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FATTY ACID COMPOSITION OF MILK OF REFUGEE KAREN AND URBAN KOREAN MOTHERS. IS THE LEVEL OF DHA IN BREAST MILK OF WESTERN WOMEN COMPROMISED BY HIGH INTAKE OF SATURATED FAT AND LINOLEIC ACID?

I. GOLFETTO*, R. MCGREADY**&, K. GHEBREMESKEL*[1],
Y. MIN*, L. DUBOWITZ***, F. NOSTEN**&, P. DRURY*#,
J.A. SIMPSON**&, R. ARUNJERDJA**AND M.A. CRAWFORD*

*[1]*The Institute of Brain Chemistry and Human Nutrition, London Metropolitan University, Holloway Road, London N7 8DB, **Shoklo Malaria Research Unit, Mae Sot., Tak Province, Thailand, &Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Oxford, UK, *** Department of Paediatrics, Imperial College of Science and Medicine, Hammersmith Hospital, London, UK, #King's Fund, Cavendish Square, London, UK.*

ABSTRACT

Background: Lower proportions of docosahexaenoic acid (DHA) and total n-3 metabolites have been reported in breast milk of European, Australian and North American women compared with milk of mothers from non-Western countries. This difference is not always explained by intakes of marine products.
Objective: We investigated the possibility that the relative composition of DHA and total n-3 metabolites in breast milk of non-Western mothers with low fat intakes is higher than the levels commonly reported in their Western counterparts.
Subjects: Mature milk of refugee Karen women from two different camps in Thailand (n=26 and n=53), and transition milk from urban Korean mothers (n=12) in Seoul was collected. In common with their respective community, the mothers have low fat intake, which is predominately of plant origin.
Results: The percentage levels of DHA and n-3 metabolites in the milk of the Karen mothers were 0.52±0.14 and 0.85±0.24 (camp 1) and 0.54±0.22 and 0.92±0.42 (camp 2). In the Korean milk, DHA was 0.96±0.21 and total n-3 metabolites 1.51±0.3.
Conclusion: We postulate that the levels of DHA and total n-3 metabolites may be compromised in breast milk of mothers on the Western high fat diet. This calls into question the use of DHA composition of such milk as a reference for the formulation of milk designed, for infant feed or, to test the function of DHA in neuro-visual development.

Key words: Breast milk, Refugee women, Docosahexaenoic acid, N-3 metabolites, Western high fat diet.

Correspondence to author: keb@kebgm.demon.co.uk

INTRODUCTION

Arachidonic (AA) and docosahexaenoic (DHA) acids are universally present in breast milk (Crawford *et al.*, 1976, Kneebone *et al.*, 1985, Prentice *et al.*, 1989, Drury and Crawford 1990, Koletzko *et al.*, 1992, Chulei *et al.*, 1995, Jensen 1996, Chen *et al.*, 1997). They are major structural lipid components of the brain, retina and blood vessels (Svnnerholm 1968, Crawford and Sinclair 1972, Fliesler and Anderson 1983, Sastry 1985). AA and DHA are found in tissue specific proportions in all cellular and sub-cellular bio-membranes. In the major inner cell membrane phospholipid, ethanolamine phosphoglycerides (EPG), of red cells of healthy individuals, the levels of AA and DHA account for about 20% and 10%. In contrast, the parent compounds linoleic (LA) and alpha-linolenic (ALA) comprise only about 6% and 0.4%.

In human cerebrum and cerebellum, over a three fold increase in AA and DHA occurs, during the third trimester and, between birth and postnatal week 12 (Martinez *et al.*, 1974, Clandinin *et al.*, 1980a, 1980b). A deficiency of DHA is marked by an increase in the synthesis and incorporation of the minor fatty acid, docosapentaenoate (22:5n-6, DPA) (Fiennes *et al.*, 1973, Holman 1977, Hornstra *et al.*, 1995). Similarly, an insufficiency of LA and AA is indicated by a relative increase in the synthesis of eicosatrienoic (20:3n-9) and docosatrienoic (22:3n-9) acids (Crawford *et al.*, 1990, Al *et al.*, 1990).

AA and DHA are selectively transferred to the fetus by the placenta from maternal circulation in utero. Post-natally, babies have to depend on breast milk or formulae for their AA and DHA. Formulae milk presently available in retail outlets contain small amounts of AA and DHA, only DHA, or none of the two fatty acids. Hence, formula fed babies may not receive sufficient amounts of these vital nutrients during their crucial period of development.

