Dietary vitamins, carotenoids and their sources in relation to age-related macular degeneration risk in China: a population-based case–control study

Hong Jiang[1,2,3], Lina Wang[3], Juan Li[4], Yahui Fan[3], Zhaofang Li[3], Mei Ma[3], Sijiao Liu[3], Baoyu Li[3], Jia Shi[3], Chao Li[3], Baibing Mi[3], Fangyao Chen[3], Duolao Wang[5]*, Cheng Pei[1]* and Le Ma[3,6]*

[1]*The First Affiliated Hospital, Xi'an Jiaotong University Health Science Center, Xi'an, People's Republic of China*
[2]*Key Laboratory of Shaanxi Province for Craniofacial Precision Medicine Research, College of Stomatology, Xi'an Jiaotong University, Xi'an, People's Republic of China*
[3]*School of Public Health, Xi'an Jiaotong University Health Science Center, Xi'an, People's Republic of China*
[4]*Shaanxi Eye Hospital, Xi'an People's Hospital (Xi'an Fourth Hospital), Affiliated Guangren Hospital, Xi'an Jiaotong University Health Science Center, Xi'an, People's Republic of China*
[5]*Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool, UK*
[6]*Key Laboratory of Environment and Genes Related to Diseases (Xi'an Jiaotong University), Ministry of Education of China, Xi'an, People's Republic of China*

Abstract

Mechanistic studies have suggested that antioxidants have beneficial effects on age-related macular degeneration (AMD). This study aimed to investigate the association between the types and sources of dietary vitamin and carotenoid intakes and AMD risk in China. A matched case–control study of 260 AMD cases and 260 matched controls was performed. The participants were interviewed for dietary information and potential confounders, and comprehensive ophthalmic examinations were performed. Conditional logistic models were used to estimate the odds ratio (OR) and 95 % confidence interval (CI) of specific vitamins and carotenoids and their main sources. When comparing the extreme quartiles, the ORs (95 % CI) were 0·30 (0·10, 0·88) for lutein and 0·28 (0·11, 0·74) for β-cryptoxanthin. The associations for other dietary vitamin and carotenoid intakes were generally weaker and non-significant. Higher intakes of spinach and egg, which are important sources of lutein, were associated with a reduced odds of AMD. ORs (95% CIs) comparing extreme categories were 0·42 (0·20, 0·88) for spinach and 0·52 (95% CI: 0·27, 0·98) for egg. Participants who were in the highest category of both egg intake and spinach intake had a much greater reduced odds of having AMD (OR: 0·23; 95% CI: 0·08, 0·71) than those in the lowest category of egg intake and spinach intake. In conclusion, a higher intake of lutein and lutein-rich foods was associated with a significantly decreased odds of AMD. These findings provide further evidence of the benefits of lutein and lutein-rich foods in the prevention of AMD.

Key words: Lutein: Carotenoid: Egg: Age-related macular degeneration

Age-related macular degeneration (AMD) is a major cause of severe irreversible visual impairment among elderly individuals in developed countries. It is estimated that approximately 8·7 % of persons over the age of 45 years worldwide were affected by this disease in 2014, and the number of persons with AMD will increase to 288 million by 2040[1]. New therapies have been shown to effectively prevent progressive vision loss in certain types of exudative AMD; however, there remains a lack of an established effective therapy for most AMD patients, and it is difficult to restore the pathologically damaged retina to a healthy state with current therapies[2]. Therefore, the identification of modifiable factors related to this disease is important to prevent the onset and progression of AMD.

Evidence from *in vitro* and animal experiments suggests that oxidative damage coupled with inflammation plays a vital role in the aetiology of AMD[3,4]. Accordingly, as they are important non-enzymatic anti-oxidative agents in the retina, vitamins and carotenoids were hypothesised to protect the retina from oxidative damage, which suggests that these nutrients play an important role in preventing the process of vision impairment[5,6]. In particular, lutein and its structural isomer, zeaxanthin, which are concentrated in the macula, have also been

---

suggested to play a role in protecting the macular region by acting as a blue light filter, thereby decreasing photochemical light damage[7].

To date, conflicting results have been reported on the association between the intakes of antioxidants, such as lutein, vitamin C and β-carotene and AMD, with some demonstrating a possible inverse association have been reported[8–13], whereas other studies showed no clear association[14–19]. Moreover, the majority of these studies were conducted in the developed countries; to our knowledge, no data are available for the Chinese population. In addition, the evidence regarding the association between the predominant source of specific vitamins and carotenoids and AMD remains controversial[9,12,13,16,18,20,21]. Moreover, the co-consumption of various carotenoid-rich sources, such as raw vegetables, fruits and eggs, may potentially affect the absorption and bioavailability of carotenoids; however, few studies have examined the joint effects of the intakes of different sources of these nutrient on the risk of AMD.

Therefore, in this study, we investigated the association of vitamins and carotenoids, as well as their main dietary sources with the risk of AMD by conducting a population-based case–control study in the Chinese population.

