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Fatty acid composition of Bengali mothers' milk : A survey based population study

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Abstract : This study is aimed to investigate the fatty acid profile of Bengali mothers' milk. Total 108 mothers were included in this study. All the mothers were belonging to the medium income group with average monthly income of Rs. 5000–10000. Mothers of Kolkata and its two adjacent areas, North and South 24-Parganas are included in this study. Their nutritional status was documented by a food frequency questionnaire which consists the usual foods taken by the mothers. The percentage of medium chain fatty acids ($C_{8:0}$ to $C_{14:0}$) are of special importance as they are increased with increasing consumption of carbohydrate rich foods. The amount of n-6 PUFA is 12.88 ± 0.83 and n-3 PUFA is 3.61 ± 0.33. Two essential fatty acids, linoleic acid (LA, $C_{18:2}$ n-6) and α-linolenic acid (ALA, $C_{18:3}$ n-3) are abundantly present in green leafy vegetables which are passed in human milk through diet of the mothers. Long chain polyunsaturated fatty acids (LCPUFAs) are metabolites of these two essential fatty acids. Oily fish and vegetable oils are recognized as dietary sources of n-3 and n-6 PUFAs respectively. Among LCPUFAs docosahexaenoic acid ($C_{22:6}$ n-3) and arachidonic acid ($C_{20:4}$ n-6) are of special importance as these two fatty acids play important roles in brain and visual acuity development in newborns.

Keywords : Human milk, socio-economic status, fatty acids, LCPUFA.

Introduction

Human breast milk is universally accepted as the best nutritious food for both term and preterm infants. Besides carbohydrate, protein and fat fractions, it contains many health factors like immunoglobulin A and G (Ig A and Ig G), various enzymes, growth factors, antibodies, lactoferrin, minerals etc. The lipid fraction of human milk provides not only the required energy to the infant, but also essential fatty acids as well as fat soluble vitamins. About 96% of human milk fat is triacylglycerols (TGs), remainder are phospholipids and cholesterol[1]. TGs are composed of different fatty acids of which *sn-2* position is generally occupied either by palmitic acid ($C_{16:0}$) or by stearic acid ($C_{18:0}$)[2]. Among various fatty acids, long chain polyunsaturated fatty acids (LCPUFAs) play important roles in different physiological functions. Essential fatty acids such as linoleic acid (LA, $C_{18:2}$ n-6) and α-linolenic acid (ALA, $C_{18:3}$ n-3) are the precursors of LCPUFAs like arachidonic acid (AA, $C_{20:4}$ n-6) and docosahexaenoic acid (DHA, $C_{22:6}$ n-3) which are very much important as their physiological functional arena is fairly large. They are engaged in various physiological processes including apoptosis, gene transcription, neurite outgrowth, membrane excitability, synthesis of prostaglandins (PGs), thromboxane formation, desaturation and elongation of fatty acids, cerebral ischemia, inflammatory and immunological events and membrane fluidity and elasticity, neurotransmitter metabolism, membrane protein function, and protection from oxidative damage[3]. These two fatty acid depositions are also found in retinal rod cells and grey matter of central nervous system, thus AA and DHA are also associated with visual acuity and cognitive function[2].

Fatty acid composition of human milk is influenced by maternal gestational period, age, stage of lactation, parity, and mainly by maternal diet and long-term food habit[4–8]. The fat in human milk is mainly come from maternal body stores, maternal dietary intake and some directly from metabolism of mother's body. For example, EPA (eicosapentaenoic acid, $C_{20:5}$ n-3) and DHA are abundant in milk of mothers those who consume marine fishes more[5]. Mothers consuming carbohydrates rich diet contain a great proportion of medium chain fatty acids (MCFA) in their milk[9]. The content of *trans* fatty acids in the milk lipid also reflect the maternal dietary intake of *trans* fats. Mainly these *trans* fats come from margarines, shortenings etc.[10]. The composition of mothers' milk from many Western, Asian and African countries has already been reported which display the direct relationship of human diet with fatty acid composition of milk[11–18]. Therefore it is expected that country like India, where more or less every state have different food habit, should have a variety of milk fatty acid composition. Moreover, variety of vegetable oil consumption is seen in India which generally adds a major gateway of fatty acids to the milk. The socio-economic status of mothers is also an important factor as their food habit fully depends on it. But still no report is available in India addressing the all above criteria. So, the objective of the present study was to initially start with by analyzing the fatty acid composition of Bengali mothers' milk having medium socio-economic background and residing in and around Kolkata (Fig. 1).

