Brief Report

Studies on the Fluidity of Milk Lipids of Mothers from Three Socioeconomic Groups of West Bengal, India

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Summary

This study evaluated the fluid character of human milk by determining the mean melting points (MMP) of fatty acids of milk lipid of Bengali mothers. Fatty acid methyl esters were analyzed by gas liquid chromatography. MMPs were calculated from the fatty acid concentration (% w/w) and their molar mass. Phospholipid content of samples was also determined. The MMPs of milk lipid of higher income group ($n = 48$), medium income group ($n = 57$) and lower income group ($n = 112$) mothers were found to be 31.33°C ± 0.61, 36.86°C ± 0.64 and 35.11°C ± 0.65, respectively, which showed a significant correlation with the fatty acid composition, with $P < 0.0001$, 0.003, 0.0001, respectively. The average MMP of milk lipid (34.43°C ± 0.63) of these three groups was below the core body temperature (37.4°C) of human beings, which perhaps helps in maintaining the milk fluidity as well as lipid digestion in breastfed infants.

Key words: *de novo* fatty acids, mean melting point, phospholipid, socioeconomic groups, *trans* fatty acids.

Introduction

Human milk is a natural and superior food for infants, containing optimal composition to meet their nutritional needs in early life, and providing essential polyunsaturated fatty acids (PUFA) to support the growth and development of the breastfed infant [1]. Triglycerides (TG) constitute 98% of the milk lipid, with the remainder being made up by phospholipid and cholesterol [2]. Molecular weight, configuration of double/triple bonds, stereochemistry and presence of polar groups, with interrelationships existing between them, regulate the melting point (MP) of fatty acids [3]. Generally, saturated fatty acids have higher melting points than unsaturated fatty acids [4]. Again, *trans* fatty acids (TFA) have higher melting points than their *cis* counterparts. Milk fat globule membranes are the main source of phospholipids in human milk [5]. The relative fluidity of milk lipid of different human milk specimens is dependent on the mole percentages of the constituent fatty acids and their respective melting points [6, 7].

The objective of this study was to determine the range and mean melting points (MMPs) of milk lipids of the three experimental groups of mothers and to evaluate and establish a correlation between fatty acid composition of milk lipid and MMPs with a further relation to phospholipid content of milk samples.

Methods

An experimental group of 217 nonvegetarian mothers, aged between 17 and 34 years, was involved in this study, which consisted of 48 higher income

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TABLE 1
Amount of foods consumed per day by individual Bengali mother of three different experimental groups

Food items	Consumption/day		
	HIG ($n = 48$)	MIG ($n = 57$)	LIG ($n = 112$)
Cereals			
Rice (parboiled) milled (g)	151.20	150.00	157.40
Wheat flour (g)	88.60	87.80	69.40
Maida (g)	4.45	4.29	4.16
Puffed rice (g)	11.66	16.34	20.69
Flaked rice (g)	5.80	1.77	9.49
Suji (g)	3.58	3.59	4.10
Pulses and legumes			
Lentil (g)	15.17	17.77	18.80
Black gram (g)	6.01	6.52	6.41
Green gram (g)	11.56	10.91	8.93
Bengal gram (g)	2.03	1.69	1.34
Root vegetables			
Potato (g)	29.40	21.40	22.20
Radish (g)	0.36	0.35	0.36
Onion (g)	4.80	5.10	4.40
Colocasia/yam (g)	0.34	0.31	0.34
Carrot (g)	0.32	0.27	0.17
Leafy vegetables			
Cabbage (seasonal) (g)	13.74	15.09	15.57
Spinach (seasonal) (g)	15.26	14.80	16.14
Amaranth (g)	1.31	1.79	2.20
Kalmi sak (seasonal) (g)	1.66	1.75	1.85
Pumpkin leaves (g)	1.87	1.67	1.97
Colocasia leaves (g)	2.59	2.91	3.04
Other vegetables			
Brinjal (g)	12.26	12.43	13.14
Cauliflower (seasonal) (g)	15.37	14.91	13.49
Ladies finger (g)	2.89	2.83	3.14
Drumstick (seasonal) (g)	1.22	1.61	1.72
Ash gourd (g)	1.54	1.69	1.99
Plantain green (g)	0.41	0.32	0.43
Papaya green (g)	0.46	0.89	1.03
Beans (g)	3.06	2.86	2.14
Pumpkins (g)	3.51	3.63	3.89
Bitter gourd (g)	0.19	0.16	0.29
Bottle gourd (g)	0.75	0.45	0.67
Parwar (g)	0.91	0.83	0.94
Fats and Oils			
Mustard oil (ml)	26.13	26.99	28.83
Rice bran oil (ml)	8.50	8.47	5.57
Sunflower oil (ml)	15.00	12.67	8.53
Soybean oil (ml)	9.80	8.40	7.53
Milk and milk products			
Cow/buffalo milk (ml)	246.00	260.00	226
Skimmed milk powder (g)	26.40	24.00	18.40
Butter (g)	1.69	1.43	1.06
Ghee (g)	1.03	1.00	0.86
Flesh foods			
Egg (piece)	3 in a week	2 in a week	2 in a week
Fish (g)	75.71	68.57	53.43
Meat (g)	34.57	30.00	22.00
Sugar and jaggery			
Sugar (g)	10.60	16.00	17.00
Jaggery (g)	Seldom	Seldom	Seldom
Common salt (g)	7.00	8.00	10.60
Seasonal Fruits	Yes	Yes	Yes
Fast foods	Seldom	Seldom	Seldom

