REVIEW

Dietary Intake and Circulating Concentrations of Carotenoids and Risk of Type 2 Diabetes: A Dose-Response Meta-Analysis of Prospective Observational Studies

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ABSTRACT

Previous meta-analysis studies have indicated inverse associations between some carotenoids and risks of metabolic syndrome, cardiovascular disease, cancer, and all-cause mortality. However, the results for associations between carotenoids and type 2 diabetes (T2D) remain inconsistent and no systematic assessment has been done on this topic. We conducted a systematic review and meta-analysis to examine the associations of dietary intakes and circulating concentrations of carotenoids with risk of T2D. We searched PubMed and Ovid Embase from database inception to July 2020. Prospective observational studies of carotenoids and T2D risk were included. Random-effects models were used to summarize the RRs and 95% CIs. Thirteen publications were included. Dietary intake of β-carotene was inversely associated with the risk of T2D, and the pooled RR comparing the highest with the lowest categories was 0.78 (95% CI: 0.70, 0.87; $I^2 = 13.7\%$; $n = 6$); inverse associations were also found for total carotenoids ($n = 2$), α-carotene ($n = 4$), and lutein/zeaxanthin ($n = 4$), with pooled RRs ranging from 0.80 to 0.91, whereas no significant associations were observed for β-cryptoxanthin and lycopene. Circulating concentration of β-carotene was associated with a lower risk of T2D, and the pooled RR comparing extreme categories was 0.60 (95% CI: 0.46, 0.78; $I^2 = 56.2\%$; $n = 7$); inverse associations were also found for total carotenoids ($n = 3$), lycopene ($n = 4$), and lutein ($n = 2$), with pooled RRs ranging from 0.63 to 0.85, whereas no significant association was found for circulating concentrations of α-carotene and zeaxanthin when comparing extreme categories. Dose-response analysis indicated that nonlinear relations were observed for circulating concentrations of α-carotene, β-carotene, lutein, and total carotenoids (all P-nonlinearity < 0.05), but not for other carotenoids or dietary exposures. In conclusion, higher dietary intakes and circulating concentrations of total carotenoids, especially β-carotene, were associated with a lower risk of T2D. More studies are needed to confirm the causality and explore the role of foods rich in carotenoids in prevention of T2D.This systematic review was registered at www.crd.york.ac.uk/prospero as CRD42020196616. *Adv Nutr* 2021;12:1723–1733.

Keywords: carotenoids, type 2 diabetes, prospective observational study, systematic review, meta-analysis

Introduction

Carotenoids are a group of natural antioxidants that are rich in many colored fruits and vegetables. Six types of carotenoids, i.e., α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin, represent ~90% of the total carotenoids in the human diet and circulation system (1, 2). According to their chemical composition, carotenoids can be classified into 2 groups: carotenes (such as α-carotene, β-carotene, and lycopene) which are formed by carbon and hydrogen atoms, and xanthophylls (such as β-cryptoxanthin, lutein, and zeaxanthin) which are composed by carbon, hydrogen, and oxygen atoms (3). Carotenoids are derived mainly from diet with varying food sources for different carotenoids (3): β-carotene can be obtained from most fruits and vegetables; α-carotene is mainly from carrots, winter squash, and pumpkin; β-cryptoxanthin is mainly from tropical fruits like mango and papaya; lutein is mainly from green vegetables such as broccoli and sprouts; lycopene is

mainly from tomatoes and tomato products; and zeaxanthin is mainly from egg yolks and corn (4). As one of the most easily obtained natural antioxidants from food, carotenoids could reduce oxidative stress, which has been reported to accelerate the development of several chronic diseases (5). Accumulating studies have suggested an inverse association between some carotenoids and risk of metabolic syndrome, cardiovascular disease, cancer, or all-cause mortality (6, 7).

However, the results of the association between carotenoids and type 2 diabetes (T2D) remain inconsistent. For example, some studies have indicated a lower risk of T2D with higher intakes or circulating concentrations of β-carotene (8–11), whereas others showed no significant association (12–18). Some clinical trials did not find any significant effect of β-carotene supplementation on prevention of T2D in male smokers ($n = 29{,}133$) after 6.1 y of intervention (15), healthy male physicians ($n = 22{,}071$) after 12.0 y of intervention (19), or women at high risk of cardiovascular disease ($n = 8171$) after 9.2 y of intervention (20); however, those trials were not designed to test diabetes prevention as the primary endpoint, and researchers need to be cautious when generalizing results from those trials to the general population with different characteristics. Although the Antioxidant Vitamins and Minerals (SU.VI.MAX) study among 3146 participants who followed a balanced diet showed no effect of additional antioxidant supplementation (120 mg vitamin C, 30 mg vitamin E, 6 mg β-carotene, 100 g Se, and 20 mg Zn/d) on fasting plasma glucose after a 7.5-y intervention, the supplementation with multiple substances made it hard to assess the independent effect of β-carotene (21). Furthermore, there are no clinical trials reporting the effect of other carotenoids on T2D.

Because of the inconsistent results in the literature, we conducted a comprehensive systematic review and meta-analysis of prospective observational studies to summarize the evidence on the association of dietary intake and circulating concentrations of carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein/zeaxanthin, and total carotenoids) with risk of T2D. To the best of our knowledge, this is the first meta-analysis to comprehensively assess associations for carotenoids and T2D.

Methods

This review was registered at PROSPERO as CRD42020196616.

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Supplemental Methods, Supplemental Tables 1–9, and Supplemental Figures 1–4 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/advances/.
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Search strategy

We searched PubMed and Ovid Embase from database inception to July 2020 in accordance with Meta-analysis Of Observational Studies in Epidemiology guidelines (22) and the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (23). The **Supplemental Methods** show the detailed search strategy.

