

Original Article

The fatty acid composition of colostrum in three geographic regions of China

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Purpose: To describe the fatty acid composition of human colostrum in three different regions of China. **Methods:** Women were recruited from river/lake, coastal and inland regions of China during late pregnancy. Their diet frequency was assessed during the 34th week of pregnancy. Colostrum samples were collected between postpartum days 3 and 5. Thirty-one different fatty acids were separated and identified in colostrum. **Results:** There were significant differences among the women in the three regions with respect to food and culinary oil consumption patterns. The mean±SD fat content (g/100 g) of colostrum from river/lake, coastal and inland regions was 2.17±1.26, 2.50±1.39 and 2.68±1.26, respectively ($p=0.265$, adjusted by pregnant women's age). The main saturated, monounsaturated, n-6 polyunsaturated and n-3 polyunsaturated fatty acids in colostrum were 16:0, 18:1n9, 18:2n6 and 18:3n3, respectively. Colostrum from river/lake region had the lowest composition of total SFA and of total n-6 PUFA and the highest composition of total MUFA and of total n-3 PUFA. The amount of DHA (22:6n3) in colostrum (mean±SD, % wt/wt) was 0.51±0.18, 0.52±0.20 and 0.35±0.13 in the river/lake, coastal and inland regions, respectively ($p=0.0002$, adjusted by pregnant women's age). Frequency of maternal marine fish intake during the late pregnancy was positively correlated with DHA in colostrum ($r=0.203$, $p=0.040$). **Conclusions:** The fatty acid composition of colostrum differs across geographical regions in China, which may be related to differences in maternal dietary pattern.

Key Words: human colostrum, fatty acids, marine fish, rapeseed oil, docosahexaenoic acid

INTRODUCTION

Colostrum is the first food consumed by breast-fed neonates, and it provides the initial postnatal nutrient supply to the exclusively breastfed infant. The fat content and fatty acid composition of human colostrum are variable around the world, and can be influenced by many factors.¹⁻³ Long chain polyunsaturated fatty acids (LCPUFAs) are important components of colostrum, and of breast milk, because they promote neural and visual development in the newborn.^{4,5} They also appear to be important in immune development.^{6,7} The content of LCPUFAs in breast milk could be modified by maternal diet; for example, increased maternal intake of the n-3 LCPUFA DHA increased human breast milk DHA content.⁸ Aquatic foods, especially marine oily fish, are good sources of n-3 LCPUFAs. Therefore, it is expected that colostrum n-3 LCPUFA composition will vary according to quantity and quality of aquatic food consumption during pregnancy and lactation.

There are a number of reports on the fatty acid composition of human colostrum worldwide^{1,3,9-22} and some studies on the fatty acid composition of human transitional and/or mature milk in China.²³⁻²⁵ However, there is

little information on the fatty acid composition of colostrum in the Chinese population²⁶ and there is no Chinese database for this. Cultural and biological differences between Chinese and Caucasians, and among Chinese from different regions, might affect the fatty acid composition of human colostrum. Therefore, we analysed the fat content and fatty acid composition of colostrum from breast feeding Chinese women living in three different regions of China.

MATERIALS AND METHODS

Subjects

Women were recruited during late pregnancy; they were free of health problems based on medical physical examinations and did not smoke. The women who took lipid-

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rich nutritional supplements such as cod liver oil during their pregnancy were exclusive to our study. The women were recruited in three regions of China. These were an inland region close to large sources of freshwater (river/lake region; Jurong; Jiangsu Province), a coastal region (Rizhao; Shandong Province), and an inland region with limited access to freshwater (Xushui; Hebei Province). All women were long-term residents of their respective region and were of the Han ethnic group. Their age before pregnancy and height were recorded and they completed an investigator-led frequency questionnaire about main lipid-rich foods in their diet, in the latest month before the 34th week of pregnancy. The consumption of culinary oil in the latest month before the 34th week of pregnancy was quantitative in the questionnaire. Our questionnaire was adapted from an earlier study,²⁷ and the main adaptation highlighted the consumption frequency of almost all species of common edible fish in China. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethical Committee of the Chinese Centre for Disease Control and Prevention. Written informed consent was obtained from all subjects.

