Medium- and long-chain triacylglycerol in human breast milk and association with maternal dietary patterns

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Abstract

This study aims to investigate the influences of maternal dietary patterns on the triacylglycerol (TAG) composition, particular medium- and long-chain triacylglycerol (MLCT) in human breast milk. A total of 180 Chinese human breast milk samples were collected and divided into three dietary patterns groups accounting to semi-quantitative food intake frequency questionnaire and a 24-hour dietary recall. Using high performance liquid chromatography combined with quadrupole time-of-flight mass spectrometry, 108 TAGs were identified and quantified. In breast milk samples, MLCTs accounted for 25.76 % (median of 24.63%; 95% CI: 24.60, 26.92) of the total TAGs. In human breast milk, the main type of MLCT is TAGs esterified with one medium-chain fatty acid and two long-chain fatty acids (MLL type) which accounted for ˜81% of total MLCTs. Among the molecular species of MLCT, O-P-La, O-L-La, and O-L-M are the three most abundant configurations in MLCT (2.28%, 2.17%, and 2.01% of total TAGs, respectively). MLCT in breast milk was positively correlated with the energy supply ratio of carbohydrates (P = 0.205), which was significantly higher than that of the other two groups (P = -0.150 and -0.232).

1. Introduction

Human milk is recognized as the best food for newborns, rich in lipids, proteins, carbohydrates, and other micronutrients to ensure the enough energy and nutrients for newborns for growth and development (Harding et al., 2017). The World Health Organization (WHO) recommends exclusive breastfeeding for the first six months of life of infants (Eidelman et al., 2012). Lipids are important nutrients in breast milk, accounting for about 3-5% and providing 50-60% of the newborn's energy (Demmelmair and Koletzko, 2018). Human milk lipids are reported as variable influenced by many factors for example individuals, lactational period, maternal dietary *etc* (Grunewald et al., 2019; Burianova et al., 2019; Gridneva et al., 2022; Ye et al., 2021).

The fatty acid (FAs) profiles in human breast milk have been well-studied worldwide (Liu et al., 2018). Recent studies show that triacylglycerols (TAGs) in human breast milk have unique molecular structure and showing important roles for infant lipid digestion and metabolism. Previously, we analyzed the TAG composition of Chinese human milk, found a group of TAG containing both medium-chain fatty acids (MCFAs) and long-chain fatty acids (LCFAs) esterified at one glycerol backbone, namely medium- and long-chain triacylglycerol (MLCT) (Yuan et al., 2020). According to the number of medium-chain fatty acid (MCFA) and long-chain fatty acids (LCFAs) on the glycerol backbone, MLCT can be divided into two types,the MLCT esterified with one MCFA and two LCFAs are the MLL type; and the MLCT esterified with two MCFAs and one LCFA are the MML type. In human milk, MLCT types of TAGs contains approximately 40 kinds of TAGs, accounting for 20-30% of total TAG (Zhang et al., 2021; Yuan et al., 2021). There are obvious differences of the MLCT content and molecular species in lipids between human milk.

Compared with LCT, MLCT has not only long-chain fatty acids, but also medium-chain fatty acids. Due to the presence of MCFA, it is more conducive to the direct absorption and utilization of infants, MLCT

will have higher lipolysis degree as well as release higher levels of free fatty acid in infant intestinal digestion (Yuan et al., 2020; Wang et al., 2022), therefore MLCT is conducive to the digestion and absorption of lipids, providing fast energy (Yu et al., 2022a). In addition, MLCT has more incorporation of long-chain fatty acids than MCT, allowing it to provide the physiological activities of the body (Lee et al., 2022).

Previous studies show that FAs in human milk are highly influenced by the maternal diet (Ward et al., 2021; Wang et al., 2010). The content of protein, and lactose in breast milk was not obviously affected by the breast milk diet, but the fatty acids in breast milk were affected by the mother 's immediate diet (Aumeistere et al., 2019). There are also studies of Nigeri mothers that high carbohydrate intake leads to higher MCFA in breast milk, while excessive intake of fat may lead to lower levels of MCFA (VanderJagt et al., 2000), Sudan (Nyuar et al., 2010), and Egypt (Bakry et al., 2021) show high content of 12:0 and 14:0, which were approximately 20% of total fatty acids. Current studies have shown that MCFAs are affected by dietary structure, but the relationship between TAG containing MCFAS and dietary patterns in breast milk is rarely studied.

This study aims to analysis the correlation between MLCT content & molecular species and maternal dietary patterns. This study collected mature breast milk samples and dietary intake data of healthy Chinese lactating women 30-90 days *postpartum* . A total of 180 volunteer mothers were divided into three groups fat group ($>$ 30 energy %, $n = 60$), carbohydrate group ($>$ 50 energy %, $n = 60$), and protein group ($>$15 energy %,$n = 60$) according to Yang (Yang, 2018). recommendations. TAGs were identified and quantified using High Performance Liquid Chromatography-Mass Spectrometry (LC-MS) following our previous studies. The composition of MLCTs in breast milk and their relationship with nutrient energy supply ratio were clarified, which provided scientific basis for developing novel structured lipids used in infant formula.

2. Materials and methods

2.1. Materials and Reagents

All lactating mothers in this study were obtained from the Maternal and Child Health and Family Planning Service Center of Jiangning District, Nanjing, Jiangsu Province, China. From November 2018 to January 2019, breast milk of 121 eligible healthy lactating mothers was included in this study. The project has been approved by the Human Research Ethics Committee of Nanjing Medical University and registered as ChiCTR1800020179 in clinicaltrials.gov. Each participant provided oral and written informed consent. Inclusion criteria: (1) 20-40 years old healthy lactating mothers, 30-50 days postpartum exclusive breast-feeding; (2) singleton pregnancy, full-term delivery ([?] 37 weeks of gestation), birth weight [?] 2500 g, [?] 4000 g infants. Mothers with any infectious disease, severe malignant disease, malnutrition, or mental illness; people diagnosed with breast diseases such as mastitis; or people with pregnancy complications such as gestational hypertension and diabetes were excluded. Mothers who had a history of drinking and smoking during pregnancy and lactation, vegetarians, mothers who consumed fatty acid supplements during lactation, or who participated in other studies in the past 30 days were also excluded.

