The investigation of fatty acid composition of breast milk and its relationship with dietary fatty acid intake in 5 regions of China

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**Abstract**

To investigate the relationship between dietary fatty acid (FA) intake and the lactate FA levels in Chinese women.

A total of 2007 samples from 5 regions of China were collected, including 431 in Shandong, 402 in Changchun, 419 in Chongqing, 398 in Guangzhou, and 357 women in Hohhot. All participants were mothers of healthy full-term newborns, collecting the foremilk from day 1 to day 7 and the mature breast milk on day 42 after delivery. The FA composition of the breast milk was analyzed by high resolution capillary gas chromatography.

The total FA content of mature breast milk in Shandong was the highest (34.95 g/L), and that was the lowest in Guangzhou (29.72 g/L). The saturated FA content of breast milk was increased from 1 to 7 days after delivery and decreased in mature breast milk. The saturated FA content of breast milk in Hohhot was the highest (37.43%), but that was the lowest in Changchun (32.80%). Maternal dietary FA composition was positively correlated with saturated FAs (SFA), polyunsaturated FAs (PUFA), and docosahexaenoic acid (DHA) (P < .001), and negatively with monounsaturated FAs (MUFA) (P > .05) in breast milk.

The FA content of breast milk is different in different regions of China, which was affected by maternal dietary FA composition. Lactating women in China should consume more foods rich in polyunsaturated FAs to ensure the growth of infant.

**Abbreviations:** AA = arachidonic acid, ALA = α-linolenic acid (omega-3 FA), DHA = docosahexaenoic acid, EPA = eicosapentaenoic acid, FA = fatty acid, FAME = FA methyl esters, LA = linoleic acid (omega-6 FA), MUFA = monounsaturated FAs, PUFA = polyunsaturated FAs, r = Pearson’s correlation coefficients, SFA = saturated FAs, TFA = total FA.

**Keywords:** breast milk, dietary intake, fatty acids, 5 main regions of China, polyunsaturated FAs

1. Introduction

The rapid growth of the newborn infant puts exceptional demands on the supply of nutrients. A lot of experiments have proved that any deficit of nutrient could affect the somatic growth and organ structural as well as functional development, especially the brain growth including cell number, organ structural, and synaptic connectivity. Breast milk contains a wide variety of proteins that provide adequate nutrition to newborn infants and it has been universally recognized as the optimal food for infants. In addition, several investigations showed that children who were breast-fed as babies could attain higher scores on intelligence tests than those who were bottle-fed. In recent years, the fatty acid (FA) of breast milk was proven to be a critical component in the development of central nervous system of infants. Several FA composition of breast milk such as arachidonic acid (AA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) have already been sufficiently studied and been thought key factors for fetal growth and also for tissue, organ development. However, the level of FA composition in breast milk differs amongst geographical locations with various dietary habits and varies substantially between lactating mothers. Therefore, understanding the role and content of key FA composition in breast milk could better guide the baby feeding.

Numerous studies indicated that maternal diet can influence FA composition of the milk lipid fraction. The clinical trial also demonstrated that FA profile in the infants’ blood and tissues depends on their diet FA pattern. Nevertheless, the actual effect of maternal diet to the breast milk lipid content during long period of lactation still has not been fully valued in China. Therefore, we investigated FA composition of breast milk in 5 regions of China and further studied the related diets to help pregnant and lactating woman obtain the important FA needed by their baby.
2. Materials and methods

2.1. Subjects and design

In this prospective study, a total of 2007 women from 5 regions of China donated the breast milk samples, including 431 women in Shandong, 402 women in Changchun, 419 women in Chongqing, 398 women in Guangzhou, and 357 women in Hohhot. Mothers who delivered healthy term newborns (gestation >37 weeks; weighing >2.5 kg) at obstetric clinics and planned to exclusively breastfeed their infants for at least 6 weeks were recruited from 5 regions of China, including Shandong, Chongqing, Changchun, Guangzhou, and Hohhot. In addition, mothers who participated in this study had no medical and alcoholic history and were not receiving any vitamin supplements, apart from iron supplements and folic acid during their pregnancy or postpartum. Mothers were approached by a member of the research group during the last 2 months of their pregnancy and were thoroughly informed about the study protocols and aims. They were asked to sign an informed consent form. Samples of breast milk were obtained from day 1 to 7 and day 42 after delivery. Ethical approval was obtained from the ethics committee of Sixth People’s Hospital of Jinan.