Colostrum samples

Colostrum samples (circa 5 ml) were collected by the women, who each exclusively breast-fed only one neonate born at term. Samples were collected at post-partum day 3 to 5. All samples were expressed directly into plastic containers and were frozen immediately at -40°C and later at -70°C. The samples were analyzed as soon as possible after collection.

Samples analysis

Total lipids were extracted from 3 g of colostrum by 45 ml chloroform-methanol (2:1, by volume, containing 30 mg/L butylated hydroxytoluene). The mixture was emulsified with 0.85% NaCl (12 ml) and the lower layer was collected. The fat was quantified gravimetrically after the lower layer was dried at 40°C. Fatty acid methyl esters (FAME) were prepared with methanol-acetyl chloride (100:15, by volume) at 70°C in a water bath for 3 hours and were dissolved in hexane. Analysis of FAME was carried out in a Shimadzu GC-14B GC fitted with a CP-SIL-88 capillary column (100 m * 0.25 mm internal diameter, 0.2 µm film thickness). Helium was used as the carrier gas, with a flow rate of 2 ml/min. The split ratio was 40:1. The injector and the flame ionization detector temperatures are both 260°C. The oven program had an

initial temperature of 140°C which subsequently increased to 240°C at a rate of 4°C/min. The peaks of FAME were identified by comparing the retention time with that of certified standards (Supelco 37 Component FAME Mix). The percentage of fatty acids was calculated by normalization method. Peaks of which area is lower than 0.05% of total area were omitted.

Statistical analysis

Data are expressed as mean and standard deviation (mean ± SD). ANOVA was used to test the difference of consumption of foods and culinary oils among regions. ANCOVA was used to test the difference of composition of each kind of fatty acid after the adjustment of age among regions. Bonferroni t test was used to test inter-group differences. Chi-square test was used to test the difference of rates of passive smoking and of alcohol intake among regions. Correlation coefficient was used to assess the relationship between consumption of rapeseed oil or frequency of marine/freshwater fish intake and colostrum content of alpha linolenic acid (ALA) or DHA. A value for $p < 0.05$ was considered to indicate statistical significance among regions. All analysis of the data was carried out by Statistical Analysis System 9.1 (SAS Institute Inc., Cary, NC, U.S.).

RESULTS

Subject characteristics

36 women from the river/lake region, 35 women from the coastal region and 32 women from the inland region were recruited. Height did not differ among regions, but women in the coastal region were older than in the other two regions. Pregnancy duration was slightly, but significantly, longer in the coastal region. There was no difference of rate of passive smoking ($p=0.538$) and of alcohol intake ($p=0.335$) among regions (Table 1). All mothers delivered only one infant. We were unable to obtain accurate weight information from these pregnant women because of the difference in latitude among the three regions that caused different climates, and increased the difficulty to accurately estimate the weight of clothes worn by these women.

Dietary intake of lipid-rich foods and culinary oils

Women in the coastal region consumed more frequency marine fish and meat than those in the other two regions, while those in the river/lake region consumed more frequency freshwater fish and milk. Women in the inland region consumed more frequency of eggs than those in

Table 1. Characteristics of the women and their pregnancies

	River/lake region (n=36)		Coastal region (n=35)		Inland region (n=32)	
	Mean	SD	Mean	SD	Mean	SD
Age before pregnancy (y) [†]	24.8 ^a	2.4	28.8 ^b	4.1	25.7 ^a	2.8
Height (cm) [†]	160	4.3	161	3.8	160	4.7
Pregnancy duration (weeks) [†]	39.4 ^a	1.1	40.1 ^b	1.0	39.8 ^a	0.9
Parity before this pregnancy [†]	0.11 ^a	0.4	0.50 ^b	0.56	0.25 ^{ab}	0.44
Rate of passive smoking (%) [‡]	25.0		31.4		37.5	
Rate of alcohol intake (%) [‡]	5.71		0.00		6.25	

[†]Different letters in the same row denote significant differences among groups ($p < 0.05$).

