RESEARCH ARTICLE

Carotenoid Intake and Esophageal Cancer Risk: a Meta-analysis

Xiao-Xiao Ge[1], Mei-Yuan Xing[2], Lan-Fang Yu[1], Peng Shen[1]*

Abstract

This meta-analysis was conducted to evaluate the association between intake of carotenoids and risk of esophageal cancer. A systematic search using PubMed, Cochrane Library, Web of Science, Scopus, CNKI, and CBM (updated to 6 May 2012) identified ten articles meeting the inclusion criteria with 1,958 cases of esophageal cancer and 4,529 controls. Higher intake of beta-carotene, alpha-carotene, lycopene, beta-cryptoxanthin, lutein, and zeaxanthin reduced esophageal cancer risk with pooled ORs of 0.58 (95% CI 0.44, 0.77), 0.81 (95% CI 0.70, 0.94), 0.75 (95% CI 0.64, 0.86), 0.80 (95% CI 0.66, 0.97), and 0.71 (95% CI 0.59, 0.87), respectively. In subgroup analyses, beta-carotene showed protective effects against esophageal adenocarcinoma in studies located in Europe and North America. Alpha-carotene, lycopene, and beta-cryptoxanthin showed protection against esophageal squamous cell cancer. This meta-analysis suggested that higher intake of carotenoids (beta-carotene, alpha-carotene, lycopene, beta-cryptoxanthin, lutein, and zeaxanthin) is associated with lower risk of esophageal cancer. Further research with large-sample studies need to be conducted to better clarify the potentially protective mechanisms of carotenoid associations risk of different types of esophageal cancer.

Keywords: Carotenoids (lycopene, b-carotene) - esophageal cancer - cancer risk - meta-analysis

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Introduction

Esophageal cancer is the eighth most common cancer worldwide (Kamangar et al., 2006). There are 17460 new cases of esophageal cancer in USA and 15070 cases are estimated to die in 2012 (Siegel et al., 2012). The 5-year mortality of esophageal cancer exceeds 85% to 90% (Ilson, 2008). Tobacco and alcohol has been established as strong risk factors for esophageal squamous cell carcinoma (Lagergren et al., 2000; Freedman et al., 2007), and obesity and tobacco are well-established risk factors for esophageal adenocarcinoma (Vaughan et al., 1995; Chow et al., 1998; Engel et al., 2003). Genetic factor was suggested to be associated with susceptibility of esophageal cancer in a recent study (Liu et al., 2011). Intake of lamb meat, fried red meat, barbecued red meat, boiled red meat, and salted meat (De Stefani et al., 2012), and micronutrient deficiencies due to low intake of fruit and vegetables appear related to the risk of esophageal cancers in some parts of the world (Glade, 1999).

There has been argument that diet be one potential modifiable protective factor against cancers. Some epidemiologic studies showed that higher intake of fruit and vegetables was associated with a lower cancer incidence (Weisburger, 1991; Soerjomataram et al., 2010; Hajizadeh et al., 2011). It is probable that some dietary micronutrients, or rather antioxidants, may reduce the effects of environmental carcinogens (Glade, 1999), or may reduce tissue DNA damage by scavenging reactive oxygen species (Clarkson et al., 2000). Carotenoids, a class of yellow, orange, or red pigments, are widespread distributed in diet, especially in fruit and vegetables. There are two major groups: the hydrocarbon class, such as beta-carotene, also known as carotenes, and the oxygenated class, or xanthophylls, such as zeaxanthin (von Lintig, 2010). In human cells carotenoids act as oxidants, which are capable to scavenge free radical and prevent oxidative damage (Demmig-Adams et al., 2002) and improve cell antioxidant protection (Porrini et al., 2005). Whether carotenoids could reduce the risk of esophageal cancer has been discussed in many studies (Ziegler et al., 1981; Negri et al., 1992; De Stefani et al., 2000; Islami et al., 2009). But the conclusions were not consistent. Meta-analysis published was focused on the association between antioxidants and adenocarcinoma of esophageal and gastric cardia, in which esophageal squamous cell cancer or separate nutrient of carotenoids was not involved (Kubo et al., 2007). Here a meta-analysis is conducted to detect whether carotenoids (beta-carotene, alpha-carotene, lycopene, beta-cryptoxanthin, lutein, and zeaxanthin) intake would reduce the risk of esophageal cancer.

Materials and Methods

Search strategy

We performed a systematic search for literature

*[1]Department of Medical Oncology, [2]Department of Library, the First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang, China *For correspondence: zyhlk@sina.cn, Shenp@zju.edu.cn*

published updated to 6 May 2012 using PubMed, Cochrane Library, Web of Science, and Scopus in English, CNKI and CBM in Chinese.

