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Lactational changes of fatty acids and fat-soluble antioxidants in human milk from healthy Chinese mothers

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Abstract

Human milk fat is specially tailored to supply the developing infant with adequate and balanced nutrients. The present study aimed to quantify the composition of fatty acids, tocopherols and carotenoids in human milk, with special emphasis on the lactational changes. Colostrum, transitional and mature milk samples were collected longitudinally from the same forty-two healthy, well-nourished Chinese mothers. Fatty acids were quantified by GC with carotenoids (carotenes and xanthophylls) and tocopherols (*α*-, *γ*-tocopherol) determined by HPLC. Total fatty acid (TFA) content increased from 15·09 g/l in colostrum to 32·57 g/l in mature milk with the percentages of DHA and arachidonic acid (ARA) decreased. The ratio of *n*-6:*n*-3 PUFA and ARA:DHA remained constant during lactation at about 11:1 and 1·3:1, respectively. Both *α*-tocopherol and *γ*-tocopherol decreased over lactation with the ratio of *α*-:*γ*-tocopherol declined significantly from 7·21:1 to 4·21:1 ($P < 0·001$). Carotenoids all dropped from colostrum to mature milk as the less polar carotenes dropped by 88·67 %, while xanthophylls only dropped by 35·92 %. Lutein was predominated in both transitional and mature milk carotenoids (51·64–52·49 %), while colostrum carotenoids were mainly composed of lycopene (32·83 %) and *β*-carotene (30·78 %). The concentrations of tocopherols and xanthophylls but not carotenes were positively associated with TFA content in milk. These results suggested that colostrum and mature milk contained divergent lipid profiles and selective transfer mechanisms related to polarity might be involved. The present outcomes provide new insights for future breast-feeding studies, which also add in scientific evidences for the design of both initial and follow-on infant formulas.

Key words: **Human milk: Fatty acids: Tocopherols: Carotenoids: Polarity: Lactation stages**

Milk fat accounts for 45–55 % of the total energy provided by human milk[1,2]. It is the essential source of bioactive fatty acids and important delivery medium for fat-soluble antioxidants such as tocopherols and carotenoids[1,2]. The composition of human milk fluctuates with the progress of lactation and can be divided into three stages as colostrum, transitional milk and mature milk. As milk total fat content increases with lactation, long-chain PUFA (LC-PUFA) and fat-soluble antioxidants such as *α*-tocopherol and *β*-carotene indicated sharp declines[3–6]. Specific mechanisms may exist in the transfer of different liphophilic components in order to satisfy the infant's requirements[3], and a detailed analysis of human milk fatty acids and fat-soluble antioxidants over lactation is imperative for better estimations of the dynamic demands.

Linoleic acid (LA, C18 : 2*n*-6) and *α*-linolenic acid (ALA, C18 : 3*n*-3) are two essential PUFA in human body and compete for the same enzymes required for the conversion into the corresponding *n*-6 and *n*-3 long-chain derivatives, respectively[7]. LC-PUFA exert a number of cognitive and immune benefits[8,9] with tocopherols providing antioxidant protections[10,11]. Recent studies have demonstrated the concern that the increasing maternal consumption of LA from vegetable oils may result in the predominance of proinflammatory arachidonic acid (ARA, C20 : 4*n*-6) in the infant's body, which impairs Th1/Th2 immune

Abbreviations: ALA, *α*-linolenic acid, C18 : 3*n*-3; ARA, arachidonic acid, C20 : 4*n*-6; LA, linoleic acid, C18 : 2*n*-6; LC-PUFA, long-chain fatty acids; TFA, total fatty acids.

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balance and neocortical development[9,12,13]. Contrarily, *n*-3 LC-PUFA help in promoting psychomotor development and lowering the risk of lifelong allergic diseases and emotional problems[8,12,14,15]. Hence, the evaluation of human milk *n*-6:*n*-3 PUFA or LA:ALA may outweigh the measurement of individual fatty acid alone. However, previous studies have rarely discussed these ratios in Chinese breast milks.

α-Tocopherol has been well documented, showing antioxidation, anti-lipid peroxidation and anti-inflammation functions. Other vitamin E isoforms were less investigated, but concerns have arisen that an increasing number of foods and formulas deliver much higher *γ*-tocopherol than *α*-tocopherol[4,16,17]. *γ*-Tocopherol also decreases lipid peroxidation (weaker than *α*-tocopherol) and neutrophilic inflammation (stronger than *α*-tocopherol), but it can promote type 2 inflammation[18,19]. The latest early life study in both Nigerian and American maternal–neonatal dyads reported the association between decreased circulating *α*-:*γ*-tocopherol and negative birth outcomes such as Caesarian sections[20]. The ratios of *α*-:*γ*-tocopherol could contribute to a more comprehensive understanding of the infant's vitamin E requirements than *α*-tocopherol alone, but the ratios in human milk still lack investigation.

