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

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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 0.40 (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 0.27 (0.08, 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. 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. 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. 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. In particular, lutein and its structural isomer, zeaxanthin, which are concentrated in the macula, have also been

Abbreviation: AMD, age-related macular degeneration.

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suggested to play a role in protecting the macular region by acting as a blue light filter, thereby decreasing photochemical light damage\(^5\).

To date, conflicting results have been reported on the association between the intakes of antioxidants, such as lutein, vitamin C and \(\beta\)-carotene and AMD, with some demonstrating a possible inverse association have been reported\(^{96-13} \), 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 \(\geq 45\) 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 \(\alpha\)-carotene, 0.52 (\(P=0.07\)) for \(\beta\)-carotene, 0.34 (\(P=0.06\)) for \(\beta\)-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
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.
The spline analysis (morphology and function of the retina and the strength of the
Fig. 2).

In contrast, weak or null associations with AMD were observed for
respectively. A similar inverse association with AMD risk was
observed for
- cryptoxanthin, with a multivariate adjusted OR
\( P_{\text{trend}} = 0.01 \); online Supplemental Fig. 3). Likewise, a
non-linear association was also observed for \( \beta \)-cryptoxanthin in
the spline analysis (\( P_{\text{for non-linearity}} < 0.01 \); 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
tomatoes, citrus fruits and eggs (Table 3). Compared with the
participants who consumed < 1 servings of spinach per week,
those who consumed \( \geq 2 \) servings of spinach per week had a
58% (95% CI: 12%, 80%; \( P_{\text{trend}} = 0.03 \)) 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 \( \leq 2 \) 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 \( \geq 1 \) serving egg per day (\( P_{\text{trend}} = 0.07 \)).
We observed a monotonically decreasing risk of AMD associated
with increasing egg intake (\( P_{\text{for non-linearity}} = 0.06 \), 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 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 multivariable
model, an increasing lutein intake was inversely associated with
the AMD risk, and the OR (95% CI) across quartiles of dietary
intake with the risk of AMD. Participants who were in the highest
category of consumption, and the OR was 0.38 (95% CI: 0.19, 0.61).
A similar inverse association with AMD risk was
observed for
- cryptoxanthin, with a multivariate adjusted OR
\( P_{\text{trend}} = 0.04 \), respectively. A similar inverse association with AMD risk was
observed for \( \beta \)-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; \( P_{\text{trend}} = 0.02 \)). 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 \( \beta \)-cryptoxanthin in
the spline analysis (\( P_{\text{for non-linearity}} < 0.01 \); 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