2.2. Dietary assessment

Dietary intake data were collected using a food record for 3 consecutive days. Subjects recorded all foods and beverages they consumed during those 3 days. The dietary protocols were completed before and after 1 week of milk collection by the participants at home. The sampling period included 2 weekdays and 1 weekend day. The average of the 3 days was used to estimate normal dietary intake. Details were introduced elsewhere. All the mothers participated in this study were asked to complete a questionnaire, focused on foods that was rich in important FA (including saturated FA [SFA], monounsaturated FA [MUFA], total polyunsaturated FA [PUFA], n–6 PUFA, and n–3 PUFA), such as fish, pork, beef, mutton and seafood, fruits, vegetables, cereals, soybean and products, eggs and oils (including flaxseed oil, sunflower oil, olive oil, and coconut oil). The foods of the questionnaire represented the typical sources of different FA in China diet. For example, the fish and other seafood are the main source of DHA and linoleic acid (LA) as well as arachidonic acid comes from the cereals and soybean. In addition, vegetables have abundant vitamin. The questionnaire covered 6-week period. The energy and nutrient intake levels for dietary data were calculated using the China Food Composition Table.

2.3. Breast milk collection

Mothers discarded the first few drops of breast milk and manually collected the actual milk sample (10 mL) into a plastic container from 09.00 to 11.00 am. All samples were frozen and stored at −20 °C until the FA was analyzed.

2.4. Breast milk FA composition analysis

The milk samples were thawed at 4 °C and the milk fat (the upper layer) was extracted. FA methyl esters (FAME) were prepared from milk fat by combining 0.2 mL of fat with 2 mL of methanol and benzene (4:1, v/v), 33 μL of daturic acid (C17:0), and 200 μL of acetyl chloride in a 10 mL glass tube. The mixture was incubated in glycerinum at 100 °C for 60 minutes. FAME samples were cooled and then 5 mL of 6% K₂CO₃ was slowly added to stop the reaction and neutralize the mixture. The solution was centrifuged for 10 minutes at 1580 × g, and then 1 μL of the upper phase was injected into a gas chromatograph with a flame ionization detector.

The samples were analyzed by gas chromatography (GC-2010 Auto Injector/Auto Sampler, Shimadzu, Japan) equipped with a capillary column (DB-23 column; 60 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent Technologies, DE). The peaks of FA were identified by comparing their retention times with those of known standard mixtures FAME 37 (Supelco, Bellefonte, PA) and 68D (NuChek Prep, Elysian), and then quantified in relation to the internal standard. The FA composition of the total lipids of breast milk was expressed as weight percentage of total FA. The retention times and peak areas were compared with the inner standard and the FA concentration was calculated in accordance with the national standard (Liu et al[15]).

2.5. Statistical analysis

The statistical analysis was performed using SPSS statistical software (Version 16.0). Normal distribution of the FA was evaluated with Analysis of Variance. Correlation analysis was performed between the FA composition of breast milk and the maternal dietary intake using Pearson test. The results are expressed as means± standard deviations. The bilateral P < .05 was considered statistically significant.

3. Results

3.1. Anthropometric characteristics of subjects

The characteristics of the participating mothers and babies, including age, gestational age, and pre-pregnancy body mass index (BMI) were summarized in Table 1.

3.2. Maternal dietary FA intake

Maternal dietary FA intake was presented in Table 2 and the relevant FA was expressed as g/d, respectively.

