• Intelligent Ophthalmology •

Analysis and comparison of retinal vascular parameters under different glucose metabolic status based on deep learning

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Received: 2023-12-19 Accepted: 2024-05-11

Abstract

● **AIM:** To develop a deep learning-based model for automatic retinal vascular segmentation, analyzing and comparing parameters under diverse glucose metabolic status (normal, prediabetes, diabetes) and to assess the potential of artificial intelligence (AI) in image segmentation and retinal vascular parameters for predicting prediabetes and diabetes.

● **METHODS:** Retinal fundus photos from 200 normal individuals, 200 prediabetic patients, and 200 diabetic patients (600 eyes in total) were used. The U-Net network served as the foundational architecture for retinal artery-vein segmentation. An automatic segmentation and evaluation system for retinal vascular parameters was trained, encompassing 26 parameters.

● **RESULTS:** Significant differences were found in retinal vascular parameters across normal, prediabetes, and diabetes groups, including artery diameter (P=0.008), fractal dimension (P=0.000), vein curvature (P=0.003), C-zone artery branching vessel count (P=0.049), C-zone vein branching vessel count (P=0.041), artery branching angle (P=0.005), vein branching angle (P=0.001), artery angle asymmetry degree (P=0.003), vessel length density (P=0.000), and vessel area density (P=0.000), totaling 10 parameters.

● **CONCLUSION:** The deep learning-based model facilitates retinal vascular parameter identification and quantification, revealing significant differences. These parameters exhibit potential as biomarkers for prediabetes and diabetes.

● **KEYWORDS:** deep learning; retinal vascular parameters; segmentation model; diabetes; prediabetes
DOI:10.18240/ijo.2024.09.02

Citation: Jiang Y, Gong D, Chen XH, Yang L, Xu JJ, Wei QJ, Chen BB, Cai YJ, Xi WQ, Zhang Z. Analysis and comparison of retinal vascular parameters under different glucose metabolic status based on deep learning. *Int J Ophthalmol* 2024;17(9):1581-1591

INTRODUCTION

Diabetes is a chronic metabolic disorder characterized by prolonged elevated blood glucose levels above the normal range, leading to damage to various organs and tissues throughout the body. Type 2 diabetes constitutes 90%-95% of the total population with diabetes. According to the World Health Organization standards, the diagnostic criteria for diabetes include fasting blood glucose levels ≥7.0 mmol/L, random blood glucose levels ≥11.1 mmol/L, or blood glucose levels ≥11.1 mmol/L two hours after an oral glucose tolerance test (OGTT). Alternatively, diabetes can be diagnosed if the glycated hemoglobin (HbA1c) level is ≥6.5% based on standardized Diabetes Control and Complications Trial (DCCT) analysis[1]. Prediabetes is a pathological condition characterized by blood glucose levels higher than normal but not reaching the diagnostic criteria for diabetes. It includes impaired fasting glucose (IFG) and impaired glucose tolerance (IGT). IFG is defined as fasting blood glucose levels in

the range of 6.1–6.9 mmol/L, while IGT is characterized by OGTT blood glucose levels in the range of 7.8–11.0 mmol/L. Although IFG and IGT themselves are not considered clinical entities, prediabetes can still lead to a range of health issues[2].

The global population with diabetes has doubled over the past 30y. According to statistics, the estimated global prevalence of diabetes was 9.3% (463 million people) in 2019 and is projected to rise to 10.2% (578 million people) by 2030 and 10.9% (700 million people) by 2045. Additionally, nearly 400 million adults worldwide have prediabetes, with China reporting a prediabetes prevalence of up to 35.7% in the adult population[3-4]. Diabetes not only significantly impacts the overall health of patients but is also closely associated with complications affecting multiple organs and systems. Diabetic retinopathy (DR) is one of the most common complications of diabetes and a major cause of blindness in the working-age population[5-6]. Research indicates that the prolonged hyperglycemic state in patients with diabetes can affect vascular structure and function through various pathways, including increased adhesiveness and permeability of endothelial cells, promotion of smooth muscle cell proliferation, and accelerated deposition of vascular wall matrix, leading to morphological changes such as vascular thickening and luminal narrowing[7-8]. Previous studies have extensively investigated DR, employing various imaging techniques such as fundus photography, scanning laser ophthalmoscope, optical coherence tomography and fluorescein fundus angiography. These studies primarily focus on pathological changes in patients with diabetes' retinal vessels, microvascular lesions, hemorrhage, and edema[9].