Materials and methods

Participants

The Xi'an Eye Study is a multicenter, randomised, controlled, parallel group clinical trial designed to evaluate the effects of oral supplementation with macular xanthophylls alone or in combination with fish on the primary and secondary prevention of AMD (ChiCTR1900028680). Potential participants were recruited from clinics and health fairs between November 2016 and December 2019 by posting advertisements and flyers. A validated questionnaire was used to obtain detailed medical history, lifestyle, usual diet and other health-related information after obtaining written informed consent from each participant. Thorough ophthalmologic examinations, including the best-corrected visual acuity, intraocular pressure, slit lamp inspection, optical coherence tomography, fundus photography and fundus autofluorescence, were performed by ophthalmologists using a standardised protocol for all participants. Overnight blood samples were drawn at the same time as the dietary information was collected.

Participants aged ≥ 45 years with available data on dietary carotenoids and vitamins at baseline were included in the present investigation. Patients were eligible for inclusion in the analysis if they were first clinically diagnosed with early AMD (soft drusen and/or pigmentary abnormalities) or late AMD (geographic atrophy or signs of exudative AMD degeneration) by eye specialists according to the Age-Related Eye Disease Study classification system[22]. Patients with a prior diagnosis of high myopia, glaucoma and clinically significant diabetic retinopathy or any other ocular disease that might affect central or parafoveal macular visual function were ineligible. In addition, those who had undergone recent cataract surgery or other intraocular surgery (within six months), followed special diets (such as vegetarians), had an unstable chronic illness or were taking photosensitising drugs (such as phenothiazines and chloroquine) were also excluded.

Using the same exclusion criteria, the same number of controls, who were matched for age and sex, were randomly selected during the same period from those without clinical signs of AMD. After applying the exclusion criteria, 260 controls and 260 cases remained for the current analysis.

The study adhered to the Declaration of Helsinki guidelines and was approved by the medical ethics committee of the Xi'an Jiaotong University Health Science Center. All participants provided written informed consent before participating in the study.

Dietary assessment

A validated semi-quantitative FFQ with eighty-six items was used to collect the dietary information. Participants were asked about the portion size and how many times, on average, they had consumed each food throughout the previous year, with five possible responses (per day, week, month, year or never). A colour food photography atlas with different portion sizes for all food items was used to produce a more accurate estimate. Nutrient and energy intakes were calculated by converting the frequency of consumption and portion size estimates to daily intake, then multiplying the daily intake with the corresponding nutrient contribution of a standard portion (100 g) for each item, summed across all foods consumed. The nutrient composition values were primarily based on the food composition tables from the China Food Composition Database and other published sources (such as scientific literature from local researchers)[23].

The FFQ used in the present analysis was validated to three consecutive 24-h dietary recalls in a subsample of participants (n 31). The value of the Spearman correlation coefficient was 0·37 ($P = 0·05$) for lutein, 0·42 ($P = 0·02$) for α-carotene, 0·32 ($P = 0·07$) for β-carotene, 0·34 ($P = 0·06$) for β-cryptoxanthin, 0·79 ($P < 0·001$) for retinol and 0·36 ($P = 0·05$) for vitamin C.

Assessment of covariates

We collected information on medical, lifestyle and other health-related factors, such as body weight, socioeconomic status, smoking status, alcohol consumption, sunlight exposure, physical activity, medication or supplement use, family history of major chronic conditions and personal history of chronic diseases. BMI was calculated as the ratio of weight in kilograms to the square of the height in metres.

Statistical analysis

For those with questionnaires with missing information for individual food data when aggregating items to calculate the composite items, we assumed that they did not consume that food[24]. Participants with incomplete dietary data (blank responses for more than ten items) or implausible reported dietary assessments (i.e. < 800 or > 6000 kcal/d for men and < 600 or > 4000 kcal/d for women) were excluded. Exposure variables were adjusted for total energy intake using
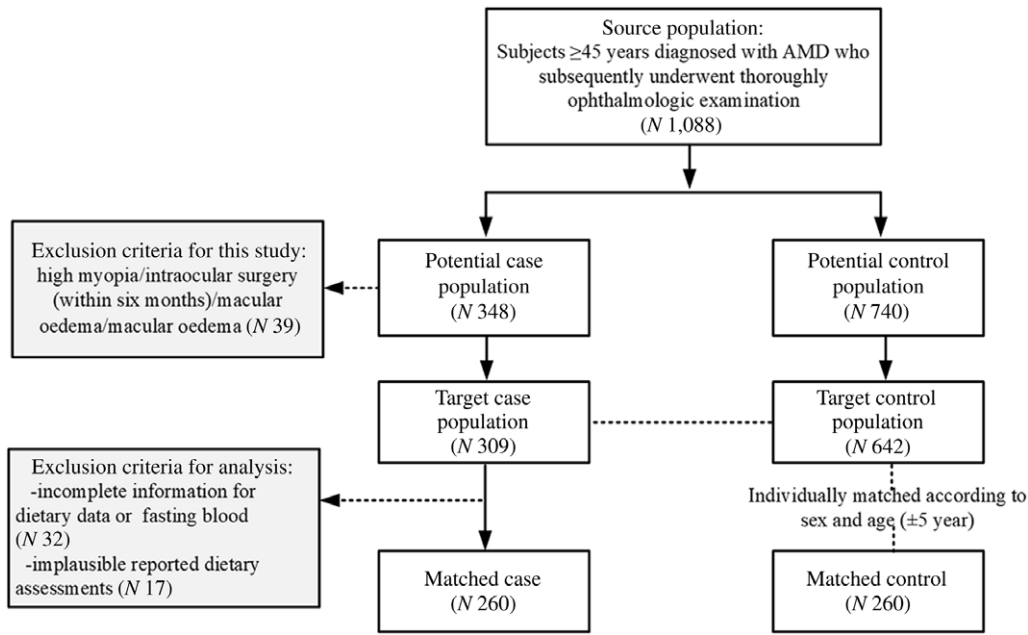
Fig. 1. Flow diagram of selection of the study sample in the matched case–control study.

