

Association between dietary folic acid intake and diabetic retinopathy among patients with diabetes: An analysis of 2005—2008 NHANES data

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ABSTRACT

Objective: Dietary folic acid (FA) intake may reduce homocysteine levels and alleviate oxidative stress, both of which are closely associated with the development and progression of diabetic retinopathy (DR). This study aims to investigate the association between dietary FA intake and DR among patients with diabetes aged ≥ 40 years in the United States.

Methods: Data from the 2005—2008 National Health and Nutrition Examination Survey (NHANES) were used. The study population comprised 3 220 patients previously diagnosed with type 2 diabetes mellitus, after excluding patients with diabetes who had missing data on dietary FA intake or DR. Multivariable logistic regression was used to evaluate the association between dietary FA intake and the prevalence of DR, with subgroup analyses and interaction tests performed. Smooth curve fitting was used to explore the potential nonlinear relationship between dietary FA intake and DR.

Results: After adjustment for potential confounders, higher dietary FA intake was significantly associated with a lower prevalence of DR ($OR=0.394$, 95% CI 0.193 to 0.804, $P=0.011$). Smooth curve fitting revealed a predominantly linear, inverse dose-response relationship between dietary FA intake and the odds of DR, with no significant non-linearity detected (P value for non-linearity=0.6535). Subgroup analyses demonstrated that the inverse association remained consistent across subgroups stratified by sex, age, body mass index (BMI), education level, smoking status, alcohol consumption, hypertension, and hypercholesterolemia (all P values for interaction >0.05).

Conclusion: Among adults with diabetes aged ≥ 40 years in the United States who were diagnosed clinically or based on biochemical testing, higher dietary FA intake is significantly associated with a lower prevalence of DR. Future prospective studies are needed to clarify the causal relationship and underlying mechanisms.

KEY WORDS

dietary folic acid; diabetic retinopathy; NHANES; cross-sectional study; type 2 diabetes mellitus

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糖尿病患者膳食叶酸摄入量与糖尿病视网膜病变的关联： 基于2005—2008年NHANES数据

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[摘要] 目的：膳食叶酸(folic acid, FA)摄入可降低同型半胱氨酸水平并减轻氧化应激，而这两者均与糖尿病视网膜病变(diabetic retinopathy, DR)的发生和发展密切相关。本研究旨在探讨美国≥40岁的糖尿病患者中膳食FA摄入量与DR之间的关联。方法：使用2005—2008年美国国家健康与营养检查调查(National Health and Nutrition Examination Survey, NHANES)的数据。研究对象为3 220名此前已被诊断为2型糖尿病的患者，并排除膳食FA和DR数据缺失的糖尿病患者。采用多变量Logistic回归评估膳食FA摄入与DR患病率之间的关联，并行亚组分析及交互作用检验；通过平滑曲线拟合探索二者间的非线性关系。结果：在调整潜在混杂因素后，膳食FA摄入升高与DR患病率降低显著相关($OR=0.394$, 95% CI 0.193~0.804, $P=0.011$)。平滑曲线拟合结果显示，膳食FA摄入量与DR患病比值比之间呈线性负向剂量-反应关系，未检测到明显的非线性效应($P_{\text{非线性}}=0.653$)。亚组分析显示，在性别、年龄、体重指数(body mass index, BMI)、教育水平、吸烟、饮酒、高血压及高胆固醇血症各亚组中，上述负向关联均保持稳定(均 $P_{\text{交互}}>0.05$)。结论：在美国≥40岁临床诊断或生化检测确诊的成年糖尿病患者中，较高的膳食FA摄入与DR患病风险降低显著相关。未来需开展更多前瞻性研究以进一步明确二者的因果关系及具体机制。

[关键词] 膳食叶酸；糖尿病视网膜病变；美国国家健康与营养检查调查；横断面研究；2型糖尿病

Diabetes mellitus has emerged as a highly prevalent chronic disease worldwide, associated with substantial mortality and reduced life expectancy^[1]. Diabetic retinopathy (DR) progresses in a stage-dependent manner following type 2 diabetes (T2D) diagnosis, with mild DR emerging at a mean latency of 8 years and subsequent transition through moderate, severe, and proliferative stages occurring within 1–2 years per stage; stage advancement correlates with escalating mortality risk, whereas optimized glycemic and blood pressure control may slow progression or, in some cases, promote regression of early-stage lesions^[2].

Retinal hypoxia and oxidative stress have been identified as key stimuli that upregulate vascular endothelial growth factor (VEGF) release in the pathogenesis of DR, contributing to early vascular alterations in the disease^[3–4]. Therefore, the development of innovative supplements targeting the axis of oxidative stress-inflammation-angiogenesis to prevent the progression of DR is of paramount importance.