In brief, we searched for prospective observational studies (cohort, nested case-control, and case-cohort studies) that reported the associations between dietary intakes and/or blood concentrations of carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein, zeaxanthin, and total carotenoids) and the risk of all-cause mortality, cardiovascular disease, coronary artery disease, stroke, and incident T2D. In this study, we only focused on the studies for T2D; the results for other outcomes were reported separately. Reference lists of retrieved articles and previous relevant reviews on this topic were also manually searched.

Study selection

Literature eligibility was independently assessed by Y-WJ and Z-HS, as well as another group of investigators, including W-WT, KY, and K-QG, with discrepancies resolved by discussion with the senior authors (GL and AP). Studies were excluded if they were *1*) unrelated to the exposures or outcome; *2*) published in non-English languages; *3*) other article types (e.g., protocol, review, cross-sectional study, case-control study, or clinical trial) or not peer-reviewed publications (e.g., meeting abstract, editorial, commentary); *4*) studies among participants with severe diseases that may heavily influence their lifestyles or their expected lifespan, such as cancer, renal failure, HIV infection, or other life-threatening illnesses; or *5*) studies without necessary or sufficient data on the effect estimates (e.g., HR, RR, or OR). For duplicate publications from the same cohort, we included the study with the longest follow-up or largest number of participants or incident cases. For the dose-response analysis, we only included studies that had quantitative measures of exposure with $\geq$3 categories.

Data extraction and quality assessment

Y-WJ and Z-HS, as well as another group of investigators, including W-WT, KY, and K-QG, extracted the following information using a predesigned collection form: study characteristics (study name, publication year, study location, follow-up years, sample size, and number of incident cases), participants' characteristics (age, sex, BMI, general health or disease status), exposure and outcome assessment (exposure sources and species, mean/median or range of exposure in each category, measurement methods of exposure and outcome), statistical analysis (statistical models and confounders in the models), and effect sizes from the most adjusted models (OR, HR, and RR). Additional information that was not available in the online publications or supplementary materials was obtained by contacting study authors via e-mails. The Newcastle-Ottawa Scale (NOS) was used to evaluate study quality based on selection of study groups,

comparability of groups, and ascertainment of exposures or outcomes (24).

Statistical methods

Random-effects models were used to summarize the RRs and 95% CIs for the highest compared with the lowest categories. Heterogeneity across studies was assessed by Q and I^2 statistics, with $P < 0.10$ or $I^2 > 50\%$ considered as statistically significant. If significant heterogeneity was observed, subgroup analyses were planned to be conducted by study characteristics (e.g., age, sex, BMI, geographic location, exposure measurement method, outcome assessment method, duration of follow-up, and number of incident T2D cases). However, because of the limited numbers of studies that were included for many exposure–outcome associations, we only did stratified analyses for associations with ≥6 studies. Publication bias was evaluated using visual inspection of funnel plots as well as Egger's tests. Duval and Tweedie's trim and fill method was used to generate "unbiased" estimates by adding hypothesized studies to make the funnel plot symmetrical.

We converted the data to milligrams per day for dietary exposure and to micromoles per liter for circulating exposure when conducting dose-response analysis. For studies that reported blood concentrations of carotenoids, α-carotene, β-carotene, or lycopene in micrograms per deciliter, we multiplied the concentration by 0.01863, for β-cryptoxanthin we multiplied by 0.01809, and for lutein or zeaxanthin we multiplied by 0.01758 (25). Linear dose-response meta-analysis was conducted using the method described by Greenland and Longnecker (26). The mean, median, or midpoint values of the upper and lower limits were used as the estimated dose amount. When the dose range was open-ended, the width of the adjacent interval was used to estimate the lower and upper limits for the category. Potential nonlinear dose–response relation was evaluated using restricted cubic splines with 3 knots at 5%, 50%, and 95% of the distribution of the exposure.

Sensitivity analyses were conducted to test the robustness of the results by excluding 1 study at a time when there were >3 included studies. Statistical power of the meta-analysis was estimated using the SAS macro %metapower, which calculates power using the methods described by Hedges and Pigott (27, 28). All statistical analyses were performed with Stata version 15 (StataCorp) except for the power analysis, which was conducted using SAS software version 9.4 (SAS Institute, Inc.). Two-tailed $P < 0.05$ was deemed statistically significant unless stated otherwise.

Results

Literature search

Figure 1 shows the literature screening procedure. The search strategy identified 17,697 unique citations. After screening titles and abstracts with the aforementioned criteria, 297 full texts were acquired and further evaluated. Of these, 192 articles were excluded after detailed examination (see Figure 1), leaving 105 relevant publications. Among them, 90 articles reported associations for other outcomes (i.e., all-cause death, cardiovascular disease, coronary artery disease, and stroke), 1 article reported the association for vitamin A (29), and 1 article used data from the same cohort study (30); therefore, these were not included in the current analysis, leaving 13 studies included in the final analysis.

Supplemental Table 1 summarizes characteristics of the 13 included studies. Overall, 7 were conducted in Europe, 5 in the United States, and 1 in Asia (Japan). All studies were of prospective design (10 cohort studies, 2 nested case-control studies, and 1 case-cohort study) with follow-up duration ranging from 4.8 to 27.0 y. All studies were performed among adults (>18 y old), with 11 of them among participants who were aged ≥50 y on average at baseline. Three studies were performed only among male participants and 3 only among females. Most studies were of high quality, with 2 studies having an NOS score ≤ 6 (**Supplemental Tables 2**, **3**).