the density method (intake per 1000 kcal). The participants were divided into quartiles according to the average vitamin or carotenoid intake, with the lowest quantile treated as the reference group. The category-specific OR and 95 % CI were estimated using conditional logistic regression analysis. In the multivariate analysis, we adjusted for smoking status (never, past or current smoker), educational level (less than college or college), regular physical activity (yes or no), total energy intake (continuous), blood cholesterol (continuous), HDL-cholesterol (continuous) and LDL-cholesterol (continuous). Trend analyses across increasing quartiles of nutrient intake were conducted by assigning a median value for each dietary exposure variable category, which was treated as a continuous variable in the regression models. In addition, restricted cubic spline regression with three knots was used to assess the non-linear dose–response relationship.

The primary exposures for this analysis were lutein, α-carotene, β-carotene, β-cryptoxanthin, retinol and vitamin C. Because significant results were observed in the primary analysis of xanthophyll, we further explored the consumption of foods rich in lutein to provide more insight into the primary results. In addition, we evaluated the joint associations of different vitamin- and carotenoid-rich foods (three categories) with the AMD risk. All statistical analyses were performed using Stata 12.0 (StataCorp), and a two-sided $P < 0.05$ was considered statistically significant.

Results

In total, 260 AMD cases and an equal number of disease-free controls were included in the current analysis (Fig. 1). The characteristics of the patients with AMD and controls included in this study are presented in Table 1. Compared with non-AMD controls, the AMD patients tended to have a lower educational status, were more likely to smoke and drink alcohol and were less likely to engage in regular exercise.

Table 1. Baseline characteristics of the study sample*
(Number and percentages; mean values and standard deviations)

Baseline characteristics	Controls (n 260)		Cases (n 260)	
	n	%	n	%
Age, year				
Mean	65·45		65·54	
SD	8·01		8·17	
Male, n (%)	42·19		42·19	
Cigarette smoking (%)				
Never	79·69		70·70	
Past	14·45		16·80	
Current	5·86		12·50	
Alcohol consumption (%)	11·33		14·45	
Education level (%)				
Less than college	35·94		57·42	
College	64·06		42·58	
Regular physical activity (%)	77·73		55·86	
Light exposure (h/w)				
<6	96·88		94·14	
6–12	3·13		5·86	
Sunlight protective gear use (%)				
Antioxidant use (%)	42·19		41·80	
BMI (kg/m²)				
Mean	23·79		24·13	
SD	2·98		2·55	
History of hypertension (%)	41·41		35·16	
History of diabetes (%)	13·28		17·19	
History of coronary heart disease (%)	13·28		10·16	
Parental history of hypertension (%)	46·18		35·94	
Parental history of diabetes (%)	20·31		14·84	
Parental history of CHD (%)	25·00		23·05	

* Categorical variables are expressed as *n* (%); the continuous variables are expressed as the mean SD values.

Table 2. Odds ratio (95 % confidence intervals) of age-related macular degeneration according to quartiles of nutrient intake
(Odd ratio and 95 % confidence intervals)

