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Carotenoids in maternal and cord blood, breast milk and their association with maternal dietary intake: a longitudinal study in Shanghai, China

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Abstract

Carotenoids are important bioactive substances in breast milk, the profile of which is seldom studied. This study aimed to explore the profile of carotenoids in breast milk and maternal/cord plasma of healthy mother–neonate pairs in Shanghai, China, and their correlation with dietary intake. Maternal blood, umbilical cord blood and breast milk samples from five lactation stages (colostrum, transitional milk and early-, mid- and late-term mature milk) were collected. Carotenoid levels were analysed by HPLC. Carotenoid levels in breast milk changed as lactation progressed ($P < 0{\cdot}001$). β-Carotene was the primary carotenoid in colostrum. Lutein accounted for approximately 50 % of total carotenoids in transitional milk, mature milk and cord blood. Positive correlations were observed between five carotenoids in umbilical cord blood and maternal blood (P all $< 0{\cdot}001$). β-Carotene levels were also correlated between maternal plasma and three stages of breast milk ($r = 0{\cdot}605$, $P < 0{\cdot}001$; $r = 0{\cdot}456$, $P = 0{\cdot}011$, $r = 0{\cdot}446$; $P = 0{\cdot}013$, respectively). Dietary carotenoid intakes of lactating mothers also differed across lactation stages, although no correlation with breast milk concentrations was found. These findings suggest the importance of exploring the transport mechanism of carotenoids between mothers and infants and help guide the development of formulas for Chinese infants as well as the nutritional diets of lactating mothers.

Keywords: **Breast milk: Carotenoids: Lactation stage: Maternal/cord blood: Maternal diet: China**

Human breast milk is the preferred source of nutrition for newborns and infants for development[1]. Exclusive breast-feeding is recommended for the first 6 months of life, along with continued breast-feeding for up to 2 years or more[2]. Breast milk from a healthy and well-nourished mother provides proteins, lipids, carbohydrates, vitamins, minerals and biologically active components that decrease the infants' risk of disease and help their immune system mature[3–5]. Carotenoids, as a category of biologically active components in breast milk, have a variety of nutritional and health effects, including antioxidative, anti-inflammatory and immunomodulatory properties[6,7]. For infants, carotenoids are important for their visual and cognitive development during early life[8,9]. The major benefits of carotenoids can be explained mechanistically in terms of their antioxidant potential, while specific carotenoids may also act through other mechanisms[10]. For example, lutein and zeaxanthin reach high concentrations in the retinal macula and absorb light at specific wavelengths to reduce blue light damage and promote infants' visual and brain development[11–13]. β-Carotene, as well as α-carotene, can be converted to vitamin A (retinol)[10]. Lycopene may help prevent cancer and CVD[14,15].

Carotenoids are a family of more than 600 fat-soluble plant pigments that cannot be synthesised by humans and must be consumed from food or through supplements[10]. Approximately 50 of these carotenoids can be found in the human diet, mainly in fruits and vegetables (especially yellow, orange, red or green), but are also found in eggs, oil and some fish[16,17]. The amount of certain nutrients in breast milk depends on the mother's dietary intake, which can affect the health of infants by influencing the composition of breast milk[18,19]. Further research is needed to determine whether the lactating mother's diet affects the carotenoid composition of breast milk. After birth, infants can only obtain carotenoids through breast milk or carotenoid-supplemented infant formula. Concentrations of carotenoids in human milk change dynamically during different lactation stages

Abbreviation: 24HDR, 24-h dietary review.

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to meet the needs of infants at different ages[5,20–26]. However, studies consistently show differences in the absorption of carotenoids in breast milk and infant formula by infants, possibly due to variations in transport mechanisms and bioavailability[27,28]. Therefore, providing additional insight into carotenoid concentrations in breast milk may help guide the addition of carotenoids to infant formula and provide more precise nutrition guidance during lactation.

The fetus acquires carotenoids through placental transfer during pregnancy[29]. Current literature reports a strong concentration between maternal plasma concentration and the concentration of carotenoids in the newborn[29–31]. However, there is a lack of reference ranges for carotenoid concentrations in breast milk and blood of mothers and infants in the Chinese population.

Much of the research on the nutrient composition of breast milk and its correlations with maternal and cord blood has been focused on macronutrients, fatty acids, minerals and vitamins in lactating Chinese mothers[32–35]. Previous studies on breast milk have rarely explored carotenoid concentrations based on lactation stage and dietary intake. We have conducted a longitudinal study in both southwestern[23] and coastal cities[20] of China, collecting maternal blood and breast milk at different stages of lactation to measure carotenoid levels. We also collected umbilical cord plasma at birth. Our findings revealed a strong correlation between the carotenoid content of umbilical cord blood and maternal blood, while regional differences were observed in the carotenoid content of breast milk. Considering the geographical distribution characteristics and urban economic development in China, different regions have varied lifestyles and dietary patterns. Thus, Shanghai was chosen as a representative coastal megacity for this study, distinguishing it from previous studies conducted in Chengdu[23] and Guangzhou[20].

This study aimed to (1) investigate the concentrations of carotenoids (lutein, zeaxanthin, *β*-carotene, lycopene and *β*-cryptoxanthin) in breast milk at different lactation stages among healthy mothers living in Shanghai; (2) analyse the relationship between carotenoids in maternal blood and those in colostrum or umbilical cord blood and (3) explore the associations between dietary carotenoid intake and carotenoid levels in breast milk. The findings could enhance our understanding of the breast milk composition in China and contribute to formulating recommendations for maternal supplements and infant formula.

Methods

Participants

This study was part of a larger initiative study, a longitudinal study designed to characterise the composition of human milk in Chinese lactating mothers. Shanghai was chosen as the representative metropolitan city of human milk based on its geographical location and economic development status. Based on previous research and practical considerations, it was calculated that a minimum of fifty longitudinal samples were needed for colostrum and transitional milk and blood samples. A sample size of fifty cases was chosen to ensure that any differences observed were not simply due to small sample sizes. At least 300 cross-sectional samples were included for mature milk analysis. A total of 322 breast-feeding women were recruited for this trial, out of which 301 completed follow-ups. This included fifty-two mothers who provided colostrum and transitional milk as well as blood samples, of whom thirty completed follow-ups and twenty-two were lost to follow-up.

In this study, healthy women were recruited from the Obstetrics Clinic of Xinhua Hospital affiliated with Shanghai Jiao Tong University School of Medicine, Shanghai, China between January 2018 and August 2019. Eligible women who met the inclusion and exclusion criteria were recruited. Inclusion criteria were (1) well-nourished mothers, aged 20–35 years, gestational week between 37 and 42 weeks, single-child delivery and planned exclusive breast-feeding and (2) healthy infants, with Apgar score > 8. Exclusion criteria were (1) mothers with metabolic diseases such as gestational diabetes mellitus, gestational hypertension and other diseases that may affect nutrient metabolism; (2) any acute or chronic infectious disease, serious heart or kidney diseases, disability and mental disorders and (3) alcohol consumption, drug abuse and smoking.

A total of 361 milk samples were collected from 5 lactation periods: 30 of colostrum, 30 of transitional milk, 101 of early-term mature milk, 101 of mid-term mature milk and 99 of late-term mature milk. Of these, ninety milk samples (thirty each of colostrum, transitional milk and early-term mature milk) were collected strictly longitudinally from the same thirty mothers who provided maternal blood and cord blood.

This study was conducted based on the declaration of Helsinki. All procedures involving human subjects were approved by the Ethics Committee of Clifford Hospital and registered in the China Clinical Trial Center (ChiCTR1800015387) as part of the Maternal Nutrition and Infant Investigation (MUAI) study. Only those participants who volunteered and signed an informed consent form were interviewed.

