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The influence of insulin on diabetic retinopathy and retinal vessel parameters in diabetes

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Abstract

Aim To investigate the associations between insulin use and diabetic retinopathy (DR), and retinal vascular parameters in type 2 diabetes (T2DM).

Methods A total of 6,374 T2DM patients, consisting of 2,231 patients receiving insulin alone and 4143 patients without any hypoglycemic medication, were included in cross-sectional analyses. Among those without DR at baseline, 791 patients were followed for three years in longitudinal analyses. Fundus photography was taken to diagnose DR and calculate central retinal arteriolar equivalent (CRAE), central retinal venular equivalent (CRVE), arteriolar-to-venular ratio (AVR), and vascular tortuosity. Inverse probability treatment-weighted analyses were performed.

Results After adjusting for gender, age, body mass index, blood pressure, blood glucose, T2DM duration, smoking, and alcohol use, insulin users showed a higher risk of DR (odds ratio (OR) = 2.27, 95% confidence interval (95%CI) = 2.08–2.48, $P < 0.001$), larger CRVE (β = 3.92, 95%CI = 2.46–5.37, $P < 0.001$), smaller AVR (β=-0.0083, 95%CI=-0.0121- -0.0046, $P < 0.001$), and larger vascular curvature (β = 0.19, 95%CI = 0.05–0.33, $P = 0.008$). After 3 years, insulin users had a higher risk of developing DR (OR = 1.94; 95% CI = 1.37–2.73, $P = 0.002$), and greater change in CRVE (β = 3.92, 95%CI = 0.96–6.88, $P = 0.009$).

Conclusions The impact of insulin on the retinal microvasculature provides support for linking insulin to the increased risk of DR, as well as cardiovascular events in T2DM.

Keywords Insulin, Diabetic retinopathy, Central retinal arteriolar equivalent, Central retinal venular equivalent, Arteriolar-to-venular ratio

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Introduction

With the rapid economic development, people's lifestyles have changed significantly over the past few decades, including increased sedentary behavior and higher calorie intake, resulting in the annual rise in the prevalence of diabetes mellitus (DM) [1]. Vascular complications, one of the most pervasive complications among people with diabetes, can contribute to a pronounced reduction in quality of life or even lead to death [2]. Diabetes-related macrovascular complications include peripheral vascular disease, myocardial infarction, and stroke, while microvascular issues encompass diabetic neuropathy, diabetic nephropathy, and diabetic retinopathy (DR) [3]. The prevalence of DR among diabetes patients is reported to be as high as 22.27% [4] globally and 18.45% [5] in China. In 2016, our study group conducted a demographic analysis of 6,978 individuals residing in Shanghai with type 2 diabetes(T2DM) and revealed that the prevalence of DR was 16.97% [6]. Between 1990 and 2010, there was a 64% and 27% increase in the incidence of visual impairment and blindness caused by DR, respectively, making it the fifth most prevalent cause of moderate to severe vision loss and blindness around the world [7].

Insulin is one of the most renowned and effective therapy choices for diabetes, as it drastically reduces blood glucose levels by acting directly on muscle and liver tissue [8]. Nevertheless, the rapid decline in blood glucose disrupts the body's metabolic environment, which can result in cognitive decline, impaired consciousness, and at times, even death [9]. The application of insulin in people with T2DM has been found to be a considerable risk factor for cardiovascular issues [10] and DR [11, 12], especially the "early worsening of DR" phenomenon that arises soon after the initiation of insulin treatment [9, 13]. Currently, it is widely accepted that the underlying mechanism of the "early worsening of DR" phenomenon may be the recurring hypoglycemia caused by insulin. When blood glucose levels drop significantly, the use of insulin could lead to oxidative stress from reactive oxygen species (ROS) and result in overexpression of vascular endothelial growth factor (VEGF), which produces pathological neovascularization that eventually leads to DR [9]. In addition, several animal studies intended to explore the mechanism by which insulin causes DR by studying the effects of insulin on retinal blood vessels. Since retinal vascular parameters, such as vessel diameter, capillary plexus density, and neovascularization are subclinical indicators of microvascular injury that can predict DR onset and progression [14–16]. Su et al. [17] demonstrated that insulin may initiate the production of nitric oxide (NO) from endothelial cells in porcine retinal arteries, leading to vasodilation. Additional research has indicated that the dilated vessels may be associated with an increase in the release of inflammatory factors, leading to the development of DR [18].

