British Journal of Nutrition (2021), 126, 1281–1287 doi:10.1017/S0007114521000027

Carotenoid profile in breast milk and maternal and cord plasma: a longitudinal study in Southwest China

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(Submitted 24 July 2020 – Final revision received 23 December 2020 – Accepted 27 December 2020 – First published online 8 January 2021)

Abstract

Carotenoids are increasingly being implicated to have an important role in brain and eye development. This study aimed to quantify the content and profile of carotenoids in human breast milk, maternal plasma and neonatal umbilical cord plasma in Chengdu, an urban area in Southwest China. In this study, fifty-four healthy mothers were enrolled. Maternal blood, umbilical cord blood, colostrum, transitional milk and mature milk were collected. Concentrations of carotenoids (lutein, zeaxanthin, β-cryptoxanthin, β-carotene and lycopene) were analysed by HPLC. We found that carotenoid concentrations decreased from colostrum to mature milk. Hydrocarbon carotenoids with weaker polarity decreased more than the polar carotenoids. Lycopene concentrations dropped by 99 %, β-carotene by 92 %, β-cryptoxanthin by 83 %, lutein by 32 % and zeaxanthin by 22 %. Lycopene and β-carotene accounted for 70 % of the total carotenoids in colostrum, and lutein predominated amongst carotenoids in transitional milk and mature milk (51–55 %). Carotenoid concentrations in maternal plasma were much higher than that in cord plasma. Lutein predominated in cord plasma. The concentrations of all carotenoids in maternal plasma were correlated with those of cord plasma and human milk. These results are consistent with selective transport mechanisms in the mammary gland related to the polarity of carotenoids, and each carotenoid has its own implications, which may have different priorities in the early life development of infants. These findings may help guide dietary recommendations for carotenoid inclusion in infant formulas.

Key words: **Human milk: Carotenoids: Plasma: Lactation stages: Lutein**

Human breast milk from healthy, well-nourished mothers is rich in nutrients, hormones and growth and immune factors necessary for infants; it is the only natural food that can satisfy the need for survival and growth of infants[1,2]. As constituents of breast milk, carotenoids play an important role in babies' brain, vision and immune system development[3–5]. Carotenoids are natural pigments synthesised by a wide variety of plants and found in many animal tissues. Carotenoids can be classified into two groups according to their chemical structure, namely, hydrocarbon carotenes (β-carotene, α-carotene and lycopene) and their hydroxylated derivatives-xanthophylls (lutein, zeaxanthin and β-cryptoxanthin). Amongst them, β-carotene, α-carotene and β-cryptoxanthin can be converted into vitamin A in the body and are therefore called pro-vitamin A carotenoids. Carotenoids can effectively quench active oxygen and are well-recognised as important antioxidants in the human body[4,6]. Lutein and zeaxanthin accumulating in macular area of eyes can resist blue light and oxidative damage and positively affect visual development and brain development of infants. β-Carotene and β-cryptoxanthin mainly serve as a source of vitamin A and have antioxidant properties. The antioxidant capacity of lycopene is two to ten times higher than that of β-carotene[6].

Carotenoids can only be obtained from the diet given that the human body cannot synthesise them. The intake of carotenoids in the breast-fed infant is highly correlated with the carotenoid concentration of human milk. In addition, carotenoid concentrations in human milk are positively correlated with maternal carotenoid intake[7–10]. Moreover, the placenta transfer of fat-soluble vitamins is thought to be limited, leading to low content in newborn infant plasma[11]. Thus, the carotenoid concentration of colostrum may be particularly important for the newborn infant.

Human lactation is usually divided into three stages: colostrum (1–7 or 10 d postpartum), transitional milk (7–15 d postpartum) and mature milk (more than 15 d after delivery). Studies

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have shown that the concentration and composition of carotenoids in breast milk change greatly over the course of these stages of lactation. Most studies reveal that colostrum carotenoid concentration is the highest followed by sequential declines as lactation proceeds[12–15].

Umbilical cord is the direct link between a mother and her fetus, and the fetus is totally dependent on maternal nutrient delivery to umbilical venous blood. The correlation between the concentration of carotenoids in maternal and cord blood could well reflect the placental carotenoid transport.