| | | Quartile of dietary intake | | | | | | | |
| | | 2 | | 3 | | 4 | | |
Nutrient	1*(ref)	Odds ratio	95 % CI	Odds ratio	95 % CI	Odds ratio	95 % CI	P_{trend}†
Lutein								
No. of cases/controls	100/65	59/65		46/64		55/66		
Median lutein intake (μg/1000 kcal)	722·402	1403·71		2062·14		3599·44		
Model 1‡	1	0·47	0·28, 0·80	0·34	0·19, 0·61	0·36	0·18, 0·69	0·002
Model 2§	1	0·44	0·18, 1·09	0·39	0·15, 1·00	0·30	0·10, 0·88	0·040
Alpha-carotene								
No. of cases/controls	80/65	67/65		61/65		52/65		
Median, μg /d (case/control)	107·77	342·13		660·42		1490·65		
Model 1‡	1	0·81	0·50, 1·29	0·74	0·46, 1·21	0·61	0·36, 1·03	0·072
Model 2§	1	1·10	0·50, 2·40	1·05	0·44, 2·48	0·76	0·30, 1·97	0·497
Beta-carotene								
No. of cases/controls	77/65	81/65		50/65		52/65		
Median, μg /d (case/contro αl)	614·45	1271·86		2058·00		3877·87		
Model 1‡	1	0·98	0·61, 1·58	0·60	0·35, 1·02	0·56	0·31, 1·02	0·023
Model 2§	1	1·00	0·47, 2·09	0·56	0·25, 1·29	0·70	0·26, 1·87	0·404
Beta-cryptoxanthin								
No. of cases/controls	128/65	48/65		42/65		42/65		
Median, μg /d (case/control)	23·70	47·84		81·98		172·58		
Model 1‡	1	0·34	0·20, 0·58	0·28	0·16, 0·50	0·27	0·15, 0·48	<0·001
Model 2§	1	0·50	0·21, 1·20	0·55	0·21, 1·45	0·28	0·11, 0·74	0·019
Retinol								
No. of cases/controls	96/65	49/65		52/65		63/65		
Median, μg RE/d (case/control)	167·80	299·24		415·63		623·36		
Model 1‡	1	0·48	0·29, 0·81	0·49	0·29, 0·84	0·55	0·31, 0·97	0·053
Model 2§	1	0·71	0·32, 1·58	0·56	0·24, 1·29	0·54	0·22, 1·35	0·176
Vitamin C								
No. of cases/controls	91/65	60/65		52/65		57/65		
Median, mg /d (case/control)	32·69	53·86		75·51		111·23		
Model 1‡	1	0·61	0·38, 0·99	0·53	0·32, 0·89	0·55	0·32, 0·94	0·043
Model 2§	1	0·51	0·23, 1·15	0·65	0·28, 1·52	0·43	0·17, 1·08	0·105

* Reference group.
† $P_{for\ trend}$ over the quintile categories used the median for each quintile category as a continuous variable.
‡ Model 1: no adjustment.
§ Model 2: adjusted for smoking status, educational level, regular physical activity, dietary total energy intake, blood cholesterol, HDL-cholesterol and LDL-cholesterol.

Table 2 exhibits the associations between dietary vitamin and carotenoid intake and AMD risk. In the age- and sex-adjusted model, lutein intake was associated with a lower AMD risk. After adjusting for confounding variables in the multivariate model, an increasing lutein intake was inversely associated with the AMD risk, and the OR (95 %CI) across quartiles of dietary lutein intake were 1·0, 0·44 (95 % CI: 0·18, 1·09), 0·39 (95 % CI: 0·15, 1·00) and 0·30 (95 % CI: 0·10, 0·88) (P_{trend} = 0·04), respectively. A similar inverse association with AMD risk was observed for β-cryptoxanthin, with a multivariate adjusted OR (95 % CI) for the highest quartile of intake compared with the lowest quartile of 0·28 (95 %CI: 0·11, 0·74; P_{trend} = 0·02). In contrast, weak or null associations with AMD were observed for the dietary intake of other antioxidant nutrients. The risk of AMD decreased rapidly with increasing dietary lutein intake until approximately-2000 μg/1000 kcal per day (P for non-linearity < 0·01; online Supplemental Fig. 1). Likewise, a nonlinear association was also observed for β-cryptoxanthin in the spline analysis ($P_{for\ non-linearity}$ < 0·01; online Supplemental Fig. 2).

Given the vital role of lutein in maintaining a normal morphology and function of the retina and the strength of the association between this carotenoid and AMD risk, we also examined the risk of AMD by major food sources of lutein, including spinach, rapeseed, broccoli, pumpkin, carrot, sweet potatoes, citrus fruits and eggs (Table 3). Compared with the participants who consumed < 1 servings of spinach per week, those who consumed ≥ 2 servings of spinach per week had a 58 % (95 % CI: 12 %, 80 %; P_{trend} = 0·03) lower risk of AMD. In contrast to spinach, the inverse association between the intakes of other lutein-rich vegetables and AMD was weak and non-significant. When compared with the participants who ate ≤ 2 servings of egg per week, the multivariable pooled OR (95 % CI) of AMD was 0·52 (95 % CI:0·27, 0·98) for those with an intake of ≥ 1 serving egg per day (P_{trend} = 0·07). We observed a monotonically decreasing risk of AMD associated with increasing egg intake ($P_{for\ non-linearity}$ = 0·06, online Supplemental Fig. 3).

We further examined the joint association of egg and spinach intake with the risk of AMD. Participants who were in the highest category of both egg and spinach intake had the greatest reduction in AMD risk in comparison with those who were in the lowest category of consumption, and the OR was 0·23 (95 % CI: 0·08, 0·71; Fig. 2).

