Xanthophylls in Human Milk and Maternal Diet: A Cross-sectional Analysis of Data from the Japanese Human Milk Study Cohort

Hiroshi M Ueno,[1] Touko Sato,[2] Satoshi Higurashi,[1] Hiroyuki Tazaki,[2] and Yasuhiro Toba[1]

[1]Research and Development Department, Bean Stalk Snow Co., Ltd, Kawagoe, Japan and [2]School of Veterinary Medicine, Nippon Veterinary and Life Science University, Musashino, Japan

ABSTRACT

Background: Maternal diet and sociodemographic factors influence xanthophyll concentration and composition in human milk. However, the importance of dietary patterns regarding the intake of fruits, vegetables, and xanthophylls remains unclear.

Objective: The aim was to determine the composition of xanthophylls in the human milk of Japanese mothers and explore associations of xanthophylls with dietary and sociodemographic factors.

Methods: This cross-sectional study was conducted in the early phase of the Japanese Human Milk Study. Xanthophyll content was measured using liquid chromatography at 30–36 d postpartum. Maternal intake of foods, nutrients, and dietary supplements was estimated using a food-frequency questionnaire. Linear regression models were established using xanthophylls, maternal diet, and sociodemographic factors.

Results: Xanthophyll concentrations were measured in human milk from 118 mothers. The xanthophyll concentration varied among individuals. The median (IQR) concentrations of lutein, zeaxanthin, and β-cryptoxanthin were 65.6 ng/mL (51.6–103.4 ng/mL), 18.6 ng/mL (12.9–25.8 ng/mL), and 15.6 ng/mL (9.0–26.0 ng/mL), respectively. In multivariate models, the lutein concentration was associated independently with dietary green vegetables, exclusive breastfeeding, and education ($r^2 = 0.153$ for the model; $\beta \pm$ SE: 0.468 ± 0.198, 25.048 ± 10.222, and 13.460 ± 6.774; standardized $\beta = 0.210$, 0.217, and 0.175; $P = 0.019$, 0.016, and 0.049 for dietary green vegetables, exclusive breastfeeding, and education, respectively). For zeaxanthin, exclusive breastfeeding was the most appropriate predictor ($r^2 = 0.085$; $\beta \pm$ SE: 7.811 ± 3.300; standardized $\beta = 0.218$; $P = 0.020$). The highest predictive power for human milk β-cryptoxanthin was obtained with dietary β-cryptoxanthin ($r^2 = 0.258$; $\beta \pm$ SE: 0.089 ± 0.015; standardized $\beta = 0.468$; $P < 0.001$), attributed to maternal citrus intake.

Conclusions: β-Cryptoxanthin in human milk was the xanthophyll most influenced by the maternal diet in Japanese women. The β-cryptoxanthin concentration in human milk was reflected by the maternal β-cryptoxanthin intake, mainly attributed to Japanese citrus consumption. This trial was registered in the Japanese Clinical Trials Registry (https://center6.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000017649) as UMIN000015494. *Curr Dev Nutr* 2022;6:nzac093.

Introduction

Breastfeeding is beneficial to mothers and infants. It reduces the risk of diabetes, breast, and ovarian cancer for mothers and the risk of infectious illness for infants (1). At 3 mo postpartum, 55% of Japanese mothers successfully continue exclusive breastfeeding for their children, while 90% of mothers breastfeed in either exclusive breastfeeding or mixed feeding (2). Early initiation of breastfeeding, early skin-to-skin contact, and the rooming-in effect support the maintenance of exclusive breastfeeding (3). Approximately 50% of the energy intake of young infants is obtained from human milk fat, which contains fatty acids, lipophilic vitamins, and bioactive components including fat-soluble phytochemicals (4). We recently conducted an analysis of 1079 samples of human milk from Japanese women and showed that 46.7% of energy in human milk was supplied from fat (5).

Carotenoids, which are bioactive phytochemicals, are a well-known natural pigment, and there are approximately 600 unique carotenoids (6). Humans cannot synthesize carotenoids de novo; thus, the intake of carotenoids is dependent on the diet. Dietary carotenoids vary among individuals, and their bioavailability is influenced by the nature of each

molecule, other dietary factors, and their food matrices ([7], [8]). Xanthophylls are oxidation products of carotenes, which is 1 of the 2 classes of carotenoids. Lutein, zeaxanthin, and β-cryptoxanthin are the representative xanthophylls ([9]). Although inadequate intake of carotenoid does not lead to carotenoid deficiency, lutein and zeaxanthin play important roles in brain and eye development both in utero and throughout life ([10]). The proportional contribution of lutein to the total composition of carotenoids is twice in pediatric brains than that found in adults ([11]). In pregnancy, lutein and zeaxanthin status may influence early visual development in offspring ([12]). β-Cryptoxanthin is a polar, pro-vitamin A xanthophyll found in some fruits such as tangerine, papaya, persimmon, and peach, with a greater bioavailability than β-carotene, among different dietary patterns ([13]). High maternal plasma β-cryptoxanthin is particularly associated with better cognitive and motor development in offspring ([14]), and thus β-cryptoxanthin is an emerging nutrient that supports better development of infants in the context of mother–human milk–infant triads. Indeed, the concentrations of β-cryptoxanthin in maternal plasma is correlated with β-cryptoxanthin of cord plasma and human milk; furthermore, plasma β-cryptoxanthin acts as a biomarker for dietary β-cryptoxanthin ([15], [16]).