As the centre of collecting samples we chose R. G. Kar Medical College and Hospital, Kolkata; Naihati State General Hospital, North 24-Parganas and Joynagar Health Centre, South 24-Parganas with an idea that all mothers who are admitting here for their children birth are belonging to middle income group.

Results and discussion

All mothers were from medium income group and their educational level was from 4th standard to 9th standard. Most of them were housewives; others were working at various paper mill or jute mill. All of them consumed heavy meals twice a day. The data of the usual food habit of mothers were made by the food frequency questionnaire. All mothers were of non-vegetarian type and they used mustard oil as the main cooking medium with sunflower oil and dalda (hydrogenated fat) in special occasion. Fish was consumed 3-4 days/week by all the mothers. Fruits consumption was very limited by all the mothers and it was limited among banana, mango, cucumber, guava and musambi. Milk and milk products were consumed occasionally.

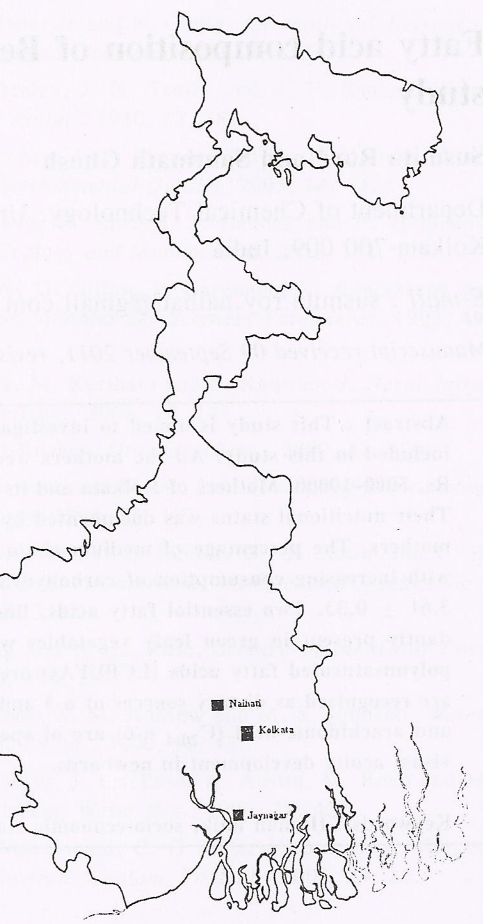

Fig. 1. Three areas of West Bengal from where the samples were collected.

Table 1 shows the average values of different fatty acids of mothers' milk of specified samples. Among the saturated fatty acids, palmitic acid ($C_{16:0}$) has the highest amount. Presence of higher amount of medium chain fatty acids (MCFA; $C_{8:0}$ to $C_{14:0}$) in Bengali mothers' milk than the mothers from Western countries signifies that Bengali mothers consume more carbohydrates rich diet than Western mothers, like mothers of Tsimane and Cincinnati (USA)[21]. It has already been documented that MCFA content increases with more consumption of carbohydrate rich diet. Not only that, but high carbohydrate consumption also can change the phospholipids subclasses of milk[22]. Monounsaturated fatty acids content is of special importance as it comprises about 32.86% of the total fatty acids of milk. Among MUFA, erucic acid ($C_{22:1}$) and nervonic acid ($C_{24:1}$) are present in significant amount in mothers' milk fatty acids. Erucic acid may derived

Table 1. Average value of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) of collected samples from Bengali mothers from in and around Kolkata