The majority of carotenoids can be classified into two groups: carotenes (*α*-carotene, *β*-carotene and lycopene) and their hydroxylated derivatives-xanthophylls (lutein, zeaxanthin and *β*-cryptoxanthin)[21,22]. Although there are reports that high doses of carotenes might act as pro-oxidants and present cancer risks[23,24], carotenoids at physiological levels have been well recognised to exert antioxidant effect[21,22]. Additionally, each carotenoid plays unique roles. For example, *β*-carotene mainly serves as vitamin A source in maternal–neonatal pairs[21,22]; Lutein and zeaxanthin contribute vitally to the macular and cognitive development[21,22]. Consequently, the dominance of individual carotenoid may indicate different priorities in early life nutrition. The levels of human milk *β*-carotene have been investigated globally, but other carotenoids still warrant further investigations. Considering that the absorption of different carotenoids interferes substantially with each other and excessive consumption may pose undesirable risks[23], it is important to elucidate the dynamic supply of human milk carotenoids to sustain a better estimate of the infant's demand. However, to date, the available data are insufficient.

Given the insufficient data and the long-term neglect of the balance of lipid nutrients in human milk, the present study aimed to explore the profiles of fatty acids, tocopherols and carotenoids simultaneously in Chinese milks collected from healthy and well-nourished mothers. Nutrients were individually and contrastively analysed to report the concentrations and ratios. The lactational tendencies were specially focused to evaluate the dynamic nutrients supply in maternal–offspring dyads.

Materials and methods

Subject recruitment

The present study included lactating mothers aged between 18 and 40 years old with singleton and full-term delivery who were recruited from 1 July 2018 to 30 September 2018 at Xinhua Hospital affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China. Both mothers and their corresponding infants were medically certified as healthy (asymptomatic and with no clinical indications). Only mothers with family income beyond 150 000 CNY (21 513 USD) were included, which can surely be recognised as middle-income or above in China. All mothers were food-secured, well-nourished and had a good dietary habit as assessed by nutritionists. Anthropometric measurements were performed to collect the general characteristics including height, body weight, parity and educational background. All procedures of the present study were approved by the Ethics Committee of Xinhua Hospital (XHEC-2017-064), and informed consent was obtained from all the participants.

Human milk collection

Colostrum (1–5 d postpartum), transitional milk (11–15 d postpartum) and mature milk (41–45 d postpartum) were collected longitudinally from the same mothers. When maternal breasts were full of milk (around 09.00–11.00 hours), both sides were evacuated with an electric pump. The milk sample was carefully mixed to ensure homogenisation, from which a proportion (5–10 ml for colostrum; 20–50 ml for transitional or mature milk) was placed into a brown sterile conical tube and transported immediately to the freezer (−80°C) via cold chain within 5 h. The rest of the milk was fed to the infant. A total of ninety-five subjects participated in the study and provided 212 milk samples all together. Among them, 126 milk samples were collected longitudinally from the same forty-two mothers at three lactation stages and were chosen for the following analyses.

Fatty acid analysis

Lipids were extracted from 2·0 ml milk samples into dichloromethane–methanol–petroleum ether (30–60°C) (2:1:2, v/v/v; 20 ml) according to the previously described method[25]. The extraction solution was then evaporated to dryness under N_2 flushing and re-dissolved in hexane. An accurate amount of tritridecanoin (C13 : 0; Nu-Chek-Prep) was added as internal standard (4·0 mg for each sample), and the mixture was then transesterified with sodium methoxide methanol solution (0·5 M, 2 ml). The generated fatty acid methyl ethers were then extracted with hexane and purified through a 0·22 μm nylon filter for the further determination.

