Micronutrients and Diabetic Retinopathy: Evidence From The National Health and Nutrition Examination Survey and a Meta-analysis

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• PURPOSE: To investigate the associations between circulating micronutrients (vitamins A, C, D, E, and carotenoids) and risk of diabetic retinopathy (DR).
• DESIGN: Cross-sectional study and meta-analysis.
• METHODS: The cross-sectional study included 517 diabetic participants aged ≥40 years in the 2005-2006 National Health and Nutrition Examination Survey. Serum vitamin D was converted to liquid chromatography-tandem mass spectrometry-equivalent results, while other micronutrients were measured using high-performance liquid chromatography. Presence of DR was determined based on non-mydriatic fundus photographs. A meta-analysis was subsequently performed, which included relevant studies published from January 01, 1990 to December 31, 2020.
• RESULTS: Of the 517 included participants, DR was identified in 159 participants (25.17%). After adjusting for multiple confounders, only serum vitamin C was associated with a lower risk of DR (odds ratio [OR]: 0.60; 95% confidence interval [CI]: 0.38-0.95). A total of 35 studies were included in the subsequent meta-analysis. Comparing 1056 participants with DR to 920 controls, the pooled weighted mean difference (WMD) of vitamin C was –11.01 (95% CI: –19.35 to –2.67). Regarding vitamins D and E, the pooled WMD was –3.06 (95% CI: –5.15 to –0.96) and –3.03 (95% CI: –4.24 to –1.82), respectively. No associations were identified between DR and circulating vitamin A or carotenoids.
• CONCLUSIONS: Lower levels of circulating vitamins C, D, and E were found in DR patients than those without. More high-quality studies are required to assess the real effects of micronutrients on DR. (Am J Ophthalmol 2022;238: 141–156. © 2022 Elsevier Inc. All rights reserved.)

INTRODUCTION

According to the Global Burden of Disease Study, diabetic retinopathy (DR) was the only cause of blindness that demonstrated an increasing trend in age-standardized prevalence between 1999 and 2020, amidst a global decreasing trend for other causes of blindness.[1] With the improving socioeconomic status, longer life expectancy and accompanying increasing burden of diabetes mellitus (DM), it is estimated that 245 million people worldwide will have DR by 2045.[2] Intravitreal anti-vascular endothelial growth factor injections, laser treatment, and vitreous surgery are proven effective treatments for severe DR; however, they are invasive in nature and associated with a high cost, posing considerable financial and health care burden on the individual and society. Thus, finding effective methods to prevent or control DR in its early stages is a critical public issue, and efforts to curb its progression are of significant importance.

Diabetes mellitus is predominantly a metabolically-driven pathogenesis. Appropriate nutritional and dietary advice forms an essential part of DM management. Micronutrients, otherwise known as vitamins and minerals, also play an important role in the maintenance of metabolic homeostasis. While the number of people taking daily micronutrient supplements is rapidly rising, micronutrient supplementation has not been adopted in general DM management due to the paucity of evidence on its benefits and potential adverse effects.[3] It has been reported that micronutrients play a protective role against the development

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of DM and its complications by modulating insulin sensitivity and maintaining pancreatic β-cell function.[4,5] The pathophysiological mechanisms of DR have not been fully clarified, but the damages caused by chronic hyperglycemia involving activation of the polyol pathway, accumulation of advanced glycation end products, increasing oxidative stress, pathological activation of protein kinase C, and inflammation are well understood.[6] Among them, oxidative stress has been implicated as a key underlying mechanism of DR; thus, it is logical to hypothesize that antioxidant micronutrients may be protective against DR.[7,8] Furthermore, since micronutrients can be easily obtained through a well-balanced diet or additional nutritional supplementation, a comprehensive analysis of the association between micronutrients and DR may have significant practicality in guiding clinical practice and patient behavior.

The association between micronutrients and DR is unclear in the literature. Patients with DR were found to have lower serum vitamin D levels, based on three previous meta-analyses,[15-17] whilst another systematic review reported no association between vitamin C or E and DR.[14] Most previous studies have focused on 1 or 2 micronutrients based on a small sample size and used questionnaires to assess daily micronutrient intake.[9-14] Compared with questionnaires, serum or plasma levels of micronutrients are less vulnerable to recall bias and can better reflect actual individual micronutrient levels.

Therefore, this study aimed to investigate the associations between multiple serum micronutrients (vitamins A, C, D, E, and carotenoids) and DR using a large representative sample of the US general population. A related systematic review and meta-analysis was also performed to comprehensively summarize current understanding of the associations between micronutrients and DR.

METHODS

• **DATA SOURCE:** This study was conducted based on the National Health and Nutrition Examination Survey (NHANES), which is a continuous series of cross-sectional surveys administered biennially by the National Center for Health Statistics of the Centers for Disease Control and Prevention.[18] A stratified, multistage probability sampling design was used to randomly select participants from the non-institutionalized US civilian population to collect data on their health and nutritional status. Personal interviews, physical examinations, and laboratory tests were conducted in every cycle of the cross-sectional survey. Details of the NHANES design and protocol can be found elsewhere.[18] The NHANES protocol is reviewed and approved by the National Center for Health Statistics ethics review board annually, and written informed consent was obtained from all participants. The survey adhered to the tenets of the Declaration of Helsinki.

• **STUDY POPULATION:** The present study included diabetic participants with available retinal imaging data from the NHANES 2005-2006. Diabetes was defined based on self-reported DM diagnosis, self-reported insulin or anti-hyperglycemic medication usage, or meeting any laboratory-confirmed diagnostic criteria for DM.[19,20] The diagnostic thresholds for DM were 7.0 mmol/L, 11.1 mmol/L, and 6.5% for fasting plasma glucose value after fasting $\geq$ 8 hours, random blood sugar level 2 hours post-glucose tolerance test, and glycosylated hemoglobin A1c (HbA1c), respectively, based on the 2020 Standards of Medical Care in Diabetes.[21]

• **ASSESSMENT OF DIABETIC RETINOPATHY:** Fundus photography examinations were performed for participants aged $\geq$40 years and without blindness, ocular infections, or eye patches on both eyes.[22] Two 45° non-mydriatic digital fundus photographs, 1 centered on the macula and the other on the optic nerve head, were obtained from both eyes using the Canon Non-Mydriatic Retinal Camera CR6-45NM (Canon, Tokyo, Japan). All images were graded by the grading team of the University of Wisconsin, Madison. The level of retinopathy was defined according to the NHANES Grading Protocol.[23] A severity level of 10 to 13 was categorized as absence of retinopathy, and level $\geq$ 14 was categorized as presence of retinopathy. Furthermore, the severity of retinopathy was classified as mild non-proliferative diabetic retinopathy (NPDR), moderate or severe NPDR, and proliferative diabetic retinopathy (PDR), corresponding to graded levels of 14-31, 41-51, and 60-80, respectively. The eye with more severe retinopathy was included in the current analysis if binocular retinal images were available. Diabetic retinopathy was defined based on presence of retinopathy on retinal images and a diagnosis of DM.