Current studies on Chinese breast milk have mainly focused on macronutrients, minerals, fatty acids and vitamins[16–19]. In contrast, carotenoids in Chinese breast milk are relatively rarely reported, especially the longitudinal changes in concentration and composition. In addition, the concentrations of carotenoids in Chinese maternal and cord plasma are unknown. This study aimed to (1) longitudinally investigate the profile of carotenoids in breast milk from healthy mothers through different lactation stages and (2) investigate the corresponding profile of carotenoids in both maternal and cord plasma.

Methods

Subjects

This study was part of the larger initiative study which was a cross-sectional study designed to characterise the human milk composition of Chinese lactating mothers. Six cities (Chengdu, Shanghai, Guangzhou, Tianjin, Changchun and Lanzhou) were chosen for the characterisation of human milk according to the geographical location and status of economic development. Based on published literature and practical feasibility, at least fifty longitudinal samples were enrolled in each city. In this study, fifty-four lactating mothers from Chengdu aged between 20 and 35 years old were enrolled; they had singleton and full-term delivery and were recruited from 1 May 2018 to 30 September 2018 at Chengdu Women's and Children's Central Hospital, Chengdu, China. Their infants were appropriate for gestational age newborn infants and had an Apgar score >8. The mothers and their infants were medically certified as healthy (asymptomatic and with no clinical indications). The mothers did not have gestational hypertension, gestational diabetes or any other diseases affecting nutrient metabolism. All mothers were well-nourished, as assessed by nutritionists through a nutritional assessment including dietary assessment, anthropometric measurement, physical examination and biochemical tests. At enrolment, nursing mothers planned to exclusively breastfeed their infant for more than 2 months. All procedures were approved by the Ethics Committee of Chengdu Women's and Children's Central Hospital (IEC-C-005-V.03), and the study was registered in the Chinese Clinical Trial Registry (ChiCTR1800015387). Written informed consent was obtained from all participants in the study.

Data collection

Anthropometric and sociological aspects of the mothers, such as height, self-reported body weight at the beginning and staff-measured weight at the end of pregnancy, number of gestational weeks at delivery, parity and educational background as well as the sex, length and weight of the babies were collected from hospital medical records and questionnaires. Maternal height and body weight at the end of pregnancy were measured with electronic body scales (SUHONG, RGZ-120) just prior to or on the day of delivery. Maternal height, body weight at the beginning and at the end of pregnancy were collected to calculate BMI and gestational weight gain.

Human milk and plasma sample collection

Colostrum (1–5 d postpartum), transitional milk (10–15 d postpartum) and mature milk (40–45 d postpartum) were collected longitudinally from the same mothers. A single full breast (08.00–11.00 hours) was emptied with an electric pump (PHILIPS AVENT SCF 301). The milk sample was carefully mixed to ensure a homogeneous mixture, 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 (AS ONE TB1500 and AS ONE TB5000) and transported to the laboratory via cold chain within 5 h and immediately frozen at –80°C. The remaining milk was fed to the infant. Each milk sample was distributed into 1·2 ml cryogenic vials (Corning 430658) under dim light on ice, labelled with subject information and immediately frozen at –80°C. A total of 162 human milk samples were collected longitudinally from fifty-four mothers at three lactation stages.

A total of fifty-four pairs of maternal blood and umbilical cord blood samples (5 ml) were collected using vacutainers (BD vacutainer, lithium heparin blood collection tubes) during parturition and then centrifuged at 1500 ***g*** centrifugal force for 15 min to gather the plasma within 30 min. Plasma samples were also frozen at –80°C.

All milk and plasma samples were transported to Abbott Nutrition Research and Development Centre, Shanghai for analysis within 5 months of collection.

Sample preparation

Carotenoids were extracted and analysed according to the method previously described by Schimpf *et al.*[20]. In summary, 4 ml water containing 0·5 g sodium ascorbate, 10 ml methanol and 1 ml tetrahydrofuran were added sequentially to 1 ml of milk or 0·5 ml plasma into a 50 ml tube to prevent oxidation. The saponification reaction was then operated after 1 ml aqueous solution of potassium hydroxide (45 %, w/w) was added and mixed under 65°C water bath for 15 min. A quantity of 10·0 ml hexane-methyl tert-butyl ether (3:1, v/v) was added and mixed to extract the carotenoids, followed by 15 ml water for delamination. The samples were centrifuged at 2000 rpm for 5 min at room temperature to clarify extracts. Then, 2·0 ml of the upper organic layer were evaporated to dryness under N_2 flushing and redissolved in 200 µl dilution solution (10 % butylated hydroxytoluene in methanol-methyl tert-butyl ether, 3:1, v/v) for further determination.