Hyperhomocysteinemia (HHcy), characterized by an abnormally elevated concentration of homocysteine (Hcy) in the blood, has been shown in experimental models to induce reactive oxygen species (ROS) generation^[5–6]. There is evidence from a Meta-analysis

that HHcy increases the odds of DR^[7].

Folate is obtained from dietary sources and supplements. Based on prior studies^[8–9], inadequate levels of folate may result in an accumulation of Hcy, which in turn raises the odds of DR. Vitamin B12 (Vit B12) and vitamin B6 (Vit B6) serve as essential cofactors in the transmethylation and transsulfuration pathways, that mediate Hcy metabolism via remethylation and transsulfuration pathways, respectively^[10]. Folate exists in 2 forms: Naturally occurring folate and folic acid (FA)^[11]. The main absorbed forms of folate are FA—added to foods and supplements—and naturally occurring tetrahydrofolate species, often in polyglutamyl form^[11]. Mammals lack the ability to endogenously produce folate^[12].

Consequently, our investigation aims to examine the association between dietary FA and the odds of DR in the participants of the National Health and Nutrition Examination Survey (NHANES) in the US. We speculated that more dietary FA intake might be correlated with relatively lower odds of DR.

A broad NHANES-based screen^[13] of 58 dietary nutrients (2003—2018) recently reported that several micronutrients, including a composite folate measure, were associated with self-reported eye disease in

diabetic adults. However, that analysis relied on a single self-reported question to ascertain DR and did not isolate dietary FA—as distinct from naturally occurring folate—as a pre-specified exposure, nor did it test for a dose—response relationship or for effect modification by demographic or clinical characteristics. The present study addresses this gap by 1) restricting analysis to the NHANES 2005—2008 cycles, the only cycles in which DR was assessed by objective retinal fundus photography rather than by self-report; 2) defining diabetes using both self-report and American Diabetes Association biochemical criteria, so as to include biochemically confirmed but previously undiagnosed cases; and 3) pre-specifying dietary FA, rather than a broader nutrient panel, as the exposure of interest, modeling it continuously, by quartile, and through non-linear smooth curve fitting, and formally testing whether the association is modified by age, sex, body mass index (BMI), education, smoking, alcohol consumption, hypertension, or hypercholesterolemia. This design allows us to evaluate, with an objectively confirmed outcome, whether the FA-DR association is robust, dose-dependent, and consistent across diabetic subpopulations.

1 Subjects and methods

1.1 Data and sample sources

For this research, we acquired information from the NHANES. The objective of the program is to gauge the health and nutritional status of the population of the US by utilizing examinations and a sequence of assessments. All NHANES protocols were approved by the Institutional Review Board of the National Center for Health Statistics (NCHS), and written informed consent was obtained from all participants prior to data collection^[14]. All questionnaire respondents provided written permission; for those under 16, consent was obtained from a parent or legal guardian. NHANES data are accessible to the public at www.cdc.gov/nchs/nhanes/.

For this research, we utilized publicly accessible data gathered from 2 NHANES cycles (2005—2006 and 2007—2008). These specific cycles were selected because they are the only ones that provide information about retinopathy through retinal imaging tests.

1.2 Study population

The criteria for excluding individuals in the study were 1) participants without T2D; 2) missing data on DR status; and 3) lack of data on dietary FA. An initial selection of 20 497 participants was made. After excluding participants who were not T2D ($n=13\,269$), those who had insufficient data on DR ($n=3\,945$), and those who had incomplete information on dietary FA ($n=63$), 3 220 eligible participants who were 40 years of age or older were included in the final analysis (Figure 1).

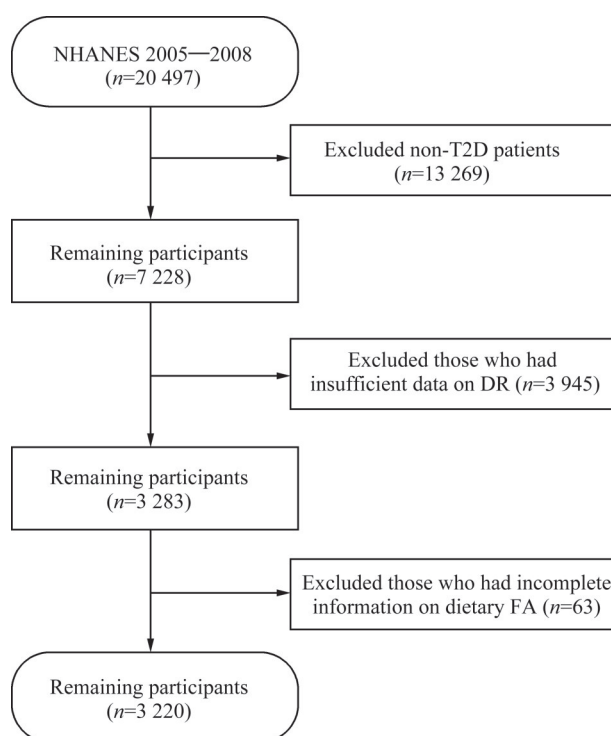


Figure 1 Flowchart of the sample selection from NHANES 2005—2008

NHANES: National Health and Nutrition Examination Survey; T2D: Type 2 diabetes; DR: Diabetic retinopathy; FA: Folic acid.

