Original Article

The Influence of Maternal Ethnic Group and Diet on Breast Milk Fatty Acid Composition

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Abstract

Introduction: Breast milk fatty acids play a major role in infant development. However, no data have compared the breast milk composition of different ethnic groups living in the same environment. We aimed to (i) investigate breast milk fatty acid composition of three ethnic groups in Singapore and (ii) determine dietary fatty acid patterns in these groups and any association with breast milk fatty acid composition. Materials and Methods: This was a prospective study conducted at a tertiary hospital in Singapore. Healthy pregnant women with the intention to breastfeed were recruited. Diet profile was studied using a standard validated 3-day food diary. Breast milk was collected from mothers at 1 to 2 weeks and 6 to 8 weeks postnatally. Agilent gas chromatograph (6870N) equipped with a mass spectrometer (5975) and an automatic liquid sampler (ALS) system with a split mode was used for analysis. Results: Seventy-two breast milk samples were obtained from 52 subjects. Analysis showed that breast milk ETA (Eicosatetraenoic acid) and ETA:EA (Eicosatrienoic acid) ratio were significantly different among the races (P = 0.031 and P = 0.020), with ETA being the highest among Indians and the lowest among Malays. Docosahexaenoic acid was significantly higher among Chinese compared to Indians and Malays. No difference was demonstrated in n3 and n6 levels in the food diet analysis among the 3 ethnic groups. Conclusions: Differences exist in breast milk fatty acid composition in different ethnic groups in the same region, although no difference was demonstrated in the diet analysis. Factors other than maternal diet may play a role in breast milk fatty acid composition.

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Introduction

Human milk is the ideal food which provides the complete nutritional requirements for infants during the first 6 months of life. The lipids accumulated in an infant represent the majority of all energy retained in the growing tissues during this crucial period of rapid growth and development. The biological importance of the fatty acid composition of human milk for an infant's growth and development has therefore prompted extensive research in this field.

The role of long-chain polyunsaturated fatty acids (LCPs) with chain length of 20 and 22 carbon atoms has received special attention because the supply of LCPs during early life may be of particular relevance for growth and maturation of the nervous system.[1,2] High concentrations of polyunsaturated fatty acids have also been shown to inhibit growth and mucus adhesion of probiotic bacteria.[3] Certain fatty acids like alpha-linolenic acid (representing the essential n-3 fatty acid) may have anti-inflammatory capacities while linoleic acid (representing the major essential fatty acid of the n-6 series) may have the reverse effect.[4] Alpha-linolenic acid is ultimately converted to docosahexaenoic acid (DHA), a fatty acid important for the development of the brain and retina of the infants.

Dietary intake, mobilisation of body fat stores and endogenous synthesis by mammary glands are the sources of fatty acids in human milk.[5] The influence of diet on

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breast milk fatty acid composition has been suggested by earlier studies.[6,7] Recent studies from Asia have shown the differences in breast milk composition from various regions within the same countries.[8,9]

However, there has been no published study to date looking at the breast milk composition of the different ethnic groups living in the same region. Singapore's population consists of 3 major ethnic groups: Chinese, Malays and Indians. Although these 3 groups live in the same environment, their culture and diets are quite distinct. This background provides us with the opportunity to explore the effects of ethnicity as well as the different ethnic diets on breast milk fatty acid composition.

In this study, we aimed to (i) investigate the fatty acid composition in the breast milk of lactating mothers in the 3 main ethnic groups in Singapore: Chinese, Malays and Indians, and (ii) determine any possible difference in the fatty acid patterns in the diet intake of the mothers and how it influences the fatty acid concentration in the breast milk.

Materials and Methods

We recruited healthy pregnant women who were attending antenatal clinics at a tertiary hospital in Singapore. Women were recruited from the outpatient obstetric clinic by one research assistant who was an experienced lactation consultant. Mothers were considered eligible for participation if they were at more than 36 weeks' gestation at the time of delivery, expressed an intention to breastfeed, and had no illness that would contraindicate breastfeeding or severely compromise its success. Women who agreed to participate were required to give written informed consent. The study was approved by the Institutional Review Board of the Yong Loo Lin School of Medicine, National University of Singapore. We conducted our study in conjunction with the Singapore Clinical Research Institute, which is an independent organisation. This unit performed the trial coordination, site monitoring, data collection and analysis for this study according to Good Clinical Practice guidelines.