Dietary carotenoids intake and risk of T2D

We observed significant inverse associations of dietary intake of α-carotene, β-carotene, lutein/zeaxanthin, and total carotenoids with risk of T2D (**Table 1**, **Figure 2**), and the pooled RRs comparing the highest with the lowest groups were 0.91 (95% CI: 0.85, 0.96; $I^2 = 0.0\%$, P-heterogeneity = 0.91) for α-carotene (4 studies; 71,293 participants; 2596 cases) (8, 9, 13, 14), 0.78 (95% CI: 0.70, 0.87; $I^2 = 13.7\%$, P-heterogeneity = 0.33) for β-carotene (6 studies; 77,643 participants; 3109 cases) (8, 9, 12–14, 31), 0.86 (95% CI: 0.76, 0.97; $I^2 = 29.8\%$, P-heterogeneity = 0.23) for lutein/zeaxanthin (4 studies; 71,293 participants; 2596 cases) (8, 9, 13, 14), and 0.80 (95% CI: 0.68, 0.95; $I^2 = 0.0\%$, P-heterogeneity = 0.34) for total carotenoids (2 studies; 42,095 participants; 1292 cases) (9, 14). No significant associations were observed for dietary intakes of β-cryptoxanthin (RR: 0.82; 95% CI: 0.61, 1.10; $I^2 = 77.7\%$, P-heterogeneity = 0.01) (9, 13, 14) and lycopene (RR: 1.00; 95% CI: 0.90, 1.11; $I^2 = 0.0\%$, P-heterogeneity = 0.44) (9, 13, 14, 32) (Table 1, Figure 2).

Egger's test indicated no evidence of publication bias (all P values ≥ 0.05, **Supplemental Figure 1**). The trim and fill method added 1 study for dietary α-carotene intake and 1 study for dietary intake of lutein/zeaxanthin. However, the results remained unchanged after adding hypothesized studies (**Supplemental Table 4**). Sensitivity analysis excluding 1 study at a time found similar results (**Supplemental Table 5**).

There was no indication of nonlinear associations for intakes of total and individual carotenoids and T2D (all P-nonlinearity ≥ 0.05; data not shown). Although not significant, inverse associations were observed for α-carotene ($P = 0.16$), β-carotene ($P = 0.08$), and lutein/zeaxanthin ($P = 0.65$) in the linear dose-response analysis; total carotenoids intake was inversely related to the risk of T2D in a linear dose-response manner ($P = 0.04$) (Table 1, **Supplemental Figure 2**).

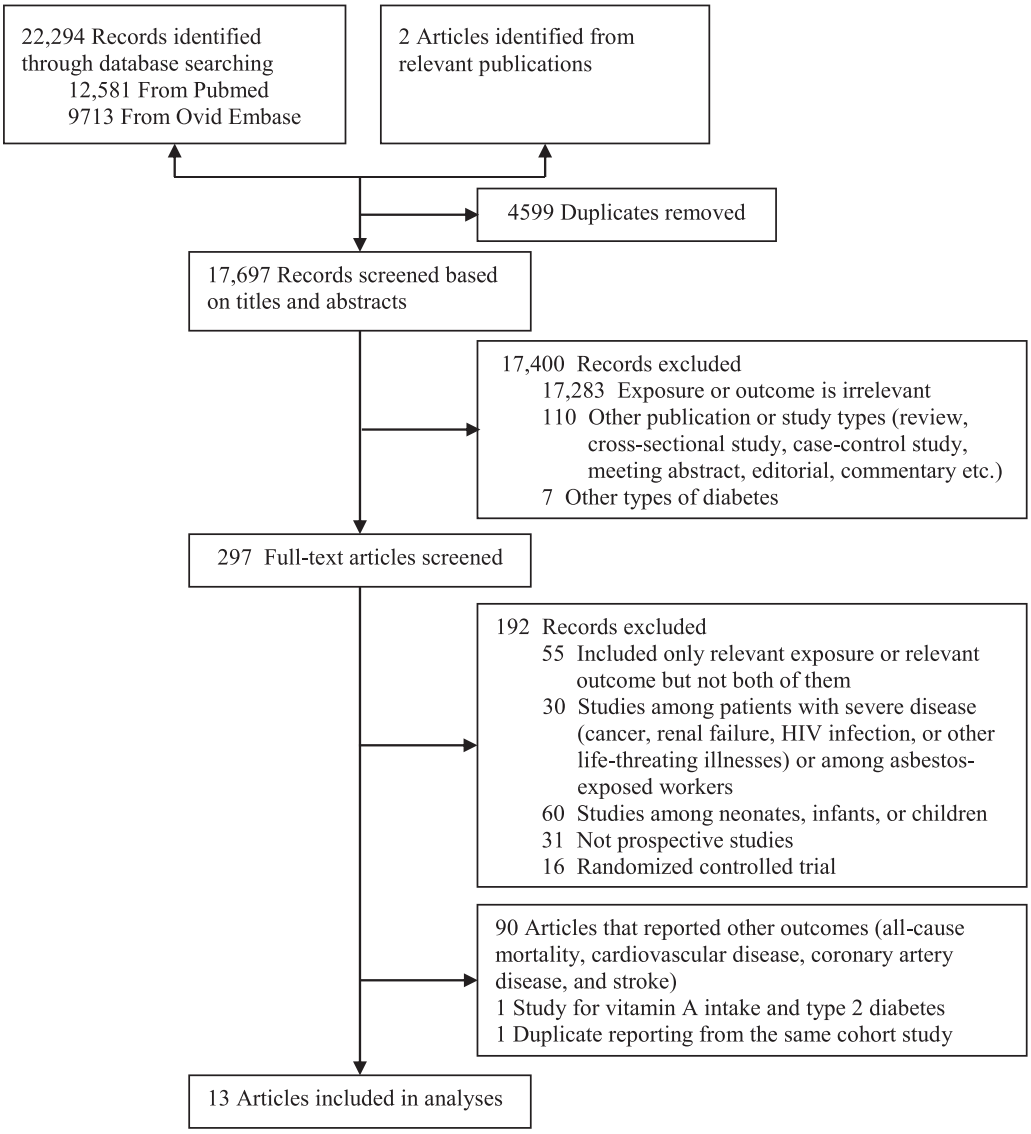

FIGURE 1 Flowchart of study selection.

