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Dietary iron intake is nonlinearly associated with the risk of diabetic retinopathy in adults with type 2 diabetes

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Abstract

Objective To elucidate the association between dietary iron intake and diabetic retinopathy (DR) in type 2 diabetes (T2D) patients.

Methods Participants from the National Health and Nutrition Examination Survey (NHANES) 2005–2008 aged over 40 years with T2D were included. Dietary iron intake was estimated from standardised questionnaires. The presence of DR and vision-threatening DR (VTDR) was determined through retinal imaging. We used logistic regression to assess the relationship between iron intake and DR, and restricted cubic splines to reveal nonlinear links.

Results The study enrolled 1172 T2D adults. We found significant nonlinear associations between dietary iron intake and DR among females ($P=0.023$), but not in males ($P=0.490$). Compared with the lowest quartile of iron intake, the third quartile (13.2–18.1 mg/d) yielded significantly lower odds of developing DR (odds ratio [OR], 0.59; 95% CI, 0.39–0.90) and VTDR (OR, 0.42; 95% CI, 0.19–0.94). Stratified logistic analyses showed that medium-high iron intake was associated with lower risks of DR in females (OR, 0.44; 95% CI, 0.24–0.81), non-Hispanic Blacks (OR, 0.38; 95% CI, 0.17–0.85), and individuals with obesity (OR, 0.45; 95% CI, 0.25–0.82), high HbA1c (OR, 0.56; 95% CI, 0.34–0.93), long diabetes duration (OR, 0.40; 95% CI, 0.21–0.76) or low blood haemoglobin (OR, 0.17; 95% CI, 0.05–0.60).

Conclusion Dietary iron intake was nonlinearly negatively associated with the prevalence of DR and VTDR, showing protective effect against retinopathy of medium-high iron intake in T2D patients. Such associations significantly vary by multiple factors such as age, ethnicity, obesity and glycaemic control.

Keywords Diabetic retinopathy, Vision-threatening diabetic retinopathy, Dietary iron intake, NHANES

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Introduction

Diabetic retinopathy (DR) is one of the major causes of vision impairment globally [1]. According to the Global Burden of Disease Study 2019, DR has been the fifth leading cause of blindness and moderate-to-worse visual impairment among individuals aged 50 years and greater [2]. Of note, it was the only cause of blindness with a globally increasing trend in age-standardised prevalence from 1990 to 2020 [2]. Among individuals with diabetes, approximately a third have any sign of DR, and a third of them might have vision-threatening diabetic retinopathy (VTDR) [3]. With the estimated global diabetes prevalence to be rising from 9.3% (463 million) in 2019 to 10.9% (700 million) by 2045 [4], along with longer life expectancy and lifestyle changes, the global burden of DR is expected to grow rapidly. Although hyperglycaemia, diabetes duration, and hypertension are considered as major risk factors for DR, they merely account for a small amount of the variation in the DR risk [5]. Several novel pathogeneses have raised that the abnormal homeostasis of trace elements including iron may associate with the progression of DR [6]. However, little is known about the association between dietary iron intake and DR.

Iron plays a critical role in dynamic redox balance, disruption of which can lead to oxidative stress and damage to target organs including the retina [7, 8]. Nevertheless, previous studies showed contradictory results regarding whether iron could protect the retina from harmful stimuli. On one hand, iron overload can exacerbate the development of retinopathy in mice, implying that iron depletion strategies may ameliorate diabetic microvascular complications [9, 10]. On the other hand, epidemiologic and clinical studies showed that iron deficiency anaemia (IDA) was related to increased risk of DR [11, 12], indicating the efficacy of anaemia treatment in decreasing retinopathy risk in type 2 diabetes (T2D). Such findings were supported by a recent study [13], in which serum iron was negatively correlated with the occurrence of DR in diabetic adults. Notably, all previous studies concentrated on the relationship of body iron status rather than dietary intake of iron and DR. The current assessment of iron status is derived from a battery of hemaetological indicators, including serum ferritin, transferrin saturation, and mean corpuscular volume, etc. However, measuring these hemaetological indicators is invasive and might be infeasible as for cost and complexity of procedure in certain settings. Thus, there is a need for a simpler and more accessible method to evaluate iron status. A complementary and easy-to-use option is to consider iron intake from diet and dietary supplements. When associations between DR risks and iron intake through nutritional assessment could be accurately estimated, it is promising to establish recommendations for appropriate dietary iron intake as to prevent DR in T2D patients. Nutritional interventions with optimal combinations of iron and other nutrients that support conventional therapies could be developed to reduce disease risk and severity in T2D patients.

Herein, we investigated the association between the level of dietary iron intake, measured by a standardised questionnaire, and the risk of DR and VTDR among patients with T2D in a series of national representative samples. We further assessed whether such associations varied in specific subgroups of population defined by major stratification factors.

Materials and methods

Study design and population

This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting statement (Supplemental Material 1). We conducted this cross-sectional study using the data from the National Health and Nutrition Examination Survey (NHANES) 2005 to 2008. NHANES is a population-based, multipurpose survey to evaluate the health and nutritional status of the US population. The National Centre for Health Statistics (NCHS) ethics review board reviewed and approved the study protocol. The study was conducted adhered to the principles of the Declaration of Helsinki, and written informed consent was obtained from each participant. Detailed data and methodology files are accessible online [14]. All potentially identifiable information has been removed to ensure the confidentiality of participants and their households. Briefly, every 2-year cycle of survey comprises of questionnaires administered at home, and a standardised physical examination in a mobile examination centre (MEC) including physical measurements and collection of biospecimens for laboratory tests. For 2005–2006 and 2007–2008 cycles, retinal photography and dietary interview were conducted to subjects older than 40 years, making this analysis available. Herein, T2D was defined according to participants' meeting one or more of the following criteria: (1) self-reported physician diagnosis of diabetes; (2) using oral glucose-lowering medicines or insulin; and (3) fasting plasma glucose level of at least 126 mg/dL, or haemoglobin A1c (HbA1c) level of at least 6.5% [15]. In this study, only those with complete data of dietary iron intake, diabetes and retinopathy were eligible.

Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Assessment of dietary iron intake

All NHANES examinees were eligible for two 24-hour dietary recall interviews. The first was performed

in-person in the MECs, and the second by telephone 3 to 10 days later. Intakes of energy, nutrients, and other components from foods and beverages were estimated from the two detailed dietary interview. Detailed questionnaire and methodology of dietary intake calculation is publicly accessible online [14]. In this study, dietary intake of iron was calculated by averaging data of two 24-hour recalls if available, otherwise the single reliable dietary recall data was used. The continuous iron intake data were further dichotomised as adequate and inadequate categories according to the Recommended Dietary Allowance (RDA) issued by the Food and Nutrition Board of the National Academies [16].

Ascertainment of diabetic retinopathy

During 2005–2008 NHANES survey, the presence of major retinal diseases including DR was tested for participants aged over 40 years by the Retinal Imaging. Two 45-degree digital retinal images for each eye were captured utilising the Canon Non-Mydriatic Retinal Camera CR6-45NM (Canon, Tokyo, Japan), one focused on the optic nerve and the other on the macula. The digital images were transferred to the University of Wisconsin Ocular Epidemiologic Reading Centre, Madison for grading retinopathies according to the standardised protocol. At least two raters graded a same set of images. If the first two graders disagreed, the third graded the image. If two of three disagreed, an adjudicator would make a final decision.

DR severity was broadly classified into 4 levels: no DR, mild non-proliferative diabetic retinopathy (NPDR), moderate/severe NPDR, and proliferative diabetic retinopathy (PDR) according to the Early Treatment Diabetic Retinopathy Study (ETDRS) classification standards [17]. In this study, we aggregated these 4 levels as no DR versus any DR (incorporating mild NPDR, moderate/severe NPDR, and PDR). We also defined VTDR as the presence of severe NPDR, PDR, or clinically significant macular oedema (CSME). Herein CSME was defined when (1) the oedema involved the fovea or within 500 microns of the fovea, and/or (2) a 1 + disc area of oedema present with at least a portion of it involving the macula. Outcomes of our study were defined according to the worse one of two eyes.

Assessment of covariates

Sociodemographic variables, including age, sex, race/ethnicity, education attainment, and poverty income ratio (PIR) was collected through a questionnaire. Race/ethnicity was self-reported according to NCHS categories (Mexican American, non-Hispanic Black, non-Hispanic White, other Hispanic, or other). We combined other Hispanic and other race/ethnicity groups as the broader "other group". Education was grouped into three categories: (1) less than high school, (2) high school or equivalent, and (3) greater than high school. PIR was classified into 3 categories: less than 1.30, 1.30 to 3.49, and 3.5 or higher. Body mass index (BMI) was calculated as weight in kilograms divided by height in metres squared, and was categorised as three groups: normal or underweight (less than 25.0 kg/m^2), overweight (25.0 to 30.0 kg/m^2), and obese (greater than 30.0 kg/m^2). Smoking status was classified into 3 categories: never (less than 100 cigarettes in lifetime), former (greater than 100 cigarettes in lifetime, but had given up at the time of interview), and current smoker (greater than 100 cigarettes in lifetime and currently smoking). Alcohol consumption was classified into 3 categories: never (never drank alcohol in lifetime and the past 12 months), former (had ever drunk in lifetime, but not in the past 12 months), and current drinker (drunk at least 12 alcoholic drinks during the past year or lifetime and consumed alcohol at least 1 day during the past year).

Duration of diabetes was self-reported by interviewees, and was dichotomised as < 10 and ≥ 10 years. Haemoglobin A1c (HbA1c) raw data were continuous, and were further grouped into < 7.0% versus ≥ 7.0%. Blood haemoglobin level was dichotomised as normal/high (> 12 g/dL) and low (< 12 g/dL) based on the standard of World Health Organization [18].

Information about medical comorbidities was obtained from physical examination and questionnaire. We defined hypertension if participants with (1) diastolic blood pressure ≥ 80 mmHg, or systolic blood pressure ≥ 130 mmHg according to the mean value of 3 measurements, or (2) self-reported hypertension history, or (3) taking blood pressure medications [19]. Individuals whose total cholesterol ≥ 240 mg/dL (6.2mmol/L), or taking lipid-lowering medications were considered as having hypercholesteraemia. Congestive heart failure, coronary heart disease, heart attack, angina/angina pectoris, and stroke were confirmed based on self-reported physician diagnosis. The full questionnaire can be found in the Supplemental Material 2.

Statistical analysis

In this study, we reported descriptive statistics as numbers (percentages) for categorical variables, and means (standard deviations, SDs) for continuous variables. We used $\chi 2$ and unpaired t-tests to assess differences in sociodemographic, clinical and dietary characteristics between groups. We established logistic regression models to evaluate odds ratios (ORs) of DR and VTDR based on iron intake quartiles after adjustment for demographic variables, lifestyle variables, DM related variables, and medical comorbidities.