Demographic data

The study gathered anthropometric and sociological data from mothers, including age, weight, height, educational level, occupation, medical history, delivery mode, gravidity and parity. This information was obtained from hospital medical records and questionnaires. Self-reported weight at the beginning and at the end of pregnancy and the number of gestational weeks at delivery were also recorded. These data were obtained to calculate the gestational weight gain and BMI.

Dietary survey

The dietary intake of lactating mothers was evaluated using the 24-hr dietary review (24HDR) questionnaire and the FFQ. Prior to the administration of the questionnaires, the surveyors received consistent training to ensure uniform criteria for assessing dietary intake. On the day of sample collection, the investigator instructed lactating mothers to complete a questionnaire or fill it out over the phone, providing information on the type, amount and frequency of all foods consumed in the 24 h before breast-feeding, as well as in the month leading up to

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breast-feeding. The surveyors carefully reviewed the collected questionnaires and reached out to the lactating mothers for verification, confirmation and correction of any issues. The carotenoid content of Chinese foods was summarised based on the latest version of the USDA Nutrient Standard Reference Database and the food groups in the 6th edition of the Chinese Food Composition Table in 2018. The questionnaires were double entered to avoid inaccuracies.

Sample collection

A total of 5 ml of maternal venous blood and the corresponding umbilical cord blood were collected into a lithium heparin anticoagulant tube during labour, immediately centrifuged at 1500 ***g*** centrifugal force for 15 min to separate the upper plasma and stored in a −80°C refrigerator in the dark.

Breast milk samples in Shanghai were collected, packed and stored in accordance with standard operating procedures. During the period of 1–5, 10–15, 40–45, 200–240 and 300–400 d after the delivery, breast milk samples were collected on any day of each period between 09.00 and 23.00 (2 h after the first feeding in the morning) to avoid circadian influence on the results. The breast pump (PHILIPS AVENT SCF 301) was used to extract all the milk from a single full breast, which was thoroughly mixed. Each sample was collected in a brown sterile centrifuge tube (AS ONE TB1500 and AS ONE TB5000), protected from light, labelled with subject information. The milk sample (5–10 ml for colostrum; 20–50 ml for transitional or mature milk) was carefully mixed to ensure a homogeneous mixture 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.

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

HPLC analysis for carotenoids levels

Carotenoids were extracted and analysed according to the method previously described by Schimpf *et al.*[36]. Briefly, 4 ml of water, 0·5 g of sodium ascorbate, 10 ml of methanol, 3 ml of aqueous potassium hydroxide (45%, w/v) and 1 ml of tetrahydrofuran were added successively to 0·5 ml of milk sample. After mixing, the tube was placed for 15 min in a shaking water bath at 60°C for saponification. The samples were then cooled down on the ice and extracted by 10·0 ml hexane–methyl tert–butyl ether (3:1, v/v). After centrifugation, 2·0 ml of the upper extract was taken, reaching a dry state under N^2 flushing, and then re-dissolved in 200 µl dilution solution (10 % butylated hydroxytoluene in methanol–methylated–butyl ether, 3:1, v/v) and waited for further assay.

All the compounds (*β*-carotene, *β*-cryptoxanthin, lutein, lycopene and zeaxanthin) were determined using a high-performance liquid chromatograph Agilent 1260 infinity with a fluorescence detector, equipped with a C30 column (4·6 mm × 250 mm × 3 µm; YMC). The injection volume of each sample was 50 µl. The flow rate was set to 1·0 ml/min. Mobile phase A was an ammonium acetate aqueous solution, and mobile phase B was an acetonitrile/ether/methanol mixed solution (76/9/15, w/w/W). The eluting gradient programme was as follows: 0–20 min, and 75 % B; 20–22 min, 78 % B; 22–22·1 min, 80 % B; 22·1–30 min, 100 % B; 30–42 min, 100 % B; 42–42·1 min, 75 % B and 42·1–55 min, 75 % B. Each carotenoid was determined and quantified by UV light, and the standard calibration curve for each compound was constructed. All determinations were made by duplicate. The difference between the two parallel samples must be below 10 % of the average of the two; otherwise, the sample needs to be re-measured.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 26 software (IBM Corp.), and $P < 0{\cdot}05$ was considered statistically significant. All figures were drawn using Origin 2021 (OriginLab Corp.). Normally distributed continuous data were expressed as mean values and standard deviations, and continuous data with non-normal distribution were presented as median and interquartile range (P25, P75). Categorical data were expressed as counts and/or percentages. As the carotenoid contents and intake were non-normally distributed data, Kruskal–Wallis test and Spearman's correlation were used to compare the difference and analyse the correlations, respectively. Pairwise comparisons were then performed using Bonferroni correction if the results of Kruskal–Wallis test were significant.

Results

Basic characteristics of the lactating mothers

A total of 301 subjects were enrolled in this study, and 361 breast milk samples were collected from 0 to 400 days, of which thirty mothers who provided maternal blood/cord blood also provided colostrum, transition milk and early-term mature milk as a strictly longitudinal sample. The rest of the samples were cross-sectional and came from different groups. The collection of breast milk samples is shown in Table 1.

Table 2 provides the baseline demographic characteristics of lactating mothers at different stages of breast-feeding. The average age of mothers was 30·17 (SD 3·00) years, and their pre-pregnancy BMI, pre-natal BMI and pregnancy weight gain were all within the normal range. More than half of the mothers were primiparous and had natural childbirth. Most of them have acquired bachelor's degrees.

Carotenoid in plasma

Figure 1 shows the difference in carotenoid content between maternal blood and cord blood. Lutein was the most abundant carotenoid in both maternal and umbilical cord plasma, with proportions of 35 and 52 %, respectively. The levels of *β*-cryptoxanthin (19 %), lycopene (12 %) and zeaxanthin (6 %) accounted for about one-third of the total carotenoid in maternal plasma, with the remaining 29 % being *β*-carotene. Both zeaxanthin (9 %) and lycopene (4 %) were less than 10 %; *β*-carotene was 15 % and cryptoxanthin was 20 % in cord plasma.

As shown in Table 3, carotenoid levels in maternal plasma were much higher than in cord plasma. In particular, lycopene and zeaxanthin were about 22 and 11-fold more abundant,

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Table 1. Sample size of this study

	Colostrum (0–5 d)	Transition milk (10–15 d)	Early-term mature milk (40–45 d)	Mid-term mature milk (200–240 d)	Late-term mature milk (300–400 d)	Total
Sample size	30*	30*	30* 71	101	99	361

* Strictly longitudinal samples.

Table 2. Baseline maternal characteristics (Numbers and percentages; mean values and standard deviations)

Characteristics		Colostrum and transitional milk ($n = 30$)		Early-term mature breast milk ($n = 101$)		Mid-term mature breast milk ($n = 101$)		Late-term mature breast milk ($n = 99$)	
		n	%	*n*	%	*n*	%	*n*	%
Age at enrolment, years									
Mean		29·63		29·64		30·23		30·89	
SD		3·08		3·37		2·92		2·51	
Education level									
	High school graduate or below	4	13·33	7	6·93	1	0·99	1	1·01
	Bachelor's degree	26	86·67	81	80·20	90	89·11	83	83·84
	Master's degree or above	0	0	13	12·87	10	9·90	15	15·15
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Pre-pregnancy BMI, kg/m^2		20·89	3·09	20·99	2·64	21·01	2·64	21·42	2·57
Pre-natal BMI, kg/m^2		26·66	3·50	26·52	3·12	26·53	3·14	26·51	4·29
Pregnancy weight gain, kg		12·37	5·30	14·52	6·62	14·46	6·63	13·26	9·90
Primiparous									
n		23		76		77		70	
%		76·67		75·24		76·2		70·70	
Vaginal birth									
n		16		64		63		61	
%		53·33		63·34		62·4		61·61	
Gestational age, weeks		39·82	1·05	39·60	1·00	39·59	1·00	39·56	0·90

Data were expressed as mean values and standard deviations for continuous variables and count (percentage) for categorical variables. BMI was calculated as body weight by height squared (kg/m^2).

