ORIGINAL ARTICLE

Calcium, iron and essential fatty acid composition of bengali mother's milk: a population based cross-sectional study

Susmita Roy[1], Amit Basu[2], Pubali Dhar[3], Mahua Ghosh[4]

[1,4]Department of Chemical Technology, University of Calcutta, Kolkata; [2]Department of Obstetrics and Gynaecology,B.R. Singh Hospital, Eastern Railway, Sealdah, Kolkata; [3]Laboratory of Food Science and Technology, Food and Nutrition Division Department of Home Science, Kolkata.

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Corresponding Author

Address for Correspondence: Dr. Susmita Roy, Oil Laboratory, Department of Chemical Technology, University of Calcutta, 92 A.P.C. Road, Kolkata- 700 009, West Bengal, India.
E Mail ID: susmita.roy.naihati@gmail.com

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Abstract

Background: Extensive literature is available that highlights only a healthy mother ensures the optimal growth of an infant. Human milk fatty acid is the only component which is influenced more by maternal diet. Beside the lipid fraction of maternal milk, micro and macro elements play major roles in execution of development of new-born. **Aims and Objectives:** For the first time, the present study entails to exhibit the relative concentration of essential nutrients of human milk of Bengali mothers with typical Bengali food habit with aims to observe (i) the level of Calcium (Ca), Iron (Fe) (ii) the composition of lipid in Bengali mothers' milk and (iii) maternal dietary habit and its influence on these nutrients. **Materials and Methods:** 19 colostrum, 14 transitional milk and 16 matured milk samples were collected from Bengali mothers, belonging to higher income group (HIG) and medium income income group (MIG). Milk lipid was extracted, and then converted to fatty acid methyl ester to analyse by gas liquid chromatography. Phospholipid content was determined spectrophotometrically. Ca and Fe contents were determined by atomic absorption microscopy. **Results:** Ca content changed in an ascending order throughout the lactation period in both HIG and MIG mothers, so as the lipid content of HIG mothers. Daily intake of Ca is higher in HIG mothers than MIG, but not Fe. Ca content is linearly correlated with maternal age and BMI. **Conclusion:** Ca, Fe and fatty acid composition of mothers' milk are influenced by maternal dietary intake. Linear correlation signifies that demand of calcium to neonate increases as maternal age progress. Eicosapentaenoic acid, arachidonic acid and docosahexaenoic acid are of great importance for neonatal growth which is solely dependent on maternal fish intake. Consumption of mustard oil results in a significant amount of nervonic acid which is an imperative component of nerve tissue.

Key Words

Human Milk; Dietary Habit; Essential Nutrients; Trace Elements; Essential Fatty Acids.

Introduction

Human milk is the exclusive natural source of energy and essential elements to the breast-fed infants. Particularly the lipid portion of maternal milk contributes an important role in physiological and neuronal growth and development of foetus and neonate in both intra-uterine and post parturition life. Macro-elements like Calcium (Ca), Iron (Fe), Zinc (Zn), Selenium (Se) are also important for proper development of the infant. The major macro element Calcium (Ca) in human milk contributes to growth of bones, muscle contraction, transmission of nerve impulses and obviously clotting of blood in wounds. Among micro elements, Iron (Fe) involves in haemoglobin for transport of oxygen and an essential component of enzymes intricate in biological oxidation (Leotsinidiset al, 2005).Human milk constituents, mainly the fatty acid composition is the only component which is influenced more by maternal diet. Fatty acids are placed within the

triacylglycerol (TG) molecules in sn-1, sn-2 and sn-3 positions, which is the determinant of the melting point (MP) of that particular TG. Apart from this stereochemistry, molecular weight, configuration of double/triple bonds, presence of polar groups and –cis or –trans positioning of fatty acids in TG molecule regulates the melting point of fatty acids, leading to a resultant mean melting point of milk lipid (Roy et al, 2013).

Milk fat globules (MFG) are the colloidal assemblies which are the vehicle of mainly TG, retinyl ester, cholesteryl ester in the core enveloped by a biological membrane called milk fat globule membrane (MFGM) consisting of phospholipids, cholesterol, membrane specific proteins and enzymes.The MFGM has gained a lot of attention recently, due to the growing interest in its nutritional, physiological and health properties. MFGM polar lipids have been reported to have various benefits for human health due to their involvement in cell function and transport systems). Zouet al (2012) reported that MFG of different stages of lactation have a substantial difference in the chemical composition. Although the fatty acid composition and distribution of milk fat have a minor impact on the microstructure of MFG, they are of great significance to infant nutrition including supply to the infant the necessary instructions for the development of the intestinal mucosa, of the immune and nervous systems as well as for metabolic activity. Moreover, some components of the MFGM have anti-infection or anti-adhesion properties, and hence can protect the new-borns from various viral and bacterial infections.