Retinal vessels are the only blood vessels in the body that can be observed directly. The central retinal arteriolar equivalent (CRAE), the central retinal venular equivalent (CRVE), the arteriole-to-venule ratio (AVR), and vascular curvature have all been shown to be indicators of retinal vascular morphology that provides information regarding the macrovascular and microvascular complications of DM. An increased risk of cardiovascular problems can be linked to a narrower CRAE and smaller AVR [19], whereas the development of DR has been associated with a wider CRVE and larger curvature [20]. However, the correlation between DR and CRAE as well as AVR remains a controversial topic in the field [20]. So far, the effects of insulin use on CRAE, CRVE, and AVR in patients with diabetes have not been examined thoroughly in population studies, and its influence is still unknown. This study was conducted based on an established study of T2DM individuals in the Shanghai community, including patients who only use insulin to control glycemia and those who did not receive any hypoglycemic drugs, in order to evaluate the associations between insulin use, retinal vasculature, and DR.

Methods

Study population

The Shanghai Cohort Study of Diabetic Eye Disease (SCODE) is a community-based cohort study (clinicaltrials.gov identifier: NCT03665090) that has been carried out since 2003. The purpose of this study is to examine the physical health (such as height, weight, blood pressure, and blood glucose levels) and eye health (such as visual acuity and the onset and progression of major eye diseases) of patients with diabetes. Previous publications have provided a detailed overview of the study's methodological approach [6]. Briefly, the inclusion criteria are individuals with (1) an age of 18 years or older, (2) the ability to successfully participate in all tests and interviews, and (3) a medical history of T2DM. On the other hand, exclusion criteria are defined as (1) presence of anterior segment diseases that seriously affect the quality of fundus examination and (2) those who underwent ophthalmic surgery in the most recent 6 months. Ethical standards in accordance with the Declaration of Helsinki were strictly followed throughout the study. All participants were required to sign an informed consent form, which was authorized by the ethics committee of the Shanghai General Hospital (approval number: 2017KY138).

Out of the initial pool of 66,033 participants in the SCODE study recruited in 2017 (from January 1, 2017 to December 31, 2017), we excluded subjects who were using more than 1 type of hypoglycemic drug (n=34,525)

and other types of hypoglycemic drugs (n=21,108) based on an interview on medication. Subjects with missing DR diagnosis (n=164) and incomplete examination data (n=3,862) were further excluded. Consequently, 6,374 participants remained for the cross-sectional analysis, including 2,231 patients who were receiving insulin alone and 4,143 patients who were not taking any hypoglycemic medication as depicted in Fig. 1.

Among the initial cohort, a total of 826 patients attended annual follow-ups and underwent fundus photography taken in 2020. We excluded those with DR at baseline and those who had changed medication usage information at follow-up (n=33), and excluded those without complete data (n=2). Ultimately, a total of 791 patients were included in the longitudinal analysis.

General information

The investigation included: (1) basic demographic information collected by researchers through questionnaires, which contain age, gender, residential address, the amount of smoking and drinking alcohol if one admitted in the most recent one month, time and frequency of exercise, duration of DM, history of hypertension and current medication; (2) basic measurements, such as height, weight, and blood pressure; (3) ocular examinations, including uncorrected and presenting visual acuity using tumbling Early Treatment Diabetic Retinopathy Study (ETDRS) charts (LCD backlit lamp, WH0701, Guangzhou Xieyi Weishikang, Guangzhou, China), slit lamp (66 Vision Tech, Suzhou) examination for both anterior segment and posterior segment with the assist of the non-contact lens 90D, and fundus photography of optic disc and macula of both eyes examined separately using non-mydriatic retinal cameras (Topcon NW400, Topcon, Tokyo, Japan); and (4) fasting blood glucose (FBG) and HbA1c.

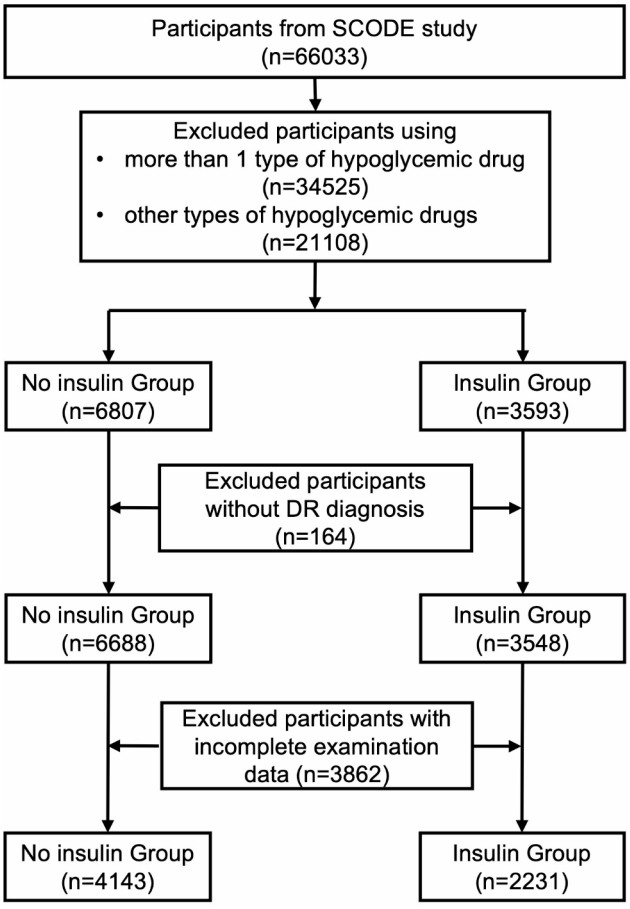

Fig. 1 Flowchart of the study participants included in the cross-sectional analysis
SCODE: the Shanghai Cohort Study of Diabetic Eye Disease; DR: diabetic retinopathy