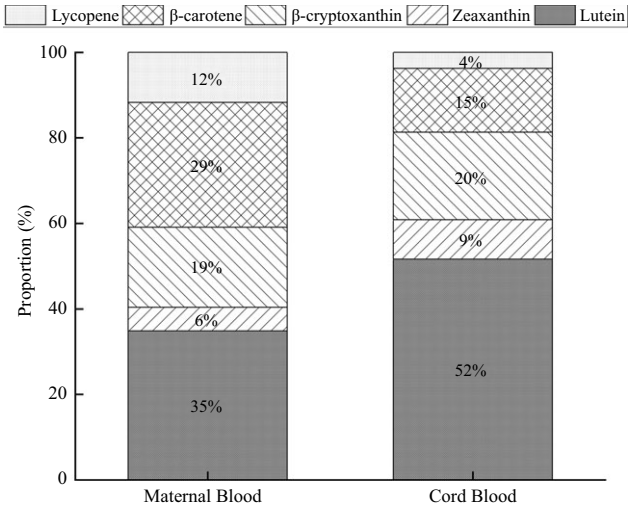

Fig. 1. Proportion of carotenoids in maternal and cord plasma (n 30).

respectively, in maternal blood than in cord blood. The concentrations of lutein, zeaxanthin and β-cryptoxanthin in maternal plasma were approximately four to six-fold higher than those in cord plasma. The interquartile range showed large individual differences in carotenoid content of maternal and cord plasma.

The correlation between carotenoids in maternal and cord plasma is also shown in Table 3. The concentration of each carotenoid in maternal plasma was positively correlated with the concentration in cord plasma. Among them, β-carotene ($r = 0{\cdot}912$; $P < 0{\cdot}001$) had the highest maternal-infant correlation, β-cryptoxanthin ($r = 0{\cdot}816$; $P < 0{\cdot}001$), lycopene ($r = 0{\cdot}631$; $P < 0{\cdot}001$), and lutein ($r = 0{\cdot}609$; $P < 0{\cdot}001$) correlations decreased in order and zeaxanthin ($r = 0{\cdot}543$; $P = 0{\cdot}002$) had the lowest maternal-infant correlation.

Carotenoid in human breast milk at different lactation

Figure 2 shows the distribution of carotenoids in breast milk at five stages. The contents of various carotenoids in colostrum differed significantly from that in transition and mature milk, with colostrum having the largest proportion of β-carotene (45 %), followed by lycopene (20 %), lutein (18 %), cryptoxanthin (13 %) and zeaxanthin (3 %). The highest proportion of lutein (47 %, 47 %, 43 %, 46 %) was found in transitional and all mature milk. Across different lactation stages of mature milk, the proportion of zeaxanthin (11–31 %) increased gradually, and the proportion of β-cryptoxanthin (11–3 %) decreased. The distribution of carotenoids in cord blood, transitional milk and early-term mature milk was very similar, with the most carotenoids being lutein and the least carotenoids being lycopene. Carotenoids were similar in mid-term mature milk and late-term mature milk.

Table 3. Maternal and umbilical cord plasma carotenoid levels (μg/l) and correlations (*n* 30) (Medians and interquartile ranges)

	Maternal plasma		Cord plasma		Percentage (cord blood *v.* maternal blood)		Correlation	
Carotenoid	Median	P25, P75	Median	P25, P75	Median	P25, P75	*r*	*P*
Lutein	423·46	316·98, 535·11	97·04	67·68, 133·51	22·69 %	19·05, 29·13 %	0·609**	<0·001
Zeaxanthin	67·91	52·22, 101·61	17·33	12·64, 23·41	25·95 %	21·93, 32·20 %	0·543*	0·002
β-Cryptoxanthin	227·33	153·20, 302·65	38·48	25·44, 48·93	16·72 %	11·75, 21·42 %	0·816**	<0·001
β-Carotene	355·32	265·16, 512·47	28·03	16·31, 50·05	8·11 %	5·89, 11·21 %	0·912**	<0·001
Lycopene	142·72	80·62, 237·20	7·07	3·80, 10·15	4·37 %	3·21, 7·74 %	0·631**	<0·001
Total	1311·73	1091·48, 1721·61	197·77	154·88, 233·66	14·19 %	12·29, 18·15 %	0·594**	<0·001

Data were expressed as medians and interquartile ranges. Spearman's correlation was performed to analyse the correlations between carotenoid levels in maternal plasma and cord plasma.
* $P < 0·05$.
** $P < 0·001$.

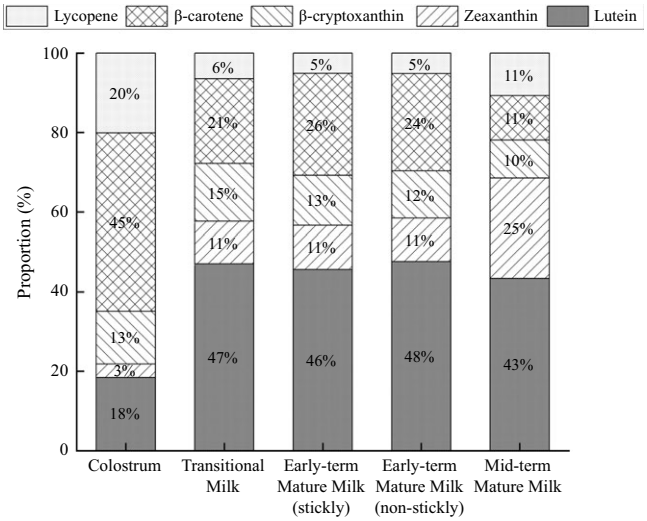

Fig. 2. Distribution of carotenoids in breast milk (*n* 30).

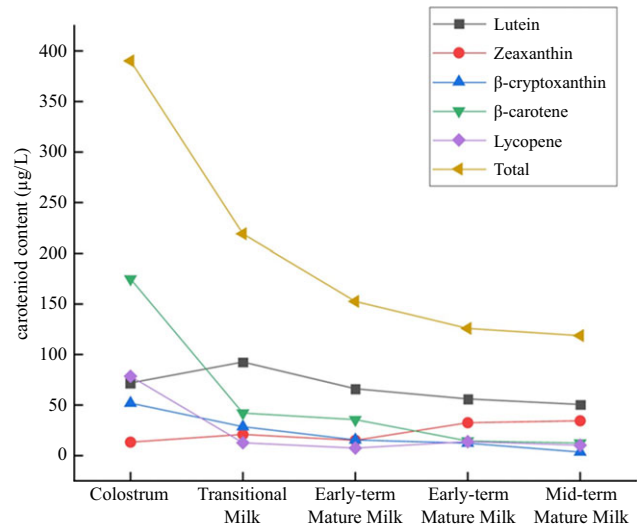

Fig. 3. Trends in carotenoid content during lactation.

