British Journal of Nutrition (2020), 124, 1311–1319 doi:10.1017/S000711452000152X

Associations between serum carotenoid levels and the risk of non-Hodgkin lymphoma: a case–control study

Shan Li[1]†, Xianglu Zhu[2]†, Lin Zhu[1], Xin Hu[1] and Shujuan Wen[1]*

[1]*Department of Lymphoma, Cancer Hospital of Xinjiang Medical University, Urumqi 830000, Xinjiang, People's Republic of China*

[2]*Department of Breast Radiotherapy, Cancer Hospital of Xinjiang Medical University, Urumqi 830000, Xinjiang, People's Republic of China*

(Submitted 24 January 2020 – Final revision received 16 April 2020 – Accepted 17 April 2020 – First published online 30 April 2020)

Abstract

Limited studies have investigated the effects of serum carotenoids on the risk of non-Hodgkin lymphoma (NHL), and the findings have been inconclusive. This study aims to assess the association between serum total or specific carotenoid levels and NHL risk. This 1:1 matched, hospital-based case–control study enrolled 512 newly diagnosed (within 1 month) NHL patients and 512 healthy controls who were matched by age (±5 years) and sex in Urumqi, China. Serum carotenoid levels were measured by HPLC. Conditional logistic regression showed that higher serum total carotenoid levels and their subtypes (e.g. *α*-carotene, *β*-carotene, *β*-cryptoxanthin and lycopene) were dose-dependently associated with decreased NHL risk. The multivariable-adjusted OR and their 95 % CI for NHL risk for quartile 4 (*v.* quartile 1) were 0·31 (95 % CI 0·22, 0·48; $P_{\text{for trend}} < 0{\cdot}001$) for total carotenoids, 0·52 (95 % CI 0·33, 0·79; $P_{\text{for trend}}$: 0·003) for *α*-carotene, 0·63 (95 % CI 0·42, 0·94; $P_{\text{for trend}}$: 0·031) for *β*-carotene, 0·73 (95 % CI 0·49, 1·05; $P_{\text{for trend}}$: 0·034) for *β*-cryptoxanthin and 0·51 (95 % CI 0·34, 0·75; $P_{\text{for trend}}$: 0·001) for lycopene. A null association was observed between serum lutein + zeaxanthin and NHL risk (OR 0·89, 95 % CI 0·57, 1·38; $P_{\text{for trend}}$: 0·556). Significant interactions were observed after stratifying according to smoking status, and inverse associations were more evident among current smokers than past or never smokers for total carotenoids, *α*-carotene and lycopene ($P_{\text{for heterogeneity}}$: 0·047, 0·042 and 0·046). This study indicates that higher serum carotenoid levels might be inversely associated with NHL risk, especially among current smokers.

Key words: **Non-Hodgkin lymphoma: Serum carotenoids: Case–control studies: Chinese patients**

Non-Hodgkin lymphoma (NHL), a solid tumour that originates in the immune system, accounts for approximately 90 % of all lymphomas[1]. According to the Global Burden of Disease Study 2015, NHL was ranked seventh for incident cancer cases and 11th for cancer-related deaths worldwide[2]. In China, the estimated numbers of NHL cases and NHL-related deaths in 2016 were 237 000 and 37 600, respectively[3]. In particular, the burden of NHL in China showed an increasing trend from 2006 to 2016, with the change in age-standardised disability-adjusted life years associated with NHL of approximately 9·18 %[3]. NHL prevention has become a public health issue in China.

Although the underlying aetiology of NHL remains largely unknown[4], several risk factors have been demonstrated to be associated with susceptibility to NHL, including immune dysregulation[5], infections[6], obesity[7], genetics[8] and environmental exposure[9]. In recent years, antioxidant nutrients have received increased attention because of their protective properties against diverse diseases[10–12] and they have also been suggested to play a role in the development of NHL[13,14].

Carotenoids, the lipid-soluble pigments that give yellow, red and orange colours to foods, are well known as natural antioxidant nutrients synthesised by plants, bacteria and some algae. Among all carotenoids that have been identified, *α*-carotene, *β*-carotene, *β*-cryptoxanthin, lycopene and lutein/zeaxanthin are the most studied because of their abundance in the diet[14]. Carotenoids can reduce the production of reactive oxygen species in cell membranes and inhibit radical species, which may result in a protective effect against DNA damage and the malignant transformation of immune cells[15,16]. Currently, most evidence focused on carotenoids, and NHL risk is mainly related to dietary carotenoid intake. A meta-analysis based on data from Western countries suggested that increased dietary intakes of *α*-carotene, *β*-carotene and lutein/zeaxanthin, but not lycopene or *β*-cryptoxanthin, were associated with a reduced risk of

Abbreviations: CLL, chronic lymphocytic leukaemia; DLBCL, diffuse large B-cell lymphoma; NHL, non-Hodgkin lymphoma; SLL, small lymphocytic lymphoma.

* **Corresponding author:** Dr Shujuan Wen, fax +86 991 7968111, email wudaq245@126.com

† These authors contributed equally to this work and should be considered as co-first authors.

