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Specific serum carotenoids are inversely associated with breast cancer risk among Chinese women: a case–control study

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Abstract

Previous epidemiological studies have revealed the anti-cancer effect of dietary circulating carotenoids. However, the protective role of specific individual circulating carotenoids has not been elucidated. The purpose of this study was to examine whether serum carotenoids, including *α*-carotene, *β*-carotene, *β*-cryptoxanthin, lycopene and lutein/zeaxanthin, could lower the risk for breast cancer among Chinese women. A total of 521 women with breast cancer and age-matched controls (5-year interval) were selected from three teaching hospitals in Guangzhou, China. Concentrations of *α*-carotene, *β*-carotene, *β*-cryptoxanthin, lycopene and lutein/zeaxanthin were measured using HPLC. Unconditional logistic regression models were used to calculate OR and 95 % CI using quartiles defined in the control subjects. Significant inverse associations were observed between serum *α*-carotene, *β*-carotene, lycopene, lutein/zeaxanthin and the risk for breast cancer. The multivariate OR for the highest quartile of serum concentration compared with the lowest quartile were 0·44 (95 % CI 0·30, 0·65) for *α*-carotene, 0·27 (95 % CI 0·18, 0·40) for *β*-carotene, 0·41 (95 % CI 0·28, 0·61) for lycopene and 0·26 (95 % CI 0·17, 0·38) for lutein/zeaxanthin. However, no significant association was found between serum *β*-cryptoxanthin and the risk for breast cancer. Stratified analysis by menopausal status and oestrogen receptor (ER)/progesterone receptor (PR) showed that serum *α*-carotene, *β*-carotene, lycopene and lutein/zeaxanthin were inversely associated with breast cancer risk among premenopausal women and among all subtypes of ER or PR status. The results suggest a protective role of *α*-carotene, *β*-carotene, lycopene and lutein/zeaxanthin, but not *β*-cryptoxanthin, in breast cancer risk.

Key words: Serum carotenoids: Breast cancer: Case–control studies: Chinese women

Carotenoids are potent anticarcinogenic substances involved in antioxidant activity, stimulation of gap-junction intercellular communication and inhibition of cellular proliferation. Besides scavenging radical substances, carotenoids may stimulate the immune system and protect against breast cancer[1].

Compared with estimates of dietary intake, serum or plasma carotenoids are better indicators of the biological availability of carotenoids. Some epidemiological studies have revealed the anti-cancer effect of circulating carotenoids. However, the protective role of individual specific serum/plasma carotenoids remains controversial. A retrospective case–control study found an inverse association[2], whereas cohort studies[3–5] were more likely to represent modest or null associations between serum carotenoids and breast cancer risk. In a pooled analysis of eight prospective studies of circulating carotenoids[6], significant negative associations with breast cancer were observed for *α*-carotene, *β*-carotene, lutein/zeaxanthin, lycopene and total carotenoids. *β*-cryptoxanthin was not significantly associated with risk.

However, seven of eight studies included in this pooled analysis were conducted in western countries. Compared with western women, in whom the median age at diagnosis is 60–64 years, the age at cancer diagnosis among Chinese women was much younger. Mean age at diagnosis is 48 years[7], and 65 % of women were premenopausal[8]. Chinese women exhibited a significantly advanced average stage on diagnosis (stage IIA *v.* stage I) on the basis of primary tumour size[9]. Moreover, although the incidence rate of female breast cancer in China was still significantly lower than that in western countries (age-standardised incidence rate of 22·1/100 000 women-years for Chinese women, 92·9/100 000 women-years for American women, 69·9/100 000 women-years for European women), breast cancer had a rapid increase in China[10]. Therefore, there is an urgent need for efficient prevention strategies among Chinese women.

Abbreviations: ER, oestrogen receptor; PR, progesterone receptor.

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To the best of our knowledge, only one previous study has investigated the association between specific circulating carotenoids and the risk for breast cancer in the Chinese population[5], and evidence for the protective effect of each individual serum carotenoid is inconsistent. People living in Guangdong, China, follow the 'traditional southern' dietary pattern[11] characterised by high intakes of vegetables and fruits, which is different from the dietary pattern in Shanghai.

The purpose of the present study was to examine whether serum carotenoids including α-carotene, β-carotene, β-cryptoxanthin, lycopene and lutein/zeaxanthin may lower the risk for breast cancer among Chinese women. As the aetiologies of breast cancer may differ by receptor status, analyses were stratified by menopausal status, oestrogen receptor (ER) status, or progesterone receptor (PR) status to examine any protective effect of each carotenoid in these subgroups.