group mothers (HIG), 57 medium income group mothers (MIG) and 112 lower income group mothers (LIG). A written and duly signed consent form was taken from each mother. This study was ethically considered by the Bioethics Committee for Animal and Human Research Studies of the University of Calcutta (reference number 3222), Kolkata, India.

About 7–10 ml of term and matured milk samples were collected from the mothers. To avoid diurnal variation in lipid content [8], hind-milk samples were collected between 11 a.m. to 11:30 a.m., just before lunch. All mothers were interviewed, and after enrollment on the study, a dietary data and other physical parameters of mothers were made by the food frequency questionnaire. Exclusion criteria were same as described in our previously published article [9].

Body mass index (BMI, kg/m^2) was calculated after measurement of weight and height by a portable weighing pan and a portable anthropometric rod, respectively.

Lipid in milk was extracted following the modified Folch method [10], as performed in our previously published article [9].

Fatty acid composition of both triglyceride and free fatty acid of milk samples was determined by gas liquid chromatography [11]. TGs were converted into their corresponding fatty acid methyl esters (FAME), as performed in the previous study [9]. FAMEs were analyzed by an Agilent 6890 N computerized gas chromatograph (network GC system G 1530 N) using DB-23 (60 m × 0.32 mm; film 0.25 μm) column.

Phospholipid content of milk samples was determined spectrophotometrically (UV-1700, Pharma Spec, Shimadzu; Tokyo, Japan) by the method of Chen *et al.* [12].

The MMPs of the lipid fractions of the human milk were calculated using the mol% of fatty acids and their respective molecular weight [6, 7]. A total of 30 fatty acids were included in this calculation. The first step was to determine the mol% of each fatty acid, which was done by dividing the mass % of each fatty acid by their respective molecular weights. Next, each mol% of fatty acid was multiplied by the MP of that particular fatty acid, and to that 100 was added. Finally, these values were summed up, and 100 was subtracted from the sum to give the final estimate of the lipid's MMP.

$$Mol\ \%\ of\ fatty\ acid = \text{Mass \% of fatty acid}/\text{Molecular weight of fatty acid}$$

$$\text{Mean melting point} = \sum_{k=0}^{n} [\text{Mol \% of each fatty} \times (\text{Melting point of that particular fatty acid} + 100)] - 100$$

All data of fatty acids were analyzed as triplicate, and the results were expressed as Mean ± SEM. Level of significance of various parameters in different groups was performed by one-way analysis of variance (Tukey's test) followed by independent two-tailed t-test; $P < 0.05$ was considered as significant. Linear regression analysis was used to examine correlations between MMPs and mol% of fatty acids, and between MMPs and phospholipids content of three experimental groups of mothers.