The mixed TAGs standards (8:0, 10:0, 12:0, 14:0 and 16:0, 17811-1AMP) were purchased from Sigma-Aldrich Limited (Shanghai, China). High performance liquid chromatography grade *n* -hexane, acetonitrile, and *iso* -propanol were purchased from Shanghai Bailingwei Technology Co., Ltd. (China, Shanghai). Ammonia, ethanol, anhydrous ethyl ether (boiling point of 30 to 60), and petroleum ether were purchased from Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Study design

Dietary assessment. The semi-quantitative Food Frequency Questionnaire (FFQ) report asks all mothers to record their usual dietary intake in the past month. The three components of a food list, how often you eat a certain food, and the amount of food you eat at a time make up the FFQ. A total of 156 food items are on the list, mainly divided into 13 categories: staple foods, vegetables, fruits, aquatic products, livestock and poultry, poultry, eggs, milk and its products, soybeans and their products, nuts, edible oils, processed foods, seasonings, and beverages. This FFQ was validated with 3-day dietary records from lactating mothers. This

information is collected by trained graduate students themselves. The original data of food amount in FFQ were input into EpiData software for verification. Then the daily intake of food group, energy and nutrition was calculated. The database was based on the *Chinese Food Composition Table (6th edition)* (Yang, 2018) and combined with the values obtained from the USDA National Nutrient Database for Standard Reference (Burke et al., 2016).

Sample collection. Breast milk (10-15 mL) was collected from each mother between 07:30 a.m. and 09:00 a.m. Breast milk samples are collected in a polypropylene tube and no preference for one breast. All sampling procedures are carried out to ensure that the light is avoided. The samples were quickly stored in a hospital refrigerator at 4 , and then transported to a laboratory refrigerator in a lightproof container with ice, stored at -20 for one week, and then stored at -70 until analysis.

Quality control. Experts were invited to review and revise the questionnaire on baseline characteristics to ensure its scientificity. All researchers received rigorous training and evaluation before conducting the study. After the experiment was completed, baseline information and dietary data were examined with the participants. In order to improve the accuracy of dietary data in FFQ, participants estimated the size of the food component with the help of a food map with sufficient visual reference. Breast milk samples were collected in strict accordance with standard sampling techniques. The instruments and containers used for sampling were disinfected and used once. TAG concentration in breast milk was detected by the same instrument and the same batch of reagents. The main data were input into EpiData software twice by two trained researchers to verify its accuracy. In statistical analysis, the appropriate statistical methods are selected strictly according to the statistical requirements and data types.

2.3. Lipid extraction from breast milk

Human milk fat was extracted using the classical Röse–Gottlieb method(Crocker et al., 1955). Briefly, 1 mL of ammonium hydroxide solution was added into 5 mL breast milk. The mixture was heated to 65 ± 5 using electric heating constant temperature oscillation water bath for 20 min. After cool to room temperature, 5 mL anhydrous ethanol, 10 mL anhydrous ether, and 10 mL petroleum ether were added into the mixture with full shaking and stand for 2 h. The supernatant was separated from water and then collected. The solvent of the supernatant was removed by nitrogen. The above operation was repeated for the second extraction. The resulting supernatant was combined with the first extract, and the solvent was blown dry to constant weight with nitrogen to obtain human milk fat. The human milk fat content was calculated with the results were expressed as g/100 mL.

2.4. Triacylglycerol composition analysis

Extracted human milk fat was dissolved in n -hexane to obtain a concentration of 0.3 mg/mL. The composition of TAGs in human milk fat was determined using UPLC-Q-TOF-MS following our previous studies (Zhang et al., 2021). Column was BEHC18 (2.1 mm x 50 mm, 1.9 μm, Waters, USA). The UPLC condition was set as: column temperature is 45 , injection temperature of 20 and injection volume of 1 μL. Mobile phase A was acetonitrile / isopropanol (1:9, v /v), and mobile phase B was acetonitrile/water (4:6, v /v). Both mobile phases contain ammonium acetate (10 mmol/L). Mobile phase of 70% A was used as the starting gradient for 1 min; then increase to 87% A at 30 min and hold for 1 min; then return to initial 70% A at 32 min and maintain for 4 min. The flow rate was 300 μL/min. After each sample, the column was washed with 5% mobile phase A for 5 min before starting detection for the next sample.

Q-TOF-MS condition was set as 10 mmol/L of ammonium formate in methanol as inclusion with a shunt ratio of 1:3 and a flow rate of 0.2 mL/min; electrospray ionization source (ESI), positive ion mode; capillary voltage of 3.5 kV, cone hole voltage of 30 V; ion source temperature 100 , desolvent temperature 400; collision gas is argon, flow rate of 50 L/h; dessolvated gas is nitrogen, flow rate of 700 L/h. The molecular weight scans ranged from 200 to 1500 m/z with a scan time of 0.2 s and a time interval of 0.02 s. TAGs were qualitative according to mass spectrometry information and quantified by area normalization. Relative concentrations were calculated by dividing the peak area of a single TAG by the sum of all peak areas within the sample.

2.5. Statistical analysis

Data from the UPLC-Q-TOF-MS were analyzed using the MassLynx V4.1 software (Milford, MA, USA). Data results are represented as mean ± standard deviation. Statistical analysis was performed using IBM SPSS Statistics 25 software (New York, USA). Groups were significant by the Duncan test in analysis of variance (ANOVA), which were considered statistically different at $P < 0.05$. The Pearson's correlation test was used to determine the relationship between breast milk triacylglycerol composition and maternal dietary intake. Boxplots were plotted using GraphPad prism 9.0 (California, USA). Correlation heat maps were plotted using TBtools software (Guangdong, China). Principal component analysis (PCA) plots were plotted using SIMCA 14.1 software (Demo Umetrics, Umea, Sweden).

3. Results and discussion

3.1. Socio-demographic characteristics of milk sample donors

In this study, a total of 180 breast milk samples were analyzed in this study. The demographic characteristics of the groups according to dietary patterns are shown in Table 1. According to the energy supply ratio of the three major nutrients of the mother, 180 cases of mature milk were divided into three groups (fat group, carbohydrate group, and protein group), with 60 samples in each group. The average ages of mothers in three group were all approximately 30, and the pregnancy periods were approximately 39 weeks. The rate of natural delivery was slightly higher than that of cesarean section. All infants were full-term infants with birth weight of approximately 3.5 kg. Male infants were more than female infants. It can be seen from the table that there is no significant difference between mothers and newborns in each group. There was no significant difference in total energy intake among the three groups of mothers.