Methods

Study subjects

Details of this ongoing hospital-based case–control study, which began in 2011, have been reported previously[12]. Female subjects aged 25–70 years were consecutively recruited from three teaching and general hospitals in Guangzhou, China. All had been histologically diagnosed with breast cancer within 3 months of the recruitment interview. Subjects were natives of Guangdong province or had lived in Guangdong for at least 5 years. Women were excluded if they had a history of other cancers. From September 2011 to May 2014, a total of 521 (96·30 %) of 541 eligible cases were included in the study.

Control subjects were females with no history of any type of cancer who had been admitted to the same hospitals during the same period as the case subjects. They were frequency-matched by age (5-year interval) and were recruited from the departments of Plastic and Reconstructive Surgery, Vascular Surgery, and Ear, Nose and Throat. In total, 521 of 537 (97·02 %) controls participated. The controls were recruited from the above departments because we had no prior reason to believe that several conditions from these departments had apparent association with a dietary cause.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by The Ethical Committee of School of Public Health, Sun Yat-sen University. A written informed consent form was signed by all study subjects.

Data collection

The data were collected by trained interviewers through face-to-face interviews. A structured and previously validated questionnaire was used[13]. Information on socio-demographic situation, current weight, height, menstrual and reproductive history, menopausal status, use of exogenous hormones, use of contraceptive drugs, family history of cancer, medical history, medication treatment, dietary habits, active and passive smoking, alcohol drinking and physical activities was obtained. BMI was calculated by dividing body weight (kg) by height (m) squared. Regular smoking was defined as smoking at least 1 cigarette/d for >6 consecutive months. Passive smoking was defined as exposure to others' tobacco smoke for at least 5 min/d in the previous 5 years. Regular drinking was defined as drinking alcohol at least once per week over the past year. Postmenopausal status was defined as at least 12 months since the last menstrual cycle. Relevant medical diagnoses and pathological findings were abstracted from the medical records.

Measurement of serum carotenoids

Fasting serum samples (5 ml) were collected in pro-coagulation tubes on the 2nd day after subjects had been admitted and kept fasting for at least 12 h. Samples were put in a box filled with dry ice and sent to the laboratory. Sera were separated from blood cells by centrifugation (3000 rpm at 4°C for 15 min) within 1 h of collection. Serum samples were stored at −80°C until analysis. Concentrations of α-carotene, β-carotene, β-cryptoxanthin, lycopene and lutein/zeaxanthin were measured using HPLC. Serum carotenoids (200 μl) were deproteinated with ethanol, and α-tocopherol acetate was added as an internal standard before extraction. After extraction with hexane-butylated hydroxytoluene (2 ml) solution, carotenoids were evaporated to dryness with N at room temperature. The extract was dissolved in acetonitrile–methanol–tetrahydrofuran–ammonium acetate (mobile phase B, 200 μl, 55:35:5:5, v/v) and then a C18 HPLC column (Shiseido) and a Waters 2998 diode-array detector (Waters) were used to detect carotenoids. Mobile phase A included acetonitrile–methanol–tetrahydrofuran–ammonium acetate (85:5:5:5, v/v). A sample was injected into the column every 30 min. Retinol and carotenoids were measured at 325 nm and 325/450 nm, respectively. All procedures were performed by the same technician, and peaks were calculated automatically. The median between-batch inter-assay CV were 7·8 % for α-carotene, 8·6 % for β-carotene, 9·7 % for β-cryptoxanthin, 10·6 % for lycopene and 8·0 % for lutein/zeaxanthin. The with-run CV were 1·40 % for α-carotene, 1·50 % for β-carotene, 4·00 % for β-cryptoxanthin, 3·30 % for lycopene and 1·70 % for lutein/zeaxanthin.

Statistical analysis

Statistical analyses were performed using SPSS 19.0, and results were considered significant when $P<0·05$ (two-sided). For continuous variables, data are shown as means and standard deviations. For categorical variables, frequencies are presented as percentages. The quartiles of the measured carotenoids were defined according to the distribution of the control subjects. The socio-demographic characteristics and potential risk factors between the two groups were compared using Student's t tests or Wilcoxon's rank-sum test for continuous variables and χ^2 tests or Fisher's exact tests for categorical variables.

Unconditional logistic regression was used to estimate the OR and 95 % CI of each quartile (Q1–Q4) of serum levels of specific carotenoids, setting the lowest quartile group as the reference. The association between the risk for breast cancer and the serum levels of specific carotenoids was further examined after adjusting for several potential confounders using multivariate logistic regression models. BMI (continuous variable), residence

(urban/rural), education level (primary school or below/junior high school/senior high school/secondary technical school/college or above), income (<2000, 2001–5000, 5001–8000, >8000 yuan/month), regular drinker (yes/no) and history of benign breast disease (yes/no) were regarded as potential confounders according to a comparison of baseline characteristics between cases and controls. Tests for trends were performed by entering the categorical variables (Q1–Q4) as continuous variables in the models.