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NHL[14]. Serum levels of carotenoids are considered valid biological markers of dietary intake[16]. However, the association between circulating carotenoid levels and NHL risk remains poorly investigated. According to a comprehensive search of the PubMed database, we only found one related study reported by Ollberding *et al.*[16], which was a nested case–control study including 271 NHL cases and 538 controls, and explored the effect of circulating biomarkers of carotenoids on NHL risk. Ollberding *et al.*[16] reported that higher pre-diagnostic serum total carotenoids, lycopene and α-cryptoxanthin, but not α-carotene, β-cryptoxanthin or *trans*-zeaxanthin, were associated with a reduced risk of NHL, which is partly consistent with the findings of the meta-analysis. More studies are needed to confirm these associations. In addition, because circulating carotenoid levels are determined by dietary intake, owing to differing dietary patterns among different populations, the associations found in Western populations may not be generalisable to the population in Asia, including China. To the best of our knowledge, no report has evaluated the relationship of serum levels of carotenoids with NHL risk in Chinese patients.

Therefore, this study aims to measure and compare the serum levels of total and specific carotenoids in Chinese patients with NHL and cancer-free participants who underwent a health examination in the same hospital to identify circulating carotenoids that may be related to NHL risk.

Materials and methods

Study design and population

In this 1:1 matched, hospital-based case–control study, patients with newly diagnosed NHL (≤1 month prior to the interview) were consecutively enrolled at the Department of Lymphoma, Cancer Hospital of Xinjiang Medical University from February 2016 to April 2019. The types of NHL mainly included the three most common subtypes: diffuse large B-cell lymphoma (DLBCL), follicular lymphoma and chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL). NHL was diagnosed histologically and confirmed by physicians. Cases were categorised based on the 2001 WHO classification, which considered CLL as a different presentation of the same disease as SLL, and staged according to Lugano classification. Patients were excluded if they (1) were ≤20 years old (twenty-four cases); (2) had started chemotherapy treatment (thirty-eight cases); (3) had chronic diseases, such as diabetes, major CVD, kidney failure, chronic liver diseases, digestive complications or other diseases/conditions that may have changed their dietary habits (seventy-five cases); (4) simultaneously had a history of any other type of tumour (three cases) or (5) changed their dietary habits significantly in the previous year (three cases). In addition, fifty-six patients refused to participate in the survey.

For each case, we enrolled a participant who underwent a general health check-up in the same hospital during the same time period as the case, and the cases and controls were matched by age (±5 years) and sex. The researchers asked each potentially eligible subject if they wanted to join the study. We also used publicity posters to recruit research subjects. In addition, some subjects were introduced by acquaintances to participate in this study. The selection criteria used for the controls were the same as those used for the cases except for a history of NHL. In addition, the subjects were symptom-free, and subjects with symptoms requiring prompt medical care were excluded from the study. As a result, all controls in this study could be considered to represent healthy individuals. If more than one subject could be matched to one of the cases, we used a random number to choose the control. Finally, 512 cancer-free controls were selected as the control group.

All participants were informed of the requirements of the study and provided written consent. The study was approved by the Ethics Committee of Cancer Hospital of Xinjiang Medical University.

Interview of participants

Before the formal interview, we used a brief questionnaire to screen the eligibility of the included participants. Then, we used a standard structured questionnaire to collect the information used in this study. All information was collected in the hospital by two trained nurses through face-to-face interviews, and these two nurses completed an equal number of interviews with cases and controls. Information in the questionnaire included sociodemographic factors (e.g. age, sex, income and marital status), lifestyle habits (e.g. smoking and alcohol consumption habits and physical activity), dietary intake, medical history and family history of diseases. In this study, a smoker was defined as someone who had smoked at least one cigarette per day for six consecutive months and a current smoker was defined as a smoker who still smoked in the year of interview or in the year before, otherwise to the participant who was considered a past smoker. Alcohol consumers were defined as those who had consumed at least one alcoholic drink daily for at least six consecutive months. Physical activity was defined as someone who performed moderate to intense physical exercise for at least 30 min each time. Generally, anthropometric measurements were conducted after the interview. Weight and height were measured with standard protocols, and BMI was calculated by dividing weight (kg) by height squared (m^2). The participants' usual dietary intakes before the year of the NHL diagnosis or control enrolment were assessed with a ninety-eight-item FFQ that was revised from the questionnaire used for the 5th Chinese National Nutrition and Health Survey. The daily intake of carotenoids was calculated based on the 2009 Chinese Food Composition Table.

Laboratory measurements

All assays were performed at the clinical laboratory of the Cancer Hospital of Xinjiang Medical University. The laboratory personnel was blinded to the case–control status of the samples. Blood samples were drawn from all participants in the morning after an overnight fast and were processed within 4 h of collection by centrifugation. Serum was collected by pipette and stored at –80°C until analysis. Serum concentrations of blood lipids, including total cholesterol and TAG, were measured using a Hitachi High-Tech 7180 automatic analyzer.