The diagnosis and staging of DR in current clinical practice primarily rely on observable signs in fundus photographs, such as microaneurysms, hemorrhages, and hard exudates[2]. However, research suggests that subtle morphological changes in retinal vasculature may occur in patients with diabetes before macroscopically visible signs, such as bleeding and exudation, manifest in fundus images. Retinal vascular parameters involve the quantitative analysis of the retinal vascular network's structure and morphological characteristics[10]. These parameters include retinal vascular equivalent, fractal dimension, curvatures tortuosity, vessel density, among others, and can provide a more accurate assessment of the functional status and pathological changes in retinal vessels[11-17]. Previous evidence indicates that retinal vascular dilation may be a risk factor for the early progression of DR[18]. A study by Sasongko *et al*[19] found that the retinal vascular system in patients with diabetes is more tortuous than in non-diabetes individuals. Additionally, the degree of arteriolar tortuosity in patients with diabetes is associated with mild and moderate DR, suggesting that the degree of retinal vascular tortuosity may serve as an early parameter of microvascular damage in diabetes.

However, there is currently insufficient research on the differential analysis of retinal vascular parameters specific to prediabetes and diabetes. Moreover, there are no clear observational parameters in clinical practice. Additionally, even experienced ophthalmologists may find it challenging to identify these subtle early changes with the naked eye. The rapid development of artificial intelligence (AI) theories and technologies, including deep learning, holds promise for addressing this issue.

In recent years, with the rapid advancement of AI in the medical field, ophthalmology stands out as one of the fastest-developing and most promising areas for the widespread application of AI technology in medicine. AI demonstrates high precision and sensitivity in identifying medical imaging data, enabling the detection of early lesions that are difficult for the naked eye to discern[20-23].

The application of AI for the automatic segmentation of novel biomarkers in retinal images and the investigation of its impact on retinal vascular parameters in prediabetes and diabetes are crucial for a better understanding of the mechanisms underlying diabetic retinal changes. It also holds significant importance for enhancing eye health management in patients with diabetes.

Therefore, this study aims to utilize AI for the automatic segmentation of retinal vessels in individuals with normal glucose metabolism, prediabetes, and diabetes without DR. By comparing the differences in retinal vascular parameters between prediabetic individuals, patients with diabetes, and those with normal glucose metabolism, the research seeks to provide insights into the impact of prediabetes and diabetes on retinal vascular parameters. The results of this study are expected to provide new clues for early diagnosis and prevention of diabetes, and also provide valuable information for eye health management, early diagnosis and early warning for people with prediabetes and diabetes.

MATERIALS AND METHODS

Ethical Approval The data for this study were obtained from participants at the Health Management Center of Peking University Shenzhen Hospital. The examination protocol included fundus photography and the collection of clinical data. The study protocol adhered to the ethical requirements of the Declaration of Helsinki, and the protocol was approved by the Research Ethics Committee of Peking University Shenzhen Hospital.

Technical Workflow The technical workflow of this study was illustrated in Figure 1.

Data Source and Detailed Sample Selection A systematic sampling method was employed to select samples from the Health Management Center. The detailed description of sample selection is as follows. Inclusion criteria: 1) participants aged

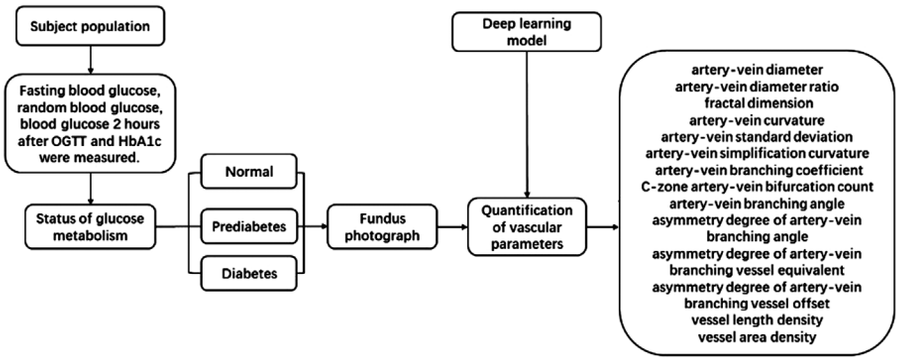

Figure 1 Workflow diagram of this study OGTT: Oral glucose tolerance test.

between 20 and 60y; 2) complete clinical data, and ophthalmic imaging data of sufficient quality. Exclusion criteria: 1) Participants whose fundus color photographs have developed DR manifestations. 2) Participants with other severe eye diseases or a history of eye surgery. 3) Participants with other systemic diseases (such as hypertension, heart disease, kidney disease, *etc*.). 4) Female participants in pregnancy status.