The natural variation in human milk xanthophylls is influenced by dietary, sociodemographic, and health factors. The concentration of xanthophylls in human milk varies according to the lactation stage ([15], [16]), delivery mode ([17]), gestational period ([7]), and maternal diet ([18–20]). Xanthophyll concentrations in human milk are high in Japan (0.157 nmol/L) and China (0.111 nmol/L) but low in the United States (0.038 nmol/L) and Philippines (0.047 nmol/L) ([21]). Furthermore, lutein, zeaxanthin, α-cryptoxanthin, and β-cryptoxanthin concentrations are higher in China than in the United States ([22]). β-Cryptoxanthin has the greatest difference, with a 9-fold variance in concentrations among the 5 major carotenoids groups, and the mean β-cryptoxanthin concentration in human milk ranges from 0.012 nmol/L in the United States to 0.080 nmol/L in Japan ([21]). In Japan, there has been a reduced consumption and change in dietary patterns of fruits and vegetables ([23], [24]), which can partly explain the reason for the decline in dietary intake of xanthophylls in the young population. Accordingly, we hypothesized that the change in dietary patterns in terms of the intake of fruits and vegetables has influenced the xanthophyll composition in human milk in Japanese women. In this study, we aimed to determine the composition of xanthophylls in human milk in a subcohort of the Japanese Human Milk Study and to explore the associations of xanthophylls with dietary and sociodemographic factors.

Methods

Participants and human milk samples

A cross-sectional study was performed as part of the Japanese Human Milk Study cohort ([25], [26]). Briefly, we recruited healthy mother–infant dyads throughout Japan from medical institutions between March 2016 and September 2017. The inclusion criteria were healthy lactating women who had delivered infants and experienced no disruptions in their breastfeeding patterns when providing human milk samples at 30–36 d postpartum for this study. The largest number of samples were included in this narrower time frame in our study compared with other time frames, to lessen the longitudinal variation in xanthophylls

throughout lactation, in the Japanese Human Milk Study population (which included those <60 d postpartum; mean ± SD: 53.8 ± 17.8 d). The exclusion criteria were as follows: *1*) hepatitis B–positive or hepatitis C–positive individuals, or patients with HIV or human T-cell leukemia virus type 1 infections; *2*) patients who were on medication for underlying illnesses; *3*) individuals who did not breastfeed exclusively or partially; and *4*) mothers or infants who were not of Japanese ethnicity. A flowchart of the disposition of the study participants and grouping in the study is shown in **Supplemental Figure 1**. The eligible participants were the largest population with narrow ages of infants from the source population.

According to the instructions in the study protocol provided via the telephone and in a documented form, the participants collected middle and hind milk samples and transported the frozen samples at approximately −18°C by local frozen transportation services. The study group was provided with milk storage bags and a manual breast pump (Yanase Waichi) at registration. The participants manually pumped the milk samples at any time after breastfeeding, once a day, for 7 d during 30–36 d postpartum, into storage bags using a manual pump. A typical volume of milk samples was approximately 20 mL per container, and the human milk samples were stored at −80°C until analysis.

Milk macronutrient and energy

We thawed and pooled the received milk samples taken for 3 d individually and then measured the macronutrients and energy in the pooled sample. Thus, all analytical results of human milk samples were obtained as a mean of the samples over 3 d per participant. The milk samples were thawed in a water bath maintained at 37–40°C, pooled, and homogenized at room temperature. Macronutrient and energy analyses were performed using mid-infrared transmission spectroscopy equipment (Miris Human Milk Analyzer; Miris).

Carotenoid analysis
Chemicals.

Lutein, α-cryptoxanthin, α-carotene, and trans-β-apo-8′-carotenal were purchased from Sigma-Aldrich. Zeaxanthin, β-cryptoxanthin, lycopene, and β-carotene were purchased from FUJIFILM Wako Pure Chemicals unless otherwise specified.

Sample preparation.

Carotenoids were extracted from the pooled milk samples and analyzed using HPLC with modifications in the method described by Levêques et al. ([27]) to implement the method in our laboratory. The analytical procedure was performed in a laboratory room with shaded windows and room lights with UV-cut filters.

Human milk (750 μL) was transferred into a glass tube with screw caps and then mixed with 5 μL BHT-ethanol solution (79 g/L) and 10 μL deferoxamine mesylate aqueous solution (10 mg/mL) to prevent oxidation of carotenoids. Then, 1 mL ethanol and 25 μL trans β-apo-8′-carotenal hexane solution as an internal standard (2.5 μg/mL) were mixed with the human milk samples. To the mixed solution, we added 2.5 mL BHT-hexane solution (350 mg/L) and centrifuged the whole solution at 1000 × *g* for 4 min at 4°C. Two milliliters of the supernatant was transferred into an amber test tube and the BHT-hexane extraction process was repeated, with the additional supernatant being mixed with the previously obtained supernatant. The extract was

dried under N_2 atmosphere; then, 70 μL dioxane-ethanol (1:1) and 70 μL acetonitrile were added and mixed, followed by centrifugation at 21,880 × g for 10 min at 25°C. The supernatant was analyzed using HPLC.

HPLC analysis.
The carotenoids were analyzed by reverse-phase HPLC using Prominence ultra-fast LC (Shimadzu) HPLC system with an Acquity UPLC HSS T3 column (1.8 μm, 2.1 mm × 150 mm; Waters) and a Van-Guard guard column (1.8 μm, 2.1 mm × 5 mm; Waters). Solvent A was 0.05 mol ammonium acetate/L, and solvent B was acetonitrile-diethylether-methanol (76:9:15, wt:wt:wt). The HPLC column was set at 45°C, and a photodiode array detector was monitored at a wavelength of 450 nm. The gradient program for solvent B was as follows: the proportion of solvent B was initially set at 75% for 0–20 min, 78% for 20–22 min, 80% for 22–22.1 min, and programmed in a linear increase to 100% for 22.1–30 min and then retained at 100% for 30–42 min, followed by a linear decrease to 75% for 42–42.1 min and retention at 75% for 42.1–55 min. The parameters for the HPLC analysis including limit of detection, limit of determination, analytical range, and linearity of analytes are summarized in **Supplemental Table 1**. All the experimental procedures were performed in duplicate.