Aims & Objectives

There are several studies revealing milk environmental contaminants in human milk worldwide, but limited studies in India, which gains consideration to do a pilot study on influence of maternal diet on milk micronutrients. Our study investigated Ca, Fe, and total lipid (TL), phospholipids (PL) contents of three kinds of milk of two groups of Bengali mothers representing different socio-economic status. A major part focuses on the dietary contribution to essential nutrients.

Material and Methods

Subjects: A total of 49 milk samples from mothers, aged between 20 to 35 years, belonging to higher income group (HIG) and medium income group (MIG), were collected from B.R. Singh Hospital, Eastern Railway, Sealdah, Kolkata. Collected samples were divided into colostral milk (CM, 0-3 days), transitional milk (TM, 4-7 days) and matured milk (MM, 7 days onwards). The socio-economic status (SES) had been assessed throughthe food frequency questionnaires (FFQ), which were filled in during the time of milk sampling and categorized on the basis of monthly income, food habit, literacy rank, occupation with importance of per head income and residential bounds of the respective family, andmothers were asked about their day-to-day food habit and a special emphasis was made on the cooking oil they used.To determine socio-economic status of the subjects, Kuppuswamy's Socio-economic Scalewas used. According to Kuppuswamy, there were different scores on different parameters in respect of various social activities, such as, education score, occupation score, and score on monthly income. These all scores were summed up to get the total score which suggested the socio-economic class of the subject. The parameters of the subjects were noted in the questionnaire and then matched with the respective points in the Kuppuswamy's method and the score was determined.

Before sample collection, the hospital was requested to pass the proposal through respective Human Ethical Committee and finally this study was approved by the Bio Ethics Committee for Animal and Human Research Studies of the University of Calcutta, Kolkata, India.

Sampling: Three to 5 ml of milk samples were collected from each mother. Most of the mothers were primiparous and some with two/three babies. Mothers with term infants and non-vegetarian food habit were included in this study. All mothers breastfed their babies exclusively and both mothers and their babies possessed good health. To avoid diurnal variation in lipid content, milk samples were collected between 10 a.m. to 11:30 a.m. from 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, then 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. Lipid was extracted within two weeks of sample collection. All chemicals and solvents for analysis were of analytical grade and procured from Merck (Mumbai, India).

Anthropometric Data: Data were collected at the time of milk sample collection. Weight and height of the mothers were measured by a portable weighing pan and a portable anthropometric rod respectively. Body Mass Index (BMI, kg/m2) was calculated from these two measurements. Weight and height were cross-checked by the hospital record (both antenatal and postnatal clinic).

Dietary Assessment: Recorded dietary data of mothers were analysed by DietSoft software (Version 1.2.0, Invincible IDeAS 2008-2009), developed by the All India Institute of Medical Sciences (AIIMS), New Delhi, India in collaboration with invincible IDeAS, Noida, India. Mothers did not have dietary restrictions during the last trimester.

Lipid Extraction: Milk lipid was extracted by modified Folch method (Folch, 1957). At first, milk samples were lipase inactivated and then 5 ml of 1(N) HCl was added to 1.5 ml of lipase inactivated milk sample for coagulation of milk proteins. Chloroform and methanol (2:1 v/v) with 0.01% of TBHQ (tertiary butyl hydroquinone) in ethanol (w/v) were mixed to this solution andhomogenized for 5 minutes at 8000 rpm. After that, 6 ml of saturated potassium/sodium chloride solution was added and centrifuged at 5000 rpm for 15 minutes. The upper aqueous phase was discarded and lower chloroform layer was taken in a separatory funnel and shaken vigorously with addition of water. After layer separation, lower chloroform phase was transferred into a flat bottom flask passing through anhydrous sodium sulfate to remove traces of water present in it and repeated for at least 4 times. The samples were made solvent free under the stream of nitrogen.