As certain risk factors for breast cancer may exert different influences on premenopausal and postmenopausal women[14], the association between specific serum carotenoids and breast cancer risk may be altered by menopausal status. Therefore, an analysis stratified by premenopausal or postmenopausal status was performed. Additionally, breast cancer defined by ER and PR status appears to be aetiologically heterogeneous[15]. Stratified analyses by ER status (ER+ or ER–) or PR status (PR+ or PR–) were carried out to assess whether breast cancer risk differs in accordance with ER or PR status. Our sample of 225 cases and 260 controls in two quartiles (Q1 and Q4) gave us 78 % power to detect an OR of 0·71 for the association between serum β-cryptoxanthin and breast cancer risk at $P<0·05$ (two-tailed). We had 100 % power to detect OR of 0·44, 0·27, 0·41 and 0·26 for the association between serum α-carotene, β-carotene, lycopene and lutein/zeaxanthin and breast cancer risk.

Results

The socio-demographic characteristics of the study subjects are presented in Table 1. Compared with control subjects, women with breast cancer were more likely to live in rural areas, have a higher BMI, be regular drinkers and have a history of benign breast disease. Case subjects were more likely to possess a lower household income and lower educational level. No significant differences were observed between the cases and controls in terms of age, number of live births, age at menarche, age at menopause, age at first live birth, menopausal status, marital status, occupation, physical activity, smoking status, history of a first-degree relative with cancer, passive smoking, oral contraceptive use or breast-feeding.

The comparison of mean concentration of serum carotenoids between cases and controls is shown in Table 2. Control subjects possessed significantly higher mean concentrations of serum α-carotene, β-carotene, β-cryptoxanthin, lycopene and lutein/zeaxanthin when compared with cases.

The OR and 95 % CI for breast cancer risk according to the serum concentration of specific carotenoids are presented in Table 3. After adjustment for various confounders, a significant inverse association was observed between serum α-carotene, β-carotene, lycopene, lutein/zeaxanthin and the risk for breast cancer. The adjusted OR for the highest quartile compared with the lowest quartile were 0·44 (95 % CI 0·30, 0·65; $P_{trend}<0·01$) for serum α-carotene, 0·27 (95 % CI 0·18, 0·40; $P_{trend}<0·01$) for β-carotene, 0·41 (95 % CI 0·28, 0·61; $P_{trend}<0·01$) for lycopene and 0·26 (95 % CI 0·17, 0·38; $P_{trend}<0·01$) for lutein/zeaxanthin. However, no significant association was found between serum β-cryptoxanthin and the risk for breast cancer, with an adjusted OR of 0·71 (95 % CI 0·48, 1·03) comparing the highest with the lowest quartile ($P_{trend}=0·07$).

Table 1. Socio-demographic and selected risk factors for breast cancer among breast cancer cases and controls
(Numbers and percentages; mean values and standard deviations)

	Cases (*n* 521)		Controls (*n* 521)		
	n	%	*n*	%	*P*
Age (years)					0·46
Mean	47·6		48·0		
SD	9·4		9·5		
Marital status					0·26
Married	492	94·4	482	92·5	
Unmarried/divorced/widowed	29	5·6	39	7·5	
Residence					0·02
Urban	373	71·6	406	77·9	
Rural	148	28·4	115	22·1	
Education level					<0·01
Primary school or below	126	24·2	126	24·2	
Junior high school	158	30·3	116	22·3	
Senior high school/secondary technical school	128	24·6	133	25·5	
College or above	109	20·9	146	28·0	
Occupation					0·76
Administrator/other white collar worker	119	22·8	125	24·0	
Blue collar worker	140	26·9	146	28·0	
Farmer/other	262	50·3	250	48·0	
Income (yuan/month)					<0·01
<2000	34	6·5	27	5·2	
2001–5000	162	31·1	128	24·5	
5001–8000	195	37·4	191	36·7	
>8001	130	25·0	175	33·6	
Physical activity (exercise for health)					0·08
Never	321	61·6	288	55·3	
Occasionally	105	20·2	113	21·7	
Often(more than once a week)	95	18·2	120	23·0	
BMI (kg/m^2)					0·03
Mean	23·1		22·6		
SD	3·2		3·1		
Regular smoker	5	1·0	5	1·0	0·60
Passive smoking	423	81·2	432	82·9	0·52
Regular drinker	45	8·6	23	4·4	<0·01
Age at menarche (years)					0·97
Mean	14·6		14·6		
SD	1·9		1·7		
Menopausal status					0·14
Premenopausal	348	66·8	326	62·6	
Postmenopausal	173	33·2	195	37·4	
Number of live births*					0·10
Mean	1·9		1·8		
SD	1·1		1·1		
Months of breast-feeding†					0·06
Mean	21·7		19·0		
SD	24·4		18·2		
Age at menopause (years)‡					0·06
Mean	49·8		49·0		
SD	4·2		4·0		
Age at first live birth (years)					0·60
Mean	25·6		25·5		
SD	3·6		3·2		
Ever used an oral contraceptive	36	6·9	28	5·4	0·37
History of benign breast disease	184	35·3	130	25·0	<0·01
First-degree relative with cancer	77	14·8	56	10·8	0·06
History of breast-feeding	426	81·8	437	83·9	0·41

