The cutoff for rejection of the null hypothesis was set at 0.05 or 5% ($\alpha \leq 0.05$) and significant values are marked with asterisks.

Results

The nursing mothers studied had a mean age of 27.9 years (±6.3), mean weight at the start of the study of 61.2 kg (±10.8), mean stature of 158.2 cm (±5.3) and a mean BMI of 24.3 kg/m^2 (±4.2). Distribution by parity revealed a predominance of multiparous mothers (61.3%). Mean gestational weight gain was 10.9 kg (±6.5). Classification by nutritional status[12] revealed a majority of well-nourished mothers (58.1%), and many overweight ones (32.3%). The infants presented a mean birth weight of 3,286 g (±456), mean stature at birth of 49.5 cm (±2.2) and their average age was 22.7 days postpartum on the first day of the study(D0).

Thirty dietary recalls were analyzed from D0, D15 and D30, with one mother being excluded because she was not in a fit condition to provide the dietary recall information.

With respect of the mothers' dietary habits prior to the study, it was observed that just five of them had been in the habit of consuming fish more than once a week, i.e. 83.3% of the nursing mothers (n = 26) were consuming less fish than is recommendable according to this research.

The analysis of the mothers' diets did not reveal any variation across the data collection dates (Table 1).

The fatty acid (FA) composition of the sardines was analyzed as a percentage of the total acids (Table 2). The FA composition of the breastmilk was analyzed as percentages of total FA at D0, D15 and D30. All of the analyses employed Friedman analysis of variance by ranks and the Wilcoxon test, using %Δ, Δ1 (D15 - D0/D0 x 100) and Δ2 (D30 - D0/D0 x 100) (Table 3). These analyses did not reveal statistical differences.

The quantities of omega-6 and omega-3 polyunsaturated fatty acids (PFA) in the breastmilk were correlated for D0, D15 and D30 using Spearman's correlation coefficient. The correlation was statistically significant for D15 (r^2 = 0.58) and D30 (r^2 = 0.59) ($p < 0.05$) (Table 3).

In response to the variations observed in how much of the sardines were consumed in each week of the study, the decision was taken to classify the mothers according the frequency of intake and interval between the last consumption prior to collection and collection itself, with the objective of verifying the possibility that differences

Table 1 - Nursing mother's dietary intake – total energy value (TEV), proteins, carbohydrates, lipids, g and % as compared to TEV, saturated fatty acids, monounsaturated and polyunsaturated at days 0, 15 and 30 (n = 30)

Diet	T0		T15		T30	
	Mean	±SD	Mean	±SD	Mean	±SD
Total energy value (Kcal)	2304	±694.7	2158.8	±828.0	2193.4	±752.2 NS *
Proteins (g)	85.1	±33.6	83.4	±32.8	89.6	±30.2 NS *
Proteins (%)	14.8	±3.5	15.6	±3.5	16.7	±4.0 NS *
Carbohydrates (g)	299.6	±85.0	293.1	±120.0	280.9	±119.3 NS *
Carbohydrates (%)	53.5	±7.1	54.4	±7.1	51.3	±10.2 NS *
Lipids (g)	82.6	±32.2	74.1	±34.1	79.4	±32.7 NS *
Lipids (%)	31.3	±29.9	29.9	±5.2	32.5	±8.3 NS *
Saturated fatty acids (g)	26.6	±22.1	22.1	±11.2	26.2	±12.5 NS *
Monounsaturated (g)	29.4	±12.8	26.3	±10.9	29.4	±12.2 NS *
Polyunsaturated (g)	17.3	±8.4	16.4	±8.5	17.9	±9.7 NS *

SD = standard deviation; NS = non significant ($p > 0.05$).
* Friedman.

Table 2 - Composition of fatty acids in fried sardine (% of the total fatty acids)

Fatty acids	%	Fatty acids	%
Saturated		**Monounsaturated**	
Total	21.47	Total	24.46
Polyunsaturated		**Polyunsaturated**	
ω6 series		ω3 series	
18:2 ω6	40.62	18:3 ω3	3.31
18:3 ω6	0.35	20:5 ω3	1.41
20:4 ω6	1.05	22:5 ω3	0.89
		22:6 ω3	6.44
Total ω6	42.02	Total ω3	12.06
Polyunsaturated			
Total ω6+ω3	54.07		

between fatty acid concentrations could be determined by these factors. The analysis was performed separately for the interval D0 to D15 and then for D0 to D30 days, with the interval taken in days.