First of all, articles about esophageal cancer were searched. The medical subject headings (MeSH) "Esophageal Neoplasms" were performed. We also used the terms "esophag*" and the British spelling form "oesophag*" with combination of any of the following terms: "cancer", "tumor", "carcinoma", "neoplasm", and "malignancy". Besides, such expression as "cancer of esophagus" was included.

During the next part, we searched for the articles about carotenoids, including alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein, zeaxanthin, lycopene, and other compounds in diet discussed by other researchers (Krinsky, 1993; Castenmiller et al., 1998; von Lintig, 2010; Hu et al., 2011). The MeSH of "carotenoids", "xanthophylls", and the six nutrients were performed if they occurred in the catalog. The terms "carotene", "carotenoid", "xanthophyll", and their plural forms were used as text words for complement. Then the terms of the relative substances were used. Aliases were all included, such as "all-trans-beta-carotene" and "β, β-carotene" for "beta-carotene". Moreover, variant forms of expression were all performed, such as the ones with or without hyphen or space between two adjacent words, the Greek or English letter instead of "beta", and the expression with or without quotation marks.

In the end we combined the two parts above to get the primary list of relevant articles. References of selected articles and "related articles" showed in databases at search were also marked to review.

Criteria for inclusion and exclusion

Articles about the association between carotenoids and esophageal cancer were included if they met all of the following criteria: (a) reported occurrence of esophagus cancer not combined with the ones of esophagogatric junction or other sites; (b) evaluated exposure of carotenoids intake rather than serum or plasm levels; (c) sufficient information provided to estimate the relative risk (RR) or odds ratio (OR) and 95% confidence intervals (CI) comparing the highest intake with the lowest, and (d) not be related with one another. The inclusion criteria were not restricted by study size, study design, population ethnicity, or publication language. For articles with same population resources or overlapping datasets, only the largest or most recent one was included.

Data extraction

Two reviewers independently assessed the articles for compliance with the inclusion criteria and extracted data using a standardized data extraction form. The discrepancy was discussed when a third reviewer or more joined to resolved disagreements until consensus achieved. Information extracted from each article included first author, publication year, location of the study, study design, number of cases and controls, sex, pathological diagnosis of cases, types of carotenoids intake, OR or RR, corresponding 95% CI for intake including the highest level versus the lowest, and potential confounding factors used for adjustment. Considering that esophageal cancer is a rare disease, the OR was assumed approximately the same as RR. For studies that reported several multivariable-adjusted ORs/RRs, we extracted the effect size (ES) that was most fully adjusted for potential confounding factors. Data from different studies in the same article without overlapping population were extracted separately.

Statistical Analysis

All analysis were conducted with the STATA statistical package (version 11, StataCorp LP, College Station, Texas, USA). The association between carotenoids intake and the risk of esophageal cancer was estimated by calculating pooled OR and 95% CI for comparing the highest with the lowest intake. The significant α level of 0.05 was used. Subgroup analyses were performed by pathological diagnosis, location, published year, and sex. I^2 score (Higgins et al., 2003) and Q test (DerSimonian et al., 1986) were adopted to assess heterogeneity among studies. I^2 values ranged between 0% and 100% and high values would show increasing heterogeneity. I^2 values of <25% were considered to be low, and values of <50% were considered to be moderate (Higgins et al., 2002; Higgins et al., 2003). In Q test, statistical significance was considered while $P<0.05$. Fixed effect model with Mantel-Haenszel was used to calculate the pooled OR when heterogeneity was not an issue. Otherwise, a random effect model was used (Fleiss, 1993). Sensitivity analyses was performed to evaluate whether the removal of one study at a time would influence the results and whether the category levels would influence the results. Publication bias was assessed by Egger's test (Egger et al., 1997) and Begg's test (Begg et al., 1994) ($P<0.05$ was considered statistically significant).