1.3 Assessment of T2D

A previous study^[13] has defined diabetic individuals by directly considering the response “Yes” to the questionnaire item “Other than during pregnancy, have you ever been told by a physician or other health professional that you have diabetes or sugar diabetes?” In this study, T2D was assessed according to the American Diabetes Association criteria^[15] and a self-administered questionnaire. Participants were diagnosed with T2D if they met any of the following

criteria^[16]: 1) Glycated hemoglobin (HbA1c) level $\geq 6.5\%$; 2) fasting blood glucose (FBG) ≥ 7.0 mmol/L; 3) 2-hour plasma glucose ≥ 11.1 mmol/L during an oral glucose tolerance test (OGTT); or 4) self-reported physician-diagnosed T2D. Using this definition, we identified an additional 5 721 T2D cases. It should be noted that we excluded participants who were currently taking insulin or oral hypoglycemic agents, because the questionnaire item “Do you take insulin or diabetes medication?” had a missing data rate exceeding 40%, and including such cases could introduce bias.

1.4 Assessment of DR

In the NHANES cohort, professional cameras and imaging systems were used for fundus photography, capturing separate images of the macula and optic nerve for each eye. Digital photographs were graded according to the Airlie House classification system. The primary outcome derived from the examination data was the “4-level retinopathy severity based on the worse eye”. The DR variable was redefined as a binary outcome, categorizing participants as having no retinopathy or retinopathy, with the latter including mild non-proliferative retinopathy (NPR), severe NPR, and proliferative retinopathy.

1.5 Assessment of dietary FA

Each participant was asked to complete 2 24-hour dietary recalls. The first recall was administered in person at the mobile examination center (MEC), and the second was conducted by telephone approximately 3 to 10 days later. Our analysis used the mean intake values from both recalls.

1.6 Definition of the covariates

Drawing on previous studies^[13, 17-20], we constructed multivariable-adjusted models that included potential covariates that might confound the association between dietary FA and the prevalence of DR. These covariates comprised demographic factors (age, gender, race/ethnicity), socioeconomic indicators [poverty income ratio (PIR), education level], lifestyle factors (smoking status, alcohol consumption), physical condition (BMI, hypercholesterolemia, hypertension), and laboratory measures, including C-reactive protein (CRP), oral glucose tolerance test (OGTT), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol

(HDL), total protein (TP), low-density lipoprotein cholesterol (LDL), Vit B6, HbA1c, and fasting blood glucose (FBG).

Alcohol consumption was defined as having consumed at least 12 alcoholic drinks in the past year. Smokers were those who had smoked >100 cigarettes in their lifetime and currently smoked daily or occasionally. BMI was categorized into 3 groups^[21]: Normal weight (<25.00 kg/m²), overweight ($25.00 - 29.99$ kg/m²), and obese (≥ 30.00 kg/m²). Hypertension was defined as a self-reported physician diagnosis of high blood pressure. Hypercholesterolemia was defined as a self-reported physician diagnosis of hypercholesterolemia. To ensure that all participants were included in the multivariable models, missing values for covariates (PIR, TP, Vit B6, BMI, CRP, TC, TG, LDL, and HDL) were imputed using the mean or median.

1.7 Statistical analysis

All statistical analyses were performed using R software (version 4.3.3). The dietary folate intake values for 2007–2008 and 2005–2006 were each calculated as the average of folate intakes from the first-day and second-day dietary samples collected in the same year, followed by pooling of data from the 2 survey cycles.

The Shapiro-Wilk test was used to examine normality of continuous variables. Given its high sensitivity to minor deviations in large samples, skewness was also evaluated ($|\text{skewness}| < 1$ as approximate symmetry). Variables meeting both criteria (Shapiro-Wilk $P > 0.05$ and $|\text{skewness}| < 1$) were presented as mean $\pm$ standard deviation; otherwise, as median (25th, 75th percentile). Categorical variables were summarized as frequencies (percentages).

Baseline characteristics were compared between participants with and without DR. For continuous variables, regardless of their underlying distribution, the Kruskal-Wallis rank-sum test was applied. Categorical variables were compared using the chi-square test; Fisher's exact test was employed when any expected cell count in the contingency table was less than 10.