Results

Foods consumed by Bengali mothers were analyzed by food frequency questionnaire. A day-to-day dietary habit and the principle foods consumed by Bengali mothers were summed-up in Table 1. Main cooking medium was mustard oil. Fish was regularly consumed by all. Green leafy vegetables were consumed in higher amount by LIG mothers. Fish, cooking oils and various green leafy vegetables were the main sources of n-3 and n-6 fatty acids in Bengali mothers' diet.

Total lipid content, phospholipids content, parity and BMI of the mothers of three experimental groups are shown in Table 2. It was interestingly noted that BMI was linearly proportional to total lipid content, whereas MMPs were linearly proportional to phospholipids content of the three experimental groups of Bengali mothers.

The fatty acid composition of the three groups of subjects is depicted in Table 3.

TABLE 2
Total lipid content (g/dl), phospholipids content (mg/dl), parity and BMI (kg/m^2) of three experimental groups of Bengali mothers

Groups	Total lipid (g/dl)	Phospholipids (mg/dl)	Parity	BMI (kg/m^2)
HIG ($n = 48$)	5.77 ± 0.42	57.84 ± 15.15	1.54 ± 0.22	25.49 ± 0.86
MIG ($n = 56$)	5.83 ± 1.49	79.70[a] ± 27.68	1.57 ± 0.12	24.35 ± 0.85
LIG ($n = 105$)	3.35[b,c] ± 0.47	60.61[c] ± 8.95	1.68 ± 0.15	21.98[b] ± 0.52

Data are represented as Mean ± SEM.
[a]Significant at <0.05 in HIG vs. MIG.
[b]Significant at <0.05 in HIG vs. LIG.
[c]Significant at <0.05 in MIG vs. LIG.

TABLE 3
Fatty acid composition (% wt/wt) of milk lipid of three experimental groups of Bengali mothers