3.2. Total energy intake to nutrient energy ratio

The purpose of this study was to explore the effect of nutrient energy supply ratio on TAG composition of breast milk by food frequency questionnaire. The total energy intake and nutrient supply ratio of the three groups are shown in Table 1. The total daily energy intake in the fat group was 2106.07 kcal in the mothers, 2189.21 kcal in the carbohydrate group, and 2246.35 kcal in the protein group. There was no significant difference in total energy intake among the three groups ($P > 0.05$). Each group had and only one nutrient function ratio was significantly higher than the other two groups. The fat energy ratio and protein energy ratio were significantly different among the three groups of mothers ($P < 0.05$). The carbohydrate energy ratio of mothers in carbohydrate group was significantly higher than that in fat group and protein group, but there was no significant difference between fat group and protein group.

Referring to the 2022 Dietary Guidelines for Chinese Residents (*Dietary Guidelines for Chinese Residents* , 2022), the total energy intake of all mothers was lower than the dietary energy requirement of lactating mothers (2300 kcal/day). Only the carbohydrate intake of lactating mothers in the carbohydrate group (˜57%) was within the acceptable range of macronutrients (50-60%), and the carbohydrate intake of the other two groups (˜45% and ˜43%) was below the lower limit of the range. In addition, the fat intake of lactating mothers in the three groups was higher than the upper limit of the acceptable range of macronutrients (20-30%), especially the fat intake of lactating mothers in the fat group and the protein group was too high (˜40% and ˜36%).

Lipids are known as a major source of energy, and a diet composed mainly of lipids can provide more energy for future generations (Mehta, 2008). A mother 's high-fat diet increases the concentration of milk fat, especially LCFA and MCFA, leading to a fuller transfer of this macronutrient to the infant (Seet et al., 2015). In an early report, it was observed that a high-fat diet can promote the development of the hippocampus by stimulating neurogenesis and reducing apoptosis (Li et al., 2017). However, some studies have shown that maternal high prenatal intake of sugar and prenatal or postpartum intake of saturated fatty acids are associated with increased obesity (Gomes et al., 2018) in offspring. But this excessive fat intake is consistent with two studies (Chen et al., 2012; Tian et al., 2019; Hui-Min et al., 2019) conducted in southeastern and northeastern China, which may be related to the nature of postpartum eating habits

in China. This postpartum practice refers to increasing the consumption of high-fat and protein-rich foods, such as chicken soup and pig hoof soup *etc* . It shows that Chinese lactating women need to follow a more balanced diet, especially emphasizing less fat intake.

3.3. Compositional characteristics of breast milk triacylglycerols

In this study, UPLC-Q-TOF-MS was used to identify the species and content of TAGs in 180 breast milk, and a total of 108 TAGs were identified based on the m/z values of precursors and fragments. As shown, Table 2 shows the TAG in breast milk and 95% confidence intervals (95% CI) in 3 groups, of which 41 TAG were significantly different ($P < 0.05$). The highest content of TAG in mature breast milk is O-P-L (median of 17.09%; 95% CI: 16.83, 17.63), followed by O-P-O (median of 12.84%; 95% CI: 12.40, 13.20), which is consistent with many previous research conclusions. Studies on TAGs in breast milk in Western countries have shown that the highest content of TAG is O-P-P, which may be related to the different preferences of various countries for edible oils, genetic and environmental factors. Pons et al. (Morera Pons et al., 2000) detected the highest content of two TAGs in Spanish breast milk as O-P-O and O-P-L, which accounted for more than 49 % of the total, and dominated in transition milk (34%), and mature milk (42%). And Ten-Domenech et al. (Ten-Domenech et al., 2015) detected 42 TAGs in mature breast milk in Spain, of which O-P-O had the highest content, followed by O-P-L and O-P-La. Zou et al. (Zou et al., 2013) detected 22 TAGs in Danish breast milk, of which the highest content was O-P-O (about 22%), followed by O-P-L (about 17%) and O-P-La (about 10%). Different from Western countries, Zhu et al. (Zhu et al., 2021) studied breast milk from 8 different cities in China. A total of 66 TAGs were detected in mature breast milk, and the maximum values of O-P-O and O-P-L were 15.7 % and 11.88 %, respectively. The difference of TAG content in breast milk in different countries may be related to the detection methods used by researchers and the dietary habits and genes of mothers in different regions, which also reflects the diversity and complexity of TAG composition in breast milk.

This study shows that the highest content of TAG is O-P-L in Chinese breast milk which fits the previous studies (Zhang et al., 2021; Xinghe et al., 2019). This may be due to the high consumption level of soybeans and soybean products in Chinese mothers (Wang et al., 2023). Soybean oil ranks first in the total consumption of Chinese cooking (Fang and Beghin, 2002). The content of O-P-L in breast milk of protein group (˜18%) was significantly higher than that of carbohydrate group (˜17%) ($P < 0.05$). In addition, the content of O-P-O in breast milk of the protein group was 13.52%, which was significantly higher than that of the carbohydrate group (˜12%) ($P < 0.05$). It can be seen that the breast milk of the protein group is rich in saturated-unsaturated-unsaturated (SUU) type, especially 1,3-diunsaturated fatty acid-2-palmitate (UPU) type TAG. However, because there are few studies on the relationship between the composition of the three major nutrients and the composition of triglycerides in breast milk, it is not clear why the content of SUU type TAG in protein group is high.