Common names	Carbon numbers	% of fatty acids (w/w)
	SFA	
Caprylic acid	$C_{8:0}$	0.73 ± 0.40
Capric acid	$C_{10:0}$	1.53 ± 0.82
Lauric acid	$C_{12:0}$	4.14 ± 1.39
Myristic acid	$C_{14:0}$	5.77 ± 1.77
Pentadecanoic acid	$C_{15:0}$	0.10 ± 0.06
Palmitic acid	$C_{16:0}$	16.80 ± 3.38
Margaric acid	$C_{17:0}$	0.29 ± 0.21
Stearic acid	$C_{18:0}$	3.25 ± 0.94
Arachidic acid	$C_{20:0}$	0.81 ± 0.46
Behenic acid	$C_{22:0}$	1.14 ± 0.71
Lignoceric acid	$C_{24:0}$	0.60 ± 0.33
	Σ SFA	35.17 ± 10.47
	MUFA	
Myristoleic acid	$C_{14:1}$ n-5	0.35 ± 0.14
Palmitoleic acid	$C_{16:1}$ n-7	5.19 ± 1.67
Oleic acid	$C_{18:1}$ n-9	24.16 ± 4.70
Eicosenoic acid	$C_{20:1}$ n-9	0.88 ± 0.45
Erucic acid	$C_{22:1}$ n-9	0.97 ± 0.55
Nervonic caid	$C_{24:1}$ n-9	1.3 ± 0.70
	Σ MUFA	32.86 ± 8.21
	PUFA	
Linoleic acid	$C_{18:2}$ n-6	10.55 ± 3.32
Linolenic acid	$C_{18:3}$ n-3	1.63 ± 0.62
Eicosadienoic acid	$C_{20:2}$ n-6	0.90 ± 0.77
Eicosatrienoic acid	$C_{20:3}$ n-6	0.48 ± 0.20
Arachidonic acid	$C_{20:4}$ n-6	0.55 ± 0.24
Eicosapentaenoic acid	$C_{20:5}$ n-3	0.61 ± 0.55
Docosaletraenoic acid	$C_{22:4}$ n-6	0.40 ± 0.21
Docosapentaenoic acid	$C_{22:5}$ n-3	0.49 ± 0.37
Docosahexaenoic acid	$C_{22:6}$ n-3	0.88 ± 0.45
	Σ PUFA	16.49 ± 6.73

Data are represented as Mean ± SD. n = 108.

from mustard oil which is the primary vegetable oil used by the Bengali mothers, and it is rich in erucic acid (about 45–55% of all fatty acids)[23] and nervonic acid is the chain elongated metabolite of erucic acid. PUFA content of Bengali mothers are lower in most of the cases than Western mothers[21]. Among LCPUFAs ($C_{20:2}$ to $C_{22:6}$ n-3), DHA and AA contents are 0.88 ± 0.08 and 0.55 ± 0.04 respectively and EPA content is 0.61 ± 0.08 in Bengali mothers' milk. Percentage of saturated fatty acids, monounsaturated fatty acids, and n-6 and n-3 PUFAs are shown in Fig. 2. The ratio of n-6/n-3 fatty acids is 3.57 in Bengali mothers' milk. This ratio plays an important role as it has various health effects[1]. The linoleic acid content of adipose tissue is inversely related with cardiovascular diseases. Some vegetables like karela (*Momordica charantia*) and snake-gourd (*Trichosanthes angvina*) contain conjugated linolenic acid (CLnA) which may transported to the milk fatty acids by consumption of these vegetables. CLnA has free radical scavenging activity and anti lipid peroxidation property[24]. It is documented that human milk contains conjugated linoleic acid (CLA) isomers in very low percentage (0.12–0.15%)[25]. The essential fatty acid content in these collected milk samples are sometimes more than the reported value of many studies[1]. Further studies of this area are needed in a broad spectrum for more specification and resolution of different fatty acid concentration and its varying degree with food habit of mothers. Study with randomly selected more subjects can give a clear idea regarding the fatty acid composition of Bengali mothers' milk.

Experimental

Milk sampling :

Total 108 mothers were included in this study, 35 from R. G. Kar Medical College Hospital, Kolkata, 30 mothers from Naihati State General Hospital (North 24-Parganas) and 43 from Joynagar Health Centre (South 24-Parganas). Both verbal and written, singed informed consent were given by the mothers and our study was approved by the Human Ethical Committee of these three institution individually and monitored by the Bioethics Committee for Animal and Human Research Studies of the University of Calcutta, West Bengal, India.