* Among women who have had a live birth.
† Among women who had breast-fed.
‡ Among menopausal women.

Table 4 shows the association between serum carotenoid and the risk for breast cancer stratified by menopausal status. An inverse association between serum levels of α-carotene,

Table 2. Concentration of serum carotenoids (μmol/l) among cases and controls in Guangzhou, China*
(Mean values and standard deviations; median values and 25th, 75th percentiles)

	Cases (*n* 521)				Controls (*n* 521)				
	Mean	SD	Median	25th, 75th percentile	Mean	SD	Median	25th, 75th percentile	*P*
α-Carotene	0·06	0·04	0·04	0·03, 0·07	0·07	0·06	0·06	0·04, 0·08	<0·01
β-Carotene	0·45	0·31	0·38	0·26, 0·56	0·57	0·37	0·48	0·32, 0·71	<0·01
β-Cryptoxanthin	0·15	0·13	0·12	0·08, 0·18	0·19	0·17	0·13	0·08, 0·23	0·01
Lycopene	0·16	0·11	0·13	0·09, 0·19	0·21	0·14	0·18	0·11, 0·26	<0·01
Lutein/zeaxanthin	0·57	0·31	0·52	0·39, 0·69	0·70	0·34	0·65	0·46, 0·86	<0·01

* Wilcoxon's rank-sum test comparing the median consumption levels between cases and controls.

Table 3. Risk of breast cancer according to quartiles of serum carotenoids
(Odds ratios and 95 % confidence intervals)

	Q1	Q2		Q3		Q4		
	OR	OR	95 % CI	OR	95 % CI	OR	95 % CI	P_{trend}
α-Carotene								
No. of cases/controls	194/130	145/131		101/130		81/130		
Crude	1	0·74	0·54, 1·03	0·52	0·37, 0·73	0·42	0·29, 0·60	<0·01
Adjusted*	1	0·75	0·54, 1·06	0·59	0·41, 0·85	0·44	0·30, 0·65	<0·01
β-Carotene								
No. of cases/controls	200/130	143/130		111/131		67/130		
Crude	1	0·72	0·52, 0·99	0·55	0·39, 0·77	0·34	0·23, 0·48	<0·01
Adjusted*	1	0·63	0·45, 0·89	0·50	0·35, 0·72	0·27	0·18, 0·40	<0·01
β-Cryptoxanthin								
No. of cases/controls	133/130	162/130		134/131		92/130		
Crude	1	1·22	0·87, 1·70	1·00	0·71, 1·41	0·69	0·48, 0·99	0·05
Adjusted*	1	1·26	0·89, 1·78	1·02	0·71, 1·46	0·71	0·48, 1·03	0·07
Lycopene								
No. of cases/controls	188/130	177/131		87/130		69/130		
Crude	1	0·93	0·68, 1·28	0·46	0·33, 0·66	0·37	0·25, 0·53	<0·01
Adjusted*	1	1·01	0·72, 1·41	0·48	0·33, 0·70	0·41	0·28, 0·61	<0·01
Lutein/zeaxanthin								
No. of cases/controls	218/130	147/131		98/130		58/130		
Crude	1	0·67	0·49, 0·92	0·45	0·32, 0·63	0·27	0·18, 0·39	<0·10
Adjusted*	1	0·69	0·50, 0·97	0·43	0·30, 0·62	0·26	0·17, 0·38	<0·10

* OR adjusted for BMI, residence, education levels, income, regular drinker and a history of benign breast disease.

β-carotene, lutein/zeaxanthin and the risk for breast cancer was found in both premenopausal and postmenopausal women. Serum *β*-cryptoxanthin was not significantly associated with the risk for breast cancer in pre- and postmenopausal women. The inverse association between serum lycopene and breast cancer risk was only observed among premenopausal women, with an adjusted OR of 0·36 (95 % CI 0·22, 0·60) comparing the highest quartile with the lowest quartile ($P_{trend} < 0·01$).

