


BMJ Open Association between metformin use and the risk of age-related macular degeneration in patients with type 2 diabetes: a retrospective study

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ABSTRACT

Objectives To investigate the effect of metformin on the decreased risk of developing age-related macular degeneration (AMD) in patients with type 2 diabetes mellitus (T2DM) for ≥ 10 years.

Design A retrospective study.

Participants Patients aged ≥ 50 with a diagnosis of T2DM no less than 10 years were included.

Methods Variables predisposing to AMD were reviewed; the potential confounders related to T2DM or AMD were selected from literature records; AMD and diabetic retinopathy (DR) were diagnosed by funduscopy, optical coherence tomography and/or fluorescein angiography. The subgroup analysis was performed in early and late AMD. The protective effect of metformin was evaluated in duration-response and dose-response patterns.

Results A total of 324 patients (115 metformin non-users and 209 users) were included in the final analysis. AMD was observed in 15.8% of metformin users and 45.2% of metformin non-users ($p < 0.0001$). The ORs for any AMD, early AMD and late AMD present in patients with DR were 0.06 (0.02–0.20), 0.03 (0.00–0.20) and 0.17 (0.04–0.75). The serum high-density lipoprotein level was positively associated with the late AMD risk ($p = 0.0054$). When analysed by the tertiles of cumulative duration, a similarly reduced risk was observed for the second (5–9 years) (OR: 0.24, 95% CI: 0.08 to 0.75) and third tertiles (≥ 10 years) (OR: 0.22, 95% CI: 0.09 to 0.52) compared with the first tertile (≤ 4 years).

Conclusion Among patients with T2DM for ≥ 10 years, metformin users were less likely to develop any AMD and early AMD than non-users; however, the late AMD was not significantly associated with the use of metformin. Also, AMD was less prevalent in patients with DR. The prolonged metformin treatment with a high cumulative dose enhanced the protective effect against AMD. Metformin significantly reduces the AMD risk when the cumulative duration is > 5 years.

INTRODUCTION

Age-related macular degeneration (AMD) is a progressive eye disorder and the leading cause of irreversible visual impairment in the population aged ≥ 50 years worldwide.^{1 2} The early AMD is characterised by the presence

Strengths and limitations of this study

- This study included elderly patients with type 2 diabetes mellitus for ≥ 10 years.
- Variables in this study included potential confounders, such as laboratory data, duration and dose of metformin use and diabetic retinopathy.
- The subgroup analysis in the early and late age-related macular degeneration (AMD) and the in-depth investigation in duration-dependent and dose-dependent response patterns were performed.
- This study was retrospective design with relatively small sample of AMD and lack of analysis of other medication histories.

of drusenoid deposits, and the late AMD involves atrophy of the retinal pigment epithelium (RPE) or the development of choroidal neovascularisation (CNV). A number of risk factors, such as cigarette smoking, obesity, hypertension, elevated lipid levels, are related to AMD.^{3–5} However, the roles of immunological process, inflammatory response and oxidative stress in the pathogenesis of AMD remain to be further clarified.^{6–8}

Metformin, a biguanide recommended as the first-line antidiabetic therapy for the management of type 2 diabetes mellitus (T2DM), possesses anti-inflammatory and antioxidant effects^{9 10} and lowers the blood glucose effect. In addition, it plays a role in extending the life span and reducing the risk of some age-associated diseases, such as cancer, diabetes mellitus (DM) and cardiovascular disease.^{11 12} Therefore, whether the use of metformin affects the risk of age-associated eye diseases, such as AMD and diabetic retinopathy (DR), which are of public health concern, deserves further investigation.

Recent clinical studies concluded a controversial association between the use of metformin and the risk of developing

AMD.^{13–16} A nested case–control study concluded that metformin did not inhibit AMD in elderly patients >60 years old.¹³ In contrast, a retrospective 1:3 case–control study suggested that patients who had taken metformin were less likely to develop AMD.¹⁴ This study did not consider the systemic risk factors associated with AMD and failed to include the dosage and duration of metformin treatment. Another retrospective cohort study also showed that patients with T2DM who used metformin were at a significantly lower risk of developing AMD; the lower AMD risk is associated with a higher dose of metformin.¹⁵ Although AMD becomes clinically evident usually after 50 years of age,¹⁷ the study comprised the largest number of participants in the under-50 age-group and did not analyse the subtypes of AMD. A recent case–control study suggested that metformin use was associated with reduced odds of developing AMD.¹⁶ This association was dose dependent, but low to moderate doses showed the greatest benefit. This article had a large sample size, but only included patients with a history of metformin use within 2 years prior to the diagnosis of AMD, and did not include medical information such as haemoglobin A1c (HbA1c) levels. Epidemiological studies of the association between systemic diseases and AMD demonstrated that any AMD and late AMD were less prevalent in patients with diabetes.¹⁸ Nonetheless, another study concluded that patients with DM were likely to have early AMD.¹⁹ Therefore, the morbidity characteristics of subtypes of AMD in patients with diabetes may be different, necessitating a subtype analysis. Furthermore, metformin reduces oesophageal cancer risk when the cumulative duration is >2 years.²⁰ Thus, prolonged metformin use is crucial for the investigation of drug effects.