All participants underwent measurements of fasting blood glucose, random blood glucose, OGTT, and HbA1c. Based on the glucose metabolism status, participants were divided into the following three groups: 1) Normal group: fasting blood glucose levels between 6.1–6.9 mmol/L or OGTT blood glucose levels between 7.8–11.0 mmol/L. 2) Prediabetes group: abnormal blood glucose levels not meeting the diagnostic criteria for diabetes. 3) Diabetes group: fasting blood glucose levels ≥7.0 mmol/L, or random blood glucose levels ≥11.1 mmol/L, or OGTT blood glucose levels ≥11.1 mmol/L, or HbA1c levels ≥6.5%. The study ultimately included an equal number of participants in each group, with 200 participants in each group, totaling 600 participants.

Measurement Method and Definition of Retinal Vascular Parameters The measurement of retinal vascular parameters involves two main steps: deep learning-based segmentation of retinal images into arteries and veins, and computation of the required retinal vascular parameters based on the obtained segmentation results.

Deep learning-based retinal artery and vein segmentation 1) Deep learning model: In this study, we utilized the commonly used U-Net architecture for retinal artery and vein segmentation. To enhance the feature extraction capability of the network, we replaced the encoder part of U-Net with the popular Inception-V3 backbone. Additionally, considering that some vascular areas with less distinct features are challenging to differentiate as arteries or veins based solely on local features during segmentation, and it requires a broader perspective based on the connectivity and similarity between vessels, we added the Atrous Spatial Pyramid Pooling module to extract larger-range features[24]. Thereafter, we used the softmax function to distinguish arteries and veins in the segmented vascular regions. The model structure is depicted in Figure 2. 2) Data and training parameters: The data used to train the artery and vein segmentation model were obtained from five publicly available datasets with vessel segmentation annotations: DRIVE[25], HRF[26], LES-AV[27], STARE[28], and CHASEDB1[29]. These datasets collectively comprise 155 fundus images. Except for the DRIVE dataset, which followed the official training and testing set divisions, the remaining data were divided on a patient basis, with a ratio of 7:1:2 for training, validation, and testing sets, respectively. The data distribution is summarized in Table 1.

Table 1 Vessel dataset statistics

Dataset	Distinguish between artery and vein	Training	Validation	Testing	Total
CHASEDB1	No	20	2	6	28
STARE	No	14	2	4	20
DRIVE	No	17	3	20	40
HRF	Yes	31	5	9	45
LES-AV	Yes	16	2	4	22
Total		98	14	43	155

During the network training process, the input size of fundus images was set to 1280×1280. Stochastic Gradient Descent was employed as the optimization method with an initial learning rate of 0.001, a batch size of 4, and momentum set to 0.9. The training utilized the cosine learning rate adjustment strategy with a period of 200 and a maximum learning rate equal to the initial learning rate, and the minimum learning rate set to 1×10^{-6}. Validation was conducted every 60 iterations, and if the validation results did not improve for 10 consecutive times, the training was stopped.

Based on artery and vein segmentation, calculation of vascular parameters Localization and segmentation of the optic disc and macula were performed in fundus images. The distance between the center of the optic disc and the center of the macular fovea was estimated to be 4.76 mm, serving as a standard for converting length or distance parameters in the parameters. The regions were defined as follows: A-zone,

the area within 0 to 0.5 times the optic disc diameter from the optic disc edge; B-zone, the area within 0.5 to 1.0 times the optic disc diameter from the optic disc edge; C-zone, the area within 1.0 to 2.0 times the optic disc diameter from the optic disc edge (Figure 3)[12]. Based on the distance from the optic disc boundary, the retina was divided into zones, and relevant vascular parameters were calculated. Detailed retinal vascular parameters were shown in Table 2[12-15,17,30-32].

Through the measurement and analysis of the above parameters, we can compare the retinal vascular parameters of individuals with different metabolic states, thereby gaining a deeper understanding of the impact of prediabetes and diabetes on retinal vascular parameters.