To investigate the association between dietary FA intake and the odds of DR, we used multivariable Logistic regression models. In the final model, age, sex, and race/ethnicity were retained as important covariates, regardless of their statistical significance, because they

are known to influence both DR and FA intake. Model 1 included no covariate adjustments. Model 2 adjusted for age, race/ethnicity, and gender. Model 3 further adjusted for sex, TC, age, race/ethnicity, TP, BMI, CRP, Vit B6, hypertension, hypercholesterolemia, HDL-C, alcohol consumption, and smoking status. To assess robustness, dietary FA intake was also analyzed as a categorical variable (quartiles) rather than a continuous one. Additional investigation into the non-linear association between dietary FA and the odds of DR was conducted utilizing smooth curve fitting. Subgroup analyses were performed using stratified multivariable Logistic regression models, stratified by sex, smoking status, age, alcohol consumption, BMI, education level, hypertension, and hypercholesterolemia. A 2-sided $P < 0.05$ was also considered statistically significant.

2 Results

2.1 Baseline characteristics of participants

The study included 3 220 participants, including 1 639 (50.901%) males and 1 581 (49.099%) females (Table 1). The age was 60 (50, 70) years. There were 534 (16.584%) patients suffering from DR, and 2 686 (83.416%) patients without DR. There were statistically significant differences in age, ethnicity, alcohol consumption, education level, hypercholesterolemia, TC, hypertension, PIR, LDL, BMI, HDL, HbA1c, OGTT, FBG, and FA between individuals with and without DR (all $P < 0.05$). There were no significant differences in gender, smoking status, CRP, TG, TP, or Vit B6 (all $P > 0.05$).

Table 1 Baseline data of study subjects

Characteristics	Total($n=3\ 220$)	NDR($n=2\ 686$)	DR($n=534$)	χ^2/H	P
Gender/[No.(%)]				$\chi^2=2.355$	0.125
Male	1 639 (50.901)	1 351 (50.298)	288 (53.933)		
Female	1 581 (49.099)	1 335 (49.702)	246 (46.067)		
Race/ethnicity/[No.(%)]				$\chi^2=49.816$	<0.001
Non-Hispanic White	1 679 (52.143)	1 461 (54.393)	218 (40.824)		
Non-Hispanic Black	675 (20.963)	507 (18.876)	168 (31.461)		
Mexican American	522 (16.211)	430 (16.009)	92 (17.228)		
Other	344 (10.683)	288 (10.722)	56 (10.487)		
Education level/[No.(%)]				$\chi^2=37.817$	<0.001
Less than high school	1 007 (31.273)	789 (29.375)	218 (40.824)		
High school or GED	812 (25.217)	679 (25.279)	133 (24.906)		
Above high school	791 (24.565)	670 (24.944)	121 (22.660)		
Others	610 (18.944)	548 (20.402)	62 (11.610)		
Smoking status/[No.(%)]				$\chi^2=2.188$	0.139
Yes	1 752 (54.410)	1 477 (54.989)	275 (51.489)		
No	1 468 (45.590)	1 209 (45.011)	259 (48.502)		
Alcohol consumption/[No.(%)]				$\chi^2=10.481$	0.001
Yes	2 119 (65.807)	1 800 (67.014)	319 (59.738)		
No	1 101 (34.193)	886 (32.986)	215 (40.262)		
Chronic conditions/[No.(%)]					
Hypercholesterolemia/[No.(%)]				$\chi^2=10.628$	0.001
Yes	1 428 (44.348)	1 157 (43.075)	271 (50.749)		
No	1 792 (55.652)	1 529 (56.925)	263 (49.251)		
Hypertension/[No.(%)]				$\chi^2=54.409$	<0.001
Yes	1 617 (50.217)	1 271 (47.319)	346 (64.794)		
No	1 603 (49.783)	1 415 (52.681)	188 (35.206)		

Table 1 (continued)