Carotenoid analysis

Carotenoids (lutein, zeaxanthin, *β*-cryptoxanthin, *β*-carotene and lycopene) were analysed by reversed-phase Agilent 1260 HPLC (Agilent Tech) on a C30 column (250 mm × 4·6 mm ×

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3 μm; YMC Carotenoids P/N CT99S032546WT) placed in a column oven set to 25°C; the autosampler was also set to 25°C.

The injection volume of the final extract was 50 μl. The flow rate was set to 1·0 ml/min, the mobile phase A was 100 % methanol, and the mobile phase B was methanol-methyl tert-butyl ether (85:15, v/v). The eluting gradient programme was as follows: 0·0–10·0 min, 100 % B; 10·0–18·0 min, 30 % A; 18·1–25·0 min, 40 % A; 25·1–30·0 min, 75 % A; 30·1–35·0 min, 100 % B. Carotenoids were detected and quantified using UV at the wavelength of 470 nm. Quantification was performed by external calibration, and the standard calibration curve for each compound was constructed by plotting the response (peak area) *v.* the corresponding concentration of pure standards (analytical standards; Sigma-Aldrich). All determinations were made by duplicate with a quality control sample (CLC16-B) accompanying each batch.

Method performance was evaluated during method validation by analysing pooled breast milk spiked with standard mixtures of three different concentrations (25, 50 and 100 % of milk concentration) in duplicate ($k = 3$). Statistical analysis showed intra-assay and inter-assay variabilities below 5 % (expressed as the CV). Average recovery rates were 95·03, 96·47, 95·11, 94·55 and 95·00 % for lutein, zeaxanthin, β-cryptoxanthin, β-carotene and lycopene, respectively.

Statistical analysis

Categorical data were expressed as percentages, and continuous data were expressed as medians and interquartile ranges (P25, P75) due to non-normal distribution assessed by the Shapiro–Wilk test. Carotenoid concentrations in human milk over different lactation stages were compared using the non-parametric Friedman test. Then, pairwise comparisons were performed using Bonferroni correction if the results were significant. Differences between maternal and cord plasma values were tested using the Wilcoxon signed-rank test. Correlations between maternal and cord plasma/milk levels of carotenoids were tested using Spearman's correlations. The analyses were carried out using SPSS Statistics 22.0 (IBM), and $P < 0·05$ was considered statistically significant.

Results

Characteristics of the lactating mothers and corresponding infants

As shown in Table 1, the mean age of the lactating women was 30·57 (SD 3·08) years. The majority of lactating women had completed high school and had a normal pre-gestation BMI and appropriate weight gain during gestation. Half of these women were primiparas. The mean birth weight and length of neonates were 3442 (SD 381) g and 49·8 (SD 1·2) cm, respectively.

Table 1. Demographic characteristics of lactating mothers and their infants*
(Mean values and standard deviations; numbers and percentages, *n* 54)

Characteristics	Mean	SD
Mothers		
Age (years)	30·57	3·08
Pre-gestation BMI (kg/m^2)	21·19	2·83
Pre-delivery BMI (kg/m^2)	26·73	2·85
Gestational weight gain (kg)	14·25	4·41
Primipara		
n	27	
%	50·00	
Education		
High school or below		
n	11	
%	20·37	
College		
n	35	
%	64·81	
Master's or above		
n	8	
%	14·82	
Infants		
Sex		
Female		
n	29	
%	53·70	
Male		
n	25	
%	46·30	
Birth weight (g)	3442	381
Birth length (cm)	49·8	1·2
Gestational age (weeks)	38·89	0·96

* Data are expressed as mean values and standard deviations for continuous variables and numbers and percentages for categorical variables.