Stratified analyses by ER and PR status showed that *α*-carotene, *β*-carotene, lycopene and lutein/zeaxanthin were inversely associated with breast cancer risk among all subtypes of ER or PR status. *β*-cryptoxanthin was not associated with breast cancer risk either in ER or in PR subjects (Table 5).

Discussion

This study investigated the relationship between serum carotenoid concentration and breast cancer risk. High levels of serum *α*-carotene, *β*-carotene, lycopene and lutein/zeaxanthin were found to be associated with lower risk for breast cancer. Serum *α*-carotene, *β*-carotene and lutein/zeaxanthin were found to be inversely associated with breast cancer risk in pre- and postmenopausal women. A stratified analysis by ER and PR status showed that serum *α*-carotene, *β*-carotene, lycopene and lutein/zeaxanthin were inversely associated with breast cancer risk among all subtypes of ER or PR status. Serum *β*-cryptoxanthin was not associated with breast cancer risk.

Previous findings regarding the protective effect of circulating carotenoids on breast cancer risk have been mixed. The protective effect of serum *α*-carotene, *β*-carotene, lycopene and lutein/zeaxanthin observed in the present study is consistent with a pooled analysis of eight prospective studies published in 2012[6]. This analysis, which included 3055 cases and 3956 matched controls, reported inverse associations between circulating *α*-carotene (highest *v.* lowest quintile, relative risk (RR) 0·87; 95 % CI 0·71, 1·05; $P_{trend} = 0·04$), *β*-carotene (highest *v.* lowest quintile, RR 0·83; 95 % CI 0·70, 0·98; $P_{trend} = 0·02$), lycopene (highest *v.* lowest quintile, RR 0·78; 95 % CI 0·62, 0·99;

Table 4. Risk of breast cancer stratified by menopausal status
(Odds ratios and 95 % confidence intervals)

	Premenopausal (*n* 674)								Postmenopausal (*n* 368)								
	Q1	Q2		Q3		Q4			Q1	Q2		Q3		Q4			
	OR	OR	95 % CI	OR	95 % CI	OR	95 % CI	P_{trend}	OR	OR	95 % CI	OR	95 % CI	OR	95 % CI	P_{trend}	$P_{interaction}$
α-Carotene																	
No. of cases/controls	140/81		91/82		60/82		57/81		63/49		46/49		38/48		26/49		
Crude	1	0·64	0·43, 0·96	0·42	0·28, 0·62	0·41	0·26, 0·63	<0·01	1	0·73	0·42, 1·26	0·62	0·35, 1·09	0·41	0·23, 0·76	<0·01	0·55
Adjusted*	1	0·69	0·45, 1·06	0·49	0·31, 0·78	0·43	0·27, 0·69	<0·01	1	0·75	0·42, 1·33	0·80	0·44, 1·47	0·42	0·21, 0·81	0·01	0·13
β-Carotene																	
No. of cases/controls	136/81		98/82		75/82		39/81		60/49		50/48		33/49		30/49		
Crude	1	0·71	0·48, 1·06	0·55	0·36, 0·83	0·29	0·18, 0·46	<0·01	1	0·85	0·49, 1·47	0·55	0·31, 0·98	0·50	0·28, 0·90	0·02	0·19
Adjusted*	1	0·62	0·40, 0·96	0·49	0·31, 0·77	0·24	0·14, 0·40	<0·01	1	1·04	0·57,1·90	0·82	0·43,1·54	0·42	0·22, 0·74	0·01	0·05
β-Cryptoxanthin																	
No. of cases/controls	98/81		100/82		87/82		63/81		44/48		48/49		53/50		28/48		
Crude	1	1·01	0·67, 1·53	0·88	0·58, 1·34	0·64	0·41, 1·00	0·50	1	1·07	0·60, 1·90	1·16	0·66, 2·03	0·64	0·34, 1·18	0·15	0·73
Adjusted*	1	1·09	0·70, 1·69	0·98	0·63, 1·54	0·65	0·41, 1·04	0·07	1	1·12	0·62, 2·03	1·06	0·59, 1·92	0·70	0·37, 1·35	0·29	0·83
Lycopene																	
No. of cases/controls	139/81		121/82		48/82		40/81		47/48		53/49		45/49		28/49		
Crude	1	0·86	0·58, 1·27	0·34	0·22, 0·54	0·29	0·18, 0·46	<0·01	1	1·11	0·63, 1·93	0·94	0·53, 1·66	0·58	0·32, 1·08	0·09	<0·01
Adjusted*	1	0·96	0·63, 1·45	0·40	0·25, 0·65	0·36	0·22, 0·60	<0·01	1	1·00	0·55,1·81	0·88	0·47, 1·62	0·56	0·29, 1·09	0·09	0·01
Lutein/zeaxanthin																	
No. of cases/controls	153/81		92/82		60/82		43/81		63/48		59/49		33/49		18/49		
Crude	1	0·59	0·40, 0·89	0·39	0·25, 0·59	0·28	0·18, 0·44	<0·01	1	0·92	0·54, 1·56	0·51	0·29, 0·92	0·28	0·15, 0·54	<0·01	0·57
Adjusted*	1	0·59	0·39, 0·90	0·36	0·23, 0·58	0·25	0·15, 0·41	<0·01	1	1·02	0·58, 1·79	0·46	0·25, 0·86	0·28	0·13, 0·57	<0·01	0·21