• **ASSESSMENT OF SERUM MICRONUTRIENTS:** Blood samples were collected from the study participants at a mobile examination center (MEC) and transported to the Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention for analysis. Serum levels of vitamins A, C, E, α-carotene, trans-β-carotene, cis-β-carotene, and combined lutein/zeaxanthin were measured using isocratic high-performance liquid chromatography. Serum levels of vitamin D (25-hydroxyvitamin D) were measured using the DiaSorin RIA kit (Stillwater, MN, USA) and converted to liquid chromatography-tandem mass spectrometry equivalent data for analysis. More details of serum assessments can be found at https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/labmethods.aspx?BeginYear=2005. The serum cut-off value for individual vitamin deficiency for vitamins A, C, D, and E was 0.7 μmol/L,[24] 11.4 μmol/L,[25] 50 nmol/L,[26] and 9 μmol/L,[25] respectively, according to previous reports. Due to the inconsistent definition of carotenoid deficiency

in the literature, the 10th percentiles of serum α-carotene (0.836 ug/dL), trans-β-carotene (4.12 ug/dL), and combined lutein/zeaxanthin (7.23 ug/dL) distributions for participants aged >40 years in the 2005-2006 NHANES were used as the cut-off values.[27] Given that the 10th percentile of serum cis-β-carotene distribution was less than the limit of detection, cis-β-carotene deficiency was not analyzed in this study.

• **COVARIATES:** Demographic and socioeconomic data were obtained from personal interviews, including: age, gender, race/ethnicity (non-Hispanic White, non-Hispanic Black, Mexican American, or other), education level (less than high school, with high school diploma, or more), poverty income ratio (< 1.0, ≥ 1.0), marital status (unmarried/other, married/with a partner), and health behavior information on smoking status (never, former, or current) and alcohol consumption (never/former, current). Weight, height, blood pressure, and total cholesterol were measured at MEC or participants' houses. Being overweight/obese was determined if the body mass index was >25 kg/m^2, which was calculated by weight in kg divided by height in m^2. Duration of DM was calculated as the reported age at screening minus the age of the subject when first told he/she had DM, and further divided into two groups: < 10 and ≥ 10 years. Insulin treatment was collected from interview data. Hypertension was defined as self-reported hypertension, usage of anti-hypertensive drugs, or a mean systolic blood pressure $\geq$ 140 mmHg and/or a mean diastolic blood pressure $\geq$ 90 mmHg after 3 to 4 measurements. History of cardiovascular disease was based on self-reported history of congestive heart failure, coronary heart disease, angina pectoris, heart attack, or stroke.

• **STATISTICAL ANALYSES:** Statistical analyses were performed by Stata (version 15.0; StataCorp, College Station, Texas, USA). According to the NHANES Tutorials,[28] full sample 2-year MEC exam weight, strata, and primary sampling units were used to account for the complex sampling design, oversampling, and non-response in the NHANES. All analyses were performed based on the SVY commands. Characteristics of participants were reported using means and standard errors (SE) for continuous data, and unweighted counts and weighted percentages for categorical data. The SEs for all estimates were obtained using the Taylor series (linearization) method. The *t* test was used for continuous data when comparing participants with and without DR, while the design-adjusted Rao-Scott Pearson χ^2 test was used for categorical data. A two-sided *P* -value $< .05$ was considered statistically significant.

Separate logistic regression analyses were used to explore the associations between each micronutrient and DR in diabetic participants. The micronutrients were analyzed as continuous and categorical variables separately. Model 1 adjusted for age, gender, and race/ethnicity. For lipid-soluble micronutrients (vitamins A, D, E, and carotenoids), the total serum cholesterol was also adjusted. Model 2 further adjusted for education level, poverty income ratio, marital status, smoking status, alcohol consumption, overweight/obesity, duration of DM, insulin treatment, HbA1c, hypertension, and cardiovascular disease. Age, HbA1c, and total serum cholesterol were added into the models as continuous variables, and the remaining as categorical variables. Separate ordinal logistic regression analyses were further conducted to explore the associations between each micronutrient and DR severity. Adjusted covariates were the same as the aforementioned variates. Subgroup analyses were stratified by gender (female/male), race (non-Hispanic, White/other), duration of DM ($\geq$ 10 years/$<$ 10 years), insulin treatment (yes/no), and glycemic control (good/poor). Good glycemic control was defined as HbA1c $< 7\%$.[29] The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for each 1 standard deviation (SD) increase in the serum level of micronutrients or for those with micronutrient deficiency compared with micronutrient sufficiency.

• **SYSTEMATIC REVIEW AND META-ANALYSIS:** The meta-analysis was reported based on the preferred reporting items for systematic review and meta-analysis (PRISMA) guideline.[30] The literature review and data extraction were conducted independently by two researchers (R.X and X.H), and any disagreements were resolved by consultations with a senior researcher (W.W).

A systematic literature search was conducted based on the MEDLINE and EMBASE datasets of studies reporting the associations between DR and micronutrients published from January 01, 1990 to December 31, 2020 with the limitations of English language and human subjects. Keywords included "micronutrients; vitamin A; ascorbic acid; vitamin D; vitamin E; carotenoids; carotene; lutein; zeaxanthin; diabetic retinopathy". The details of the search strategy are outlined in Supplemental Table 1. The reference lists of important reviews were also checked. Studies were included if they were cross-sectional or case-control in design, conducted on diabetic participants, and reported mean with SD for the comparison of serum or plasma levels of vitamins A, C, D, E, α-carotene, β-carotene, and combined lutein/zeaxanthin as the exposure of interest among participants with and without DR. The exclusion criteria included: (1) randomized clinical trials, cohort studies, case reports, reviews, letters, comments and abstracts of meetings; (2) studies that only considered dietary or supplementary micronutrients as exposure; (3) studies that only conducted comparison among participants with DR and healthy controls; and (4) studies with insufficient data. Unpublished studies, except for the present analysis, were also excluded. If the same dataset had been published in more than one publication, only the one with the largest number of participants was included.

For each included study, data on the name of the first author, publication year, study design, age, gender, type of

DM, duration of DM, HbA1c, severity of DR, exposure, method used for exposure assessment, sample sizes in the group with and without DR, and mean with SD of each exposure in the two groups were extracted. For studies that reported mean with SD separately for diabetic participants with different severity of DR, the estimates were combined to obtain an overall estimate and then included in the final meta-analysis. Levels of micronutrients were converted into μmol/L or nmol/L.

Means and SDs extracted in each study were used to calculate the overall weighted mean difference (WMD) with 95% CI to evaluate the association between circulating levels of micronutrients and DR. A negative WMD indicated a lower level of micronutrient in participants with DR than those without, and a positive WMD indicated the opposite. Heterogeneity was assessed using the I^2 statistic. Random effects models were chosen due to I^2 values >50%, suggesting significant heterogeneity among studies. The risk of bias was analyzed using the Newcastle-Ottawa Quality Assessment Scale for case-control studies and the Agency for Healthcare Research and Quality methodology checklist for cross-sectional studies.[31] The Newcastle-Ottawa Quality Assessment Scale consists of three categories: selection, comparability, and exposure.[32] The quality of each study was assessed by the total number of awarded stars (0 to 9). The Agency for Healthcare Research and Quality methodology checklist contains 11 items, with "Yes", "No", or "Unclear" being assigned to each item to assess the quality of studies.[33] Potential publication biases were evaluated by the Egger's tests. Sensitivity analyses were conducted using the leave-one-out method. Statistical analyses were conducted using the Stata version 15.0.