3.3. Total FA concentration of 5 main regions

Total FA (TFA) content of colostrum in 5 regions was all low day 1 after delivery (11.99 ± 9.18 g/L), and increased on day 7. TFA...
level increased to 32.26 ± 15.42 g/L on day 42. The TFA content of colostrum on day 1–7 of Guangzhou was the lowest in 5 regions. On day 42, the TFA level was the highest in Shandong and the lowest in Guangzhou (Table 3).

3.4. SFA concentration of 5 main regions

The SFA content of human milk is relatively stable and constantly increases from day 1 to day 7 and slightly decreased on day 42. The SFA content in Hohhot (37.43 ± 4.28%) on day 42 was the highest among regions and that of Changchun (32.80 ± 4.88%) was the lowest. In addition, the content of palmitic acid of human milk from day 1 to 7 decreased in 5 regions and had showed the lowest level in Changchun (17.94 ± 2.09%). However, the content of LA and myristic acid increased. The highest LA level (3.98 ± 1.66%) came from Hohhot. The highest SFA content of human milk in 5 areas was palmitic acid (19.52 ± 1.54%), followed by stearic acid (5.45 ± 1.07%), LA (4.55 ± 1.49%), and myristic acid (3.52 ± 1.62%) (Table 4).

3.5. MUFA concentration of 5 main regions

The MUFA content of human milk decreased from day 1 to 7 in 5 regions, while increased on day 42 in 4 regions except Changchun. The MUFA content on day 42 in Chongqing (44.06 ± 3.45%) was the highest among 5 regions, and that in Changchun (32.57 ± 4.05%) was the lowest. The highest MUFA content of human milk on day 42 was oleic acid. In 5 regions, the area showed the highest oleic acid content was Chongqing, and Changchun was the lowest (Table 5).

3.6. PUFA concentration of 5 main regions

The mean LA level of mature milk in 5 regions was 23.32 ± 6.59%. The LA content of mature milk in Changchun was the highest, and that in Chongqing was the lowest. The LA content continuously increased in 4 regions except Hohhot. The mean ALA level of mature milk in 5 regions was 1.72 ± 1.32%, with the highest level in Changchun and the least level in Guangzhou. The mean EPA level in 5 regions was 0.18 ± 0.22%, with the highest level in Shandong. The mean DHA level in 5 regions was 0.31 ± 0.18%, and Shandong and Guangzhou showed the highest level. The mean AA level of mature milk in 5 regions was 0.55 ± 0.12%, and the highest level appeared in Guangzhou (Tables 6 and 7).

3.7. The comparison of LA/ALA and AA/DHA in 5 regions

The LA/ALA data of human milk were different in these 5 regions. The area showing the highest LA/ALA data was Guangzhou, and the lowest was Chongqing. The AA/DHA data
of 5 regions were also different, with the highest ratio in Shandong and the lowest in Guangzhou (Table 8).

### 3.8. Correlation analysis between breast milk FA composition and maternal dietary FA composition

Table 9 showed that correlation analysis between breast milk FA composition and maternal dietary FA composition. Maternal dietary FA composition was positively correlated with SFA, PUFA, and DHA ($P < .001$), and negatively with MUFA ($P > .05$) in breast milk.

## 4. Discussion

Breast milk provides energy and some important FAs that is highly beneficial for infant health and development. In this study, the levels of TFA in colostrum in 5 regions were low on the first day after delivery, but increased over time. This result is consistent with the reported literature.[12] These characteristics of human milk fat may be suitable for physiological needs of newborn babies, because the capacity of fat digestion and absorption of newborn babies is relatively poor. The SFA content in colostrum in Hohhot is the highest and that in Shandong is the lowest. This result may be associated with the traditional dietary pattern of this area because Hohhot is well known for abundant sheep and cow. The area that possessed the highest SFA content in colostrum is Chongqing, and the oleic acid content in Chongqing is obviously higher than other regions possibly due to more colza oil intake of pregnant women there. The SFA and MUFA content of mature milk in 5 regions were similar to other countries. PUFA is a structural component of cellular membrane and the precursor of thromboxane, prostaglandin, and leukotriene. PUFAs especially AA and DHA ensure adequate fetal growth and the development of tissue, organ, and nervous system.[13] In addition, healthy brain tissue consists of about 60% structural fat including about 25% DHA and 15% AA.[14] In neonates, AA