Characteristics	Total(<i>n</i> =3 220)	NDR(<i>n</i> =2 686)	DR(<i>n</i> =534)	χ^2/H	<i>P</i>
Age/years	60 (50, 70)	60 (49, 69)	63 (54, 71.750)	<i>H</i> =31.764	<0.001
PIR/%	2.340 (1.290, 4.220)	2.340 (1.330, 4.380)	2.175 (1.240, 3.410)	<i>H</i> =16.340	<0.001
BMI/(kg·m ⁻²)	28.84 (25.28, 33.05)	28.68 (25.04, 32.82)	29.71 (26.36, 34.68)	<i>H</i> =21.381	<0.001
CRP/(mg·dL ⁻¹)	0.240 (0.100, 0.550)	0.240 (0.100, 0.540)	0.240 (0.120, 0.590)	<i>H</i> =2.447	0.118
TC/(mg·dL ⁻¹)	197 (170, 226)	198 (172, 227)	191 (162, 221)	<i>H</i> =16.107	<0.001
TG/(mg·dL ⁻¹)	121 (94, 158)	121 (92, 161)	121 (110.250, 151.750)	<i>H</i> =2.590	0.108
HDL/(mg·dL ⁻¹)	51 (42, 62)	51 (42, 62)	50 (41, 58)	<i>H</i> =8.264	0.004
LDL/(mg·dL ⁻¹)	118.093 (99, 134)	118.093 (100, 135)	118.093 (99, 120)	<i>H</i> =8.377	0.004
TP/(g·dL ⁻¹)	7.100 (6.900, 7.400)	7.100 (6.900, 7.400)	7.153 (6.900, 7.475)	<i>H</i> =1.579	0.209
Vit B6/(nmol·L ⁻¹)	28.500 (18.675, 52.800)	28.500 (18.700, 52.500)	28.500 (18.625, 56.600)	<i>H</i> =0.437	0.509
HbA1c/%	5.700 (5.400, 6.300)	5.600 (5.300, 6.100)	6.600 (5.700, 8.200)	<i>H</i> =287.434	<0.001
OGTT/(mg·dL ⁻¹)	121 (97, 157)	120 (97, 155)	135 (100.500, 193.500)	<i>H</i> =12.002	<0.001
FBG/(mg·dL ⁻¹)	103 (96, 116)	103 (95, 113)	114 (98, 152)	<i>H</i> =81.505	<0.001
FA/(mg·d ⁻¹)	0.124 (0.065, 0.217)	0.126 (0.066, 0.225)	0.115 (0.060, 0.193)	<i>H</i> =5.825	0.016

NDR: Non-diabetic retinopathy; DR: Diabetic retinopathy; GED: General Educational Development; PIR: Ratio of family income to poverty; BMI: Body mass index; CRP: C-reactive protein; TC: Total cholesterol; TG: Triglycerides; HDL: High-density lipoprotein-cholesterol; LDL: Low-density lipoprotein-cholesterol; TP: Total protein; Vit B6: Serum vitamin B6; HbA1c: Glycated hemoglobin; OGTT: Oral glucose tolerance test; FBG: Fasting blood glucose; FA: Folic acid. The Shapiro-Wilk test was used to examine normality of continuous variables. Variables meeting both criteria (Shapiro-Wilk $P>0.05$ and $|\text{skewness}|<1$) were presented as mean±standard deviation; otherwise, as median (25th, 75th percentile). Categorical variables were summarized as frequencies (percentages). For continuous variables, the Kruskal-Wallis rank-sum test was applied. Categorical variables were compared using the chi-square test; Fisher's exact test was employed when any expected cell count in the contingency table was less than 10.

2.2 Association between dietary FA and the odds of DR

Higher dietary FA level was correlated with reduced odds of DR ($OR=0.394$, 95% *CI* 0.193 to 0.804, $P=0.011$). The connection was statistically significant in both our crude model ($OR=0.330$, 95% *CI* 0.166 to 0.656, $P=0.002$) and our model adjusted for basic covariates ($OR=0.424$, 95% *CI* 0.210 to 0.854, $P=0.016$). The fully adjusted model still showed an inverse association between dietary FA and odds of DR ($OR=0.394$, 95% *CI* 0.193 to 0.804, $P=0.011$). This suggests that each increment of dietary FA was associated with a 60.6% decrease in the odds of DR. By splitting the dietary FA data into quartiles, we transformed it from a continuous to a categorical variable. The odds of DR were substantially reduced by 27.9% in individuals with the greatest amount of dietary FA intake compared to those with the lowest dietary FA intake, which was statistically significant ($OR=0.721$, 95% *CI* 0.543 to

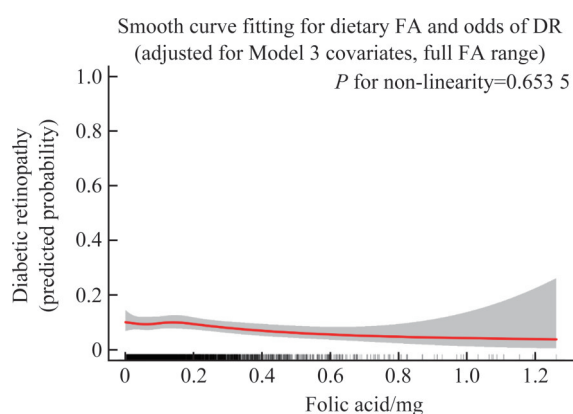
0.958, $P=0.024$; Table 2).