RESULTS

• **STUDY POPULATION CHARACTERISTICS:** Among the 10,348 participants in the 2005-2006 NHANES survey, the following participants were further excluded: 7,292 aged <40 years, 2,377 with missing information on or without DM, and 162 with missing or ungradable retinal photography in both eyes. The remaining 517 diabetic participants were included in the present analysis (the weighted population size was 18,214,473). Retinopathy was identified in 159 participants (weighted prevalence: 25.17%), including 96 with mild NPDR, 50 with moderate/severe NPDR, and 13 with PDR. The mean age of the participants was 60.9 ± 0.73 years, and 263 were male (weighted percentage: 48.7%). Table 1 shows the characteristics of included participants with different DR statuses. Compared with those without DR, DR participants were more likely to be non-Hispanic Black, less educated, use insulin, have longer duration of DM, and higher level of HbA1c (all with $P < .05$). Mean serum level of vitamin C was significantly lower in participants with DR than those without (43.4 ± 2.17 μmol/L vs 50.2 ± 1.75 μmol/L; $P = .031$). A higher rate of vitamin C deficiency was found in participants with DR than those without (10.56% vs 3.83%; $P = .017$). A lower rate of α-carotene or trans-β-carotene deficiency was found in participants with DR (7.57% vs 15.18%; $P = .019$ for α-carotene; 11.10% vs 19.57%; $P = .016$ for trans-β-carotene). No significant differences were observed for other demographic, socioeconomic, health-related characteristics, or levels of other serum micronutrients.

• **MICRONUTRIENTS AND DIABETIC RETINOPATHY:** The associations between each serum micronutrient and DR evaluated by multivariate logistic regression analyses are demonstrated in Table 2. Model 1 showed that serum vitamin C was significantly associated with a lower risk of DR (OR: 0.71, 95% CI: 0.54-0.92), whereas the associations of serum vitamins A, D, E, α-carotene, trans-β-carotene, cis-β-carotene, and combined lutein/zeaxanthin with DR were insignificant. After further adjustment for other factors in Model 2, the significant association between serum vitamin C and DR persisted, representing a 40% reduction in the DR risk for every 1 SD increase in serum level of vitamin C (OR: 0.60, 95% CI: 0.38-0.95). Moreover, participants with vitamin C deficiency had a 7-fold higher risk of DR than those without (OR: 7.41, 95% CI: 1.43-38.34). Table 3 shows the associations between each serum micronutrients and DR severity. A significant association was found between vitamin C and the severity of DR (OR: 0.72, 95% CI: 0.56-0.93 in Model 1; OR: 0.65, 95% CI: 0.46-0.93 in Model 2). Results of the subgroup analyses are summarized in Supplemental Table 2. The protective effect of vitamin C against DR was significant in males, non-Hispanic White participants, and participants with either good or poor glycemic control, but not in other subgroups.

• **ELIGIBLE ARTICLES FOR META-ANALYSIS:** Overall, 2411 potentially relevant articles were identified in the initial search. After screening of titles, abstracts, and full texts, 34 articles were eligible for inclusion (Figure 1).[15,34-66] Thirteen of them reported the effect sizes for circulating vitamin C,[34,35,38,39,42,47,48,59-64] 17 for vitamin D,[15,37,40,43-46,48-52,54-58] 6 for vitamin E,[35,36,48,62-64] 4 for vitamin A,[35,36,65,66] and 2 for carotenoids.[41,53]

• **CHARACTERISTICS OF THE STUDIES INCLUDED IN THE META-ANALYSIS:** Characteristics of the included articles are summarized in Table 4. Twenty-three studies were conducted on Asians, ten on Caucasians, and one on Africans. The number of participants with and without DR ranged from 10 to 1407 and 20 to 1738, respectively. The age of participants with and without DR ranged from 28 to 69 and 13 to 68 years, respectively. One study included only males, while the remaining included both genders. The duration of DM among participants with DR ranged from 7 to 27 years, and the HbA1c level ranged from 6.7% to 12.3%. Among participants without DR, the range of DM duration

TABLE 1. Characteristics of Participants Without and With Diabetic Retinopathy in The 2005-2006 National Health and Nutrition Examination Survey

Characteristic	Overall ($N = 517$)	Without DR ($N = 358$)	With DR ($N = 159$)	*P*-value
Mean age (SE), yrs.	60.9 (0.73)	60.3 (0.84)	62.7 (1.24)	.108
Sex, No. (%)				
Men	263 (48.7)	182 (47.9)	81 (51.1)	.517
Women	254 (51.3)	176 (52.1)	78 (48.9)	
Race, No. (%)				
Non-Hispanic White	222 (69.3)	172 (73.1)	50 (58.0)	**.038**
Non-Hispanic Black	150 (14.7)	88 (12.1)	62 (22.4)	
Mexican American	116 (8.08)	78 (7.22)	38 (10.7)	
Other	29 (7.95)	20 (7.60)	9 (8.98)	
Education, No. (%)				
$<$ High school	190 (23.8)	117 (19.9)	73 (35.3)	**.018**
$\geq$ High school	327 (76.3)	241 (80.2)	86 (64.7)	
Poverty income ratio, No. (%)				
At or above poverty line ($\geq$1.00)	72 (22.2)	55 (24.1)	17 (16.5)	.079
Below poverty line ($<$1.00)	417 (77.8)	286 (75.9)	131 (83.5)	
Marital status, No. (%)				
Unmarried or other	200 (33.1)	143 (34.8)	57 (28.1)	.309
Married or living with a partner	317 (66.9)	215 (65.2)	102 (71.9)	
Smoker, No. (%)				
Never	227 (46.3)	148 (43.3)	79 (55.3)	.243
Former	200 (35.4)	142 (37.4)	58 (29.4)	
Current	89 (18.3)	67 (19.3)	22 (15.4)	
Alcohol consumption, No. (%)				
Never/Former	206 (37.8)	141 (37.1)	65 (39.6)	.745
Current	295 (62.3)	205 (62.9)	90 (60.4)	
Overweight/Obesity, No. (%)				
No	75 (12.8)	52 (13.2)	23 (11.8)	.694
Yes	437 (87.2)	301 (86.8)	136 (88.2)	
Duration of diabetes, No. (%)				
$<$ 10 years	192 (59.5)	151 (76.2)	41 (27.9)	**<**
$\geq$ 10 years	154 (40.5)	56 (23.8)	98 (72.1)	**.001**
Insulin treatment, No. (%)				
No	421 (82.6)	326 (91.9)	95 (55.1)	**<**
Yes	96 (17.4)	32 (8.13)	64 (44.9)	**.001**
Mean HbA1c (SE), %	6.84 (0.11)	6.56 (0.11)	7.65 (0.19)	**< .001**
Mean total cholesterol (SE), mg/dL	192 (3.53)	192 (3.48)	190 (5.71)	.619
Hypertension, No. (%)				
No	148 (30.4)	112 (31.7)	36 (26.1)	.287
Yes	357 (69.6)	241 (68.3)	116 (73.9)	
Cardiovascular disease, No. (%)				
No	363 (73.1)	264 (76.0)	99 (64.5)	.072
Yes	154 (26.9)	94 (24.0)	60 (35.5)	
Mean Vitamin A (SE), μmol/L	2.25 (0.03)	2.24 (0.05)	2.27 (0.10)	.838
Mean Vitamin C (SE), μmol/L	48.4 (1.44)	50.2 (1.75)	43.4 (2.17)	**.031**
Mean Vitamin D (SE), nmol/L	52.9 (1.03)	53.3 (1.30)	51.7 (2.12)	.549
Mean Vitamin E (SE), μmol/L	31.5 (1.06)	32.0 (1.01)	30.1 (1.54)	.116
Mean α-carotene (SE), μmol/L	0.06 (0.006)	0.06 (0.006)	0.06 (0.007)	.421
Mean trans-β-carotene (SE), μmol/L	0.26 (0.02)	0.26 (0.02)	0.26 (0.02)	.999
Mean cis-β-carotene (SE), μmol/L	0.02 (0.0009)	0.02 (0.0011)	0.02 (0.0009)	.278
Mean lutein and zeaxanthin (SE), μmol/L	0.29 (0.01)	0.28 (0.01)	0.32 (0.03)	.147
Vitamin A deficiency, No. (%)				
No	476 (99.28)	329 (99.16)	147 (99.64)	.513
Yes	3 (0.72)	2 (0.84)	1 (0.36)	