Figure 3 and Table 4 present carotenoid concentrations and trends in human milk. Carotenoid concentrations decreased varied throughout the period of 0–400 d of lactation. Specifically, lutein decreased by 30 %, lycopene by 87 % and *β*-cryptoxanthin and *β*-carotene experienced the most decrease, by 93 %. Total carotenoids decreased from 390·19 μg/l in colostrum to 219·10 μg/l in transition milk (56 % of colostrum) and further decreased to 152·63 μg/l in early-term mature milk, 125·87 μg/l in mid-term mature milk and 118·75 μg/l in late-term mature milk (30 % of colostrum). The concentration of *β*-carotene declined steepest from colostrum to transitional milk. Zeaxanthin content increased to 2·5 times that of colostrum within 400 d. The levels of *β*-cryptoxanthin and *β*-carotene decreased continuously during lactation, but lutein increased from 71·96 μg/l in colostrum to 92·71 μg/l in transition milk and then gradually decreased in mature milk. Lycopene level was lowest in early-term mature milk (only 9·43 % of colostrum) and increased in mid- and late-term mature milk, but still below colostrum levels. In online Supplementary Table S1 and S2, we compared the carotenoid levels among thirty strictly longitudinal breast milk samples of colostrum, transition milk and early mature milk and evaluated three cross-sectional samples of mature milk that excluded the thirty longitudinal samples, and the results did not change significantly. Our previous reports from different Chinese locations also reported longitudinal trends[20,23,37–39].

Relationship between carotenoid concentrations in maternal blood and breast milk

Table 5 shows the correlation for all carotenoids between maternal plasma and human milk. *β*-Carotene, zeaxanthin and lycopene in colostrum and maternal blood were positively correlated ($r = 0·605$, $P < 0·001$; $r = 0·591$, $P < 0·001$; $r = 0·546$, $P = 0·002$). The concentration of each carotenoid in maternal plasma was positively correlated (P all $< 0·05$) with that in transition milk, but the correlations weakened or disappeared in mature milk. Correlations between cord blood and breast milk showed a similar trend.

Dietary carotenoid intake

As shown in Table 6, a total of 329 valid questionnaires from five lactation periods were returned for the 24HDR method and 254 valid questionnaires from four lactation periods were returned for the FFQ. Due to the COVID-19 epidemic, FFQ data are missing for breast-feeding mothers 10–15 d postpartum.

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Table 4. Comparison of carotenoids in breast milk at different period (μg/l) (Medians and interquartile ranges)

Carotenoid	Colostrum (0–5 d) *n* 30		Transitional milk (10–15 d) *n* 30		Early-term mature milk (40–45 d) *n* 101		Mid-term mature milk (200–240 d) *n* 101		Late-term mature milk (300–400 d) *n* 99		*P*
	Median	P25, P75	Median	P25, P75	Median	P25, P75	Median	P25, P75	Median	P25, P75	
Lutein	71·96 ^{ac}	65·36, 106·42	92·71^{a}	63·20, 126·24	66·12^{ac}	44·97, 101·82	56·11^{bc}	32·33, 78·54	50·68^{b}	32·62, 73·84	<0·001*
Zeaxanthin	13·29^{c}	9·17, 20·67	21·00^{bc}	15·30, 28·23	15·05^{c}	11·81, 23·55	32·53^{ab}	17·22, 44·47	34·41^{a}	22·15, 53·19	<0·001*
β-Cryptoxanthin	51·9^{a}	36·33, 84·58	28·63^{ab}	19·11, 44·97	15·50^{b}	9·55, 27·94	12·37^{c}	1·54, 20·17	3·46^{d}	0, 11·70	<0·001*
β-Carotene	174·95^{a}	83·51, 289·02	42·11^{ab}	26·80, 58·91	35·63^{b}	17·93, 51·40	14·53^{c}	10·33, 22·56	12·49^{c}	9·52, 17·42	<0·001*
Lycopene	78·58^{a}	43·45, 139·47	12·70^{b}	6·08, 20·72	7·41$_{c}$ (0, 13·40)		13·83^{b}	9·04, 22·64	10·29^{b}	7·29, 15·01	<0·001*
Total	390·19^{a}	235·69, 564·08	219·10^{ab}	151·79, 283·83	152·63^{bc}	96·82, 215·80	125·87^{c}	84·73, 200·20	118·75^{c}	80·28, 168·00	<0·001*

Data were expressed in median (P25, P75).
* Indicated a significant difference among five periods ($P < 0·05$). ^{abc}Different superscript letters in the same row were significantly different ($P < 0·05$).

The dietary carotenoid intakes of lactating mothers estimated by the 24HDR and the FFQ are shown in Tables 7 and 8, respectively. Data collected by the 24HDR questionnaire showed that only *β*-cryptoxanthin did not significantly differ in dietary intake between 0 and 400 d postpartum ($P = 0·361$). The dietary intake of total carotenoids in Shanghai lactating mothers declined gradually from 0 to 400 d postpartum, with the lowest total carotenoid intake (1625·00 μg/d) in the days of 300–400. Lactating mothers had the lowest dietary intake of lycopene and the highest intake of *β*-carotene throughout the 0–400-d lactation period. The data collected by the FFQ showed significant differences ($P < 0·05$) in dietary intake of each carotenoid between 0 and 400 d postpartum. The daily dietary intakes of carotenoids estimated by the FFQ were all higher than the 24HDR. The interquartile spacing and the maximum and minimum values showed that there were large individual differences in the daily dietary intakes of the five carotenoids. As shown in Table 9, the daily carotenoid intake of lactating mothers obtained by the two dietary survey methods was analysed for correlation. The results showed no association between the carotenoid dietary intake data measured by either method, except for lutein and zeaxanthin.

The correlation between the dietary intake of carotenoids and the carotenoid levels in breast milk estimated by the two methods is shown in Table 10 and Table 11, respectively. Among the five carotenoids, only a weak positive correlation was identified between the dietary intake of *β*-cryptoxanthin estimated by 24HDR and levels in early-term and mid-term mature milk ($r = 0·231$, $P < 0·05$; $r = 0·212$, $P < 0·05$) but not for other carotenoids. There was also a weak positive correlation between total carotenoid intake and levels in mid-term mature milk ($r = 0·197$, $P < 0·05$). Table 11 showed that no significant correlation was found between dietary carotenoid intake estimated by FFQ ($P > 0·05$) and carotenoid content in breast milk.

Discussion

To the best of our knowledge, this is the first comprehensive research to longitudinally study the trends in carotenoids in breast milk from 0 to 400 d and their relationship with maternal and cord blood, as well as maternal dietary intake, in Shanghai, which serves as a representative metropolitan city in China. Located in the southeastern coastal region, Shanghai has the highest level of economic development in China, leading to a distinct dietary pattern compared with other cities. Consequently, these findings contribute to a better understanding of carotenoid content in breast milk at various lactating stages, providing scientific data for future studies and development of infant formula. Additionally, by integrating dietary surveys, we were able to establish potential associations between mothers' dietary intake and the composition of breast milk, offering a reference for the nutrition of lactating mothers and infants.

In this study, we found that carotenoid concentrations and distributions differed across the five lactation stages, with the highest concentrations of carotenoids observed in colostrum.

Table 5. Correlations of carotenoid levels in maternal plasma and breast milk (*n* 30)

	Lutein	Zeaxanthin	β-Cryptoxanthin	β-Carotene	Lycopene
Colostrum	0·316	0·591**	0·261	0·605**	0·546*
P	0·089	<0·001	0·163	<0·001	0·002
Transitional milk	0·416*	0·418*	0·673**	0·456*	0·639*
P	0·022	0·022	<0·001	0·011	<0·001
Early-term mature milk	0·212	0·236	0·570**	0·446*	0·022
P	0·261	0·208	<0·001	0·013	0·972

Spearman's correlation was performed to analyse the correlations between carotenoid levels in maternal plasma and breast milk.
* $P < 0·05$.
** $P < 0·001$.