Circulating carotenoids concentrations and risk of T2D

As Table 1 and **Figure 3** present, circulating total carotenoids concentrations showed significant inverse association with risk of T2D (3 studies; 25,264 participants; 8412 cases) (10, 11, 17), and the pooled RR was 0.63 (95% CI: 0.42, 0.96) with moderate heterogeneity (I^2 = 59.9%, *P*-heterogeneity = 0.08). As for subtypes of carotenoids, circulating concentrations of β-carotene, lycopene, and lutein were found to be inversely associated with risk of T2D. The pooled RR comparing extreme categories was 0.60 (95% CI: 0.46, 0.78; I^2 = 56.2%, *P*-heterogeneity = 0.03) for β-carotene (7 studies; 34,234 participants; 9421 cases) (10, 11, 15–18, 31), 0.85 (95% CI: 0.76, 0.96; I^2 = 0.0%; *P*-heterogeneity = 0.40) for lycopene (4 studies; 26,204 participants; 8882 cases) (10, 11, 17, 18), and 0.65 (95% CI: 0.55, 0.77; I^2 = 0.0%; *P*-heterogeneity = 0.97) for lutein (2 studies; 20,771 participants; 8264 cases) (11, 17). There was no significant association of circulating concentrations of α-carotene (4 studies; RR: 0.71; 95% CI: 0.44, 1.16; I^2 = 85.7%; *P*-heterogeneity < 0.001) (10, 11, 17, 18), β-cryptoxanthin (4 studies; RR: 0.80; 95% CI: 0.60, 1.06; I^2 = 56.1%; *P*-heterogeneity = 0.08) (10, 11, 17, 18), or zeaxanthin (2 studies; RR: 0.82; 95% CI: 0.63, 1.05; I^2 = 2.3%; *P*-heterogeneity = 0.31) (11, 17) with risk of T2D.

Subgroup analysis of circulating concentration of β-carotene and T2D (comparing the highest group with the lowest) showed no evidence of heterogeneity resulting from age, sex, BMI, geographic location, number of T2D cases, or outcome assessment method (all *P*-heterogeneity between-subgroup ≥ 0.05). However, there was significant heterogeneity by follow-up duration (*P*-heterogeneity between-subgroup = 0.04), with a weaker association among studies with ≥10 y follow-up than among those with <10 y follow-up (**Supplemental Table 6**). Egger's test did not

TABLE 1 Relations of dietary carotenoids and circulating carotenoids with risk of type 2 diabetes: pooled analyses comparing the highest with the lowest categories and dose-response analyses

Exposure	Studies, *n*	Participants, *n*	Cases, *n*	RR (95% CI)	I^2, %	*P*-heterogeneity	References
Total carotenoids							
Dietary							
High vs. low	2	42,095	1292	0.80 (0.68, 0.95)	0.0	0.34	(9, 14)
Per 1.00 mg/d	2	42,095	1292	0.98 (0.95, 1.00)	0.0	0.42	(9, 14)
Blood concentrations							
High vs. low	3	25,264	8412	0.63 (0.42, 0.96)	59.9	0.08	(10, 11, 17)
Per 2.00 μmol/L	3	25,264	8412	0.64 (0.44, 0.93)	66.8	0.05	(10, 11, 17)
α-carotene							
Dietary							
High vs. low	4	71,293	2596	0.91 (0.85, 0.96)	0.0	0.91	(8, 9, 13, 14)
Per 1.00 mg/d	3	67,600	1952	0.88 (0.73, 1.06)	19.6	0.29	(9, 13, 14)
Blood concentrations							
High vs. low	4	26,204	8882	0.71 (0.44, 1.16)	85.7	<0.001	(10, 11, 17, 18)
Per 0.20 μmol/L	3	21,711	8734	0.65 (0.33, 1.26)	81.7	0.004	(11, 17, 18)
β-carotene							
Dietary							
High vs. low	6	77,643	3109	0.78 (0.70, 0.87)	13.7	0.33	(8, 9, 12–14, 31)
Per 1.00 mg/d	5	73,950	2465	0.95 (0.90, 1.01)	26.5	0.25	(9, 12–14, 31)
Blood concentrations							
High vs. low	7	34,234	9421	0.60 (0.46, 0.78)	56.2	0.03	(10, 11, 15–18, 31)
Per 0.50 μmol/L	5	29,434	9167	0.65 (0.48, 0.89)	69.7	0.01	(11, 15, 17, 18, 31)
β-cryptoxanthin							
Dietary							
High vs. low	3	67,600	1952	0.82 (0.61, 1.10)	77.7	0.01	(9, 13, 14)
Per 0.01 mg/d	3	67,600	1952	0.99 (0.96, 1.03)	85.1	0.001	(9, 13, 14)
Blood concentrations							
High vs. low	4	26,204	8882	0.80 (0.60, 1.06)	56.1	0.08	(10, 11, 17, 18)
Per 0.50 μmol/L	3	21,721	8734	0.85 (0.76, 0.94)	0.0	0.87	(11, 17, 18)
Lycopene							
Dietary							
High vs. low	4	103,383	3496	1.00 (0.90, 1.11)	0.0	0.44	(9, 13, 14, 32)
Per 1.00 mg/d	4	103,383	3496	1.00 (0.99, 1.01)	0.0	0.54	(9, 13, 14, 32)
Blood concentrations							
High vs. low	4	26,204	8882	0.85 (0.76, 0.96)	0.0	0.40	(10, 11, 17, 18)
Per 0.50 μmol/L	3	21,721	8734	0.83 (0.74, 0.92)	0.0	0.77	(11, 17, 18)
Lutein/zeaxanthin							
Dietary							
High vs. low	4	71,293	2596	0.86 (0.76, 0.97)	29.8	0.23	(8, 9, 13, 14)
Per 1.00 mg/d	3	67,600	1952	0.97 (0.83, 1.12)	41.9	0.18	(9, 13, 14)
Blood concentrations							
High vs. low	2	5433	618	0.99 (0.81, 1.20)	0.0	0.35	(10, 18)
Per 0.20 μmol/L	1	940	470	1.22 (0.81, 1.83)	—	—	(18)
Lutein							
Blood concentrations							
High vs. low	2	20,771	8264	0.65 (0.55, 0.77)	0.0	0.97	(11, 17)
Per 0.20 μmol/L	2	20,771	8264	0.79 (0.72, 0.86)	0.0	0.75	(11, 17)
Zeaxanthin							
Blood concentrations							
High vs. low	2	20,771	8264	0.82 (0.63, 1.05)	2.3	0.31	(11, 17)
Per 0.20 μmol/L	2	20,771	8264	0.67 (0.41, 1.10)	0.0	0.41	(11, 17)