The fitted spline curve demonstrated a generally monotonically negative association between dietary FA intake and the predicted probability of DR across the observed range (0–1.262 mg/d). At the lowest dietary FA intake level, the predicted probability was approximately 0.100 (95% *CI* 0.068 to 0.145), whereas at the highest level it decreased to approximately 0.039 (95% *CI* 0.006 to 0.206), consistent with the negative association observed in the multivariable Logistic regression analysis (Table 2). The likelihood ratio test comparing the restricted cubic spline model with the corresponding linear model yielded no statistically significant evidence of non-linearity (deviance=1.626, $\Delta df=3$, P for non-linearity=0.654), indicating that a linear dose-response relationship adequately described the association between dietary folate intake and DR risk, without evidence of a threshold or curvilinear effect.

Table 2 Association between dietary FA and odds of DR

Dietary FA intake	Model 1 (Crude)		Model 2 (Minimally adjusted)		Model 3 (Fully adjusted)	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Continuous	0.330 (0.166 to 0.656)	0.002	0.424 (0.210 to 0.854)	0.016	0.394 (0.193 to 0.804)	0.011
Categories						
Quartile level 1	Reference		Reference		Reference	
Quartile level 2	0.975 (0.755 to 1.258)	0.843	0.994 (0.767 to 1.289)	0.966	0.971 (0.747 to 1.260)	0.827
Quartile level 3	1.004 (0.778 to 1.294)	0.979	1.041 (0.802 to 1.350)	0.764	1.023 (0.786 to 1.332)	0.867
Quartile level 4	0.680 (0.518 to 0.893)	0.006	0.743 (0.561 to 0.983)	0.038	0.721 (0.543 to 0.958)	0.024

In order to assess its robustness, dietary FA is transformed from a continuous variable to a categorical (quartile) variable. Model 1: No covariates were adjusted. Model 2: Adjusted for gender, age, and race. Model 3: Adjusted for gender, age, race, TC, TP, BMI, CRP, Vit B6, hypertension, hypercholesterolemia, HDL-C, alcohol consumption, and smoking status.

**Figure 2 Smooth curve fitting for dietary FA and odds of DR**

2.3 Subgroup analysis

Subgroup analyses were performed to evaluate the association between dietary FA intake and odds of DR in different subgroups (Table 3).

In the age-stratified analysis, a statistically significant association was observed only among participants aged 40 – 59 years ($OR=0.297$, 95% CI

0.092 to 0.961, P for trend=0.043). Regarding gender, a significant inverse association was detected exclusively in males ($OR=0.363$, 95% CI 0.143 to 0.924, P for trend=0.033).

Additionally, significant inverse associations were found in subgroups of participants with a high school diploma or GED ($OR=0.092$, 95% CI 0.018 to 0.478, P for trend=0.005), alcohol drinkers ($OR=0.279$, 95% CI 0.110 to 0.709, P for trend=0.007), and with hypercholesterolemia ($OR=0.346$, 95% CI 0.121 to 0.988, P for trend=0.047).

For the remaining subgroups across all stratified categories, no statistically significant associations were observed, although negative trends were generally noted (all P for trend >0.05).

In this study, no significant interaction effects were observed across all stratified subgroups (all P for interaction >0.05), indicating that the inverse associations were generally consistent across different population strata.

Table 3 Subgroup analysis for the association between dietary FA and odds of DR

Subgroups	OR (95% CI)	P	P for interaction
Age/years			0.859
40–59	0.297 (0.092 to 0.961)	0.043	
≥60	0.421 (0.171 to 1.035)	0.059	
Gender			0.986
Male	0.363 (0.143 to 0.924)	0.033	
Female	0.416 (0.134 to 1.294)	0.130	
BMI/(kg·m ⁻²)			0.619
<25.00	0.203 (0.031 to 1.333)	0.097	
25.00–29.99	0.449 (0.136 to 1.487)	0.190	
≥30.00	0.437 (0.159 to 1.203)	0.109	

Table 3 (continued)

Subgroups	OR (95% CI)	P	P for interaction
Education			0.331
Less than high school	0.868 (0.281 to 2.685)	0.806	
High school or GED	0.092 (0.018 to 0.478)	0.005	
Above high school	0.421 (0.110 to 1.613)	0.207	
Others	0.487 (0.073 to 3.235)	0.457	
Smoking status			0.660
Yes	0.452 (0.170 to 1.201)	0.111	
No	0.359 (0.125 to 1.027)	0.056	
Alcohol consumption			0.268
Yes	0.279 (0.110 to 0.709)	0.007	
No	0.646 (0.207 to 2.019)	0.453	
Hypercholesterolemia			0.777
Yes	0.346 (0.121 to 0.988)	0.047	
No	0.446 (0.167 to 1.195)	0.108	
Hypertension			0.927
Yes	0.445 (0.174 to 1.136)	0.091	
No	0.386 (0.125 to 1.192)	0.098	

OR (95% CI) and P values represent Logistic-regression results for continuous FA and DR within each subgroup, adjusted for all covariates in Model 3 except the stratifying variable. P-interaction denotes the overall P-value for the interaction test of the corresponding stratifying variable. None of the strata, including gender, age, BMI, education level, smoking status, alcohol consumption status, hypertension, and hypercholesterolemia significantly affected the negative association of dietary FA with odds of DR.