Among the 41 TAGs with significant differences, 22 were MLCT type. L-L-Ca (median of 0.19%; 95% CI: 0.19, 0.22) and O-L-Ca (median of 0.64%; 95% CI: 0.62, 0.68) are typical MLL-type TAG. As shown in Table 3, the contents of MLCT for example L-L-Ca and O-L-Ca in breast milk of carbohydrate group were 0.24% and 0.71%, respectively, which were significantly higher than those of fat group and protein group ($P< 0.05$), and their contents were 0.17% and 0.60%. Similarly, the contents of L-L-La (median of 0.70%; 95% CI: 0.73, 0.85), O-L-La (median of 2.06%; 95% CI: 2.05,2.28) and L-Po-M (median of 0.12%; 95% CI: 0.12, 0.14) in breast milk of carbohydrate group (0.96%, 2.46% and 0.15%) were significantly higher than those of fat group (0.75%, 2.1% and 0.13%) and protein group (0.66%, 1.93% and 0.12%) ($P <0.05$). In addition, it can also be seen that the content of MML type TAG such as L-La-Ca (median of 0.22%; 95% CI: 0.22, 0.25), L-M-Ca (median of 0.16%; 95% CI: 0.16, 0.18) and L-La-La (median of 0.36%; 95% CI: 0.36, 0.42) and UUU such as L-L-L (median of 1.71%; 95% CI: 1.83, 2.20) and O-L-L (median of 3.82%; 95% CI: 3.94, 4.79) in the breast milk of the carbohydrate group was also significantly higher than that of the other two groups ($P< 0.05$). This study confirmed that the higher the proportion of carbohydrate energy supply in mothers in Nanjing, China, the higher the content of MLCT in breast milk.

The contents of SSU such as O-P-P (median of 2.89%; 95% CI: 2.96, 3.28) and S-O-P (median of 3.58%;

95% CI: 3.44, 3.86) in breast milk of the fat group were slightly higher, which were 3.31 % and 4.01 %, respectively. Table 2 shows that the composition and composition of TAGs in breast milk fat are correlated with maternal diet. This observation should inform future research and can be taken into account when discussing diet with breastfeeding mothers.

3.4. Comparation of triacylglycerol composition in breast milk of different dietary groups

In order to intuitively show the difference of MLCT composition and UPU composition in different groups of breast milk, their compositions were made into a box line diagram, as shown in Fig.1. According to Fig.1, the carbohydrate group had the highest MLCT (˜27%) and the lowest UPU (˜38%) in breast milk. On the contrary, the content of UPU (˜41%) in breast milk of the proteome group was higher than that of other groups, and the content of MLCT (˜23%) was lower than that of other groups. This is consistent with the positive correlation of the protein energy ratio with the amount of UPU in the breast milk. The total content of MLCT and UPU in breast milk of the three groups was very close, maintained at ˜65%, and there was no significant difference, indicating that the total content of MLCT and UPU in breast milk was not affected by dietary structure.

Principal component analysis (PCA) was performed on the MLCT components in breast milk of the three groups to further show the differences of MLCT components in breast milk under different dietary patterns. From Fig.2, it can be seen that although some breast milk samples from different dietary groups are clustered together, their distribution is significantly different, indicating that there are significant differences in the composition of MLCT in breast milk from different dietary patterns. For example, the breast milk samples of carbohydrate group were mainly distributed in the first, third and fourth quadrants, while the other two groups were distributed in four quadrants. The distribution of carbohydrate group was significantly different from that of the other two groups, indicating that the composition of MLCT in carbohydrate group was significantly different from that of the other two groups. The fat group was very close to the protein group, almost overlapping, both concentrated in the second and third quadrants, but the breast milk samples of fat group were more dispersed. The difference in the composition of MLCT in breast milk is related to the dietary structure of the mother, but further studies are needed to clarify.

The 21 kinds of TAGs with a content greater than 1 % in the three groups of breast milk were made into a cluster heat map to show the differences more intuitively in TAG content in breast milk of different dietary groups. The results are shown in Fig.3, and the differences in TAG composition in breast milk of different dietary groups were further analyzed. As can be seen in Fig.3, the carbohydrate group breast milk is rich in MLCT such as O-L-La, O-P-La, and O-L-M, the fat group breast milk is rich in SSU type, such as O-P-P, S-P-L and S-P-O, while the protein group breast milk is rich in SUU type especially UPU type, such as O-P-L and O-P-O. About 70 % of palmitic acid is distributed in the*sn* -2 position of TAG, and stearic acid, oleic acid, linoleic acid and linolenic acid are mainly distributed in the *sn* -1,3 position, which is conducive to the absorption and utilization of fat and minerals (Watkins et al., 2003). However, due to the current research on the relationship between dietary structure and breast milk TAG composition, it is not clear why there are differences in breast milk TAGs in different dietary groups. Future studies should focus on the effect of maternal diet on TAG composition in breast milk to better guide the diet of lactating women. This study confirmed that the energy supply ratio of carbohydrate in Chinese mothers was positively correlated with the content of MLCT in breast milk ($P < 0.05$), the energy supply ratio of protein was positively correlated with the content of UPU ($P < 0.01$), and the energy supply ratio of fat was positively correlated with the content of SSU ($P < 0.05$).

3.5. Correlation between energy supply ratio of three major nutrients and triacylglycerol composition of breast milk

Pearson correlation coefficient analysis was performed on the energy supply ratio of the three nutrients and breast milk TAGs. As shown in Tables 3, MMM in breast milk was weakly positively correlated with carbohydrate energy supply ratio ($P < 0.05$), MML was strongly negatively correlated with protein energy supply ratio ($P < 0.05$), and weakly positively correlated with carbohydrate energy supply ratio ($P < 0.05$).

The contents of MLCT and MLL in breast milk were strongly positively correlated with carbohydrate energy supply ratio ($P < 0.05$), strongly negatively correlated with protein energy supply ratio ($P < 0.05$), and weakly negatively correlated with fat energy supply ratio ($P < 0.05$). It also can be seen from Table 4 that the content of 8:0-MLL, 10:0-MLL and 12:0-MLL in breast milk of carbohydrate group was significantly higher than that of protein group ($P < 0.05$). In particular, 12:0-MLL in breast milk was strongly positively correlated with carbohydrate energy ratio ($P < 0.01$), strongly negatively correlated with protein energy ratio ($P < 0.01$), and weakly negatively correlated with fat energy ratio ($P < 0.05$). UPU was strongly positively correlated with protein energy ratio ($P< 0.05$), strongly negatively correlated with carbohydrate energy ratio ($P < 0.05$), and weakly positively correlated with fat energy ratio ($P < 0.05$).