The present study included patients with ≥ 10 years of T2DM and had taken into account the systemic risk factors associated with AMD, the subtypes of AMD and the presence of DR. Also, a correlation was established between the duration–response and dosage–response. Thus, understanding the effect of metformin on the development of AMD might improve the knowledge regarding its pathogenesis, prevent the disease by adjusting anti-glycaemic drugs, and subsequently reduce the morbidity of AMD in patients with T2DM.

MATERIALS AND METHODS

In this, retrospective study, we reviewed the medical records of 1891 patients diagnosed with T2DM and followed-up at the Ophthalmology Department of China-Japan Friendship Hospital, Beijing, China, from September 2015 to August 2020. We identified patients with diagnosis of T2DM (ICD-10 : E11.2–E11.9) and AMD (ICD-10: H35.30–H35.31) using the International Classification of Disease, Tenth Revision, Clinical Modification codes. The exclusion criteria are as follows: diagnosis of AMD before T2DM; refractive medium opacity affected the examination and diagnosis of fundus disease; diagnosed with other diseases related with macular and posterior pole; history of vitreoretinal surgery, intravitreal injection or retinal photocoagulation treatment. The final study cohort included 324 patients aged ≥ 50 years old, were diagnosed as T2DM for ≥ 10 years, had complete information with respect to baseline characteristics and medical history (figure 1). This study complied with the Declaration of Helsinki. No patients and the public

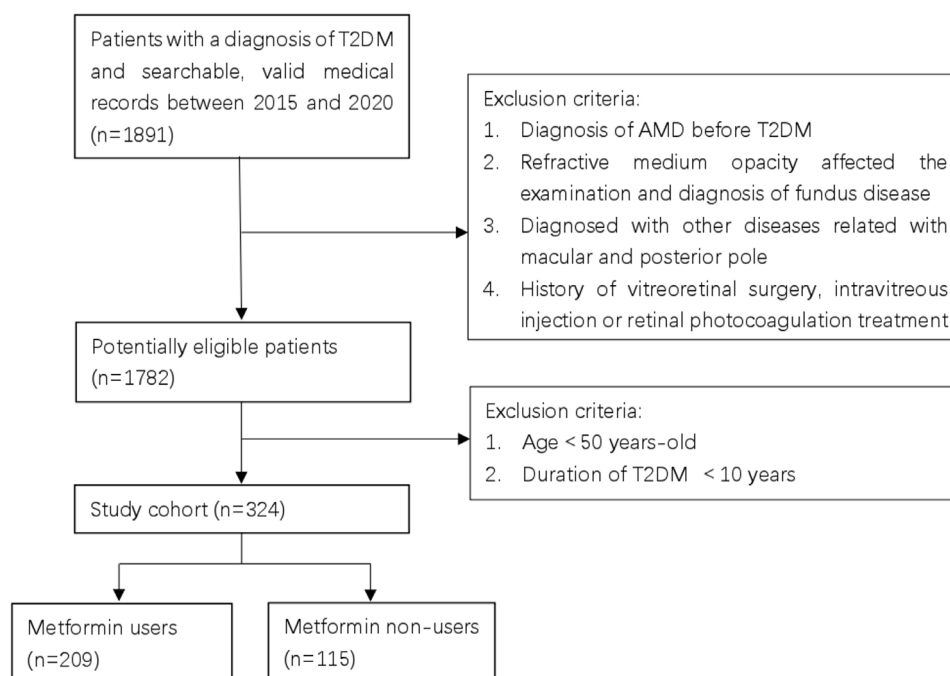


Figure 1 Flowchart of inclusion and exclusion criteria. AMD, age-related macular degeneration; T2DM, type 2 diabetes mellitus.

were involved in the design, or conduct, or reporting or dissemination plans in our research.

AMD was graded by ophthalmologists using the International Age-related Maculopathy Epidemiological Study Group grading system.²¹ Early AMD was defined by the presence of drusen (discrete whitish–yellow spots located external to the neuroretina or RPE) or drusen with RPE abnormalities (areas of hyperpigmentation or hypopigmentation). Late AMD was defined by the presence of geographical atrophy within the grid or neovascular AMD (subretinal haemorrhage, subretinal fibrous scar, RPE detachment or serous detachment of sensory retina).^{19 22} DR was graded using the early treatment DR study scale.²³ The primary diagnosis on a medical record was adopted. The onset of AMD and DR has been confirmed by funduscopy, optical coherence tomography (OCT) and/or fluorescein angiography. Each fundus and OCT image was graded twice for the presence and type of AMD and DR and stratified into stages based on the grading in the worst eye.

The potential confounders, such as age, gender, smoking, body mass index (BMI), hypertension, hyperlipidaemia and diabetes have been selected based on the reports of significant associations between DR and AMD. BMI (kg/m^2) was calculated as $\text{weight}/\text{height}^2$ using the measured values. The plasma was evaluated from the blood samples collected at the most recent endocrinology visit. Laboratory data regarding glucose control, plasma lipids and kidney function, including fasting blood glucose (FBG), HbA1c, total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), uric acid (UA) and creatinine (Cr), were obtained from the Biochemistry Laboratory of the China-Japan Friendship Hospital.