Body mass index (BMI) was then calculated based on the recorded data using the formula BMI(kg/m^2)=weight (kg) / height^2 (m^2). Following standardized training protocols, experienced ophthalmologists made the diagnosis of DR and proliferative diabetic retinopathy (PDR) based on the fundus images of both eyes using the worldwide clinical diagnostic criteria as published by the International Academy of Ophthalmology [21]. Patients with the presence of any signs of DR or PDR in either eye were judged as DR or PDR.

Measurement of retinal vascular parameters

An automated region of interest extraction-based method of universal pixel pitch calibration for fundus cameras was utilized [22]. The method offers the advantage of reducing image variability and can be applied to all models of fundus cameras. Additionally, it provides precise measurements with a deviation of less than 5% when compared to manual pixel pitch measurements [22].

Before analysis, the quality of the fundus photos is carefully assessed, and only those with clear images, qualified for utilization with the artificial intelligence algorithm, are included. The measurement of retinal vessels is uniformly performed using the fundus photo of the patient's right eye, which is centered on the optic disc. The program then automatically calculated the diameters of the six largest retinal arteries and veins as well as the average curvature of the retinal vessels within a region of 0.5 to 1 optic disc diameter from the edge of the optic disc using deep learning semantics. The modified Parr-Hubbard formula [23, 24] for iterative calculation of CRAE and CRVE was used, which are subsequently divided to calculate AVR [24]. CRAE and CRVE abbreviate the average caliber of small arteries and veins in the eye, respectively. Retinal vessel curvature was defined as the squared curvature along the vessel path divided by the actual path length, and a lesser value of curvature suggested a straighter vessel [25]. The retinal arterioles and venules

were automatically recognized and extracted by the computer algorithm, as shown in Fig. 2.

Medication use

Face-to-face interviews were performed by the researchers to collect data on the present utilization of hypoglycemic drugs. The information obtained during the interviews consisted of the number of medications, medication type, name, single-use medication dose, frequency, and route of administration. The researchers identified the drug types based on the clinical practice recommendations published by the Endocrine Society [26]. Only individuals who had been receiving exogenous insulin for at least three months were eligible for inclusion in the study. As for patients who use non-pharmacological methods for diabetes management, a meticulous inquiry was conducted to understand the specific strategies they employ for blood glucose control. This investigation encompassed detailed information on dietary choices, exercise routines, and lifestyle interventions.

Propensity scoring and weighting

To address confounding by indication (the clinical indication for treatment also affects the outcome), inverse probability treatment weighted (IPTW) analyses with weights calculated from propensity scores were performed [27]. The propensity score was computed by using logistic regression with the dependent variable being the receipt of insulin, and the independent variables (covariates) being gender, age, duration of DM, BMI, blood pressure, FBG, HbA1c, smoking, and alcohol use. The propensity score was the probability of each patient being predicted to use insulin. Weighted samples using IPTW were used to estimate the average effect of insulin. This approach helps to minimize the influence of confounding factors and enhances the comparability of the groups, thereby strengthening the validity of our findings [27].

Statistical analyses

Baseline characteristics between the two groups were compared using the chi-square test for categorical data, presented as frequency (%), and t-test for continuous data, presented as mean (95% confidence interval (95%CI)]. Logistic regression models were utilized to analyze the effect of insulin use on the prevalence and incidence of DR. Furthermore, linear regression analyses were utilized to examine the relationship between insulin use and retinal vascular parameters, specifically CRAE, CRVE, AVR, and vascular curvature. For the follow-up patients, changes in retinal vascular parameters were analyzed using the values of 2020 minus those of 2017. The multivariate analyses accounted for potential confounding factors, including gender, age, BMI, blood pressure, FBG, HbA1c, duration of DM, smoking, and alcohol use.

Subgroup analyses were conducted to explore whether the impact of insulin on the prevalence of DR and the retinal blood vessels was consistent in various subgroups.