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### Table 5

MUFA concentration of 5 main cities.

|          | 1 day (%) | 2 days (%) | 3 days (%) | 4 days (%) | 5 days (%) | 6 days (%) | 7 days (%) | 42 days (%) | $P$   |
|----------|-----------|------------|------------|------------|------------|------------|------------|-------------|-------|
| Hohhot   | 38.54 ± 3.15 | 38.19 ± 3.06 | 37.54 ± 3.38 | 36.28 ± 3.75 | 35.57 ± 3.70 | 36.13 ± 3.45 | 35.70 ± 3.74 | 37.42 ± 4.35 | .00   |
| Changchun| 35.90 ± 2.40 | 36.01 ± 2.50 | 35.39 ± 2.73 | 34.94 ± 3.09 | 35.30 ± 2.64 | 34.68 ± 3.56 | 35.13 ± 2.50 | 32.57 ± 4.05 | .00   |
| Chongqing| 46.19 ± 5.57 | 45.89 ± 3.24 | 45.28 ± 3.32 | 43.82 ± 3.26 | 43.68 ± 2.77 | 42.85 ± 2.78 | 42.34 ± 2.97 | 40.06 ± 3.45 | .00   |
| Shandong | 36.81 ± 3.23 | 36.05 ± 3.86 | 34.63 ± 3.37 | 33.37 ± 3.75 | 32.68 ± 3.91 | 32.76 ± 4.14 | 31.11 ± 4.04 | 35.85 ± 3.74 | .00   |
| Guangzhou| 40.69 ± 2.40 | 41.85 ± 3.65 | 41.98 ± 4.19 | 41.47 ± 3.26 | 40.49 ± 3.22 | 40.00 ± 3.45 | 38.95 ± 3.86 | 39.22 ± 3.98 | .00   |

Results are presented as mean ± SD. MUFA = monounsaturated fatty acids.

### Table 6

PUFA concentration of 5 main cities.

|          | 1 day (%) | 2 days (%) | 3 days (%) | 4 days (%) | 5 days (%) | 6 days (%) | 7 days (%) | 42 days (%) | $P$   |
|----------|-----------|------------|------------|------------|------------|------------|------------|-------------|-------|
| Hohhot   | 25.06 ± 3.41 | 24.76 ± 3.55 | 24.82 ± 3.62 | 25.33 ± 5.12 | 25.52 ± 4.58 | 24.59 ± 3.91 | 24.61 ± 4.68 | 25.15 ± 5.24 | .98   |
| Changchun| 29.29 ± 3.64 | 27.90 ± 4.27 | 27.63 ± 4.55 | 27.56 ± 4.38 | 27.33 ± 3.98 | 26.27 ± 3.03 | 27.53 ± 3.82 | 34.63 ± 6.53 | .00   |
| Chongqing| 20.66 ± 3.04 | 20.31 ± 3.03 | 20.57 ± 3.27 | 20.14 ± 4.52 | 18.87 ± 3.77 | 18.65 ± 3.79 | 18.38 ± 3.79 | 22.04 ± 3.80 | .00   |
| Shandong | 27.37 ± 4.60 | 28.63 ± 3.29 | 29.58 ± 4.20 | 29.79 ± 5.05 | 29.81 ± 5.42 | 29.61 ± 5.48 | 28.91 ± 5.00 | 31.07 ± 6.25 | .51   |
| Guangzhou| 21.28 ± 2.68 | 21.37 ± 3.45 | 21.51 ± 4.11 | 21.86 ± 3.76 | 22.16 ± 3.66 | 21.97 ± 3.59 | 22.52 ± 3.72 | 23.61 ± 3.19 | .24   |

Results are presented as mean ± SD. PUFA = polyunsaturated fatty acids.