The total content of MLCT and UPU in breast milk was not affected by dietary structure, indicating that the total content of MLCT and UPU in breast milk was relatively stable. Our previous study (Yu et al., 2022b) also pointed out that the sum of MLCT and UPU was not affected by region and lactation and remained stable. By changing the proportion of the three major nutrients in the diet, changes in the TAG composition of breast milk can be achieved, especially MLCT type TAG. In the face of infants with poor absorption of medium and long chain fatty acids or feeding intolerance, beneficial consequences can be produced by adjusting the mother 's diet.

4. Conclusion

This study introduced a previously developed UPLC-Q-TOF-MS method for the qualitative and quantitative detection of TAGs in 180 Chinese breast milk samples. According to the proportion of the three major nutrients (fat, carbohydrate, and protein) in the maternal diet, they were divided into three groups to explore the potential correlation between fat composition in Chinese breast milk and maternal dietary factors. Lactation is a physiological state, which poses a major challenge to maternal energy homeostasis due to its high demand for energy.

Maternal carbohydrate energy ratio was positively correlated with the amount of MLCT in breast milk ($P < 0.05$), protein with UPU triglycerides ($P < 0.01$) and fat with SSU ($P< 0.05$). However, the total content of MLCT and UPU were not significantly associated with the energy supply ratio of the three major nutrients. Comparison of the triglyceride composition in different groups showed that the total amount of MLCT in breast milk was significantly higher in the carbohydrate group than in the other two groups ($P < 0.05$). Further, the content of 8:0-MLL, 10:0-MLL and 12:0-MLL in the carbohydrate group was significantly higher than that in the proteome ($P < 0.05$). The total amount of UPU in the proteome breast milk was significantly higher than that in the carbohydrate and fat groups ($P < 0.05$). There was no significant difference in total content of MLCT and UPU in different groups.

There are two limitations of the current study that should be deeper studied in the future. Firstly, in the dietary recall survey, many participants were unable to identify specific cooking vegetable oil types or report the use of mixed oils; which limits the further analysis of the effect of each vegetable oil on the fat composition of breast milk. Secondly, the recruitment of lactating women only focused on the Jiangsu Province which lacking representation. Therefore, a diverse and larger population needs to be studied in the future to confirm these results, considering possible interactions and confounding factors. The digestive characteristics of different types of MLCT in the gastrointestinal tract of infants also need further study.

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Abbreviation used

TAG, triacylglycerol; MLCT, medium- and long-chain triacylglycerol; MLL, one medium-chain fatty acid and two long-chain fatty acids; MML,two medium-chain fatty acid and one long-chain fatty acids; FA, fatty acid; MCFA, medium-chain fatty acid; LCFA, long-chain fatty acids; LC-MS, high performance liquid Chromatography-Mass Spe; FFQ, food frequency questionnaire; UPLC, Ultra performance liquid chromatography; Q-TOF-MS, Quadrupole-time of flight-mass spectrometry; ESI, electrospray ionization source;

APCA, principal component analysis; SUU, saturated-unsaturated-unsaturated; UPU, 1,3-diunsaturated fatty acid-2-palmitate.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1. Demographic characteristics of groups by dietary pattern.

Characteristics	Fat group	Carbohydrate group	Protein grou
n=	60	60	60
Maternity information	*Maternity information*	*Maternity information*	*Maternity in*
Gestational age (weeks)	39.29 ± 1.06	39.48 ± 1.34	39.49 ± 1.12
Maternal age (years)	29.49 ± 3.74	30.03 ± 4.03	30.57 ± 3.62

Characteristics	Fat group	Carbohydrate group	Protein grou
Delivery type (vaginal/caesarean)	33/27	38/22	35/25
Baby information	*Baby information*	*Baby information*	*Baby inform*
Baby birth weight (kg)	3.43 ± 0.40	3.39 ± 0.42	3.40 ± 0.42
Baby gender (male/female)	35/25	39/21	32/28
Energy ratios of three nutriments[a]	*Energy ratios of three nutriments*[a]	*Energy ratios of three nutriments*[a]	*Energy ratio*
Energy (kcal/d)	2106.07 ± 439.81	2189.21 ± 411.14	2246.35 ± 4(
Fat (g)	92.03 ± 23.19	71.25 ± 14.68	89.95 ± 17.6
Fat (energy %)	39.67 ± 5.32[a]	29.42 ± 3.22[c]	36.46 ± 4.72
Carbohydrate (g)	236.55 ± 61.93	312.22 ± 63.52	249.02 ± 69.
Carbohydrate (energy %)	44.82 ± 6.43[b]	56.97 ± 3.78[a]	43.84 ± 5.97
Protein (g)	87.59 ± 21.07	81.06 ± 19.6	116.25 ± 26.
Protein (energy %)	15.51 ± 1.84[b]	13.61 ± 1.66[c]	19.70 ± 2.13

Different lower-case letters indicate significant differences between different dietary groups, P <0.05. [a]The contribution to total energy intake by macronutrient (mean daily intake). In China, the recommendations are 20-30% from fat and 50-65% from carbohydrates of total energy intake. For protein intake, lactating mothers are 25 g/d more than the reference value of the same age group, and the recommended intake is 80 g/d.

Table 2. Triacylglycerol composition (wt%) of in human breast milk.