Results

Characteristics of included studies

The process of identifying related articles was illustrated in Figure 1. After exclusion by two reviewers independently, ten articles (Decarli et al., 1987; Hu et al., 1994; Terry et al., 2000; Franceschi et al., 2000; Mayne et al., 2001; Bollschweiler et al., 2002; Chen et al., 2002; De Stefani et al., 2006; Wright et al., 2007; Jessri et al., 2011) including a total of 1958 cases of esophageal cancer and 4529 controls fulfilled our inclusion criteria. The

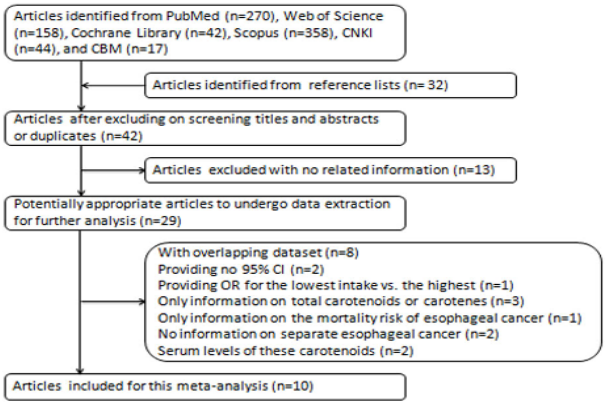

Figure 1. Flow Diagram of Article Identifying Process

Table 1. Characteristics of the Included Studies of Carotenoids Intake and Risk of Esophageal Cancer

Reference	Location	Year	Design	Number of cases (pathological type)	Number of controls	Carotenoids involved	Adjusted confounding factors[a]	Carotenoids intake assessment
Decarli et al. (1987)	Italy	1987	Hospital based case-control	105	348	Beta-carotene	a, b, c, d, e, g, s, u	Questionnaire
Hu et al. (1994)	China	1994	Hospital based case-control	196	392	Beta-carotene	c, d, i, q, x	Interview
Terry et al. (2000)	Sweden	2000	Populaion based case-control	350 (Adenocarcinoma 185, Squamous cell carcinoma 165)	815	Beta-carotene	a, b, c, s, u	Questionnaire
Franceschi et al. (2000)	Italy	2000	Hospital based case-control	304 (Squamous cell carcinoma)	743	Beta-carotene, Alpha-carotene, Lycopene, Beta-cryptoxanthin, Lutein and Zeaxanthin	a, b, c, d, e, f, m, s, w	Questionnaire
Mayne et al. (2001)	USA	2001	Populaion based case-control	488 (Adenocarcinoma 282, Squamous cell carcinoma 206)	687	Beta-carotene	a, b, c+f, d, e, g, i, m, r, s, t	Questionnaire
Bollschweiler et al. (2002)	Germany	2002	Hospital-population based case-control	99 (Adenocarcinoma 47, Squamous cell carcinoma 52)	50	Beta-carotene	c, d	Questionnaire
Chen et al. (2002)	USA	2002	Populaion based case-control	124 (Adenocarcinoma)	449	Beta-carotene, Alpha-carotene, Lycopene, Beta-cryptoxanthin, Lutein and Zeaxanthin	a, a*a, b, c, d, e, h, n, s, t, v	Questionnaire
De Stefani et al. (2006)	Uruguay	2006	Hospital based case-control	234 (Squamous cell carcinoma)	936	Beta-carotene, Alpha-carotene, Lycopene, Beta-cryptoxanthin	a, b, c, d, f, k, l, j, o, p, s	Questionnaire
Wright et al. (2007)	Finland	2007	Prospective cohort	11	13	Beta-carotene	a, c, e, z	Daily suppliment from researchers
Jessri et al. (2011)	Iran	2011	Hospital based case-control	47 (Squamous cell carcinoma)	96	Beta-carotene	a, b, c, e, f, s, t, w, y	Questionnaire

[a]The confounding factors involved in the articles: a, age; a*a, square of age; b, bmi; c, smoking; d, alcohol; e, education; f, energy; g, social status; h, history of family; i, income; j, years since stopping smoking; k, all dietary constituents; l, urban or rural status; m, city site; n, respondent type; o, current number of cigarettes per day; p, cigarette mate consumption; q, occupation; r, race; s, sex; t, micronutrients or mineral intake; u, dietary vitamin; v, vitamin supplement use; w, physical activity; x, with energy and nutrients; y, gastroesophageal reflux disease; z, dietary nitrate inside