[‡]The rates of passive smoking and alcohol intake mean percentage of subjects in each region who smoke passively or have the habit of alcohol intake.

Table 2. Frequency of consumption of lipid-rich foods and culinary oils by women in the different regions in the latest month before the 34th week of pregnancy

	River/lake region (n=36)		Coastal region (n=35)		Inland region (n=32)	
	Mean	SD	Mean	SD	Mean	SD
Marine fish (times/week)	0.40 ^a	0.60	4.26 ^b	2.35	0.66 ^a	0.62
Freshwater fish (times/week)	3.60 ^a	1.99	1.14 ^b	1.05	2.13 ^c	2.64
Meat (times/week)	5.47 ^a	4.34	10.7 ^b	4.41	7.98 ^c	4.37
Poultry (times/week)	2.25	3.55	1.28	0.94	1.43	1.04
Eggs (times/week)	6.19 ^a	3.34	5.74 ^a	2.96	9.52 ^b	7.55
Milk (times/week)	10.1 ^a	5.83	5.98 ^b	3.77	6.01 ^b	6.91
Bean (times/week)	4.73 ^a	4.80	6.10 ^a	2.95	10.8 ^b	9.18
Nuts (times/week)	5.38 ^a	6.08	5.51 ^a	6.11	10.1 ^b	8.92
Total culinary oil (100 g/month)	13.4	5.95	13.4	4.25	12.7	3.25
Total vegetable oil (100 g/month)	13.0	5.85	13.4	4.25	11.9	3.13
Rapeseed oil (100 g/month)	9.97 ^a	5.12	0.00 ^b	0.00	0.46 ^b	0.94
Arachis oil (100 g/month)	0.17 ^a	1.04	12.6 ^b	5.31	3.54 ^c	3.95
Soybean-salad oil (100 g/month)	0.41 ^a	1.71	0.11 ^a	0.63	4.30 ^b	5.39

Different letters in the same row denote significant differences among groups ($p < 0.05$).

the other two regions. Use of culinary oil was different among the three regions with high use of rapeseed oil in the river/lake region ($p < 0.0001$), but there were no differences of total culinary oils ($p = 0.806$) and total vegetable oil ($p = 0.395$) consumption among regions. Our questionnaire also shown that some families consumed more than one kind of culinary oil simultaneously, and the culinary oil included not only vegetable oil, but also animal oil, eg, lard. The tiny difference between consumption of total culinary oil and of total vegetable oil indicated that the consumption of animal oil is negligible (Table 2).

Fat content and fatty acid composition of colostrum

The mean \pm SD fat content (g/100 g colostrum) from the river/lake, coastal and inland region was 2.17 ± 1.26 , 2.50 ± 1.39 and 2.68 ± 1.26 , respectively; there were no differences among regions after the adjustment by pregnant women's age ($p = 0.265$).

The main SFA, MUFA, n-6 PUFA and n-3 PUFA in colostrum in all regions were 16:0, 18:1n-9, 18:2n-6 and 18:3n-3, respectively. Colostrum from the river/lake region had the lowest content of total SFA, but the highest content of 18:1n-9 and total MUFA and of ALA. Colostrum from the inland region had the highest content of 18:2n-6 and the lowest content of EPA and DHA (Table 3). The amount of DHA in colostrum (mean \pm SD, % wt/wt) was 0.51 ± 0.18 , 0.52 ± 0.20 and 0.35 ± 0.13 in the river/lake, coastal and inland regions, respectively ($p = 0.0002$, adjusted by pregnant women's age).

Maternal rapeseed oil intake in the latest month before the 34th week of pregnancy was positively correlated with ALA in colostrum ($r = 0.562$, $p < 0.0001$). Frequency of maternal marine fish intake in the latest month before the 34th week of pregnancy was positively correlated with colostrum DHA content ($r = 0.203$, $p = 0.040$), but freshwater fish intake frequency was not ($r = -0.045$, $p = 0.655$).