To explore nonlinear association between DR and iron intake, we used restricted cubic spline (RCS) logistic

regression analyses after adjusting the abovementioned confounders. We set four knots (5th, 35th, 65th, and 95th percentiles of iron intake) according to Harrell's recommendation that four knots can offer an adequate fit of the model and well balance flexibility and imprecision caused by overfitting [20]. The R package plotRCS (version 0.1.4) was used to visualise splines [21]. Logistic regression and RCS analysis were stratified by sex, race/ethnicity, weight status, HbA1c level (< 7.0% or ≥ 7.0%), duration of diabetes (< 10 years or ≥ 10 years), and blood haemoglobin level (normal/high or low). We performed sensitivity analyses to evaluate the robustness of major results by applying the following strategies: (1) excluding participants with outlying iron intake data (≥ 3SD from the mean value), (2) reconstructing logistic regression and RCS models through ignoring missing data rather than multiple imputation (i.e., complete case analysis). All data analyses were performed by R statistical package (R Core Team, Vienna, Austria, version 4.2.3). Variables with missing values were imputed through multiple imputation approach using mice package (version 3.15.0) [22]. *P* value less than 0.05 was considered to be statistically significant.

Results

Participant characteristics

Of all participants from the NHANES 2005–2006 ($n = 10348$) and 2007–2008 ($n = 10149$), 18,923 participants younger than 40 years ($n = 13416$) or without diabetes ($n = 5507$) were excluded. Participants with ungradable images ($n = 374$) or without dietary iron intake data ($n = 28$) were excluded as well, leaving 1172 participants ultimately included (Fig. 1). Nine covariates of the dataset had missing values over 1% of observations (Supplemental Figure S1), and PIR had the highest proportion (8%). To take into account the effect of missing data, we handled missing data with multiple imputation technique for the subsequent analyses.

The general characteristics of study population by DR status were presented in Table 1. There were 358 participants (30.5%) with any DR, among whom 188 were males (52.5%). Compared with disease-free individuals, DR patients had comparable average age, sex, education, marriage status, PIR, smoking status, and alcohol consumption, but were more likely to be non-Hispanic black, Mexican American, and have higher BMI. DR population showed poorer general health condition, longer diabetes duration, higher levels of HbA1c, lower levels of blood haemoglobin, as well as higher prevalence of systemic

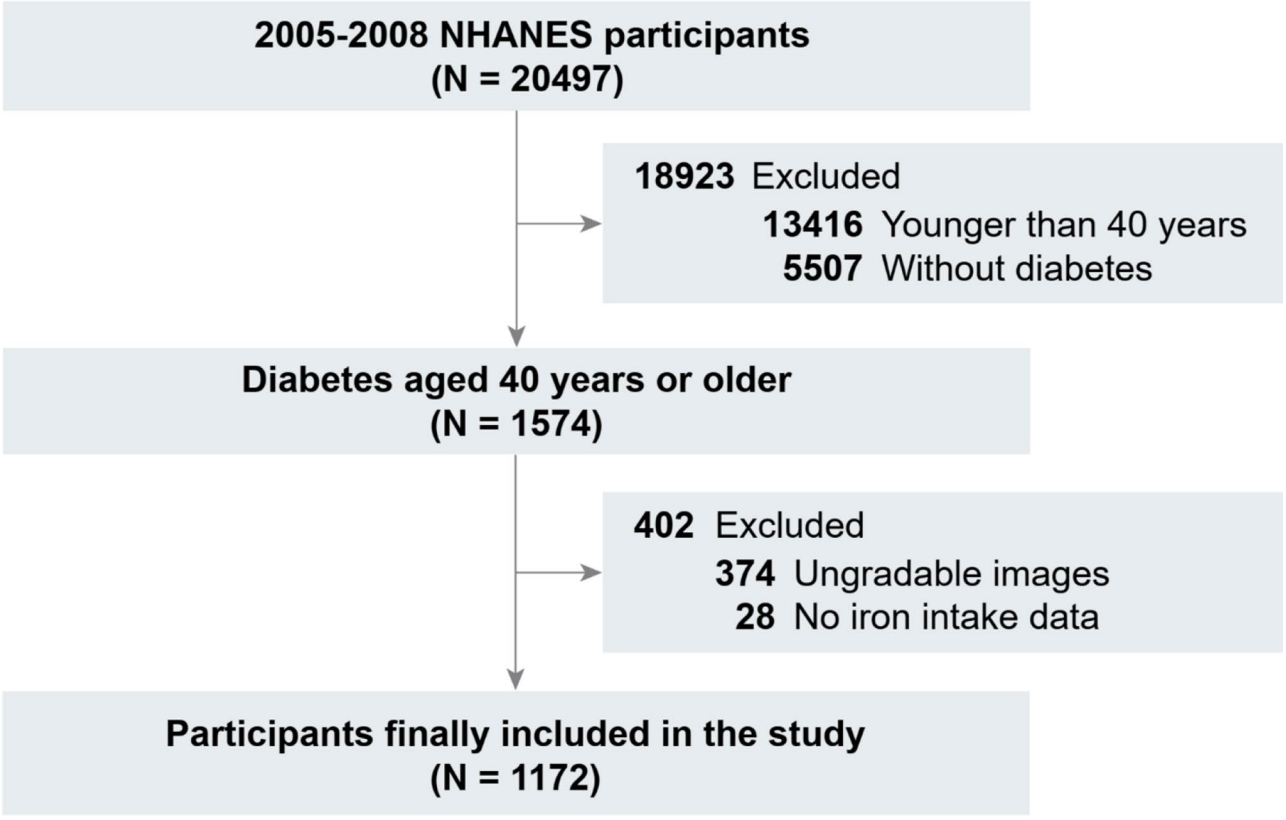

Fig. 1 Flow diagram of participant enrolment of this study

Table 1 Characteristics of the study population according to status of diabetic retinopathy