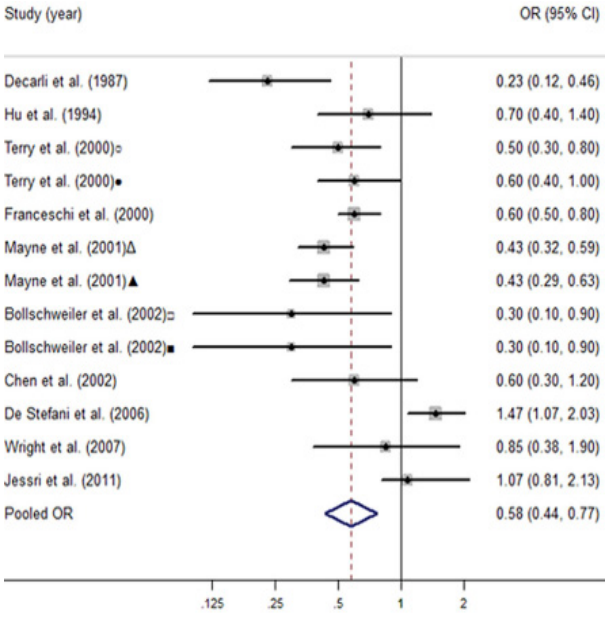

Figure 2. Forest Plot of OR and 95% CI for Highest Versus Lowest Category of Beta-carotene Intake and Risk of Esophageal Cancer. O The study of beta-carotene intake and risk of esophageal adenocarcinoma by Terry et al. (2000). ● The study of beta-carotene intake and risk of esophageal squamous cell carcinoma by Terry et al. (2000). Δ The study of beta-carotene intake and risk of esophageal adenocarcinoma by Mayne et al. (2001). ▲ The study of beta-carotene intake and risk of esophageal squamous cell carcinoma by Mayne et al. (2001). □ The study of beta-carotene intake and risk of esophageal adenocarcinoma by Bollschweiler et al. (2002). ■ The study of beta-carotene intake and risk of esophageal squamous cell carcinoma by Bollschweiler et al. (2002)

characteristics of the articles were shown in Table 1. There were 24 studies involved, none of which was randomized controlled trial (RCT). Of all the ten articles, five were conducted in Europe (Decarli et al., 1987; Franceschi et al., 2000; Terry et al., 2000; Bollschweiler et al., 2002; Wright et al., 2007), two in Asia (Hu et al., 1994; Jessri et al., 2011), two in North America (Mayne et al., 2001; Chen et al., 2002), and one in South America (De Stefani et al., 2006). Seven articles (Decarli et al., 1987; Hu et al., 1994; Terry et al., 2000; Mayne et al., 2001; Bollschweiler et al., 2002; Wright et al., 2007; Jessri et al., 2011) consisted of studies on beta-carotene only, one (De Stefani et al., 2006) consisted of beta-carotene, alpha-carotene, lycopene, and beta-cryptoxanthin, and two (Franceschi et al., 2000; Chen et al., 2002) consisted of beta-carotene, alpha-carotene, lycopene, beta-cryptoxanthin, lutein, and zeaxanthin. There were five articles on hospital based case-control studies (Decarli et al., 1987; Hu et al., 1994; Franceschi et al., 2000; De Stefani et al., 2006; Jessri et al., 2011), three on population based case-control studies (Terry et al., 2000; Mayne et al., 2001; Chen et al., 2002), one on hospital-population based case-control studies (Bollschweiler et al., 2002), and one article on a cohort study (Wright et al., 2007). Three articles provided data on esophageal squamous cell carcinoma only (Franceschi et al., 2000; De Stefani et al., 2006; Jessri et al., 2011), one on esophageal adenocarcinoma only (Chen et al., 2002), three on both pathological types with separate data from separate studies (Terry et al., 2000; Mayne et al., 2001; Bollschweiler et al., 2002), and three on both types within the same study (Decarli et al., 1987; Hu et al., 1994; Wright et al., 2007). Most of the studies provided OR adjusted for cigarette, alcohol, and body mass index (BMI). There was one article without adjustment for cigarette (Mayne et al., 2001), three without alcohol (Terry et al., 2000; Wright et al., 2007; Jessri et al., 2011), and three without BMI (Hu et al., 1994; Bollschweiler et al., 2002; Wright et al., 2007), all of which were on beta-carotene only. Subgroup analyses were performed among different confounding factors involved. Two articles (Bollschweiler et al., 2002; Wright et al., 2007) with 63 cases in all restricted to males only, while other articles (Decarli et al., 1987; Hu et al., 1994; Franceschi et al., 2000; Terry et al., 2000; Mayne et al., 2001; Chen et al., 2002; De Stefani et al., 2006; Jessri et al., 2011) did not have restriction to sex or separate information on males or females. Therefore, stratified analyses by sex were not conducted.