Expert Committees have recommended that formula milk for babies, term and preterm (BNF 1992, FAO/WHO 1994, ISSFAL 1994 and CDEC 1996) and preterm (ESPGAN 1991), should be modelled on breast milk with respect to the long-chain polyunsaturated fatty acids: AA and DHA. However, there is no clear recommendation with regard to the chemical form and amounts of AA and DHA, which milk formulae, ought to contain.

We have previously studied the fatty acid composition of breast milk of healthy mothers from Hungary (Szeged) and Thailand (Khon Kaen) between 1982 and 1984. The Thailand mothers had higher DHA and total n-3 metabolites ($p<0.05$) and total n-6 metabolites ($p>0.05$) at week 12 and 24 post partum. These findings were intriguing as the Thailand mothers were from an in-land community with a low fat intake. A summary of the relevant data is shown in table 1. Similarly, Chueli *et al.* (1995) have reported high proportions of DHA and n-3 metabolites in milk of women from pastoral and rural regions of China with negligible fish and shellfish intake.

TABLE 1

Meant (SD) total milk lipid and percent fatty acid composition of breast milk of Hungarian (Szeged) and Thailand (Khon Kaen) mothers at week 12 and 24.

	Hungarian Mothers (n = 26)		**Thailand Mothers (n = 57)**	
	Week 12	Week 24	Week 12	Week 24
Total lipid	6.49±2.1	6.44±2.3	5.07±1.7	4.70±1.3
Fatty acids				
18:2n-6	9.88±3.0	11.0±3.0	6.70±2.8	7.28±3.0
20:2n-6	0.30±0.07	0.27±0.08	0.27±0.08	0.28±0.11
20:3n-6	0.30±0.08	0.26±0.08	0.38±0.08	0.37±0.11
20:4n-6	0.53±0.08	0.49±0.08	0.52±0.05	0.60±0.17
22:4n-6	0.12±0.04	0.12±0.04	`	0.13±0.19
0.14±0.09				
22:5n-6	0.07±0.05	0.04±0.06	0.16±0.19	0.14±0.09
ΣN-6 metablites	**1.30±0.22**	**1.22±0.21**	**1.47±0.35**	**1.54±0.32**
18:3n-3	0.49±0.19	0.40±0.09	0.36±0.17	0.44±0.27
20:5n-3	0.03±0.05	0.02±0.05	0.10±0.06	0.12±0.09
22:5n-3	0.13±0.05	0.14±0.05	0.19±0.07	0.22±0.09
22:6n-3	0.17±0.07	0.13±0.06	0.47±0.15	0.53±0.17
ΣN-3 metabolites	**0.32±0.25**	**0.29±0.09**	**0.75±0.24**	**0.85±0.31**

This study was conducted to establish if the relative composition of DHA and total n-3 metabolites in breast milk of non-Western mothers with low fat intakes is higher than the levels commonly reported in their Western counterparts.

MATERIALS AND METHODS

Subjects

Mature milk, after post-partum week 4, was collected from refugee Karen women from two different refugee camps in Thailand (n = 26 and n = 53) near the Burmese boarder between 1996 and 1997. Samples of transition milk, 4–7 days post partum, was also obtained from urban Korean women (n = 12) in Seoul 1997. All of the mothers delivered at term and had no health complications before or after delivery. The age range of the Karen was 16-40 and the Korean 22–41. At least one hour after the last feeding, about 10 ml of milk was expressed from the other breast while the child was suckling. The milk specimen was immediately frozen and subsequently transported to the United Kingdom for analysis.

In larger hospitals in South Korea, mother and baby are kept in separate rooms between delivery and hospital discharge. During this period, it is customary to feed babies on formula rather than maternal milk. Three brands of Korean formulae for babies aged 4 months and under which are commonly provided in major hospitals in Seoul: Maeil Mamma D&A-1 (Maeil Dairy Industry Co. Ltd.), Low-Heat 1 (Pasteur Milk Co. Ltd) and Ahkisarang 1 (Namyang Dairy Product Ltd.) were analysed for comparison with breast milk.