Patients with T2DM were divided into two groups of metformin non-users and metformin users. Patients who had administered metformin with a daily average dose >250 mg were categorised as ‘with metformin treatment.’ The use of other oral antidiabetic medications and insulin treatment was also assessed. For subgroup analysis, we classified exposure specifically according to the daily average metformin dose and duration. The cumulative duration was calculated as total months of metformin treatment without intermittent days or irregular medication. The cumulative dose was obtained by multiplying the daily average dose by the treatment duration. The data for statistical analysis are recorded according to the rounding principle; for example, 1–5 months is counted as 0.5 years, 6–17 months is counted as 1 year and 18–29 months is counted as 2 years.

The categorical variables were expressed as number (%), and the comparison between groups was evaluated by χ^2 test. The continuous variables were expressed as median (IQR) and compared between groups by Mann-Whitney U test. ORs with 95% CIs were estimated by logistic regression models in univariable and multivariable analyses. A stepwise method was adopted to screen the risk factors that were significantly associated with

the occurrence of AMD. Furthermore, the interaction between the duration of metformin use and the daily average dose of metformin was explored. The cumulative duration and cumulative dose of metformin were categorised into three tertiles to analyse their effects on reducing the risk of AMD. The second and third tertiles were compared with the corresponding first tertile to derive adjusted ORs and p values. All statistical analyses were performed using SAS software (Cary V.9.4). A p value <0.01 was considered statistically significant.

RESULTS

Among 324 enrolled patients, 209 (65%) had received metformin treatment. Metformin non-users contained four types of treatment situations: 46 patients had taken other types of glucose-lowering drugs, 35 patients had used other types of glucose-lowering drugs and insulin, 24 patients had treated with insulin only and 10 patients had not received medication. The demographic and clinical characteristics of the patients are described in [table 1](#). Metformin users were likely to have hyperlipidaemia ($p=0.0048$) and a high BMI value ($p=0.0077$), and a high HbA1c value ($p=0.0053$). AMD was diagnosed in 15.8% of metformin users and 45.2% of metformin non-users ($p<0.0001$). However, the presence of DR ($p=0.4198$) and the number of personal and medical variables were similar in both groups. Also, no significant difference was detected in the levels of FBG, serum UA and plasma lipids in the two groups.

Variables that are significantly associated with AMD risk are presented in [table 2](#). Patients treated with metformin prior to their ophthalmology visit were less likely to progress to any AMD diagnosis (OR: 0.23, 95% CI: 0.13 to 0.38, $p<0.0001$). The AMD diagnosis is also significantly associated with the age (OR: 1.10, 95% CI: 1.06 to 1.14, $p<0.0001$), DR (OR: 0.06, 95% CI: 0.02 to 0.20, $p<0.0001$), HbA1c (OR: 0.76, 95% CI: 0.65 to 0.89, $p=0.0009$) and HDL (OR: 3.55, 95% CI: 1.49 to 8.43, $p=0.0041$). In the multivariable analysis, patients who took metformin (OR: 0.24, 95% CI: 0.13 to 0.42, $p<0.0001$) or had DR (OR: 0.07, 95% CI: 0.02 to 0.23, $p<0.0001$) were significantly less likely to be diagnosed with AMD.

The subgroup analysis was performed in early AMD and late AMD separately. After adjusting for the potential confounding factors, early AMD was found to be significantly associated with the use of metformin (OR: 0.17, 95% CI: 0.09 to 0.34, $p<0.0001$). However, late AMD was not significantly associated with the use of metformin (OR: 0.43, 95% CI: 0.18 to 1.04, $p=0.0619$). Similarly, the presence of DR, the value of BMI and the level of HbA1c were associated with early AMD ($p=0.0004$, $p=0.0074$ and $p=0.0005$, respectively) but not with late AMD ($p=0.0193$, $p=0.0991$ and $p=0.3615$, respectively). The level of serum HDL cholesterol was significantly associated with late AMD ($p=0.0054$). Age was a significant risk factor for AMD in univariate and multivariate analyses. Elderly patients were prone to AMD (OR: 1.08, 95% CI: 1.04

Table 1 Characteristics of patients with diabetes who used and did not use metformin