indicate any significant publication bias (all *P* values $\geq$ 0.05, **Supplemental Figure 3**). The trim and fill test did not significantly alter the findings except that the association with α-carotene became statistically significant (pooled RR: 0.61; 95% CI: 0.39, 0.97) (**Supplemental Table 7**). Sensitivity analysis excluding 1 study at a time (**Supplemental Table 8**) showed similar results.

Nonlinear relations with T2D were observed for circulating concentrations of α-carotene, β-carotene, lutein, and total carotenoids (all *P*-nonlinearity $<$ 0.05) (**Figure 4**). Although the circulating β-cryptoxanthin concentration showed a nonsignificant inverse association with T2D when comparing extreme categories ($P = 0.12$), linear dose-response analysis found a significant association

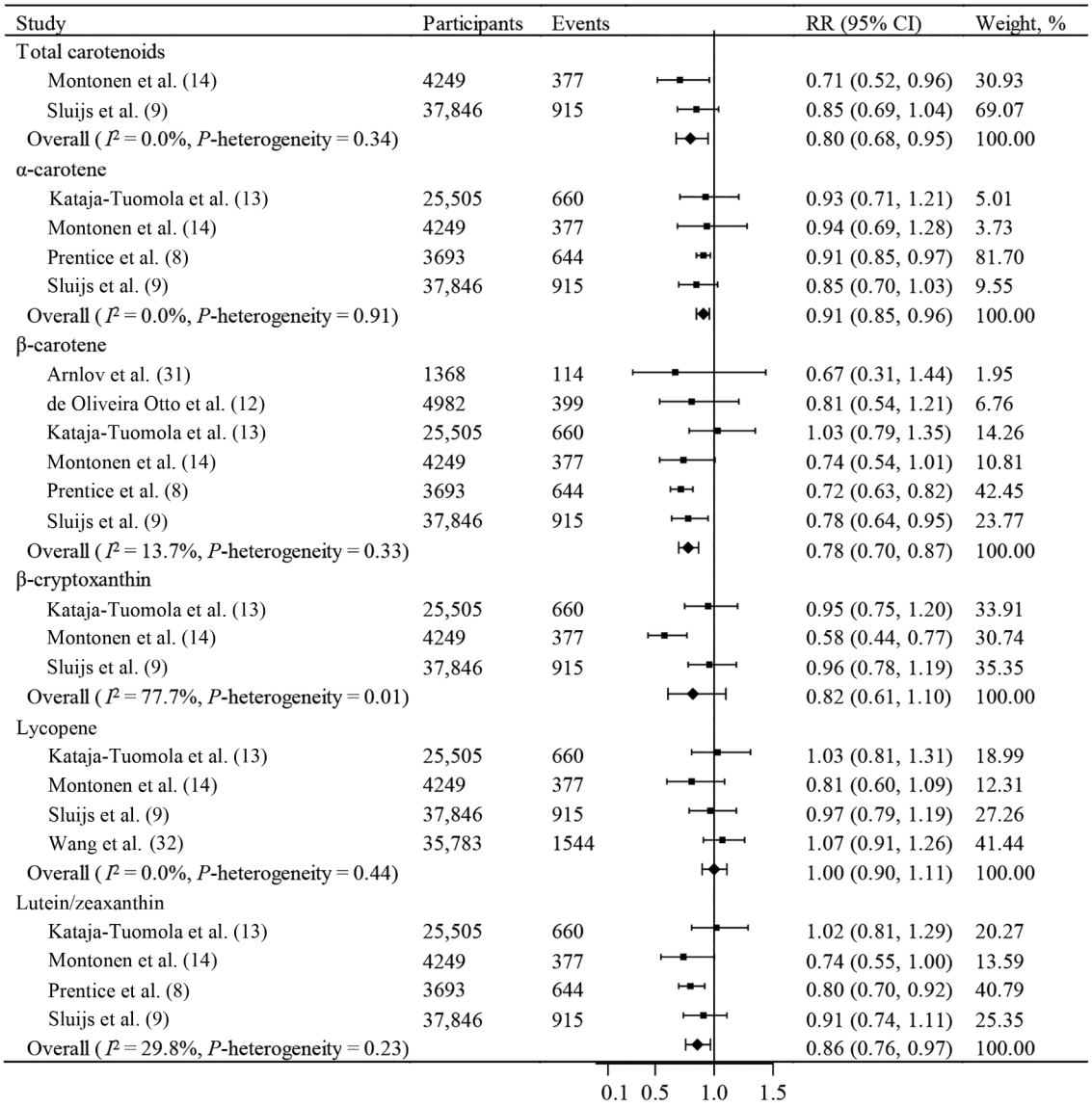

FIGURE 2 Meta-analyses of dietary carotenoids and risk of type 2 diabetes comparing the highest with the lowest categories. Summary estimate effects were calculated using a random-effects model.

(RR for per 0.50-μmol/L increment: 0.85; 95% CI: 0.76, 0.94; I^2 = 0.0%; P-heterogeneity = 0.87) (Table 1, **Supplemental Figure 4**). Similarly to the results comparing extreme categories, linear dose-response analysis also showed significant inverse associations for circulating β-carotene, lycopene, lutein, and total carotenoids (Table 1, Supplemental Figure 4).