Ethical approval was granted by the Karen Ethics Committee and the Ethical Committee of Mahidol University for the study of the Karen women. Similarly, approval was obtained from the Ethical Committee of the Asan Medical Centre, Seoul, South Korea.

Fatty acid analysis

Total lipid in the milk was extracted by the method of Folch and Stanley (1957) by homogenising the samples in chloroform and methanol (2:1 v/v) containing 0.01% butylated hydroxytoluene (BHT) as an antioxidant under N_2. Fatty acid methyl esters (FAME) were prepared by heating the lipid extract with 5ml of 15% acetyl chloride in methanol in a sealed vial at 70°C for 3 hr under N_2. FAME were separated by a gas liquid chromatograph (HRGC MEGA 2 Series, Fisons Instruments, Italy) fitted with a capillary column (25m × 0.32mm ID, 0.25μ film, BP20). Hydrogen was used as a carrier gas, and the injector, oven and detector temperatures were 235, 210 and 260°C. The FAMEs were identified by comparison of retention times with authentic standards and calculation of equivalent chain length values. Peak areas were quantified by a computer chromatography data system (EZChrom Chromatography Data System, Scientific Software, Inc., San Ramon, CA).

Data analyses

Data are expressed as mean and standard deviation (s.d.). Un-paired t-test was use to compare the differences in fatty acids composition of milk of the different mothers. A non-parametric test was used to compare the mean composition of the formulae with that of the Korean mothers. The strength of association (Pearson Correlation Coefficient, **r**) between DHA and the other n-3 fatty acids, and between AA and the n-3 fatty acids was calculated by linear regression analysis. A statistical package, SPSS for Windows (Release 7), was used to analyse the data.

RESULTS

The Karen women in common with most of refugee population in Thailand rely primarily on ration foods for their dietary intake. These foods are low in total and saturated fat, and marginal in a number of essential nutrients (Riedel and Menefee 1997). Moreover, as there is an overlap in dietary habit between the Karen and the Thai populations, we were able to compare the fatty acid composition of the milk of the Karen and those of the Thai studied previously.

Korean women are generally reluctant to provide breast milk after their discharge from a hospital. Hence, we were only able to obtain transition milk between days 4 and 7 post partum from twelve mothers. Nevertheless, the data provided information on AA and DHA composition in breast milk of the Koreans during the transition stage of lactation. It also enabled us to compare the fatty acid composition of the milk of the mothers with those of the formulae, which are commonly provided to neonates between birth and hospital discharge.

TABLE 2

Percent fatty acid composition of mature breast milk of Karen women from two different camps.

Fatty acids	Karen (Camp 1, n=36)	Karen (Camp 2, n=53)
10:0	0.92±0.43	1.42±0.45
12:0	9.30±3.6	9.17±2.76
14:0	10.43±3.7	10.66±4.0
16:0	26.74±2.7	27.00±2.48
18:0	4.26±0.67	4.39±1.1
20:	0.15±0.06	0.13±0.03
22:0		
24:0	0.04±0.03	0.05±0.03
Σ Saturates	**51.53±6.0**	**53.13±5.80**
16:1n-7	5.20±1.3	4.51±1.2
18:1n-9	29.01±4.4	28.58±4.7
20:1n-9	0.42±0.09	0.32±0.08
22:1n-9	0.07±0.03	0.06±0.05
24:1n-9	0.05±0.08	0.07±0.08
Σ Monoenes	**34.72±4.5**	**33.70±4.8**
18:2n-6	7.84±1.9	7.99±1.7
20:2n-6	0.21±0.06	0.19±0.05
20:3n-6	0.37±0.08	0.38±0.07
20:4n-6	0.49±0.10	0.48±0.07
22:4n-6	0.13±0.03	0.12±0.02
22:5n-6	0.09±0.05	0.09±0.03
Σ N-6 metabolites	**1.29±0.24**	**1.26±0.42**
18:3n-3	0.52±0.29	0.44±0.19
20:5n-3	0.16±0.08	0.18±0.3
22:5n-3	0.17±0.09	0.20±0.06
22:6n-3	0.52±0.14	0.54±0.22
Σ N-3 metabolites	**0.85±0.24**	**0.92±0.42**

Refugee Karen mothers

Data of the fatty acid composition of breast milk of the two groups of Karen mothers are shown in Table 2. There was no difference in the percentage levels of AA, DHA, total n-6 and n-3 metabolites and saturated and monounsaturated fatty acids between the mothers from the two camps ($p>0.05$). The proportions of AA, DHA, and total n-3 and n-6 metabolites between the rural Thai from Khon Kaen we studied previously (Table 1) and the refugee Karen mothers were not significantly different ($p>0.05$). However, the Karen women, similar to the Thai, had significantly higher DHA and n-3 metabolites compared with Hungarians ($p<0.05$).