Fatty acids	Common names	Percentage (wt/wt)		
		LIG	HIG	MIG
Saturated fatty acid (SFA)				
Caprylic acid	$C_{8:0}$	0.3 ± 0.06	$0.60^a \pm 0.08$	$0.61^b \pm 0.07$
Capric acid	$C_{10:0}$	0.8 ± 0.18	$1.47^a \pm 0.19$	$1.24^b \pm 0.15$
Lauric acid	$C_{12:0}$	1.39 ± 0.23	$4.33^a \pm 0.31$	$3.11^b \pm 0.22$
Myristic acid	$C_{14:0}$	4.43 ± 0.45	6.75 ± 0.49	4.81 ± 0.26
Pentadecanoic acid	$C_{15:0}$	0.55 ± 0.14	0.29 ± 0.05	$0.09^b \pm 0.007$
Palmitic acid	$C_{16:0}$	8.13 ± 1.49	$16.63^a \pm 0.65$	$17.49^b \pm 0.60$
Margaric acid	$C_{17:0}$	0.41 ± 0.06	0.5 ± 0.11	$0.29^c \pm 0.03$
Stearic acid	$C_{18:0}$	2.0 ± 0.30	$3.25^a \pm 0.20$	$3.39^b \pm 0.12$
Arachidic acid	$C_{20:0}$	1.12 ± 0.14	1.26 ± 0.13	0.83 ± 0.07
Behenic acid	$C_{22:0}$	0.55 ± 0.06	0.72 ± 0.09	$1.38^b \pm 0.10$
Lignoceric acid	$C_{24:0}$	0.54 ± 0.09	0.67 ± 0.08	0.61 ± 0.04
	Σ SFA	20.30 ± 3.20	$36.48^a \pm 2.38$	$33.85^b \pm 1.67$
Monounsaturated fatty acid (MUFA)				
Myristoleic acid	$C_{14:1n\text{-}5}$	2.98 ± 0.35	$0.28^a \pm 0.02$	$0.93^b \pm 0.02$
Palmitoleic acid	$C_{16:1n\text{-}7}$	12.81 ± 1.27	$3.68^a \pm 0.38$	$4.59^b \pm 0.22$
Palmitelaidic acid	$C_{16:1n\text{-}9}$ *trans*	1.51 ± 0.18	2.73 ± 0.16	2.89 ± 0.06
Oleic acid	$C_{18:1n\text{-}9}$	28.37 ± 1.81	27.04 ± 0.88	26.43 ± 0.86
Elaidic acid	$C_{18:1n\text{-}9}$ *trans*	1.92 ± 1.42	2.31 ± 0.30	$3.12^b \pm 0.23$
Eicosenoic acid	$C_{20:1n\text{-}9}$	1.33 ± 0.14	0.87 ± 0.08	0.85 ± 0.06
Erucic acid	$C_{22:1n\text{-}9}$	0.59 ± 0.06	1.02 ± 0.12	0.99 ± 0.06
Nervonic caid	$C_{24:1n\text{-}9}$	0.66 ± 0.13	1.21 ± 0.11	$1.42^b \pm 0.07$
	Σ MUFA	50.17 ± 5.36	$39.14^a \pm 2.05$	$41.22^b \pm 1.58$
PUFA				
Linoleic acid (LA)	$C_{18:2n\text{-}6}$	16.58 ± 1.43	15.35 ± 0.80	$11.86^{b,c} \pm 0.48$
α-Linolenic acid (ALA)	$C_{18:3n\text{-}3}$	4.80 ± 0.54	2.48 ± 0.21	$1.86^b \pm 0.10$
Stearidonic acid	$C_{18:4n\text{-}3}$	0.68 ± 0.08	0.54 ± 0.05	0.29 ± 0.02
Eicosadienoic acid	$C_{20:2n\text{-}6}$	0.86 ± 0.12	0.65 ± 0.09	0.77 ± 0.03
Dihomo-γ-linolenic acid	$C_{20:3n\text{-}6}$	0.98 ± 0.11	0.51 ± 0.03	0.52 ± 0.03
Arachidonic acid	$C_{20:4n\text{-}6}$	0.64 ± 0.06	0.57 ± 0.05	0.59 ± 0.03
Eicosapentaenoic acid	$C_{20:5n\text{-}3}$	0.89 ± 0.12	0.54 ± 0.06	0.33 ± 0.02
Docosatrienoic acid	$C_{22:3n\text{-}3}$	0.83 ± 0.13	0.88 ± 0.10	0.24 ± 0.02
Adrenic acid	$C_{22:4n\text{-}6}$	0.45 ± 0.10	0.47 ± 0.10	0.41 ± 0.03
Docosapentaenoic acid	$C_{22:5n\text{-}3}$	0.56 ± 0.09	0.54 ± 0.07	0.52 ± 0.05
Docosahexaenoic acid	$C_{22:6n\text{-}3}$	0.66 ± 0.09	0.54 ± 0.06	$1.03^c \pm 0.07$
	Σ PUFA	27.93 ± 2.87	23.07 ± 1.62	$18.42^{b,c} \pm 0.88$
Σ PUFA:Σ SFA		1.38 ± 0.90	0.63 ± 0.68	0.54 ± 0.53
LA:ALA	$C_{18:2\ n\text{-}6}$:$C_{18:3\ n\text{-}3}$	3.45 ± 2.65	$6.19^a \pm 3.81$	$6.38^b \pm 4.80$
Σ *n*-6:Σ *n*-3		2.32 ± 1.73	3.18 ± 1.95	3.31 ± 0.07
Σ *de novo* fatty acid	$C_{8:0} + C_{10:0} + C_{12:0} + C_{14:0}$	6.96 ± 0.92	13.15 ± 1.07	9.77 ± 0.70
Σ *trans* fatty acid		3.43 ± 1.60	5.04 ± 0.46	6.01 ± 0.29

Data are represented as Mean ± SEM.
HIG $n = 48$, MIG $n = 57$, LIG $n = 112$.
[a]Significant at <0.05 in HIG vs. MIG.
[b]Significant at <0.05 in HIG vs. LIG.
[c]Significant at <0.05 in MIG vs. LIG.

MMPs of Bengali mothers' milk lipid are shown in Table 4.