Statistical power of the meta-analysis

Supplemental Table 9 shows the statistical power in the meta-analysis comparing extreme categories. The power varied for different carotenoids, with statistical power >80% only for dietary intakes of α-carotene, β-carotene, and total carotenoids and for circulating concentrations of β-carotene and lutein.

Discussion

In this synthesis of evidence from prospective observational studies, we found that dietary intakes and circulating concentrations of total carotenoids were associated with a lower risk of T2D. β-Carotene and lutein were also consistently reported to be inversely associated with risk of T2D in analysis of both dietary intakes and circulating biomarkers. The associations with other types of carotenoids were not entirely consistent but all were in the direction of inverse association. To our knowledge, this is the first meta-analysis of prospective studies investigating relations of dietary intake and circulating concentrations of total and 6 individual carotenoids with T2D risk.

Previous meta-analysis studies have shown prospective inverse associations for carotenoids and risks of

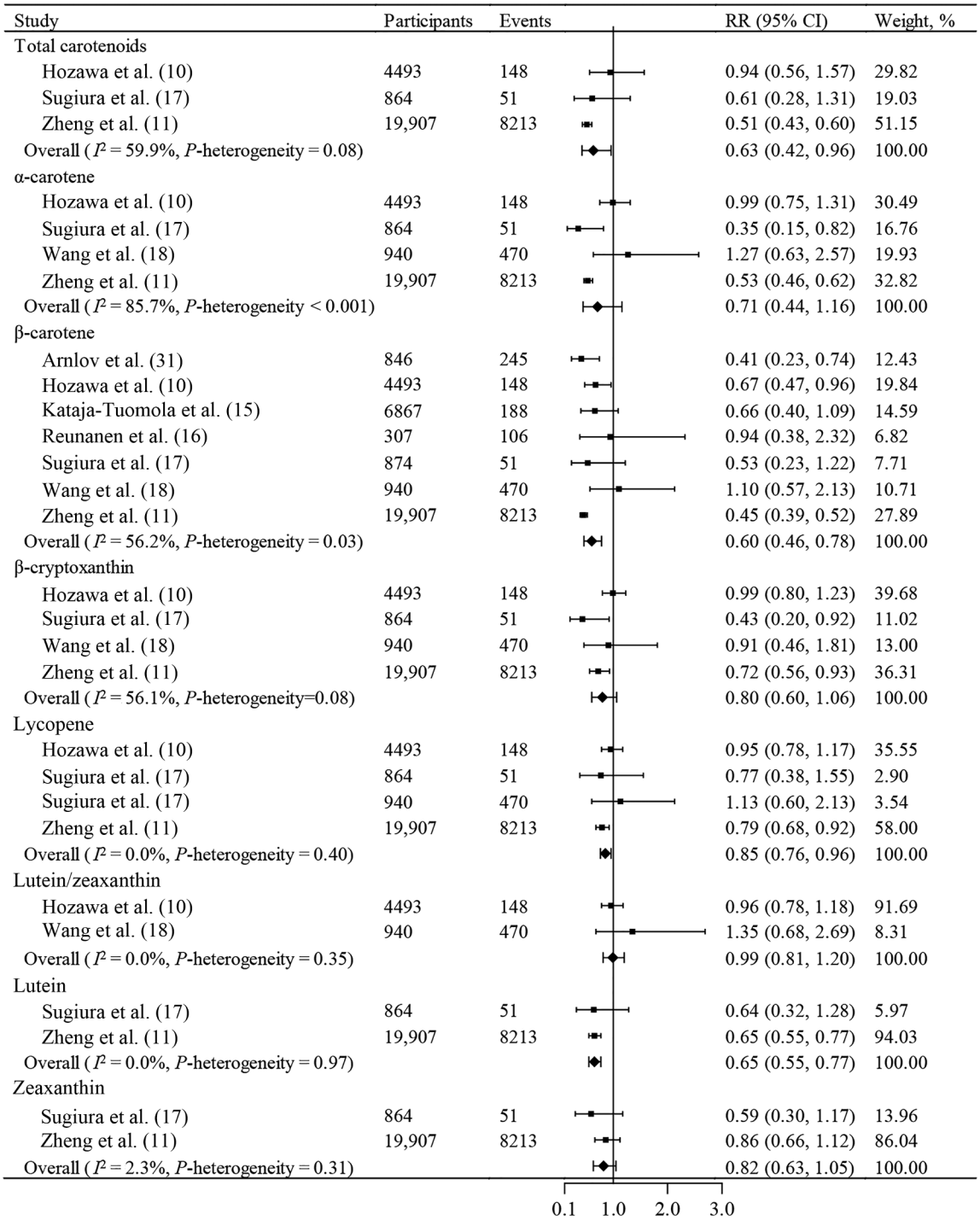

FIGURE 3 Meta-analyses of circulating carotenoids and risk of type 2 diabetes comparing the highest with the lowest categories. Summary estimate effects were calculated using a random-effects model.

cardiovascular disease, cancer, and all-cause mortality (7). However, prospective studies about carotenoids and other cardiometabolic conditions (such as insulin resistance, hyperglycemia, and metabolic syndrome) are lacking. A meta-analysis of 11 cross-sectional studies published in 2019 found that each SD increment of serum total carotenoids was related to 34% (95% CI: 22%, 44%) lower odds of metabolic syndrome (6). Similarly to our results, this meta-analysis found inverse associations for different types of carotenoids. However, the cross-sectional design of the included studies has compromised the causal inference.