Characteristic	Total Participants (*n* = 1172)	Without DR (*n* = 814)	With DR (*n* = 358)	*P* value [c]
Sex, n (%)				0.442
Male	594 (50.7%)	406 (49.9%)	188 (52.5%)	
Female	578 (49.3%)	408 (50.1%)	170 (47.5%)	
Age, mean (SD), y	63.2 (10.8)	63.0 (10.8)	63.7 (10.8)	0.250
Race/ethnicity, n (%)				0.002
Non-Hispanic White	485 (41.4%)	360 (44.2%)	125 (34.9%)	
Non-Hispanic Black	337 (28.8%)	210 (25.8%)	127 (35.5%)	
Mexican American	226 (19.3%)	153 (18.8%)	73 (20.4%)	
Other	124 (10.6%)	91 (11.2%)	33 (9.2%)	
Education, n (%)				0.056
Less than high school	467 (39.8%)	306 (37.6%)	161 (45.0%)	
High school	308 (26.3%)	224 (27.5%)	84 (23.5%)	
College or higher	397 (33.9%)	284 (34.9%)	113 (31.6%)	
Marital status, n (%)				0.578
Married or living with partner	726 (61.9%)	509 (62.5%)	217 (60.6%)	
Not married	446 (38.1%)	305 (37.5%)	141 (39.4%)	
Poverty income ratio, n (%)				0.455
< 1.85	573 (48.9%)	390 (47.9%)	183 (51.1%)	
1.85 to 3.50	304 (25.9%)	211 (25.9%)	93 (26.0%)	
≥ 3.50	295 (25.2%)	213 (26.2%)	82 (22.9%)	
Weight status by BMI, n (%)				0.030
Normal or underweight (< 25.0)	152 (13.0%)	107 (13.1%)	45 (12.6%)	
Overweight (25.0 to 30.0)	332 (28.3%)	212 (26.0%)	120 (33.5%)	
Obese (≥ 30.0)	688 (58.7%)	495 (60.8%)	193 (53.9%)	
Smoking status, n (%)				0.052
Never smoker	532 (45.4%)	351 (43.1%)	181 (50.6%)	
Former smoker	442 (37.7%)	323 (39.7%)	119 (33.2%)	
Current smoker	198 (16.9%)	140 (17.2%)	58 (16.2%)	
Alcohol consumption, n (%)				0.229
Never drinker	213 (18.2%)	147 (18.1%)	66 (18.4%)	
Former drinker	179 (15.3%)	115 (14.1%)	64 (17.9%)	
Current drinker	780 (66.6%)	552 (67.8%)	228 (63.7%)	
General health status, n (%)				0.003
Excellent to good	640 (54.6%)	468 (57.5%)	172 (48.0%)	
Fair or poor	532 (45.4%)	346 (42.5%)	186 (52.0%)	
Duration of diabetes, n (%)				< 0.001
< 10 years	786 (67.1%)	655 (80.5%)	131 (36.6%)	
≥ 10 years	386 (32.9%)	159 (19.5%)	227 (63.4%)	
HbA1c, mean (SD)	7.25 (1.68)	6.94 (1.49)	7.98 (1.86)	< 0.001
HbA1c level, n (%)				< 0.001
< 6.5	411 (35.1%)	340 (41.8%)	71 (19.8%)	
≥ 6.5	761 (64.9%)	474 (58.2%)	287 (80.2%)	
Blood haemoglobin level, n (%) [a]				0.004
Normal/High	997 (85.1%)	709 (87.1%)	288 (80.4%)	
Low	175 (14.9%)	105 (12.9%)	70 (19.6%)	
History of comorbidities, n (%)				
Hypertension	965 (82.3%)	660 (81.1%)	305 (85.2%)	0.105
Hypercholesteraemia	643 (54.9%)	432 (53.1%)	212 (59.2%)	0.059
Congestive heart failure	123 (10.5%)	64 (7.9%)	59 (16.5%)	< 0.001
Coronary heart disease	138 (11.8%)	88 (10.8%)	50 (14.0%)	0.148
Angina/angina pectoris	97 (8.3%)	64 (7.9%)	33 (9.2%)	0.509
Heart attack	139 (11.9%)	82 (10.1%)	57 (15.9%)	0.006

Table 1 (continued)

Characteristic	Total Participants (*n* = 1172)	Without DR (*n* = 814)	With DR (*n* = 358)	*P* value [c]
Stroke	127 (10.8%)	74 (9.1%)	53 (14.8%)	0.005
Dietary iron intake, mg/d, Mean (SD)	13.9 (6.62)	14.3 (6.76)	13.2 (6.24)	0.008
Adequate intake of iron by RDA, n (%) [b]				0.176
Adequate	245 (20.9%)	161 (19.8%)	84 (23.5%)	
Inadequate	927 (79.1%)	653 (80.2%)	274 (76.5%)	

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in metres squared); DR, diabetic retinopathy; RDA, recommended dietary allowance; SD, standard deviation