Table 3. Odds ratio (95 % confidence intervals) of age-related macular degeneration by frequency of consumption of foods rich in carotenoids

Food item	1*(ref)	2		3		P_{trend}†
		Frequency of consumption				
Spinach	≤3 servings/mo	1 serving/week		≥2 servings/week		
No. of cases/controls	139/108	49/61		72/91		
Model 1‡	1	0·56	0·35, 0·90	0·53	0·33, 0·83	0·012
Model 2§	1	0·62	0·29, 1·32	0·42	0·20, 0·88	0·026
Egg	≤2 servings/week	3–6 servings/week		≥7 servings/week		
No. of cases/controls	138/67	44/57		78/136		
Model 1‡	1	0·36	0·21, 0·62	0·30	0·19, 0·45	<0·001
Model 2§	1	0·42	0·18, 0·99	0·52	0·27, 0·98	0·070
Rapeseed	≤3 servings /mo	1–4 servings/week		≥5 servings/week		
No. of cases/controls	100/112	129/110		30/38		
Model 1‡	1	1·31	0·91, 1·89	0·90	0·50, 1·61	0·833
Model 2§	1	1·32	0·66, 2·64	0·93	0·34, 2·59	0·799
Watermelon	< 1 serving/mo	1–3 servings/mo		≥1 serving/week		
No. of cases/controls	177/162	52/60		31/38		
Model 1‡	1	0·82	0·53, 1·25	0·75	0·44, 1·27	0·265
Model 2§	1	0·83	0·42, 1·68	0·67	0·28, 1·59	0·562
Broccoli	≤3 serving/mo	1 serving/week		≥2 servings/week		
No. of cases/controls	157/127	43/67		60/66		
Model 1‡	1	0·54	0·34, 0·84	0·75	0·50, 1·15	0·160
Model 2§	1	0·51	0·25, 1·05	0·73	0·36, 1·46	0·818
Pumpkin	≤3 serving /mo	1–4 servings/week		≥5 servings/week		
No. of cases/controls	163/139	84/92		13/29		
Model 1‡	1	0·73	0·50, 1·08	0·30	0·13, 0·68	0·003
Model 2§	1	0·81	0·43, 1·53	0·45	0·14, 1·46	0·459
Carrot	≤3 serving /mo	1–4 servings/week		≥5 servings/week		
No. of cases/controls	126/105	109/117		25/38		
Model 1‡	1	0·78	0·53, 1·15	0·54	0·30, 0·97	0·039
Model 2§	1	0·90	0·50, 1·61	0·61	0·25, 1·50	0·944
Sweet potatoes	≤1 servings/week	2–4 servings/week		≥5 servings/week		
No. of cases/controls	177/153	66/65		17/42		
Model 1‡	1	0·86	0·57, 1·30	0·32	0·17, 0·62	0·001
Model 2§	1	1·34	0·68,2·66	0·37	0·14, 1·00	0·336
Citrus fruits	≤3 serving/mo	1–3 servings/week		≥4 servings/week		
No. of cases/controls	174/129	50/86		36/45		
Model 1‡	1	0·43	0·28, 0·66	0·55	0·32, 0·93	0·037
Model 2§	1	0·43	0·20, 0·96	0·58	0·25, 1·39	0·649

* Reference group.
† $P_{for\ trend}$ over the quintile categories used the median for each quintile category as a continuous variable.
‡ Model 1: no adjustment.
§ Model 2: adjusted for smoking status, educational level, regular physical activity, dietary total energy intake, blood cholesterol, HDL-cholesterol and LDL-cholesterol.
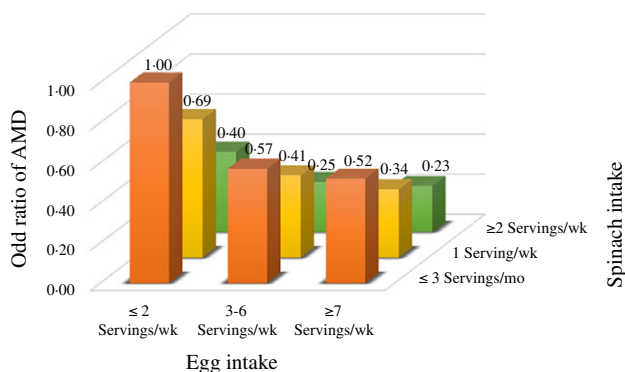
British Journal of Nutrition

Fig. 2. Joint association of spinach and egg intake with risk of age-related macular degeneration. OR were calculated in conditional logistic models after adjusting for smoking status, educational level, regular physical activity, dietary total energy intake, blood cholesterol, HDL-cholesterol and LDL-cholesterol.

Discussion

In the present study, a higher intake of dietary lutein and β-cryptoxanthin was associated with a lower risk of AMD. The consumption of lutein-rich foods, such as spinach or eggs, was also inversely associated with the risk of AMD. The decrease in the risk of AMD was more pronounced among participants who had a higher combined consumption of spinach and eggs. These findings provide further evidence for the benefits of lutein and lutein-rich foods in preventing of AMD.