(*continued on next page*)

TABLE 1. (*continued*)

Characteristic	Overall ($N = 517$)	Without DR ($N = 358$)	With DR ($N = 159$)	*P*-value
Vitamin C deficiency, No. (%)				
No	448 (94.44)	315 (96.17)	133 (89.44)	**.017**
Yes	33 (5.57)	17 (3.83)	16 (10.56)	
Vitamin D deficiency, No. (%)				
No	227 (55.55)	171 (57.73)	56 (49.17)	.251
Yes	261 (44.45)	167 (42.27)	94 (50.83)	
Vitamin E deficiency, No. (%)				
No	478 (99.67)	331 (100.00)	147 (98.71)	.088
Yes	1 (0.33)	0 (0.00)	1 (1.29)	
α-carotene deficiency, No. (%)				
No	421 (86.76)	289 (84.82)	132 (92.43)	**.019**
Yes	58 (13.24)	42 (15.18)	16 (7.57)	
Trans-β-carotene deficiency, No. (%)				
No	409 (82.59)	278 (80.43)	131 (88.90)	**.016**
Yes	70 (17.41)	53 (19.57)	17 (11.10)	
Lutein and zeaxanthin deficiency, No. (%)				
No	438 (86.94)	300 (85.72)	138 (90.50)	.432
Yes	41 (13.06)	31 (14.28)	10 (9.50)	

Abbreviations: DR = diabetic retinopathy, SE = standard error

TABLE 2. Association Between Antioxidant Micronutrients and Diabetic Retinopathy in The 2005-2006 National Health and Nutrition Examination Survey

Micronutrient	Per SD increase				Deficiency or not			
	Model 1*		Model 2**		Model 1*		Model 2**	
	OR	*95% CI*	*OR*	*95% CI*	*OR*	*95% CI*	*OR*	*95% CI*
Vitamin A	1.02	0.70-1.49	0.91	0.60-1.38	0.51	0.03-7.77	-	-
Vitamin C	**0.71**	**0.54-0.92**	**0.60**	**0.38-0.95**	**3.15**	**1.17-8.48**	**7.41**	**1.43-38.34**
Vitamin D	0.99	0.71-1.38	1.00	0.58-1.73	1.23	0.68-2.21	1.06	0.40-2.80
Vitamin E	0.84	0.66-1.07	0.78	0.56-1.09	-	-	-	-
α-carotene	0.88	0.67-1.16	0.75	0.49-1.14	0.47	0.24-0.91	0.62	0.19-2.06
trans-β-carotene	0.94	0.68-1.31	1.10	0.71-1.69	0.55	0.29-1.05	0.57	0.26-1.22
cis-β-carotene	0.80	0.54-1.19	0.79	0.45-1.38	-	-	-	-
Lutein and zeaxanthin	1.18	0.98-1.42	0.92	0.63-1.34	0.81	0.24-2.71	2.39	0.25-22.62

Abbreviations: CI = confidence interval, DR = diabetic retinopathy, OR = odds ratio, SD = standard deviation

*Model 1: adjusted for age, gender, race, and total cholesterol (except vitamin C)

**Model 2: adjusted for age, gender, race, and total cholesterol (except vitamin C), education, marital status, poverty, smoking status, alcohol consumption, overweight/obesity, duration of diabetes, insulin treatment, HbA1c, hypertension, and cardiovascular disease

and HbA1c was 3 to 14 years and 5.7% to 9.8%, respectively. Twenty-nine studies described associations between micronutrients and DR in participants with type 2 or non-insulin dependent DM, two in participants with type 1 DM, and the remaining three in any type of DM. In total, 24 studies directly reported the effect sizes for DR, whereas 10 studies separately reported different severity of DR. Presence of DR was confirmed based on fundus photography in 7 studies and fluorescein angiography in 1 study, while DR assessment was based on fundoscopy, ophthalmoscopy, or ophthalmologist judgment in other studies.

• **SYSTEMIC REVIEW:** Ten of 13 studies reported significantly lower vitamin C levels in participants with DR compared with those without,[34,38,39,47,48,59,60,62-64] and the other three reported insignificant differences.[35,42,61] Regarding vitamin D, 8 of 17 reported significantly lower levels in those with DR,[15,40,45,48,49,54-56] while the remaining 9 indicated comparable findings.[37,43,44,46,50-52,57,58] Five of 6 studies reported that participants with DR had significantly lower serum levels of vitamin E;[35,48,62-64] the remaining 1 found no significant association.[36] With respect to vitamin A, 2 of 4 studies reported significantly lower vitamin A lev-

TABLE 3. Association Between Antioxidant Micronutrients and Severity of Diabetic Retinopathy in The 2005-2006 National Health and Nutrition Examination Survey

Micronutrient	Model 1*		Model 2**	
	OR	*95% CI*	*OR*	*95% CI*
Vitamin A, per SD	1.06	0.73-1.55	1.16	0.80-1.68
Vitamin C, per SD	**0.72**	**0.56-0.93**	**0.65**	**0.46-0.93**
Vitamin D, per SD	0.98	0.71-1.34	0.87	0.57-1.32
Vitamin E, per SD	0.83	0.66-1.04	0.77	0.53-1.12
α-carotene, per SD	0.87	0.67-1.14	0.67	0.42-1.07
trans-β-carotene, per SD	0.95	0.69-1.30	0.93	0.61-1.42
cis-β-carotene, per SD	0.82	0.55-1.21	0.75	0.46-1.24
Lutein and zeaxanthin, per SD	1.11	0.97-1.28	0.83	0.62-1.09

Abbreviations: CI = confidence interval, DR = diabetic retinopathy, OR = odds ratio, SD = standard deviation

*Model 1: adjusted for age, gender, race, and total cholesterol (except vitamin C)

**Model 2: adjusted for age, gender, race, and total cholesterol (except vitamin C), education, marital status, poverty, smoking status, alcohol consumption, overweight/obesity, duration of diabetes, insulin treatment, HbA1c, hypertension, and cardiovascular disease