Variable	All (n=324)	Subgroup		P value
		No metformin group (n=115)	Metformin group (n=209)	
Men	175 (54.0)	57 (49.6)	118 (56.5)	0.2335
Median age	67 (62.0, 73.0)	68 (63.0, 77.0)	66 (61.0, 75.0)	0.0299*
Duration of DM (years)	17 (12.0, 20.0)	18 (12.0, 21.0)	16.0 (12.0, 20.0)	0.2349
Presence of hypertension	210 (64.8)	76 (66.1)	134 (64.1)	0.7221
Presence of hyperlipidaemia	141 (43.5)	38 (33.0)	103 (49.3)	0.0048*
Smoking	103 (31.8)	31 (27.0)	72 (34.4)	0.1658
Body mass index (kg/m ²)	25.0 (22.9, 27.2)	24.3 (22.2, 26.2)	25.3 (23.1, 27.9)	0.0077*
Metformin				
Duration (years)	N.A.	10.0 (6.0, 15.0)	N.A.	
Average dosage (mg/day)	N.A.	1500 (1000.0, 1500.0)	N.A.	
Insulin users	161 (49.7)	59 (51.3)	102 (48.8)	0.6667
Diabetic retinopathy	91 (28.1)	30 (26.1)	61 (29.2)	0.4198
AMD	85 (26.2)	52 (45.2)	33 (15.8)	<0.0001*
Early	63 (19.4)	42 (36.5)	21 (10.0)	
Late	22 (6.8)	10 (8.7)	12 (5.7)	
HbA1c (%)	7.6 (6.5, 9.1)	7.3 (6.1, 8.8)	7.8 (6.8, 9.2)	0.0053*
FBG (mmol/L)	7.5 (6.1, 9.8)	7.4 (5.7, 9.8)	7.6 (6.2, 9.9)	0.2814
Total cholesterol (mmol/L)	3.9 (3.2, 4.7)	3.9 (3.4, 4.8)	3.9 (3.1, 4.7)	0.1453
Triglycerides (mmol/L)	1.4 (1.0, 2.1)	1.4 (0.8, 1.9)	1.4 (1.0, 2.1)	0.1285
HDL cholesterol (mmol/L)	1.0 (0.8, 1.2)	1.0 (0.9, 1.3)	1.0 (0.8, 1.2)	0.1549
LDL cholesterol (mmol/L)	2.3 (1.8, 2.9)	2.5 (1.9, 3.1)	2.3 (1.7, 2.8)	0.1062
Blood uric acid (µmol/L)	324.0 (268.5, 393.0)	324.0 (258.0, 399.0)	324.0 (272.0, 392.0)	0.8705
Blood creatinine (µmol/L)	67.9 (56.4, 82.5)	70.9 (61.1, 89.4)	66.0 (55.3, 80.8)	0.0398*

Data represent median (IQR) or n (%).

*P value <0.01 (independent t-test or χ^2 test).

AMD, age-related macular degeneration; DM, diabetes mellitus; FBG, fasting blood glucose; HbA1c, haemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

to 1.12, $p < 0.0001$), both early AMD (OR: 1.07, 95% CI: 1.03 to 1.12, $p < 0.0001$) and late AMD (OR: 1.15, 95% CI: 1.08 to 1.23, $p < 0.0001$). These results suggested that metformin in glucose-lowering therapy protects against the development of early AMD in patients with T2DM, while there was no significant protection effect against the development of late AMD. On the other hand, patients with T2DM with DR are less likely to develop AMD; this protective effect is more pronounced in early AMD than late AMD. In contrast, the elevated levels of serum HDL cholesterol constitute the risk factor for the development of AMD, and this risk is more pronounced in late AMD than in early AMD.

A subsequent analysis was performed in metformin users to evaluate the effects of treatment duration and dose on the risk of early and late AMD. Table 3 shows that the metformin users with prolonged duration and daily average dose of at least 1500mg were less likely to develop AMD, and the effects were pronounced in the early AMD (OR: 0.79, 95% CI: 0.70 to 0.88, $p < 0.0001$ and OR: 0.18, 95% CI: 0.07 to 0.48, $p = 0.0007$). The interaction

effects of duration and average dosage were significantly associated with the development of any AMD (OR: 0.87, 95% CI: 0.81 to 0.94, $p = 0.0003$) and early AMD (OR: 0.77, 95% CI: 0.66 to 0.90, $p = 0.0010$). Conversely, the risk of developing late AMD was not associated with either the metformin treatment duration ($p = 0.8828$) or the average dosage ($p = 0.1744$). The level of serum UA was associated with the risk of early AMD (OR: 0.99, 95% CI: 0.99 to 1.00, $p = 0.0098$). The age and the serum HDL cholesterol level were significantly associated with the risk of late AMD ($p < 0.0001$ and $p = 0.0054$, respectively) in overall patients.

Figure 2 shows the risk of AMD corresponding to the metformin exposure. A trend of decreasing incidence with both prolonged duration (p value for trend = 0.0007) and high cumulative dose (p value for trend = 0.0007) was observed. When analysed by the tertiles of cumulative duration, reduced risk was observed similarly for the second tertile (OR: 0.24, 95% CI: 0.08 to 0.75) and third tertile (OR: 0.22, 95% CI: 0.09 to 0.52) compared with the first tertile (≤ 4 years). When analysed by the tertiles of cumulative dose, the ORs for AMD risk in the second

Table 2 Univariate and multivariable logistic regression models assessing risk of developing AMD in metformin users and non-users