To date, observational data related to specific dietary vitamin and carotenoid intake and AMD risk have been predominantly analyzed in Western populations. In the Nurses' Health Study and the Health Professionals Follow-up Study, Wu *et al.* reported that individuals with a higher intake of bioavailable lutein, β-cryptoxanthin, and α-carotene were had an approximately 30 %–40 % lower risk of advanced AMD[10]. Similarly, in a

population of 4519 men and women aged 60 to 80 years in the Age-Related Eye Disease Study, SanGiovanni *et al.* observed that lutein intake was inversely associated with the risk of advanced AMD and large or extensive intermediate drusen[22]. Our previous meta-analyses, which were mainly based on studies carried out in Western populations, also suggested that a high dietary intake of lutein yielded a 32 % reduction in the risk of late AMD[25]. In agreement with these findings, our results suggest that increasing the intakes of vitamins and carotenoids, especially lutein, was inversely associated with the risk of AMD. In addition, a similar inverse association between spinach consumption and lutein intake observed in our study supports the hypothesis that lutein in spinach may be responsible for the potential beneficial effect of spinach on AMD risk.

Several potential mechanisms for specific antioxidants constituents reducing biological pathways related to AMD development have been suggested, including the quenching of singlet oxygen, inhibition of lipid peroxidation and reduction of the inflammatory response[26]. The retina is particularly susceptible to oxidative damage due to its continuous intense exposure to short-wavelength visible light, in combination with its high concentration of oxygen and PUFA[27]. The uncontrolled oxidative injury could contribute to lipofuscin formation and induce a chain reaction of lipid peroxidation[28]. In addition to disruption of the structure of retina stability and integrity directly, the accumulation of lipofuscin could increase the susceptibility of retina components to phototoxicity, which has been implicated in the pathophysiology of AMD[29]. Vitamins and carotenoids can attenuate photo-oxidative injury through the scavenging of reactive oxygen species, thereby counteracting the process of complement activation and inflammation[30]. *In vitro* studies have also revealed that ARPE-19 cells with carotenoid supplementation exhibited increased viability and less accumulation of lipid hydroperoxides, indicating the importance of the carotenoids in the efficient protection against oxidative damage induced by photosensitised reactions[31]. Vitamins and carotenoids may be related to the capacity to modulate the expression of inflammation-related genes involved in chronic local inflammatory responses within the retina, which are believed to play a critical role in the pathophysiology of drusen[32,33]. Using a murine model of laser-induced choroidal neovascularisation, lutein supplementation led to substantial inhibition of macrophage infiltration into the retina choroidal neovascularisation area by suppressing tumour necrosis factor α-induced nuclear factor (NF)-kB activation and NF-kappaB p65 nuclear translocation *in vivo* and *in vitro*[33]. Leung *et al.* observed that carotenoids exhibited benefits in reducing the non-enzymatic oxidation of *n*-6 PUFA and appeared to regulate inflammatory lipid mediators[34]. In addition, the macular xanthophylls were uniquely concentrated at the macula, and the absorbance spectrum peak of these macular pigment coincides with the absorbance spectrum of short-wavelength visible light, which protects the macular region by filtering damaging blue light, thereby possibly attenuating photochemical light damage[35].

It should be noted that the inverse association was suggested to be stronger for the additive intakes of spinach and egg in the joint analysis. Although eggs contain markedly less lutein than dark-green leafy vegetables, lutein bioavailability from eggs is higher than that from other food sources, possibly owing to the presence of the lipid matrix in egg yolk[36]. In a crossover trial, Chung *et al.* observed that lutein-enriched egg consumption led to the highest serum lutein response compared with lutein supplements and spinach[37]. In addition, the influence of egg consumption on the incidence of AMD may be potentially mediated by other nutrients and compounds in the diet.

The egg yolk matrix, which has a high lipoprotein content, could increase the bioaccessibility and bioavailability of lutein[38]. It has been indicated that co-consuming eggs could enhance the absorption of total carotenoids from other carotenoid-rich foods such as raw vegetables[39]. From a public health prospective, as eggs are a low-cost, widely available and easily digestible source of many nutrients, it would feasible to encourage a moderate egg consumption, as a factor in the context of a healthy diet that can help to prevent AMD.

Our results must be interpreted within the context of several limitations. First, given the nature of case–control studies, our results cannot establish causality between the intakes of carotenoids and vitamins and the risk of AMD, as the possibility of unmeasured or residual confounding cannot be ruled out and these confounders could theoretically affect the observed associations. However, several established and potential risk factors for AMD were statistically controlled for the present study, which may minimise the potential impact of residual confounding to some extent. Second, although the FFQ used in the present analysis was validated against dietary records, the assessment of diet was still inevitably prone to measurement error. Moreover, individuals' eating patterns and food composition levels may change over time, so a single measurement may not fully represent the variability of carotenoids and vitamins in the development of AMD, which has a long aetiologic period. However, in addition to a reasonably high correlation between repeated measures on the same samples over a one-year period, individuals are less likely to switch their habitual diet or lifestyle as a result of the diagnosis because the relatively high quality of vision may mask the effect of AMD for many years. Third, nutrients in the diet may have additive or synergistic effects on the occurrence of AMD; therefore, we cannot rule out the potential synergistic effects of lutein and other dietary factors on AMD risk. In addition, detailed information regarding food storage conditions and cooking methods were not collected by the present FFQ, and this may introduce additional measurement errors. Therefore, the associations between circulating biomarker status and AMD risk among the same population should be considered in future research before considering the possible implications for public health. Finally, although the participants in the present study were recruited from different centers, the study population was only composed of residents with Chinese ancestry, which may reduce the generalisability of our results to the non-Chinese population.