Carotenoid profile in breast milk over lactation

As shown in Table 2, total carotenoid concentration decreased from colostrum to mature milk. The concentrations of β-cryptoxanthin, β-carotene and lycopene decreased rapidly from colostrum to transitional milk and then continuously declined to mature milk (β-cryptoxanthin: 95·85, 23·95 and 15·44 μg/l; β-carotene: 262·57, 46·63 and 22·03 μg/l; lycopene: 246·42, 16·98 and 3·37 μg/l). Lutein remained stable from colostrum (104·06 μg/l) to transitional milk (119·27 μg/l) and then significantly decreased in mature milk (70·73 μg/l) ($P < 0·05$). The concentration of zeaxanthin rose from colostrum (19·80 μg/l) to transitional milk (23·95 μg/l) and then declined in mature milk (15·44 μg/l) ($P < 0·05$). From colostrum to mature milk, lycopene level dropped by 99 %, β-carotene by 92 %, β-cryptoxanthin by 83 %, lutein by 32 % and zeaxanthin by 22 %.

Carotenoid composition in human milk over lactation

As shown in Fig. 1, the proportions of carotenoids in colostrum were as follows: lutein 14 %, zeaxanthin 3 %, β-cryptoxanthin 13 %, β-carotene 36 % and lycopene 34 %. The proportions of carotenoids in transitional milk were as follows: lutein 51 %, zeaxanthin 10 %, β-cryptoxanthin 12 %, β-carotene 20 % and lycopene 7 %. And the proportions of carotenoids in mature milk were as follows: lutein 55 %, zeaxanthin 12 %, β-cryptoxanthin 13 %, β-carotene 17 % and lycopene 3 %.

Carotenoid levels in plasma

As shown in Table 3, the concentration of carotenoids in maternal plasma was markedly higher than that in cord plasma ($P < 0·001$). In maternal plasma, the concentration of lutein was the highest, followed by β-carotene, β-cryptoxanthin,

Table 2. Concentrations of carotenoids (μg/l) in human milk over lactation (Medians and 25th–75th percentiles (P25, P75), *n* 54)

	Colostrum	Transitional milk	Mature milk
Lutein			
Median	104·06[a]	119·27[a]	70·73[b]
P25, P75	58·71, 184·44	87·55, 181·98	45·18, 92·99
Min, max	6·13, 388·25	25·15, 258·50	20·15, 190·59
Zeaxanthin			
Median	19·80[a]	23·95[b]	15·44[c]
P25, P75	13·52, 26·99	19·53, 35·25	10·62, 21·90
Min, max	2·34, 60·07	7·76, 60·98	3·86, 40·74
β-Cryptoxanthin			
Median	95·85[a]	28·35[b]	15·92[c]
P25, P75	55·10, 181·96	22·11, 41·85	11·80, 20·87
Min, max	9·30, 427·64	8·41, 142·35	4·43, 166·16
β-Carotene			
Median	262·57[a]	46·63[b]	22·03[c]
P25, P75	128·27, 464·98	30·19, 61·72	13·13, 31·18
Min, max	14·37, 1201·21	10·60, 212·63	6·00, 58·21
Lycopene			
Median	246·42[a]	16·98[b]	3·37[c]
P25, P75	123·87, 385·25	10·62, 22·62	1·18, 9·23
Min, max	12·09, 1204·69	3·32, 61·18	0·00, 25·76

Min, minimum; max, maximum.
[a,b,c] Median values within a row with unlike superscript letters were significantly different ($P < 0·001$). The concentrations of carotenoids in human milk over different lactation stages were compared using the non-parametric Friedman test. Pairwise comparisons were performed using Bonferroni correction.

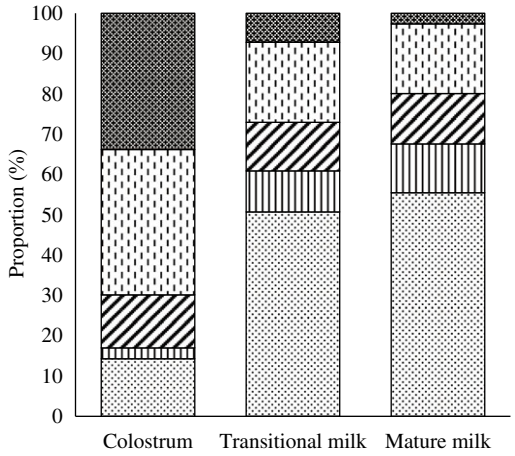

Fig. 1. Proportions of carotenoids in human milk over lactation (*n* 54). ⊡, Lutein; ◪, *β*-cryptoxanthin; ⊡, *β*-carotene; ◫, zeaxanthin; ▩, lycopene.

lycopene and zeaxanthin. The concentration of lutein in cord plasma was also the highest, followed by *β*-cryptoxanthin, *β*-carotene and zeaxanthin, and lycopene. The concentrations of lutein, zeaxanthin and *β*-cryptoxanthin in maternal plasma were approximately four to seven times higher than those in cord plasma. In addition, the levels of *β*-carotene and lycopene were 14–20 times higher than those in cord plasma.