To date, more than 700 carotenoids have been identified[14], but six (α-carotene, β-carotene, β-cryptoxanthin, lycopene and lutein/zeaxanthin) represent more than 95 % of the total

carotenoids in human plasma[17]. Therefore, we considered the sum of these six carotenoids as the total serum carotenoids in this study. HPLC, according to previous studies[18,19], was used to obtain carotenoid concentrations. Briefly, carotenoids were extracted from 200 μl of serum using hexane-butylated hydroxytoluene and dissolved in 100 μl of acetonitrile methanol tetrahydrofuran ammonium acetate. A 20 μl aliquot was used to detect the concentration of carotenoids using a C18 HPLC column (Agilent 1200). The concentrations of carotenoids were quantified by determining peak areas in the HPLC chromatogram and were calibrated against known amounts of standards. In this assay system, lutein/zeaxanthin was measured together because of their peaks at the same sites. All samples were analysed in a random order. Intra-assay CV (percentages) were 6·9 % for α-carotene, 7·2 % for β-carotene, 8·9 % for β-cryptoxanthin, 9·4 % for lycopene and 7·4 % for lutein/zeaxanthin.

Statistical analysis

Baseline characteristics of cases and controls are presented as frequency (percentage) for categorical variables and as the mean values and standard deviations or median values and interquartile ranges for continuous variables and were compared using χ^2 tests for categorical data, *t* tests for normally distributed continuous data and the Wilcoxon rank-sum test for non-normally distributed data. Spearman correlation analysis was used to analyse the correlation between intake and serum concentrations, as well as the correlation between each serum carotenoid with adjustment for age and sex.

Conditional logistic regression with matched sets as strata was used to assess the association between serum carotenoids and NHL risk and calculate the corresponding OR and 95 % CI. To optimise the distribution of cases and controls within strata, quartiles were used to divide the exposure into four groups based on the distribution of serum carotenoids in the total study population. The lowest carotenoid group served as the reference. We also analysed the risk estimates for each standard error increase in serum carotenoids. Model 1 was adjusted for age (continuous), and model 2 was further adjusted for BMI (continuous), marital status (married/others), education level (primary or below/secondary/senior high/college or above), income level (≤2000/2001–4000/4001–6000/>6000 yuan/month per person), physical activity (never/1–2/≥3 time/week), smoking history (current/past/never), alcohol intake (yes/no), family history of cancer (yes/no), total cholesterol (continuous) and TAG (continuous). Tests for trends were conducted by entering the categorical variables as continuous variables in the models.

As certain risk factors for NHL may have a different influence according to NHL subtypes, we applied stratification analyses between serum carotenoids and the risk of NHL by subtype. Additionally, chronic cigarette smokers have been suggested to have lower concentrations of serum carotenoids than non-smokers[20]; thus, the associations between serum carotenoids and NHL risk might be different among current, past or never smokers. Therefore, an analysis stratified by smoking status was performed. Heterogeneity across different NHL subtypes or smoking status was estimated by adding interaction terms according to the likelihood ratio test. Because matching was impossible for smoking status, unconditional logistic regression analyses adjusting for the confounders in model 2 were performed.

All analyses were performed using SPSS version 23 (SPSS Inc.). All tests were two-sided; a *P* value of <0·05 was considered statistically significant.

Results

Subject characteristics

A total of 512 cases (245 DLBCL cases, 152 CLL/SLL cases and 115 follicular lymphoma cases) were successfully interviewed and had blood samples available, and the participation rate among eligible cases was 90·1 %. According to the Lugano classification, 12·5 % were diagnosed in stage I, 31·3 % in stage II, 24·6 % in stage IIE and 31·6 % in stage IV. Moreover, there were no significant differences in basic characteristics, such as age, sex, marital status, education, smoking and alcohol consumption habits, or BMI, between participants and non-participants (all $P > 0·05$, data not shown).

The basic characteristics of NHL cases and matched controls are presented in Table 1. The mean age at enrolment was 60·9 (SD 6·66) years for cases and 60·9 (SD 6·62) controls ($P = 0·584$). Patients with NHL were more likely to be current smokers (33·7 *v.* 24·6 %) and alcohol consumers (25·8 *v.* 20·3 %) than controls. Higher proportions of NHL cases were found to have a family history of cancer than cancer-free controls (5·5 *v.* 2·7 %). No differences in BMI, marital status, education level, income, physical activity, total cholesterol or TAG were detected between cases and controls (all $P > 0·05$).

For the subjects (189 controls and 254 cases) who completed the dietary questionnaire, the Spearman correlation coefficients (r_s) between intake and serum concentrations of α-carotene, β-carotene, β-cryptoxanthin and lutein + zeaxanthin were 0·32, 0·25, 0·20 and 0·23 for cases and 0·27, 0·30, 0·21 and 0·18 for controls (all $P < 0·05$).

Distributions of serum carotenoid levels among cases and controls

Both the mean and standard deviations and median with 25th and 75th percentile values of serum carotenoid levels are presented in Table 2. The mean levels of total carotenoids in cases were significantly lower than those in controls (14·19 (SD 8·12) *v.* 16·56 (SD 9·12) μmol/dl, $P < 0·001$). For specific carotenoids, the serum concentrations of α-carotene, β-carotene and lycopene were significantly lower for cases than for controls (all $P < 0·05$); the differences in levels according to the case–control status did not reach statistical significance for β-cryptoxanthin ($P = 0·105$) or lutein/zeaxanthin ($P = 0·884$). Significant correlations were observed among these carotenoids (r_s range 0·230–0·608).