Covariate	Any AMD						Early AMD						Late AMD					
	Univariate analysis		Multivariable analysis		Univariate analysis		Multivariable analysis		Univariate analysis		Multivariable analysis		Univariate analysis		Multivariable analysis			
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value		
Metformin use (yes vs no)	0.23 (0.13 to 0.38)	<0.0001	0.24 (0.13 to 0.42)	<0.0001	0.18 (0.10 to 0.33)	<0.0001	0.17 (0.09 to 0.34)	<0.0001	0.43 (0.18 to 1.04)	0.0619	0.43 (0.18 to 1.04)	0.0619	0.43 (0.18 to 1.04)	0.0619	0.43 (0.18 to 1.04)	0.0619		
Sex (female vs male)	1.13 (0.69 to 1.86)	0.6284			1.10 (0.63 to 1.92)	0.7305			1.21 (0.51 to 2.91)	0.6649	1.21 (0.51 to 2.91)	0.6649	1.21 (0.51 to 2.91)	0.6649	1.21 (0.51 to 2.91)	0.6649		
Age	1.10 (1.06 to 1.14)	<0.0001	1.08 (1.04 to 1.12)	<0.0001	1.09 (1.05 to 1.13)	<0.0001	1.07 (1.03 to 1.12)	<0.0001	1.15 (1.08 to 1.22)	<0.0001	1.15 (1.08 to 1.22)	<0.0001	1.15 (1.08 to 1.22)	<0.0001	1.15 (1.08 to 1.23)	<0.0001		
DM	1.03 (0.99 to 1.07)	0.1056			1.03 (0.99 to 1.08)	0.1391			1.04 (0.96 to 1.11)	0.3400	1.04 (0.96 to 1.11)	0.3400	1.04 (0.96 to 1.11)	0.3400	1.04 (0.96 to 1.11)	0.3400		
Hypertension	1.02 (1.00 to 1.04)	0.0955			1.02 (0.99 to 1.04)	0.1885			1.03 (0.99 to 1.07)	0.1820	1.03 (0.99 to 1.07)	0.1820	1.03 (0.99 to 1.07)	0.1820	1.03 (0.99 to 1.07)	0.1820		
Hyperlipidaemia	0.97 (0.93 to 1.01)	0.1063			0.95 (0.90 to 1.00)	0.0340			1.01 (0.95 to 1.07)	0.7237	1.01 (0.95 to 1.07)	0.7237	1.01 (0.95 to 1.07)	0.7237	1.01 (0.95 to 1.07)	0.7237		
Smoking	0.98 (0.96 to 1.00)	0.0242			0.47 (0.24 to 0.92)	0.0266			0.53 (0.19 to 1.50)	0.2318	0.53 (0.19 to 1.50)	0.2318	0.53 (0.19 to 1.50)	0.2318	0.53 (0.19 to 1.50)	0.2318		
DR (Yes vs no)	0.06 (0.02 to 0.20)	<0.0001	0.07 (0.02 to 0.23)	<0.0001	0.03 (0.00 to 0.20)	0.0004	0.03 (0.00 to 0.23)	0.0007	0.17 (0.04 to 0.75)	0.0193	0.17 (0.04 to 0.75)	0.0193	0.17 (0.04 to 0.75)	0.0193	0.17 (0.04 to 0.75)	0.0193		
BMI	0.95 (0.88 to 1.02)	0.1483			0.88 (0.81 to 0.97)	0.0074			1.11 (0.98 to 1.25)	0.0991	1.11 (0.98 to 1.25)	0.0991	1.11 (0.98 to 1.25)	0.0991	1.11 (0.98 to 1.25)	0.0991		
HbA1c	0.76 (0.65 to 0.89)	0.0009			0.71 (0.58 to 0.86)	0.0005			0.89 (0.69 to 1.15)	0.3615	0.89 (0.69 to 1.15)	0.3615	0.89 (0.69 to 1.15)	0.3615	0.89 (0.69 to 1.15)	0.3615		
FBG	0.95 (0.87 to 1.03)	0.2127			0.95 (0.86 to 1.05)	0.3081			0.93 (0.79 to 1.09)	0.3757	0.93 (0.79 to 1.09)	0.3757	0.93 (0.79 to 1.09)	0.3757	0.93 (0.79 to 1.09)	0.3757		
CHO	0.99 (0.96 to 1.02)	0.6719			0.99 (0.95 to 1.04)	0.7200			1.00 (0.96 to 1.03)	0.8619	1.00 (0.96 to 1.03)	0.8619	1.00 (0.96 to 1.03)	0.8619	1.00 (0.96 to 1.03)	0.8619		
TG	0.86 (0.69 to 1.08)	0.1997			0.85 (0.65 to 1.10)	0.2163			0.91 (0.62 to 1.33)	0.6125	0.91 (0.62 to 1.33)	0.6125	0.91 (0.62 to 1.33)	0.6125	0.91 (0.62 to 1.33)	0.6125		
HDL	3.55 (1.49 to 8.43)	0.0041			2.68 (1.00 to 7.18)	0.0492			6.79 (1.76 to 26.13)	0.0054	6.79 (1.76 to 26.13)	0.0054	6.79 (1.76 to 26.13)	0.0054	6.79 (1.76 to 26.13)	0.0054		
LDL	1.21 (0.90 to 1.63)	0.2167			1.02 (0.73 to 1.43)	0.9136			1.95 (1.16 to 3.29)	0.0117	1.95 (1.16 to 3.29)	0.0117	1.95 (1.16 to 3.29)	0.0117	2.14 (1.21 to 3.77)	0.0088		
UA	1.00 (1.00 to 1.00)	0.2031			1.00 (0.99 to 1.00)	0.0833	1.00 (0.99 to 1.00)	0.0332	1.00 (1.00 to 1.01)	0.7303	1.00 (1.00 to 1.01)	0.7303	1.00 (1.00 to 1.01)	0.7303	1.00 (1.00 to 1.01)	0.7303		
Cr	1.00 (0.99 to 1.01)	0.6582			1.00 (0.99 to 1.01)	0.6798			2.29 (0.47 to 11.18)	0.3056	2.29 (0.47 to 11.18)	0.3056	2.29 (0.47 to 11.18)	0.3056	2.29 (0.47 to 11.18)	0.3056		