Milk Fatty Acid Analyses: Fatty acid composition was determined by gas liquid chromatography (Morrison, 1964) after trans-methylation of milk fatty acids to Fatty acid methyl esters (FAME) by boron-trifluoride methanol (14% BF3 in methanol) after saponification of lipid portion. FAMEs were analyzed by an Agilent 6890 N computerized gas chromatograph (network GC system- G 1530 N). A glass capillary column, DB-23 (30 meter × 0.32 mm; film 0.25 µm) was used as the stationary phase. Temperature of analysis was programmed as follows: oven temperature starting at 150 ºC for 2 minutes and rising up to 200 ºC immediately after injection, followed by linear heating (15 ºC/ min) up to 250 ºC and held at this temperature for 10.33 minutes; finally oven temperature was maintained at 270 ºC for 16.67 minutes rising with 4 ºC/ min. Carrier gas was N2 (flow rate 1ml/ min) and injection mode was splitless. Calibration of peaks was done by using Chemstation software and results were expressed in % wt/wt of total fatty acids. The gas chromatograph was calibrated prior to sample injection on each day, and all chemical methods were validated before sample analysis.

Milk Phospholipids Analyses: Phospholipids content of milk samples was determined spectrophotometrically (UV-1700, Pharma Spec, Shimadzu; Tokyo, Japan) by the method of Chen et. al. (1956).

Determination of Calcium and Iron Content of Milk: Calcium and Iron content of mothers' milk were analysed by atomic absorption spectrophotometer (AAS). The raw milk samples were converted to ash at first and an acidic solution was prepared which was analysed in AAS. The ash was prepared by the method of Roig et. al. (1999).

Statistical Analysis: All data were analyzed as triplicate and the results were expressed as Arithmetic Mean ± SEM (standard error of mean). A normality test (one-way ANOVA) was done for all groups to estimate the normal distributions of the samples. A one- tailed t test was used for comparison of means at different fatty acids with the samples of different groups. Potential factors like maternal BMI, energy, carbohydrates, protein and fat intake were selected as independent variables while the mean fat content of breast milk was chosen as the dependent variable (Nikniaz et. al., 2009). OriginLab 8 software was used for statistical analysis. In all measurements, p value less than 0.05 was considered as significant. Linear regression analysis was used to examine correlations between milk lipid content Vs BMI, dietary lipid, Ca and Fe contentsVs milk lipid, Ca and Fe contents, maternal ageVsCa and Fe contents, and between Ca and Fe contentsVs phospholipids content of three types of milk ofboth experimental groups of Bengali mothers.

Results

Table 1 shows the anthropometric measurement data, e.g. maternal age and BMI, and total lipid content, phospholipid content and Ca & Fe contents of collected milk samples.

Table 2 depicts the daily consumption of nutrients from the diet by the mothers of both experimental groups. All the mothers were non-vegetarian in food-nature. None of the women was on strict reducing diets during the third trimester of

pregnancy. At least one serving was rice and others were mainly roti based on wheat flour.

Table 3 describes the detailed fatty acid composition of the collected milk samples. Lauric acid ($C_{12:0}$), which has antimicrobial effect (monolaurate), concentrations of three types of milk of Bengali mothers were noticeably higher than world reported values. Palmitic acid accounts the highest concentration among all saturated fatty acids in three types of human milk of Bengali mothers, whereas, oleic acid makes the same in monounsaturated fatty acids (MUFA).

A Comparison of the total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), n-6 and n-3 polyunsaturated fatty acids (PUFA) of three types of milk lipid of Bengali mothers belonging to two different socio-economic groups are depicted in Table 4. Total n-6 polyunsaturated fatty acid (PUFA) of three type's milk is higher in HIG mothers than MIG mothers, and also for n-3 PUFA.

Maternal age and Ca content has a positive correlation coefficient (R^2= 0.8087) which indicates a linear relationship of Ca content in human milk with mothers' age. Linear correlation signifies that demand of calcium to neonate increases as maternal age progress. Correlations of Ca and Fe with maternal age are shown in Figure 1.

A positive linear correlation of maternal fat intake (g/day) with total lipid content (mg/dL) of mothers' milk (R^2= 0.9515) is also shown in Figure 2.

Discussion

BMI of HIG mothers were higher than that of MIG mothers except transitional milk collected group of mothers. Transitional milk of MIG mothers accounted for highest content of total lipid, otherwise the trend of changing total lipid content was same in other two groups. Changing pattern of phospholipid content was just reverse as lipid content with a significantly plunging tendency from colostrum to matured milk. Ca content increases from colostrum to matured milk in both groups of mothers, but not significantly. Fe content also differed from each group but not significantly.