3 Discussion

In our cross-sectional investigation of 3 220 people, we found that those who had more dietary FA had lower odds of DR. The subgroup analysis and interaction test demonstrated that this association was consistent across several subgroups. Our analysis suggested that higher dietary FA was associated with reduced odds of DR.

Intake of nutrients such as vitamin A, alpha-carotene, and folate has been associated with a decreased likelihood of retinopathy^[13]. Using the 2003—2018 NHANES data, Zhang, et al^[13] screened 58 dietary nutrients against self-reported eye disease and identified dietary folate, among 9 other nutrients, as associated with the odds of DR. The present study differs from that work in several respects beyond the NHANES cycle used. First, that study^[13] ascertained DR using a single self-reported question, whereas we used objective, photograph-based grading (Airlie House classification), which is available only in the 2005—2008 NHANES

cycles and may provide higher sensitivity for detecting early, asymptomatic disease compared with self-report. Second, their definition of diabetes relied solely on self-report, whereas we additionally applied American Diabetes Association biochemical criteria (HbA1c, FBG, OGTT) to capture previously undiagnosed cases. Third, their composite folate (DFE) measure combines naturally occurring folate and FA and was examined as one of 58 nutrients in an exploratory screen using quintile comparisons only; we instead pre-specified dietary FA—the synthetic, fortification-derived form most directly implicated in the homocysteine-lowering pathway—as the sole exposure of interest, and modeled it both continuously and by quartile, with additional non-linear dose-response characterization via smooth curve fitting. Of note, the 95% CI widened substantially at dietary FA intakes above approximately 0.7 mg/d, attributable to the limited sample size in this range (n= 40, representing 1.24% of the cohort, with only 1 DR case). The sparse data at the upper end of the dietary FA distribution reduced the precision of the spline

estimates; therefore, interpretation of the curve beyond approximately 0.7–0.8 mg/d should be made with caution. Nevertheless, within the main distribution range with adequate data, the overall linear trend remained stable and statistically supported. Fourth, we performed pre-specified subgroup analyses across 8 demographic and clinical strata with formal interaction testing, which was not reported in their study; none of the interaction terms were significant, supporting the consistency of the FA and DR association across diabetic subpopulations. Finally, we applied imputation for missing covariate data to preserve statistical power. Taken together, these differences mean the 2 studies address related but distinct questions: Their work asked which of many nutrients correlate with self-reported eye disease, whereas we asked whether dietary FA specifically shows a dose-dependent, modifier-independent association with objectively confirmed DR.

In addition, we applied multiple imputation techniques to handle missing quantitative data, thereby preserving the sample size for analysis. Given the limitations of the available evidence, our analysis provides valuable supplementary insights. While the mechanisms underlying the inverse association between dietary FA and DR odds remain unclear, our findings lend support to this protective relationship.

High FA intake may reduce cellular toxicity and mitigate the body's inflammatory response and other related pathological processes^[22]. It has been hypothesized that increasing FA intake could reduce cardiovascular mortality^[23]. Endothelium-dependent vasodilation has been shown to improve with FA supplementation at doses of 10 mg/d^[24] and 5 mg/d^[25]. Under high-glucose conditions, retinal microvascular endothelial cells exhibit increased global DNA methylation and hydroxymethylation levels, an effect that can be attenuated by FA treatment^[26]. Furthermore, FA has been shown to influence the activity of related proteins, including Yes-associated protein 1 (YAP1), transcriptional enhanced associate domain 1 (TEAD1), Delta-like canonical Notch ligand 1 (DLL1), and neurogenic locus notch homolog protein 2 (Notch2). Molecular docking was also used to investigate how FA interacts with these proteins, and research shows that FA can form stable ligand-receptor complexes with them, supporting its regulatory effects^[27]. Notably, the function

of the FA transport protein is decreased under hyperglycemic and diabetic conditions, potentially leading to FA deficiency in the retina^[28].