The correlation between MMPs and mol% of fatty acids in HIG, MIG and LIG mothers are shown in Fig. 1A–C, respectively, and they showed a good correlation with a $P < 0.0001$, 0.003, 0.0001, respectively in HIG, MIH and LIG mothers. There was also a significant correlation between MMPs and phospholipids of the milk of three groups of Bengali mothers ($P < 0.0001$, $r^2 = 0.940$).

Discussion

This study was designed to evaluate the MMPs of the different fatty acids elucidating the fluidity of the milk lipids, and its correlation with the mol% of those fatty acids along with the phospholipids content of the three experimental groups of mothers. Average MMP of these three groups of mothers was found to be 34.43°C, which happened to be several degrees lower than the core body temperature

TABLE 4
MMPs of three experimental groups of Bengali mothers

Groups	MMP (°C)
HIG ($n = 48$)	31.33 ± 0.61
MIG ($n = 56$)	$36.86^a \pm 0.64$
LIG ($n = 105$)	$35.11^b \pm 0.65$
Average	34.43 ± 0.63

Data are represented as Mean ± SEM.
[a]significant at <0.05 in HIG vs. MIG.
[b]significant at <0.05 in HIG vs. LIG.

(37.4°C). MMPs of milk fatty acids from different ethnic groups in Nigeria and Nepal were found to be 35.6°C [6], which was about 1° higher than the result obtained by our investigation. The difference of dietary pattern of Bengali mothers and those of Nigerian and Nepalese women may be responsible for the lowering of MMP.

Linear proportionality of MMPs with the phospholipid contents was studied, where with greater the amounts of milk phospholipid, higher MMP values were observed. Also higher the total *de novo* fatty acids in the milk, higher the MMP values were observed. In the present study, we observed that total *de novo* fatty acids amounted highest in MIG mothers' milk, which reflected the more consumption of dietary carbohydrates by the MIG mothers [7, 13]. This result indicated the ability of mammary glands to synthesize triacylglycerol (TAG), which would be liquid at body temperature [7].

No significant correlation was observed between *trans* fatty acids and MMP of milk lipid.