All the mothers were consuming mustard oil as the major cooking oil along with sunflower oil, soybean oil and hydrogenated fat (vanaspati) in a very small amount. All mothers consumed cow milk and/or milk powder with tea twice every day. Fish was consumed by every mother regularly. Seasonal fruits were consumed by every mother, but higher in HIG mothers. Sugar and iodized salt were common components in all groups of mothers in regular basis. Calcium intake was higher in HIG mothers (2277.73mg) whereas MIG mothers also met the everyday need of calcium by consuming 1152.07mg (Recommended Dietary Allowances, RDA 1000 mg/d) (Gopalan et al., 2007). Free folic acid consumed by MIG mothers (139.93µg) was a little bit higher than that of HIG mothers (136.43µg). In both cases, consumption does not meet the daily requirement (RDA 150µg/d). Iron consumed daily by HIG and MIG mothers, were 47.71 mg and 42.87 mg respectively, though the RDA for iron was quiet lower (30 mg/d). Carbohydrate, protein and fat consumed by both groups of mothers also differ but not significantly.

An important observation was the presence of Nervonic acid ($C_{24:1}$) in Bengali mothers' milk in a significant amount (average value 1.085%, 1.025% and 0.84% respectively in CM, TM and MM). Nervonic acid (NA, $C_{24:1}$n-9) is the chain elongation product of erucic acid ($C_{22:1}$n-9) which is present abundantly in mustard oil (45%-50%), the main cooking medium of Bengali mothers. NA is a very crucial fatty acid as it has an important functional role in forming the myelin sheath covering the neurone which increases the conducting activity of nerves. Among PUFAs LA ($C_{18:2}$n-6) and ALA ($C_{18:3}$n-3) concentrations in Bengali mothers' milk were comparable with other countries as AA, EPA and DHA concentrations were a little bit higher than many countries as reported (Kabir et al, 2003; Ruan et al, 1995; Layea, 1995; Schmeits, 1999; Smit et al, 2001; Sanders et al, 1992; Olafsdottir et al, 2006; Jensen et al, 1995;Scopesi et al, 2001). Countries with more coastal regions, such as Japan, Philippines, and Chile have higher AA and DHA concentrations in mothers' milk lipid than that of Bengali mothers (Yuhaset. al., 2006).

Conclusion

This pilot study evaluates a relationship of maternal diet with every composition of maternal milk. The dietary habits of the Bengali mothers which included fish lipid and mustard oil in a fair amount regulated the fatty acid composition of the milk lipids in TG molecules which otherwise regulates the quality of the maternal milk. Study with more samples can infer more suitably.

Recommendation

This study may have impact on further social/demographic study of Indian mothers regionally. Also it has an importance in maternal and child health data in Indian perspective.

Limitation of the study

Collection of milk samples was a very important as well as robust job for this study. Many superstitions and religious bindings were there among mothers especially in villages, which had to be overcome first to collect samples.

Relevance of the study

The entire study is a part of the thesis work. It may add the current status of maternal and child nutrition to the current knowledge. Also it has a great relevance in nutritional fortification of infant formula in another way.

Authors Contribution

SR: carried out the whole work, analysed data and wrote the article. AB: monitored especially the sample collection part of the said hospital. PD: helped to analyse statistical data and edited the manuscript. MG: mentored over the work and also helped to edit the manuscript.

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Tables

TABLE 1: ANTHROPOMETRIC PARAMETERS, TOTAL LIPID CONTENT, PHOSPHOLIPID CONTENT, CALCIUM AND IRON CONTENTS OF HIG AND MIG BENGALI MOTHERS.