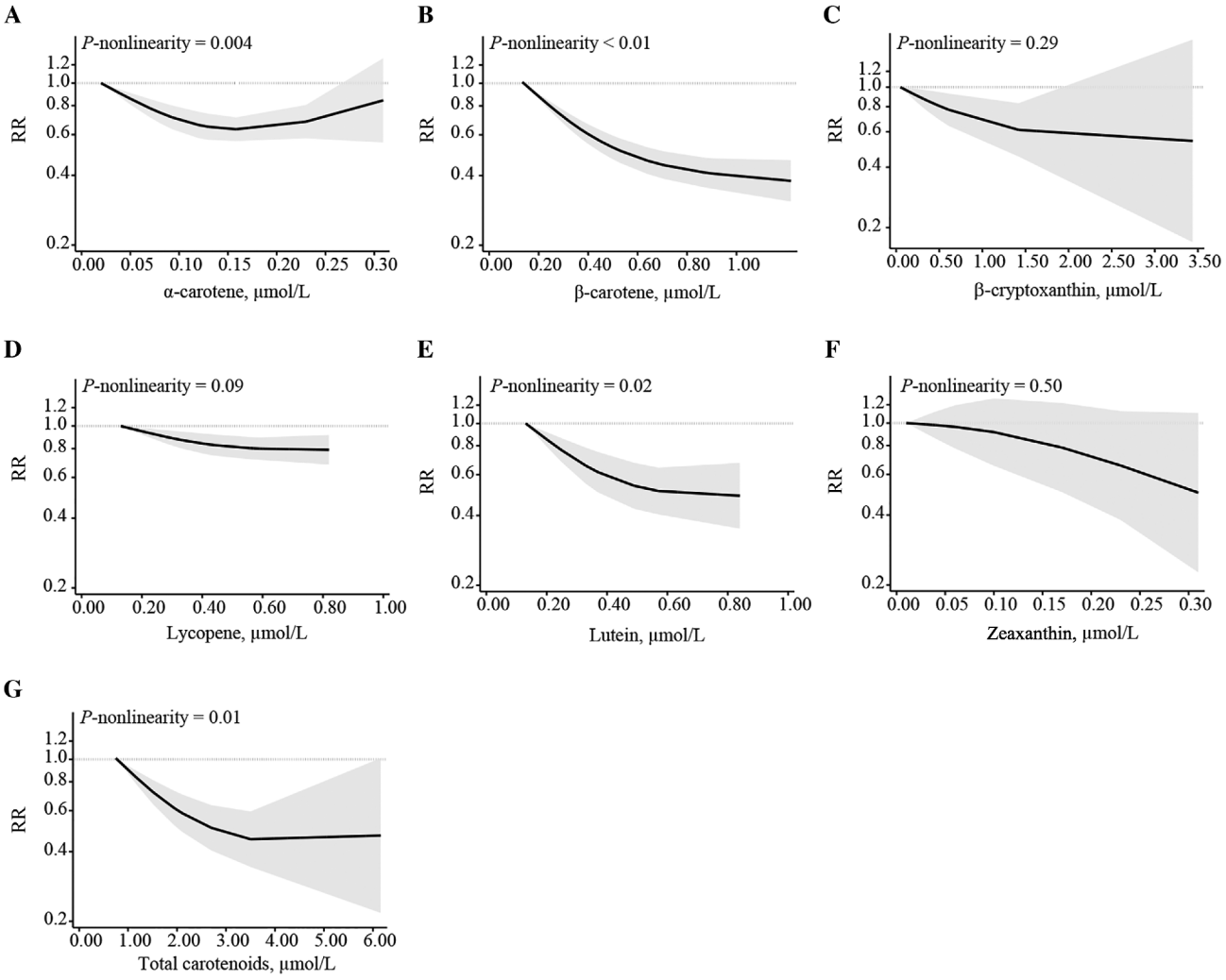

FIGURE 4 Nonlinear dose-response meta-analyses for circulating carotenoids and risk of type 2 diabetes using restricted cubic splines. Relations for circulating concentrations of (A) α-carotene, (B) β-carotene, (C) β-cryptoxanthin, (D) lycopene, (E) lutein, (F) zeaxanthin, and (G) total carotenoids. Solid lines represent the fitted nonlinear trend and lines with long dashes represent the pointwise 95% CIs.

Our findings did not reveal consistent associations between dietary intakes and circulating concentrations for some carotenoids. For example, no significant association was found for dietary intakes of lycopene, but a significantly inverse association was found for circulating concentrations of lycopene. One of the possible reasons may be that, compared with dietary intake, circulating concentrations reflect many more factors that influence bioavailability of carotenoids, including food processing, lipid co-consumption, interactions with smoking or alcohol consumption, body composition, variation in relevant genes, and so on (33). Therefore, the blood concentrations may better reflect the carotenoids that directly participated in human physiological processes and metabolism in the circulation. Moreover, measurement error and recall bias may exist for dietary assessment, which may obscure the relation between intakes of carotenoids and T2D. Previous studies have found that the correlations between dietary and circulating concentrations for different carotenoids varied across studies and were relatively low in general (correlation coefficients $\leq$ 0.50 in many studies) (34–38). Hence, it seemed unlikely that the discrepancy of findings for dietary intakes and circulating concentrations of carotenoids was explained by the low correlations.