Milk DHA of the Karen mothers correlated positively with alpha-linolenic acid ($r = 0.39$, $p = 0.004$), docosapentaenoic acid, 22:5n-3 ($r = 0.71$, $p = 0.0001$) and AA ($r = 0.44$, $p = 0.001$), and negatively with linoleic/ alpha-linolenic ratio ($r = -0.42$, $p = 0.002$). Similarly, docosapentaenoic acid (22:5n-3) correlated positively with alpha-linolenic acid ($r = 0.29$, $p = 0.03$) and negatively with linoleic/alpha-linolenic acid ratio ($r = -0.32$, $p = 0.018$). There was no correlation between linoleic acid and AA ($p>0.05$).

Korean mothers

Fatty acid Data of the Korean milk and formulae are shown in Table 3. The urban Korean women had significantly lower saturates and higher monoenes ($p<0.0001$) compared with their Karen and Thai counterparts. They also had higher proportions of AA, DHA and total n-6 and n-3 fatty acids ($p<0.0001$) than the Hungarians, Karen and Thai mothers.

Korean milk and formulae

Fatty acid composition of the Korean human milk was compared with the mean values of the three formulae. Percentage levels of total saturates, and capric (C10:0), lauric (C12:0) and myristic (C12:0) acids were higher and monenes, palmitoleic (16:1n-7) and oleic (C18:1n-9) acids lower ($p<0.05$) in the formulae compared with the human milk. Similarly, the proportion of linoleate was higher and that of AA, DHA, total n-6 and n-3 metabolites and total n-3 lower in the formulae in comparison to the human milk ($p<0.05$). In contrast to the breast milk of the Korean women, the formulae were devoid of 20:2n-6, 20:3n-6, 22:4n-6, 22:5n-6 and 22:5n-3.

TABLE 3

Percent fatty acid composition of transition breast milk of Korean women (n=12) and three formulae milk which are commonly sold in Seoul, South Korea.

Fatty acids	Mothers	Mamma D&A-1	Ahkisarang 1	Low-Heat 1
8:0	0.32±0.3	0.41	0.23	0.16
10:0	0.24±0.36	1.72	1.08	1.97
12:0	2.21±1.4	11.56	9.57	3.06
14:0	4.47±1.4	7.00	5.99	9.09
16:0	24.89±1.5	23.38	24.48	25.72
18:0	5.50±0.51	6.12	6.42	10.63
20:	0.17±0.04	0.26	0.29	0.22
22:0	0.11±0.03	0.19	0.19	0.25
24:0	0.12±0.21			
Σ Saturates	**37.76±2.9**	**50.23**	**48.01**	**50.94**
16:1n-7	3.52±0.69	0.51	0.59	1.33
18:1n-9	33.19±1.81	27.40	28.86	24.53
20:1n-9	0.76±0.15	0.18	0.07	0.40
22:1n-9	0.18±0.05			
24:1n-9	0.27±0.08			
Σ Monoenes	**40.41±2.9**	**28.20**	**29.75**	**27.03**
18:2n-6	14.57±1.63	17.29	18.96	16.70
20:2n-6	0.94±0.16			
20:3n-6	0.65±0.21	0.05		
20:4n-6	0.87±0.28	0.11	0.07	0.17
22:4n-6	0.48±0.20			
22:5n-6	0.07±0.04			
Σ N-6 metabolites	**2.98±0.72**	**0.11**	**0.12**	**0.17**
18:3n-3	0.75±0.24	1.43	0.97	0.62
20:5n-3	0.17±0.05	0.12	0.14	0.15
22:5n-3	0.43±0.12			
22:6n-3	0.96±0.21	0.26	0.20	0.15
Σ N-3 metabolites	**1.51±0.33.**	**0.38**	**0.34**	**0.3**

DISCUSSION

The milk of the refugee Karen mothers had high percentage levels of the medium-chain fatty acids (C10:0, C12:0 and C14:0) and lower total monoenes, and oleic and linoleic(LA) acids than of milk of European women. These are consistent with their high carbohydrate and low fat diet. In contrast, the Korean mothers had low proportions of the medium-chain fatty acids and higher oleate and LA. Plant based foods are still the major source of lipid for the majority of the Korean population (Moon *et al.*, 1997). With affluence, there has been an increase in the consumption of fat particularly in the urban areas and this is reflected in the higher levels of oleate and LA in the milk.