Statistical Analysis Before comparing the differences between groups in metric data, a test for homogeneity of variances is conducted. When the homogeneity of variances is not significantly different, analysis of variance (ANOVA) is directly used for comparison. If the homogeneity of variances test indicates a significant difference, Welch ANOVA is employed for comparison. Statistical analysis is performed using SPSS version 26.0, and a significance level of $P<0.05$ is considered statistically significant.

RESULTS

Basic Statistical Data This study included a total of 600 fundus color photographs, with 200 in the normal group, 200 in the prediabetes group, and 200 in the diabetes group. The statistics encompassed 26 parameters, including artery caliber/vein caliber, fractal dimension (FD), arteriovenous ratio (AVR), artery curvature/vein curvature, standard deviation of vessel widths (BSTD) of artery, vein BSTD, artery simple curvatures/vein simple curvatures, artery branching coefficient/vein branching coefficient, artery number of first branching (Num1stBa)/vein Num1stBa, artery branching angle/vein branching angle, artery angle asymmetry/vein angle asymmetry, artery Length-diameter ratio (LDR)/vein LDR, artery asymmetry ratio/vein asymmetry ratio, artery junctional exponent deviation (JED)/vein JED, vessel length density and vessel area density. The basic statistical descriptions of these parameters are presented in Table 3.

Homogeneity of Variance Test among Groups A homogeneity of variance test was conducted to examine whether there were significant differences in the fluctuation (standard deviation) of data among different groups. The results indicated that for 15 parameters, including artery curvature, vein curvature, artery BSTD, artery branching coefficient, artery Num1stBa, vein Num1stBa, vein branching angle, vein angle asymmetry, artery LDR, vein LDR, artery JED, vein JED, vessel area density, no significant differences were observed ($P>0.05$). This suggests consistent data fluctuation among groups, indicating homogeneity of variance,

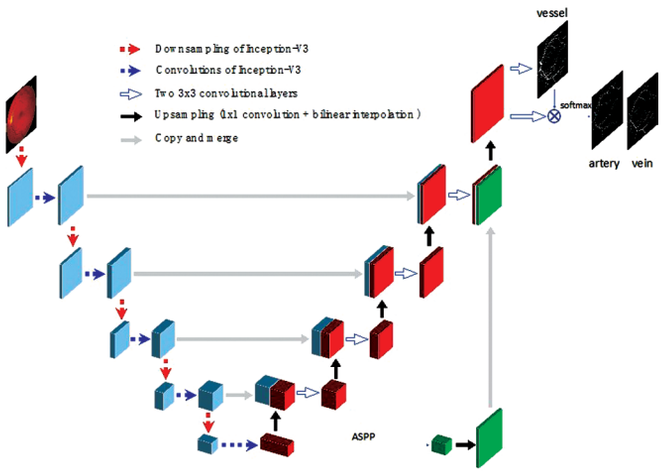

Figure 2 Illustration of the retinal segmentation model of the retinal artery and vein.

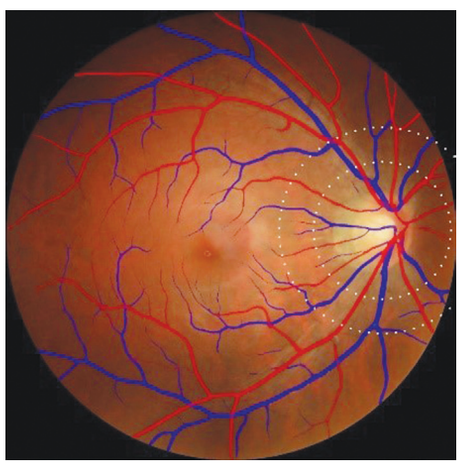

Figure 3 Vascular automatic recognition and segmentation mode image (B-zone defined as the area within 0.5 to 1.0 times the optic disc diameter from the optic disc edge; C-zone defined as the area within 1.0 to 2.0 times the optic disc diameter from the optic disc edge).

making it suitable for further analysis using variance analysis to study differences.

Additionally, for 13 parameters, including artery caliber, vein caliber, FD, AVR, vein BSTD, artery simple curvatures, vein simple curvatures, vein branching coefficient, artery branching angle, artery angle asymmetry, artery asymmetry raito, vein asymmetry raito, vessel length density, significant differences were observed ($P<0.05$). This implies inconsistent data fluctuation among the aforementioned parameters, indicating heterogeneity of variance. Therefore, Welch ANOVA tests were conducted to investigate the relationship between these parameters.