After informed written consent was obtained, a trained research coordinator then profiled their diet using a standard validated 3-day food diary. After delivery, these mothers were helped to initiate, establish and continue breastfeeding. A breastfeeding diary was given to the mothers to record their breastfeeding practice. Breast milk (5 mL) was collected from mothers at 1 to 2 weeks, 6 to 8 weeks, 3, 6 and 12 months after delivery (as long as the mother was breastfeeding).

Experimental Details

Calibrators: The fatty acids chosen for analysis were the n3 fatty acids: Docosahexaenoic acid (DHA), α-Linolenic acid (ALA), Eicosatrienoic acid (EA) and the n6 fatty acids: Linoleic acid (LA), Arachidonic acid (AA), Eicosatetraenoic acid (ETA) and Eicosadienoic acid (EDA). The calibrators, mixtures containing major fatty acids (SIGMA Aldrich Sweden AB, Stockholm, Sweden), were prepared in various concentrations (1 mg/ml, 0.8 mg/ml, 0.7 mg/ml, 0.5 mg/ml, 0.4 mg/ml, 0.2 mg/ml 0.1mg/ml, 50 ppm, 10 ppm, 5 ppm and 1 ppm). The calibrators were prepared in matrix; a mixture of 1 g/L Bovine serum albumin and 1 g/L of sodium azide. The calibration curves were obtained. The intra-day and inter-day calculations were carried out and the accuracy of the method was determined. AA:DHA, ALA: AA and ETA: EA ratios were also calculated.

Instrumentation: Agilent gas chromatograph (6870N) equipped with a mass spectrometer (5975) and an automatic liquid sampler (ALS) system with a split mode was used for the analysis. Capillary column: 30-m length and 0.32 mm i.d; Omegawax-320 (Supelco, Bellefonte, PA) were used for the separation of the methyl esters. Helium was used as the carrier gas with a linear velocity of 60 cm/sec.

Analysis of the breast milk samples: To 100 µl of the breast milk sample, 50 µl of deuterium substituted (d_{33}) Heptadecanoic acid (0.8 mg/ml in hexane) was added as internal standard. The breast milk mixture was then mixed with 2 ml of methanol: acetyl chloride (20:1 v/v) in a glass tube closed with teflon-lined caps. The glass tube was incubated at 70°C for 90 minutes. After the tube was cooled, 5 ml of 6% K_2CO_3 was added. To this mixture, 0.4 ml of hexane was added and after vortexing, the upper layer containing the fatty acid esters (FAMEs) was removed. The removed fatty acid esters were purified by centrifugation at 13000 RPM for 8 minutes and then transferred to the automatic injector vials equipped with glass inserts.[10] Relative concentration of the fatty acid esters were determined by running the samples in a SCAN mode in Gas Chromatography-Mass Spectrometry.

Results

In total, there were 72 breast milk samples obtained from 52 subjects. These breast milk samples were collected from 5 days to 417 days after delivery. The mean values for each fatty acid item in different time range were listed in Tables 1, 2 and 3. One significant finding from the analysis was the decreasing trend of Arachidonic acid (AA) (n6 fatty acid) and Arachidonic acid: Docosahexaenoic acid ratio (AA:DHA) with time after delivery. The other fatty acid which showed significant trend with time was (ETA) (n6 fatty acid) (Table 1).

The breast milk composition was then compared among the 3 ethnic groups of Chinese, Malays and Indians. The different time-points measured were 1 to 2 weeks, 6 to 8 weeks, 3, 6 and 12 months after delivery. Analysis at 1 to 2 weeks showed that ETA and ETA:EA of breast milk