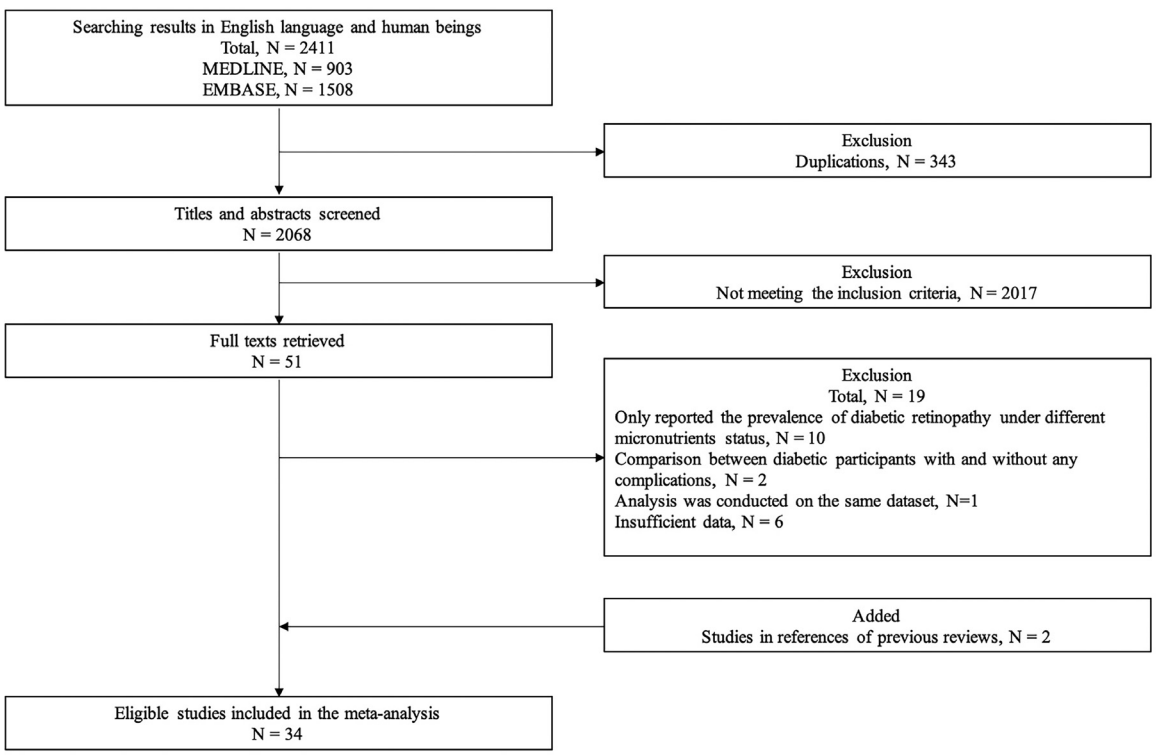

FIGURE 1. Overview of the research strategy.

els in participants with DR,[35,65] while the other 2 reported the opposite.[36,66] One of 2 studies illustrated that participants with DR had higher plasma levels of α-carotene compared with those without,[41] while the other reported significantly lower serum levels of α-carotene in participants with DR in non-smokers.[53] Other carotenoid levels were not significantly different in the two groups.[41,53]

• **META-ANALYSIS RESULTS:** Results of the meta-analysis are displayed in Figure 2. Fourteen studies (including the 13 previous studies and the current study) investigating vitamin C and DR were included in the meta-analysis. The numbers of participants with and without DR were 1056 and 920, respectively. The pooled WMD of vitamin C between the two groups was –11.01 (95% CI: –19.35 to –2.67). Significantly lower serum levels of vitamin D were detected in the 4617 participants with DR, compared with the 6794 participants without DR (WMD: –3.06, 95% CI: –5.15 to –0.96). As for vitamin E, 7 studies were analyzed. The sample sizes of participants with and without DR were 469 and 705, respectively. Significantly decreased vitamin E levels were observed in patients with DR (WMD: –3.03, 95% CI: –4.24 to –1.82). Significant heterogeneity was observed with an I^2 of 97.8%, 77.1%, and 85.1% for vitamins C, D, and E, respectively. No significant differences were observed for vitamin A, α-carotene, β-carotene, and combined lutein/zeaxanthin.

• **ASSESSMENT OF RISK OF BIAS AND PUBLICATION BIAS:** Risk of bias assessment was performed and summarized in Supplement Table 3 and Supplement Table 4. The significance of identified associations remained in the leave-one-out analyses (Supplemental Figure 1, Supplemental Figure 2, Supplemental Figure 3, and Supplemental Figure 4). Egger's tests confirmed no clear evidence of publication bias ($P > .05$). As fewer than 5 studies were included in the meta-analysis for carotenoids, sensitivity analysis and Egger's tests were not performed.

DISCUSSION

This cross-sectional study found that a higher serum vitamin C level was associated with a lower risk of DR in the diabetic population. No significant associations were observed between DR and serum vitamins A, D, E, or carotenoids. A meta-analysis confirmed the significantly lower circulating vitamin C levels in DR participants compared with controls. Lower serum vitamin D and vitamin E levels were also significantly associated with a higher DR risk in the meta-analysis.

The inverse association between circulating vitamin C and DR has been consistently reported in previous studies.[34,38,39,47,48,59,60,62-64] Null association between vitamin C and DR was concluded in a prior systematic review of six