Table 6. Valid questionnaires returned in Shanghai (unit: copies)

Questionnaire type	0–5 d	10–15 d	40–45 d	200–240 d	300–400 d	Total
24HDR	27	28	74	101	99	329
FFQ	28	/	55	97	74	254

The trends we found regarding carotenoid concentrations in breast milk were consistent with previous findings in Beijing, Shanghai and Guangzhou, China[20,23,25]. Total carotenoids, β-cryptoxanthin, β-carotene and lycopene in breast milk decreased over the course of lactation, with the highest levels found in colostrum and lowest levels in late-term mature milk[20,22,23,25,39]. Consistent with previous studies, we observed a slight increase in lutein and zeaxanthin levels in transition milk (10–15 d) compared with levels in colostrum[20,23]. However, zeaxanthin levels were the lowest in early mature milk and then gradually approached the levels observed in colostrum at 200–400 d of lactation. According to other longitudinal studies, these changes generally occurred between colostrum and early-term mature milk, and mature milk maintained a stable level of carotenoids[5,20,22,23,39,40]. The pattern of changes in carotenoids in breast milk throughout lactation may be related to the nutritional requirements of infants at different stages of growth and development.

Previous studies conducted in China, Mexico, Canada, Europe and the USA have demonstrated that the dominant types of carotenoids present in breast milk vary across different periods[22,25,39–44]. In our study, β-carotene was the primary carotenoid in colostrum and then decreased rapidly, and lutein gradually became the dominant carotenoid in transitional and mature milk. Our results correspond to the findings of previous data. We hypothesised that the high concentration of β-carotene in colostrum may act as an antioxidant for neonates. Neonates have a high postnatal oxidative stress response but low concentrations of antioxidants in their bodies after the transition from the uterine hypoxic environment to the air[45]. The fetal liver is only able to store a small amount of vitamin A during pregnancy, and almost all babies are born with marginal vitamin A deficiency[46]. After birth, infants receive a high concentration of β-carotene in colostrum, which corrects the abnormally low plasma β-carotene concentrations in newborns and replenishes pro-vitamin A to meet the nutritional requirement[41]. As infants gradually adapt to their environment, lutein gradually dominates carotenoids as an important factor in the development of cognitive ability and vision. Non-polar β-carotene is generally transported via LDL[47], whereas the higher polar lutein tends to be transported via HDL[48]. Previous studies[22,29,38] have found that the pattern of carotenoids in colostrum was similar to the maternal plasma and LDL fractions, whereas in mature milk the pattern was similar to the HDL fraction. We speculated that the rapid decrease in β-carotene concentration in transition milk may be influenced by changes in lipoprotein content in breast milk. These findings may indicate different priorities in early-life nutrition.

Multinational studies have been done on the carotenoid content of human milk and discovered that the concentrations differed greatly among the nations[5,39,41,42,49]. Some researchers found that Chinese mothers' milk was distinguished with a high concentration of lutein[39,41,50]. Another study compared the total carotenoid contents of breast milk from China, the USA and Mexico and discovered that China had the highest levels[42]. Intra-country variability was also found when compared with the results of previous studies of longitudinal breast milk in Guangzhou and Chengdu. The total carotenoids, lutein and β-carotene in breast milk from Shanghai were at lower levels, and the levels of lycopene and zeaxanthin were more abundant in the middle and late mature milk[20,23]. Some research results suggest that dietary carotenoid intake may affect the carotenoid content of breast milk[51] and that the main sources of carotenoids are plant-originated foods, influenced by the availability of vegetable and fruit types, climate and season. The pre-natal serum carotenoid content of Shanghai nursing mothers was also lower in Shanghai lactating mothers compared with the Chengdu and Guangzhou areas, but the content of lutein, β-carotene and β-cryptoxanthin in neonatal umbilical cord blood was the highest among the three areas[20,23]. The dietary carotenoid intake of lactating mothers in Shanghai was higher than that in Guangzhou[20]. There are several possible explanations for these differences: for example, differences in dietary habits between regions and populations due to differences in geographical and ethnic distribution; or differences in bioavailability due to individual genetic variability; or the fast pace of life and the stressful nature of life in Shanghai consuming a considerable amount of antioxidative components.

Table 7. Carotenoids intake estimated by 24HDR in five stages of lactation (Medians and interquartile ranges)

	Dietary intake median (P25, P75) Min–Max															
	0–5 d			10–15 d			40–45 d			200–240 d			300–400 d			
Carotenoids (μg/d)	Median	P25, P75	Min–Max	Median	P25, P75	Min–Max	Median	P25, P75	Min–Max	Median	P25, P75	Min–Max	Median	P25, P75	Min–Max	*P*
Lutein+Zeaxanthin	708·60^{ab}	251·50, 1109·90	0–13 241·50	640·40^{ab}	451·62, 1181·59	110·90–7645·50	859·37^{a}	492·87, 2132·00	0–14 419·00	675·50^{ab}	281·50, 1411·00	0–44 248·00	446·50^{b}	251·50 868·12	0–3593·50	<0·001*
β-Cryptoxanthin	9·00	4·50, 26·00	0–512·68	17·70	5·62, 32·62	0–619·50	22·25	9·00, 106·12	0–1008·80	22·50	4·50, 120·00	0–2757·50	23·50	4·50, 115·75	0–375·50	0·361
β-Carotene	2693·00^{a}	551·70, 5767·90	0–9400·25	2855·60^{a}	104·65, 5265·24	0–13 245·45	906·75^{a}	154·57, 3149·17	0–12 450·00	675·00^{ab}	159·00, 1546·50	0–51 209·00	449·00^{b}	137·50, 877·00	0–7540·00	<0·001*
Lycopene	0^{ab}	0, 1286·50	0–5146·00	0^{ab}	0, 2573·00	0–5158·00	0^{ab}	0, 2573·00	0–18 128·00	0^{b}	0, 0	0–17 762·50	0^{a}	0, 2573·00	0–11 944·00	<0·001*
Total carotenoids	4118·74^{ab}	1325·50, 7063·50	0–24 980·50	4994·17^{ab}	1001·06, 8126·94	0–20 314·85	4218·50^{a}	983·30, 8165·62	0–23 628·00	2228·00^{ab}	664·75, 5043·75	0–98 214·50	1625·00^{b}	452·62, 955·12	0–22 931·00	<0·001*

24HDR, 24-h dietary review.
Data were expressed as median (P25, P75), min–max.
* Indicated a significant difference among five periods ($P < 0·05$). ^{abc}Different superscript letters in the same row were significantly different (*$P < 0·05$).

Table 8. Dietary carotenoids intake estimated by FFQ in four stages of lactation (Medians and interquartile ranges)

	Dietary intake median (P25, P75) Min–Max												
	0–5 d			40–45 d			200–240 d			300–400 d			
Carotenoids (μg/d)	Median	P25, P75	Min–Max	Median	P25, P75	Min–Max	Median	P25, P75	Min–Max	Median	P25, P75	Min–Max	*P*
Lutein+Zeaxanthin	18 719·52^{a}	3097·72, 19 108·55	405·50–37 480·11	18 561·87^{a}	4258·86, 19 072·75	110·90–7645·50	1939·64^{b}	957·09, 4096·44	176·51–23 499·73	1852·08^{b}	1077·53, 2532·97	54·50–15 018·90	<0·001*
β-Cryptoxanthin	32·25^{bc}	22·50, 126·92	6·75–637·50	24·75^{c}	17·14, 33·64	1·61–617·25	122·85^{ab}	47·64, 180·05	3·23–617·64	125·23^{a}	63·95, 184·57	10·00–554·36	<0·001*
β-Carotene	8818·00^{a}	5458·91, 11 260·09	317·68–21 678·69	8495·62^{a}	2000·03, 9185·00	47·79–21 158·64	2108·51^{b} (1004·83, 3800·47		72·21–13 851·03	1741·64^{b}	916·22, 4566·70	79·78–14 566·43	<0·001*
Lycopene	0^{b}	0, 5·71	0–18 128·00	0^{b}	0, 1·00	0–6798·00	1846·50^{a}	831·29, 4099·54	0–26 448·64	3147·21^{a}	1430·79, 4605·31	0–27 806·00	0·043*
Total carotenoids	27 555·50^{a}	12 435·47, 39 518·76	868·86–54 566·53	27 113·42^{a}	9037·00, 31 917·00	378·58–58 626·00	6465·34^{b}	3947·87, 12 677·43	501·36–40 372·11	8052·06^{b}	4844·38, 12 778·55	443·17–42 550·77	0·004*

Data were expressed as median (P25, P75), min–max.
* Indicated a significant difference among five periods ($P < 0·05$). ^{abc}Different superscript letters in the same row were significantly different (*$P < 0·05$).