Beta-carotene

Ten articles on the association between beta-carotene intake and the risk of esophageal cancer were included (Decarli et al., 1987; Hu et al., 1994; Franceschi et al., 2000; Terry et al., 2000; Mayne et al., 2001; Bollschweiler et al., 2002; Chen et al., 2002; De Stefani et al., 2006; Wright et al., 2007; Jessri et al., 2011). Three of them analyzed esophageal squamous cell carcinoma and adenocarcinoma in separate studies (Terry et al., 2000; Mayne et al., 2001; Bollschweiler et al., 2002). Thus, 12 case-control studies and 1 cohort study in all entered

Table 2. Stratified Analysis of the Association Between Beta-carotene Intake and Risk of Esophageal Cancer

Subgroup	Number of studies	Number of cases	Pooled OR	95% CI	Heterogeneity test		P value for publication bias	
					I^2 score (%)	P value of Q test	Egger's test	Begg's test
Pathological type								
Adenocarcinoma	4	638	0.46	0.36, 0.58	0	0.697	0.962	1
Squamous cell carcinoma	6	1008	0.69	0.45, 1.07	85.2	0	0.801	0.851
Publication year								
Before/in 2000	5	955	0.52	0.39, 0.70	49	0.098	0.382	0.142
After 2000	8	1003	0.62	0.39, 0.99	84.2	0	0.624	0.902
Study design								
Hospital based case-control	5	886	0.71	0.42, 1.22	88.3	0	0.8	0.327
Populaion based case-control	5	962	0.48	0.39, 0.57	0	0.711	0.114	0.142
Prospective cohort	1	11	0.85	0.38, 1.90	—	—	—	—
Study location								
Europe	7	869	0.49	0.37, 0.66	43.9	0.098	0.198	0.133
Asia	2	243	0.91	0.61, 1.36	9.5	0.293	—	0.317
North America	3	612	0.45	0.36, 0.56	0	0.673	0.21	0.117
South America	1	234	1.47	1.07, 2.02	—	—	—	—
Adjusted for smoking	11	1470	0.62	0.45, 0.86	76.7	0	0.279	0.436
Not adjusted for smoking	2	488	0.43	0.34, 0.55	0	1	—	0.317
Adjusted for BMI	9	1652	0.59	0.42, 0.83	84.3	0	0.63	1
Not adjusted for BMI	4	306	0.55	0.34, 0.91	24.6	0.264	0.156	0.308
Adjusted for alcohol	9	1550	0.52	0.36, 0.76	83.5	0	0.379	0.754
Not adjusted for alcohol	4	408	0.71	0.49, 1.01	44.7	0.143	0.772	1

the meta-analysis. Heterogeneity was observed among the studies (I^2 = 78.2%; *P* for heterogeneity 0.000), and random effect model was adopted. When the highest intake was compared with the lowest, higher intake of beta-carotene reduced the esophageal cancer risk with pooled OR of 0.58 (95% CI 0.44, 0.77) (Figure 2).

To analyze the heterogeneity among studies, stratified analyses were proposed. The results of them were presented in Table 2. The statistically significant association between beta-carotene intake and risk of esophageal cancer remained in studies of adenocarcinoma cases with pooled OR of 0.46 (95% CI 0.36, 0.58) and population based case-control studies with pooled OR of 0.48 (95% CI 0.39, 0.57). The factors of pathological type and study design reduced the heterogeneity in the studies with statistically significant results, whereas increased that in others. Likewise, inconsistency was observed among the subgroups by study location. Studies located in Europe and North America showed that high intake of beta-carotene reduced esophageal cancer risk by 0.49 (95% CI 0.37, 0.66) and 0.45 (95% CI 0.36, 0.56), respectively. The heterogeneity alleviated in all the four subgroups by study location. The association was not altered by publication year in both subgroups, although one of them was marginal with pooled OR of 0.62 (95% CI 0.39, 0.99). The heterogeneity in two subgroups remained moderate to significant. Additionally, the positive association and significant heterogeneity remained in studies adjusted for alcohol with pooled OR of 0.52 (95% CI 0.36, 0.76). Moderate heterogeneity and negative association and were observed in those without adjustment. Whether or not the studies were adjusted by smoking or BMI, positive association revealed in each group. Heterogeneity in studies not adjusted for the two factors were low, while significant in the other studies.

In sensitivity test, the removal of one study at a time had no influence on the pooled ORs of the associations between beta-carotene and esophageal cancer.

