

Lipid content and fatty acids composition of mature human milk in rural North China

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(Received 8 May 2009 – Revised 15 September 2009 – Accepted 19 September 2009 – First published online 14 October 2009)

To determine the lipid content and fatty acid (FA) composition of breast milk from fifty-two lactating women between ninth and twelfth lactation weeks in rural North China. The mothers were questioned on their dietary habits. Total milk lipids extracts were transmethylated and analysed using GLC to determine FA contents. The mean lipid content was 40.21 (SD 1.43) g/l. SFA constituted 35.92 % of the total FA. Medium-chain and long-chain SFA presented levels of 10.91 and 25.01 %, respectively. MUFA and PUFA constituted 32.59 and 19.97 % of the total FA, respectively. Oleic, linoleic and α -linolenic acid (ALA) presented contents of 31.26, 17.73 and 1.03 %, respectively. Arachidonic acid had a content of 0.30 %, while DHA content was 0.19 %. Not any form of *trans* FA were found in human milk samples. A maternal diet transition is proceeding in China. Further investigation on the analysis of human milk FA composition is needed to upgrade the human milk database in China.

Human milk: Fatty acids: Dietary habits: Lipid content

Human milk provided by healthy and well-nourished mothers is considered the optimal form of nourishment for infants during the first 6 months of life⁽¹⁾. In terms of its macronutrients, the lipids in human milk represent the major source of energy for the newborn and provide essential nutrients such as essential fatty acids (FA), i.e. ALA and long-chain PUFA (LC PUFA)⁽²⁾. The biological significance of the FA composition of human milk for newborns and their development has led to widespread research^(2–5).

There are three sources of FA in human milk: diet; mammary gland synthesis; tissue mobilisation⁽⁶⁾. It is generally known that the worldwide human milk FA composition is subjected to inter-individual biological variation, mostly because of different maternal diets^(4,7,8). Although there have been several reports about the FA compositions of human milk in China^(5,9–12), according to data obtained from the China Health and Nutrition Survey (2002) and the China National Nutrition Survey (1982 and 1992), within the last two decades, the Chinese have entered a new stage of the nutrition transition that is shifting towards a high-fat, high-energy density and low-fibre diet⁽¹³⁾. Meanwhile, China has experienced great socio-economic development processes and social environmental changes⁽¹⁴⁾, thus it is essential to update the human milk database for Chinese people.

The purpose of the present study is to prospectively evaluate the lipid content and FA composition of human milk, from 9 to 12 weeks of lactation and to roughly relate these findings with the breast-feeding mothers' diet in Northern China.

Methods

Characteristics and dietary habits of subjects

From May to July 2007, fifty-two women between ages 19 and 35 years who had been lactating for 1–3 months were recruited into the study. The exclusion criteria were maternal use of tobacco, use of immunosuppressive drugs, pregnancy, and diabetes mellitus. All mothers agreed to provide samples of breast milk at 9–12 weeks postpartum. A face-to-face interview was conducted with mothers using a structured questionnaire by trained investigator at the mothers' homes. All mothers completed the 24-h dietary recall before the collection of the milk. Computer Dietary Guide Service System 4.0 (CDGSS 4.0, Beijing, China) is utilised to analyse the nutrient intake. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Medical Ethics Research Board

Abbreviations: ALA, α -linolenic acid; FA, fatty acid; LC PUFA, long-chain PUFA; MC SFA, medium-chain SFA; TFA, *trans* FA.

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(Approval no. IRB00001052-07037) of Peking University. Written informed consent was obtained from all subjects.

Collection of human milk

A small volume of breast milk (approximately 1–5 ml) was collected adhering to Neville & Picciano⁽⁸⁾. The samples were prepared and stored according to Bitman *et al.*⁽¹⁵⁾ Analyses were performed within 3 months of collection.

Fatty acids analysis

The preparation of the FA methyl esters was conducted by the method described by Hartman & Lago⁽¹⁶⁾. Briefly, aliquots of extracted lipids were saponified and boron trifluoride–methanol (14%, w/v) was used for transesterification of the fractions⁽¹⁷⁾. FA methyl esters were separated and identified by SHIMADZU GC-2010 gas chromatograph (Kyoto, Japan). Each FA was identified by comparing the retention time with that of internal standard (heptadecanoic acid methyl esters, Sigma-Aldrich Co., St Louis, MO, USA). The final FA profile was expressed as percentage of total fat for each FA. Peaks lower than 0.01% of the total areas were omitted.

Statistical analysis

Results were presented as mean values and standard deviations. All data were analysed using Statistical Packages for Social Sciences version 11.0 (SPSS Inc., Chicago, IL, USA).