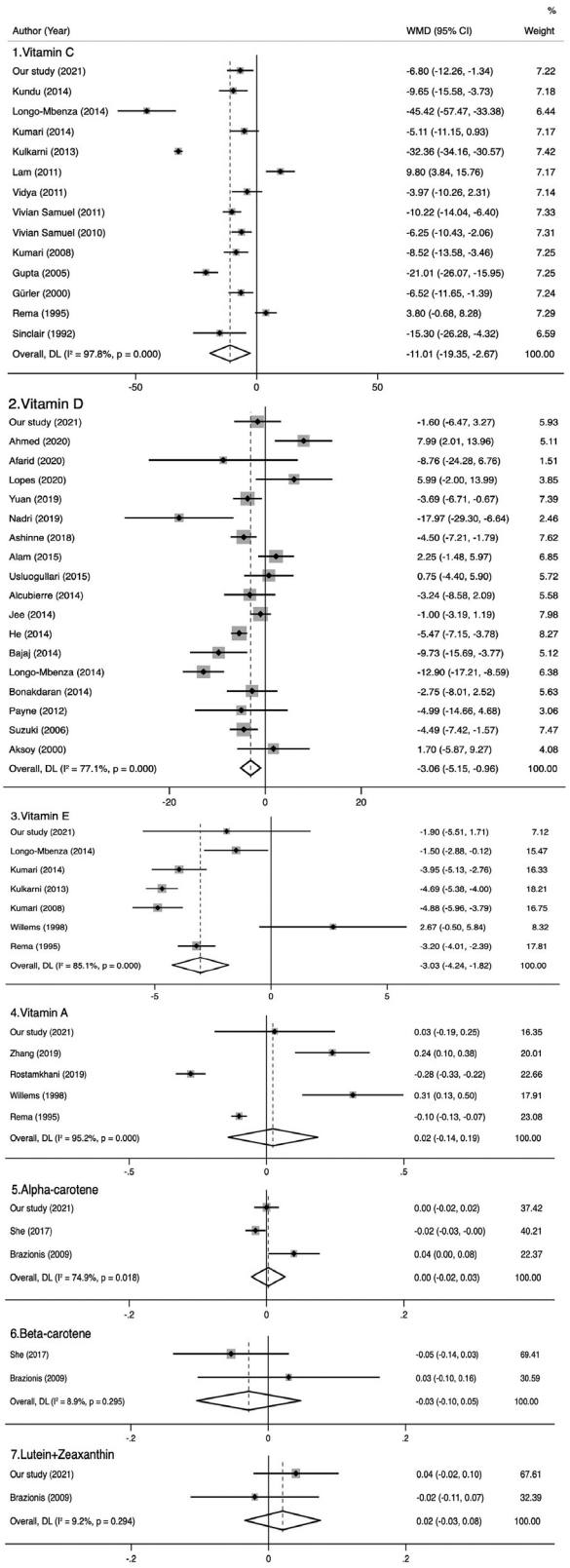

FIGURE 2. The overall weighted mean difference in circulating micronutrients between diabetic retinopathy participants and controls. Diamonds represent the pooled estimates from random effects analyses. CI = confidence interval.

TABLE 4. Characteristics of The Studies Included in This Meta-Analysis

Author	Year	Study design	Country	DM	Group	Sample	Age(years)*	Sex(M/F)	DM duration (year)*	HbA1c (%)*	Exposure	Exposure measurement	DR diagnosis
Sinclair	1992	Cross-sectional	UK	Type 2	DR	25	69.1 ± 8.0	12/13	9.2 ± 6.3	NA	Serum vitamin C	HPLC	Fundoscopy
					NDR	25	68.4 ± 7.5	13/12	10.1 ± 6.5	NA			
Rema	1995	Cross-sectional	Indian	NIDDM	DR	83	46 ± 6	NA	NA	11 ± 2.8	Plasma vitamins A, C and E	Colorimetric method	NA
					NDR	63	42 ± 9	NA	NA	9.6 ± 1.7			
Gürler	2000	Cross-sectional	Turkey	NIDDM	DR	25	54.6 ± 6.9	17/8	7.6 ± 2.9	9.1 ± 2.0	Serum vitamin C	DNPH	Ophthalmoscopy
					NDR	34	51.8 ± 7.7	21/13	3.7 ± 2.1	8.8 ± 1.5			
Gupta	2005	Case-control	Indian	NIDDM	NPDR	20	47 ± 6	9/11	11.2 ± 1.9	12.2 ± 1.4	Plasma vitamin C	Phosphotungstic acid	Ophthalmoscopy
					PDR	22	46 ± 5	10/12	11.9 ± 2.1	12.3 ± 1.2			
					NDR	40	40 ± 6	27/13	6.4 ± 2.3	9.3 ± 1.9			
Kumari	2008	Case-control	India	Type 2	DR	50	63.0 ± 4.8	NA	11.4 ± 1.8	NA	Serum vitamins C and E	DNPH, spectrophotometry	NA
					NDR	36	55.3 ± 6.5	NA	6.4 ± 1.8	NA			
Vivian Samuel	2010	Case-control	India	NIDDM	DR	30	49.5 ± 11.7	NA	NA	NA	Serum vitamin C	DNPH	NA
					NDR	30	50.6 ± 10.5	NA	NA	NA			
Vivian Samuel	2011	Case-control	India	NIDDM	DR	10	NA	NA	NA	NA	Serum vitamin C	DNPH	NA
					NDR	49	NA	NA	NA	NA			
Vidya	2011	Case-control	India	Type 2	DR	50	53.6 ± 9.3	NA	NA	8.3 ± 1.1	Plasma vitamin C	DNPH	NA
					NDR	25	48.6 ± 10.5	NA	NA	8.0 ± 1.4			
Lam	2011	Cross-sectional	China	Type 2	BDR	161	58.2 ± 10.6	NA	8.7 ± 6.3	7.5 ± 1.3	Plasma vitamins C and E	Comet assay	Mydriatic fundus photography
					NPDR	207	59.6 ± 9.1	NA	8.8 ± 6.1	7.6 ± 1.3			
					PDR	6	66.8 ± 12.7	NA	8.2 ± 10.8	7.5 ± 0.3			
					NDR	46	59.9 ± 11.9	NA	7.9 ± 5.8	7.6 ± 1.3			
Kulkarni	2013	Case-control	India	DM	DR	50	NA	NA	NA	NA	Serum vitamins C and E	DNPH, spectrophotometry	NA
					NDR	50	NA	NA	NA	NA			
Kumari	2014	Case-control	India	Type 2	DR	42	64.1 ± 6.2	NA	NA	NA	Serum vitamins C and E	DNPH, spectrophotometry	NA
					NDR	30	56.3 ± 6.3	NA	NA	NA			
Longo-Mbenza	2014	Case-control	Congo	Type 2	DR	66	53.4 ± 13.6	26/40	NA	9.8 ± 4.3	Serum vitamins C, D and E	HPLC	Ophthalmoscopy
					NDR	84	56.6 ± 12.4	39/45	NA	9.8 ± 4.3			
Kundu	2014	Cross-sectional	Indian	Type 2	DR	50	58.6 ± 9.1	36/14	15.4 ± 1.1	7.9 ± 1.0	Plasma vitamin C	DNPH	Ophthalmoscopy and fundus photography
					NDR	50	56.2 ± 9.9	39/11	4.8 ± 1.2	7.2 ± 1.0			
Aksoy	2000	Cross-sectional	Turkey	NIDDM	BDR	15	56.9 ± 9.8	7/8	7.4 ± 6.2	8.1 ± 2.1	Serum vitamin D	RIA	Ophthalmologists
					NPDR	14	57.1 ± 5.8	7/7	9.7 ± 4.5	8.4 ± 1.4			
					PDR	17	58.8 ± 6.1	9/8	12.1 ± 4.6	9.5 ± 3.9			
					NDR	20	57.1 ± 9.4	12/8	4.3 ± 5.2	6.5 ± 2.6			

(*continued on next page*)

TABLE 4. (*continued*)