Carotenoids are a group of natural antioxidants. Previous mechanism studies have indicated that oxidative stress could lead to insulin resistance and apoptosis of pancreatic β cells through the NF-κB pathway and various protein kinase pathways (39, 40). Therefore, dietary antioxidant capacity may be considered to play a role in the inverse association between carotenoids and risk of T2D. In accordance with this hypothesis, epidemiological studies among humans have found inverse associations between dietary antioxidant capacity and fasting glucose concentration, serum insulin concentrations, and insulin resistance (HOMA-IR) (41–43). However, the antioxidant function of a specific carotenoid

itself may not completely explain the observed associations for carotenoids. For example, dietary β-carotene and lycopene both have high antioxidant properties with comparable bioavailability (44–46) and similar intake amounts (average intakes ranging from 0.1 to 16.8 mg/d across groups in different studies for lycopene and from 0.5 to 5.5 mg/d for β-carotene), but we observed a significant inverse relation with T2D for β-carotene intake and no significant association for lycopene intake. One reason for the potential disparity between β-carotene and lycopene could be due to the dietary sources. The main sources of lycopene are from tomato and derived products, whereas β-carotene derives from a wide range of fruits and vegetables, including all green vegetables, carrots, red bell peppers, oranges, sweet potatoes, and broccoli (3). In accordance with this hypothesis, previous cohort studies have found higher intake of vegetables and fruit was associated with a lower risk of T2D (47, 48), whereas no significant association was found for intakes of tomato products (32). It is probable that β-carotene could indicate an overall antioxidant capacity originating from a dietary pattern rich in fruits and vegetables, which could be related to a lower risk of T2D (49). In addition, β-carotene might interact with other components from fruits and vegetables in relation to a lower risk of T2D. Considering that nutrients from the same food sources could highly correlate with each other, we could not rule out the possibility that the observed association could be due to confounding factors from other nutrients in the foods that also are dietary sources of β-carotene. Although the EPIC (European Prospective Investigation into Cancer and Nutrition) study (Netherlands) consisting of 37,846 adults aged 21–70 y showed a significant inverse association of β-carotene intake with T2D risk after adjustment for other dietary factors including intakes of fiber and vitamin E (9), other studies did not fully adjust for those nutrients that may be correlated with β-carotene intake. Thus, more studies are needed to exclude the probability of confounding from other nutrients and phytochemicals.

Our study showed inconsistent results with previous clinical trials, which did not find a beneficial effect of β-carotene supplementation on prevention of T2D. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study showed no significant effect of 20 mg/d of β-carotene supplementation on prevention of T2D after a 6.1-y intervention among 29,133 male smokers (15). Similarly, 2 intervention studies in US health professionals (the Physicians' Health Study and the Women's Antioxidant Cardiovascular Study) also did not show significant beneficial effects of 50 mg β-carotene supplementation every other day on risk of incident T2D during 15 and 9.2 y of follow-up, respectively (19, 20). The discrepancies between these clinical trials and prospective observational studies may be explained by several reasons. First, as aforementioned, these trials did not treat diabetes as the primary endpoint and generalization of results from them to the general population with diverse characteristics should be cautious. Second, the dosage of β-carotene supplements used in the clinical trials (20 mg/d or 50 mg every other day) was much higher than those in most cohort studies (the estimated dose ranged from 0.5 to 5.5 mg/d across groups in different studies). Free radicals have been reported to play a dual biological function in the progression of many diseases, both harmful and beneficial (50, 51). Supplying too much in the way of carotenoids could have over-eliminated free radicals and led to adverse influences. Third, the natural forms of carotenoids from foods may vary in biological activity and health-promoting properties compared with the synthetic compounds used in supplements (52, 53). Fourth, the natural forms of carotenoids may accompany other antioxidants and nutrients from the food sources, which may have an interactive beneficial effect for the prevention of T2D.

Besides antioxidant capacity, carotenoids have been reported to play roles in regulating adiposity, including controlling adipogenesis, production of adipokines and inflammatory mediators, as well as regulating glucose/insulin homoeostasis (54, 55). In addition, gut microbiota might also be potential mediators between carotenoids and diabetes risk. The association between dysbiosis of gut microbiota and T2D has been well established (56). Studies have shown that supplementation of carotenoids contributed to gut immune homeostasis by directly regulating IgA production and further preventing the dysbiosis of intestinal flora (57). However, the exact mechanisms are unclear and multiple pathways could be involved.

Our meta-analysis has some strengths. First, this is the first meta-analysis that we know of to summarize the evidence for 6 major subtypes of carotenoids from prospective observational studies, which could enlarge the sample size and provide more statistical power to detect associations. Second, the prospective design of the included studies could minimize the possibility of recall bias. Moreover, most included studies had high quality with NOS scores $\geq$ 7. Third, we conducted analysis comparing the extreme groups to provide the possibility of association, as well as dose-response analysis to show the strength and shape of the dose–response relations. Finally, we analyzed the association with T2D for both dietary intakes and circulating concentrations of carotenoids, which provided comprehensive evidence for this topic.

Our study also had several limitations. First, many studies only had a single-time measurement of dietary intake or circulating concentrations, and thus the exposure estimates may not represent the long-term levels, especially for blood concentrations of carotenoids, whose half-lives were reported to be relatively short (1–11 d for carotenes, including α-carotene, β-carotene, and lycopene; 12–39 d for β-cryptoxanthin; and 15–76 d for lutein and zeaxanthin) (33). Moreover, measurement errors were also possible, particularly in the diet assessment. Second, unmeasured or residual confounding is possible considering that the included observational studies may have made inadequate adjustment for potential confounders. For example, most studies did not adjust for other vitamins or nutrients from fruit and vegetables and social-economic status, which may result in confounding bias for the associations. Third, we only included publications in English. Fourth, most studies

were performed in the United States or among European countries with mean age ≥ 50 y and mean BMI ≥25 kg/m^2 at baseline, which may limit the generalizability of the results to populations in other countries, younger adults, or those with lower BMI. Fifth, the sample size for some studies was relatively small and the number of studies for each exposure–outcome analysis was generally small, which limited the power to detect associations and the possibility for further exploration of effect modification, heterogeneity, and publication bias. Power analysis showed a statistical power >80% for both dietary intake and circulating concentration for β-carotene only. Hence, the interpretation of results for other carotenoids needs to be cautious. More powerful research for other carotenoids on this topic is still needed in the future.

In conclusion, we found that higher dietary intakes and circulating concentrations of total carotenoids, especially β-carotene, were associated with a lower risk of T2D. More studies are needed to confirm the causality and explore the role of foods rich in carotenoids in prevention of T2D.

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