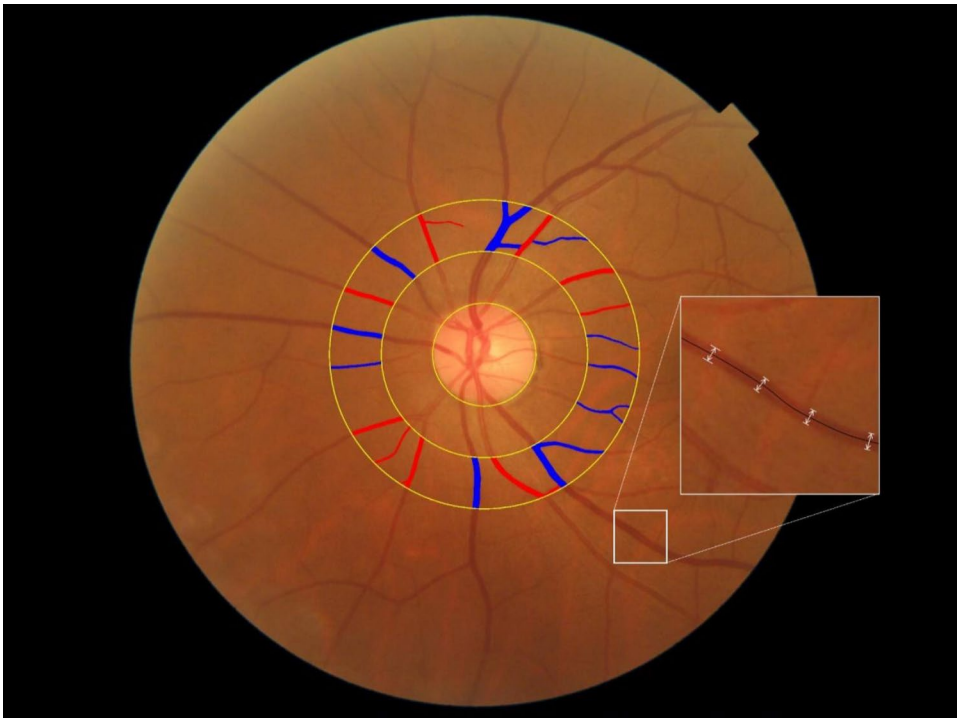

Fig. 2 The measurement area and retinal arterioles and venules automatically identified and extracted

The participants were stratified into 2 groups by: (1) gender; (2) age with a cutoff of 65 years as defined for elderly by World Health Organization [28]; (3) BMI with a cutoff of 23 kg/m^2 for overweight [29]; 4) duration of diabetes with a cutoff of the median value (6.17 years); 5) SBP/DBP with a cutoff of 130/80 mmHg for well/poor BP control [30]; 6) FBG levels of 7.2 mmol/L and HbA1c levels of 7% for well/poor glucose control [31].

A *P*-value less than 0.05 (two-tailed) indicated statistical significance. All statistical analyses were performed using the SAS, version 9.4 (SAS Institute, Cary, NC) and GraphPad Prism 9 (GraphPad Software, San Diego, USA).

Results

Out of the 6,374 patients with T2DM included in the cross-sectional analysis, 2,231 (35.00%) patients were using insulin. As shown in Table 1, individuals using insulin had a longer duration of DM, higher HbA1c, and higher FBG levels compared to those who were not receiving any hypoglycemic drugs.

In the unadjusted data, the prevalence of DR was considerably higher in the Insulin group compared with the No insulin group, and the difference was also significant for the prevalence of PDR (Table 1). In terms of retinal vessels, the Insulin group exhibited larger CRVE, smaller AVR, and larger vascular curvature than the No insulin group. However, the difference in CRAE was not statistically significant (Table 1).

After performing propensity score weighting using the IPTW method, we achieved a relatively balanced distribution of baseline characteristics between the No insulin and the Insulin group (Table 1). While certain differences remained statistically significant, the disparities in the mean values were substantially reduced and lacked clinical significance.

The logistic regression analyses for DR and PDR in the weighted analyses were shown in Table 2. The insulin group showed a significantly higher prevalence of DR (OR=2.27; 95% CI=2.08–2.48, $P<0.001$) and PDR (OR=2.78; 95% CI=1.66–4.64, $P<0.001$) compared with those who did not receive any hypoglycemic drugs after adjusted for gender, age, duration of diabetes, body mass

Table 1 Characteristics of study participants according to the use of insulin in cross-sectional analyses ($n=6,374$)