Food-frequency questionnaire
The participants completed a food-frequency questionnaire at the time of milk collection for this study (25). Briefly, we used the Brief-type self-administered Diet History Questionnaire (BDHQ) to estimate the dietary intake of the lactating women during the previous month (28, 29). The BDHQ is a short version of a self-administered diet history questionnaire that was developed in Japan and asks the frequency of consumption in the food list based on the National Health and Nutrition survey. We adopted the energy-adjusted intakes for energy, nutrients, and foods (per day and 1000 kcal) using an ad hoc computer algorithm for further analyses, based on the BDHQ validation study (28, 30). **Supplemental Table 2** lists the major nutrients listed in the BDHQ. The types of fruits included citrus, seasonal citrus, strawberry, and seasonal persimmon. The types of vegetables included carrot, tomato, green vegetables, root vegetables, and leafy vegetables. In addition, grain types included bread, potato, pasta, and noodles. Micronutrients included vitamin A, α-carotene, β-carotene, and β-cryptoxanthin. Another food-frequency questionnaire was administered to obtain information about the use of botanicals supplements and multivitamin supplements containing vitamins A, C, and E.

Sociodemographic, anthropometric, and birth-related questionnaires
Information on sociodemographic, anthropometric, feeding methods, and birth-related environmental factors was obtained using a questionnaire that included queries on the dyads. Information on age, maternal education, household income, maternal current and prepregnancy BMI, infant birth weight and length, delivery, gestation, parity, and sex was used for the analyses.

Ethics
The study protocol was approved by the Internal Review Board of Fukuda Clinic (IRB20140621-03) and registered in the Japanese Clinical Trials Registry (UMIN000015494). The study was performed in accordance with the 1975 Declaration of Helsinki, as revised in 1983. All study participants provided written informed consent at the time of their enrollment in the Japanese Human Milk Study, which included future uses of their data in published research. Participants may stop participating and continuing in the Japanese Human Milk Study at any time when health problems occur, and the participants could consult health care professionals for further assistance or from the study team. All data were anonymized during the preparation of the electronic dataset for the paper-based questionnaires and when making aliquots from the received frozen donor milk. A financial compensation (∼27 US dollars of gift cards) was offered for the donation of human milk samples and for completing the questionnaires. Participants were informed of the results of the study through an e-mail and corporate press releases on websites provided by the sponsor.

Statistical analysis
The scarcity of quantitative data on milk xanthophyll content and dietary patterns in Japanese women precluded power calculation in this exploratory study. The sample size was initially set at 100 subjects, according to the estimated feasibility of measurement during this study period and 10 participants per predictor for the possible dietary, maternal, and birth-related factors in the final multivariate models and, at most, 10 possible predictor allowances estimated for this study. Descriptive statistics were used to describe participant characteristics. For the milk xanthophyll content, all detected samples were included in the data analysis. Data of continuous variables are presented as medians and IQRs (quartile 1, quartile 3) because of the nonnormal distribution of the data (tested graphically and using the Shapiro-Wilk test). Differences in characteristics between the groups were investigated using the Mann-Whitney U test. Linear regression analyses were performed to identify the predictors of xanthophyll content in milk. Crude univariate models were developed to explore the potential predictors of milk xanthophylls as background factors. Factors found in the univariate models were further applied to multivariate linear regression analyses to identify the most appropriate prediction for milk xanthophyll concentration. SPSS Statistics, version 27.0 (IBM Corporation), and R 4.1.1 (R Foundation for Statistical Computing) were used for statistical analyses. Statistical significance was set at $P < 0.05$.

Results

Participant characteristics and maternal diet
The eligible participants were 118 mother–infant dyads in this study. The median age and gestational period were 32 y and 39 wk for mothers, and the median birth weight was 2990 g for infants. One hundred and thirteen infants were delivered at term gestations, except for 3 who were delivered preterm at 36 wk and 2 infants missing information on gestational age. Overall, 15.3% of infants were delivered by cesarean section, and the median age was 32 d. The median energy-adjusted dietary carotenoid intakes were 105 μg · 1000 kcal^{-1} · d^{-1} of β-cryptoxanthin and 1.92 mg · 1000 kcal^{-1} · d^{-1} of β-carotene (**Table 1**). The intakes of other antioxidant vitamins are listed in Table 1, whereas only 9 and 6 mothers reported the use of botanicals and antioxidant vitamin supplements.

TABLE 1 Maternal and infant characteristics of participants[1]

Variable	n	Median (IQR) or n (%)
Maternal characteristics		
Age, y	118	32 (29–35)
BMI, kg/m^2		
Current	117	21.9 (20.2–23.4)
Prepregnancy	118	20.4 (19.1–22.3)
Education, n (%)	118	
JHS/HS/others		18 (15.3)
Some college/technical		58 (49.2)
Four-year college/graduate degree		42 (35.6)
Household income (JPY/y), n (%)	116	
<1,999,999		2 (1.7)
2,000,000–3,999,999		23 (19.5)
4,000,000–5,999,999		48 (40.7)
6,000,000–7,999,999		19 (16.1)
8,000,000–9,999,999		12 (10.2)
≥10,000,000		12 (10.2)
Maternal nutrient intake from diet		
Energy, kcal/d	117	1795.1 (1523.3–2109.3)
Fat, g/1000 kcal	117	32.5 (28.0–35.8)
Antioxidant vitamins and carotenoids		
Vitamin A, μg RE/1000 kcal	117	164.2 (133.5–275.8)
β-Carotene, μg/1000 kcal	117	1922 (1236–2531)
α-Carotene, μg/1000 kcal	117	265 (172–367)
β-Cryptoxanthin, μg/1000 kcal	117	105 (55–197)
Maternal food intake from diet		
Grain, potato, and noodles		
Bread, g/1000 kcal	117	23.4 (13.1–33.0)
Potato, g/1000 kcal	117	21.8 (12.3–39.8)
Udon noodles, g/1000 kcal	117	8.3 (4.7–14.5)
Pasta, g/1000 kcal	117	6.2 (4.5–10.9)
Ramen noodles, g/1000 kcal	117	4.9 (0.0–9.4)
Vegetables and seaweed		
Cooked vegetables		
Green vegetables		
Carrot and pumpkin, g/1000 kcal	117	13.9 (9.0–19.3)
Other, g/1000 kcal	117	20.8 (10.9–32.6)
Root vegetables		
Radish, g/1000 kcal	117	7.6 (3.2–16.8)
Others, g/1000 kcal	117	25.3 (14.8–34.4)
Leafy vegetables, cooked, g/1000 kcal	117	16.9 (9.3–32.3)
Raw vegetables		
Leafy vegetables, raw, g/1000 kcal	117	12.5 (7.9–22.1)
Any cooking method		
Tomato, g/1000 kcal	117	12.3 (5.1–23.1)
Seaweed, g/1000 kcal	117	3.4 (1.3–8.4)
Fruits		
Citrus		
Annual, g/1000 kcal	117	6.5 (2.4–18.2)
Winter-seasonal, g/1000 kcal	117	6.3 (3.5–11.5)
Persimmon, autumn-seasonal, g/1000 kcal	117	0.9 (0.0–2.5)
Strawberry, winter-seasonal, g/1000 kcal	117	3.5 (1.6–7.4)
Others, g/1000 kcal	117	21.0 (7.5–37.3)
Egg, g/1000 kcal	117	17.1 (11.7–25.7)
Botanicals supplement use, n (%)	113	9 (7.6)
Antioxidant vitamin supplement use, n (%)	113	6 (5.3)
Vitamin A, n (%)	113	2 (1.8)
Vitamin C, n (%)	113	6 (5.3)
Vitamin E, n (%)	113	2 (1.8)