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

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Quartile of dietary intake</th>
<th>1*(ref)</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Odds ratio</td>
<td>95% CI</td>
<td>Odds ratio</td>
<td>95% CI</td>
</tr>
<tr>
<td>Lutein</td>
<td></td>
<td>100/65</td>
<td>59/65</td>
<td>46/64</td>
<td>55/66</td>
</tr>
<tr>
<td>Median lutein intake (µg/1000 kcal)</td>
<td>722-402</td>
<td>1403-71</td>
<td>2062-14</td>
<td>3599-44</td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td></td>
<td>1</td>
<td>0.47</td>
<td>0.28, 0.80</td>
<td>0.34</td>
</tr>
<tr>
<td>Model 2‡</td>
<td></td>
<td>1</td>
<td>0.44</td>
<td>0.18, 1.09</td>
<td>0.39</td>
</tr>
<tr>
<td>Alpha-carotene</td>
<td></td>
<td>80/65</td>
<td>67/65</td>
<td>61/65</td>
<td>52/65</td>
</tr>
<tr>
<td>Median, µg/d (case/control)</td>
<td>107-77</td>
<td>342-13</td>
<td>660-42</td>
<td>1490-65</td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td></td>
<td>1</td>
<td>0.81</td>
<td>0.50, 1.29</td>
<td>0.74</td>
</tr>
<tr>
<td>Model 2‡</td>
<td></td>
<td>1</td>
<td>1.10</td>
<td>0.50, 2.40</td>
<td>1.05</td>
</tr>
<tr>
<td>Beta-carotene</td>
<td></td>
<td>77/65</td>
<td>81/65</td>
<td>50/65</td>
<td>52/65</td>
</tr>
<tr>
<td>Median, µg/d (case/control)</td>
<td>614-45</td>
<td>1271-66</td>
<td>2058-00</td>
<td>3877-87</td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td></td>
<td>1</td>
<td>0.98</td>
<td>0.61, 1.58</td>
<td>0.60</td>
</tr>
<tr>
<td>Model 2‡</td>
<td></td>
<td>1</td>
<td>1.00</td>
<td>0.47, 2.09</td>
<td>0.56</td>
</tr>
<tr>
<td>Beta-cryptoxanthin</td>
<td></td>
<td>128/65</td>
<td>48/65</td>
<td>42/65</td>
<td>42/65</td>
</tr>
<tr>
<td>Median, µg/d (case/control)</td>
<td>23-70</td>
<td>47-84</td>
<td>81-98</td>
<td>172-58</td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td></td>
<td>1</td>
<td>0.34</td>
<td>0.20, 0.58</td>
<td>0.28</td>
</tr>
<tr>
<td>Model 2‡</td>
<td></td>
<td>1</td>
<td>0.50</td>
<td>0.21, 1.20</td>
<td>0.55</td>
</tr>
<tr>
<td>Retinol</td>
<td></td>
<td>96/65</td>
<td>49/65</td>
<td>52/65</td>
<td>63/65</td>
</tr>
<tr>
<td>Median, µg RE/d (case/control)</td>
<td>1657-90</td>
<td>299-24</td>
<td>415-63</td>
<td>623-36</td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td></td>
<td>1</td>
<td>0.48</td>
<td>0.29, 0.81</td>
<td>0.49</td>
</tr>
<tr>
<td>Model 2‡</td>
<td></td>
<td>1</td>
<td>0.71</td>
<td>0.32, 1.58</td>
<td>0.56</td>
</tr>
<tr>
<td>Vitamin C</td>
<td></td>
<td>91/65</td>
<td>60/65</td>
<td>52/65</td>
<td>57/85</td>
</tr>
<tr>
<td>Median, mg/d (case/control)</td>
<td>32-69</td>
<td>50-46</td>
<td>75-11</td>
<td>110-6</td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td></td>
<td>1</td>
<td>0.81</td>
<td>0.38, 0.99</td>
<td>0.53</td>
</tr>
<tr>
<td>Model 2‡</td>
<td></td>
<td>1</td>
<td>0.51</td>
<td>0.23, 1.15</td>
<td>0.65</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Food item</th>
<th>Frequency of consumption</th>
<th>1*(ref)</th>
<th>2</th>
<th>3</th>
<th>( P_{\text{trend}} )‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spinach</td>
<td>≤3 servings/mo</td>
<td>1 serving/week</td>
<td>≥2 servings/week</td>
<td>139/108</td>
<td>49/61</td>
</tr>
<tr>
<td></td>
<td>No. of cases/controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1</td>
<td>0.56</td>
<td>0.35, 0.90</td>
<td>0.53</td>
<td>0.33, 0.83</td>
</tr>
<tr>
<td>Model 2§</td>
<td>1</td>
<td>0.62</td>
<td>0.29, 1.32</td>
<td>0.42</td>
<td>0.20, 0.88</td>
</tr>
<tr>
<td>Egg</td>
<td>≤2 servings/week</td>
<td>3–6 servings/week</td>
<td>≥7 servings/week</td>
<td>138/67</td>
<td>44/57</td>
</tr>
<tr>
<td></td>
<td>No. of cases/controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1</td>
<td>0.36</td>
<td>0.21, 0.62</td>
<td>0.30</td>
<td>0.19, 0.45</td>
</tr>
<tr>
<td>Model 2§</td>
<td>1</td>
<td>0.42</td>
<td>0.18, 0.99</td>
<td>0.52</td>
<td>0.27, 0.98</td>
</tr>
<tr>
<td>Rapeseed</td>
<td>≤3 servings/mo</td>
<td>1–4 servings/week</td>
<td>≥5 servings/week</td>
<td>100/112</td>
<td>129/110</td>
</tr>
<tr>
<td></td>
<td>No. of cases/controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Watermelon</td>
<td>&lt; 1 serving/mo</td>
<td>1–3 servings/mo</td>
<td>≥1 serving/week</td>
<td>177/162</td>
<td>52/60</td>
</tr>
<tr>
<td></td>
<td>No. of cases/controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1</td>
<td>1.31</td>