DISCUSSION

We surveyed the pregnant women's dietary information and analysed fatty acid composition of their colostrum after delivery in our study, and our study indicated that there were significant differences in dietary patterns and fatty acid composition in colostrum among the regions.

A similar report was relatively rare in China.

We determined frequency of dietary intakes in the latest month before the 34th week of pregnancy and it is unlikely that these alter during lactation.²⁸ Pregnant women were recruited from three representative geographic regions of China, because we hypothesised that food and nutrient intakes would differ among those regions. This was found to be the case with differences in consumption of freshwater fish, marine fish, meat, eggs, milk, beans, nuts and culinary oils among the regions. These differences translated into differences in intake of a number of fatty acids.

The percentage of ALA in colostrum from all three regions was higher than reported in some previous studies conducted mainly in Europe and Australia (0.33-0.77 g/100 g total fatty acids),⁹⁻¹⁶ and it is in general accordance with the value reported in a Swedish study (0.99 g/100 g total fatty acids),¹⁷ but our data are uncomparable with the Chinese study mentioned above²⁶ because of the different unit of measurement of fatty acids. One reason for the high ALA content in colostrum in the river/lake region may be the high use of rapeseed oil in this region.²⁹

Linoleic acid (LA), ALA, EPA and DHA contents of colostrum varied across the three regions. This may in part reflect dietary differences, but the data suggest that other factors are involved. For example, marine fish intake frequency was much higher in the coastal region than in the other two regions, where frequencies were similar. However, colostrum DHA was similar in the river/lake and coastal regions and greater than in the inland region. The lack of a strong relationship between dietary intake of certain DHA-rich food, like marine fish, and the content of DHA in colostrum, might reflect differences in maternal stores or in fatty acid metabolism,^{15,18,30} that might for some reason be characteristic of the region. Women in the river/lake region had a high intake of ALA-rich rapeseed oil, while soybean-salad oil intake in the inland region was also quite high ($p < 0.0001$); additionally, the intake of LA-rich arachis oil in the coastal region was much higher than in river/lake and inland regions, which could cause the inhibition of biosynthesis of DHA from ALA, because ALA and LA compete with

Table 3. Fatty acids in the colostrum of women in the different regions (% wt/wt, comparison among regions was adjusted by age)