Carotenoid composition in plasma

As shown in Fig. 2, the proportions of carotenoids in maternal plasma were as follows: lutein 33 %, zeaxanthin 6 %, *β*-cryptoxanthin 18 %, *β*-carotene 25 % and lycopene 18 %. The proportions of carotenoids in cord plasma were as follows: lutein 47 %, zeaxanthin 11 %, *β*-cryptoxanthin 21 %, *β*-carotene 14 % and lycopene 7 %. Lutein was the most common carotenoid in both maternal and cord plasma.

Table 3. Carotenoid levels (μg/l) in plasma (Medians and 25th–75th percentiles (P25, P75), *n* 54)

	Maternal plasma	Cord plasma	*P**
Lutein			
Median	465·53	84·46	<0·001
P25, P75	337·36, 679·99	63·55, 123·77	
Min, max	134·53, 1247·39	17·23, 226·94	
Zeaxanthin			
Median	80·75	20·43	<0·001
P25, P75	66·60, 99·91	13·41, 24·26	
Min, max	35·41, 196·99	5·76, 37·96	
β-Cryptoxanthin			
Median	255·72	37·10	<0·001
P25, P75	192·47, 341·40	30·29, 51·61	
Min, max	94·11, 738·34	11·28, 152·52	
β-Carotene			
Median	348·93	25·30	<0·001
P25, P75	271·23, 475·47	14·83, 33·94	
Min, max	69·12, 1957·99	4·74, 94·13	
Lycopene			
Median	261·79	13·04	<0·001
P25, P75	184·01, 406·72	7·07, 23·71	
Min, max	58·08, 1202·81	0·74, 67·63	

Min, minimum; max, maximum.
* Differences between carotenoid levels in maternal plasma and cord plasma were tested using the Wilcoxon signed-rank test.

Associations between plasma and human milk carotenoid concentrations

Correlations for all carotenoids between plasma and human milk are reported in Tables 4 and 5. The concentration of each carotenoid in maternal plasma was strongly correlated ($P < 0·01$) with that in cord plasma. In different lactation stages, positive correlations were observed between maternal plasma and both colostrum and transitional milk, but the correlations weakened or disappeared in mature milk.

Discussion

Longitudinal studies on the carotenoid content of Chinese breast milk and plasma are rarely reported. The present study is the first systematic longitudinal study, to our knowledge, to report the carotenoid levels in three stages of breast milk and both maternal and cord plasma carotenoid concentrations in Southwest China.

Our study revealed significant differences in both the concentrations and patterns of carotenoids in human milk during three stages of lactation. Consistent with some existing report[12,14,15,21,22], carotenoids decreased with lactation stage. However, the pattern of change varied among the carotenoids. We found that polar carotenoids exhibited less change over the lactation stages compared with the hydrocarbon carotenoids. This was especially true during the early lactation period (0–15 d). The isomers, lutein and zeaxanthin showed an identical pattern; both compounds rose slightly in transitional milk compared with colostrum and then declined in mature milk. In contrast, *β*-cryptoxanthin, *β*-carotene and lycopene rapidly decreased from colostrum to transitional milk and then decreased

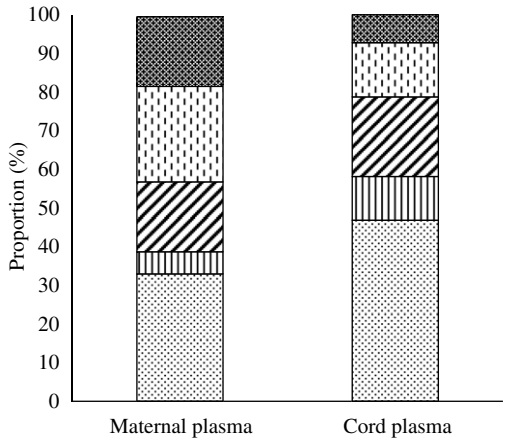

Fig. 2. Proportions of carotenoids in plasma (*n* 54). ⊡, Lutein; ◪, *β*-cryptoxanthin; ▯, *β*-carotene; ◫, zeaxanthin; ▩, lycopene.