Associations between serum carotenoids and non-Hodgkin lymphoma risk

Age-adjusted conditional logistic regression analyses showed a dose-dependent inverse association between NHL risk and serum total carotenoids ($P_{for\ trend} < 0·001$), α-carotene ($P_{for\ trend} = 0·005$), β-carotene ($P_{for\ trend} = 0·030$) and lycopene ($P_{for\ trend} = 0·001$),

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Table 1. Basic characteristics of non-Hodgkin lymphoma cases and matched controls*
(Mean values and standard deviations; numbers and percentages)

Characteristics	Cases (*n* 512)		Controls (*n* 512)		
	n	%	*n*	%	*P*
Age (years)					
Mean	60·9		60·9		0·584
SD	6·66		6·62		
BMI (kg/m^2)					
Mean	23·5		23·5		0·939
SD	3·32		3·24		
Marital status					
Married	354	69·1	335	65·4	0·231
Unmarried/divorced/ widowed	158	30·9	177	34·6	
Education level					
Primary school or below	146	28·5	152	29·7	0·170
Secondary school	178	34·8	165	32·2	
Senior high school	118	23·0	102	19·9	
College or above	70	13·7	93	18·2	
Income (yuan/ month/person)					
≤2000	21	4·1	20	3·9	0·088
2001–4000	230	44·9	206	40·2	
4001–6000	209	40·8	208	40·6	
>6000	52	10·2	78	15·2	
Physical activity†					
Never	287	56·1	275	53·7	0·684
1–2 times/week	164	32·0	177	34·6	
≥3 times/week	61	11·9	60	11·7	
Smoking‡					
Current	174	34·0	126	24·6	<0·001
Past	55	10·7	46	9·0	
Never	283	55·3	339	66·4	
Alcohol drinking§					
Yes	132	25·8	104	20·3	0·038
No	379	74·2	408	79·7	
Family history of cancer					
Yes	28	5·5	14	2·7	0·039
No	485	94·5	498	97·3	
Total cholesterol (mmol/l)					
Mean	5·62		5·67		0·509
SD	1·13		1·16		
TAG (mmol/l)					
Mean	2·60		2·54		0·596
SD	1·35		2·09		

* Continuous variables were described by mean values and standard deviations, and categorical variables were described by *n* and percentage values.
† Smokers were defined as someone who had smoked at least one cigarette per d more than six consecutive months.
‡ Alcohol drinkers were defined as someone who had drunk at least once daily for at least six consecutive months.
§ Physical activity was defined as someone who had done moderate to intense exercises at least 30 min each time.

but no association was observed for β-cryptoxanthin ($P_{for\ trend} = 0{\cdot}142$) or lutein/zeaxanthin ($P_{for\ trend} = 0{\cdot}833$) (Table 3). With adjustments further made for BMI, marital status, education level, income level, physical activity, smoking history, alcohol intake, family history of cancer, total cholesterol and TAG, significant associations remained and a significantly inverse association emerged for β-cryptoxanthin. The multivariate OR and their 95 % CI for the highest quartile compared with the lowest quartile were 0·32 (95 % CI 0·22, 0·48) for total carotenoids, 0·52 (95 % CI 0·33, 0·79) for α-carotene, 0·63 (95 % CI 0·42, 0·94) for β-carotene, 0·73 (95 % CI 0·49, 1·05) for β-cryptoxanthin, 0·89 (95 % CI 0·57, 1·38) for lutein/zeaxanthin and 0·51 (95 % CI 0·34, 0·75) for lycopene (Table 4). In addition, for each SD increase, the multivariate OR and their 95 % CI were 0·64 (95 % CI 0·55, 0·75) for total carotenoids, 0·69 (95 % CI 0·60, 0·81) for α-carotene, 0·72 (95 % CI 0·63, 0·83) for β-carotene, 0·84 (95 % CI 0·72, 0·98) for β-cryptoxanthin, 0·95 (95 % CI 0·81, 1·11) for lutein/zeaxanthin and 0·69 (95 % CI 0·59, 0·81) for lycopene (Table 4).

Stratification analyses for the associations between serum carotenoids and non-Hodgkin lymphoma risk

After stratification by NHL subtype, the inverse association of higher serum levels of total carotenoids and lycopene was limited to DLBCL and CLL/SLL (Table 5). The *P* values for trend were significant for all three types of NHL for total carotenoids and for DLBCL and CLL/SLL for lycopene, but no significant findings were observed for other strata.

After stratification by smoking status, statistically significant inverse associations were generally observed in current smokers but not in never or past smokers (Table 5). Compared with never smokers, for current smokers, lower OR were obtained from total carotenoids ($OR_{Q4\ v.\ Q1}$ 0·15 *v.* 0·58, $P_{for\ heterogeneity} = 0{\cdot}047$), α-carotene ($OR_{Q4\ v.\ Q1}$ 0·14 *v.* 0·78, $P_{for\ heterogeneity} = 0{\cdot}042$) and lycopene ($OR_{Q4\ v.\ Q1}$ 0·21 *v.* 0·89, $P_{for\ heterogeneity} = 0{\cdot}046$).