The fatty acid methyl ethers were then separated in a capillary column (Supelco 2560, 100 m × 0·25 mm × 0·20 μm; Sigma-Aldrich) and quantified by Agilent 7890B GC with flame ionisation detector (Agilent Tech.)[26]. The carrier gas was He with a flow rate of 0·8 ml/min and a split ratio of 1:20. Both the injector and detector temperature were 250°C. The oven programme was as follows: 100°C for the first 13 min, increased by 10°C/min until 180°C, maintained for 6 min, increased by 1°C/min until 200°C, maintained for 20 min, increased by 4°C/min until 230°C and maintained at this temperature for 13·5 min. A mixed fatty acid methyl ethers standard (GLC-746; Nu-Chek-Prep) was used to identify the fatty acid methyl ethers in milk samples. All of the twenty-eight kinds of fatty acids were quantified, and the presented results included eight kinds of major fatty acids (≥1 % of

total fatty acids (TFA)) and seven kinds of LC-PUFA. All determinations were conducted by duplicate with a mixed mature milk control sample accompanying each batch.

Tocopherols and carotenoids analysis

Tocopherols and carotenoids were extracted and analysed according to the method described previously with some modifications[27]. Briefly, 4 ml water containing 0·5 g sodium ascorbate, 10 ml methanol and 1 ml tetrahydrofuran was added successively to 0·5 ml of milk sample to prevent oxidation. Saponification reaction was then operated with 1 ml aqueous solution of potassium hydroxide (45 %, w/w) under 65°C water bath for 15 min, and the tocopherols and carotenoids were extracted by 10·0 ml hexane–methyl tert–butyl ether (3:1, v/v). Then, 4·0 ml of the upper organic layer was evaporated to dryness under N_2 flushing and re-dissolved in 400 µl dilution solution (10 % butylated hydroxytoluene in Methanol–methyl tert–butyl ether, 3:1, v/v) for further determination.

Tocopherols (α, γ-tocopherol) and carotenoids (lutein, zeaxanthin, β-cryptoxanthin, β-carotene, lycopene) were then analysed by reversed-phase Agilent 1260 HPLC (Agilent Tech.) on a C30 column (250 mm × 4·6 mm × 3 µm; YMC). The flow rate was set at 1·0 ml/min and mobile phase A was 100 % methanol and mobile phase B was 100 % methyl tert–butyl ether. The eluting gradient programme was: 0·0–10·0 min, 100 % A; 10·0–10·1 min, 90 % A; 10·1–20·0 min, 90 % A; 20·0–20·1 min, 62 % A; 20·1–29·0 min, 62 % A; 29·0–29·1 min, 30 % A; 29·1–40·0 min, 30 % A; 40·0–40·1 min, 100 % A; 40·1–46·0 min, 100 % A. Tocopherols were determined with fluorescence detection (Ex 290 nm, Em 327 nm; Agilent Tech.), and carotenoids were quantified with multiple wavelength detection (445 ± 4 nm; Agilent Tech.). External standards (analytical standards; Sigma-Aldrich) were used for quantification. All determinations were made by duplicate with a formula control sample (SRM 1849a, NIST) accompanying each batch.

Statistical analysis

Categorical data were expressed as percentages, and continuous data were expressed as medians and interquartile ranges (P25, P75) due to abnormal distribution assessed by the Shapiro–Wilk test. Data were log-transformed to meet normality, and indexes in human milk over different lactation stages were then compared using one-way ANOVA. Pairwise comparisons were then performed using Bonferroni correction if the results of ANOVA were significant. All of the analyses were carried out using SPSS Statistics 22.0 (IBM), and $P < 0·05$ was statistically significant. Figures were made by GraphPad Prism 6.0 (GraphPad Software).

Results

Basic characteristics of the lactating mothers and corresponding infants

As shown in Table 1, the forty-two lactating mothers were aged between 21 and 37 years with the median age 29 years. All mothers were Han Chinese and had completed high school education or above. The majority of the mothers had a normal pre-gestation BMI and appropriate weight gain during gestation. Most mothers were primiparas. Neonates all had an Apgar score of 10 and good growth parameters.

Table 1. Basic characteristics of the paired mothers and neonates (n 42) (Medians and ranges (minimum, maximum) for continuous variables; numbers and percentages for categorical variables)

	Parameters			
Characteristics	Median	n	Ranges (minimum, maximum)	%
Mothers				
Age (years)	29		21, 37	
Pre-gestation BMI (kg/m^2)	20·20		15·60, 28·34	
Pre-delivery BMI (kg/m^2)	27·37		19·20, 32·03	
Education				
High school		2		4·80
University		34		81·00
Postgraduate		6		14·30
Delivery mode				
Vaginal		21		50·00
Caesarean		21		50·00
Parity				
Primipara		32		76·20
Multipara		10		23·80
Neonates				
Gestational age (weeks)	39·43		37·14, 41·00	
Body length (cm)	50		46, 52	
Body weight (g)	3450		2740, 4360	
Head circumstance (cm)	34·5		33·0, 37·5	
Sex				
Female		24		57·10
Male		18		42·90