	Overall Cohort			IPTW Cohort		
	No insulin ($n=4143$)	Insulin ($n=2231$)	*P*	No insulin ($n=4143$)	Insulin ($n=2231$)	*P*
Gender (%)						
Male	1732 (41.81)	996 (44.64)	0.029	2854.26 (43.16)	2784.74 (44.18)	0.241
Female	2411 (58.19)	1235 (55.36)		3759.59 (56.84)	3518.36 (55.82)	
Age (years)	66.11 (65.9, 66.32)	65.68 (65.38, 65.97)	0.019	65.96 (65.74, 66.19)	66.24 (65.94, 66.55)	0.148
Duration of diabetes (years)	5.85 (5.72, 5.99)	10.82 (10.56, 11.09)	< 0.001	8.06 (7.87, 8.25)	7.61 (7.37, 7.85)	0.004
BMI (kg/m^2)	24.51 (24.41, 24.6)	24.29 (24.17, 24.41)	0.004	24.36 (24.27, 24.46)	24.37 (24.25, 24.49)	0.905
SBP (mmHg)	129.9 (129.6, 130.2)	129.9 (129.5, 130.3)	0.891	129.9 (129.6, 130.1)	129.9 (129.6, 130.1)	0.620
DBP (mmHg)	78.41 (78.22, 78.6)	78.39 (78.14, 78.65)	0.904	78.28 (78.09, 78.48)	78.24 (77.98, 78.5)	0.778
HbA1c (%)	6.58 (6.54, 6.61)	7.3 (7.24, 7.37)	< 0.001	6.94 (6.9, 6.99)	6.82 (6.76, 6.88)	0.002
FBG (mmol/L)	6.50 (6.47, 6.53)	6.98 (6.91, 7.04)	< 0.001	6.84 (6.79, 6.89)	6.72 (6.67, 6.77)	0.001
Cigarette smoking (%)						
Yes	3904 (94.23)	2099 (94.08)	0.810	6207.12 (93.85)	5937.93 (94.21)	0.393
No	239 (5.77)	132 (5.92)		406.73 (6.15)	365.17 (5.79)	
Alcohol drinking (%)						
Yes	3994 (96.4)	2138 (95.83)	0.254	6370.87 (96.33)	6073.94 (96.36)	0.908
No	149 (3.6)	93 (4.17)		242.98 (3.67)	229.16 (3.64)	
DR (%)						
Yes	525 (12.67)	742 (33.26)	< 0.001	1067.87 (16.15)	1779.29 (28.23)	< 0.001
No	3618 (87.33)	1489 (66.74)		5545.98 (83.85)	4523.81 (71.77)	
PDR (%)						
Yes	10 (0.24)	21 (0.94)	< 0.001	20.774 (0.31)	52.0457 (0.83)	< 0.001
No	4133 (99.76)	2210 (99.06)		6593.08 (99.69)	6251.06 (99.17)	
CRAE	134.89 (134.36, 135.42)	135.24 (134.48, 136.00)	0.459	134.59 (134.06, 135.13)	135.27 (134.48, 136.06)	0.166
CRVE	223.95 (223.06, 224.84)	227.95 (226.71, 229.19)	< 0.001	223.53 (222.61, 224.45)	227.34 (226.11, 228.57)	< 0.001
AVR	0.607 (0.605, 0.609)	0.598 (0.595, 0.601)	< 0.001	0.607 (0.605, 0.610)	0.599 (0.596, 0.602)	< 0.001
Retinal vascular curvature (×10^4)	10.747 (10.662, 10.832)	10.981 (10.861, 11.100)	0.002	10.764 (10.679, 10.850)	10.944 (10.826, 11.062)	0.016

Continuous variables were presented as mean (95%CI), and categorical variables were presented as No.(%)

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBG: fasting blood glucose; DR: diabetic retinopathy; PDR: proliferative diabetic retinopathy; CRAE: central retinal arteriolar equivalent; CRVE: central retinal venular equivalent; AVR: arteriole-to-venule ratio

Table 2 Associations of insulin use with the prevalence of DR and PDR, and with retinal vascular parameters (n=6,374)

	Univariate regression[a]		Multivariate regression[a, b]	
Logistic analyses	OR (95%CI)	*P* value	OR (95%CI)	*P* value
DR	2.04 (1.88, 2.23)	< 0.001	2.27 (2.08, 2.48)	< 0.001
PDR	2.64 (1.59, 4.40)	< 0.001	2.78 (1.66, 4.64)	< 0.001
Linear analyses	β (95%CI)	*P* value	β (95%CI)	*P* value
CRAE	0.68 (-0.23, 1.58)	0.142	0.71 (-0.18, 1.61)	0.120
CRVE	3.81 (2.34, 5.28)	< 0.001	3.92 (2.46, 5.37)	< 0.001
AVR	-0.0083 (-0.012, -0.0046)	< 0.001	-0.0083 (-0.0121, -0.0046)	< 0.001
Vascular curvature	0.18 (0.04, 0.32)	0.011	0.19 (0.05, 0.33)	0.008

[a] Weighted by IPTW

[b] Adjusted for gender, age, duration of diabetes, body mass index, blood pressure, fasting blood glucose, HbA1c, smoking, and alcohol use

OR: odds ratio; CI: confidence interval; DR: diabetic retinopathy; PDR: proliferative diabetic retinopathy; CRAE: central retinal arteriolar equivalent; CRVE: central retinal venular equivalent; AVR: arteriole-to-venule ratio