Alpha-carotene, lycopene, and beta-cryptoxanthin

Three article (Franceschi et al., 2000; Chen et al., 2002; De Stefani et al., 2006) with 662 cases and 2128 controls reported the association between the risk of esophageal cancer and alpha-carotene, lycopene, beta-crytoxanthin intake respectively. The three studies were located in different areas, one in Europe (Franceschi et al., 2000), one in North America (Chen et al., 2002), and another in South America (De Stefani et al., 2006). Two hospital based case-control studies (Franceschi et al., 2000; De Stefani et al., 2006) were on esophageal squamous cell carcinoma with 538 cases and one population based case-control study (Chen et al., 2002) on adenocarcinoma with 124 cases. There was one study (Franceschi et al., 2000) published in 2000 with 304 cases, and two (Chen et al., 2002; De Stefani et al., 2006) after 2000 with 358 cases. All of the three studies (Franceschi et al., 2000; Chen et al., 2002; De Stefani et al., 2006) on alpha-carotene intake reported OR less than 1, however, only one (De Stefani et al., 2006) suggested statistical association. The heterogeneity was little (I^2 = 0.0%; *P* for heterogeneity 0.678), and the fixed effect model was adopted. Comparing the highest intake with the lowest, the intake of alpha-carotene reduced the esophageal cancer risk with pooled OR of 0.81 (95% CI 0.70, 0.94) (forest plot not shown). In further subgroup analyses (forest plots not shown), the statistical association were reported in hospital based case-control studies with esophageal squamous cell cancer with pooled OR of 0.82 (95% CI 0.70, 0.95; I^2 = 0.0%, *P* for heterogeneity 0.467), and in studies published after 2000 with pooled OR of 0.78 (95% CI 0.66, 0.93; I^2 = 0.0%, *P* for heterogeneity 0.700). Sensitivity test showed inconsistent result which implicated there wasn't protective effect of alpha-carotene intake with pooled OR of 0.85 (95% CI 0.65, 1.12) after removal of the study that

reported statistical association (De Stefani et al., 2006). No influence on the result was caused by removal any of the other two studies.

Two (Franceschi et al., 2000; Chen et al., 2002) of the three studies on lycopene intake reported large magnitude of 95% CI covering 1. There was little evidence of heterogeneity among the three studies (I^2 = 0.0%; *P* for heterogeneity 0.957), and the fixed effect model was conducted. Comparing the highest intake with the lowest, the intake of lycopene reduced the esophageal cancer risk with pooled OR of 0.75 (95% CI 0.64, 0.86) (forest plot not shown). In subgroup analyses (forest plots not shown), the statistically significant results were observed in hospital based case-control studies with esophageal squamous cell cancer with pooled OR of 0.74 (95% CI 0.64, 0.86; I^2 = 0.0%, *P* for heterogeneity 0.830), and studies after 2000 with pooled OR of 0.74 (95% CI 0.64, 0.86; I^2 = 0.0%, *P* for heterogeneity 0.830). Sensitivity test showed inconsistent result which implicated there was no protective effect of lycopene intake with pooled OR of 0.80 (95% CI 0.49, 1.31) after removal of the study that reported statistical association(De Stefani et al., 2006). No influence on the result was caused by removal any of the other two studies.

Three studies (Franceschi et al., 2000; Chen et al., 2002; De Stefani et al., 2006) on beta-cryptoxanthin intake all reported OR less than 1, however, only one (De Stefani et al., 2006) suggested statistically marginal association. There was moderate heterogeneity among the three studies (I^2 = 50.9%; *P* for heterogeneity 0.130), and the random effect model was conducted. Comparing the highest intake with the lowest, the intake of beta-cryptoxanthin reduced the esophageal cancer risk with pooled OR of 0.80 (95% CI 0.66, 0.97) (forest plot not shown). In subgroup analysis, the statistically marginal association remained in hospital based case-control studies with esophageal squamous cell cancer with pooled OR of 0.83 (95% CI 0.72, 0.97; I^2 = 42.6%; *P* for heterogeneity 0.187). Sensitivity test showed inconsistent result which implicated there was no protective effect of lycopene intake after removal of the study by De Stefani et al. (2006) with pooled OR of 0.73 (95% CI 0.42, 1.27).

Lutein and zeaxanthin

There were 2 case-control studies (Franceschi et al., 2000; Chen et al., 2002) with 428 cases and 1192 controls in all on the association of lutein and zeaxanthin intake and the risk of esophageal cancer. One hospital based case-control studies (Franceschi et al., 2000) with 304 cases of esophageal squamous cell carcinoma reported statistically marginal result, while the other population based case-control study (Chen et al., 2002) with 124 cases of adenocarcinoma did not. There was little heterogeneity among studies (I^2 = 0.0%; *P* for heterogeneity 0.527), and fixed effect model was adopted. Comparing the highest intake with the lowest, lutein and zeaxanthin intake reduced the esophageal cancer risk with pooled OR of 0.71 (95% CI 0.59, 0.87) (forest plot not shown). Considering the number of studies included, subgroup analyses and sensitivity test were not conducted.