TAGs	Fat group (n=60)	Carbohydrate group (n=60)	Protein group (n=60)	Median, 95 CI (n=180)
La-La-Ca	0.04 ± 0.04	0.05 ± 0.05	0.04 ± 0.04	0.03 (0.03, 0.05)
M-La-Ca	0.04 ± 0.04	0.05 ± 0.05	0.04 ± 0.03	0.03 (0.04, 0.05)
La-La-La	0.06 ± 0.06	0.07 ± 0.07	0.05 ± 0.05	0.04 (0.05, 0.07)
L-P-Bu	0.03 ± 0.03[ab]	0.04 ± 0.03[a]	0.03 ± 0.02[b]	0.03 (0.03, 0.04)
O-P-Bu	0.10 ± 0.08	0.10 ± 0.08	0.08 ± 0.06	0.08 (0.09, 0.11)
P-La-Ca	0.08 ± 0.08	0.10 ± 0.08	0.08 ± 0.06	0.07 (0.08, 0.10)
M-La-La	0.15 ± 0.13	0.18 ± 0.16	0.13 ± 0.08	0.13 (0.13, 0.17)
O-L-Bu	0.03 ± 0.03[ab]	0.04 ± 0.03[a]	0.02 ± 0.02[b]	0.03 (0.03, 0.03)
L-La-Ca	0.23 ± 0.12[b]	0.28 ± 0.13[a]	0.20 ± 0.10[b]	0.22 (0.22, 0.25)
O-La-Ca	0.42 ± 0.18	0.46 ± 0.20	0.39 ± 0.16	0.40 (0.39, 0.45)
P-M-Ca	0.13 ± 0.10	0.15 ± 0.12	0.12 ± 0.07	0.11 (0.12, 0.15)
M-M-La	0.25 ± 0.20	0.29 ± 0.25	0.23 ± 0.13	0.22 (0.23, 0.29)
O-L-Co	0.05 ± 0.04[ab]	0.06 ± 0.04[a]	0.04 ± 0.03[b]	0.04 (0.04, 0.06)
L-M-Ca	0.16 ± 0.08[b]	0.20 ± 0.10[a]	0.15 ± 0.07[b]	0.16 (0.16, 0.18)
L-La-La	0.38 ± 0.18[b]	0.46 ± 0.22[a]	0.35 ± 0.16[b]	0.36 (0.36, 0.42)
O-M-Ca	0.31 ± 0.15[ab]	0.34 ± 0.17[a]	0.28 ± 0.12[b]	0.29 (0.29, 0.34)
O-La-La	0.54 ± 0.27	0.59 ± 0.29	0.52 ± 0.24	0.51 (0.51, 0.59)
Po-M-La	0.14 ± 0.07[ab]	0.15 ± 0.08[a]	0.12 ± 0.06[b]	0.13 (0.13, 0.15)
S-M-Ca	0.07 ± 0.05	0.08 ± 0.05	0.07 ± 0.04	0.06 (0.06, 0.08)
S-La-La	0.06 ± 0.04	0.06 ± 0.05	0.06 ± 0.03	0.05 (0.05, 0.07)
P-P-Ca	0.07 ± 0.05	0.08 ± 0.06	0.07 ± 0.04	0.06 (0.07, 0.08)
P-M-La	0.27 ± 0.18	0.29 ± 0.23	0.24 ± 0.12	0.22 (0.24, 0.29)
M-M-M	0.02 ± 0.02	0.02 ± 0.03	0.01 ± 0.02	0.01 (0.01, 0.02)
O-L-Cy	0.08 ± 0.05[ab]	0.09 ± 0.05[a]	0.07 ± 0.04[b]	0.08 (0.08, 0.09)
L-Po-Ca	0.05 ± 0.03[ab]	0.05 ± 0.03[a]	0.04 ± 0.02[b]	0.05 (0.04, 0.05)
L-P-Ca	0.47 ± 0.42	0.51 ± 0.51	0.35 ± 0.37	0.47 (0.38, 0.51)