(*Continued*)

TABLE 1   (*Continued*)

Variable	n	Median (IQR) or n (%)
Birth and infant characteristics		
Cesarean delivery, n (%)	118	18 (15.3)
Gestational period, wk	116	39 (38–40)
Age, d	118	32 (31–34)
Birth length, cm	118	49 (48–50)
Birth weight, g	118	2990 (2746–3256)
Sex, boys, n (%)	118	53 (44.9)
Parity, n	118	2 (1–3)
Breastfeeding characteristics at sample collection		
Exclusive breastfeeding, n (%)	117	82 (69.5)

[1]HS, high school; JHS, junior high school; JPY, Japanese yen; RE, retinol equivalents.

Milk macronutrients and xanthophyll profiles

Data on other macronutrients, energy, and concentrations of lutein, zeaxanthin, and β-cryptoxanthin are presented in **Table 2**. The median total lipid content was 3.8 g/100 mL. The median concentrations of lutein, zeaxanthin, and β-cryptoxanthin were 65.6 ng/mL (IQR: 51.6–103.4 ng/mL), 18.6 ng/mL (12.9–25.8 ng/mL), and 15.6 ng/mL (9.0–26.0 ng/mL), respectively. All xanthophylls were higher in the human milk from women who exclusively breastfed than in women who partly breastfed (median: 74.0 vs. 53.7 ng/mL, $P < 0.001$ for lutein; 20.5 vs. 12.9 ng/mL, $P = 0.001$ for zeaxanthin; 17.1 vs. 10.7 ng/mL, $P = 0.044$ for β-cryptoxanthin, respectively). The milk xanthophyll content was not different between women users and nonusers of botanical supplements (**Supplemental Table 3**).

Regression analyses

Data from crude univariate and multivariate models of milk xanthophylls are summarized in **Table 3**. In crude univariate regression analyses, associations were observed between maternal diet and milk xanthophylls, as follows: milk lutein was associated with maternal intake of green vegetables and potato (standardized $\beta = 0.265$ and 0.198, $r^2 = 0.070$ and 0.039, $P = 0.004$ and 0.032 for green vegetables and potato, respectively); milk zeaxanthin concentration was associated with the maternal intake of bread (standardized $\beta = 0.207$, $r^2 = 0.043$, $P = 0.025$) and potato (standardized $\beta = 0.187$, $r^2 = 0.035$, $P = 0.044$); and the concentration of β-cryptoxanthin was associated with the maternal intake

TABLE 2   Macronutrients, energy, and xanthophyll composition in human milk[1]

Variable	Median (IQR)
Macronutrients and energy	
Fat, g/100 mL	3.8 (2.9–4.4)
Crude protein, g/100 mL	1.3 (1.2–1.5)
True protein, g/100 mL	1.1 (1.0–1.2)
Carbohydrate, g/100 mL	7.8 (7.3–8.1)
Total solid, g/100 mL	12.9 (12.0–13.7)
Energy, kcal/100 mL	70 (64–78)
Xanthophylls, ng/mL	
Lutein	65.6 (51.6–103.4)
Zeaxanthin	18.6 (12.9–25.8)
β-Cryptoxanthin	15.6 (9.0–26.0)

[1]n = 118.

of annual and winter-seasonal citrus and autumn-seasonal persimmon (standardized $\beta = 0.452$, 0.192, and 0.204, $r^2 = 0.204$, 0.037, and 0.042, $P < 0.001$, 0.038, and 0.028 for annual citrus, winter-seasonal citrus, and autumn-seasonal persimmon, respectively).

There were 2 significant associations between maternal intakes of xanthophyll and fat-soluble vitamins; milk lutein was associated with maternal β-carotene intake and milk β-cryptoxanthin with maternal β-cryptoxanthin intake (standardized $\beta = 0.247$ and 0.487, $r^2 = 0.061$ and 0.237, $P = 0.007$ and < 0.001, respectively). In addition, maternal education was positively associated with milk lutein concentrations (standardized $\beta = 0.211$, $r^2 = 0.044$, $P = 0.022$).

Exclusively breastfeeding mothers showed associations with milk lutein and zeaxanthin concentrations (standardized $\beta = 0.264$ and 0.245, $r^2 = 0.070$ and 0.060, $P = 0.004$ and 0.008, respectively). Other variables potentially related to xanthophyll intake, including carotenoid, carotenoid-containing foods, and the use of dietary supplements that were recorded in the food-frequency questionnaire, were not associated significantly with milk xanthophyll concentrations.