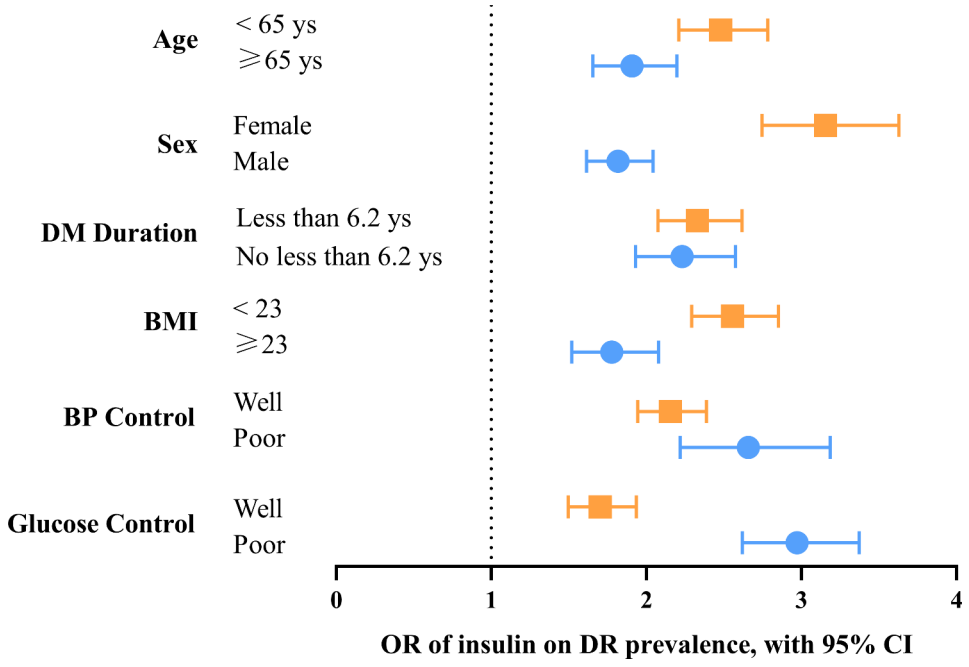

Fig. 3 Outcomes of subgroup analyses for the impact of insulin use on the prevalence of DR
The ORs for the use of insulin on the prevalence of DR were calculated for each subgroup using logistic regression models adjusting for gender, age, gender, duration of diabetes, body mass index, blood pressure, fasting blood glucose, HbA1c, smoking, and alcohol use based on the IPTW analyses
DR: diabetic retinopathy; IPTW: inverse probability treatment weighted; DM: diabetes mellitus; BMI: body mass index; BP: blood pressure

index, blood pressure, fasting blood glucose, HbA1c, smoking, and alcohol use (Table 2). Subgroup analyses revealed that the association between insulin use and increased risk of DR was consistent in all subgroups (All P<0.001, Fig. 3).

The linear regression analyses for CRAE, CRVE, AVR, and vascular curvature in the weighted analyses were also shown in Table 2. The insulin group showed significantly larger CRVE (β=3.92; 95% CI=2.46–5.37, P<0.001), smaller AVR (β=-0.0083; 95% CI=-0.0121- -0.0046, P<0.001), and increased vascular curvature (β=0.19; 95% CI=0.05–0.33, P=0.008) compared with those who did not receive any hypoglycemic drugs after adjusted for all confounders (Table 2). However, no significant association was observed between insulin treatment and CRAE in the univariable (P=0.14) or multivariable (P=0.120) analysis.

In the subgroup analyses, the associations between insulin use and CRAE were all insignificant (all P>=0.05) in almost all subgroups, except for the subgroup with well glucose control (β=1.68, 95% CI=0.50–2.85, P=0.005) (see Fig. S1 in the Supplement). The associations between insulin use and increased CRVE were all significant (all P<0.05) in almost all subgroups, except for the subgroup with poor glucose control (β=0.38, 95% CI= -1.92-2.67, P=0.747) (see Fig. S2 in the Supplement). Regarding AVR (see Fig. S3 in the Supplement), insulin use was significantly associated with reduced AVR in the

various subgroups of BMI, blood pressure, and gender. However, this association was only significant in the subgroups characterized by well glucose control (β=-0.011; 95% CI=-0.016- -0.0062, $P<0.001$), older age (β=-0.011; 95% CI=-0.016- -0.0056, $P<0.001$) and longer DM duration (β=-0.011; 95% CI=-0.016- -0.0055, $P<0.001$). The impact of insulin use on vascular curvature showed variations across different subgroups (see Fig. S4 in the Supplement). In particular, insulin use significantly increased retinal vascular curvature in subgroups with well glucose control (β=0.17, $P=0.048$), higher BMI (β=0.19, 95% CI=0.015–0.36, $P=0.03$), lower blood pressure (β=0.27, 95% CI=0.004–0.54, $P=0.047$), and female gender (β=0.34, 95% CI=0.17–0.52, $P<0.001$).