Table 9. Correlations of dietary intake of lactating mothers investigated by two methods

	Lutein+Zeaxanthin	β-Cryptoxanthin	β-Carotene	Lycopene	Total carotenoids
r	0·135	0·021	0·112	–0·084	0·101
P	0·032*	0·738	0·075	0·184	0·107

Spearman's correlation was performed to analyse the correlations between two methods.
* $P < 0·05$.

Table 10. Correlation between dietary intake estimated by 24HDR and carotenoid levels in breast milk

	Colostrum		Transitional milk		Early-term mature milk		Mid-term mature milk		Late-term mature milk	
Carotenoids	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Lutein+Zeaxanthin	0·129	0·520	–0·135	0·493	0·158	0·177	0·073	0·470	0·015	0·879
β-Cryptoxanthin	–0·260	0·191	0·174	0·375	0·231*	0·047	0·212*	0·033	–0·088	0·388
β-Carotene	–0·373	0·055	–0·290	0·135	–0·083	0·484	0·037	0·713	0·088	0·386
Lycopene	–0·234	–0·241	0·352	0·067	0·048	0·685	0·026	0·796	–0·043	0·669
Total carotenoids	–0·334	0·089	–0·255	0·190	0·012	0·919	0·197*	0·048	0·120	0·237

24HDR, 24-h dietary review.
Spearman's correlation was performed to analyse the correlations between dietary intake and carotenoid levels in breast milk.
*$P < 0·05$.

Table 11. Correlation between dietary intake estimated by FFQ and carotenoid levels in breast milk

	Colostrum		Early-term mature milk		Mid-term mature milk		Late-term mature milk	
Carotenoids	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Lutein+Zeaxanthin	0·063	0·755	0·081	0·557	–0·018	0·865	–0·099	0·445
β-Cryptoxanthin	–0·145	0·469	–0·138	0·316	–0·068	0·507	–0·022	0·851
β-Carotene	0·016	0·936	–0·017	0·902	–0·054	0·601	–0·127	0·281
Lycopene	0·186	0·353	–0·03	0·829	0·08	0·439	–0·074	0·530
Total carotenoids	–0·081	0·687	0·045	0·743	0·061	0·551	–0·122	0·302

Spearman's correlation was performed to analyse the correlations between dietary intake and carotenoid levels in breast milk.

We found that carotenoid concentrations in cord blood were highly correlated with maternal blood. The concentration of carotenoids in maternal blood is much higher than in cord blood. Our results correspond to previous studies[20,23,39,43]. There are various considerations for the differences observed in carotenoid concentrations in maternal blood and cord blood. The level of carotenoids in the maternal/cord blood may correlate with the level of lipoproteins in blood[52,53]. Pregnant women are in a state of high oxidative stress, so maternal carotenoid concentrations need to be higher to cope with the depletion of antioxidants in the placenta or fetus during critical stages of development[54]. Likewise, high levels of oxidative stress in the newborn just after delivery can deplete the fetus' own reserves of antioxidant nutrients, resulting in low levels of carotenoids[55]. The lowest percentage of lycopene in umbilical cord blood may suggest depletion of oxidising nutrients during the stressful state of delivery. The results of the persistent correlation of carotenoids between maternal blood and breast milk suggest that the carotenoid concentration in the mother's body reflects a relatively stable level for 10–45 d after delivery. Since carotenoids are not equally distributed among the lipoprotein fractions in plasma, this transfer mechanism related to lipoproteins can lead to differences in breast milk during the progression of lactation. Therefore, more research is needed to investigate the mechanisms of placental transport of carotenoids, as well as the mechanisms of delivery in cord blood and breast milk, to improve understanding of nutrition in early life.

We did not find a strong correlation between mothers' dietary carotenoid intake and breast milk carotenoid concentrations. We only found a weak correlation between dietary intake of β-cryptoxanthin estimated by the 24HDR and early- and mid-term mature milk. None of the carotenoid daily intakes estimated by FFQ was associated with the carotenoids of breast milk. Previous studies suggested carotenoids in breast milk were associated with maternal dietary intake[5,26,56]. However, Xue *et al.*[25] found no significant correlation between dietary intake and carotenoids, which is consistent with our findings. Consumption of lutein and zeaxanthin and breast milk levels were not shown to be significantly correlated in recent research on Chinese lactating women[24]. Another Brazilian study[57] found no correlations between pro-vitamin A intake and levels of β-carotene in breast milk. Some intervention studies clearly show an increase in carotenoid concentrations in breast milk following the consumption of foods high in carotenoids (e.g., carrots or tomato paste, chlorella) or dietary supplements[58,59,60].

The difference between the results of this study and those of previous studies may be due to several factors. The absorption and metabolism of dietary carotenoids are influenced by many intrinsic and extrinsic factors[61]. First, the bioavailability of

carotenoids varies depending on the source from which they are consumed, which makes it challenging to accurately interpret dietary intake data[58]. Second, the sampling period spanned a large period, and the latter stages of mature milk collection occurred during the winter months. This affected the intake of fruits and vegetables by the lactating mothers due to seasonal variations[59]. Moreover, when mothers resume work after their maternity leave, they often have a limited amount of time and energy to devote to meal preparation, which can lead them to opt for more convenient and easily accessible food options. This shift towards a limited choice of food items may result in reduced consumption of carotenoid-rich foods. Lastly, the absence of a comprehensive database on carotenoid intake in China might have influenced the study results due to the differences in the types of food consumed[60]. At present, there are few studies on the dietary carotenoid intake of lactating mothers in China, and further research is needed on the influence of food sources and dietary habits on breast milk nutrition.

Compared with cross-sectional studies, the primary advantage of this study is that our longer follow-up period, covering breast milk data from 0 to 400 d, provided a more sensitive and reliable picture of the carotenoid profile in the breast milk of mothers in Shanghai. Second, we used both the FFQ and 24HDR methods to explore the dietary intake of lactating women in the study to provide more accurate information on carotenoid intake on the day before breast-feeding, as well as on the long-term dietary habits of lactating mothers. Third, mothers who provided maternal blood and cord blood also provided colostrum, transitional milk and early-mature milk, which may reduce variance. There were some limitations to the present study. First, the standard 3-consecutive 24HDR was not used, and daily dietary intakes were self-reported, which could lead to some bias. Second, the FFQ questionnaire had a low recall rate. Third, as the carotenoid food composition database was calculated based on the U.S. food composition database, some Chinese specialty foods in the survey could not be included in the database.