Furthermore, we chose the potential confounders for milk xanthophylls based on crude models with $P < 0.05$. The multivariate models predicted that the milk lutein was associated independently with maternal intake of green vegetables, exclusive breastfeeding, and maternal education ($r^2 = 0.153$ for the model; $\beta \pm$ SE: 0.468 ± 0.198, 25.048 ± 10.222, and 13.460 ± 6.774; standardized $\beta = 0.210$, 0.217, and 0.175; $P = 0.019$, 0.016, and 0.049 for dietary green vegetables, exclusive breastfeeding, and maternal education, respectively). For zeaxanthin, exclusive breastfeeding was the most appropriate predictor after adjustment for dietary bread intake ($r^2 = 0.085$; $\beta \pm$ SE: 7.811 ± 3.300; standardized $\beta = 0.218$; $P = 0.020$ for exclusive breastfeeding). The highest predictive power for milk β-cryptoxanthin was obtained with dietary β-cryptoxanthin adjusted for dietary raw leafy vegetables ($r^2 = 0.258$; $\beta \pm$ SE: 0.089 ± 0.015; standardized $\beta = 0.468$; $P < 0.001$ for dietary β-cryptoxanthin).

Discussion

In this study, we described the effects of dietary patterns on the xanthophyll profiles in the human milk of the Japanese population. The results showed that the xanthophyll concentrations were comparable to a series of recent results from mature milk studies (7, 15, 17, 19–22,

TABLE 3 Associations between xanthophyll concentrations in human milk, dietary intake, and routine antioxidant vitamin supplementation[1]

	β	(95% CI) SE	Standardized β	r²	P
(A) Lutein					
Crude univariate models					
Maternal dietary intake, foods					
Green vegetables, foods other than carrot and pumpkin	0.589	(0.194, 0.985) 0.200	0.265	0.070	0.004
Potato	0.555	(0.048, 1.061) 0.256	0.198	0.039	0.032
Maternal dietary intake, nutrients					
β-Carotene	0.010	(0.003, 0.017) 0.004	0.247	0.061	0.007
Sociodemographics					
Maternal education	16.048	(2.350, 29.746) 6.916	0.211	0.044	0.022
Infant and lactation					
Exclusive breastfeeding	30.168	(9.831, 50.505) 10.267	0.264	0.070	0.004
Final multivariate model[2]				0.153	<0.001
Maternal dietary intake, foods					
Green vegetables, foods other than carrot and pumpkin	0.468	(0.077, 0.860) 0.198	0.210		0.019
Sociodemographics					
Maternal education	13.460	(0.039, 26.882) 6.774	0.175		0.049
Infant and lactation					
Exclusive breastfeeding	25.048	(4.794, 45.303) 10.222	0.217		0.016
(B) Zeaxanthin					
Crude univariate models					
Maternal dietary intake, foods					
Bread	0.222	(0.028, 0.416) 0.098	0.207	0.043	0.025
Potato	0.162	(0.004, 0.321) 0.080	0.187	0.035	0.044
Maternal health					
Current BMI	−1.128	(−2.189, −0.067) 0.536	−0.193	0.037	0.037
Infant and lactation					
Exclusive breastfeeding	8.697	(2.351, 15.044) 3.204	0.245	0.060	0.008
Final multivariate model[2]				0.085	0.007
Maternal dietary intake, foods					
Bread	0.172	(−0.036, 0.380) 0.105	0.151		0.104
Infant and lactation					
Exclusive breastfeeding	7.811	(1.273, 14.348) 3.300	0.218		0.020
(C) β-Cryptoxanthin					
Crude univariate models					
Maternal dietary intake, foods					
Raw leafy vegetable	−0.385	(−0.725, −0.045) 0.172	−0.205	0.042	0.027
Citrus, annual	0.703	(0.447, 0.959) 0.129	0.452	0.204	<0.001
Citrus, winter-seasonal	0.614	(0.035, 1.192) 0.292	0.192	0.037	0.038
Persimmon, autumn-seasonal	0.971	(0.109, 1.833) 0.435	0.204	0.042	0.028
Maternal dietary intake, nutrients					
β-Cryptoxanthin	0.092	(0.062, 0.123) 0.015	0.487	0.237	<0.001
Final multivariate model[2]				0.258	<0.001
Maternal dietary intake, nutrients					
β-Cryptoxanthin	0.089	(0.058, 0.120) 0.015	0.468		<0.001
Maternal dietary intake, foods					
Raw leafy vegetables	−0.277	(−0.579, 0.026) 0.153	−0.147		0.073

[1]n = 118. Linear model considering milk xanthophylls in breast milk as the dependent variable and the factors listed as independent variables.
[2]Adjusted models were developed using factors in crude univariate models to obtain the most appropriate prediction.

31). Our study showed high median lutein and zeaxanthin concentrations in mature milk (**Supplemental Table 4**; 65.6 ng/mL vs. 22–65 ng/mL for lutein, 18.5 ng/mL vs. 8–17 ng/mL for zeaxanthin). Consideration of possible variations by methodological inconsistency across studies showed that the lutein and zeaxanthin concentrations in human milk appeared to be high in our study population. In crude analysis, an association was found between maternal intake of potato and lutein and zeaxanthin concentrations. Some potatoes, including yellow-fleshed potato and sweet potato, contain lutein and zeaxanthin; however, little attention has been paid to the dietary sources of lutein and zeaxanthin (32). Sweet potato is a nutritious food, as a source of starch, dietary fiber, vitamins, and minerals. Habitual intake of potatoes could influence the dietary intake of lutein and zeaxanthin in lactating women, and this could provide information on maternal xanthophyll status. Another association was found between the maternal intake of bread and zeaxanthin concentration in a crude model. Certain cereals

contain relatively high amounts of lutein and zeaxanthin as major carotenoids (33). Additionally, selective transfer mechanisms related to polarity can be involved in fat and fat-soluble nutrients in human milk (34). The absorption of β-cryptoxanthin is considered to be better than other common carotenoids, such as β-carotene, for the following reasons (35). Provitamin A carotenoids are incorporated into plasma membranes along with genetic features of genotypes and haplotypes in scavenger receptor class B type I (SR-B1) and cluster determinant 36 genes (36). SR-B1 prefers incorporations of xanthophylls to carotenes along with the hydrophobicity of molecules (35). Xanthophylls are distributed in the milk fat globule membranes according to their amphiphilic molecular characteristics. Thus, the molecular hydrophobicity of β-cryptoxanthin seems to be preferable to the localization in the inner part of the milk fat membrane. Likewise, the molecular uptake from the aqueous environment of the intestine is preferable to the amphiphilic environment of the milk fat globule membrane and hydrophobic environment of the fat in human milk. In addition, the genetic features of the Asian population might also affect the facilitating process of the absorption of β-cryptoxanthin (37). Taken together, dietary pattern was a potential predictor of xanthophyll profiles in human milk, as a combination of molecular and genetic mechanisms in the absorption and distribution of xanthophylls.