In conclusion, the results of this study indicate that the dietary intake of vitamins and carotenoids, especially lutein, as well as its major food sources, is significantly and inversely associated with AMD risk, which supports the dietary recommendations to increase consumption of lutein-rich foods to facilitate the prevention of AMD. Along with our findings on the importance of increasing the bioaccessibility and bioavailability of lutein, the

joint beneficial effects of different lutein-rich foods on the risk of AMD warrant further investigation in future studies.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114522002161

References

1. Wong WL, Su X, Li X, *et al.* (2014) Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *Lancet Glob Health* **2**, 106–116.
2. Mehta H, Tufail A, Daien V, *et al.* (2018) Real-world outcomes in patients with neovascular age-related macular degeneration treated with intravitreal vascular endothelial growth factor inhibitors. *Prog Retin Eye Res* **65**, 127–146.
3. Abokyi S, To CH, Lam TT, *et al.* (2020) Central role of oxidative stress in age-related macular degeneration: evidence from a review of the molecular mechanisms and mnimal models. *Oxid Med Cell Longev* **2020**, 7901270.
4. Liu RT, Gao J, Cao S, *et al.* (2013) Inflammatory mediators induced by amyloid-beta in the retina and RPE *in vivo*: implications for inflammasome activation in age-related macular degeneration. *Invest Ophthalmol Vis Sci* **54**, 2225–2237.
5. Murugeswari P, Firoz A, Murali S, *et al.* (2020) Vitamin-D3 (α-1, 25(OH) 2D3) protects retinal pigment epithelium from hyperoxic insults. *Invest Ophthalmol Vis Sci* **61**, 4.
6. Kamoshita M, Toda E, Osada H, *et al.* (2016) Lutein acts via multiple antioxidant pathways in the photo-stressed retina. *Sci Rep* **6**, 30226.
7. Bernstein PS, Li B, Vachali PP, *et al.* (2016) Lutein, zeaxanthin, and meso-zeaxanthin: the basic and clinical science underlying carotenoid-based nutritional interventions against ocular disease. *Prog Retin Eye Res* **50**, 34–66.
8. Nidhi B, Mamatha BS, Padmaprabhu CA, *et al.* (2013) Dietary and lifestyle risk factors associated with age-related macular degeneration: a hospital based study. *Indian J Ophthalmol* **61**, 722–777.
9. Seddon JM, Ajani UA, Sperduto RD, *et al.* (1994) Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye disease case-control study group. *JAMA* **272**, 1413–1420.
10. Wu J, Cho E, Willett WC, *et al.* (2015) Intakes of lutein, zeaxanthin, and other carotenoids and age-related macular degeneration during 2 decades of prospective follow-up. *JAMA Ophthalmol* **133**, 1415–1424.
11. Aoki A, Inoue M, Nguyen E, *et al.* (2016) Dietary *n*-3 fatty acid, α-Tocopherol, zinc, vitamin D, vitamin C, and β-carotene are associated with age-related macular degeneration in Japan. *Sci Rep* **6**, 20723.
12. Kim EK, Kim H, Vijayakumar A, *et al.* (2017) Associations between fruit and vegetable, and antioxidant nutrient intake and age-related macular degeneration by smoking status in elderly Korean men. *Nutr J* **16**, 77.
13. Kim EK, Kim H, Kwon O, *et al.* (2018) Associations between fruits, vegetables, vitamin A, β-carotene and flavonol dietary intake, and age-related macular degeneration in elderly women in Korea: the Fifth Korea National Health and Nutrition Examination Survey. *Eur J Clin Nutr* **72**, 161–167.
14. Tan JS, Wang JJ, Flood V, *et al.* (2008) Dietary antioxidants and the long-term incidence of age-related macular degeneration: the Blue Mountains Eye Study. *Ophthalmology* **115**, 334–341.
15. van Leeuwen R, Boekhoorn S, Vingerling JR, *et al.* (2005) Dietary intake of antioxidants and risk of age-related macular degeneration. *JAMA* **294**, 3101–3107.
16. VandenLangenberg GM, Mares-Perlman JA, Klein R, *et al.* (1998) Associations between antioxidant and zinc intake and the 5-year incidence of early age-related maculopathy in the Beaver Dam Eye Study. *Am J Epidemiol* **148**, 204–214.
17. Flood V, Smith W, Wang JJ, *et al.* (2002) Dietary antioxidant intake and incidence of early age-related maculopathy: the Blue Mountains Eye Study. *Ophthalmology* **109**, 2272–2278.
18. Cho E, Seddon JM, Rosner B, *et al.* (2004) Prospective study of intake of fruits, vegetables, vitamins, and carotenoids and risk of age-related maculopathy. *Arch Ophthalmol* **122**, 883–892.
19. Morris MS, Jacques PF, Chylack LT, *et al.* (2007) Intake of zinc and antioxidant micronutrients and early age-related maculopathy lesions. *Ophthalmic Epidemiol* **14**, 288–298.
20. Moeller SM, Parekh N, Tinker L, *et al.* (2006) Associations between intermediate age-related macular degeneration and lutein and zeaxanthin in the Carotenoids in Age-related Eye Disease Study (CAREDS): ancillary study of the Women's Health Initiative. *Arch Ophthalmol* **124**, 1151–1162.
21. Smith W, Mitchell P, Webb K, *et al.* (1999) Dietary antioxidants and age-related maculopathy: the Blue Mountains Eye Study. *Ophthalmology* **106**, 761–767.
22. Age-Related Eye Disease Study Research Group (1999) The Age-Related Eye Disease Study (AREDS): design implications. AREDS report no. 1. *Control Clin Trials* **20**, 573–600.
23. Yang YX, Wang GY & Pan XC (2009) *China Food Composition*, 2nd ed. Beijing: Peking University Medical Press.
24. Joshipura KJ, Ascherio A, Manson JE, *et al.* (1999) Fruit and vegetable intake in relation to risk of ischemic stroke. *JAMA* **282**, 1233–1239.
25. Ma L, Dou HL, Wu YQ, *et al.* (2012) Lutein and zeaxanthin intake and the risk of age-related macular degeneration: a systematic review and meta-analysis. *Br J Nutr* **107**, 350–359.
26. Ozawa Y (2020) Oxidative stress in the light-exposed retina and its implication in age-related macular degeneration. *Redox Biol* **37**, 101779.