Table 4. Correlations of carotenoid levels in maternal plasma and cord plasma (*n* 54)

Carotenoids	Correlation	*P**
Lutein	0·781	<0·001
Zeaxanthin	0·655	<0·001
β-Cryptoxanthin	0·687	<0·001
β-Carotene	0·717	<0·001
Lycopene	0·765	<0·001

* Spearman's correlation was performed to analyse the correlations between carotenoid levels in maternal plasma and cord plasma.

further in mature milk. This might be due to the differences in the polarity between the xanthophylls and hydrocarbon carotenoids and their distribution in lipoproteins. More polar carotenoids, such as lutein and zeaxanthin, are distributed more or less equally in LDL and HDL, whereas carotenes and *β*-cryptoxanthin are primarily associated with the LDL fraction[23,24]. Different transfer mechanisms, such as selective uptake of HDL, may be involved during colostrogenesis and later lactation stages[12,13].

Higher plasma carotenoid levels were observed in our study compared with those in other countries[11,25–27]. Consistent with this observation, the concentrations of carotenoids in breast milk were higher than the values reported by Macias *et al.* in Cuba[14] and the colostrum carotenoid levels were similar to those observed by Schweigert *et al.* in Berlin[12]. Moreover, our study reported higher lutein levels (20·15–190·59 μg/l) in mature milk than in milk from European and American donors (15–50 μg/l) reported in a previous multinational study by Canfield *et al.*[28]. This geographic impact is consistent with other reported Chinese breast milk studies. The carotenoid concentrations in the three stages of lactation measured here were higher than those previously reported by Xue *et al.* for Beijing, Suzhou and Guangzhou donors[15]. In addition, the levels of *β*-cryptoxanthin, *β*-carotene and lycopene in transitional milk we report are similar to those reported by Lipkie *et al.*[21] for shanghai donors. The differences compared with other studies may be due to dietary habits caused by geographic distribution, bioavailability of carotenoids by genomic impact and/or the testing method and sample collection procedure differences.

The magnitude and pattern of decrease in milk carotenoid concentration appeared to correspond to the polarity of the carotenoids. In colostrum, the proportions of the less polar hydrocarbon carotenoids (*β*-carotene and lycopene) were approximately 66 % of total carotenoids. In transitional and mature milk, the polar carotenoids including lutein became the main component amongst carotenoids. Its isomer zeaxanthin followed the same pattern at a lower concentration. We speculate that the pattern change in carotenoids as a function of lactation stage may relate to their importance to the infant. Newborn infants are particularly susceptible to oxidative stress due to high metabolic rates, low antioxidant concentrations and increased production of free radicals caused by increased postnatal oxygen pressure in plasma[25,29,30]. High concentrations of *β*-carotene and lycopene in colostrum may act as antioxidants in infants. In subsequent lactation stages, the infant gradually adapts to the environment and the impact of oxidative stress gradually decreases. Meanwhile, lutein, which promotes the development of the baby's brain and vision[3,31–33], dominates in the carotenoids. The specific carotenoid profiles in the different lactation stages might suggest that each carotenoid has its own implications and may be of different priorities in the early life.

We observed that carotenoid concentrations in cord plasma and breast milk were correlated with those of maternal plasma. These correlations indicate that maternal blood carotenoid concentration is predictive of the newborn infant's carotenoid concentration. In addition, the persistent correlation between human milk in three lactation stages and maternal plasma suggests that the carotenoid concentration in maternal plasma reflects a relatively stable level that is affected by long-term factors.

Carotenoid levels in maternal plasma were reported to be markedly higher than those of cord plasma. This finding is in accordance with previous studies[29,34]. There is some hypothesis. The antioxidant activity of the mother would increase to resist the ensuing stresses of pregnancy; this condition might reduce the amount of antioxidants circulating to the neonate[25,35]. Alternatively, oxidative stress during childbirth or in the placenta reduced the neonate's ability to scavenge free radicals which, in turn, would exhaust antioxidant reserves in the fetal compartment[36–39]. More likely, the low levels of carotenoids in cord plasma could be due to a decreased transport capacity for lipophilic compounds caused by low levels of circulating lipoproteins in fetal blood[27,40,41].