Groups	Colostrum		Transitional Milk		Matured Milk	
Parameters	HIG (n= 11)	MIG (n= 8)	HIG (n= 9)	MIG (n= 5)	HIG (n= 7)	MIG (n= 9)
Maternal age (Years)	26.17±1.51	24.25±1.90	28.33±1.60	28.78±2.19	30.67±1.86	27.00±1.05
BMI (kg/m2)	28.02±1.32	24.42±1.51	24.02±1.50	24.84±1.88	27.36±1.24	25.14±0.51
Total Lipid (g/dL)	4.70±0.88	4.22±0.90	4.94±1.03	5.14±1.07	5.36±1.11	3.87±0.67
Phospholipid (mg/dL)	110.27±23.69	53.23±23.42	78.03±51.91	44.15±25.88	33.31±6.90	25.95±2.38
Calcium (ppm)	2.923±0.88	2.454±0.87	4.253±1.42	3.941±1.76	5.274±1.99	5.065±1.69
Iron (ppm)	0.142±0.04	0.167±0.06	0.143±0.05	0.144±0.06	0.162±0.06	0.173±0.06

- Data are represented as Arithmetic Mean±SEM.
- Superscripts represent significant difference among groups, $p<0.05$.

TABLE 2: AVERAGE DAILY CONSUMPTION OF NUTRIENTS BY EACH MOTHER OF HIG AND MIG MOTHERS.

Nutrients	Higher Income Group (HIG) (n = 27)	Medium Income Group (MIG) (n = 22)
Calcium (mg)	2277.73	1152.07
Carbohydrate (g)	270.31	265.96
Chloride (mg)	126.29	143.93
Choline (mg)	630.20	630.20
Chromium (mg)	0.06	0.07
Copper (mg)	3.36	3.59
Energy (kcal)	2253.92	2131.09
Fat (g)	67.84	68.73
Fibre (g)	13.67	14.35
Folic Acid (µg)	449.84	458.61
Folic Acid, Free (µg)	136.43	139.93
Iron (mg)	41.71	42.87
Magnesium (mg)	461.02	484.54
Manganese (mg)	3.61	3.72
Mineral (g)	22.70	19.72
Moisture (g)	1090.70	1033.79
Molybdenum (mg)	0.62	0.62
Niacin (mg)	19.42	19.62
Phosphorus (mg)	1941.87	1773.87
Potassium (mg)	2415.04	2373.60
Protein (g)	104.92	90.65
Riboflavin (mg)	1.56	1.52
Sodium (mg)	243.47	228.30
Sulphur (mg)	553.72	583.12
Thiamine (mg)	1.69	1.70
Vitamin-A (µg)	1070.61	1063.89
Vitamin-C (mg)	398.33	397.33
Zinc (mg)	7.03	7.30

TABLE 3: FATTY ACID COMPOSITION (% W/W) OF COLOSTRUM, TRANSITIONAL MILK AND MATURED MILK OF HIG AND MIG BENGALI MOTHERS.

Fatty Acids (w/w)	Colostrum		Transitional Milk		Matured Milk	
	HIG (n= 11)	MIG (n= 8)	HIG (n= 9)	MIG (n= 5)	HIG (n= 7)	MIG (n= 9)