Publication bias

During the systematic search, no restriction of the publication type was set, although unpublished conference articles were not specifically searched for. No language restriction was imposed and two Chinese databases were also included, even though only English articles met the inclusion criteria for the meta-analysis in the end. Publication bias was not observed in the pooled estimates for any of the carotenoids by Egger's linear regression (*P* value range 0.114-0.962) or Begg's test (*P* value range 0.117-1.000).

Discussion

This meta-analysis showed the intake of carotenoids, including beta-carotene, alpha-carotene, lycopene, beta-cryptoxanthin, lutein and zeaxanthin, had an inverse association with the incidence risk of esophageal cancer. Furthermore, beta-carotene significantly reduced the risk of adenocarcinoma, while alpha-carotene, lycopene, and beta-cryptoxanthin reduced that of squamous cell cancer of the esophagus.

In this meta-analysis, beta-carotene intake was most protective when only adenocarinoma was analyzed and was weakened when squamous cell cancer was also taken into consideration. Of the 13 studies included, there were 1008 cases of esophageal squamous cell cancer and 638 cases of adenocarcinoma. The size difference between two pathological types might strengthen the statistical power of the protective effect among adenocarcinoma to some extend. Moreover, the incidence of esophageal cancer represents a 16-fold difference between high-incidence, such as Asia, and low-incidence regions (Jemal et al., 2011). In the other hand, there were only 2 case-control studies located in Asia and 1 in South America included. Taking the relative small size into consideration, the results showing no association between beta-carotene and cancer risk in these areas should be accepted conservatively.

The prevention action of beta-carotene was thought to be related to the role of antioxidant in vitro. Kozuki et al. (Kozuki et al., 2000) showed that beta-carotene was able to inhibit the invasion of rat ascites hepatoma to rat mesentery derived hepatoma cells, AH109A, and the reactive oxygen generation during the process. In a research with ex vivo model, it had been demonstrated that the addition of beta-carotene could inhibit DNA oxidation and decrease lymphocyte DNA damage (Fabiani et al., 2001). In vivo studies also provided evidence of the similar capability as antioxidant. A group of Iranian men that had high levels of malonaldehyde (MDA), a thiobarbituric acid-reactive substance, were supplemented with beta-carotene. Ten weeks later, the levels of MDA reduced significantly (Meraji et al., 1997). In General Population Nutrition Intervention Trial conducted in Linxian, China from 1985 to 1991, daily supplementation with beta-carotene, vitamin E and selenium led to lower overall cancer mortality. As to esophageal cancer, 17% reduction of deaths was observed in participants younger than 55 while 14% increase among those aged 55 years or older (Qiao et al., 2009). Since this trial aimed at the risk of mortality and conducted in older

patients, the contradictory result might indicate greater benefit earlier in the course of carcinogenesis, beyond which supplementation may be harmful other than useful.

Studies were not more than three that involved in the analyses of the association between the other five compounds of carotenoids intake and esophageal cancers. Besides, such studies with more statistical power as cohort studies and clinical trials were lacked in this meta-analysis. Whereas the strength of the results could be alleviated due to the limits of the studies included, the protective effect showed in this meta-analysis was consist with preclinical studies and some case series studies on cancers of other sites.

Alpha-carotene was found to induce G0-G1 arrest in cell cycle by Murakoshi et al. (1989), which meant that it might have cancer preventive activity. Moreover, anticarcinogenic activity of alpha-carotene was found to be higher than that of beta-carotene in skin, lung, and liver (Murakoshi et al., 1992). Lycopene was suggested to prevent carcinogenesis by protecting critical biomolecules such as DNA and proteins (Basu et al., 2007), and to induce apoptosis in cancer cells by altering mitochondrial function (Hantz et al., 2005). Epidemiological study in elderly Americans indicated that high tomato intake was associated with 50% reduction of mortality from cancers at all sites (Colditz et al., 1985). Weekly tomato consumption was reported to be associated with a 40% reduction in risk for esophageal cancer in a case-control study (Cook-Mozaffari et al., 1979). In an in vitro research, beta-cryptoxanthin suppressed TPA-induced expression of early antigen of Epstein–Barr virus in Raji cells at the highest potency among carotenoids tested (Tsushima et al., 1995). Lutein, dihydroxy-form of alpha-carotene, and zeaxanthin, dihydroxy-form of beta-carotene, both had high uptake and secretion values in a Caco-2 cell model, which reflected high relative availability of carotenoids from food sources and synthetic formulations (O'Sullivan et al., 2007).