Fatty acids

As shown in Table 2, the amount of TFA doubled from colostrum (15·09 g/l) to transitional milk (29·95 g/l) and then remained relatively stable to mature milk (32·57 g/l). SFA (36·96–40·85 %) was the dominant fatty acids in human milk, followed by MUFA (32·53–34·95 %), while PUFA (24·85–26·86 %) occupied the least proportion. The percentage of LA remained stable over lactation, while ALA percentage increased. Consequently, the ratio of LA:ALA decreased from 19·37:1 in colostrum to 16·34:1 in transition, and 16·11:1 in mature milk. The ratio of n-6:n-3 PUFA remained constant as (10·80–11·65):1 over lactation. LC-PUFA including ARA, EPA (C20 : 5n-3) and DHA, only consisted a tiny part of milk TFA and showed significant decreases with lactation progress. The ratio of ARA:DHA was set at (1·27–1·37):1 among different lactation stages.

Concentrations of tocopherols and carotenoids

As shown in Table 3, milk tocopherols declined from 947·97 µg/100 ml in colostrum to 526·01 µg/100 ml in transitional milk and then declined to 361·01 µg/100 ml in mature stage. Both α-tocopherol and γ-tocopherol showed continuous decreases over different lactation stages. Compared with colostrum content, mature milk α-tocopherol declined by 65·10 % and γ-tocopherol declined by 42·11 %.

Similarly, total carotenoids in milk decreased from 34·42 µg/100 ml in colostrum to 20·36 µg/100 ml in transitional

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Table 2. Fatty acids in human milk over lactation (*n* 42)
(Medians and ranges (P25–P75))

	Colostrum		Transitional milk		Mature milk		
Fatty acids	Median	P25–P75	Median	P25–P75	Median	P25–P75	*P*
TFA (g/l)	15·09[a]	11·47–19·51	29·95[b]	24·59–36·23	32·57[b]	26·14–42·11	<0·001
Fatty acids (%)							
C12 : 0	2·65[a]	1·71–4·31	6·68[b]	5·77–7·67	4·36[c]	3·49–5·51	<0·001
C14 : 0	4·86[a]	3·77–5·83	5·97[b]	4·86–7·13	3·96[c]	3·09–5·29	<0·001
C16 : 0	22·77[a]	22·14–24·03	20·50[b]	19·45–21·82	21·06[b]	19·94–22·89	<0·001
C16 : 1	1·84[a]	1·50–2·02	1·95[b]	1·67–2·35	2·11[b]	1·89–2·46	0·010
C18 : 0	5·31[a]	4·76–5·61	4·87[b]	4·38–5·59	5·92[a,b]	5·2–6·63	0·002
C18 : 1*n*-9	31·96[a]	30·19–34·16	29·88[a]	28·16–32·42	31·61[a]	30·2–34·37	0·148
C18 : 2*n*-6	20·69[a]	19·34–22·76	20·53[a]	18·15–23·41	21·96[a]	18·75–24·33	0·895
C18 : 3*n*-3	1·10[a]	0·84–1·31	1·21[a,b]	0·96–1·60	1·34[b]	1·06–1·74	0·011
C20 : 2*n*-6	1·29[a]	1·13–1·49	0·68[b]	0·58–0·72	0·44[c]	0·4–0·48	<0·001
C20 : 4*n*-6	0·89[a]	0·78–1·01	0·73[b]	0·59–0·82	0·56[c]	0·47–0·66	<0·001
C20 : 5*n*-3	0·07[a]	0·05–0·09	0·07[a]	0·06–0·09	0·08[a]	0·05–0·12	0·079
C22 : 4*n*-6	0·55[a]	0·44–0·69	0·20[b]	0·18–0·25	0·14[c]	0·11–0·16	<0·001
C22 : 5*n*-6	0·16[a]	0·13–0·22	0·12[b]	0·09–0·14	0·10[b]	0·07–0·12	<0·001
C22 : 5*n*-3	0·35[a]	0·25–0·44	0·20[b]	0·17–0·23	0·16[b]	0·14–0·21	<0·001
C22 : 6*n*-3	0·67[a]	0·53–0·81	0·60[a]	0·47–0·73	0·44[b]	0·31–0·71	0·007
SFA	36·96[a]	35·14–41·31	40·85[b]	38·63–43·58	38·61[a]	35·75–41·21	0·003
MUFA	34·95[a]	33·2–37·7	32·53[a]	30·77–35·41	34·71[a]	32·62–38·05	0·124
PUFA	26·86[a]	24·99–28·75	24·85[a]	23·06–28·45	26·31[a]	22·97–28·60	0·218
PUFA *n*-3	2·18[a]	2·00–2·40	2·18[a]	1·83–2·49	2·17[a]	1·83–2·85	0·958
PUFA *n*-6	24·78[a]	23·10–26·36	22·84[a]	21·00–26·09	24·04[a]	20·77–26·23	0·183
n-6:*n*-3 PUFA	11·65[a]	10·20–12·93	10·90[a]	8·97–13·03	10·80[a]	8·58–13·97	0·848
LA:ALA	19·37[a]	16·84–23·25	16·34[b]	13·20–21·76	16·11[b]	12·64–20·66	0·016
ARA:DHA	1·37[a]	1·13–1·63	1·27[a]	1·00–1·53	1·34[a]	1·00–1·74	0·686