TAGs	Fat group (n=60)	Carbohydrate group (n=60)	Protein group (n=60)	Median, 95 CI (n=180)
L-M-La	0.26 ± 0.23	0.28 ± 0.28	0.19 ± 0.20	0.25 (0.21, 0.28)
O-P-Ca	1.40 ± 0.53	1.44 ± 0.60	1.34 ± 0.50	1.30 (1.31, 1.47)
O-M-La	0.74 ± 0.31	0.76 ± 0.35	0.66 ± 0.25	0.66 (0.68, 0.77)
S-P-Ca	0.16 ± 0.09	0.16 ± 0.10	0.14 ± 0.07	0.14 (0.14, 0.17)
S-M-La	0.15 ± 0.09	0.15 ± 0.10	0.14 ± 0.06	0.13 (0.13, 0.16)
P-P-La	0.23 ± 0.13	0.23 ± 0.15	0.21 ± 0.10	0.20 (0.21, 0.24)
P-M-M	0.05 ± 0.03	0.05 ± 0.03	0.04 ± 0.02	0.04 (0.04, 0.05)
L-L-Ca	0.20 ± 0.12^{ab}	0.24 ± 0.13^{a}	0.17 ± 0.11^{b}	0.19 (0.19, 0.22)
O-L-Ca	0.65 ± 0.21^{ab}	0.71 ± 0.21^{a}	0.60 ± 0.18^{b}	0.64 (0.62, 0.68)
L-Po-La	0.10 ± 0.03^{ab}	0.11 ± 0.03^{a}	0.09 ± 0.03^{b}	0.10 (0.09, 0.10)
S-L-Ca	0.14 ± 0.05^{ab}	0.15 ± 0.06^{a}	0.12 ± 0.04^{b}	0.13 (0.13, 0.15)
O-O-Ca	0.10 ± 0.04^{ab}	0.11 ± 0.05^{a}	0.09 ± 0.03^{b}	0.09 (0.09, 0.10)
L-P-La	1.69 ± 0.59^{ab}	1.90 ± 0.71^{a}	1.58 ± 0.53^{b}	1.65 (1.63, 1.82)
L-M-M	0.20 ± 0.08^{ab}	0.22 ± 0.09^{a}	0.18 ± 0.07^{b}	0.19 (0.19, 0.21)
O-P-La	2.31 ± 0.95	2.35 ± 1.01	2.17 ± 0.92	2.09 (2.14, 2.42)
O-M-M	0.69 ± 0.40	0.68 ± 0.38	0.58 ± 0.31	0.58 (0.60, 0.70)
S-P-La	0.33 ± 0.19	0.30 ± 0.17	0.30 ± 0.17	0.28 (0.28, 0.34)
S-M-M	0.07 ± 0.04	0.06 ± 0.04	0.06 ± 0.04	0.06 (0.06, 0.07)
P-P-M	0.08 ± 0.06	0.08 ± 0.05	0.07 ± 0.05	0.07 (0.07, 0.09)
L-Ln-La	0.05 ± 0.06^{ab}	0.06 ± 0.06^{a}	0.03 ± 0.04^{b}	0.03 (0.04, 0.06)
L-L-La	0.75 ± 0.35^{b}	0.96 ± 0.49^{a}	0.66 ± 0.34^{b}	0.70 (0.73, 0.85)
O-L-La	2.10 ± 0.68^{b}	2.46 ± 0.85^{a}	1.93 ± 0.65^{b}	2.06 (2.05, 2.28)
L-Po-M	0.13 ± 0.05^{b}	0.15 ± 0.06^{a}	0.12 ± 0.04^{b}	0.12 (0.12, 0.14)
Po-Po-Po	0.02 ± 0.01^{b}	0.02 ± 0.01^{a}	0.02 ± 0.01^{b}	0.02 (0.02, 0.02)
Ed-M-M	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.02 (0.02, 0.02)
O-O-La	1.33 ± 0.38	1.42 ± 0.43	1.28 ± 0.36	1.31 (1.28, 1.40)
O-Po-M	0.10 ± 0.03	0.11 ± 0.03	0.10 ± 0.03	0.10 (0.10, 0.11)
S-L-La	0.50 ± 0.14	0.53 ± 0.16	0.48 ± 0.14	0.49 (0.48, 0.52)
L-P-M	0.79 ± 0.23	0.85 ± 0.26	0.76 ± 0.22	0.78 (0.77, 0.84)
S-O-La	0.40 ± 0.16	0.38 ± 0.16	0.39 ± 0.18	0.35 (0.36, 0.41)
O-P-M	1.50 ± 0.59	1.41 ± 0.58	1.44 ± 0.68	1.31 (1.36, 1.54)
S-P-M	0.15 ± 0.08	0.13 ± 0.07	0.14 ± 0.08	0.13 (0.13, 0.15)
P-P-P	0.11 ± 0.06	0.10 ± 0.05	0.10 ± 0.06	0.09 (0.09, 0.11)
O-P-Pa	0.02 ± 0.02	0.01 ± 0.02	0.02 ± 0.04	0.01 (0.01, 0.02)
Et-L-La	0.02 ± 0.02^{ab}	0.02 ± 0.02^{a}	0.01 ± 0.02^{b}	0.01 (0.01, 0.02)
L-Ln-M	0.03 ± 0.03^{ab}	0.04 ± 0.03^{a}	0.02 ± 0.03^{b}	0.02 (0.02, 0.03)
L-L-M	0.71 ± 0.30^{b}	0.83 ± 0.37^{a}	0.68 ± 0.29^{b}	0.69 (0.69, 0.79)
O-L-M	2.01 ± 0.50	2.07 ± 0.56	1.95 ± 0.55	1.98 (1.93, 2.09)
O-Po-Po	0.4 ± 0.10	0.41 ± 0.10	0.39 ± 0.10	0.38 (0.39, 0.41)
L-P-Po	0.33 ± 0.09	0.34 ± 0.10	0.33 ± 0.09	0.32 (0.32, 0.35)
O-O-M	1.48 ± 0.33	1.36 ± 0.32	1.48 ± 0.45	1.41 (1.38, 1.49)
O-P-Po	0.83 ± 0.18^{a}	0.76 ± 0.16^{b}	0.84 ± 0.21^{a}	0.79 (0.78, 0.84)
S-L-M	0.28 ± 0.06	0.26 ± 0.06	0.28 ± 0.09	0.27 (0.26, 0.28)
L-P-P	2.09 ± 0.50^{ab}	1.95 ± 0.49^{b}	2.19 ± 0.53^{a}	2.01 (2.00, 2.15)
S-O-M	0.50 ± 0.17^{a}	0.42 ± 0.15^{b}	0.49 ± 0.16^{a}	0.44 (0.45, 0.50)
O-P-P	3.31 ± 1.15^{a}	2.79 ± 0.96^{b}	3.25 ± 1.05^{a}	2.89 (2.96, 3.28)
S-P-P	0.30 ± 0.16^{a}	0.25 ± 0.14^{b}	0.29 ± 0.14^{ab}	0.25 (0.26, 0.30)
O-L-Pa	0.06 ± 0.04	0.05 ± 0.03	0.06 ± 0.04	0.05 (0.05, 0.06)
L-Ha-P	0.11 ± 0.07	0.09 ± 0.07	0.11 ± 0.08	0.09 (0.09, 0.12)