Additionally, a threshold of approximately 14.0 $\mu\text{mol/L}$ was identified in the relationship between the serum Hcy levels and the odds of DR. Above this level, each 1 $\mu\text{mol/L}$ increase in serum Hcy was an independent predictor of a 66% increase in odds of DR^[29]. FA and Vit B12 supplementation has been shown to reduce Hcy levels^[30], and this effect has been confirmed in both animal models and clinical trials. By decreasing or preventing DR progression, mitigating hyperglycemia-induced damage to retinal microvasculature and neurons, and alleviating inflammatory responses interleukin-1 beta (IL-1 β), oxidative stress [4-hydroxynonenal (4-HNE), Vav guanine nucleotide exchange factor (Vav), NADPH oxidase 4 (NOX4)], and angiogenesis [cluster of differentiation 31 (CD31), vascular endothelial growth factor receptor (VEGFR)] in both the circulation and the retina, FA supplementation may exert beneficial effects on DR-related outcomes^[31].

Malaguarnera, et al^[32] observed that patients diagnosed with T2D, regardless of the presence of DR, demonstrated significantly low levels of plasma FA and red blood cell folate. Patients taking metformin have been observed to exhibit a notable reduction in peripheral folate (PF) concentrations and a significant rise in Hcy levels, resulting in hindered absorption of Vit B6 and B12^[33–34]. Currently, the majority of diabetic patients commonly use metformin, which can result in a deficiency of FA and Vit B12, exacerbating Hcy metabolism abnormalities^[35–36]. This may also be a factor leading to DR progression. The inverse association between FA and Hcy suggests that FA and Vit B6/12 might have a protective impact on DR. The combination of FA and Vit B6/B12 is currently recommended for treatment because it facilitates the metabolism of harmful one-carbon metabolites^[30]. The prescription medicine Metanx is composed of *L*-methylfolate calcium, which contains the biologically active forms of folate, along with Vit B6 and Vit B12. It is employed to alleviate discomfort associated with diabetic neuropathy in patients with diabetes. In recent decades, it has been discovered that Metanx ameliorates endothelial dysfunction^[37]. In patients with nonproliferative DR,

Metanx treatment appeared to alleviate retinal edema and enhance light sensitivity, according to a pilot study^[38]. Metanx was demonstrated to inhibit oxidative stress and inflammation in an animal experiment, and it may therefore be beneficial in the prevention of visual dysfunction caused by diabetes^[39].

Nevertheless, the findings of this investigation should be cautiously interpreted due to a number of constraints. We deliberately restricted our analysis to the 2005–2008 NHANES cycles because these are the only cycles in which DR was assessed by retinal fundus photography rather than by self-report; the fundus-photography module was discontinued after 2007–2008, so a directly comparable replication of our photograph-confirmed outcome in a more recent, larger NHANES sample is not currently possible with public data. This is also why we treat the self-report-based findings of Zhang, et al^[13] and similar studies that used more recent NHANES cycles as complementary rather than directly comparable evidence.

First, we could not identify a causal relationship, which was due to the cross-sectional study design. Prospective research having a larger number of participants is still essential to establish causality. Second, Vit B12 was not included as a covariate due to incomplete data on Vit B12 levels during 2005–2008 and the absence of information on metformin use in the NHANES database. Consequently, the potential influence of Vit B12 levels on the outcomes was not entirely eliminated. Third, in the data processing stage, the determination of smoking, alcohol consumption, hypertension, and hypercholesterolemia was oversimplified, with only unequivocal affirmative responses considered positive, while missing or uncertain responses were deemed negative. Fourth, the NHANES data failed to differentiate between type 1 diabetes (T1D) and T2D, preventing further assessment of the correlation within subgroups stratified by T1D and T2D. Fifth, dietary FA intake in the present study was assessed based on 2 24-hour dietary recalls. Given the inherent limitations of this short-term dietary assessment method, the collected data may not fully reflect the long-term habitual FA intake of the study participants, which could introduce measurement bias. Furthermore, the study was unable to completely eliminate the impact of additional possible confounding

variables, although we adjusted a few potential covariates. In the present study, missing covariate values were imputed using the mean or median, as appropriate. Single imputation may underestimate the variability of the data and introduce bias into statistical estimates. Given that multiple imputation was not performed, our findings should be interpreted with caution. Since normality was re-assessed after imputation, the baseline characteristics of the study population may not fully reflect the distribution of the original data; therefore, the baseline table should be interpreted with caution. Finally, because our study population was restricted to individuals with diagnosed or biochemically confirmed diabetes, the observed association between dietary FA and DR pertains specifically to odds of DR within a diabetic population and should not be generalized to FA and eye disease relationships in non-diabetic adults.

Among individuals with diabetes aged ≥ 40 years in this NHANES sample, higher dietary FA intake was inversely associated with the odds of DR. These findings should not be extrapolated to non-diabetic populations. Additional extensive prospective investigations are required to authenticate our findings.

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