To evaluate the long-term impact of insulin therapy on the incidence of DR and the change in retinal vascular parameters, we conducted a cohort study of 791 T2DM patients. Following a three-year follow-up period, we identified 74 individuals who were newly diagnosed with DR, resulting in a cumulative incidence rate of 9.62%. Through multivariate logistic analysis, we reaffirmed that insulin therapy significantly and independently contributed to the risk of developing DR (OR=1.94; 95% CI=1.37–2.73, $P=0.002$) after adjusting for all covariates. Regarding retinal vessels, only change in CRVE remained significantly larger in the group receiving insulin treatment (β=3.92, 95% CI=0.96–6.88, $P=0.009$). However, we did not observe a significant association between use of insulin and change in CRAE (β=1.29, 95% CI=-0.58-3.15, $P=0.176$), AVR (β= -0.0062, 95% CI= -0.0168-0.0044, $P=0.252$), or vascular curvature (β=0.12, 95% CI=-0.16-0.39, $P=0.401$).

Discussion

It has been established that an extended duration of diabetes and inadequate glycemic control are principal risk factors for DR development in China, while the potential impact of hyperlipidemia, blood pressure, obesity, smoking, and alcohol use on the progression of DR remains a subject of ongoing debate. Moreover, advancements in genetic research, such as genome-wide association studies (GWAS) and candidate gene-based association studies, have identified over 60 genes globally linked to DR. Nonetheless, the interpretation of the influence of genetic risk factors on the onset and severity of DR encounters limitations due to the lack of standardization across populations and DR phenotypes. The existence of numerous genetic variants across diverse signaling pathways suggests a complex and multifactorial etiology and pathology underlying the progression of DR [32]. In the context of this study, we revealed a higher risk of DR in patients with diabetes using insulin for glycemic control, which was unaffected by age, gender, DM duration, BMI, blood pressure, or glucose control. Furthermore, we discovered that insulin therapy was associated with retinal microvasculature.

Diabetes patients often rely on insulin injections to lower their blood sugar levels, especially type 1 diabetes with complete insulin insufficiency and type 2 diabetics who show inadequate response to oral hypoglycemic medications [26]. On the one hand, the use of insulin can effectively reduce blood glucose, decrease the time spent in hyperglycemic states, and lower the risk of diabetic ketoacidosis and other complications; on the other hand, the larger and faster waves of blood glucose lowering by insulin can increase blood glucose variability, which has been found in several studies to be an independent risk factor for diabetic complications [33]. The Age, Gene/Environment Susceptibility–Reykjavik Study [11] in Sweden confirmed a significant association between insulin use and DR at any stage in patients with diabetes. Additionally, the Spectrum of Eye Disorders in Diabetes Study in India reported a 2.55-fold increase in the risk of retinopathy after insulin treatment [12], while similar studies conducted in the United States [34] have also demonstrated this correlation. Our study has also identified this phenomenon of 'early worsening of DR' after insulin use, which reinforces the existing body of evidence supporting the association between insulin therapy and DR. The results suggested that there may be a more direct contribution of insulin to DR, possibly related to retinal oxidative stress and pathological retinal neovascularization. Wu et al. [35] reported that insulin use under hyperglycemic conditions led to the production of ROS and the expression of VEGF, while insulin-induced hypoglycemic episodes also increased ROS production in mitochondria [9].

In addition, we found that insulin use was significantly associated with wider CRVE (β=3.92, $P<0.001$), increased vascular curvature (β=0.19, $P=0.008$), and smaller AVR (β=-0.0083, $P<0.001$) in the cross-sectional analyses. In the longitudinal analyses, the change in CRVE remained significantly larger in the group receiving insulin treatment (β=3.92, $P=0.009$). However, no significant association was observed between insulin treatment and CRAE in both cross-sectional ($P=0.120$) and longitudinal ($P=0.176$) analyses. To date, only one study [17] on porcine retinal arteries has found that the intraluminal injection of exogenous insulin causes Ca^{2+} channel opening and vasodilation, which is suggested to be NO-mediated. Notably, NO release from endothelial cells is the primary mechanism that regulates vascular tone and leads to vascular dilation in the vascular system [24, 36]. Bäck et al. [37] also suggested a preferential functional role of NO in porcine pulmonary veins, originating in a greater production of nitric oxide by veins as opposed to arteries. This finding provides a potential explanation for the observed wider CRVE and

the absence of changes in CRAE following insulin treatment. The reason that insulin use was not found to be associated with the change in AVR or vascular curvature in longitudinal analyses is probably that the absolute differences were too small and studies with a larger sample size or longer follow-up period were needed to reach significant relationships.

Previous research has shown a correlation between changes in retinal vascular caliber and diabetic complications. Decreases in CRAE and AVR have predicted the onset of cardiovascular disease, while increases in CRVE have been associated with the onset and progression of stroke, cardiac diseases, and DR [19, 20]. The underlying mechanism for the disease pathogenesis may be that wider CRVE was associated with higher serum high-sensitivity C-reactive protein, interleukin 6, and amyloid A levels [18, 38]. Taken together, the alterations in retinal vascular parameters observed in our study provide additional support for linking insulin use to the onset and development of DR, as well as the increased risk of cardiovascular events [10–12, 19, 20].