TFA, total fatty acids; LA, linoleic acid; ALA, α-linolenic acid; ARA, arachidonic acid.
[a,b,c] Median values within a row with unlike superscript letters were significantly different ($P < 0·05$).

Table 3. Tocopherols and carotenoids (μg/100 ml) in human milk over lactation (*n* 42)
(Medians and ranges (P25–P75))

	Colostrum		Transitional milk		Mature milk		
Antioxidants	Median	P25–P75	Median	P25–P75	Median	P25–P75	*P*
Tocopherols							
α-Tocopherol	840·44[a]	671·18–1157·02	418·68[b]	322·39–535·20	290·65[c]	223·98–382·00	<0·001
γ-Tocopherol	110·07[a]	72·93–165·14	76·78[b]	47·27–113·42	57·28[c]	35·60–84·70	<0·001
Total tocopherols	947·97[a]	749·60–1339·20	526·01[b]	410·39–667·09	361·01[c]	273·30–486·16	<0·001
Carotenoids							
Lutein	7·12[a]	5·13–13·03	9·49[b]	6·77–13·1	4·57[c]	2·95–7·56	<0·001
Zeaxanthin	2·15[a]	1·27–3·34	2·21[a]	1·33–2·95	1·11[b]	0·7–1·93	<0·001
β-Cryptoxanthin	3·90[a]	1·54–7·02	1·99[b]	1·31–3·56	0·82[c]	0·46–2·08	<0·001
β-Carotene	11·14[a]	6·47–23·27	3·13[b]	1·78–5·3	1·77[c]	1·03–3·07	<0·001
Lycopene	11·88[a]	7·09–17·43	1·26[b]	0·86–2·52	0·58[c]	0·29–0·99	<0·001
Total carotenoids	34·42[a]	23·29–63·69	20·36[b]	14·12–24·29	10·43[c]	6·50–14·82	<0·001

[a,b,c] Median values within a row with unlike superscript letters were significantly different ($P < 0·05$).

milk, and 10·43 μg/100 ml in mature milk. The concentrations of β-cryptoxanthin, β-carotene and lycopene all decreased continuously over different lactations. Lutein reached the highest level at transitional stage (7·12 μg/100 ml in colostrum; 9·49 μg/100 ml in transitional milk) and declined to the lowest level at mature stage (4·57 μg/100 ml). Zeaxanthin remained stable from colostrum to transitional stage and then significantly decreased at mature stage (2·15, 2·21 and 1·11 μg/100 ml, successively). Compared with colostrum content, mature milk lycopene indicated the biggest fall by 94·60 %, followed by β-carotene by 82·40 %, β-cryptoxanthin by 74·17 %, zeaxanthin by 39·47 % and lutein by 30·00 %. Carotenes decreased by 88·67 % and xanthophylls decreased by 35·92 %.

Ratio of α-:γ-tocopherol over lactation

As presented in Fig. 1, the ratio of α-:γ-tocopherol reached the highest level at 7·21:1 in colostrum, dropped to 5·29:1 in transitional milk, and further decreased to 4·21:1 in mature milk ($P < 0·001$).

Proportions of individual carotenoids over lactation

As shown in Fig. 2, the proportions of carotenoids in colostrum were listed as follows: lycopene 32·83 %, β-carotene 30·78 %, lutein 19·67 %, β-cryptoxanthin 10·78 % and zeaxanthin 5·94 %. Compared with colostrum, transitional milk and mature milk

contained much higher percentages ($P < 0{\cdot}001$) of lutein (51·64–52·49 %) and zeaxanthin (12·22–12·54 %) but much lower percentages ($P < 0{\cdot}001$) of β-carotene (17·31–20·00 %) and lycopene (6·55–6·97 %). The percentages of β-cryptoxanthin in colostrum and the later lactation stages (9·27–11·01 %) were alike.