### Table 7

The concentration of LA, ALA, AA, EPA, and DHA in 5 cities.

|          | Hohhot | Changchun | Chongqing | Shandong | Guangzhou | $P$   |
|----------|--------|-----------|-----------|----------|-----------|-------|
| LA 1–7 day, g/L | 20.11 ± 3.82 | 23.54 ± 3.81 | 15.66 ± 3.33 | 24.40 ± 4.61 | 17.55 ± 3.46 | .00   |
| 42nd day g/L    | 21.09 ± 4.90 | 20.82 ± 6.35 | 17.40 ± 3.56 | 27.62 ± 6.21 | 20.82 ± 3.12 | .00   |
| ALA 1–7 day, g/L | 1.85 ± 1.54 | 1.09 ± 0.37 | 1.10 ± 0.58 | 1.23 ± 0.62 | 0.56 ± 0.34 | .00   |
| 42nd day g/L    | 1.99 ± 1.30 | 2.12 ± 0.76 | 1.88 ± 0.58 | 1.42 ± 0.63 | 0.79 ± 0.50 | .00   |
| AA 1–7 day, g/L | 0.85 ± 0.21 | 0.93 ± 0.19 | 0.82 ± 0.18 | 0.88 ± 0.29 | 1.05 ± 0.62 | .00   |
| 42nd day g/L    | 0.52 ± 0.11 | 0.58 ± 0.15 | 0.53 ± 0.11 | 0.53 ± 0.10 | 0.61 ± 0.10 | .00   |
| EPA 1–7 day, g/L | 0.17 ± 0.06 | 0.19 ± 0.07 | 0.15 ± 0.05 | 0.22 ± 0.06 | 0.19 ± 0.07 | .00   |
| 42nd day g/L    | 0.09 ± 0.08 | 0.08 ± 0.02 | 0.08 ± 0.05 | 0.10 ± 0.05 | 0.10 ± 0.06 | .37   |
| DHA 1–7 day, g/L | 0.39 ± 0.14 | 0.52 ± 0.20 | 0.50 ± 0.17 | 0.67 ± 0.29 | 0.60 ± 0.24 | .00   |
| 42nd day g/L    | 0.19 ± 0.08 | 0.28 ± 0.13 | 0.29 ± 0.20 | 0.41 ± 0.17 | 0.41 ± 0.19 | .00   |

Results are presented as mean ± SD. AA = arachidonic acid, ALA = α-linolenic acid, DHA = docosahexaenoic acid, EPA = eicosapentaenoic acid, LA = linoleic acid.
and DHA can be synthesized by chain elongation and desaturation of essential FA, such as LA and LNA, respectively.[15] All above suggest that PUFA is a key factor in the baby’s growth and development. However, this process changes because of more intake of n-6 FA and less intake of n-3 FA and this unbalance could show in human milk.[11,20] Although the level of n-6/n-3 FA in Shandong and Guangzhou is high, it may still require further research to determine whether ALA intake should be increased in both areas. Different dietary habits are largely the drivers behind the different FA profiles among the different regions. The changes observed in the concentrations of individual FAs were small, but it could potentially be physiologically significant.[16] Our study showed strong correlations between the maternal dietary FA composition and the concentrations of SFA, PUFA and DHA in breast milk, but it had no impact on breast milk MUFA levels.

In conclusion, the study shows that lactating mothers in different area indeed have different level of FA including TFA, SFA, MUFA, and PUFA. The content of PUFA and DHA varies greatly among regions. The pregnant and lactating women should eat a certain amount of seafood that is rich DHA and less amount of soybean oil, sunflower oil or peanut oil, and some mutton and beef. Furthermore, Chinese women should avoid traditional misunderstandings such as eating more food that contains less protein and more lipids. In addition, fresh vegetables and fruits also should not be ignored. Fruits and vegetables contain folic acid and all kinds of vitamins which have great influence on people’s growth. Moreover, the importance of long chain PUFA intake should also be more highlighted for Chinese pregnant women and Chinese should have their initiative to eat more PUFA-rich diet.