Author	Year	Study design	Country	DM	Group	Sample	Age(years)*	Sex(M/F)	DM duration (year)*	HbA1c (%)*	Exposure	Exposure measurement	DR diagnosis
Suzuki	2006	Cross-sectional	Japan	Type 2	Simple DR	107	61.6 ± 11.5	317/264	11.8 ± 8.6	7.7 ± 1.4	Serum vitamin D	RIA	Ophthalmologists
					PDR	122							
					NDR	350							
Payne	2012	Cross-sectional	US	Type 2	NPDR	40	68.3 ± 10.0	21/19	18.9 ± 11.1	7.4 ± 1.2	Serum vitamin D	CL	Mydriatic fundus photography
					PDR	42	59.8 ± 12.0	21/21	22.0 ± 10.5	8.1 ± 1.9			
					NDR	41	62.4 ± 11.3	21/20	7.4 ± 7.8	7.5 ± 2.0			
Jee	2014	Cross-sectional	Korea	DM	DR	375	61.2 ± 11.6	206/169	11.0 ± 7.7	8.1 ± 0.1#	Serum vitamin D	RIA	Mydriatic fundus photography
					NDR	1,738	59.7 ± 12.5	945/793	6.9 ± 8.3	7.3 ± 0.0#			
Bonakdaran	2014	Cross-sectional	Iran	Type 2	NPDR	64	56.6 ± 7.1	13/51	7.4 ± 6.0	9.3 ± 1.5	Serum vitamin D	RIA	Ophthalmologists
					PDR	18	57.9 ± 7.3	2/16	9.2 ± 7.2	9.6 ± 2.1			
					NDR	153	54.2 ± 10.3	27/126	3.2 ± 4.2	8.4 ± 1.8			
Alcubierre	2014	Case-control	Spain	Type 2	DR	139	60.3 ± 8.9	71/68	13.9 ± 9.3	8.3 ± 1.4	Serum vitamin D	CL	Ophthalmologists
					NDR	144	58.1 ± 10.3	74/70	7.2 ± 5.5	7.3 ± 1.2			
He	2014	Cross-sectional	China	Type 2		562	58.9 ± 11.4	286/276	10.3 ± 7.1	8.9 ± 2.2	Serum vitamin D	CL	Non-mydriatic fundus photography
					NSTDR								
					STDR	333	60.7 ± 12.5	170/163	11.7 ± 7.3	9.0 ± 2.3			
					NDR	625	58.3 ± 11.4	317/308	8.3 ± 6.9	8.9 ± 2.7			
Bajaj	2014	Case-control	Indian	Type 2	DR	54	NA	NA	NA	NA	Serum vitamin D	NA	Ophthalmologists
					NDR	104	NA	NA	NA	NA			
Usluogullari	2015	Retrospective	Turkey	Type 2	DR	73	55.2 ± 10.9	296/261	5 ± 3.3	7.7 ± 1.7	Serum vitamin D	HPLC	Ophthalmoscopy
					NDR	238							
Alam	2015	Cross-sectional	UK	Type 1 and 2	BDR	243	58.8 ± 13.3	121/122	18.7 ± 11.7	8.6 ± 1.7	Serum vitamin D	CL	Medical record database
					NPDR	135	60.8 ± 10.9	65/70	21.0 ± 9.8	8.9 ± 1.6			
					PDR	22	55.1 ± 13.6	12/10	19.7 ± 10.0	8.9 ± 1.5			
					NDR	257	59.8 ± 13.8	131/126	11.3 ± 8.7	8.2 ± 1.6			
Ashinne	2018	Retrospective	India	Type 2	DR	1,407	56.7 ± 9.0	954/453	15.7 ± 7.5	9.1 ± 2.0	Serum vitamin D	LC-MS/MS	Ophthalmologists
					NDR	1,647	54.1 ± 11.0	934/713	8.4 ± 6.7	8.3 ± 2.0			
Nadri	2019	Cross-sectional	India	Type 2	NPDR	22	53.7 ± 6.9	16/6	9.8 ± 5.3	8.2 ± 2.0	Serum vitamin D	CL	Ophthalmologists
					PDR	22	53.6 ± 8.3	13/9	10.8 ± 4.6	8.8 ± 2.9			
					NDR	22	53.2 ± 5.9	9/13	7.3 ± 5.6	8.0 ± 2.4			
Yuan	2019	Cross-sectional	China	Type 2	DR	273	58.1 ± 10.8	143/130	NA	9.3 ± 4.0	Serum vitamin D	ECL	Fundus photography
					NDR	616	57.7 ± 11.5	362/254	NA	8.6 ± 4.0			
Lopes	2020	Retrospective	Portugal	Type 1	DR	103	46.7 ± 13.9	51/52	27.0 ± 10.9	8.5 ± 1.7	Serum vitamin D	Clinical data	Fundoscopy
					NDR	79	37.1 ± 13.2	35/44	11.6 ± 8.2	8.0 ± 1.7			
Ahmed	2020	Cross-sectional	Qatar	Type 2	DR	184	55 ± 10	227/233	NA	7.9 ± 1.8	Serum vitamin D	LC-MS/MS	Fundoscopy
					NDR	274							

(*continued on next page*)

TABLE 4. (*continued*)

Author	Year	Study design	Country	DM	Group	Sample	Age(years)*	Sex(M/F)	DM duration (year)*	HbA1c (%)*	Exposure	Exposure measurement	DR diagnosis
Afarid	2020	Cross-sectional	Iran	Type 2	DR	30	58.6 ± 5.8	15/15	NA	7.9 ± 2.3	Serum vitamin D	Solid phase ELISA	Ophthalmoscopy
					NDR	30	58.3 ± 7.2	14/16	NA	7.2 ± 1.6			
Willems	1998	Cross-sectional	Belgium	Type 1	DR	19	28 ± 6.5	60/50	20 ± 6.5	6.7 ± 1.3	Serum vitamins A and E	HPLC	Fluorescein angiography
					NDR	84	13 ± 6.4		4.7 ± 3.7	7.2 ± 1.6			
She	2017	Cross-sectional	China	Type 2	DR	272	65.3 ± 8.5	136 /136	12.7 ± 7.6	7.5 ± 1.8	Serum β-carotene and α -carotene	HPLC	Mydriatic fundus photography
					NDR	190	67.9 ± 7.2	80/110	14.4 ± 6.7	6.8 ± 1.2			
Brazionis	2009	Cross-sectional	Australia	Type 2	DR	33	65 ± 10.3	33/0	16 ± 10.3	8.8 ± 1.8	Plasma β-carotene, α -carotene and lutein+zeaxanthin	HPLC	Mydriatic fundus photography
					NDR	78	63 ± 6.8	78/0	9 ± 6.8	7.9 ± 1.6			
Zhang	2019	Case-control	China	Type 2	DR	43	57.9 ± 13.0	24/19	NA	NA	Serum vitamin A	HPLC	NA
					NDR	43	54.1 ± 16.1	20/23	NA	NA			
Rostamkhani	2019	Cross-sectional	Iran	Type 2	NPDR	20	64.1 ± 8.9	18/42	NA	NA	Serum vitamin A	HPLC	Ophthalmologists
					PDR	20	58.6 ± 8.4		NA	NA			
					NDR	20	58.4 ± 7.1		NA	NA			