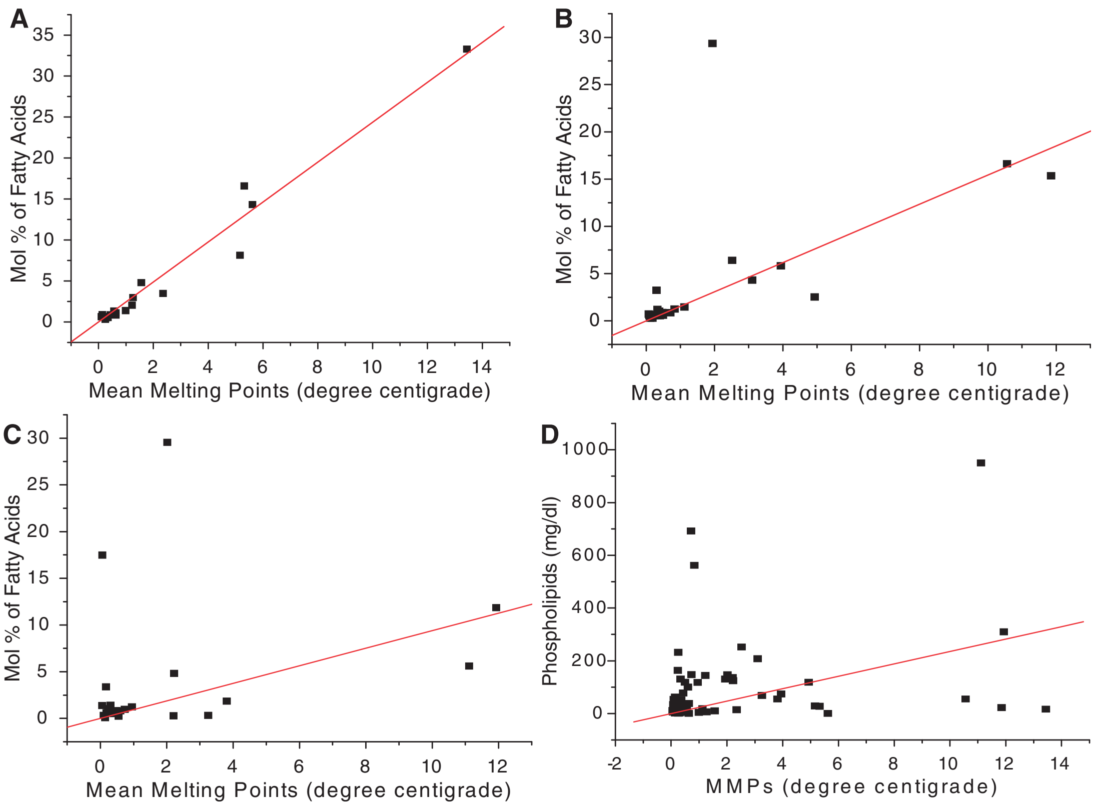

FIG. 1. Correlation between mol% of fatty acids and their respective MMPs in three different experimental groups of Bengali mothers. (A) HIG mothers. $y = 2.471\times - 0.2274$, $P < 0.0001$, $r^2 = 0.968$. (B) MIG mothers. $y = 1.3299\times + 1.4775$, $P < 0.003$, $r^2 = 0.365$. (C) LIG mothers. $y = 2.0732\times + 0.0428$, $P < 0.0001$, $r^2 = 0.927$. (D) Correlation between average values of MMPs of fatty acids and their respective phospholipids in three experimental groups of Bengali mothers' milk lipid. $y = 15.55\times - 55.69$, $P < 0.0001$, $r^2 = 0.940$.

The observations of the study were significant because they elucidated the importance of the biological concept of a set point for the MMP of the fatty acids of the triacylglycerols of human 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, leading to the setting of MMP. The positional distribution of different fatty acids in the triacylglycerol back bone may regulate the fluidity of Bengali mothers' milk and requires further investigation.

References

1. Innis SM. Human milk: maternal dietary lipids and infant development. Proc Nutr Soc 2007;66: 397–404.
2. Innis SM. Dietary triacylglycerol structure and its role in infant nutrition. Adv Nutr 2011;2:275–83.
3. Knothe G, Dunn RO. A Comprehensive evaluation of the melting points of fatty acids and esters determined by differential scanning calorimetry. J Am Oil Chem Soc 2009;86:843–56.
4. Svennerholm L. The chemical structure of normal human brain and Tay-Sachs gangliosides. Biochem Biophys Res Comm 1962;9:436–41.
5. Rustan AC, Drevon CA. Fatty acids: structures and properties. In: Encyclopedia of Life Sciences. Oslo, Norway: John Wiley & Sons, Ltd., 2005. www.els.net.
6. Glew RH, Elliott JA, Huang YS, *et al*. Constancy of the fluidity of the milk lipids of three different human populations. Nutr Res 2002;22:1231–41.
7. Jensen R, Patton S. The effect of maternal diets on the mean melting points of human milk fatty acids. Lipids 2000;35:1159–61.
8. Jensen RG. Lipids in Human Milk. Lipids 1999;34: 1243–71.
9. Roy S, Dhar P, Ghosh S. Comparative evaluation of essential fatty acid composition of mothers' milk of some urban and suburban regions of West Bengal, India. Int J Food Sci Nutr 2012;63:895–901.
10. Folch J, Lees M, Sloane-Stanley GH. A simple method for the isolation, and purification of total lipids from animal tissues. J Biol Chem 1957;226:497–509.
11. Morrison WR, Smith LM. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron trifluoride methanol. J Lipid Res 1964;5:600–8.
12. Chen PS, Toribara TY, Warner H. Microdetermination of phosphorus. Anal Chem 1956;28:1756–8.
13. van Beusekom CM, Martini IA, Rutgers HM, *et al*. A carbohydrate-rich diet not only leads to incorporation of medium-chain fatty acids (6:0-14:0) in milk triglycerides but also in each milk-phospholipid subclass. Am J Clin Nutr 1990;52:326–34.