This meta-analysis suggested that the protective effect of alpha-carotene, lycopene, and beta-cryptoxanthin remained among squamous cell cancer other than adenrocarcinoma. Two of the three studies that entered meta-analysis were hospital based case-control studies conducted with 568 cases of squamous cell cancer and one population based case-control study with 124 cases of adenrocarcinoma. Therefore, the conclusion the lack of beneficial effect among adenrocarcinoma could not be drawn definitely. Secondly, the results of the analyses of the three nutrients could be more authoritative with more studies included, and the subgroup analyses by publication year or pathological type might not be stable or representative enough. Besides, the sensitivity test showed altered results after removal of one of the articles in the analyses of the three nutrients, which could also reflect the limitation of the results mentioned above. Likewise, the analysis of lutein and zeaxanthin might have some similar complication. Therefore, the results of analyses of alpha-carotene, lycopene, beta-cryptoxanthin, lutein and zeaxanthin might have the same statistical effect with those of beta-carotene, and the subgroup analyses results should be accepted conservatively.

The significant heterogeneity existed in the studies on beta-carotene. The followings may explain the heterogeneity in beta-carotene studies. First of all, locations of the studies contributed to the heterogeneity. The subgroups in North America, Asia, Europe showed decreased heterogeneity with I^2 value of 0.0%, 9.5% and 43.9%, respectively. In the second place were the different pathological types, confounding factors of cigarette, and BMI. The value of I^2 dropped to lower than 25% in one side of the stratification, while increased in the other side. Finally, the publication year and confounding factor of alcohol brought slight heterogeneity. Studies not adjusted for alcohol and published before or in 2000 showed I^2 value of 44.7% and 49.0% respectively, which were a little lower than those before stratification.

The pooled ORs were estimated based on all the studies obtained from systematic search, which could provide relatively high statistical power. However, certain limitation should be considered in this meta-analysis. Firstly, as meta-analysis itself, no original data could be analyzed. Since esophageal cancer has been considered to be associated with many factors, the confounding factors, such as smoking status, alcohol assumption, obesity, ethnicity, traditional customs, socioeconomic status, and interactions among the six nutrients, should be taken into consideration to make the conclusions more reliable and representative. Although the stratified analyses were performed among studies with or without adjustment for cigarette, BMI, and alcohol, the potential confounding effect of these factors could not be accurately and thoroughly assessed without sufficient original data. Secondly, there were five articles conducted in Europe (Decarli et al., 1987; Franceschi et al., 2000; Terry et al., 2000; Bollschweiler et al., 2002; Wright et al., 2007), while two in Asia (Hu et al., 1994; Jessri et al., 2011), two in North America (Mayne et al., 2001; Chen et al., 2002), and only one in South America (De Stefani et al., 2006). Although during systematic search, different databases were used to cover publication from different areas, the moderate imbalance still existed among the location of the articles included. Thirdly, as mentioned above, there were more cases of esophageal squamous cell cancer than those of adenocarcinoma in the articles included. The size difference between two pathological types might have influence on the results. Fourthly, there were three studies on alpha-carotene, lycopene, and beta-cryptoxanthin, and two studies on lutein and zeaxanthin. Although detailed review and meta analyses were both conducted, the quantity and size of the studies might limit the statistical effect of the results. Moreover, of all the studies, only one cohort with 11 cases and 13 controls was selected, and none RCT met the inclusion criteria. Given the common mismatch in results obtained from cohort and case-control studies with data from RCTs, there would be more statistically power in the results. Finally, participants with high intake levels of antioxidants might also have higher intake of other nutrients with anticancer activity, or might possess other behaviors that may alter the incidence risk of esophagus cancer and account for results observed.

In summary, a meta-analysis suggested that higher intake of carotenoids (beta-carotene, alpha-carotene,

lycopene, beta-cryptoxanthin, lutein and zeaxanthin) could reduce the incidence risk of esophageal cancer. Subgroup analyses showed that beta-carotene could prevent from esophageal adenrocarcinoma, alpha-carotene, lycopene, and beta-cryptoxanthin might protect from squamous cell cancer. Additionally, studies adjusted for cigarette and BMI or not all suggested positive association, whereas the association remained in the studies adjusted for alcohol only. Taking the limits of this study into consideration, the results of subgroup analyses should be accepted conservatively. Further researches and large-sample studies need to be conducted to better clarify the potentially protective mechanism in carotenoids and the relationship between carotenoids intake and the risk of esophageal cancer

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