<td>0.91, 1.89</td>
<td>0.90</td>
<td>0.50, 1.61</td>
</tr>
<tr>
<td>Model 2§</td>
<td>1</td>
<td>1.32</td>
<td>0.66, 2.64</td>
<td>0.93</td>
<td>0.34, 2.59</td>
</tr>
<tr>
<td>Broccoli</td>
<td>≤3 serving/mo</td>
<td>1 serving/week</td>
<td>≥2 servings/week</td>
<td>157/127</td>
<td>43/67</td>
</tr>
<tr>
<td></td>
<td>No. of cases/controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1</td>
<td>0.54</td>
<td>0.34, 0.84</td>
<td>0.75</td>
<td>0.50, 1.15</td>
</tr>
<tr>
<td>Model 2§</td>
<td>1</td>
<td>0.51</td>
<td>0.25, 1.05</td>
<td>0.73</td>
<td>0.36, 1.46</td>
</tr>
<tr>
<td>Pumpkin</td>
<td>≤3 serving/mo</td>
<td>1–4 servings/week</td>
<td>≥5 servings/week</td>
<td>163/139</td>
<td>84/92</td>
</tr>
<tr>
<td></td>
<td>No. of cases/controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1</td>
<td>0.73</td>
<td>0.50, 1.08</td>
<td>0.30</td>
<td>0.13, 0.68</td>
</tr>
<tr>
<td>Model 2§</td>
<td>1</td>
<td>0.81</td>
<td>0.43, 1.53</td>
<td>0.45</td>
<td>0.14, 1.46</td>
</tr>
<tr>
<td>Carrot</td>
<td>≤3 serving/mo</td>
<td>1–4 serving/week</td>
<td>≥5 servings/week</td>
<td>126/105</td>
<td>109/117</td>
</tr>
<tr>
<td></td>
<td>No. of cases/controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1</td>
<td>0.78</td>
<td>0.53, 1.15</td>
<td>0.54</td>
<td>0.30, 0.97</td>
</tr>
<tr>
<td>Model 2§</td>
<td>1</td>
<td>0.90</td>
<td>0.50, 1.61</td>
<td>0.61</td>
<td>0.25, 1.50</td>
</tr>
<tr>
<td>Sweet potatoes</td>
<td>≤1 serving/week</td>
<td>2–4 servings/week</td>
<td>≥5 servings/week</td>
<td>177/153</td>
<td>66/65</td>
</tr>
<tr>
<td></td>
<td>No. of cases/controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1</td>
<td>0.86</td>
<td>0.57, 1.30</td>
<td>0.32</td>
<td>0.17, 0.62</td>
</tr>
<tr>
<td>Model 2§</td>
<td>1</td>
<td>1.34</td>
<td>0.68, 2.66</td>
<td>0.37</td>
<td>0.14, 1.00</td>
</tr>
<tr>
<td>Citrus fruits</td>
<td>≤3 serving/mo</td>
<td>1–3 serving/week</td>
<td>≥4 servings/week</td>
<td>174/129</td>
<td>50/86</td>
</tr>
<tr>
<td></td>
<td>No. of cases/controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1</td>
<td>0.43</td>
<td>0.28, 0.66</td>
<td>0.55</td>
<td>0.32, 0.93</td>
</tr>
<tr>
<td>Model 2§</td>
<td>1</td>
<td>0.43</td>
<td>0.20, 0.96</td>
<td>0.58</td>
<td>0.25, 1.39</td>
</tr>
</tbody>
</table>

* Reference group.
† \( P_{\text{trend}} \) 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.

Discussion

In the present study, a higher intake of dietary lutein and \( \beta \)-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 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, \( \beta \)-cryptoxanthin, and \( \alpha \)-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(23). 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(24). 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 PUFAs(25). The uncontrolled oxidative injury could contribute to lipofuscin formation and induce a chain reaction of lipid peroxidation(26). 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(27). Vitamins and carotenoids can attenuate photo-oxidative injury through the scavenging of reactive oxygen species, thereby counteracting the process of complement activation and inflammation(28). In vitro studies have also revealed that ARPE-19 cells with carotenoid supplementation increased viability and decreased accumulation of lipid hydroperoxides, indicating the importance of the carotenoids in the efficient protection against oxidative damage induced by photosensitised reactions(29). 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(30). 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)-κB activation and NF-kappaB p65 nuclear translocation in vitro and in vivo(31). 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(32). 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(33).

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(34). 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(35). 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(36). It has been indicated that co-consuming eggs could enhance the absorption of total carotenoids from other carotenoid-rich foods such as raw vegetables(37). 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 aetiological 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.

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Supplementary material

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