AMD, age-related macular degeneration; BMI, body mass index; CHO, total cholesterol; Cr, creatinine; DM, diabetes mellitus; DR, diabetic retinopathy; FBG, fasting blood glucose; HbA1c, haemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides; UA, uric acid.

Table 3 Univariate and multivariable logistic regression models assessing risk of developing AMD in metformin users

Covariate	Any AMD				Early AMD				Late AMD			
	Univariate analysis		Multivariable analysis		Univariate analysis		Multivariable analysis		Univariate analysis		Multivariable analysis	
	OR (95% CI)	P value	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Sex (female vs male)	0.95 (0.45 to 2.01)	0.8879			0.64 (0.25 to 1.67)	0.3645			1.80 (0.55 to 5.89)	0.3312		
Age	1.09 (1.04 to 1.15)	0.0008	1.09 (1.03 to 1.15)	0.0019	1.04 (0.98 to 1.10)	0.2378			1.24 (1.12 to 1.38)	<0.0001	1.26 (1.11 to 1.42)	0.0002
DM	1.03 (0.97 to 1.10)	0.3494			0.97 (0.89 to 1.07)	0.5787			1.13 (1.02 to 1.25)	0.0195		
Hypertension	1.00 (0.97 to 1.04)	0.8580			0.97 (0.93 to 1.02)	0.2886			1.05 (0.99 to 1.10)	0.0951		
Hyperlipidaemia	0.97 (0.92 to 1.02)	0.2711			0.88 (0.78 to 1.00)	0.0455			1.03 (0.97 to 1.10)	0.3121		
Smoking	0.98 (0.96 to 1.01)	0.1672			0.99 (0.96 to 1.02)	0.3348			0.98 (0.93 to 1.02)	0.2835		
Metformin use												
Duration	0.87 (0.80 to 0.94)	0.0006			0.79 (0.70 to 0.88)	<.0001			0.99 (0.88 to 1.11)	0.8828		
Daily average dosage (≥ 1500 mg vs <1500 mg)	0.25 (0.12 to 0.55)	0.0005			0.18 (0.07 to 0.48)	0.0007			0.44 (0.14 to 1.43)	0.1744		
Interaction (duration and average dosage)	0.87 (0.81 to 0.94)	0.0003	0.88 (0.81 to 0.94)	0.0005	0.77 (0.66 to 0.90)	0.0010			0.96 (0.88 to 1.05)	0.3402		
BMI	1.02 (0.91 to 1.13)	0.7653			0.97 (0.84 to 1.12)	0.6586			1.10 (0.93 to 1.31)	0.2709		
HbA1c	0.88 (0.70 to 1.10)	0.2607			0.92 (0.71 to 1.19)	0.5207			0.81 (0.55 to 1.18)	0.2628		
FBG	1.00 (0.88 to 1.14)	0.9849			1.02 (0.87 to 1.19)	0.8286			0.97 (0.79 to 1.20)	0.7947		
CHO	0.99 (0.96 to 1.03)	0.7574			0.99 (0.92 to 1.06)	0.8086			1.00 (0.96 to 1.04)	0.8813		
TG	0.92 (0.70 to 1.21)	0.5701			0.91 (0.64 to 1.29)	0.5908			0.95 (0.64 to 1.41)	0.8067		
HDL	3.96 (1.12 to 14.01)	0.0330			1.71 (0.34 to 8.54)	0.5140			13.89 (2.18 to 88.57)	0.0054		
LDL	1.48 (0.95 to 2.23)	0.0846			1.12 (0.64 to 1.97)	0.6992			2.22 (1.16 to 4.26)	0.0160		
UA	1.00 (0.99 to 1.00)	0.2691			0.99 (0.99 to 1.00)	0.0098			1.00 (1.00 to 1.01)	0.1301		
Cr	1.00 (0.99 to 1.02)	0.5372			0.99 (0.96 to 1.01)	0.2682			1.02 (1.00 to 1.03)	0.0259	1.02 (1.00 to 1.04)	0.0314

AMD, age-related macular degeneration; BMI, body mass index; CHO, total cholesterol; Cr, blood creatinine; DM, diabetes mellitus; DR, diabetic retinopathy; FBG, fasting blood glucose; HbA1c, haemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides.