Group 1 (regular consumption of fish and last intake within 3 days of milk collection): the nursing mother consumed fish regular two to three times a week and the maximum interval between the intake of sardines and milk collection was 3 days;

Group 2 (irregular consumption of fish and last intake 4 days or more before milk collection): consumption was irregular, predominantly during the first week, with little left for the week when milk was collected, and the minimum interval between breastmilk collection and the previous intake of sardines was a minimum of 4 days.

Table 3 - Composition of breastmilk – total fats (g/dl), fatty acids (AG) (% of total fatty acids) – T0, T15 and T30 (n = 31)

Fatty Acids	T0 Mean±SD	T15 Mean±SD	T30 Mean±SD	
Saturated				
10:0	1.72±1.00	1.79±0.84	1.85±0.61	NS *
12:0	7.00±2.59	7.34±2.95	6.71±2.31	NS *
14:0	7.56±3.08	7.80±2.60	7.33±3.34	NS *
16:0	20.31±1.78	20.01±2.34	19.09±2.13	NS *
18:0	5.90±1.15	6.13±1.61	6.46±1.58	NS *
Total	42.50±5.62	43.07±6.18	41.44±6.49	NS *
Monounsaturated				
16:1	3.09±0.88	3.07±0.80	4.06±1.52	NS *
18:1	30.52±3.99	30.30± 4.95	30.26±5.13	NS *
Total	33.61±4.14	33.37±5.43	34.32±5.40	NS *
Polyunsaturated ω6 series				
18:2ω6	20.72±3.78	20.36±5.68	20.95±5.10	NS *
18:3ω6	0.26±0.08	0.19±0.08	0.21±0.06	NS *
20:4ω6	0.56±0.14	0.53±0.13	0.51±0.10	NS *
Total série ω6	21.55±3.77	21.07±4.66	21.66±5.14	NS *
Polyunsaturated ω3 series				
18:3ω3	1.67±0.58	1.74±0.53	1.82±0.48	NS *
20:5ω3	0.08±0.07	0.07±0.05	0.10±0.06	NS *
22:5ω3	0.24±0.05	0.23±0.08	0.22±0.07	NS *
22:6ω3	0.46±0.11	0.52±0.28	0.47±0.11	NS *
Total ω3 series	2.34±0.68	2.49±0.82	2.57± 0.68	NS *
Total ω6+ω3	23.89±4.03	23.56±5.43	24.23±5.65	NS *
Total fats (g/dL)	3.52±1.58	3.58±1.55	3.31±1.40	NS *
Relation				
ω6 total:ω3 total	7.50	7.00	7.10	NS *
18:2 ω6:18:3 ω3 †	14.00	12.00	11.80	NS *
20:4 ω6:22:6 ω3	1.68	1.63	1.43	NS *
Correlation ω6:ω3 ‡		$r^2 = 0.58$	$r^2 = 0.59$	§

NS = non significant; * Friedman; † Relation ω3/ω6 5-15 (ESPGAN[21]); ‡ Spearman correlation; § $p < 0.05$

The omega-3 and omega-6 series PFA were related in terms of regularity of sardine intake and interval between breastmilk collection and D15 (%Δ1) and D30 (%Δ2). These analyses were performed using the Mann-Whitney test (Table 4).

The analysis revealed that the regular sardine intake and maximum 3-day interval group had significantly higher levels of docosapentaenoic acid (22:5 omega-3 – DPA) and of docosahexaenoic acid (22:6 omega-3 – DHA) at D15 and of DPA, DHA and total omega-3 PFA (PFA-omega-3) art D30 ($p < 0.05$) than the irregular sardine intake and minimum 4-day interval group.

Discussion

Maternal nutrition is of great importance to the correct development of the fetus and infant. Over recent years studies have been, and continue to be, undertaken to investigate the factors that can affect the quality of breastmilk and the possible consequences of this.[7,19]

In this study the interrelationship between nursing mothers' diets and the composition of their breastmilk can be observed. Firstly, studying these mothers' nutritional intake, evaluated by means of the 3 data collection days, it can be observed that the distribution of macronutrients across the total energy value (TEV) of the diet was in conformity with that described by Philippi et al.,[20] and also that nutritional intake did not vary across different sample dates.