There was a significant positive association between AA and DHA in the milk of the Karen women. AA and DHA as do LA and ALA have different primary dietary sources (Ackman 1982, Tinoco 1982) and there is no evidence of high correlation between AA and DHA in foods. Hence, the observed relationship between the two fatty acids in the milk suggests the involvement of a physiological selection process rather than diet in order to meet the needs of the baby.

The most remarkable aspect of the refugee Karen, the rural Thailand and urban Korean data is the high proportions of DHA and total n-3 metabolites. As transitional milk tends to have higher proportions of DHA and total n-3 metabolites compared with matured milk, the elevated levels in the Korean milk could in part be explained by the stage of lactation. Nevertheless, these values are still high compared to European transitional milk data (Pita *et al*., 1985) and are consistent with the 0.65% DHA and 1.60% total n-3 metabolites in matured Korean milk (Lee 1997).

Lower proportions of DHA have been reported in breast milk of European (Koletzko *et al*., 1988, Luukkainen *et al*., 1994, Presa-Owens *et al*., 1996), Australian (Gibson and Kneebone 1981, Makrides *et al*., 1995) and North American (Bitman *et al*., 1983, Finley *et al*., 1985, Specker *et al*., 1987, Francois *et al*., 1998) women. In contrast, breast milk from outside Europe and North America (Kneebone *et al*., 1985, van Beusekom *et al*., 1990, Boersma *et al*., 1991, Koletzko *et al*., 1992, Chulei *et al*., 1995, Jensen 1996, Chen *et al*., 1997) have higher proportions of DHA and total n-3 metabolites.

Maternal diet is thought to influence the fatty acid composition of breast milk. Nevertheless, questions need to be asked about the very low values of DHA (<0.3%) reported in some American and European mothers. Could it be that DHA in breast milk of 'well nourished' Western women is compromised by their dietary habits? Makrides *et al*. (1995) observed a relative decline in the level of DHA in breast milk in Australian women between 1981 and 1995. The authors speculated that 'a shift in Australian diet toward less animal products and increased use of LA-rich oils is being reflected in breast milk composition of the mothers'.

It possible that a high dietary LA may reduce the level of DHA in breast milk. Indeed, there was 7.84% LA (low relative to Western data), and a negative correlation between LA/alpha-linolenic acid ratio and DHA ($r = -0.42$) in the milk of the Karen women. However, both LA (14.57%) and DHA (0.96%) were high in the Korean mothers. LA constitutes about 12% of the energy intake in Israeli population (Yam *et al*., 1996) and the milk has about 20% LA, 0.37% DHA and 0.75% total n-3 metabolites (Budowski *et al*., 1994). These findings suggest that there may be components other than LA in the diet of Western women, which compromise the level of DHA in breast milk.

Energy intake from dietary fat in lactating Korean women ranges between 16 and 19.9% (Lee *et al*., 1995) and in the refugee Karen and rural Thailand mothers less than 15%. Since plant based foods are the major source of dietary lipid of these women, their intake of saturated fat would be less than 4% which is well below the European 15 to 25% range. We postulate that high total and saturated fat intakes of Western women reduce the DHA content of their milk. This postulation is consistent with our experimental study (Ghebremeskel *et al.*, 1998), which showed that suckling pups born to rats fed high total and saturated fat diet, akin to western foods, during pregnancy and lactation had about 25% lower DHA in liver CPG and EPG compared with the controls.

Should infant formula, like human milk, incorporate AA and DHA in order to meet requirements and maintain balance between them? Or, is it sufficient to add the parent compounds LA and ALA in the hope that requirements for AA and DHA will be achieved indirectly? Is it safe to incorporate AA and DHA in infant formula?