Discussion

In this case–control study conducted among Chinese adults, we found that higher levels of serum total carotenoids were associated with a decreased risk of NHL. Particularly, risk estimates for specific carotenoids, such as α-carotene, β-carotene and lycopene, also suggested inverse associations. According to stratified analyses, these inverse associations were generally confined to DLBCL and CLL/SLL and were more evident in current smokers.

In recent years, the burden of NHL in China has increased more significantly than the global burden[3]. Although the launch of rituximab and anthracyclines has improved the prognosis of B-cell origin NHL[20], extra immunochemotherapy still puts economic pressure on low-income NHL patients, especially those living in less-developed provinces in China. Determining affordable lifestyle factors that can reduce the risk of NHL is necessary. Given the antioxidant properties of carotenoids and their abundance in foods (e.g. α-carotene and β-carotene in carrots, β-cryptoxanthin in tangerines or lutein + zeaxanthin in dark green vegetables)[19], more attention has been paid to studies defining their potential role in NHL.

To the best of our knowledge, most studies on the potential role of carotenoids in NHL have focused on carotenoid intake. A meta-analysis pooled the current evidence including seven case–control and three cohort studies that demonstrated that higher intakes of α-carotene, β-carotene and lutein + zeaxanthin might protect against NHL development, but this protective effect of α-carotene and β-carotene was limited to DLBCL[14]. Similar to studies focused on dietary carotenoid intake and NHL risk, high serum carotenoids were also suggested to be associated with lower NHL risk. A nested case–control study by Ollberding *et al.*[16] comparing 271 NHL cases with 538 controls reported that higher total serum carotenoids were associated with a lower risk of NHL, which was consistent with our

Table 2. Serum carotenoid levels among cases and control subjects (μmol/dl)*
(Mean values and standard deviations; median values and 25th, 75th percentiles)

	Cases (*n* 521)				Controls (*n* 521)				
	Mean	SD	Median	25th, 75th	Mean	SD	Median	25th, 75th	*P*
Total carotenoids	14·19	8·12	14·16	10·49, 21·21	16·56	9·12	16·62	12·46, 21·56	<0·001
α-Carotene	0·55	0·26	0·54	0·21, 0·69	0·60	0·30	0·60	0·20, 0·72	0·004
β-Carotene	4·83	2·29	4·22	2·15, 7·34	5·12	2·38	5·10	2·01, 8·31	0·047
β-Cryptoxanthin	1·36	0·98	1·36	0·66, 2·37	1·46	1·01	1·45	0·60, 2·38	0·105
Lutein/zeaxanthin	6·69	3·78	6·72	3·14, 10·14	6·72	3·73	6·15	3·14, 10·99	0·884
Lycopene	1·23	0·83	1·22	0·57, 3·11	1·40	0·89	1·42	0·53, 3·43	0·001

* Wilcoxon rank-sum test comparing the median concentration between cases and controls.

Table 3. Association between serum carotenoid levels and non-Hodgkin lymphoma risk
(Odds ratios and 95 % confidence intervals)

	Quartiles of serum carotenoid levels								
	Q1: OR	Q2		Q3		Q4			Per 1 SD increase
		OR	95 % CI	OR	95 % CI	OR	95 % CI	$P_{for\ trend}$	OR	95 % CI
Total carotenoids									
n (case/control)	132/124		150/108		122/134		108/146		
OR1*	1·00	0·89	0·61, 1·27	0·61	0·41, 0·86	0·34	0·23, 0·49	<0·001	0·65	0·57, 0·75
OR2†	1·00	0·88	0·59, 1·30	0·62	0·41, 0·94	0·32	0·22, 0·48	<0·001	0·64	0·55, 0·75
α-Carotene									
n (case/control)	147/109		133/123		127/129		105/151		
OR1*	1·00	0·76	0·54, 1·05	0·73	0·50, 1·07	0·56	0·38, 0·81	0·005	0·68	0·59, 0·78
OR2†	1·00	0·70	0·48, 1·02	0·68	0·43, 1·03	0·52	0·33, 0·79	0·003	0·69	0·60, 0·81
β-Carotene									
n (case/control)	145/112		123/131		126/131		118/138		
OR1*	1·00	0·74	0·52, 1·03	0·76	0·54, 1·06	0·66	0·47, 0·94	0·030	0·71	0·62, 0·81
OR2†	1·00	0·76	0·51, 1·12	0·73	0·50, 1·06	0·63	0·42, 0·94	0·031	0·72	0·63, 0·83
β-Cryptoxanthin									
n (case/control)	138/122		133/120		118/139		123/131		
OR1*	1·00	0·99	0·70, 1·37	0·75	0·53, 1·06	0·83	0·58, 1·16	0·143	0·88	0·76, 1·02
OR2†	1·00	0·93	0·64, 1·34	0·63	0·43, 0·92	0·73	0·49, 1·05	0·034	0·84	0·72, 0·98
Lutein + Zeaxanthin									
n (case/control)	132/124		125/131		125/131		130/126		
OR1*	1·00	0·89	0·63, 1·25	0·90	0·62, 1·31	0·95	0·63, 1·43	0·833	0·95	0·82, 1·10
OR2†	1·00	0·85	0·58, 1·24	0·80	0·54, 1·23	0·89	0·57, 1·38	0·556	0·95	0·81, 1·11
Lycopene									
n (case/control)	142/112		139/120		124/131		107/149		
OR1*	1·00	0·93	0·66, 1·30	0·75	0·53, 1·06	0·55	0·39, 0·79	0·001	0·70	0·60, 0·81
OR2†	1·00	0·90	0·61, 1·31	0·71	0·48, 1·04	0·51	0·34, 0·75	0·001	0·69	0·59, 0·81