Fatty acid	River/lake region (n=36)		Coastal region (n=35)		Inland region (n=32)	
	mean	SD	mean	SD	mean	SD
8:0	0.04 ^a	0.06	0.04 ^a	0.04	0.11 ^b	0.12
10:0	0.50 ^a	0.47	0.64 ^a	0.43	1.11 ^b	0.91
12:0	2.79 ^a	1.49	4.37 ^{ab}	2.47	4.47 ^b	2.56
13:0	0.01 ^a	0.01	0.02 ^{ab}	0.01	0.02 ^b	0.03
14:0	3.34 ^a	1.12	4.41 ^b	1.51	4.15 ^{ab}	1.69
15:0	0.12	0.02	0.14	0.04	0.12	0.04
16:0	21.5 ^a	2.34	22.2 ^{ab}	1.75	22.9 ^b	2.15
17:0	0.21	0.06	0.25	0.06	0.38	0.76
18:0	4.48 ^a	0.92	5.35 ^b	0.74	5.01 ^{ab}	0.94
20:0	0.15 ^{ab}	0.05	0.19 ^b	0.05	0.15 ^b	0.07
22:0	0.08	0.04	0.10	0.06	0.08	0.07
24:0	0.14	0.06	0.16	0.07	0.12	0.09
SFA	33.5 ^a	3.13	37.9 ^b	3.99	38.6 ^b	5.02
14:1n5	0.05	0.02	0.06	0.03	0.06	0.04
16:1n7	2.04	0.57	2.04	0.57	2.05	0.51
17:1n7	0.10 ^a	0.02	0.10 ^{ab}	0.04	0.12 ^b	0.02
18:1n9 trans	0.14	0.10	0.12	0.10	0.12	0.09
18:1n9 cis	34.9 ^a	2.13	30.3 ^b	2.89	28.7 ^b	2.69
20:1n9	0.95 ^a	0.20	0.65 ^b	0.14	0.55 ^b	0.18
22:1n9	0.29 ^a	0.08	0.17 ^b	0.05	0.12 ^b	0.06
24:1n9	0.44 ^a	0.17	0.27 ^b	0.09	0.22 ^b	0.13
MUFA	38.9 ^a	2.28	33.7 ^b	3.10	31.9 ^c	2.76
18:2n6 trans	0.004	0.01	0.01	0.01	0.01	0.02
18:2n6 cis	15.8 ^a	1.74	18.4 ^b	2.77	19.7 ^b	3.88
18:3n6	0.04 ^a	0.03	0.06 ^a	0.04	0.10 ^b	0.08
20:2n6	1.03	0.22	1.04	0.26	1.04	0.44
20:3n6	0.63	0.19	0.58	0.16	0.65	0.21
20:4n6	0.83	0.19	0.79	0.16	0.81	0.18
22:2n6	0.27	0.07	0.24	0.08	0.22	0.10
n-6 PUFA	18.6 ^a	1.59	21.2 ^b	2.94	22.6 ^b	4.18
18:3n3	1.33 ^a	0.38	0.82 ^b	0.29	0.77 ^b	0.32
20:3n3	0.18 ^a	0.05	0.09 ^b	0.04	0.09 ^b	0.04
20:5n3	0.07 ^{ab}	0.03	0.09 ^a	0.08	0.04 ^b	0.03
22:6n3	0.51 ^a	0.18	0.52 ^a	0.20	0.35 ^b	0.13
n-3 PUFA	2.09 ^a	0.40	1.51 ^b	0.38	1.24 ^b	0.37
LA/ALA	12.6 ^a	3.11	25.1 ^b	9.43	28.0 ^b	7.35
n-6/n-3	9.18 ^a	1.79	14.6 ^b	2.96	19.2 ^c	4.87

Different letters in the same row denote significant differences among groups ($p < 0.05$).

each other for the same enzymes for biosynthesis of LCPUFA.³⁰ Therefore, it is possible that women in river/lake and inland regions are synthesizing DHA from its precursor ALA more effectively and that this is why the DHA content of colostrum in the three regions is more similar than dietary difference would indicate they might be. Moreover, young women seem to be fairly efficient at converting ALA to DHA, at least compared with men.^{31, 32} All these indicate either that the women are mobilizing stored DHA during lactation or that they are synthesizing DHA from ALA. The average (mean or median) DHA contents of human colostrum collected between day 0 to day 3 post-partum from previous studies is from 0.32 to 0.56 g/100 g total fatty acids,¹⁸⁻²⁰ while for colostrum collected at days 3 to 5 post-partum it is 0.11 to 1.1 g/100 g total fatty acids.^{9-17,21,22} The average contents for the three regions reported in the current study are in accordance with these earlier reports.

Just like DHA,^{33,34} ARA is important for infant development.³⁵ An earlier study²⁷ showed that egg was main food source of ARA in China. However, there was no association between maternal intake frequency of egg and

the percentage of ARA in colostrum ($r = -0.166$, $p = 0.095$), and the colostrum ARA content was similar in all three regions though the egg intake frequency varied across the three regions. Once again this may reflect mobilization of body stores or an ability to synthesize ARA from its precursor LA.

An appropriate LA/ALA ratio in breast milk (and infant formula) is advantageous for growth of term infants,^{36,37} because the two fatty acids compete with each other for the same enzymes for biosynthesis of LCPUFA.³⁰ The former recommended ratio is between 5:1 and 15:1,³⁸ but up-to-date study indicates "there is no compelling rationale for the continued recommendation of a specific ratio of n-6 to n-3 fatty acids or LA to ALA".³⁹ In the current study, the mean ratio in the coastal and inland regions is well above the mean ratio of river/lake region. This high ratio appears to involve an excess of LA-rich foods or culinary oils in the diet (e.g.: arachis oil). The influence of the high ratio on infant growth and development deserve to be investigated.