* OR adjusted for BMI, residence, education levels, income, regular drinker and a history of benign breast disease.

Table 5. Risk of breast cancer stratified by oestrogen receptor (ER) or progesterone receptor (PR) status (Odds ratios and 95 % confidence intervals)

	ER– (*n* 130)			ER+ (*n* 365)			PR – (*n* 166)			PR+ (*n* 329)		
		Adjusted*			Adjusted*			Adjusted*			Adjusted*	
	No. of cases/controls	OR	95 % CI	No. of cases/controls	OR	95 % CI	No. of cases/controls	OR	95 % CI	No. of cases/controls	OR	95 % CI
α-Carotene												
Q1	48/130	1		136/130	1		65/130	1		119/130	1	
Q2	34/131	0·69	0·41, 1·17	106/131	0·78	0·54, 1·12	47/131	0·72	0·45, 1·15	93/131	0·77	0·53, 1·12
Q3	28/130	0·66	0·38, 1·15	66/130	0·55	0·37, 0·83	31/130	0·55	0·32, 0·92	63/130	0·60	0·39, 0·90
Q4	20/130	0·40	0·21, 0·74	57/130	0·44	0·29, 0·67	23/130	0·35	0·19, 0·62	54/130	0·47	0·31, 0·73
P_{trend}			<0·01			<0·01			<0·01			<0·01
β-Carotene												
Q1	54/130	1		138/130	1		72/130	1		120/130	1	
Q2	31/130	0·46	0·27, 0·79	103/130	0·65	0·45, 0·95	37/130	0·43	0·26, 0·70	97/130	0·70	0·47, 1·03
Q3	28/131	0·45	0·26, 0·78	76/131	0·51	0·34, 0·76	34/131	0·41	0·25, 0·69	70/131	0·54	0·36, 0·81
Q4	17/130	0·24	0·12, 0·45	48/130	0·28	0·18, 0·43	23/130	0·25	0·14, 0·44	42/130	0·27	0·17, 0·43
P_{trend}			<0·01			<0·01			<0·01			<0·01
β-Cryptoxanthin												
Q1	28/130	1		101/130	1		43/130	1		86/130	1	
Q2	46/130	1·89	1·09, 3·27	110/130	1·11	0·76,1·62	53/130	1·36	0·83, 2·22	103/130	1·20	0·82, 1·74
Q3	31/131	1·12	0·62, 2·02	95/131	0·98	0·67, 1·45	39/131	0·92	0·54, 1·55	87/131	1·00	0·68, 1·47
Q4	25/130	0·97	0·52, 1·80	59/130	0·58	0·38, 0·89	31/130	0·77	0·45, 1·33	53/130	0·62	0·41, 0·94
P_{trend}			0·93			<0·01			0·35			0·02
Lycopene												
Q1	53/130	1		128/130	1		71/130	1		110/130	1	
Q2	39/131	0·78	0·47, 1·30	128/131	1·09	0·76, 1·56	52/131	0·81	0·51, 1·28	115/131	1·10	0·76, 1·61
Q3	20/130	0·35	0·19, 0·65	64/130	0·53	0·35, 0·79	25/130	0·33	0·19, 0·58	59/130	0·56	0·37, 0·85
Q4	18/130	0·36	0·19, 0·68	45/130	0·39	0·25, 0·61	18/130	0·28	0·15, 0·51	45/130	0·45	0·28, 0·70
P_{trend}			<0·01			<0·01			<0·01			<0·01
Lutein/zeaxanthin												
Q1	52/130	1		156/130	1		75/130	1		133/130	1	
Q2	39/131	0·77	0·47, 1·27	99/131	0·66	0·46, 0·95	45/131	0·63	0·40, 1·00	93/131	0·72	0·50, 1·05
Q3	25/130	0·49	0·28, 0·85	70/130	0·43	0·29, 0·63	28/130	0·38	0·22, 0·63	67/130	0·48	0·32, 0·72
Q4	14/130	0·27	0·14, 0·52	40/130	0·25	0·16, 0·39	18/130	0·24	0·13, 0·43	36/130	0·26	0·17, 0·42
P_{trend}			<0·01			<0·01			<0·01			<0·01

* OR adjusted for BMI, residence, education levels, income, regular drinker and a history of benign breast disease.