Correlations of milk fat and fat-soluble nutrients

Table 4 details the correlations between the milk fat profile (TFA content and fatty acid percentages) and tocopherols and carotenoids (absolute concentrations). After adjustment for lactation stages, milk TFA content correlated positively with tocopherols (r 0·275–0·349, $P < 0{\cdot}01$) and xanthophylls (r 0·236–0·433, $P < 0{\cdot}01$) but not with carotenes. The PUFA percentage showed positive correlations with tocopherols (r 0·178–0·200, $P < 0{\cdot}05$) but not with carotenoids.

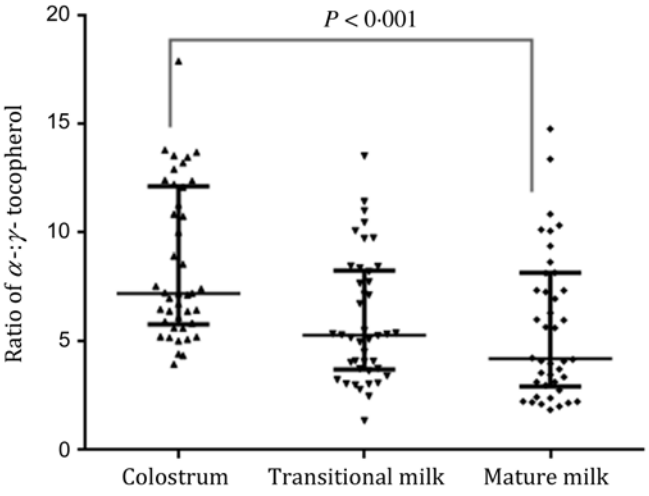

Fig. 1. Ratio of α-:γ-tocopherol in human milk (n 42) decreased from colostrum (7:21:1) to mature milk (4·21:1). Data are medians and interquartile ranges.

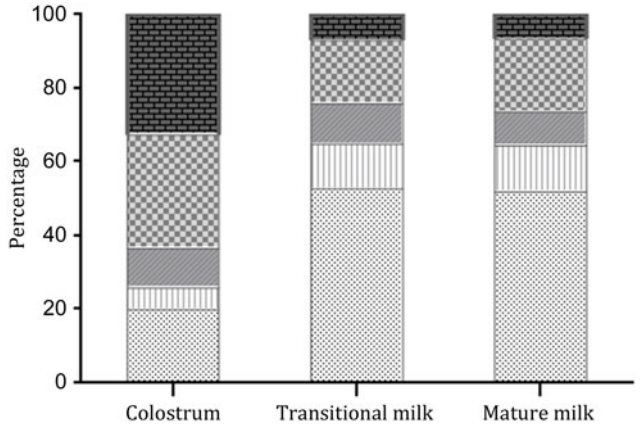

Fig. 2. Proportions of milk carotenoids over different lactations (n 42). Colostrum was predominated by lycopene (32·83 %) and β-carotene (30·78 %), while transitional milk and mature milk were mainly composed of lutein (51·64–52·49 %). , Lycopene; , β-carotene; , β-cryptoxanthin; , zeaxanthin; , lutein.

Discussion

Fatty acids

Chinese human milk studies[5,28,29] including our present one all reported a typically higher percentage of PUFA (22·40–30·04 %) than those (17·25–21·50 %) in the developed Asian countries[30,31] and those (14·68–19·71 %) in the Western countries[32–34], which was mainly due to the higher levels of human milk LA (18·90–25·10 %) in China[5,28,29] than in developed countries (11·48–16·59 %)[30–34]. Consequently, a fairly high ratio of LA:ALA (16:1) was observed in the mature milk of our study, which was on the top of the range of (9·26–16·50):1 in the latest reports from China[5,28,29]. This was much higher than the Chinese level of 7·62:1, dated back to 2006[30]. Previous Chinese milk studies rarely emphasised the rising trend. According to literature[35], the replacement of saturated animal fats with vegetable oils, such as sunflower oil, maize oil and soyabean oil in maternal diet, may be mainly responsible for the uptrend that we observed in the present study. Since n-6 series-derived eicosanoids are associated with proinflammatory properties, the predominance of n-6 PUFA is suggestive of higher risks of childhood allergic diseases, such as asthma and atopic eczema[9,15]. Also, the imbalance between n-6 and n-3 PUFA has been correlated with impaired cognitive and emotional development via epoxy metabolites and endocannabinoid system[12,13,36]. More investigations are warranted to confirm whether the increasing ratio of LA:ALA or n-6:n-3 PUFA in human milk is harmful to the short-term and long-term health of human infants.