* OR1: adjusted for age.
† OR2: adjusted for age, BMI, marital status, education level, income level, physical activity, smoking history, alcohol intake, family history of cancer, total cholesterol and TAG.

findings. However, when referring to the individual carotenoids, few differences among associations were observed. In a previous study, an inverse association was reported for lycopene and α-cryptoxanthin, but our results suggested that these associations were limited to α-carotene, β-carotene and lycopene.

Multiple underlying mechanisms by which carotenoids might be inversely associated with NHL risk have been proposed. Some carotenoids can function as provitamin A, which would have an effect on cellular differentiation and proliferation[21,22]. In addition, carotenoids can function as potent antioxidants and therefore may reduce DNA damage and the malignant transformation of lymphoid cells through the inhibition of reactive oxygen species and other free radicals[15,23,24]. According to the current evidence, SNP in genes related to the oxidative stress pathway increase NHL susceptibility[25,26] and high carotenoid levels may limit oxidative stress, thereby reducing the risk of NHL. Furthermore, carotenoids may also be implicated in NHL promotion through immune-mediated pathways[23]. Carotenoids might alter the activation of the arachidonic acid cascade and modulate the production of PG E_2. In the context of lymphoma, a subsequent increase in PG concentration allows cancer cells to evade the immune response, contributing to metastases[27]. In addition, carotenoids may suppress constitutive NF-κB activation, which has been recognised as a critical pathogenetic factor in lymphoma development, by reducing the DNA-binding activity of NF-κB[28,29].

The inverse associations for individual carotenoids have shown significant differences within studies and between studies. Although natural lipid-soluble pigments are categorised as carotenoids, the different biological functions and chemical

Table 4. Conditional logistic regression analyses of lymphoma according to quartiles of serum carotenoids by subtypes*
(Odds ratios and 95 % confidence intervals)

	Quartiles of serum carotenoid levels												
	Q1		Q2			Q3			Q4				
	n (case/control)	OR	*n* (case/control)	OR	95 % CI	*n* (case/control)	OR	95 % CI	*n* (case/control)	OR	95 % CI	$P_{for\ trend}$	$P_{for\ heterogeneity}$
Total carotenoids													
DLBCL	74/66	1·00	74/55	1·04	0·58, 1·85	60/65	0·63	0·33, 1·18	37/59	0·28	0·15, 0·52	<0·001	0·722
CLL/SLL	34/30	1·00	48/37	0·35	0·14, 0·84	29/34	0·41	0·18, 0·91	41/51	0·17	0·06, 0·46	0·002	
Follicular	24/28	1·00	28/16	3·71	0·73, 18·88	33/35	1·02	0·23, 4·44	30/36	0·41	0·10, 1·73	0·043	
α-Carotene													
DLBCL	75/63	1·00	67/49	0·72	0·41, 1·26	59/64	0·64	0·35, 1·19	44/69	0·58	0·29, 1·13	0·106	0·206
CLL/SLL	44/32	1·00	37/45	0·59	0·27, 1·30	38/31	0·44	0·18, 1·10	33/44	0·47	0·26, 1·27	0·191	
Follicular	28/14	1·00	29/29	0·51	0·13, 2·07	30/34	0·46	0·16, 1·97	28/38	0·45	0·23, 2·22	0·259	
β-Carotene													
DLBCL	65/58	1·00	67/65	1·07	0·61, 1·88	62/58	1·16	0·65, 2·06	51/64	0·98	0·54, 1·80	0·964	0·124
CLL/SLL	43/27	1·00	34/41	0·46	0·20, 1·04	38/42	0·78	0·35, 1·72	37/42	0·40	0·16, 1·01	0·164	
Follicular	37/27	1·00	22/25	0·55	0·13, 2·22	26/31	0·19	0·05, 0·66	30/32	0·74	0·23, 2·41	0·273	
β-Cryptoxanthin													
DLBCL	71/58	1·00	69/64	0·98	0·57, 1·67	50/61	0·71	0·39, 1·29	55/62	0·86	0·49, 1·52	0·429	0·910
CLL/SLL	33/33	1·00	44/31	0·84	0·40, 1·77	37/50	0·59	0·28, 1·27	38/38	0·71	0·32, 1·57	0·240	
Follicular	37/27	1·00	22/25	2·22	0·63, 7·79	26/31	0·73	0·26, 2·06	30/32	0·73	0·27, 1·98	0·399	
Lutein + zeaxanthin													
DLBCL	77/77	1·00	64/62	0·57	0·32, 1·01	58/61	1·19	0·67, 2·12	46/45	1·26	0·61, 2·61	0·410	0·289
CLL/SLL	39/33	1·00	34/34	1·05	0·50, 2·23	37/39	0·72	0·32, 1·62	42/46	0·84	0·35, 2·02	0·565	
Follicular	16/14	1·00	27/35	0·91	0·24, 2·03	30/31	0·86	0·21, 1·90	42/35	0·58	0·11, 1·54	0·236	
Lycopene													
DLBCL	67/44	1·00	69/56	0·58	0·32, 1·06	56/68	0·69	0·39, 1·22	53/77	0·46	0·25, 0·84	0·028	0·956
CLL/SLL	45/42	1·00	41/32	0·93	0·45, 1·91	42/35	0·77	0·37, 1·63	24/43	0·38	0·17, 0·85	0·023	
Follicular	30/26	1·00	29/32	0·98	0·29, 3·34	26/28	0·75	0·23, 2·46	30/29	0·47	0·20, 1·56	0·119	