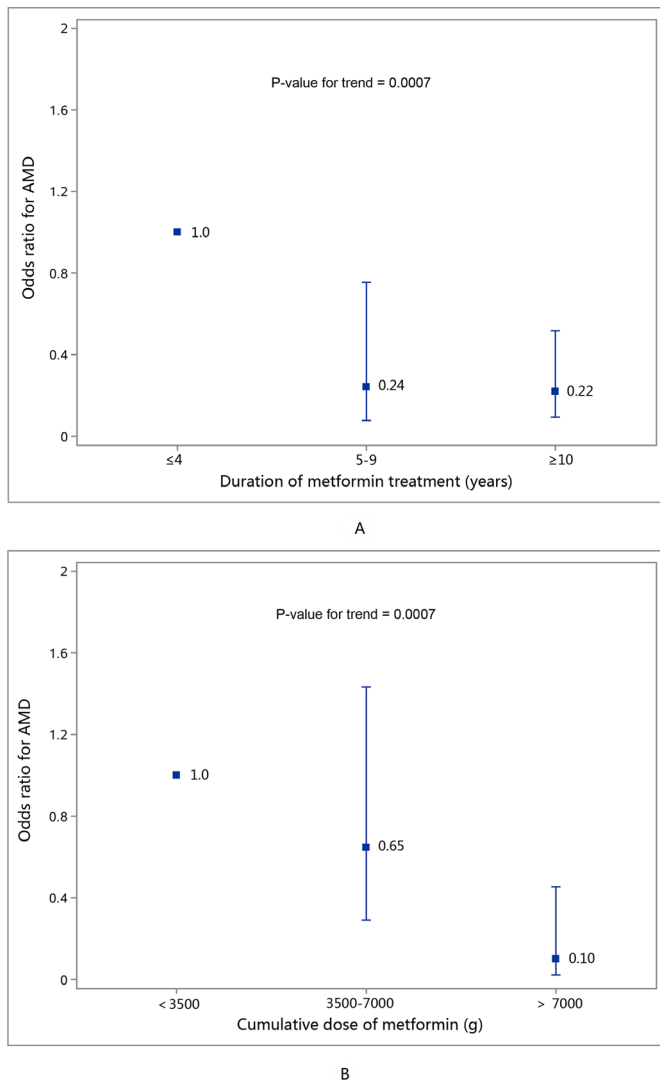


Figure 2 Risk of AMD according to (A) treatment duration and (B) cumulative dose of metformin. AMD, age-related macular degeneration.

(3500–7000 g) and third (>7000 g) tertiles compared with the first tertile (<3500 g) were 0.65 (95% CI: 0.29 to 1.43) and 0.10 (95% CI: 0.02 to 0.45), respectively.

DISCUSSION

The present retrospective study investigated the hypothesis that treatment with metformin is associated with the risk of AMD in patients with T2DM aged no less than 50 years old. We reviewed the data containing the potential confounding factors for developing AMD from patients with T2DM for ≥ 10 years. In this study, metformin users were less likely to develop any early AMD than non-users, however the late AMD was not significantly associated with the use of metformin. Next, we investigated the protective effect of metformin on AMD in duration-response and dose-response patterns. The metformin treatment with prolonged duration and the high cumulative dose provided an optimal protective effect against AMD. Metformin reduces the AMD risk when the cumulative

duration is more than >5 years or the cumulative dose is >3500 g, which further confirmed the dose-dependent association between metformin use and AMD.

This protective effect of metformin on AMD was independent of DM, as the present study found an insignificant association between the duration of DM and the risk of developing AMD. Currently, there is no consistently observed association between diabetics and AMD; several previous studies have reported similar results,^{24 25} while some others suggested a positive correlation^{19 26 27} or negative correlation^{18 28 29} between DM and AMD. Moreover, age is a recognised risk factor for AMD; thus, the susceptibility to AMD varies with the age interval of the study population, especially since patients aged <50 years old do not constitute the main population for developing AMD. In addition, if diabetes treatment medications are associated with the development of AMD, differences are noted in the risk of AMD among patients with diabetes receiving varied glucose-lowering treatments. Therefore, this study targeting patients with diabetes >50 years old and considering the effects of glucose-lowering medications provided a better understanding of the association between DM and AMD.

The mechanisms underlying the protective effect of metformin that reduces the risk of AMD remain to be explored. Typically, metformin exerts its anti-inflammatory and antioxidative effects through the activation of AMP-activated protein kinase (AMPK),^{10 30} inhibition of nuclear factor-kappa B,^{31 32} decrease in reactive oxygen species,³³ and inhibition of the mammalian target of rapamycin.³⁴ AMPK is a metabolic-sensing Ser/Thr kinase expressed in all cell types and plays a central role in the regulation of energy homeostasis and metabolic stress. AMPK regulates the dysfunction of the metabolic ecosystem, which might be involved in AMD pathogenesis.^{35 36} In addition, the protective effect of metformin against oxidative damage and mitochondrial dysfunction is also considered as a potential mechanism for AMD^{37 38}; this effect was further confirmed on the RPE cells. Metformin was found to protect RPE cells from oxidative damage by stimulating autophagy via the activation of the AMPK pathway.¹⁰ Also, metformin protects photoreceptors and RPE cells from acute injury and delayed inherited retinal degeneration. This protective effect is associated with decreased oxidative stress and increased mitochondrial energy production.³⁹ Moreover, metformin exerts an anti-inflammatory effect on the RPE by attenuating the proinflammatory and adhesion molecule genes.³¹ Early AMD characterised by drusen formation is speculated to be the product of inflammatory reaction due to RPE damage.^{40 41} Therefore, the anti-inflammatory and antioxidant effects of metformin protect the RPE from the lesions of early AMD. CNV is a characteristic sign of late AMD and is found to be associated with the upregulation of vascular endothelial growth factor (VEGF) expression. However, the effect of metformin on VEGF expression is yet controversial.^{42 43} Strikingly, metformin is unlikely to confer a preventive effect against CNV in patients with AMD.