[a] Blood haemoglobin level was classified according to haemoglobin concentrations for the diagnosis of anaemia and assessment of severity released by World Health Organization in 2011

[b] Intake recommendations for iron were developed by the Food and Nutrition Board (FNB) at the Institute of Medicine (IOM) of the National Academies (formerly National Academy of Sciences)

[c] Comparisons were made by the use of the chi-square for categorical variables and the2-sample *t* test for continuous variables

Table 2 Association of dietary iron intake with diabetic retinopathy and vision-threatening diabetic retinopathy

Iron intake (mg/d)	No [a]	No (%) [b]	Crude model		Model 1 [c]		Model 2 [d]		Model 3 [e]	
			OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value
For diabetic retinopathy										
Quartiles										
Q1 (1.81–9.60)	324	118 (36.4)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
Q2 (9.61–13.1)	304	97 (31.9)	0.82 (0.59–1.14)	0.234	0.86 (0.62–1.21)	0.385	0.84 (0.60–1.18)	0.324	0.99 (0.67–1.46)	0.945
Q3 (13.2–18.1)	279	69 (24.7)	0.57 (0.40–0.82)	0.002	0.60 (0.42–0.86)	0.006	0.60 (0.42–0.87)	0.006	0.59 (0.39–0.90)	0.014
Q4 (18.2–52.9)	265	74 (27.9)	0.68 (0.48–0.96)	0.029	0.72 (0.50–1.05)	0.086	0.72 (0.50–1.06)	0.094	0.74 (0.48–1.15)	0.179
Adequate intake [f]										
Yes	245	84 (34.2)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	
No	927	274 (29.6)	0.80 (0.60–1.08)	0.153	0.82 (0.60–1.13)	0.223	0.79 (0.57–1.09)	0.148	0.83 (0.58–1.21)	0.335
For vision-threatening diabetic retinopathy										
Quartiles										
Q1 (1.81–9.60)	238	32 (13.4)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-
Q2 (9.61–13.1)	222	15 (6.8)	0.47 (0.25–0.89)	0.020	0.52 (0.27-1.00)	0.049	0.51 (0.26–0.99)	0.048	0.77 (0.35–1.72)	0.526
Q3 (13.2–18.1)	224	14 (6.3)	0.43 (0.22–0.83)	0.012	0.51 (0.26-1.00)	0.050	0.50 (0.25–0.98)	0.044	0.42 (0.19–0.94)	0.036
Q4 (18.2–52.9)	204	13 (6.4)	0.44 (0.22–0.86)	0.016	0.62 (0.31–1.27)	0.192	0.60 (0.29–1.24)	0.167	0.80 (0.33–1.94)	0.628
Adequate intake [f]										
Yes	182	21 (11.5)	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	-	1.00 (reference)	
No	706	53 (7.5)	0.62 (0.36–1.06)	0.082	0.78 (0.44–1.36)	0.377	0.71 (0.40–1.26)	0.246	0.86 (0.42–1.76)	0.680

Abbreviations: CI, confidence interval; OR, odds ratio

[a] Number of participants with T2D in NHANES 2005–2008

[b] Number of participants with T2D and diabetic retinopathy or vision-threatening diabetic retinopathy in NHANES 2005–2008

[c] Model 1: Adjusted for demographic variables (age, sex, race/ethnicity, education, marital status, poverty income ratio)

[d] Model 2: Adjusted for demographic and lifestyle variables (smoking, drinking, body mass index)

[e] Model 3: Adjusted for demographic, lifestyle, diabetes related variables (duration of diabetes, HbA1c level), and medical comorbidity variables (general health condition, history of angina/angina pectoris, congestive heart failure, coronary heart disease, heart attack, hypercholesteraemia, hypertension, and stroke)

[f] Adequate dietary iron intake is defined according to Recommended Dietary Allowance (RDA) developed by the Food and Nutrition Board (FNB) at the Institute of Medicine (IOM) of the National Academies (formerly National Academy of Sciences)

comorbidities including congestive heart failure, heart attack and stroke (all $P < 0.05$, Table 1). The mean dietary iron intake of subjects with DR was 13.2 ± 6.24 mg/d, which was significantly lower than that of subjects without DR (14.3 ± 6.76 mg/d, $P = 0.008$). However, the proportion of participants meeting the sufficient daily iron intake was comparable between two groups (23.5% vs. 19.8%, $P = 0.176$, Table 1). When analysed according to the severity of DR, the iron intake in the VTDR subgroup was significantly lower than that in the groups without DR and without VTDR ($P = 0.007$, Supplemental Figure S2).

Association of dietary iron intake and DR and VTDR

Table 2 presents the results of multivariable logistic regression analyses. In crude models, participants in the third and fourth quartiles showed significantly lower risk of DR as compared with the first (reference) group (quartile 3: OR = 0.57; 95%CI, 0.40–0.82; quartile 4: OR = 0.68; 95%CI, 0.48–0.96). After adjustment for multiple

covariates, significant associations remained for the third quartile in all logistic regression models (Table 2; for the saturated model: OR = 0.59, 95%CI, 0.39–0.90). Besides, no significant relationship was shown between the second quartile and the reference group in any models (all $P > 0.05$). When dietary iron intake was dichotomised as adequate versus inadequate by RDA, no significant relation was found between iron intake and DR (Table 2, all $P > 0.05$).