Conclusion

The profile of five carotenoids in breast milk samples, maternal plasma, cord plasma and maternal dietary intake over five different lactation stages was studied in Shanghai, China. We found that breast milk carotenoid levels varied with the stage of lactation, with the highest levels found in colostrum, followed by the fastest decline in carotenoid levels from colostrum to transition milk, which then stabilised in mature milk. A strong positive correlation was found between the carotenoid content of maternal blood and umbilical cord blood. There was also a correlation between the three stages of breast milk and maternal blood. *β*-Carotene was the predominant carotenoid in colostrum, whereas lutein was the predominant carotenoid in maternal/cord blood, transitional milk and mature milk. Total carotenoids, *β*-carotene and lutein in Shanghai were lower in breast milk and maternal blood compared with other areas in China, and lycopene and zeaxanthin were relatively higher in transition milk and mature breast milk. However, no correlation was found between dietary carotenoid intake and breast milk carotenoid levels. Further research is necessary to explore these results and improve the knowledge of the correlation between carotenoids in breast milk and diet. Additionally, further exploration of carotenoid transfer between mothers and infants is warranted. Finally, further research is needed to provide further information for the design of infant formulae that simulate the composition of Chinese mothers' breast milk to better meet the nutritional needs of Chinese infants.

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L. Z., J. C., Y. Z., X, X., Y. M. and X. L. conceived and designed the study protocols. X. D., H. S., H. W., L. T. and A. S. conducted the subject recruitment, the sample collection and the sample determinations. A. S. wrote the manuscript. M. K. supported on data analysis and revision. Y. M. and X. L. were primarily responsible for the final contents. All authors have read and agreed to the published version of the manuscript.

The authors declare no conflict of interest. X. L., X. X., Y. Z. and Y. M., were employees of Abbott Laboratories Ltd. when this research was completed.

Supplementary material

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S000711452300257X

References

1. Section on Breastfeeding, Eidelman AI, Schanler RJ, *et al.* (2012) Breastfeeding and the use of human milk. *Pediatrics* **129**, e827–e841.
2. Fund WHOUNCs (2003) Global Strategy for Infant and Young Child Feeding. Geneva, Switzerland: World Health Organization.
3. Ríos J, Valero-Jara V & Thomas-Valdés S (2022) Phytochemicals in breast milk and their benefits for infants. *Crit Rev Food Sci Nutr* **62**, 6821–6836.
4. Andreas NJ, Kampmann B & Mehring Le-Doare K (2015) Human breast milk: a review on its composition and bioactivity. *Early Hum Dev* **91**, Suppl. 11, 629–635.
5. Zielinska MA, Hamulka J & Wesolowska A (2019) Carotenoid content in breastmilk in the 3rd and 6th month of lactation and its associations with maternal dietary intake and anthropometric characteristics. *Nutrients* **11**, 193.
6. Cocate PG, Natali AJ, Alfenas RC, *et al.* (2015) Carotenoid consumption is related to lower lipid oxidation and DNA damage in middle-aged men. *Br J Nutr* **114**, 257–264.
7. Ciccone MM, Cortese F, Gesualdo M, *et al.* (2013) Dietary intake of carotenoids and their antioxidant and anti-inflammatory effects in cardiovascular care. *Mediators Inflamm* **2013**, 782137.

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8. Zaidi Y, Stroh R & Moran NE (2022) Systematic review of carotenoid concentrations in human milk and infant blood. Nutr Rev 80, 2029–2050.
9. Victora CG, Bahl R, Barros AJ, *et al.* (2016) Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. *Lancet* **387**, 475–490.
10. Eggersdorfer M & Wyss A (2018) Carotenoids in human nutrition and health. *Arch Biochem Biophys* **652**, 18–26.
11. Institute of Medicine Panel on Dietary A, Related C (2000). Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington, DC: National Academies Press (US) Copyright 2000 by the National Academy of Sciences. All rights reserved.
12. Mares J (2016) Lutein and zeaxanthin isomers in eye health and disease. *Annu Rev Nutr* **36**, 571–602.
13. Walk AM, Khan NA, Barnett SM, *et al.* (2017) From neuropigments to neural efficiency: the relationship between retinal carotenoids and behavioral and neuroelectric indices of cognitive control in childhood. *Int J Psychophysiol* **118**, 1–8.
14. Wan L, Tan HL, Thomas-Ahner JM, *et al.* (2014) Dietary tomato and lycopene impact androgen signaling- and carcinogenesis-related gene expression during early TRAMP prostate carcinogenesis. *Cancer Prev Res (Phila)* **7**, 1228–1239.
15. Cheng HM, Koutsidis G, Lodge JK, *et al.* (2019) Lycopene and tomato and risk of cardiovascular diseases: a systematic review and meta-analysis of epidemiological evidence. *Crit Rev Food Sci Nutr* **59**, 141–158.
16. Saini RK, Nile SH & Park SW (2015) Carotenoids from fruits and vegetables: chemistry, analysis, occurrence, bioavailability and biological activities. *Food Res Int* **76**, 735–750.
17. Zielińska MA, Wesołowska A, Pawlus B, *et al.* (2017) Health effects of carotenoids during pregnancy and lactation. *Nutrients* **9**, 838.
18. Barrera C, Valenzuela R, Chamorro R, *et al.* (2018) The impact of maternal diet during pregnancy and lactation on the fatty acid composition of erythrocytes and breast milk of Chilean women. *Nutrients* **10**, 839.
19. Bravi F, Wiens F, Decarli A, *et al.* (2016) Impact of maternal nutrition on breast-milk composition: a systematic review. *Am J Clin Nutr* **104**, 646–662.
20. Dai X, Yin H, Zhang J, *et al.* (2022) Carotenoid profile in maternal/cord plasma and changes in breast milk along lactation and its association with dietary intake: a longitudinal study in a coastal city in Southern China. *Nutrients* **14**, 1989.
21. Macias C & Schweigert FJ (2001) Changes in the concentration of carotenoids, vitamin A, *a*-tocopherol and total lipids in human milk throughout early lactation. *Ann Nutr Metab* **45**, 82–85.
22. Schweigert FJ, Bathe K, Chen F, *et al.* (2004) Effect of the stage of lactation in humans on carotenoid levels in milk, blood plasma and plasma lipoprotein fractions. *Eur J Nutr* **43**, 39–44.
23. Sun H, Wu T, Mao Y, *et al.* (2021) Carotenoid profile in breast milk and maternal and cord plasma: a longitudinal study in Southwest China. *Br J Nutr* **126**, 1281–1287.
24. Xu X, Zhao X, Berde Y, *et al.* (2019) Milk and plasma lutein and zeaxanthin concentrations in Chinese breast-feeding mother-infant dyads with healthy maternal fruit and vegetable intake. *J Am Coll Nutr* **38**, 179–184.
25. Xue Y, Campos-Giménez E, Redeuil KM, *et al.* (2017) Concentrations of carotenoids and tocopherols in breast milk from urban Chinese mothers and their associations with maternal characteristics: a cross-sectional study. *Nutrients* **9**, 1229.
26. Zhang Y, Yang J, Huang N, *et al.* (2019) Changes in breast milk lutein concentrations and their associations with dietary lutein intake: a 12-week prospective analytical study. *Br J Nutr* **122**, 1033–1039.
27. Rubin LP, Chan GM, Barrett-Reis BM, *et al.* (2012) Effect of carotenoid supplementation on plasma carotenoids, inflammation and visual development in preterm infants. *J Perinatol* **32**, 418–424.
28. Bettler J, Zimmer JP, Neuringer M, *et al.* (2010) Serum lutein concentrations in healthy term infants fed human milk or infant formula with lutein. *Eur J Nutr* **49**, 45–51.
29. Zielińska MA, Wesołowska A, Pawlus B, *et al.* (2017) Health effects of carotenoids during pregnancy and lactation. *Nutrients* **9**, 838.
30. Scaife AR, McNeill G, Campbell DM, *et al.* (2006) Maternal intake of antioxidant vitamins in pregnancy in relation to maternal and fetal plasma levels at delivery. *Br J Nutr* **95**, 771–778.
31. Thoene M, Anderson-Berry A, Van Ormer M, *et al.* (2019) Quantification of lutein + zeaxanthin presence in human placenta and correlations with blood levels and maternal dietary intake. *Nutrients* **11**, 134.
32. Elwakiel M, Boeren S, Hageman JA, *et al.* (2019) Variability of serum proteins in Chinese and Dutch human milk during lactation. *Nutrients* **11**, 499.
33. Tian HM, Wu YX, Lin YQ, *et al.* (2019) Dietary patterns affect maternal macronutrient intake levels and the fatty acid profile of breast milk in lactating Chinese mothers. *Nutrition* **58**, 83–88.
34. Yang T, Zhang Y, Ning Y, *et al.* (2014) Breast milk macronutrient composition and the associated factors in urban Chinese mothers. *Chin Med J (Engl)* **127**, 1721–1725.
35. Yang T, Zhang L, Bao W, *et al.* (2018) Nutritional composition of breast milk in Chinese women: a systematic review. *Asia Pac J Clin Nutr* **27**, 491–502.
36. Schimpf KJ, Thompson LD & Pan SJ (2018) Determination of carotenoids in infant, pediatric, and adult nutritionals by HPLC with UV-Visible detection: single-laboratory validation, first action 2017.04. *J AOAC Int* **101**, 264–276.
37. Liu S, Cai X, Wang J, *et al.* (2021) Six oligosaccharides' variation in breast milk: a study in South China from 0 to 400 days postpartum. *Nutrients* **13**, 4017.
38. Su Y, Mao Y, Tian F, *et al.* (2022) Profile of folate in breast milk from Chinese women over 1–400 days postpartum. *Nutrients* **14**, 2962.
39. Wu K, Zhu J, Zhou L, *et al.* (2020) Lactational changes of fatty acids and fat-soluble antioxidants in human milk from healthy Chinese mothers. *Br J Nutr* **123**, 841–848.
40. Lipkie TE, Morrow AL, Jouni ZE, *et al.* (2015) Longitudinal survey of carotenoids in human milk from urban cohorts in China, Mexico, and the USA. *PLoS One* **10**, e0127729.
41. Song BJ, Jouni ZE & Ferruzzi MG (2013) Assessment of phytochemical content in human milk during different stages of lactation. *Nutrition* **29**, 195–202.
42. Canfield LM, Clandinin MT, Davies DP, *et al.* (2003) Multinational study of major breast milk carotenoids of healthy mothers. *Eur J Nutr* **42**, 133–141.
43. Jackson JG & Zimmer JP (2007) Lutein and zeaxanthin in human milk independently and significantly differ among women from Japan, Mexico, and the UK. *Nutr Res* **27**, 449–453.
44. de Azeredo VB & Trugo NMF (2008) Retinol, carotenoids, and tocopherols in the milk of lactating adolescents and relationships with plasma concentrations. *Nutrition* **24**, 133–139.
45. Xavier AAO, Garrido-López JE, Aguayo-Maldonado J, *et al.* (2019) *In vitro* digestion of human milk: influence of the lactation stage on the micellar carotenoids content. *Antioxidants (Basel)* **8**, 291.