A series of regression analyses showed the highest standardized β-coefficient between β-cryptoxanthin and human milk and maternal diet in crude analyses (Table 3). β-Cryptoxanthin concentration varies in human milk among studies from different settings (Supplemental Table 4). This association was hypothesized to reflect the easier absorption of β-cryptoxanthin compared with other common carotenoids from the maternal diet in accordance with the results of most in vitro studies, animal models, and human studies (35). Mandarin orange is one of the most popular fruits in Japan and has abundant concentrations of β-cryptoxanthin (38). A typical Satsuma mandarin contains 10 times more β-cryptoxanthin (1800 μg/100 g) than a commercially available Valencia orange in Japan (39). β-Cryptoxanthin was also associated with annual and winter seasonal citrus consumption in the crude univariate models. Because β-cryptoxanthin is also abundant in persimmon (35), maternal seasonal intake of persimmon was associated with β-cryptoxanthin in the crude model. Consequently, maternal β-cryptoxanthin intake can be attributed to β-cryptoxanthin–abundant fruit consumption, such as citrus and persimmon, which resulted in increased β-cryptoxanthin concentrations. In addition, maternal intake of vitamin A had no association with xanthophylls in crude models ($P \geq 0.05$; data not shown). Vitamin A supplementation does not affect β-cryptoxanthin and β-carotene status in human milk (40). Accordingly, maternal intake of vitamin A might have no substantial effect on β-cryptoxanthin status in human milk in the general population. In addition, the statistical significance remained consistent in all of the multivariate models, except for maternal intake of green vegetables for lutein in milk fat ($P = 0.064$ for the multivariate model; **Supplemental Table 5**), as the concentration of xanthophylls was determined with per fat content of human milk instead of per volume. Considering that the variation in fat content in human milk is primarily influenced by fore and hind milk as well as the localization of carotenoids in milk fat globule membrane, the fat content in human milk may also contribute to the variation in xanthophyll concentration in human milk.

Because of the nature of nonessential micronutrients, maternal carotenoid intake has not been specified in the Dietary Reference Intakes or reported in the National Health and Nutrition Survey in Japan (41, 42). However, a better understanding of the significance of carotenoids can improve xanthophyll concentrations in human milk, as shown by the association between maternal education and lutein concentration seen in this study. Maternal educational level and household income were associated with β-carotene in human milk in this study (standardized $\beta = 0.334$ and 0.228, $r^2 = 0.111$ and 0.052, $P < 0.001$ and 0.014, respectively), implying that high socioeconomic status is involved in the dietary habit of consuming more β-carotene–containing foods, such as fruits and vegetables (43). For the duration of breastfeeding, smoking status, low birth weight in infants, and maternal perceptions of insufficient human milk supply were negatively influenced in terms of continuing breastfeeding in Japanese women, while support from husbands/partners was associated with continued breastfeeding (44). Another report showed that dietary restriction results in the cessation of breastfeeding in Polish women (45). Accordingly, a less-picky diet, including green vegetables, may partly reflect the associations between exclusive breastfeeding and xanthophylls in human milk. Moreover, maternal behavior and concerns to improve their diet for exclusive breastfeeding might affect more intakes of fruits and vegetables. From the viewpoint of dietary pattern, the consumption of fruits and vegetables is a variable that potentially increases the carotenoid content in human milk. The xanthophyll concentrations were positively associated with dietary green vegetables and citrus (β-cryptoxanthin) in the final multivariate model, adjusted for other possible confounders found in crude models. In addition, maternal education remained significant in the multivariate model of lutein. Collectively, maternal diet could be influenced by socioeconomic status and vice versa, resulting in the xanthophyll profile in human milk.

This study had some possible limitations. First, our experimental conditions may have been optimized to determine polar carotenoids, such as xanthophyll. The extraction and analytical conditions influence the carotenoid concentration in human milk (46). In this study, the positive rates of β-carotene and lycopene in human milk were 63.6% and 54.2%, and the median (IQR) concentrations of β-carotene and lycopene were 7.6 ng/mL (0.0–12.7 ng/mL) and 7.7 ng/mL (0.0–12.3 ng/mL), respectively. Five other unidentified peaks were detected, while the α-carotene and α-cryptoxanthin peaks could not be detected (data not shown). β-Carotene is commonly found in human milk, and lycopene is sometimes not detected in human milk (31). Considering the variations dependent on the experimental condition among studies, component-specific and detailed validation studies would be required to determine the true variations in human milk carotenoids, in addition to systematic reviews and meta-analysis. There were some associations between lycopene concentration in human milk and maternal intake of tomato as well as β-carotene concentration in human milk and maternal intake of β-carotene ($\beta = 0.187$ and 0.199, $r^2 = 0.035$ and 0.040, $P = 0.044$ and 0.031, respectively), suggesting that, to some extent, the experimental results reflected the maternal intake of food and carotenes. Second, the use of dietary supplements, including botanicals and antioxidant vitamin supplements, was queried to explore the possible associations between dietary supplement use and xanthophyll concentrations in human milk. However, there were few users of supplements in this study ($n = 2$–9 for botanicals and vitamins A, C, and E; Table 1),

and we found no significant differences in xanthophyll concentrations according to supplementation status ($P \geq 0.05$; Supplemental Table 3). The absence of difference may be due to a lower statistical power for the use of supplements; thus, further studies are required to clarify the effect of botanicals and antioxidant vitamin supplementation.