Among the risk factors included in the current study, age is significantly positively associated with both early and late AMD, while BMI is negatively associated with early AMD. Some studies showed that increasing BMI is a risk factor for AMD,^{22 44} whereas others failed to establish this correlation.^{14 45} Higher BMI also reported a protective effect on retinopathy, which could be ascribed to a beta cell reserve.⁴⁶

The presence of DR in patients with DM has shown an inconsistent role in the development of AMD. We found that AMD was less prevalent in patients with DR, although the presence of DR was not associated with metformin treatment. Similar results have been observed in previous studies.^{18 22 47} Cummings and Cunha-Vaz found that the coexistence of DR and AMD is relatively uncommon, and the underlying alterations to the blood-retinal barrier caused by the two diseases are different.⁴⁸ DR affects predominantly the inner retina whereas AMD affects the outer retina, they alter the macula probably through different mediation mechanisms.²² Other studies have found the opposite outcome, suggesting that DR may increase the risk of developing AMD.^{16 49} The increased blood glucose levels, impaired glucose tolerance and more advanced glycation end products are considered to predispose the emergence of macular RPE abnormalities.⁵⁰ Fan *et al* observed not only typical vascular and neural damage but also sub-RPE drusenoid lesions in a non-human primate model of early-stage diabetic retinopathy.⁵¹ However, they also found that the RPE drusenoid lesion required complement activation to establish an environment of chronic inflammation circumstance, and the inflammation involvement and immune cell recruitment may play an important role in this mechanism. Therefore, we hypothesise that the anti-inflammatory effect of metformin might be more significant in the protection against RPE drusenoid lesion, than against diabetic vascular damage. In addition, DR might be induced by poor glycaemic control as indicated by increased HbA1c. The HbA1c levels may be useful in separating the antihyperglycaemic effect of metformin from its anti-inflammatory or other effects.¹⁶ Thus, the association of elevated HbA1c levels with low risk of AMD might suggest that the antihyperglycaemic effect of metformin is not the primary role in protecting against AMD.

The analysis of the laboratory data related to AMD and DM revealed that serum HDL levels were positively correlated with the risk of AMD, especially late AMD. Conflicting results have been reported with respect to the associations of AMD with elevated HDL cholesterol.^{22 52 53} Reduced HDL levels may predispose to increased lipid deposits in the macula and may be associated with CNV.⁵² However, other serum lipids, such as TC, LDL and TG, investigated in this study, did not show any significant associations with AMD. The protective effect of UA on early AMD was observed in metformin users, although further investigation is necessary.

Our study had several strengths. First, we investigated the association of metformin treatment with AMD risk

in elderly patients with T2DM for ≥ 10 years. Long duration reduces the susceptibility disparity of fundus lesions brought about by the large difference in DM duration and reflects the effect of long-term use of metformin. Second, the variables in this study included potential confounders, such as laboratory data, duration and dose of metformin use and DR. The application of OCT makes the diagnosis of AMD and DR reliable, as it overcomes the difficulty of discriminating small drusen from hard exudates in DR by fundus photographs. Moreover, the subgroup analysis in the early and late AMD was performed separately. An in-depth investigation in duration-dependent and dose-dependent response patterns confirmed the protective effect of metformin on AMD, and rendered it valuable for the assessment of clinical efficacy.

Nevertheless, the present study has some limitations. The relatively small sample with AMD and retrospective design are the major limitations. Thus, large randomised controlled prospective studies are needed for a complete assessment of the association. Other limitations include a lack of actual measurement data for confounders, such as smoking, family history, lifestyle, genetics, other medication history and cardiovascular factors. In addition, the current study only revealed the association but could not account for the correlation. Therefore, the mechanisms underlying these associations need to be further elucidated and explained by basic research. Furthermore, the population of this study was limited to individuals with diabetes. Thus, future prospective clinical studies are needed to investigate the protective effects of metformin on non-diabetic population and evaluate the safety and efficacy of metformin in the treatment of AMD.

Contributors JJ, YC and ZW conceived the concept for this study. YC, HZ, WY, TZ, NW and DZ involved in clinical diagnosis and data acquisition. GF performed the statistical analysis. JJ drafted the manuscript. ZW acted as guarantor. All authors approved the final manuscript.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This study was approved by the Clinical Research Ethics Committee of China-Japan Friendship Hospital, No. 2021-79-K47. The study was a retrospective study with absolute confidentiality of patients' personal information and did not contain any information that would allow access to participants' identity-linked data. The study was approved by the ethics committee for exemption from informed consent.

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