TAGs	Fat group (n=60)	Carbohydrate group (n=60)	Protein group (n=60)	Median, 95 CI (n=180)
O-Ha-P	0.05 ± 0.04	0.04 ± 0.04	0.05 ± 0.04	0.04 (0.04, 0.05)
L-L-Po	0.26 ± 0.12	0.28 ± 0.12	0.28 ± 0.11	0.28 (0.26, 0.29)
L-Ln-P	0.56 ± 0.25	0.59 ± 0.26	0.58 ± 0.23	0.58 (0.54, 0.61)
O-L-Po	0.82 ± 0.25	0.87 ± 0.25	0.81 ± 0.25	0.86 (0.80, 0.87)
L-L-P	6.61 ± 1.93	7.09 ± 2.08	6.63 ± 1.90	6.82 (6.48, 7.07)
O-L-P	17.05 ± 2.73^{ab}	16.67 ± 2.67^{b}	17.97 ± 2.63^{a}	17.09 (16.83, 17.63)
O-O-P	13.13 ± 2.89^{a}	11.76 ± 2.41^{b}	13.52 ± 2.43^{a}	12.84 (12.40, 13.20)
S-L-P	4.35 ± 0.96^{a}	3.90 ± 0.80^{b}	4.48 ± 0.81^{a}	4.26 (4.11, 4.38)
S-O-P	4.01 ± 1.61^{a}	3.12 ± 1.11^{b}	3.82 ± 1.34^{a}	3.58 (3.44, 3.86)
S-S-P	0.14 ± 0.10^{a}	0.10 ± 0.08^{b}	0.12 ± 0.08^{ab}	0.10 (0.11, 0.14)
O-L-He	0.02 ± 0.02	0.02 ± 0.02	0.02 ± 0.02	0.01 (0.02, 0.02)
O-L-Ha	0.08 ± 0.05	0.07 ± 0.05	0.07 ± 0.05	0.06 (0.06, 0.08)
O-O-Ha	0.04 ± 0.03	0.03 ± 0.03	0.04 ± 0.04	0.04 (0.04, 0.04)
L-L-Ln	0.20 ± 0.18	0.27 ± 0.28	0.19 ± 0.18	0.17 (0.19, 0.25)
L-L-L	1.88 ± 1.16^{b}	2.34 ± 1.39^{a}	1.83 ± 1.18^{b}	1.71 (1.83, 2.20)
O-L-L	3.98 ± 2.71^{b}	5.16 ± 3.03^{a}	3.96 ± 2.71^{b}	3.82 (3.94, 4.79)
O-O-L	2.76 ± 2.67	3.05 ± 2.90	3.46 ± 2.94	0.92 (2.67, 3.51)
Et-O-P	1.29 ± 1.03	1.30 ± 1.06	1.10 ± 0.96	1.22 (1.08, 1.38)
S-L-L	2.70 ± 1.47	2.77 ± 1.49	2.49 ± 1.30	2.26 (2.45, 2.87)
S-O-L	3.04 ± 1.14	2.99 ± 1.16	3.28 ± 1.27	2.98 (2.92, 3.28)
O-O-O	0.82 ± 0.56	0.79 ± 0.57	0.75 ± 0.55	0.66 (0.71, 0.87)
S-S-L	0.64 ± 0.32	0.57 ± 0.32	0.64 ± 0.39	0.57 (0.57, 0.67)
S-O-O	0.80 ± 0.38^{ab}	0.69 ± 0.30^{b}	0.87 ± 0.37^{a}	0.73 (0.74, 0.84)
E-O-P	0.05 ± 0.03^{a}	0.04 ± 0.02^{b}	0.05 ± 0.03^{a}	0.04 (0.04, 0.05)
S-S-O	0.13 ± 0.08^{a}	0.09 ± 0.07^{b}	0.12 ± 0.08^{ab}	0.10 (0.10, 0.13)
DHA-L-P	0.01 ± 0.02	0.01 ± 0.01	0.01 ± 0.02	0.00 (0.01, 0.01)
Et-O-L	0.05 ± 0.04^{ab}	0.03 ± 0.03^{b}	0.05 ± 0.04^{a}	0.03 (0.04, 0.05)
De-O-P	0.07 ± 0.08	0.07 ± 0.09	0.07 ± 0.07	0.04 (0.06, 0.08)
E-O-L	0.11 ± 0.14	0.12 ± 0.20	0.14 ± 0.19	0.06 (0.10, 0.15)
Da-O-P	0.08 ± 0.14	0.10 ± 0.23	0.14 ± 0.26	0.02 (0.08, 0.14)
DHA-O-L	0.04 ± 0.05	0.02 ± 0.03	0.04 ± 0.04	0.01 (0.03, 0.04)
DHA-S-L	0.03 ± 0.05	0.01 ± 0.03	0.03 ± 0.05	0.01 (0.02, 0.03)
MLCT	25.82 ± 7.53^{ab}	27.35 ± 8.64^{a}	24.12 ± 7.13^{b}	24.63 (24.60, 26.92)
MLL-MLCT	20.88 ± 5.46^{ab}	21.98 ± 6.28^{a}	19.67 ± 5.52^{b}	20.36 (19.98, 21.70)
MML-MLCT	4.95 ± 2.17^{ab}	5.37 ± 2.49^{a}	4.45 ± 1.74^{b}	4.55 (4.60, 5.24)

Different lower-case letters indicate significant differences between different dietary groups, P <0.05. Abbreviations of fatty acids: Bu: 4:0; Co: 6:0; Cy: 8:0; Ca: 10:0; La: 12:0; M: 14:0; Mo: 14:1; P: 16:0; Po: 16:1; S: 18:0; O: 18:1; L: 18:2; Ln: 18:3; E: 20:0; Eo: 20:1; Ed: 20:2; Et: 20:3; ARA: 20:4; EPA: 20:5; Da:22:1; Dt: 22:4; De:22:4; DPA: 22:5; DHA: 22:6.

Table 3. Pearson correlation coefficients of the energy and three nutriments with TAGs profiles by fatty acyl chain-length in human breast milk.

TAGs	Energy	Fat	Carbohydrates	Protein
	(kcal/d)	(energy %)	(energy %)	(energy %)
MMM	0.131	-0.135	0.158*	-0.141
MLCT	0.014	-0.150*	0.205**	-0.232**
MLL-MLCT	0.017	-0.155*	0.212**	-0.240**
MML-MLCT	0.005	-0.128	0.175*	-0.197**
8:0-MLL	-0.003	-0.167*	0.201**	-0.188*
10:0-MLL	0.000	-0.164*	0.218**	-0.238**
12:0-MLL	0.036	-0.204**	0.264**	-0.277**
14:0-MLL	0.009	0.036	0.077	-0.127
LLL	-0.018	0.151*	-0.205**	0.229**

*, P <0.05; **,P <0.01. M, medium-chain fatty acid; L, long-chain fatty acid.

Legends to Figures :

Fig. 1. Contents (%) of medium- and long-chain triacylglycerol, di-unsaturated fatty acyl-palmitoyl-glycerols in human breast milk of different dietary pattern.

Different lower-case letters indicate significant differences between different dietary groups, P <0.05.

Fig. 2. Principal component analysis of medium- and long-chain triacylglycerol in human breast milk from different dietary pattern groups.

Fig. 3. Cluster heat map of triacylglycerols (1% of total triacylglycerols) in human breast milk from different dietary pattern groups.

Fig. 1.

Fig. 2.

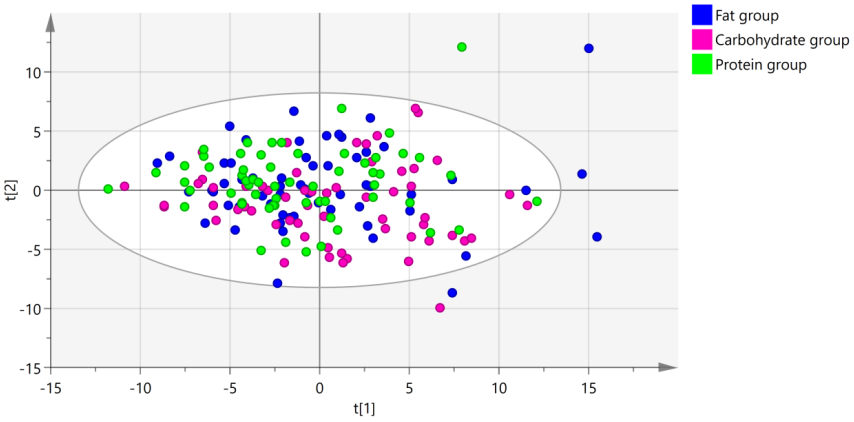

Fig. 3.