The analysis of the fatty acids in the breastmilk of the mothers under study revealed a pattern that is distinct from that described in other reports, reflecting the different feeding habits of the different study populations.

Our research found baseline values (D0) for DHA where the omega-6:omega-3 ratio was not unacceptable, but was borderline, similar to figures found in western populations where much industrialized produce is consumed and seafood and vegetable consumption is reduced.[9] Our nursing mothers exhibited a baseline omega-6:omega-3 ratio of 15:1, which is very close to the upper limit.[21] On days 15 and 30, however, the omega-6:omega-3 ratio was close to 12:1, which is compatible with countries where fish is consumed regularly or the Mediterranean where the ratios are from 10 to 12:1.

The percentage of fatty acids polyunsaturated found on days 15 and 30 of this study were similar to the results of work carried out in Panama (linoleic 19.87%, α-linolenic 1.72%, eicosapentaenoic [EPA] 0.09% and total omega-3 2.53%) and in the Congo (DPA 0.24%, DHA 0.55% and total PFA-omega-3 2.39%),[7] and higher than figures found among populations in Europe and North America.[5,9]

Studies in Brazil evaluating linoleic and linolenic acids in colostrum and mature breastmilk have observed constant concentrations irrespective of maternal conditions, despite differences in socioeconomic conditions vegetable oils are widely used, whether extracted from soy or corn.[22-24]

Analyses of these fatty acids in breastmilk in other studies have revealed that when the levels of linoleic acid are elevated (18:2 omega-3), the levels of α-linolenic also are (18:3 omega-3), which appears to reflect the extent to which synthesis adapts to maintain the two acids in balance.[9,19,23]

Table 4 - Composition of polyunsaturated fatty acids in breastmilk - T0, T15 e T30 (mean of the total of fatty acids±SD) Group 1 (regular consumption of fish and last intake within 3 days of milk collection) and Group 2 (irregular consumption of fish and last intake 4 days or more before milk collection) (n = 22)

Polyunsaturated fatty acids	T0 Group 1	T0 Group 2	T15 Group 1	T15 Group 2	T30 Group 1	T30 Group 2
18:2 ω6	21.48±3.66	21.05±4.00	22.59±4.00	19.63±6.84	24.11±5.16	20.88±4.46
18:3 ω6	0.22±0.12	0.20±0.10	0.19±0.06	0.18±0.07	0.18±0.09	0.24±0.21
20:4 ω6	0.55±0.16	0.57±0.12	0.56±0.17	0.49±0.12	0.46±0.10	0.54±0.11
Total ω6 series	22.25±3.65	21.81±3.94	23.34±4.07	20.30±6.91	24.74±5.22	21.65±4.45
18:3 ω3	1.71±0.59	1.77±0.70	1.86±0.45	1.85±0.64	1.92±0.67	1.92±0.40
20:5 ω3	0.06±0.04	0.09±0.11	0.07±0.03	0.07±0.05	0.17±0.08	0.06±0.02
22:5 ω3	0.16±0.03	0.25±0.10	0.22±0.08 *	0.20±0.03	0.24±0.11 *	0.23±0.07
22:6 ω3	0.35±0.12	0.44±0.25	0.61±0.51 *	0.45±0.20	0.67±0.47 *	0.41±0.21
Total ω3 series	2.22±0.57	2.56±0.69	2.74±0.70	2.57±0.57	3.00±0.89 *	2.62±0.39

Mann-Whitney test * $p < 0.05$.

There is evidence that the omega-6 and omega-3 fatty acids compete, since they employ the same desaturation method to form their homologues. Articles report that in situations in which there is inadequate α-linolenic acid desaturation, there may be an insufficient supply of the acid or the high levels of linoleic acid, common in infant formulae, could be inhibiting or limiting the capacity to desaturate and elongate α-linolenic acid into its products.[25] In our study a positive correlation can be observed between the omega-6 and omega-3 fatty acids. The higher levels of linoleic and α-linolenic acids found reflect the constant and elevated consumption of soy oil by the mothers before and during the data collection periods, including for preparation of the sardines as all of the nursing mothers chose to eat them fried. These polyunsaturated fatty acids did not exhibit significant differences between the three data points.