The impact of insulin use on retinal vascular parameters could vary among different metabolic environments. In the subgroup analyses, significant associations between insulin use and larger CRAE, larger CRVE, smaller AVR, and larger vascular curvature were observed in the subgroup with lower FBG and HbA1c levels (Figure S1-S4.), indicating that in hypoglycemic settings, the retinal vasculature proves to be more sensitive to insulin. Previous experimental researches showed that exposure of microvessels to insulin under normal glucose conditions could lead to a significant increase in NO levels. However, this increase was notably suppressed when the microvessels were incubated under high glucose conditions [39], which could explain the results of the present study. In addition, in the subgroups of older age and longer duration of DM, insulin use was significantly associated with decreased AVR, suggesting that these subgroups might be more susceptible to insulin use compared with patients with younger age and shorter duration of DM. The finding is consistent with the higher incidence of cardiovascular complications in these specific populations as reported in previous studies [10].

In this study, we compared individuals who did not take any hypoglycemic drugs with those who only used insulin to eliminate the influence of other hypoglycemic medications. We excluded patients who received other drug treatment regimens primarily to avoid confounding effects from multi-drug therapy, as this could mask the specific effects of insulin therapy that we aimed to investigate. Including patients taking multiple medications might introduce variability in the results, complicating their interpretation. However, we acknowledge that while excluding these patients may help isolate the impact of insulin therapy, it also limits the applicability of our findings to a broader patient population, as most patients often use multiple medications to manage their condition. Comparative analyses that include these patients can indeed provide valuable insights. Additionally, we only focused on insulin usage, without investigating other variables such as type, dosage, frequency, timing, and administration route, which might also influence the outcomes. Future research should consider incorporating patients on multiple medications and variables to gain a more comprehensive understanding of treatment efficacy. This approach would enhance the generalizability of the results and more accurately reflect real-world clinical scenarios.

To the best of our knowledge, this study is the first population-based study to examine the precise relationship between exogenous insulin injections and retinal vascular parameters. Utilizing data from community-based diabetes populations in the SCODE database, we benefited from a relatively large sample size in the cross-sectional analyses. In order to minimize the influence of confounding factors and enhance the comparability between the two groups, IPTW analyses were used to estimate the effect. Moreover, to avoid reverse causality, we conducted longitudinal analyses. All these approaches helped to strengthen the validity of our findings. Despite the promising results, several limitations exist in our study. Firstly, we only used fundus photographs to evaluate the six major blood vessels in the patient's retina. Other variables obtained through Optical Coherence Tomography Angiography, such as neovascularization, nonperfusion areas, and different levels of capillary layers, were not examined despite their potential for early detection and assessment of DR [15, 16]. Second, the rate of loss of follow-up was high and the sample size was relatively small for the longitudinal analyses, which might cause false negative and false positive results. Finally, the study was carried out on T2DM patients who were engaged in community-based diabetes management and received regular examinations. However, a more detailed and comprehensive assessment of our patients' diabetes was lacking in the study. Notably, individuals with compromised islet function, Latent Autoimmune Diabetes in Adults (LADA), and insulin deficiency may potentially present with more severe forms of diabetes, thereby possibly elevating the risk of diabetic retinopathy. It is essential to recognize that, as a consequence of the community-based management and regular examinations, these individuals exhibited relatively better control of their blood glucose levels. Therefore, caution should be exercised when extrapolating the results of this study to other populations.

In general, this study highlights the association between insulin use and the risk of developing DR in type 2 diabetic patients. The study also found that insulin use

is linked to larger CRVE, smaller AVR, and larger vascular curvature, which may potentially contribute to the onset of DR and cardiovascular complications. It is essential to establish scientific treatment guidelines and standardized management processes to improve treatment effectiveness and reduce the incidence of diabetes-related complications when using insulin. Physicians should avoid excessive use of insulin for diabetes management and adjust doses timely and appropriately based on the patient's condition. Patients using insulin should undergo routine fundus examinations to detect early signs of DR.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13098-024-01476-9.

Supplementary Material 1

Author contributions

T.Y.: formal analysis and original draft. S.L.: formal analysis, visualization of the data, and revision of the manuscript. H.Z. and Y.M.: conceptualization, review and editing, project administration, and funding acquisition. Y.X., L.L.: project administration and data collection and supervision; M.C., Y.W., Q.Y.: data collection and supervision; S.L., D.Z.: data and figure analyses; Y.S.: project administration, data collection, and curation. All authors contributed to the article and approved the submitted version.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Ethical standards in accordance with the Declaration of Helsinki were strictly followed throughout the study. All participants were required to sign an informed consent form, which was authorized by the ethics committee of the Shanghai General Hospital (approval number: 2017KY138).

Competing interests

The authors declare no competing interests.

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