We also found that LC-PUFA presented sharp decreases and mature milk showed the percentage of DHA as 0·44 % and ARA as 0·56 %, relatively comparable to the latest Chinese reports (DHA: 0·20–0·55 %; ARA: 0·50–0·89 %)[5,28,29] and the Chinese report back to 2006 (DHA: 0·35 %; ARA: 0·49 %)[30]. Since diets high in n-6 PUFA can result in lower endogenous conversion of ALA to n-3 LC-PUFA and impair tissue accumulation of n-3 LC-PUFA, there have been concerns that the rising ratio of LA:ALA may consequently have a negative influence on n-3 LC-PUFA levels in human milk[14,37]. However, speculated from our findings, the human milk LC-PUFA may come from the maternal intake of preformed LC-PUFA more than the endogenous synthesis.

Tocopherols and carotenoids

The latest three Chinese studies demonstrated obviously lower levels of α-tocopherol (colostrum: 426·4–783·5 μg/100 ml; mature milk: 126·4–206·0 μg/100 ml)[6,38,39] than Western reports (colostrum: 886·0–2455·0 μg/100 ml; mature milk: 100·0–568·5 μg/100 ml)[40]. However, dated back in 2002, the reported levels of α-tocopherol in Chinese milk were 898 μg/100 ml in colostrum and 331 μg/100 ml in mature milk[41], which were used in the calculation of the Dietary Recommended Intakes for Chinese infants (the latest 2013 version)[42]. Given the improving nutritional status of Chinese women, the lower levels in recent reports seemed to be illogical and potential confounding factors may exist. Nevertheless, our present data (colostrum: 840·44 μg/100 ml, mature milk: 290·65 μg/100 ml) were relatively close to the 2002 report[41] and Western ranges[40]. More

Table 4. Correlations between the milk fat profile and concentrations of tocopherols and carotenoids (n 42)*

Correlation	α-Tocopherol	γ-Tocopherol	Ratio of α-:γ-tocopherol	Lutein	Zeaxanthin	β-Cryptoxanthin	β-Carotene	Lycopene
TFA	0·275†	0·349†	0·022	0·421†	0·433†	0·236†	0·085	−0·019
SFA	−0·152	−0·065	−0·066	0·009	0·060	−0·015	−0·108	−0·135
MUFA	0·085	0·054	−0·013	−0·004	−0·015	0·029	0·066	0·053
PUFA	0·178‡	0·200‡	0·007	0·056	0·095	0·048	0·112	0·040
LA	0·175	0·220‡	−0·015	0·073	0·106	0·057	0·104	0·010
ALA	−0·008	0·157	−0·238†	0·072	0·203‡	0·061	0·108	0·024
ARA	−0·073	−0·065	0·073	−0·055	−0·087	−0·114	−0·171	−0·110
EPA	0·048	−0·113	0·232†	−0·005	−0·006	0·081	0·052	0·017
DHA	−0·016	−0·176	0·206†	−0·053	−0·001	0·052	0·023	−0·018
LA:ALA	0·053	−0·091	0·207‡	−0·070	−0·203‡	−0·084	−0·082	−0·029
ARA:DHA	−0·007	0·200‡	−0·226‡	−0·005	−0·115	−0·112	−0·091	0·008

TFA, total fatty acids; LA, linoleic acid; ALA, α-linolenic acid; ARA, arachidonic acid.
* Partial analysis adjusted for lactational stages.
‡ Correlations were significant at $P < 0·05$, two-tailed.
† Correlations were significant at $P < 0·01$, two-tailed.

high-quality studies are warranted. Human milk γ-tocopherol[4,39] and carotenoids[3,43–45] were less investigated, and available data showed large disparities among global published data, which may be mainly due to different intakes of these nutrients.