Caprylic acid	0.44 ± 0.14	0.67 ± 0.20	0.40 ± 0.20	0.53 ± 0.22	0.56±0.24	0.65±0.12
Capric acid	1.12 ± 0.33	1.12 ± 0.24	0.89 ± 0.16	1.34 ± 0.32	2.28±0.68	1.81±0.33
Lauric acid	2.27a ± 0.46	4.48a± 0.89	2.25b ± 0.18	3.41b ± 0.48	4.77±0.68	5.23±0.37
Myristc acid	3.63c ± 0.55	5.87c ± 0.96	3.99 ± 0.18	3.94 ± 0.38	4.16d±0.49	6.16d±0.53
Myristoleic acid	0.28 ± 0.08	0.32 ± 0.07	0.28±0.03	0.26±0.03	0.31±0.04	0.39±0.04
Pentadecanoic acid	0.49e ± 0.14	2.76e ± 1.14	0.32±0.04	0.49±0.18	0.19±0.03	0.25±0.06
Palmitic acid	19.55f ± 0.69	19.96f± 2.83	19.86±0.47	18.04±1.14	18.65±0.68	17.58±0.85
Palmitoleic acid	1.55± 0.21	0.87 ± 0.31	1.90±0.18	1.84±0.28	2.05g±0.24	3.97g±0.51
Palmitelaidic acid	1.94± 0.24	2.37± 0.31	2.55±0.20	2.66±0.27	2.41±0.29	2.22±0.21
Heptadecanoic acid	0.39 ± 0.11	0.52 ± 0.09	0.32±0.05	0.60±0.28	0.44±0.17	0.39±0.12
Stearic acid	4.35 ± 0.43	3.35 ± 0.63	3.68±0.18	3.28±0.38	3.36±0.16	3.68±0.21
Oleic acid	26.58h± 0.76	26.24h± 3.89	27.01±0.62	24.94±1.68	25.90±0.94	23.24±0.70
Elaidic acid	6.16i± 0.55	3.58i± 1.01	6.07±0.40	5.87±0.54	5.88j±0.71	9.31j±0.68
Linoleic acid	10.99 ± 1.17	8.31 ± 1.98	12.03 ±0.48	9.91 ±0.73	10.85k ±0.68	6.64k ±0.81
trans Linoleic acid	4.69l± 0.50	2.77l± 0.76	3.82±0.18	3.61±0.38	3.65±0.49	2.61±0.40
Linolenic acid	2.91± 0.86	1.46 ± 0.30	1.78±0.12	2.27±0.41	2.39±0.45	1.32±0.14
transLinolenic acid	1.10± 0.24	0.89± 0.24	0.69±0.11	0.79±0.17	0.51±0.15	0.53±0.06
Stearidonic acid	0.92± 0.29	0.71 ± 0.33	0.81m±0.10	0.41m±0.07	0.53±0.09	0.54±0.03
Arachidic acid	1.70 ± 0.20	0.74 ± 0.18	1.47±0.14	1.41±0.26	1.19±0.16	1.10±0.15
Eicosenoic acid	0.95 ± 0.20	0.47 ± 0.09	0.77±0.12	0.82±0.13	0.68±0.15	0.79±0.15
Eicosadienoic acid	0.89 ± 0.16	0.72 ± 0.19	1.21±0.13	1.06±0.19	0.79±0.10	0.80±0.16
Dihomo-γ-linolenic acid (DGLA)	0.59n± 0.08	1.19n± 0.33	0.62±0.06	0.78±0.11	0.54±0.09	0.58±0.07
Arachidonic acid (AA)	0.82 ± 0.17	0.77 ± 0.28	0.88±0.09	0.65±0.06	0.65±0.06	0.46±0.05
Eicosapentaenoic acid (EPA)	0.78± 0.21	0.50 ± 0.15	0.63±0.10	0.59±0.09	0.53±0.07	0.41±0.04
Behenic acid	0.49± 0.07	0.31 ± 0.09	0.51±0.05	0.45±0.06	0.56±0.09	0.58±0.09
Erucic acid	1.41 ± 0.38	0.52 ± 0.11	0.67±0.13	0.93±0.25	0.64±0.13	0.82±0.16
Docosatrienoic acid	0.54 ± 0.15	1.15± 0.53	0.43±0.10	0.52±0.11	0.68±0.17	0.72±0.10
Adrenic acid	0.27 ± 0.06	0.57 ± 0.22	0.59±0.13	0.52±0.23	0.35±0.13	0.36±0.04
Docosapentaenoic acid (DPA)	0.31 ± 0.08	0.34 ± 0.21	0.39±0.08	0.30±0.10	0.62±0.13	0.49±0.09
Docosahexaenoic acid (DHA)	0.43± 0.04	0.32± 0.05	0.48±0.07	0.31±0.04	0.51±0.12	0.51±0.08
Lignoceric acid	0.64 ± 0.21	0.63 ± 0.32	0.62±0.15	0.47±0.14	0.92±0.28	0.63±0.11
Nervonic acid	1.41 ± 0.23	0.76± 0.15	0.99±0.10	1.06±0.16	0.92±0.11	0.76±0.09

- Data are represented as Arithmetic Mean±SEM.
- Superscripts represent significant difference among groups, p<0.05.

TABLE 4: TOTAL SFA, MUFA, PUFA AND VARIOUS RATIOS OF FATTY ACIDS OFCOLOSTRUM, TRANSITIONAL MILK AND MATURED MILK OF HIG AND MIG BENGALI MOTHERS.