*Presented as mean ± standard deviation

#Indicated the standard error

Abbreviations: BDR = background diabetic retinopathy, CL = chemiluminescence, DM = diabetes mellitus, DNPH = 2,4-dinitrophenyl hydrazine, DR = diabetic retinopathy, ECL = electrochemiluminescence, ELISA = enzyme-linked immunosorbent assay, F = female, HPLC = high-performance liquid chromatography, LC-MS/MS = liquid chromatography-tandem mass spectrometry, M = male, NA = not available, NDR = non-diabetic retinopathy, NIDDM = non-insulin dependent diabetes mellitus; NPDR = non-proliferative diabetic retinopathy; NSTDR = non-sight-threatening diabetic retinopathy; PDR = proliferative diabetic retinopathy, RIA = radioimmunoassay, STDR = sight-threatening diabetic retinopathy

studies,[14] whereas Tabatabaei-Malazy et al. reported similar inverse associations based on 10 studies in a more recent systematic review.[67] One study using data extracted from the NHANES III cycle found lower serum levels of vitamin C in DR participants than those without, after excluding users taking vitamin C supplements.[12] Levels of vitamin C in the vitreous, aqueous humor, and serum were all lower in patients with PDR vs controls after pars plana vitrectomy, suggesting significant oxidative stress and vitamin C depletion in the retina among DR patients.[68] In a study of 62 participants who received vitamin C supplementation 1 g/day for 6 weeks, the serum vitamin C levels increased during the first 3 weeks and decreased afterwards among diabetic participants, but remained stable in healthy controls.[69] The potential protective role of vitamin C on DR has been shown in various *in vivo* and *in vitro* studies.[70,71] One potential underlying mechanism is that vitamin C can directly act as an antioxidant agent via scavenging reactive oxygen species, preventing the breakdown of nitric oxide and decreasing lipid oxidation.[72] Alternative mechanisms include its effect on retinal blood flow via reducing platelet aggregation,[73] and prevention of retinal pericyte/endothelial cell apoptosis.[74]

No significant association was detected between serum vitamin D and DR in the current cross-sectional study, which is in line with some other observational studies.[37,43,44,46,50-52,57,58,75] In contrast, 4 meta-analyses, including the current one, demonstrated positive associations between vitamin D deficiency and DR in diabetic patients.[15-17] Yuan et al. found that the serum vitamin D level was 1.68 ng/mL lower in patients with DR than those without, and the risk of PDR in patients with vitamin D deficiency increased by 60% compared with those without.[15] They also suggested potential physiological mechanisms underlying this association, which include angiogenesis prevention,[76,77] inflammation mitigation in the retina,[78,79] and systemic effects on insulin secretion and function.[5] The inconsistent findings regarding the vitamin D-DR association in existing studies may be due to the lack of adjustments for DM severity or different HbA1c levels, as suggested by another NHANES study.[29] However, subgroup analyses in the current study failed to demonstrate this finding. Another possible explanation is that the risk of DR increases only when the serum vitamin D level reduces to a certain threshold, although a comparable rate of vitamin D deficiency was detected among participants with and without DR in the current analysis. Alternative explanations for the inconsistency include differences in the DR diagnosis criteria and serum vitamin D measurement methods, ethnic differences in vitamin D metabolism, differences in geographical locations, seasons or times of vitamin D measurement, and failure to adjust for potential confounders, such as the blood calcium concentration, diseases status, and vitamin D supplements. Therefore, although increasing evidences suggest that serum vitamin D may be a potential biomarker of DR, further investigations are needed to confirm the findings and reveal any dose-response relationship.

This study supports a previous systematic review[67] and 5 original studies[35,48,62-64] that found significantly lower vitamin E levels in patients with DR. In the current meta-analysis consisting of 7 studies, circulating vitamin E was 3.03 μmol/L lower in DR patients compared with those without DR. However, some studies, including a systematic review of 4 studies, demonstrated no significant association between circulating level of vitamin E and DR.[14,36,42,80] Beyond its antioxidant effect, vitamin E could normalize the retinal blood flow by inhibiting the hyperglycemia-induced activation of the protein kinase C pathway,[81] reducing the production of vascular endothelial growth factor and intercellular cell adhesion molecule-1.[82] However, it was noticed that the majority of studies reporting a protective effect of vitamin E on DR were conducted in one country (India) and the sample size for each study was limited; thus, it remains inconclusive whether vitamin E can be used as a biomarker for DR in different ethnic populations.

The association between circulating vitamin A and DR appears to be controversial. The levels of circulating vitamin A in patients with DR have been reported to be comparable, higher,[36,66] or lower[35,65] compared with patients without DR. The inconsistency may be attributed to differences in ethnicity, types of DM, or patient behaviors.[83] The association between circulating carotene and DR was also inconsistently reported. No significant associations between DR and α-carotene or β-carotene were observed, while another two cross-sectional studies reported significantly lower and higher levels of α-carotene in DR patients, respectively.[41,53] However, retinol-binding protein 4[84] and the ratio of the plasma levels of non-provitamin A carotenoids (lutein, zeaxanthin, lycopene) to provitamin A carotenoids (α-carotene, β-carotene, β-Cryptoxanthin)[41] were suggested to be predictors of DR. As previously demonstrated, vitamin A and its metabolism have an effect on the regulation of glucose and lipid metabolism,[85-87] antioxidant,[88] anti-inflammation,[89] and the retardation of neovascularization and retinal pigment epithelium cell proliferation.[90] Given the essential role of vitamin A and its metabolites in systematic and visual pathways, it is meaningful to investigate their real contribution to the pathogenesis of DR.

Lutein and zeaxanthin also have the potential to prevent or control the progression of DR, considering the effect on scavenging reactive oxygen species, augmenting neuroprotection, and attenuating inflammation, as shown in animal studies.[91] However, an inconclusive association between DR and lutein/zeaxanthin has been observed among humans, based on existing studies.[91] Insignificant differences in the serum levels of combined lutein/zeaxanthin were found between diabetic participants with and without DR, which is consistent with previous studies.[41,53] Macular pigment (MP)—which comprises lutein, zeaxanthin, and meso-zeaxanthin—accounts for 20% to 30% of total

carotenoids in serum.[92] It has been reported that lutein and zeaxanthin intake can increase MP density.[93,94] Several studies have also evaluated the association between DR and macular pigment optical density (MPOD). Two studies suggested an inverse association between MPOD and DR,[95,96] whereas 3 studies suggested no significant difference in MPOD between diabetic patients with different retinopathy statuses.[97-99] Given that measurements of MPOD are direct and non-invasive, it is recommended that the possibility of MPOD as a biomarker of DR should be explored in the future.

It is believed that the present study is the first to provide a comprehensive analysis and summary of the associations between circulating micronutrients and DR. Other strengths include a population-based national sample that is likely generalizable to the overall US population, standardized DR grading based on fundus photography, and detailed information on potential confounders. Several limitations should also be noted. First, a causal association could not be established due to the cross-sectional design and inclusion of only cross-sectional studies in the meta-analysis. Second, subgroup analyses to investigate the effect on different types of DM could not be performed due to a lack of data. Third, the circulating micronutrient levels in this analysis and studies included in the meta-analysis were all based on a single measurement. Given that the circulating levels of micronutrients can be affected by many factors—including disease status, food consumption, and medication exposure—multiple measurements of micronutrients during a long follow-up are suggested in future research. Fourth, differences in the study population, measurements of DR, and circulating micronutrients could influence the pooled results. Finally, a dose-response relationship between circulating micronutrients and the severity of DR was not analyzed due to a lack of data.

In conclusion, this study demonstrated a significant inverse association between circulating vitamin C level and DR using a population-based dataset. The comprehensive meta-analysis also suggested a lower circulating level of vitamins D and E in patients with DR than those without. Consumption of foods rich in vitamins C, D, and E is thus recommended in the general diabetic population to prevent DR risk, but future prospective studies and randomized clinical trials are needed before formal adoption in long-term DM management strategies. There is still insufficient evidence to recommend routine supplementation of multiple micronutrients (such as multi-vitamin tablets) for DR management.

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