British Journal of Nutrition

27. Tao JX, Zhou WC & Zhu XG (2019) Mitochondria as potential targets and initiators of the blue light hazard to the retina. *Oxid Med Cell Longev* **2019**, 6435364.

28. Maeda A, Maeda T, Golczak M, *et al.* (2009) Involvement of all-trans-retinal in acute light-induced retinopathy of mice. *J Biol Chem* **284**, 15173–15183.

29. Fanjul-Moles ML & López-Riquelme GO (2016) Relationship between oxidative stress, circadian rhythms, and AMD. *Oxid Med Cell Longev* **2016**, 7420637.

30. Li SY, Fu ZJ & Lo AC (2012) Hypoxia-induced oxidative stress in ischemic retinopathy. *Oxid Med Cell Longev* **2012**, 426769.

31. Wrona M, Rózanowska M & Sarna T (2004) Zeaxanthin in combination with ascorbic acid or α-tocopherol protects ARPE-19 cells against photosensitized peroxidation of lipids. *Free Radic Biol Med* **36**, 1094–1101.

32. Bian Q, Gao S, Zhou J, *et al.* (2012) Lutein and zeaxanthin supplementation reduces photo-oxidative damage and modulates the expression of inflammation-related genes in retinal pigment epithelial cells. *Free Radic Biol Med* **53**, 1298–1307.

33. SanGiovanni JP & Neuringer M (2012) The putative role of lutein and zeaxanthin as protective agents against age-related macular degeneration: promise of molecular genetics for guiding mechanistic and translational research in the field. *Am J Clin Nutr* **96**, 1223S.

34. Leung HH, Galano JM, Crauste C, *et al.* (2020) Combination of lutein and zeaxanthin, and DHA regulated polyunsaturated fatty acid oxidation in H_2O_2-stressed retinal cells. *Neurochem Res* **45**, 1007–1019.

35. Barker FM, Snodderly DM, Johnson EJ, *et al.* (2011) Nutritional manipulation of primate retinas, V: effects of lutein, zeaxanthin, and *n*-3 fatty acids on retinal sensitivity to blue-light-induced damage. *Invest Ophthalmol Vis Sci* **52**, 3934–3942.

36. Battaglia Parodi M, Brunoro A, Tomasso L, *et al.* (2020) Benefits of micronutrient supplementation for reducing the risk of wet age-related macular disease and diabetic retinopathy: an update. *Eur J Ophthalmol* **30**, 780–794.

37. Chung HY, Rasmussen HM & Johnson EJ (2004) Lutein bioavailability is higher from lutein-enriched eggs than from supplements and spinach in men. *J Nutr* **134**, 1887–1893.

38. Kelly D, Nolan JM, Howard AN, *et al.* (2017) Serum and macular response to carotenoid-enriched egg supplementation in human subjects: the Egg Xanthophyll Intervention clinical Trial (EXIT). *Br J Nutr* **117**, 108–123.

39. Kim JE, Gordon SL, Ferruzzi MG, *et al.* (2015) Effects of egg consumption on carotenoid absorption from co-consumed, raw vegetables. *Am J Clin Nutr* **102**, 75–83.