All mothers were from medium income group (MIG, average monthly income Rs. 5000–10000) and their ages ranged between 20 and 30 years. All mothers breastfed their babies exclusively and both mothers and their babies possessed good health. Milk samples were of 7–20 days postpartum and most of the mothers were primiparous, some had two children. Mothers having term infants and non-vegetarian food habit were included in this study. To avoid diurnal variation in fat content of samples, they were collected between 10 a.m. to 11.30 a.m. from

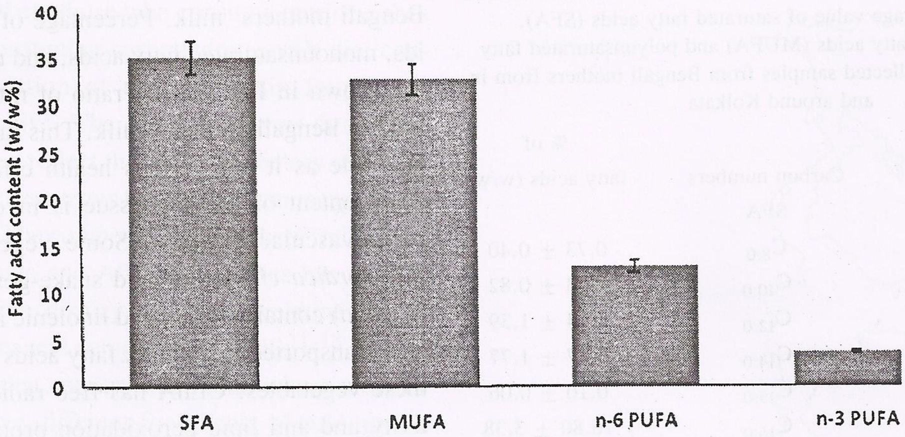

Fig. 2. Saturated, monounsaturated, and n-6 and n-3 polyunsaturated fatty acids of collected milk samples from Bengali mothers in and around Kolkata.

mothers after feeding their baby once in the morning. Samples were manually expressed from one breast at a time into an acid washed sterile glass vial, capped and immediately placed in an ice bucket for transport to the laboratory where they were stored at –20 °C until analysis without any preservative. Dietary data of mothers were made by the food frequency questionnaire. Mothers were asked their day-to-day food habit and a special emphasis was made on the cooking oil they used. Most of the mothers used mustard oil as their main cooking medium, though some used refined sunflower oil for special purpose twice or thrice in a month.

Lipid extraction :

Samples collected from mothers were kept in ice and transferred to laboratory within 3 h and then placed at –20 °C and lipid was extracted by modified Folch method[19]. At the time of lipid analysis, samples were thawed in cold water and heated to 100 °C in boiling water and held it at this temperature for about 1–2 min for lipase inactivation and then vortexed.

1.5 ml of lipase inactivated sample was taken to which 5 ml of 1 (*N*) HCl was added for coagulation of milk proteins. Then 30 ml of chloroform : methanol (2 : 1) was added and 0.01% of TBHQ (tertiary butyl hydroquinone) was mixed to this solution to prevent the oxidation of unsaturated fatty acids. Then it was homogenized for 5 min at 8000 rpm. Then 6 ml of saturated potassium/sodium chloride solution was added to this and centrifuged at 5000 rpm for 15 min at room temperature. The upper aqueous phase was removed. Lower chloroform layer was taken in a separatory funnel and added water to this and shaken vigorously. After layer separation, lower chloroform phase was transferred into a flat bottom flask after drying over anhydrous sodium sulfate. This step was repeated for at least 4 times. After this, the solvent was evaporated to dryness under the stream of nitrogen. All samples were extracted within 2 days of collection.

Milk fatty acid analyses :

Milk contains about 96% of triacylglycerols (TGs) and remaining of the total lipid is free fatty acids. Fatty acids were determined by gas liquid chromatography. For this analysis, TGs were first converted into their corresponding fatty acid methyl esters (FAME) following the method described by Morrison and Smith[20]. FAME were then analyzed by gas chromatograph (Make : Agilent Technologies; 6890 N) with a computerized gas chromatograph network (GC system – G 1530 N) fitted with a glass capillary column, INOWAX (30 meter × 0.32 mm; film 0.25 μm). The temperature was programmed as follows : oven temperature starting at 150 °C for 2 min and rising up to 200 °C immediately after injection, followed by linear heating (15 °C/min) up to 250 °C and held at this temperature for 10.33 min; finally oven temperature was maintained at 270 °C for 16.67 min rising with 4 °C/min. Carrier gas was N_2 flow rate 1 ml/min). The gas chromatograph was calibrated prior to sample injection on each day, and all chemical methods were validated before sample analysis.

Satistical analysis :

All data were analyzed as triplicate and the results were expressed as Mean ± SD (standard deviation).

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