46. Perez M, Robbins ME, Revhaug C, *et al.* (2019) Oxygen radical disease in the newborn, revisited: oxidative stress and disease in the newborn period. *Free Radical Biol Med* **142**, 61–72.
47. Strobel M, Tinz J & Biesalski H-K (2007) The importance of β-carotene as a source of vitamin A with special regard to pregnant and breastfeeding women. *Eur J Nutr* **46**, 1–20.
48. Shete V, Costabile BK, Kim YK, *et al.* (2016) Low-density lipoprotein receptor contributes to β-carotene uptake in the maternal liver. *Nutrients* **8**, 765.
49. Connor WE, Duell PB, Kean R, *et al.* (2007) The prime role of HDL to transport lutein into the retina: evidence from HDL-deficient WHAM chicks having a mutant ABCA1 transporter. *Invest Ophthalmol Vis Sci* **48**, 4226–4231.
50. Kim H, Bae TJ, Jung BM, *et al.* (2017) Association between lutein intake and lutein concentrations in human milk samples from lactating mothers in South Korea. *Eur J Clin Nutr* **71**, 681.
51. Nguyen MTT, Kim J, Lee H, *et al.* (2020) A comparison of vitamin and lutein concentrations in breast milk from four Asian countries. *Nutrients* **12**, 1794.
52. Strobel M, Tinz J & Biesalski HK (2007) The importance of beta-carotene as a source of vitamin A with special regard to pregnant and breastfeeding women. *Eur J Nutr* **46**, I1–20.
53. Herrera E, Amusquivar E, López-Soldado I, *et al.* (2006) Maternal lipid metabolism and placental lipid transfer. *Horm Res* **65**, 59–64.
54. Franke AA, Lai JF, Morrison CM, *et al.* (2013) Coenzyme Q10, carotenoid, tocopherol, and retinol levels in cord plasma from multiethnic subjects in Hawaii. *Free Radic Res* **47**, 757–768.
55. Al-Gubory KH, Fowler PA & Garrel C (2010) The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. *Int J Biochem Cell Biol* **42**, 1634–1650.
56. Schoots MH, Gordijn SJ, Scherjon SA, *et al.* (2018) Oxidative stress in placental pathology. *Placenta* **69**, 153–161.
57. Lu Z, Chan YT, Lo KK, *et al.* (2022) Carotenoids and vitamin A in breastmilk of Hong Kong lactating mothers and their relationships with maternal diet. *Nutrients* **14**, 2031.
58. Haftel L, Berkovich Z & Reifen R (2015) Elevated milk β-carotene and lycopene after carrot and tomato paste supplementation. *Nutrition* **31**, 443–445.
59. Turner T, Burri BJ, Jamil KM, *et al.* (2013) The effects of daily consumption of β-cryptoxanthin–rich tangerines and β-carotene–rich sweet potatoes on vitamin A and carotenoid concentrations in plasma and breast milk of Bangladeshi women with low vitamin A status in a randomized controlled trial. *Am J Clin Nutr* **98**, 1200–1208.
60. Sherry CL, Oliver JS, Renzi LM, *et al.* (2014) Lutein supplementation increases breast milk and plasma lutein concentrations in lactating women and infant plasma concentrations but does not affect other carotenoids. *J Nutr* **144**, 1256–1263.
61. Meneses F & Trugo NMF (2005) Retinol, β-carotene, and lutein + zeaxanthin in the milk of Brazilian nursing women: associations with plasma concentrations and influences of maternal characteristics. *Nutr Res* **25**, 443–451.
62. Moran NE, Mohn ES, Hason N, *et al.* (2018) Intrinsic and extrinsic factors impacting absorption, metabolism, and health effects of dietary carotenoids. *Adv Nutr* **9**, 465–492.
63. Fernández-García E, Carvajal-Lérida I, Jarén-Galán M, *et al.* (2012) Carotenoids bioavailability from foods: from plant pigments to efficient biological activities. *Food Res Int* **46**, 438–450.
64. Watanabe S, Zhuo XG & Kimira M (2004) Food safety and epidemiology: new database of functional food factors. *Biofactors* **22**, 213–219.
65. Hamulka J, Hallmann E & Zielinska-Pukos MA (2022) Assays for carotenoids: linking breastmilk and maternal intakes. In *Biomarkers in Nutrition*, pp. 259–289 [VB Patel & VR Preedy, editors]. Cham: Springer International Publishing.

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