Compared with the first quartile, all other quartiles yielded significantly lower odds of VTDR in crude models (quartile 2: OR = 0.47; 95%CI, 0.25–0.89; quartile 3: OR = 0.43; 95%CI, 0.22–0.83; quartile 4: OR = 0.44; 95%CI, 0.22–0.86). With adjustment of covariates, this relationship remained significant for the third quartile in all models (Table 2; for the saturated model: OR = 0.42, 95%CI, 0.19–0.94). Nonetheless, correlation between VTDR risk and the fourth quartile of iron intake became insignificant after multiple adjustments (all $P > 0.05$). Inadequate intake of iron according to RDA showed no added risk of VTDR in either crude or multi-adjusted models (all $P > 0.05$).

Stratified analyses for association of dietary iron intake and DR

Table 3 demonstrated the associations between dietary iron and DR by various stratifying factors, including sex, race/ethnicity, weight status, HbA1c level, duration of diabetes, and blood haemoglobin level. Compared with the first quartile, subjects within the third quartile yielded significantly lower odds of DR in female, non-Hispanic Black, obese, HbA1c ≥ 6.5%, and DM duration ≥ 10 years groups. Remarkably, for individuals with low blood haemoglobin, both the third and the fourth quartiles showed decreased risks of DR (quartile 3: OR = 0.17; 95%CI, 0.05–0.60; quartile 4: OR = 0.23; 95%CI, 0.06–0.90), but were insignificant in those with normal or high blood haemoglobin. No significant interaction between any stratification factors and dietary iron intake was found (Table 3, all $P > 0.05$).

Nonlinear relationship between dietary iron intake and DR

The non-monotonic relationship revealed by logistic regression analysis promoted us to further investigate the nonlinear relationship of DR and dietary iron intake. RCS analyses showed nonlinear associations between DR and dietary iron intake varied by sex (Fig. 2). In males, no significant relationship was found between dietary

Table 3 Stratified analyses for association of dietary iron with diabetic retinopathy*

Variable	Dietary iron intake, OR (95% CI), mg/d				*P* value for interaction
	Quartile 1 (1.81–9.60)	Quartile 2 (9.61–13.1)	Quartile 3 (13.2–18.1)	Quartile 4 (18.2–52.9)	
Sex					0.471
Male	1 [Reference]	1.22 (0.66–2.26)	0.82 (0.44–1.53)	0.81 (0.44–1.48)	
Female	1 [Reference]	0.84 (0.49–1.42)	0.44 (0.24–0.81)	0.76 (0.37–1.56)	
Race/ethnicity					0.288
Non-Hispanic White	1 [Reference]	1.05 (0.51–2.15)	0.82 (0.39–1.72)	1.10 (0.52–2.34)	
Non-Hispanic Black	1 [Reference]	0.79 (0.39–1.59)	0.38 (0.17–0.85)	0.74 (0.31–1.75)	
Mexican American	1 [Reference]	1.51 (0.61–3.78)	0.61 (0.23–1.61)	0.84 (0.31–2.25)	
Other	1 [Reference]	0.72 (0.07–7.71)	0.97 (0.09–11.1)	0.01 (0.00-0.55)	
Weight status					0.982
BMI < 30	1 [Reference]	1.20 (0.64–2.24)	0.73 (0.38–1.38)	0.64 (0.32–1.30)	
BMI > 30	1 [Reference]	0.88 (0.52–1.49)	0.45 (0.25–0.82)	0.82 (0.46–1.46)	
HbA1c					0.841
< 6.5%	1 [Reference]	0.75 (0.34–1.66)	0.56 (0.24–1.32)	0.45 (0.19–1.06)	
≥ 6.5%	1 [Reference]	1.10 (0.69–1.76)	0.56 (0.34–0.93)	0.90 (0.53–1.53)	
Duration of diabetes					0.422
< 10-y	1 [Reference]	0.95 (0.56–1.61)	0.78 (0.44–1.38)	0.84 (0.46–1.38)	
≥ 10-y	1 [Reference]	1.08 (0.57–2.07)	0.40 (0.21–0.76)	0.65 (0.33–1.27)	
Blood haemoglobin †					0.183
Normal/High	1 [Reference]	0.99 (0.64–1.52)	0.67 (0.42–1.07)	0.81 (0.50–1.32)	
Low	1 [Reference]	0.57 (0.20–1.65)	0.17 (0.05–0.60)	0.23 (0.06–0.90)	

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in metres squared); OR, odds ratio

* Analyses were adjusted for age, sex (except for sex stratification), race/ethnicity (except for race/ethnicity stratification), body weight status (except for weight status stratification), duration of diabetes (except for diabetes duration stratification) and medical comorbidity variables (general health condition, history of angina/angina pectoris, congestive heart failure, coronary heart disease, heart attack, hypercholesteraemia, hypertension, and stroke)

† Blood haemoglobin level was classified according to haemoglobin concentrations for the diagnosis of anaemia and assessment of severity released by World Health Organization in 2011