$P_{trend} = 0{\cdot}02$), lutein/zeaxanthin (highest *v.* lowest quintile, RR 0·84; 95 % CI 0·70, 1·01; $P_{trend} = 0{\cdot}05$) and breast cancer risk. However, circulating β-cryptoxanthin was not related to breast cancer risk. Other prospective studies also showed that serum/plasma α-carotene[3,16,17], β-carotene[3,16,18], lycopene[18,19] and lutein/zeaxanthin[3,16,19] was associated with decreased risk for breast cancer. Most previous studies[2–4,6,16–19] have pointed to a protective role for at least one individual serum/plasma carotenoid. However, three[5,20,21] of those recently published studies[5,20–22], including a study in Shanghai, China[5], indicated that none of the individual circulating carotenoids (including α-carotene, β-carotene, β-cryptoxanthin, lycopene and lutein/zeaxanthin) reduce breast cancer risk. One hospital-based case–control study even reported the deleterious effect of plasma β-carotene on Korean women[23].

Those studies that failed to find evidence for a protective role of specific serum or plasma carotenoids may have been limited by a relatively small number of cases (201 cases)[22] or restricted to a certain group of subjects, such as highly educated volunteers[20] or middle-aged or older female subjects[5,21], rather than the general population. Contrary to our findings, the previous study in Shanghai, China[5], observed no association between specific plasma carotenoids and the risk for breast cancer. The distinct differences in dietary habits between Shanghai and Guangdong females may be responsible for the inconsistent reports. Vegetables, particularly dark green leafy vegetables, are good sources of carotenoids. Compared with Shanghai women[24], Guangdong women[25] were more likely to eat vegetables (total vegetables: Shanghai women *v.* Guangdong women, 304 (SD 174) *v.* 458 (SD 252) g/d), especially dark green leafy vegetables, which are rich in α-carotene, β-carotene and lutein/zeaxanthin (dark green vegetables: Shanghai women *v.* Guangdong women, 92 (SD 65) *v.* 244 (SD 163) g/d). This may account for the finding that specific carotenoids were observed to protect against breast cancer among women in Guangdong, but not among those in Shanghai. It has been reported that the association between β-carotene and cancer risk is likely to be influenced by the source of β-carotene (food or supplement) and the doses involved[26]. In our study, most subjects attained β-carotene from dietary sources rather than supplements. This may partially explain the discrepant results.

Our study showed that serum β-cryptoxanthin was not statistically related to the risk for breast cancer. Previous reports on the association between circulating β-cryptoxanthin and breast cancer risk have been inconsistent. Several studies[5,6,16,18,23] reported no inverse associations for circulating β-cryptoxanthin and breast cancer risk after adjustment for other carotenoids or risk factors. In contrast, one recent nested case–control study conducted in French women showed a protective effect of plasma β-cryptoxanthin on breast cancer risk[4]. Mean serum β-cryptoxanthin was 0·15 (SD 0·13) in our study, which was markedly lower than that in the Education Nationale-European Prospective Investigation into Cancer and Nutrition study (mean 0·23 (SD 0·16))[4]. Relatively low serum β-cryptoxanthin concentration among the general population in China might help to explain the discrepancies. It is also known that carotenoids provide particular protection against breast cancer among smokers, because of the aggravated oxidative stress among this group[27]. In the present study, the smoking rate of subjects was extremely low (1 %). The null association between β-cryptoxanthin and breast cancer risk may be related to the limited number of Chinese women smokers.

Consistent with our study, some previous studies have shown that serum α-carotene, β-carotene[28], lycopene[29] and lutein[22] were inversely associated with breast cancer risk in both pre- and postmenopausal women. However, the protective role of serum lycopene was only observed among premenopausal women in our study. The Shanghai Women's Health Study[5] also reported the parallel effect of plasma lycopene on breast cancer risk only among premenopausal women, with an OR (95 % CI) of 0·36 (0·16, 0·80) comparing the highest with the lowest quartile ($P_{trend} = 0{\cdot}06$). Dissimilar characteristics (such as oestrogen exposure and oxidative stress status) between pre- and postmenopausal women may account for this finding[30]. Lycopene inhibits cancer cell proliferation under the influence of oestrogen exposure[31] and was found to be protective in premenopausal subjects, who have higher levels of oestrogen. Premenopausal women were also shown to be more susceptible to the protection afforded by antioxidants (including lycopene) because of aggravated oxidative stress, in comparison with postmenopausal women[32].