For millions of years, the human infant has been nurtured on breast milk with its integral constituents AA and DHA during the time of rapid cerebral expansion. Hence, the question of safety is only pertinent with regard to the chemical form and the minimum and maximum amounts which need to be incorporated in the milk formulation. A lower growth curve has been reported in preterm infants fed formula supplemented with fish oil as a source of DHA (Carlson *et al.*, 1996a, Montalto *et al.*, 1996). The formulae used in these studies were devoid of AA and contained a high level of EPA and thus were not comparable to breast milk. In subsequent studies, in which both AA and DHA were used, the adverse effect on growth was not observed.

It is well established that the human neonate can synthesise AA and DHA from LA and ALA respectively (Poisson *et al.*, 1993, Carnielli *et al.*, 1996, Salem *et al.*, 1996, Sauerwald *et al.*, 1997). The question is, does the rate of conversion is fast enough to keep pace with the rapid growth and demand of the brain, endothelium and cell membranes and other vital tissues? Studies in young adult and adult male subjects show that the conversion of LA to AA and ALA to DHA does not meet daily requirements in US diet (Emken *et al.*, 1994, Emken *et al.*, 1998). Koletzko *et al.*, (1996), by the use of isotope balance equation, have estimated that endogenous synthesis contributed only about 23% of total plasma AA in human infants born at full term. Similarly, Gerster (1998) has reported that the conversion of ALA to EPA and DHA was 6% and 3.8% respectively when the background diet is high in saturated fat in human adults. In a study of preterm neonates, Leaf *et al.* (1992) have observed that the plasma AA is reduced to a third of its initial value despite a three-fold increase in its precursor LA between birth and three weeks of age.

Studies show that breast fed infants have significantly higher levels of DHA in their cerebral cortex compared with those fed formula milk devoid of the fatty acid (Farquharson *et al.*, 1992, 1995, Makrides *et al.*, 1994). These studies question the safety of omitting DHA from formula milk since experimental animal data show an association between reduction of brain DHA and impaired development.

Babies fed breast milk (Makrides *et al.*, 1993, Birch *et al.*, 1993, Jorgensen *et al.*, 1996) and formula with DHA, or DHA and AA had better visual acuity and cognitive ability (Makrides *et al.*, 1995, Carlson *et al.*, 1996ab, Birch *et al.*, 1992, 1998 and Willatts *et al.*, 1998) compared with those fed standard formula milk which do not contain AA and DHA. In contrast, Auestad *et al.*, (1995) did not find any difference in pattern sweep-visual evoked potential and in preferential looking acuity between babies fed breast milk, formula milk with or without AA and DHA or formula milk with DHA only. One of the difference of the study of Auestad *et al.* (1995) compared with that of Birch *et al.* (1998) and Willatts *et al.* (1998) was the DHA content of the formulae. The two formulae used by Auestad and colleagues contained 0.12% (AA+DHA group) and 0.2% DHA (DHA group). The rationale for incorporating small amount of DHA is that apparently the breast milk of some Western women contains about 0.1% DHA. Koletzko *et al.* (1988) reported 0.18% DHA and 0.35% AA in breast milk of German nursing women. However, even in these mothers the total n-6 (Σ20:2 + 20:3 + 20:4 + 22:4) and n-3 (Σ20:3+20:5 + 22:5 + 22:6) metabolites were 0.85% and 0.41% respectively.

In some hospitals in South Korea, soon after delivery, mother and baby are kept in different rooms and the infants are commonly fed on formula milk. Our data show that the formulae are significantly inferior in AA, DHA and total n-6 and n-3 metabolites compared with the Korean breast milk. It is obvious that the babies are being denied maternal milk rich in these essential nutrients during their crucial period of development.

In agreement with some of the previous published human milk data from the non-Western countries, our investigation shows higher levels of DHA and total n-3 metabolites in the milk of refugee Karen, rural Thai and urban Korean mothers. In contrast, lower proportions of these nutrients have been documented in Western women. This difference is not explained by intakes of marine products. We suggest that the levels of DHA and total n-3 metabolites may be compromised in breast milk of mothers on the Western high fat diet. Moreover, our finding questions the use of DHA composition of such human milk as a reference for the formulation of milk designed, for infant feed or, to test the function of DHA in neuro-visual development.

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CONFLICT OF INTEREST

The Authors have no conflict of interest to declare.

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