In agreement with previous human milk studies worldwide[3,4,39,40,43–45], fat-soluble antioxidants in our study all indicated significant decreases, ranging between 30·00 and 94·60 % from colostrum to mature milk. Additionally, the present study demonstrated a down-regulated ratio of α-:γ-tocopherol from 7·21:1 to 4·21:1 with the progress of lactation, which is similar to previous milk studies (calculated by reported individual data)[4,39]. Polarity may be involved because α-tocopherol possessing lower polarity indicated bigger decreases than γ-tocopherol over lactation. As positive roles have been attributed to α-tocopherol in bronchopulmonary dysplasia and childhood asthma, γ-tocopherol indicates more harmful effects through pro-inflammatory properties than its antioxidant benefits[19]. However, infant formulas, calculated by the published data, provide a much lower ratio of α-:γ-tocopherol as (0·68–2·51):1[4,10]. Whether the balance of α-:γ-tocopherol in human milk exerts special significance on the offspring health needs further investigations and the extensive additions of γ-tocopherol in formulas should also be reconsidered.

Among carotenoids, we observed that less polar carotenes were more subjected to changes, which was supported by other studies[3,21]. Consequently, lycopene and β-carotene predominated in colostrum carotenoids, while mature milk was more enriched with lutein. From our perspective, none of the former studies figured out the special dominance mode of carotenoids during different lactation stages, but after calculation by the reported levels[3,21,43], similar dominance was observed ignoring the distinct absolute concentrations from our study. Polarity has been found to influence the distribution of carotenoids among lipoprotein, carotenes are generally transported via LDL and enriched in LDL receptor-rich tissues, such as liver and adrenal gland, while xanthophylls indicating higher polarity tend to be more associated with HDL and enriched in the retina and nervous system[3,21,43]. Similar to the previous study[43], colostrum carotenoids pattern resembled those of maternal plasma and the LDL fraction, whereas in mature milk, the pattern was similar to the HDL fraction. Supported by these findings, different transfer mechanisms may be involved during colostrogenesis and later lactation stages. Each fat-soluble nutrient has its own implications and may be of different priorities in the early life nutrition. As reported in literature[46], infant food formulas contained limited levels of carotenoids (0–23·3 μg/100 ml) and the carotenoids profile indicated no obvious differences from the initial formulas to the follow-on formulas. Hence, bottle-fed infants may not benefit from the uniquely tailored human milk. Future formula design must pay close attention to the lipid patterns.

Correlations between lipid components in human milk

Based on the opposite altered trends of TFA content and the levels of carotenoids and tocopherols in human milk, it can be assumed that the transfer of lipophilic nutrients into milk is not a mere reflection of the transfer of TAG. However, we found that milk tocopherols and xanthophylls but not carotenes correlated positively with milk total fat content after adjustment for lactation stages. These findings supported our above speculation that milk fat might serve as the transfer vehicle for lipophilic nutrients via selective mechanisms, possibly the lipoprotein-associated processes involving polarity. We also found that tocopherol levels but not carotenoids were positively associated with PUFA percentages in human milk, which was consistent with the theory that vitamin E may protect PUFA from oxidation and the infant's requirements of vitamin E are in relation to PUFA intake[10,11].

Limitations of the study

The limitations of the present study should be noted. Firstly, data on maternal dietary intakes were gathered but not calculated yet since the Chinese Food Composition Database lacks the data of γ-tocopherol and carotenoids. We are working on a comprehensive composition database using the US Department of Agriculture (USDA) Food and Nutrient Database, and further reports of dietary consumption can be expected. However, food security could not be an issue for the participants because the study inclusion criteria limited the study subjects to healthy, well-nourished women with balanced dietary habit and good socio-economic status. Secondly, only a single milk sample

(09.00–11.00 hours) was collected and may not be sufficient to reflect the daily content. This limitation was shared by most milk studies due to the feasibility and compliance concerns[47]. The time interval was specially chosen because in our pre-survey, mothers were likely to undergo complete breast fullness during this period and there would be enough time for milk collection and intraday transportation.

Conclusions

The present study was a comprehensive report of human milk lipid profiles including fatty acids, tocopherols and carotenoids over three different lactation stages. Mature milk contained a distinct lipid profile from colostrum with higher total fat content but lower LC-PUFA, antioxidants and α-:γ-tocopherol ratio. Meanwhile, mature milk mainly consisted of lutein, whereas lycopene and β-carotene predominated among carotenoids in colostrum. Polarity-associated lipoprotein transfer mechanisms may be involved in the transformation of milk content and composition. In spite of the distinct absolute concentrations among different studies, the balances of nutrients and their variation tendency during lactation were rather comparative. The ratios of nutrients might indicate more significance than the measurements of individual nutrients.

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None of the authors has any conflicts of interest to declare.

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