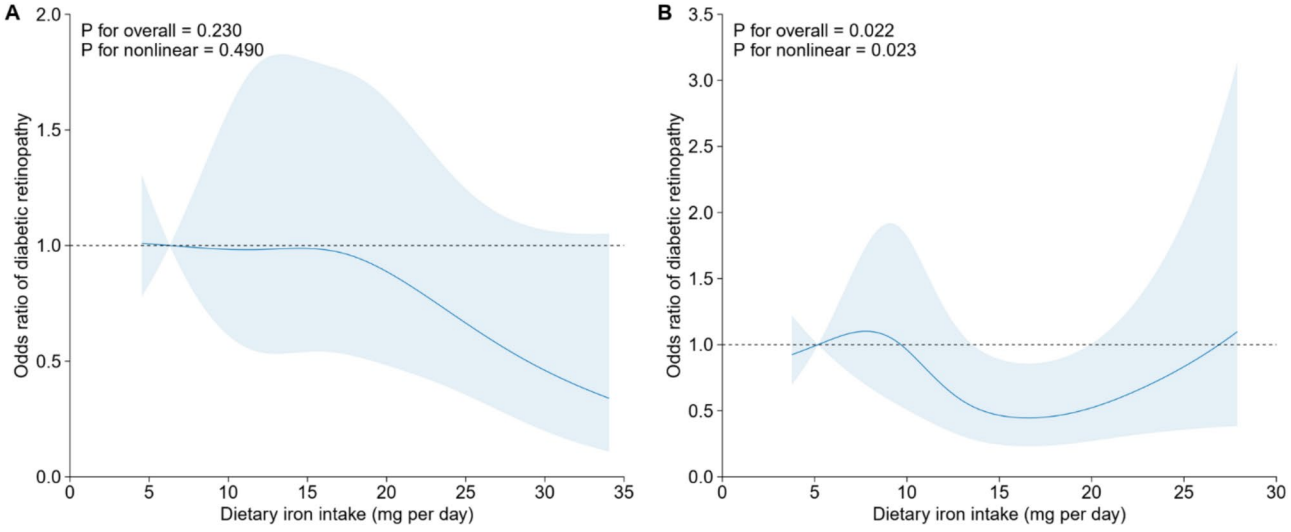

Fig. 2 Associations between dietary iron intake and diabetic retinopathy by sex using restricted cubic spline model. **A**. Male; **B**. Female. Graphs show odds ratio (OR) for any DR according to dietary iron level adjusted for age, race, education, marital status, poverty income ratio, body mass index, smoking status, alcohol consumption, duration of diabetes, HbA1c level, hypertension, hypercholesterolaemia, history of comorbidities, including congestive heart failure, heart attack and stroke. Data were fitted by a logistic regression model, and the model was conducted with 4 knots at the 5th, 35th, 65th, 95th percentiles of iron intake (reference is the 5th percentile). Solid lines indicate ORs, and shadow shape indicate 95% CIs. OR, odds ratio; CI, confidence interval

iron and DR risk ($P_{overall}$=0.230, $P_{nonlinear}$=0.490) (Fig. 2A). In females, however, an approximately U-shaped relationship was revealed; only moderate level of dietary iron intake was associated with decreased risk of DR ($P_{overall}$=0.022, $P_{nonlinear}$=0.023) (Fig. 2B). No significant nonlinear association was found in subgroups by stratification factors other than sex (Supplemental Figure S3).

Sensitivity analyses

Sensitivity analyses showed the robustness of our major results. The association between DR and quartiles of iron intake did not substantially change when participants with outlying data were excluded (Supplemental Table S1 for any DR, Supplemental Table S2 for VTDR). Such associations remained significant when we removed missing data instead of adopting multiple imputation (Supplemental Table S3 for any DR, Supplemental Table S4 for VTDR). This indicated that the outliers did not significantly deflate or inflate the mean of the sample, and had minimal influence on the association derived from the mean. Similarly, when excluding the participants with outlying data (Supplemental Figure S4) or ignoring missing data (Supplemental Figure S5), the RCS analysis results did not substantially change, indicating that missing data caused little noise or bias to estimation.

Discussion

In this large-scale, nationally representative T2D cohort, we found significantly lower daily dietary iron intake in individuals with DR, particularly those with VTDR. After adjustment for major confounding factors, medium-high level of dietary iron intake (13.2–18.1 mg/d) was associated with 59% risk reduction for DR, and 42% risk reduction for VTDR. Of note, beneficial effects of adequate iron intake were more profound in several specific subpopulations, including females, non-Hispanic Blacks, individuals with longer diabetes duration, higher level of HbA1c, concurrent obesity, or anaemia. Spline regression analysis demonstrated that there was a nonlinear U-relationship between the daily iron intake amount and DR risk in females, but not in males. Sensitivity analyses confirmed the robustness of the above findings.

As one of the essential minerals, iron is vital for maintaining the normal structures and functions of a number of macromolecules in cells. Dysregulation of iron homeostasis, either excess or deficiency, might lead to a variety of chronic diseases including diabetes. In patients with pre-existing diabetes, iron deficiency anaemia (IDA) can exacerbate retinopathy through inducing long-term hypoxia in the retina [23]. Additionally, increased lipid peroxidation induced by IDA could elevate HbA1c level [24], which is a strong indicator to predict the onset and progression of DR. Luckily, elevated HbA1c in T2D patients with IDA could be ameliorated by 3-month iron supplementation therapy [25]. Iron overload, however, generates various oxygen and nitrogen species via Fenton reaction, which is one of the major causative factors for diabetes and its complications [26]. Recently, an animal study demonstrated that excessive iron can exacerbate the development of DR by increasing retinal renin expression in mice [9]. Another study also verified that iron accumulation induced the diabetic-related pericyte

loss in eyes and aggravated diabetic microvascular complications [10]. Collectively, ensuring a balanced iron status in the body is critical for preventing the occurrence and progression of diabetic ocular complications. Our findings in this study were well consistent with this concept, and for the first time we revealed possible beneficial effects of medium-high dietary iron intake on preventing DR and VTDR; neither higher nor lower amount was significantly associated with the occurrence of DR.