In conclusion, the β-cryptoxanthin concentration in human milk was the most influencing xanthophyll by maternal diet in Japanese women. The β-cryptoxanthin concentration in human milk was reflected by the maternal β-cryptoxanthin intake, mainly attributed to Japanese citrus consumption. Our results suggest that the xanthophyll concentration in human milk from Japanese mothers reflects current fruit and vegetable consumption, including seasonal variation, sustained exclusive breastfeeding, and higher maternal education, which have considerable potential for maintaining xanthophyll concentration in human milk. Further research is warranted to investigate the cause–effect relationships of the clinical implications in the development of infants.

Data Availability

Data described in the manuscript, codebook, and analytic code will be made available upon request. Conflict of interest: H.M.U., S.H., and Y.T. are employees of Bean Stalk Snow Co., Ltd. Bean Stalk Snow participated in constructing the study protocol; and provided the materials, human resources, and infrastructure in the study. Bean Stalk Snow sells formulas and supplements for infants and mothers in Japan. T.S. and H.T. have no conflicts of interest to declare.

References

1. Victora CG, Bahl R, Barros AJD, França GVA, Horton S, Krasevec J, et al. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. Lancet 2016;387(10017):475–90.
2. Ministry of Health, Labour and Welfare. National nutrition survey on preschool children [in Japanese]. [Internet]. [Cited 2022 Jan 11]. Available from: https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/0000134208.html.
3. Inano H, Kameya M, Sasano K, Matsumura K, Tsuchida A, Hamazaki K, Japan Environment and Children's Study (JECS) Group, et al. Factors influencing exclusive breastfeeding rates until 6 months postpartum: the Japan Environment and Children's Study. Sci Rep 2021;11(1):6841.
4. Demmelmair H, Koletzko B. Lipids in human milk. Best Pract Res Clin Endocrinol Metab 2018;32(1):57–68.
5. Nojiri K, Higurashi S, Takahashi T, Tsujimori Y, Kobayashi S, Toba Y, et al. Cohort profile: Japanese human milk study, a prospective birth cohort: baseline data for lactating women, infants and human milk macronutrients. BMJ Open 2021;11(12):e055028.
6. Hammond BR, Jr, Renzi LM. Carotenoids. Adv Nutr 2013;4(4):474–6.
7. Redeuil K, Lévêques A, Oberson JM, Bénet S, Tissot E, Longet K, et al. Vitamins and carotenoids in human milk delivering preterm and term infants: implications for preterm nutrient requirements and human milk fortification strategies. Clin Nutr 2021;40(1):222–8.
8. Chacón-Ordóñez T, Carle R, Schweiggert R. Bioaccessibility of carotenoids from plant and animal foods. J Sci Food Agric 2019;99(7):3220–39.
9. Thomas SE, Johnson EJ. Xanthophylls. Adv Nutr 2018;9(2):160–2.
10. Mares J. Lutein and zeaxanthin isomers in eye health and disease. Annu Rev Nutr 2016;36(1):571–602.
11. Johnson EJ. Role of lutein and zeaxanthin in visual and cognitive function throughout the lifespan. Nutr Rev 2014;72(9):605–12.
12. Lai JS, Veetil VO, Lanca C, Lee BL, Godfrey KM, Gluckman PD, et al. Maternal lutein and zeaxanthin concentrations in relation to offspring visual acuity at 3 years of age: the GUSTO study. Nutrients 2020;12(2):274.
13. Olmedilla-Alonso B, Beltran-de-Miguel B, Estevez-Santiago R, Cuadrado-Vives C. Markers of lutein and zeaxanthin status in two age groups of men and women: dietary intake, serum concentrations, lipid profile and macular pigment optical density. Nutr J 2014;13:52.
14. Lai JS, Cai S, Lee BL, Godfrey KM, Gluckman PD, Shek LP, et al. Higher maternal plasma β-cryptoxanthin concentration is associated with better cognitive and motor development in offspring at 2 years of age. Eur J Nutr 2021;60(2):703–14.
15. Song BJ, Jouni ZE, Ferruzzi MG. Assessment of phytochemical content in human milk during different stages of lactation. Nutrition 2013;29(1): 195–202.
16. Macias C, Schweigert FJ. Changes in the concentration of carotenoids, vitamin a, alpha-tocopherol and total lipids in human milk throughout early lactation. Ann Nutr Metab 2001;45(2):82–5.
17. Xue Y, Campos-Giménez E, Redeuil KM, Lévêques A, Actis-Goretta L, Vinyes-Pares G, et al. Concentrations of carotenoids and tocopherols in breast milk from urban Chinese mothers and their associations with maternal characteristics: a cross-sectional study. Nutrients 2017;9(11):1229.
18. Zielinska MA, Hamulka J, Wesolowska A. Carotenoid content in breastmilk in the 3rd and 6th month of lactation and its associations with maternal dietary intake and anthropometric characteristics. Nutrients 2019; 11(1):193.
19. Kim H, Yi H, Jung JA, Chang N. Association between lutein intake and lutein concentrations in human milk samples from lactating mothers in South Korea. Eur J Nutr 2018;57(1):417–21.
20. Sherry CL, Oliver JS, Renzi LM, Marriage BJ. Lutein supplementation increases breast milk and plasma lutein concentrations in lactating women and infant plasma concentrations but does not affect other carotenoids. J Nutr 2014;144(8):1256–63.
21. Canfield LM, Clandinin MT, Davies DP, Fernandez MC, Jackson J, Hawkes J, et al. Multinational study of major breast milk carotenoids of healthy mothers. Eur J Nutr 2003;42(3):133–41.
22. Lipkie TE, Morrow AL, Jouni ZE, McMahon RJ, Ferruzzi MG. Longitudinal survey of carotenoids in human milk from urban cohorts in China, Mexico, and the USA. PLoS One 2015;10(6):e0127729.
23. Mo X, Gai RT, Sawada K, Takahashi Y, Cox SE, Nakayama T, et al. Coronary heart disease and stroke disease burden attributable to fruit and vegetable intake in Japan: projected DALYS to 2060. BMC Public Health 2019;19(1):707.
24. Oude Griep LM, Stamler J, Chan Q, Van Horn L, Steffen LM, Miura K, et al. Association of raw fruit and fruit juice consumption with blood pressure: the INTERMAP study. Am J Clin Nutr 2013;97(5):1083–91.