The long chain fatty acid composition may be altered by the frying process, but the figures returned by analysis were satisfactory and in line with what would be expected for this type of fish. If the study population were not in the habit of consuming soy oil regularly in the preparation of their food and for frying, it could influence linoleic and α-linolenic acid levels. During the frying process small quantities of fatty acids with 20 and 22 carbons may be absorbed by the oil, but this did not affect the results of consuming the fried fish. It is probable that the habit of regularly consuming fried food is common in this population.

It is known that changes to the quality of maternal dietary fat content can change the fatty acid content of breastmilk in 2 to 3 days.[26] Presa-Owens et al.,[5] studying the FA composition of milk from 40 nursing mothers in Spain, reported that despite the fact that fish contains a greater proportion of EPA (22:5 omega-3) than DHA (22:6 omega-3), the composition of the breastmilk of women who consume large quantities of fish includes increased levels of DHA. A study by Henderson et al.[11] that employed fish oil as a supplement for nursing mothers found that consuming 1 g a day of long chain omega-3 PFA resulted in mean levels of 0.08% EPA, 0.14% DPA and 0.37% DHA in breastmilk. These figures were similar to those found in the breastmilk of the nursing mothers studied in this project, using an easily accepted foodstuff of low cost and demonstrating that an average consumption of three sardines a day (100-120 g) two to three times a week resulted in increased levels of these fatty acids without the need for oil-based supplements.

Studies report that there is a positive correlation between DHA and AA levels in milk. [2,19] There is evidence that there are homeostatic mechanisms that regulate the serum concentration of fatty acids of a given series, as occurs with the fatty acids DHA, DPA and EPA,[19,25] and when they are supplemented in the diet, the first two of these are predominantly directed towards stores and the third made available in serum concentrations.[3,27]

The analysis of breastmilk composition broken down according to the pattern of fish intake revealed that the frequency with which the sardines were ingested and the interval between consumption and milk collection were factors that determined higher levels of omega-3 FA, the regularity of consumption, (two to three times a week) and the interval of up to 3 days between intake and collection resulted in significantly higher values for DPA, DHA total omega-3. This last exhibited statistically higher levels at D30. These findings can be explained by the considerations explained above about the preference for incorporating the two fatty acids into storage tissues.

A large proportion of the polyunsaturated fatty acids secreted in breastmilk originate from endogenous synthesis and from maternal stocks, by means of regulatory mechanisms.[3,4,19] Thus, it is believed that lower levels of EPA in breastmilk are a reflection of the regulation of this fatty acid's entry into the mammary glands so that the appropriate omega-6 to omega-3 ratio is maintained, since maternal deposits would be contributing the omega-3 series in the form of DHA and DPA.[25,27] Indeed, it is known that consumption of EPA is primarily reflected in serum levels and not in deposits.[3,27,28] On the other hand, serum EPA has a special role in the immune system, benefiting serum lipid levels and reducing the risks of platelet aggregation, atherosclerotic diseases and thrombotic complications.[25,28]

Huang et al.,[29] in an experimental study, observed that the use of fish oil in feed given to suckling rats promoted an increase in the incorporation of DHA (22:6 omega-3) and DPA (22:5 omega-3) with the phospholipids of their offspring's hearts. The EPA concentration (20:5 omega-3) in the organ was lower and the article pointed out that the levels of this fatty acid are less sensitive to dietary modifications. Other studies supplementing nursing mothers with fish oil found satisfactory levels of omega-3 fatty acids in the breastmilk produced, suggesting that the presence of α-linolenic acid and EPA in the diet of these mothers may have contributed to their breastmilk having significantly elevated DHA levels.[8,10,11]

Harris et al.[30] concluded in their article that in order to achieve the desirable levels of DHA in breastmilk, fish consumption should be extremely elevated, since to attain 0.5 to 1 g of DHA it would be necessary to consume from 350 to 750 g of fish with 1% fat or 75 to 150 g of fish with 10% fat per day. In our study, the baseline levels of DHA in breastmilk increased with an average consumption of 300 g of sardines (5% fat) per week, when the consumption was regular and took place soon before collection (a interval of less than 3 days between consumption and milk collection). Therefore, we suggest that in order to maintain their breastmilk omega-3 series levels constantly elevated, mothers should eat saltwater fish two to three times a week.

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