Results

Mother's dietary habits

Table 1 lists foods currently consumed by the mothers. Rice, noodle, pastry, green leaf vegetables, eggs, fats and oils were consumed everyday by a majority of mothers. As for the 24-h dietary recall study, the average of the energy intake is 652.81 kJ. The mean of fat intake is 104.93 g/d, the energy intake of it is 225.71 kJ/d, which takes up 34.56% of the total energy intake. The average of carbohydrate intake is 369.26 g/d, which means 54.05% of the energy intake is from the carbohydrate, 353.02 kJ/d.

Breast milk lipid content and fatty acid concentrations

The total lipid content and the FA composition of the human milk samples are shown in Table 2. The mean lipid content was 40.21 g/l. Twenty-five FA were observed, representing more than 88% of the total FA. The main FA in the mature breast milk samples were oleic acid (18:1n-9, 31.26%), palmitic acid (16:0, 18.68%), linoleic acid (18:2n-6, 17.73%). Not any form of *trans* FA (TFA) were found in human milk samples. Arachidonic acid and DHA represented 0.3 and 0.19% of total FA, respectively. The 18:2n-6/18:3n-3 was 17.22, which was relatively high.

Discussion

The mean lipid content of human milk of the lactating mothers was 40.21 g/l, which is near the upper limit of the usually reported range for human milk (35–40 g/l)⁽¹⁸⁾. It has been recognised that the total lipid content of human milk is influenced by a series of factors such as the gestational age at birth, mothers' dietary habits and diurnal rhythm^(6,19). In our present study, mothers' eating habits may be one reason to influence the lipids contents in milk. Along with the development of the China's national economy, the traditional Chinese diet is shifting towards a diet with high-fat, high-energy density and low-dietary fibre⁽¹³⁾, this may predominantly contribute to the high level of the lipid content in the breast milk. It can also be seen from the frequency of food consumption (Table 1), most of the lactating mothers consume meat, fat and oils daily, which are main sources for the lipid in human milk. Consistently, fat accounts for 34.56% of the total energy intake according to 24-h dietary recall survey.

SFA accounted for 35.92% of the total FA (Table 2). Among them, medium-chain SFA (MC SFA) represented 10.91% of total FA, which are in harmony with those in the most affluent countries^(20,21). However, MC SFA in our study are notably lower than those reported in some developing countries^(17,18,22). Relative to the long-chain SFA, MC SFA are preferentially absorbed and metabolised by neonates⁽²³⁾. The higher portion of MC SFA might be advantageous for the fat and Ca absorption, and they represent a rich source of energy⁽²⁴⁾. High-carbohydrate, low-fat diets commonly consumed in many developing countries are regarded as enhancing endogenous MC SFA biosynthesis in the mammary gland during lactation⁽²⁵⁾. In our present

Table 1. Frequency of food consumption by the mothers (% of subjects, *n* 52)

Food	Frequency of consumption				
	Every day	≥ twice a week	Once a week	≥ once ≤ thrice a month	Seldom or never
Rice, noodle, pastry etc. (staple food)	85.0	15.0	–	–	–
Chinese potato, yam etc. (rootstock food)	22.5	37.5	5.0	10.0	25.0
Pumpkin, carrot, tomato etc.	35.0	25.0	7.5	12.5	20.0
Green leaf vegetables	65.0	7.5	10.0	10.0	7.5
Pea, soyabean and bean products	10.0	10.0	20.0	12.5	47.5
All kinds of fruits	47.5	22.5	12.5	5.0	12.5
Eggs	60.0	20.0	2.5	2.5	15.0
Dairy products	30.0	20.0	10.0	12.5	27.5
Meat and meat products	52.5	27.5	10.5	–	10.0
Fats and oils	90.0	2.5	–	–	7.5

Table 2. Total lipid content, fatty acid (FA) composition and FA ratios of mature breast milk from the lactating women(Mean values and standard deviations, *n* 52)