Another interesting finding of this study is that sex can potentially modify the relationship between dietary iron intake and DR. Both multivariate logistic regression and spline analysis models demonstrated that medium-high daily iron intake was associated with a reduced risk of DR in females but not in males. The underlying mechanism is difficult to interpret but may be related to sex differences in iron homeostasis under the influences of hormonal, genetic, and dietary factors. Females have lower iron storages than males because of menstruation. On average, females lose about 20–60 mg of iron per menstrual cycle, an amount comparable to daily dietary iron intake requirements for males. Besides, differences in dietary habit by sex may also affect iron absorption: men consume more haem iron, while women consume more non-haem iron. Haem iron is more available for absorption from the diet than non-haem iron, which may substantially affect iron status and health outcomes [27].

The stratified analysis in this study found that factors other than sex may also modify the relation between dietary iron intake and DR. Participants who are non-Hispanic Black, obese, with low haemoglobin levels, with poorer glycaemic control (HbA1c ≥ 6.5%) and having a longer duration of diabetes (≥ 10 years) may benefit more from sufficient iron intake than others. A possible reason is that T2D patients with obesity, of non-Hispanic Black ethnicity, and unsatisfactory glycaemic control are more likely to have concurrent iron deficiency or IDA [28–30]. In response, our study also found a significant inverse association between dietary iron intake and the risk of DR in T2D patients with anaemia. Given that anaemia is an established risk factor for DR [11, 12], it is particularly important for the abovementioned at-risk populations to routinely evaluate and maintain sufficient iron intakes.

The findings of our study are relevant for clinical practice, and provide implications for future research as well. To help prevent DR, we encourage adults with T2D to consume a diet with sufficient iron. The optimal amount of daily iron intake showing protective effects on DR (13.2–18.1 mg/d) is higher than the RDA for male adults (8 mg/d) and close to that for premenopausal women (18 mg/d). As this range of daily iron intake is far below the upper limits established by the Food and Nutrition Board (FNB) for adults (< 45 mg/d), it poses very little risk of iron overload and toxicity. Higher iron intake than 18.2 mg/d may not be recommended because excessive iron intake is not associated with further reduced DR risk, but may cause other health problems like neurological disorders. Moreover, due to the nonlinear relationship between dietary iron and DR, our study also indicated that dichotomised cut-off values of RDA may not be capable of guiding iron intake for diabetes patients in terms of minimising retinopathy risk. In the future, a more sophisticated "protective dietary pattern" incorporating iron intake against diabetic retinopathy is to be developed and validated.

There are several strengths of our study. Compared with previous studies (Supplemental Table S5), this is the first study to illustrate the nonlinear relationships between dietary iron intake and DR using spline analyses, a powerful technique to delineate the nonlinear nature of many phenomena in clinical research. Moreover, the NHANES data are high-quality and of great representativeness which ensured the generalisability of our findings and conclusions. The comprehensive survey data allowed us to adjust potential confounders in statistical models as well.

Nevertheless, this study has several limitations. Firstly, due to the cross-sectional design, we only identified correlation rather than causation between dietary iron intake and DR. Further dietary trials are needed to establish causality and to test the efficacy of dietary iron interventions. Secondly, the dietary data in NHANES were acquired by two 24-hour recalls, largely depending on participants' memory. Recall bias could not be excluded, and the actual daily nutrient intake level might slightly differ from the self-reported data. Thirdly, due to the unavailability of data on supplement intake of iron, our study only included dietary iron intake but not on metal supplements. Fourthly, although we adjusted for a comprehensive range of confounding factors, residual or unknown confounding cannot be entirely excluded. Fifthly, cultural and dietary differences across the population have the potential to impact the findings. Sixthly, although the sample population is highly representative and with large size, the prevalence of VTDR was relatively low which may have contributed to the non-statistically significant results.

In summary, our study found a nonlinear association between dietary iron intake and DR risk. Medium-high level of iron intake was associated with a reduced risk of DR and VTDR, especially for females, non-Hispanic Blacks, obese people, those with HbA1c ≥ 6.5%, and diabetes duration ≥ 10 years. High or low levels of iron intake may not be conducive to preventing the development of DR. A precise efficacy of dietary iron intervention strategy and its impact on DR and VTDR are to be determined in longitudinal studies and controlled trials.

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

Supplementary Material 8

Supplementary Material 9

Supplementary Material 10

Supplementary Material 11

Supplementary Material 12

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None.

Author contributions
X.C. and W.X. was responsible for the concept, design, and supervision. Y.F. and H.S. collected the data from the database. W.L. and W.Y. conducted data analyses and visualisation. Y.F. and H.S. wrote the original draft. X.C. and W.X. revised the manuscript critically. All authors reviewed the manuscript.

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Data availability
All original data related to this study are available in the public, open access NHANES data repository (https://wwwn.cdc.gov/nchs/nhanes/Default.aspx).

Declarations

Ethical approval
The NHANES protocol was approved by the National Center for Health Statistic (NCHS) Research Ethics Review Board (Protocol #2005-06 for NHANES 2005–2006; Continuation of Protocol #2005-06 for NHANES 2007–2008). The Ethics Review Board approval information is accessible at: https://www.cdc.gov/nchs/nhanes/irba98.htm (accessed on 8 August, 2024).

Competing interests
The authors declare no competing interests.

Clinical trial number
Not applicable.

Patient consent for publication
Not applicable.

Additional contributions
None reported.

Role of the funder/sponsor
The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

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