DLBCL, diffuse large B-cell lymphoma; CLL, chronic lymphocytic leukaemia; SLL, small lymphocytic lymphoma.
* Follicular lymphoma (*n* 115 pairs); CLL/SLL (*n* 152 pairs); DLBCL (*n* 245 pairs). Adjusted for age, BMI, marital status, education level, income level, physical activity, smoking history, alcohol intake, family history of cancer, total cholesterol and TAG.

Table 5. Unconditional logistic regression analyses of lymphoma according to quartiles of serum carotenoids by smoking status*
(Odds ratios and 95 % confidence intervals)

	Quartiles of serum carotenoid levels												
	Q1		Q2			Q3			Q4				
	n (case/control)	OR	*n* (case/control)	OR	95 % CI	*n* (case/control)	OR	95 % CI	*n* (case/control)	OR	95 % CI	$P_{for\ trend}$	$P_{for\ heterogeneity}$
Total carotenoids													
Current smoking	63/23	1·00	52/21	0·53	0·19, 1·44	36/44	0·43	0·16, 1·16	23/38	0·15	0·07, 0·30	<0·001	0·047
Past smoking	19/10	1·00	17/11	1·15	0·31, 4·26	10/10	0·70	0·23, 2·11	9/15	0·41	0·15, 1·14	0·078	
Never smoking	46/79	1·00	80/76	1·64	0·82, 3·28	76/85	0·85	0·51, 1·43	81/99	0·58	0·35, 0·97	0·005	
α-Carotene													
Current smoking	77/23	1·00	50/31	0·22	0·10, 0·62	31/35	0·11	0·05, 0·33	16/37	0·14	0·08, 0·37	<0·001	0·042
Past smoking	14/11	1·00	16/12	0·97	0·33, 2·69	12/12	0·96	0·34, 2·68	10/12	0·54	0·19, 1·51	0·112	
No smoking	61/75	1·00	67/78	0·95	0·57, 1·60	77/82	1·02	0·61, 1·71	75/104	0·78	0·47, 1·28	0·142	
β-Carotene													
Current smoking	47/20	1·00	54/33	0·16	0·10, 0·42	39/36	0·15	0·07, 0·44	34/37	0·27	0·12, 0·78	0·030	0·167
Past smoking	18/11	1·00	14/14	0·75	0·27, 2·05	11/10	0·72	0·26, 1·96	9/11	0·68	0·25, 1·85	0·342	
No smoking	80/80	1·00	58/77	0·84	0·51, 1·38	75/89	0·85	0·52, 1·40	70/93	0·87	0·53, 1·43	0·812	
β-Cryptoxanthin													
Current smoking	40/26	1·00	45/29	0·36	0·15, 0·95	42/28	0·48	0·20, 1·21	47/43	0·31	0·12, 0·84	0·033	0·189
Past smoking	16/9	1·00	13/9	1·05	0·38, 2·86	14/17	0·69	0·25, 1·88	12/11	0·80	0·29, 2·18	0·724	
No smoking	82/87	1·00	75/82	1·20	0·73, 1·97	62/93	0·69	0·42, 1·13	64/77	0·99	0·60, 1·63	0·912	
Lutein + zeaxanthin													
Current smoking	54/27	1·00	41/35	0·32	0·15, 0·88	43/32	0·36	0·13, 0·92	36/32	0·37	0·15, 1·04	0·091	0·529
Past smoking	17/12	1·00	15/11	1·01	0·37, 2·76	9/11	0·99	0·36, 2·70	12/12	0·98	0·34, 2·86	0·934	
No smoking	60/78	1·00	60/95	1·11	0·68, 1·82	72/94	1·19	0·73, 1·95	91/72	1·21	0·74, 1·98	0·388	
Lycopene													
Current smoking	54/17	1·00	41/19	0·35	0·14, 0·87	33/36	0·36	0·15, 0·95	46/54	0·21	0·08, 0·60	0·008	0·046
Past smoking	21/10	1·00	18/13	0·99	0·36, 2·73	7/9	0·85	0·31, 2·35	9/14	0·40	0·14, 1·12	0·148	
No smoking	33/72	1·00	98/101	1·04	0·64, 1·70	91/94	0·99	0·60, 1·63	72/61	0·89	0·58, 1·36	0·420	