### Table 8

The comparison of LA/ALA and AA/DHA in 5 cities.

|       | 1 day | 2 days | 3 days | 4 days | 5 days | 6 days | 7 days | 42 days |
|-------|-------|--------|--------|--------|--------|--------|--------|---------|
| LA/ALA|       |        |        |        |        |        |        |         |
| Hohhot| 18.44 ±10.06 | 16.14 ±6.96 | 15.10 ±7.08 | 14.67 ±7.34 | 12.72 ±7.06 | 12.95 ±7.63 | 13.25 ±7.98 | 14.28 ±9.69 |
| Changchun | 25.56 ±5.28 | 25.10 ±5.07 | 23.63 ±6.12 | 22.05 ±6.40 | 23.03 ±8.51 | 22.16 ±8.46 | 21.95 ±6.01 | 15.96 ±5.81 |
| Chongqing | 21.75 ±14.84 | 17.78 ±4.76 | 15.11 ±4.38 | 14.68 ±3.90 | 14.94 ±5.11 | 14.37 ±6.13 | 15.57 ±7.00 | 10.86 ±5.29 |
| Shandong | 29.18 ±7.23 | 24.48 ±6.07 | 22.09 ±6.93 | 22.09 ±7.75 | 21.18 ±7.80 | 21.09 ±7.93 | 21.07 ±8.18 | 23.36 ±11.65 |
| Guangzhou | 43.11 ±13.02 | 42.27 ±12.97 | 40.84 ±16.02 | 36.62 ±15.91 | 35.35 ±15.04 | 34.21 ±13.41 | 30.55 ±13.37 | 33.44 ±14.86 |
| AA/DHA|       |        |        |        |        |        |        |         |
| Hohhot | 2.66 ±0.61 | 2.39 ±0.48 | 2.30 ±0.49 | 2.23 ±0.48 | 2.24 ±0.46 | 2.19 ±0.44 | 2.22 ±0.54 | 2.93 ±0.84 |
| Changchun | 1.97 ±0.49 | 1.90 ±0.47 | 1.85 ±0.44 | 1.82 ±0.44 | 1.94 ±0.41 | 1.98 ±0.47 | 1.99 ±0.47 | 2.26 ±0.63 |
| Chongqing | 2.03 ±0.42 | 1.92 ±0.41 | 1.82 ±0.39 | 1.72 ±0.44 | 1.56 ±0.40 | 1.51 ±0.51 | 1.57 ±0.47 | 2.14 ±0.61 |
| Shandong | 1.66 ±0.47 | 1.59 ±0.34 | 1.52 ±0.35 | 1.41 ±0.42 | 1.36 ±0.45 | 1.27 ±0.36 | 1.23 ±0.48 | 1.46 ±0.54 |
| Guangzhou | 2.14 ±0.98 | 1.93 ±0.80 | 1.73 ±0.50 | 1.66 ±0.42 | 1.67 ±0.42 | 1.62 ±0.41 | 1.62 ±0.45 | 0.82 ±0.82 |

Results are presented as mean ±SD. AA = α-linolenic acid, ALA = α-linolenic acid, DHA = docosahexaenoic acid, LA = linoleic acid.

### Table 9

Correlation (r) between breast milk FA composition and maternal dietary FA composition.

|       | SFA      | MUFA     | PUFA     | DHA     |
|-------|----------|----------|----------|---------|
| Hohhot | 0.245*** | –0.027*  | 0.24***  | 0.44*** |
| Changchun | 0.27*** | –0.031*  | 0.33***  | 0.53*** |
| Chongqing | 0.26*** | –0.03*   | 0.31***  | 0.51*** |
| Shandong | 0.29*** | –0.033*  | 0.35***  | 0.56*** |
| Guangzhou | 0.225*** | –0.025*  | 0.21***  | 0.42*** |

DHA = docosahexaenoic acid, FA = fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, r = Pearson correlation coefficients, SFA = saturated fatty acids.

*** P < .001.
* P > .05.
Acknowledgments
The authors wish to thank to the women who participated in this study in different areas of China. Authors also very thank for the contribution of all research groups in 5 regions of China.

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