The relatively few epidemiological studies that have examined associations between individual serum carotenoids and the risk for breast cancer stratified by ER or PR status have reported differing results. The significant protective effect of serum α-carotene, β-carotene, lycopene and lutein/zeaxanthin for all subtypes of ER and PR status in the present study was consistent with some previous findings. Tamimi *et al.*[16] found a negative association with plasma α-carotene for ER– (highest *v.* lowest quintile, OR 0·50; 95 % CI 0·28, 0·91; $P_{trend} = 0{\cdot}05$) and ER+ (highest *v.* lowest quintile, OR 0·72; 95 % CI 0·50, 1·04; $P_{trend} = 0{\cdot}03$) breast cancer. A prospective study[18] conducted in the USA suggested that β-carotene, lycopene and lutein were protective in both ER– and ER+ breast cancer patients. In contrast, a pooled analysis[6] reported that α-carotene and β-carotene reduced breast cancer risk in ER– patients but not in ER+ patients. No association between plasma lycopene and breast cancer risk was observed among ER+ and PR+ women in the Shanghai Women's Health Study[21]. Although the anti-cancer role of carotenoids was supported by an experimental study indicating that carotenoids inhibited proliferation of different hormone-defined breast cancer cell lines[33], the protective effect of serum β-cryptoxanthin on breast cancer risk was found neither in ER– nor in PR– women. The relatively small sample size might have caused a chance result or insufficient statistical power in the analysis stratified by ER or PR status. Studies with a larger sample size are warranted to clarify this association.

A protective role for carotenoids in breast cancer aetiology is biologically plausible. Carotenoids may protect against DNA damage by neutralising oxygen species[34] and activating the antioxidant response element transcription system[35]. Besides their antioxidant potential, some carotenoids such as α-carotene, β-carotene and β-cryptoxanthin are metabolised to retinol, which is involved in cell differentiation[36,37]. Carotenoids also contribute to intercellular communication[38],

cell proliferation[39] and cell apoptosis regulation[40,41]. In addition, carotenoids influence carcinogenesis through genetic mechanisms[42–44].

Our study had several strengths. To the best of our knowledge, only one previous study has examined the association between circulating carotenoids and breast cancer among Chinese women[5]. This study contributes evidence for the protective role of each carotenoid in women with different menopausal and ER/PR statuses. Furthermore, serum carotenoids were chosen as the biomarkers, to reflect carotenoid levels without being influenced by food patterns, racial differences and other environmental factors[45]. More objective results can be delivered by measuring serum carotenoids instead of estimating dietary intake.

Nevertheless, the current study had some limitations that should be taken into account when interpreting the results. First, the study design did not allow causal associations to be confirmed. Serum samples were collected after the diagnosis of breast cancer, and breast cancer itself might influence circulating carotenoids levels. However, a prospective study[46] found no variation in single serum carotenoid levels between breast cancer survivors and control subjects. Second, selection and information biases could have distorted the results. To minimise selection bias, we were careful to exclude all control subjects with any diagnoses related to breast cancer or habitual dietary changes. The similar catchment areas and length of hospitalisation of all subjects, and the relatively high response rate, also reduced selection bias. To minimise information bias, serum carotenoid measurements were performed by the same trained technician. In addition, the lower inter-assay and intra-assay CV showed that the measurement of each carotenoid was relatively accurate and precise. Third, a single-sample measurement may be defective. However, a previous study found that serum carotenoid measurements were reasonably consistent over time because carotenoids are lipid soluble and relatively stable[47]. Therefore, a single sample is adequately representative of an individual's long-term exposure[48]. Finally, potential confounding variables may not have been adequately excluded. It is possible that lifestyles were different among cases and controls. However, a wide range of known predictors was considered, including active smoking, regular drinking and family history.

In conclusion, this study supports the hypothesis of a protective role of α-carotene, β-carotene, lycopene and lutein/zeaxanthin but not β-cryptoxanthin on breast cancer risk. Circulating α-carotene, β-carotene, lycopene and lutein/zeaxanthin were observed to be inversely associated with breast cancer risk among all subtypes of ER or PR status.

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The authors' responsibilities were as follows: B. Y. conducted the data collection, analysed the data and wrote this paper. M.-S. L. conducted the laboratory measurement. X.-F. M. was responsible for connecting and coordinating the field work. L. W., W.-P. L. and Y.-F. D. participated in the data collection. C.-X. Z. constructed the project design, supervised and contributed to the manuscript writing.

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