	Mean	SD
Total lipid content (g/l)	40.21	1.43
FA (% by weight of total FA)		
SFA		
4:0	0.60	0.11
6:0	0.07	0.02
8:0	0.21	0.07
10:0	1.39	0.46
12:0	4.71	1.06
13:0	0.01	0.01
14:0	3.92	0.97
15:0	0.09	0.05
16:0	18.68	3.98
17:0	0.25	0.13
18:0	5.63	2.36
20:0	0.03	0.02
22:0	0.05	0.02
23:0	0.28	0.08
24:0	0.02	0.01
Total SFA	35.92	7.34
MUFA		
14:1 <i>n</i> -5	0.04	0.02
16:1 <i>n</i> -7	1.29	0.63
18:1 <i>n</i> -9 <i>cis</i>	31.26	3.72
Total MUFA	32.59	7.21
<i>n</i> -6 PUFA		
18:2 <i>n</i> -6	17.73	4.24
18:3 <i>n</i> -6	0.33	0.09
20:2 <i>n</i> -6	0.17	0.04
20:3 <i>n</i> -6	0.23	0.12
20:4 <i>n</i> -6	0.30	0.15
Total <i>n</i> -6 PUFA	18.75	4.56
<i>n</i> -3 PUFA		
18:3 <i>n</i> -3	1.03	0.19
22:6 <i>n</i> -3	0.19	0.08
Total <i>n</i> -3 PUFA	1.22	0.29
FA ratios		
18:2 <i>n</i> -6/18:3 <i>n</i> -3	17.22	4.78
20:4 <i>n</i> -6/22:6 <i>n</i> -3	1.56	0.35

study, 85% of the investigated mothers do have eaten rice, noodle, pastry etc. (staple food) every day. Thus, further studies are needed to elucidate the reasons for the relatively lower MC SFA compared with some developing countries.

Confusingly, we did not detect any form of TFA, and it should be considered as beneficial to the infant's nutrition⁽²⁶⁾. TFA typically represent 2–5% of the FA in dairy fats and ruminant meats⁽²⁷⁾. According to the eating habits of the donors in our work, they indeed frequently consume ruminant meat, which is also a major source of TFA. It is conceivable that probably there are some peculiarities for the Chinese lactating women's diets, which result in the decrease of the *trans* isomers in human milk. However, this will await further elucidation. Also, it is noteworthy that boron trifluoride–methanol does generate much *trans* fats in comparison to other methylation methods⁽²⁸⁾. This may contribute to the lack of TFA in our detection.

MUFA accounted for 32.59% of the total FA in the milk lipid of the lactating women. The major fraction was formed by *cis* monoenoic FA. The dietary intake of monoenoic FA may have physiologic effects by influencing membrane fluidity, cholesterol metabolism and FA peroxisomal

β -oxidation^(29,30). In relation to the lactating women's eating habits, the main source of these FA was probably vegetable oils, such as soyabean oil, which contributed to their final content.

PUFA were 19.97% of the total FA. The levels of PUFA were slightly higher than those found for the North American and European populations (10.0–16.6%)^(21,31–33). The essential FA, linoleic (18:2*n*-6), with a content of 17.73%, was similar to the range (8.2–17.2%) reported by Schmeits *et al.*⁽¹⁷⁾ for populations in different regions of the world. The main source of fat for the rural Chinese women is vegetable oil, mainly soyabean oil, which means that the concentrations of linoleic acid are high in human milk.

The essential FA ALA (18:3*n*-3) presented a content of 1.03%. It could be inferred that the ALA level found in the milk in the present study was rather high if range values reported for the world population (0.10–1.00%)^(9,17,34) are considered. Of note, although the high content of 18:3*n*-3, the 18:2*n*-6/18:3*n*-3 ratio was 17.22, which was higher than the desirable range (5–15:1) among the reported investigations. There is a worldwide concern about polyunsaturated oil consumption increase and, consequently, linoleic acid content increase, which may harm *n*-3 LC PUFA biosynthesis through elongation–desaturation mechanism⁽³⁵⁾. This work suggests that the high levels of linoleic acid found in the milk lipid may interfere in the biosynthesis of this FA group. Meanwhile, it is indicated that the diet of Chinese mothers was less balanced with regard to the levels of *n*-6 and *n*-3 PUFA.

LC PUFA showed a content of 0.88% in the milk lipid of the donors. Except for 20:4 (arachidonic acid), small proportions of *n*-6 LC PUFA (20:2 and 20:3) were also identified. Among the *n*-3 LC PUFA, the only FA identified was DHA (0.19%). A representative range being generally accepted for DHA was with 0.20–0.30%⁽²⁰⁾. The DHA concentration in breast milk is associated with mothers' food intake, especially that of fish⁽³⁶⁾. Thus, it should be recommended that lactating women in this area should eat more fish.

In conclusion, the milk from rural Northern China lactating mothers contained high levels of oleic, linoleic and ALA, but low in medium-chain SFA compared to the reports in other countries. The present findings also reflect the maternal diet transition that is proceeding in China.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (nos 30471449, 30271119, 30671759 and 30872115). There are no conflicts of interest.

Z.-X. W. performed the FA measurement, data analyses and drafted the paper. X.-L. W. designed the study and contributed to draft the manuscript. L. X. and Q. G. aided with the FA measurement. Y. Z. was the chief of the study and was responsible for the whole project.

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