Table 1. Mean Values for Breast Milk Fatty Acids across Different Time-points

Fatty Acid Mean (SD) (percentage concentrations)	1-2 weeks(n = 26)	6-8 weeks(n = 19)	3 months(n = 10)	6 months(n = 11)	12 months(n = 6)	Trend(*P* value)
LA(n6)	51.7 (11.6)	56.4 (10.6)	48.8 (13.9)	46.8 (9.8)	42.4 (5.5)	-(0.052)
AA(n6)	4.3 (1.7)	4.0 (1.6)	3.4 (2.5)	3.2 (2.3)	1.5 (0.4)	-(0.002)
DHA(n3)	4.3 (1.8)	4.3 (2.7)	3.5 (2.4)	4.7 (4.0)	3.7 (1.7)	-(0.590)
ALA(n3)	4.6 (2.0)	5.7 (3.4)	5.1 (3.1)	6.0 (2.3)	4.6 (1.4)	+(0.627)
ETA(n6)	3.9 (1.8)	2.1 (0.7)	2.4 (1.4)	4.1 (2.9)	1.1 (0.6)	-(0.043)
EDA(n6)	3.3 (1.2)	1.6 (0.7)	2.0 (1.8)	2.4 (1.4)	3.1 (2.6)	+(0.864)
EA(n3)	1.2 (0.5)	1.7 (1.2)	1.5 (1.3)	2.3 (1.7)	1.1 (0.7)	+(0.500)
AA:DHA(n3)	1.1 (0.4)	1.1 (0.5)	1.2 (0.8)	0.8 (0.4)	0.5 (0.3)	-(0.006)
ALA:AA(n6)	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)	+(0.079)
ETA:EA(n3)	3.5 (1.4)	1.8 (1.4)	2.2 (1.2)	2.6 (2.2)	1.5 (1.4)	-(0.110)

fatty acid appeared to have significant difference among the three different ethnic groups ($P = 0.031$ and $P = 0.020$), with ETA being the highest among Indians and the lowest among Malays (Table 2).

Breast milk analysis at 6 to 8 weeks showed that LA (n6 fatty acid), AA (n6 fatty acid), DHA (n3 fatty acid), ETA (n6 fatty acid), EA (n3 fatty acid) and ETA:EA ratio were significantly different among the ethnic groups. ETA continued to be the highest among Indians and the lowest among Malays. DHA (n3 fatty acid) was significantly higher among Chinese compared to Indians and Malays (Table 3).

In conjunction with the study, food diet analysis was performed for 42 out of the 52 subjects. No statistically significant difference was demonstrated in the content of n3 and n6 among the three ethnic groups. Statistically significant differences were found in the protein and saturated fat content among the ethnic groups (*P* values 0.035 and <0.017 respectively). The protein content of the diet was highest among the Chinese and lowest among the Malays whereas the saturated fat content was highest among the Malays and lowest among the Chinese.

Smaller numbers of breast milk samples were collected after 6 weeks postdelivery. Breast milk fatty acid analysis at the time points of 3 months, 6 months and 12 months did not show any statistically significant results.

Discussion

This is the first study to demonstrate differences in the fatty acid composition of breast milk among different ethnic groups staying in the same environment. ETA (Eiocosatetraenoic acid) which is a n6 fatty acid was highest among the Indians and lowest among the Malays. This statistically significant difference was demonstrated from 1 to 2 weeks after delivery to 6 to 8 weeks postnatally. This

Table 2. Breast Milk Fatty Acid Analysis at 1 to 2 Weeks after Delivery

Fatty Acid Mean (SD) (percentage concentrations)	Chinese (n = 14)	Malay (n = 5)	Indian (n = 4)	Kruskal-Wallis Test (*P*)
LA(n6)	53.1 (12.5)	46.6 (10.4)	53.1 (10.4)	0.563
AA(n6)	4.1 (1.7)	4.0 (1.8)	5.6 (1.7)	0.353
DHA(n3)	4.5 (1.8)	3.9 (1.9)	4.2 (1.9)	0.667
ALA(n3)	4.8 (2.3)	4.5 (1.6)	4.0 (2.0)	0.883
ETA(n6)	4.2 (1.3)	2.3 (0.7)	5.4 (3.2)	0.031
EDA(n6)	3.5 (1.3)	2.2 (0.9)	3.5 (1.0)	0.122
EA(n3)	1.1 (0.4)	1.3 (0.6)	1.3 (0.5)	0.819
AA:DHA(n3)	1.0 (0.5)	1.1 (0.2)	1.4 (0.4)	0.110
ALA:AA(n6)	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)	0.628
ETA:EA(n3)	3.9 (1.1)	2.0 (1.1)	4.3 (1.4)	0.020