Group	Colostrum		Transitional Milk		Matured Milk	
Parameters	**HIG (n= 11)**	**MIG (n=8)**	**HIG (n= 9)**	**MIG (n= 5)**	**HIG (n= 7)**	**MIG (n= 9)**
Total SFA	35.07 ± 3.33	40.41 ± 7.46	34.31 ± 1.40	33.96 ± 3.84	37.08 ± 3.66	38.06 ± 2.94
Total MUFA	40.28 ± 2.65	35.13 ± 5.85	40.24 ± 1.78	38.38 ± 3.34	38.79 ± 2.61	41.50 ± 2.54
Total n-6 PUFA	18.25 ± 2.14	14.33 ± 3.76	19.15 ± 1.07	16.53 ± 1.70	16.83a ± 1.55	11.45a ± 1.53
Total n-3 PUFA	6.99 ± 1.87	5.37 ± 1.81	5.21 ± 0.58	5.19 ± 0.99	5.77 ± 1.18	4.52 ± 0.51
LA/ALA (C18:2/C18:3)	3.91 ± 0.14	4.71 ± 0.18	6.42 ± 0.92	4.42 ± 0.13	5.00 ± 0.18	5.00 ± 0.19
AA/DHA	1.91 ± 0.08	2.41 ± 0.11	1.83 ± 0.03	2.10 ± 0.05	1.27 ± 0.05	0.90 ± 0.02
Σn-6/Σn-3	2.61 ± 0.09	2.67 ± 0.10	3.68 ± 0.16	3.18 ± 0.13	2.92 ± 0.02	2.53 ± 0.03

- Data are represented as Arithmetic Mean±SEM.
- Superscripts represent significant difference among groups, p<0.05.

Figures

FIGURE 1: RELATIONSHIP OF MATERNAL AGE WITH CA AND FE CONTENT OF MOTHERS' MILK. R2 VALUES ARE SHOWN INSIDE THE GRAPH WHICH SUGGESTS CA AND MATERNAL AGE LINEARLY STRONGLY CORRELATES (R2= 0.8087).

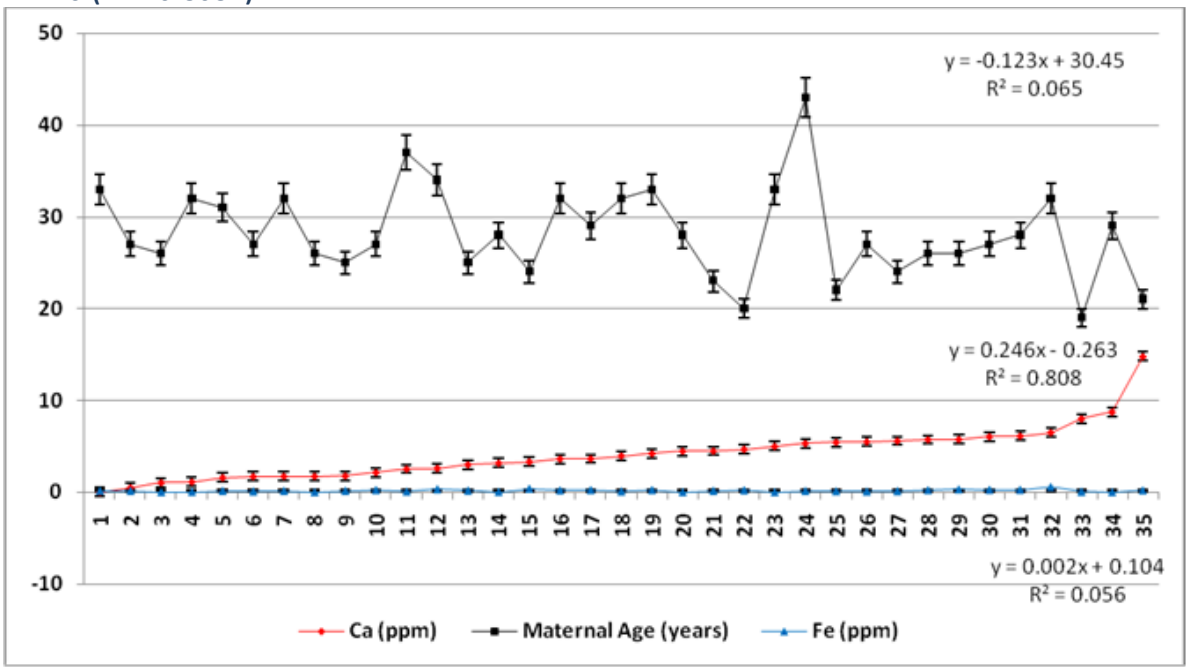

FIGURE 2: LINEAR CORRELATION OF MATERNAL FAT INTAKE (G/DAY) WITH TOTAL LIPID CONTENT (G/DL) OF MOTHERS' MILK (R^2= 0.9515).