It was recommended that erucic acid content would not exceed 1% of total fat content in infant formula, this

guidance was based on data of the composition of human milk from healthy and well-nourished women, because erucic acid has “no known nutritional benefit for infants”.⁴⁰ Our data show that even the maximum percentage of erucic acid in colostrum (0.44% of total fatty acid, from river/lake region) was far lower than the recommended upper limit. Just like ALA, the high use of rapeseed oil could be the reason for the highest erucic acid content of colostrum in the river/lake region.⁴¹ Indeed, a positive correlation between rapeseed oil intake in the latest month before the 34th week of pregnancy and colostrum erucic acid content was found ($r=0.572$, $p<0.0001$).

The food consumption frequency could reflect local dietary pattern to a certain extent in our opinion. In addition, it is unconvincing to obtain accurate quantitative data about common food consumption in a food frequency questionnaire. Therefore, we consider that it is feasible to calculate the correlation coefficient between food frequency and specific fatty acid in colostrum.

The fatty acid composition in human milk is complex. Some studies used special fatty acid as internal standard, such as C17:0, C21:0, C23:0, etc.,^{15,25,26} but other studies showed that these fatty acids existed in human milk.^{19,42} Therefore, we calculated the percentage of fatty acids in normalization method without internal standard. The value of amount of each kind of fatty acid (in form of mg/100 g colostrum) could be estimated because we reported the amount of total fat content and esterified FA provides circa 88% of the total lipid in human milk.⁴³

While the fat content of breast milk changes during a feed, the fatty acid composition remains fairly stable.^{3,9,30} In the current study the colostrum was collected between days 3 to 5 post-partum and we did not distinguish between hindmilk and midstream milk in order to suit the mothers. Almost all mothers fed their neonate directly and did not use feeding bottle. That makes it difficult to estimate the total volume or weight of colostrum during a feed. However, all mothers lactated abundantly, no neonate needed extra formula.

The overall health implications of colostrum consumption for infants among regions would deserve to be surveyed, but we are unable to continue our study due to various reasons such as fund limitation and the consent of mothers and their families.

In summary, the current study indicates that differences in dietary fish intake and of several other lipid-rich foods exist among pregnant women from three different regions in China and that these differences have associations with differences in n-3 PUFA composition of colostrum. It appears that marine fish intake by pregnant women might actively affect DHA concentration in their colostrum.

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AUTHOR DISCLOSURES

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Original Article

The fatty acid composition of colostrum in three geographic regions of China

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中国三个不同地理区域人初乳脂肪酸构成

目的：描述中国三个不同区域人初乳脂肪酸构成。方法：募集来自于淡水河湖地区、沿海及内陆地区的妊娠晚期孕妇。调查妊娠第 34 周前一个月的膳食消费频率。采集产后 3 至 5 天内初乳样品。分析初乳中 31 种脂肪酸的含量。结果：三地区孕妇膳食与食用油消费模式存在显著差异。淡水河湖地区，沿海和内陆地区产妇的初乳总脂含量(均值±标准差，g/100 g 初乳)分别为 2.17±1.26、2.50±1.39 和 2.68±1.26 (经孕妇年龄校正后 $p=0.265$)。初乳中主要饱和、单不饱和、n-6 多不饱和及 n-3 多不饱和脂肪酸分别为 16:0、18:1n9、18:2n6 和 18:3n3。河湖地区初乳脂肪中总 SFA 和总 n-6PUFA 的百分含量最低，而总 MUFA 和总 n-3PUFA 的百分含量最高。三地区初乳 DHA 含量(均值±标准差，%)分别为 0.51±0.18、0.52±0.20 和 0.35±0.13 (经孕妇年龄校正后 $p=0.0002$)。妊娠晚期海鱼摄入频率与初乳中 DHA 构成呈正相关($r=0.203$ ， $p=0.040$)。结论：中国三个不同地理区域，人初乳脂肪酸构成存在差异，并与母体膳食模式相关。

关键词：初乳、脂肪酸、海鱼、菜籽油、二十二碳六烯酸