Table 3. Breast Milk Fatty Acid Analysis at 6 to 8 Weeks after Delivery

Fatty Acid Mean (SD) (percentage concentrations)	Chinese) (n = 11)	Malay (n = 4)	Indian (n = 3)	Kruskal-Wallis Test (*P*)
LA(n6)	61.3 (8.1)	42.8 (5.8)	56.4 (8.4)	0.017
AA(n6)	4.8 (1.1)	2.1 (1.0)	3.9 (1.8)	0.019
DHA(n3)	5.6 (2.6)	2.2 (0.7)	2.2 (1.5)	0.008
ALA(n3)	6.6 (3.7)	3.0 (1.2)	6.3 (2.8)	0.039
ETA(n6)	2.0 (0.5)	1.8 (0.8)	2.9 (0.3)	0.049
EDA(n6)	1.5 (0.4)	1.2 (0.5)	2.6 (1.2)	0.123
EA(n3)	2.4 (1.1)	0.7 (0.3)	0.8 (0.3)	0.004
AA:DHA(n3)	0.9 (0.3)	0.9 (0.2)	1.9 (0.6)	0.052
ALA:AA(n6)	0.1 (0.0)	0.1 (0.0)	0.1 (0.1)	0.234
ETA:EA(n3)	0.9 (0.3)	2.6 (0.8)	3.9 (1.3)	0.003

finding translated to significant difference in ETA to EA ratio (representing n6 to n3 ratios). The scientific basis for these ethnic differences is unclear. One postulation is that the inherent genetic factors in each ethnic group influence the composition of breast milk for their infants.

Statistically significant differences were demonstrated in a greater number of breast milk fatty acids by 2 months postpartum compared to 2 weeks after delivery at which time only ETA was different. The differences in fatty acid composition in breast milk were likely to be more significant during the 6 to 8 weeks compared to 1 to 2 weeks analysis, due to the cumulative effects of breastfeeding over the two months period. We are also the first group to demonstrate the decreasing trend of Arachidonic acid (AA) (n6 fatty acid) and Arachidonic acid: Docosahexaenoic acid ratio (AA:DHA) with time after delivery.

Perinatal supply of polyunsaturated fatty acids is believed to be essential during pregnancy and lactation.[11] As DHA has been postulated to improve visual and cognitive development in term infants, intense research investigating the levels and significance of breast milk DHA in various regions of the world has been generated.[12-14] An interesting finding from our study was the significantly higher level of DHA among the Chinese compared to the Indians and Malays at 6 to 8 weeks.

Our study did not show any significant difference in the n3 and n6 levels in the food diet analysis among the three ethnic groups. Our results were consistent with the study done by Jirapinyo et al[9] which showed that the DHA content in breast milk of mothers from one area in Thailand were higher than those from other areas but no correlations between history of DHA intake and DHA content in the breast milk could be demonstrated. Francois et al[15] also showed that supplementation of flaxseed oil, which is a rich source of DHA in the diet, did not lead to an increase in breast milk DHA contents.

These results therefore raised the postulation that the differences in the level of DHA and other fatty acids in the breast milk could be related to other factors such as genetic predisposition rather than the food consumed. Although our data did not demonstrate any difference in the n3 level from the food diet nutrient analysis between the different race groups, this could be limited by the small sample size. This question remains to be answered in future trials. The lack of definite correlation between breast milk DHA levels and normal dietary DHA intake also leads to controversies about the beneficial effects of DHA supplementation in milk powder for lactating mothers or infants. However, we did not study women who specifically took DHA supplements. Breast milk fatty acid analysis at the time points of 3 months, 6 months and 12 months did not show any statistically significant results. This could be the true effects or related to the smaller number of breast milk samples collected at these time points.

A limitation of our study is the small sample size. However, the results of our study could be used as a basis for future research work. Deeper understanding of the factors which can influence breast milk lipid composition among different ethnic groups can form the basis of an important infant and child health promoting strategy.

Conclusion

Our study has shown for the first time that differences exist in breast milk fatty acid composition in different ethnic groups staying in the same environment, although no difference was demonstrated in the diet analysis. The differences in the DHA level in breast milk among the different racial groups will encourage larger trials and allow various new hypotheses to be generated and knowledge to be widened.

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