Proportions of carotenoids in cord plasma consisted of 25 % (zeaxanthin), 18 % (lutein), 15 % (*β*-cryptoxanthin), 7 % (*β*-carotene) and 5 % (lycopene) of maternal plasma levels. The profile of carotenoids in neonate cord plasma was similar to that of transitional and mature milk, but different from that of the maternal plasma. Godel *et al.* suggested that cord plasma retinol levels at 50–60 % of the maternal values may represent a normal range for newborn infants[42]. Therefore, the levels of carotenoids in cord blood may also represent normal values for neonates[27]. Sherry *et al.* found that the profile of carotenoids in the plasma of infants was similar to that in breast milk, in which lutein and zeaxanthin levels were the highest[8]. As shown in our study result, lutein, which promotes the development of the baby's brain and vision[3,31–33], dominates in carotenoids in Chinese breast milk and cord plasma. Enrichment of lutein in cord blood and breast

Table 5. Correlations of carotenoid levels in maternal plasma and human milk† (*n* 54)

	Lutein	Zeaxanthin	*β*-Cryptoxanthin	*β*-Carotene	Lycopene
Colostrum	0·578**	0·289*	0·603**	0·612**	0·319*
P	<0·001	0·038	0·001	<0·001	0·021
Transitional milk	0·589**	0·446**	0·694**	0·627**	0·392**
P	<0·001	0·001	<0·001	<0·001	0·004
Mature milk	0·220	0·352*	0·119	0·418**	0·175
P	0·118	0·010	0·402	0·002	0·214

Significant correlations: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$ (two-tailed).
† Spearman's correlation was performed to analyse the correlations between carotenoid levels in maternal plasma and milk.

milk may imply a greater demand and might be consistent with an important role of lutein in the growth and development of Chinese infants.

At present, supplementation of infant formula and follow-on formula with lutein is allowed in China, but other carotenoids have not been approved. Only a few brands have marketed infant formula fortified with lutein. Along with this, very little data on the levels of carotenoids in Chinese milk over different lactation stage are available. Breast milk is the best reference for the design of infant formula, and the fortification of specific carotenoids may impact infant health. Revision of the regulations on carotenoid fortification in infant formula would likely refer to data describing carotenoid concentrations in Chinese breast milk published in recent years. Therefore, our study can provide data that could be further used to support carotenoid fortification in infant and follow-on formulas in China.

Strengths of this study include the longitudinal sampling of the donors by lactation stage. In this case, the trends of carotenoid levels across lactation stages were clearer and more reliable than in cross-sectional studies. The limitations of the present study should be noted. The data on maternal dietary intake of carotenoids were not reported. Collection of blood samples is challenging; thus, we only collected cord plasma in childbirth. We were unable to collect maternal and infant blood on the same day as colostrum, transitional milk and mature milk were donated.

Conclusions

This study reports the concentrations of five carotenoids in maternal plasma, cord plasma and human milk over three different lactation stages in Southwest China. Carotenoid levels tended to decrease with the progression of lactation, and the patterns of individual carotenoid varied across lactation stages. Lutein predominated amongst carotenoids in transitional and mature milk, and lycopene and *β*-carotene were the main components in colostrum. The data show that the levels of carotenoids were significantly higher in maternal plasma than in cord plasma. Carotenoid concentrations in cord plasma and breast milk correlated with those in maternal plasma.

Acknowledgements

Abbott Nutrition Research and Development (R&D) Centre Shanghai sponsored the study and provided all analytical facility and support. The authors specially thank Matthew J. Kuchan from Abbott Nutrition R&D Columbus Headquarter for providing valuable suggestion on the study design and data interpretation.

This study was funded by Abbott Nutrition R&D Centre, Shanghai, China.

J. C., Y. Z., F. T., Y. M., X. C., K. M. J. and L. Z. conceived and designed the study protocols. H. S. and T. W. conducted the subject recruitment and the sample collection. H. S. conducted the sample determinations. H. S. wrote the manuscript. J. C. and Y. Z. were primarily responsible for the final contents. All the authors read and approved the final manuscript.

Y. Z., F. T., Y. M., X. C. and K. M. J. are employed by Abbott Nutrition.

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British Journal of Nutrition