* Current smoking (*n* cases/controls = 102/80); past smoking (*n* cases/controls = 55/46); no smoking (*n* cases/controls = 283/339). Adjusted for age, BMI, marital status, education level, income level, physical activity, smoking history, alcohol intake, family history of cancer, total cholesterol and TAG.

structures between individual carotenoids may result in the variation in their associations with NHL risk. Due to the different dietary traditions of the various populations, the average level of serum individual carotenoids differs substantially across studies; therefore, the inconsistent exposure dose of specific carotenoids may weaken comparability between studies. Moreover, a study by Ollberding *et al.*[16] demonstrated that serum carotenoid levels do not represent the same exposure as FFQ-based dietary intakes. To assess the associations between individual serum carotenoids and NHL risk, more studies are needed.

Differences in the mechanism of action for carotenoids between NHL subtypes are still unknown. Stratified by NHL subtypes, our finding of significant risk estimates was primarily confined to DLBCL and CLL/SLL. However, based on the current evidence, significant inverse associations with carotenoids were mainly found in DLBCL[16,30–32]. The small sample size of DLBCL (245) and CLL/SLL (152) cases and limited evidence of heterogeneity across the different NHL subtypes examined in our analysis might limit the power to detect significant heterogeneity. Thus, these findings should be interpreted with care.

Interestingly, in our study, the inverse associations between serum carotenoids and NHL risk were more significant in current smokers. A similar finding was also obtained by Ollberding *et al.*[33] who found that higher intakes of carotene-rich vegetables and α-carotene were associated with better overall survival of NHL among smokers. The previous study proposed a hypothesis that high carotenoid intake may improve the survival of smokers by mitigating smoking-related effects that place smokers at increased risk of death[33]. Based on this, we suppose that tobacco-related carcinogen exposure may increase the body's response to higher serum carotenoid concentrations and act together to reduce NHL susceptibility. However, the null association in past smokers might be due to the limited sample size for each strata, which needs further investigation.

Up to date, most research assessing the potential role of carotenoids in NHL has been conducted in Western countries, and few studies were based on the Chinese population which has a generally different diet pattern. The present study on the associations between serum carotenoid levels and the risk of NHL may fill gaps in this field, which could be important for NHL prevention. Besides, comparing with studies focusing on the associations between carotenoid intakes and NHL risk, we used serum carotenoid levels as substitute to reduce the influence of other nutrients derived from the consumption of whole foods. In particular, intra-individual circulating carotenoid levels seemed to be relatively stable over time as opposed to other nutrients[34,35].

There were also limitations that should be taken into consideration. First, reverse causality could not be fully excluded in a case–control study because the serum carotenoid levels were measured after the diagnoses of NHL. It is possible that some of the patients might have changed their diets, as well as the occurrence or treatment of the diseases might affect the concentrations of the serum biomarkers. We minimised this possibility by recruiting only cases diagnosed within 1 month and by meticulously excluding individuals with essential changes in their diet habits within 1 year preceding the onset of NHL or the interview in the controls. Second, selection bias should be taken into consideration. For example, the proportion of CLL/SLL in our study is significantly higher, while the proportion of DLBCL is lower, than those in other Asian populations[36]. Third, information bias of the measurements is difficult to rule out. To reduce this bias, the serum samples of cases and controls were examined blindly and randomly at the same time by the same staff, during which quality controls were applied. Fourth, the controls were recruited from healthy participants who came to the hospital for health check-ups which might introduce selection bias. Controls with well education and high income are more tended to come to hospital for a health check-up. In this study, we found that the income among the controls was borderline higher than among cases ($P = 0{\cdot}088$). No differences were observed for BMI, marital status, education level, physical activity and blood lipids. Although covariates such as education and income were adjusted as confounding factors in the logistic regression models, the selection bias may still not be eliminated. Fifth, the stage of cancer and co-morbidity status between cases cannot be controlled, which may lead to false-positive results, especially in stratified analyses. Sixth, the correlations between intakes and serum concentrations were significant but generally low (r_s range 0·18–0·32). These findings were consistent with previous reports[37,38], which may be partly due to significant recall bias due to FFQ. Finally, we could not assess the concentration of lutein and zeaxanthin separately and did not take other carotenoids previously identified into consideration. Therefore, to picture the full sight of the associations between serum carotenoids and NHL risk and elucidate the potential role of carotenoids for NHL prevention, detailed prospective studies using large sample are needed.

In conclusion, our study showed lower serum carotenoid levels in patients with NHL than in cancer-free people and higher levels of serum carotenoids were associated with a lower risk of NHL. In particular, these inverse associations were more significant among current smokers. Consumption of carotenoid-rich foods or carotenoid supplement might be an optional and effective strategy for preventing NHL.

Acknowledgements

This work was supported in part by the National Natural Science Foundation of China (grant number: 81860042).

The authors' responsibilities were as follows: S. W.: study concept and design; S. L. and X. Z.: conducted the research, performed the statistical analysis and wrote the paper; and all authors: assisted in the revision of the manuscript.

The authors declare that there are no conflicts of interest.

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British Journal of Nutrition