25. Nojiri K, Kobayashi S, Higurashi S, Takahashi T, Tsujimori Y, Ueno HM, et al. Maternal health and nutrition status, human milk composition, and growth and development of infants and children: a prospective Japanese human milk study protocol. Int J Environ Res Public Health 2020;17(6):1869.

26. Ueno HM, Higurashi S, Shimomura Y, Wakui R, Matsuura H, Shiota M, et al. Association of DHA concentration in human breast milk with maternal diet and use of supplements: a cross-sectional analysis of data from the Japanese Human Milk Study cohort. Curr Dev Nutr 2020;4(7):nzaa105.

27. Levêques A, Oberson JM, Tissot EA, Redeuil K, Thakkar SK, Campos-Giménez E. Quantification of vitamins A, E, and K and carotenoids in submilliliter volumes of human milk. J AOAC Int 2019;102(4):1059–68.

28. Kobayashi S, Honda S, Murakami K, Sasaki S, Okubo H, Hirota N, et al. Both comprehensive and brief self-administered diet history questionnaires satisfactorily rank nutrient intakes in Japanese adults. J Epidemiol 2012;22(2):151–9.

29. Shiraishi M, Haruna M, Matsuzaki M, Murayama R, Sasaki S. Availability of two self-administered diet history questionnaires for pregnant Japanese women: a validation study using 24-hour urinary markers. J Epidemiol 2017;27(4):172–9.

30. Kobayashi S, Murakami K, Sasaki S, Okubo H, Hirota N, Notsu A, et al. Comparison of relative validity of food group intakes estimated by comprehensive and brief-type self-administered diet history questionnaires against 16 d dietary records in Japanese adults. Public Health Nutr 2011;14(7):1200–11.

31. Duan B, Shin JA, Qin Y, Kwon JI, Lee KT. A study on the relationship of fat content in human milk on carotenoids content and fatty acid compositions in Korea. Nutrients 2019;11(9):2072.

32. Burgos G, Muñoa L, Sosa P, Bonierbale M, Felde T, Díaz C. In vitro bioaccessibility of lutein and zeaxanthin of yellow fleshed boiled potatoes. Plant Foods Hum Nutr 2013;68(4):385–90.

33. Humphries JM, Khachik F. Distribution of lutein, zeaxanthin, and related geometrical isomers in fruit, vegetables, wheat, and pasta products. J Agric Food Chem 2003;51(5):1322–7.

34. Wu K, Zhu J, Zhou L, Shen L, Mao Y, Zhao Y, et al. Lactational changes of fatty acids and fat-soluble antioxidants in human milk from healthy Chinese mothers. Br J Nutr 2020;123(8):841–8.

35. Burri BJ, La Frano MR, Zhu C. Absorption, metabolism, and functions of β-cryptoxanthin. Nutr Rev 2016;74(2):69–82.

36. Borel P, Lietz G, Goncalves A, Szabo FE, Lecompte S, Curtis P, et al. CD36 and SR-BI are involved in cellular uptake of provitamin A carotenoids by Caco-2 and HEK cells, and some of their genetic variants are associated with plasma concentrations of these micronutrients in humans. J Nutr 2013;143(4):448–56.

37. Yabuta S, Urata M, Kun RYW, Masaki M, Shidoji Y. Common SNP rs6564851 in the BCO1 gene affects the circulating levels of β-carotene and the daily intake of carotenoids in healthy Japanese women. PLoS One 2016;11(12):e0168857.

38. Unno K, Sugiura M, Ogawa K, Takabayashi F, Toda M, Sakuma M, et al. Beta-cryptoxanthin, plentiful in Japanese mandarin orange, prevents age-related cognitive dysfunction and oxidative damage in senescence-accelerated mouse brain. Biol Pharm Bull 2011;34(3):311–7.

39. Nakamura M, Sugiura M, Shibata Y, Ojima T. Effect of β-cryptoxanthin–rich Satsuma mandarin juice supplementation on pulse wave velocity: a randomized controlled trial. J Nutr Intermed Metab 2017;8: 8–13.

40. Turner T, Burri BJ, Jamil KM, Jamil M. 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 2013;98(5):1200–8.

41. Ministry of Health, Labour and Welfare. Overview of dietary reference intakes for Japanese. 2015. [Internet]. [Cited 2022 Jan 11]. Available from: http://www.mhlw.go.jp/file/06-Seisakujouhou-10900000-Kenkoukyoku/Overview.pdf.

42. National Institute of Health and Nutrition. National Health and Nutrition Survey. 2021 [Internet]. [Cited 2021 Mar 18]. Available from: https://www.nibiohn.go.jp/eiken/kenkounippon21/en/eiyouchousa/.

43. Kvaavik E, Totland TH, Bastani N, Kjøllesdal MK, Tell GS, Andersen LF. Do smoking and fruit and vegetable intake mediate the association between socio-economic status and plasma carotenoids? Eur J Public Health 2014;24(4):685–90.

44. Inoue M, Binns CW, Otsuka K, Jimba M, Matsubara M. Infant feeding practices and breastfeeding duration in Japan: a review. Int Breastfeed J 2012;7(1):15.

45. Karcz K, Lehman I, Królak-Olejnik B. The link between knowledge of the maternal diet and breastfeeding practices in mothers and health workers in Poland. Int Breastfeed J 2021;16(1):58.

46. Stout MA, Benoist DM, Drake MA. Technical note: simultaneous carotenoid and vitamin analysis of milk from total mixed ration-fed cows optimized for xanthophyll detection. J Dairy Sci 2018;101(6):4906–13.