Comparison of Retinal Vascular Parameters among Different Glycemic States The results of the ANOVA demonstrated significant differences ($P<0.05$) among different groups for 10 parameters, including artery caliber, FD, vein curvature, artery Num1stBa, vein Num1stBa, artery branching angle, vein branching angle, artery angle asymmetry, vessel

Table 2 Vascular parameters and descriptions

Retinal measure	Abbreviation	Description	Zone
Central retinal artery equivalent/central retinal vein equivalent	Artery caliber/vein caliber	Summary measures of vascular equivalent caliber representing the equivalent single-vessel parent width for the six largest arterioles and venules. Based on the Knudston-Parr-Hubbard formula (1)[13].	B
Fractal dimension	FD	Global summary measure of branching complexity of the retinal vascular tree reflecting how thoroughly the branching pattern fills two-dimensional spaces. Larger values represent a more complex pattern. Calculated from the skeletonized line tracing using the box-counting method[14].	C
Arteriovenous ratio	AVR	The ratio between summarized arteriolar caliber measurements (CRAE) with respect to the summarized venular caliber (CRVE). Formula: AVR=CRAE/CRVE[13].	B
Curvatures tortuosity	Artery curvature/vein curvature	Integral of the curvature squared along the vessel path, normalized by the total path length. Measurements are summarized to represent the average tortuosity of arterioles and venules separately with smaller values reflecting straighter vessels[30].	C
Standard deviation arteriole	Artery BSTD	The standard deviation of arteriolar widths[14].	B
Standard deviation venule	Vein BSTD	The standard deviation of venular widths[14].	B
Simple tortuosity	Artery simple curvatures/ vein simple curvatures	Simple tortuosity is estimated as the actual path length of the vessel segment divided by the straight line length. Measurements are summarized to represent the average tortuosity of arterioles and venules separately[30].	C
Branching coefficient	Artery branching coefficient/ vein branching coefficient	Calculated from average number of first branching vessels measurements. Branching coefficient reflects the relationship between parent vessels and branches. Defined as the summed square of the mean vessel widths of each branch or daughter vessel divided by the square of the mean width of the parent vessel[12].	C
Number of first branching	Artery Num1stBa /vein Num1stBa	The number of arterioles and venules with a first bifurcation, branch, or daughter vessel in zone C[14].	C
Branching angle	Artery branching angle/vein branching angle	The first angle subtended between 2 daughter vessels at each vascular bifurcation[12].	C
Angle asymmetry	Artery angle asymmetry/vein angle asymmetry	The difference between 2 daughter (branching) vessel angles[12].	C
Length-diameter ratio	Artery LDR/vein LDR	Length to diameter ratio is the length of the vessel from the midpoint of one bifurcation to the midpoint of the next bifurcation. It is expressed as a ratio to the diameter of the parent vessel at the first bifurcation[15].	C
Asymmetry ratio	Artery asymmetry raito/vein asymmetry raito	The cross-sectional area of the smaller branch divided by that of the larger[31].	C
Junctional exponent deviation	Artery JED/vein JED	Calculated from average number of first branching vessels measurements. Junctional exponent deviation reflects the deviation from the optimum ratio of vessel widths[31].	C
Vessel length density	Vessel length density	The vessel length density was defined as vessel length per unit area based on the skeletonized image[32].	C
Vessel area density	Vessel area density	The vessel density was defined as the proportion of vessel area with blood flow over the total area measured[17].	C

length density, and vessel area density. This suggests that there are distinct variations in the retinal vascular parameters among individuals with different glycemic states, as illustrated in Table 4 and Figure 4.

DISCUSSION

Numerous studies have previously indicated that retinal vascular parameters can serve as biomarkers for future cardiovascular diseases, neurodegenerative disorders, metabolic status, and ocular diseases[14,33-34]. However, there is still insufficient research on predictive studies regarding the progression of prediabetes and diabetes. Nevertheless, due to the challenges associated with the observation and quantification of retinal vascular parameters, traditional measurement methods are evidently inadequate, and AI-based image segmentation techniques hold promise in addressing this issue.

This study aimed to automatically identify and quantify retinal vascular parameters through AI image segmentation techniques. In the preliminary stages of the research, the U-Net network was chosen as the foundational architecture for arterial-venous segmentation. We replaced the encoder part of U-Net with the Inception-V3 backbone network and introduced the Atrous Spatial Pyramid Pooling (ASPP) module to extract a broader range of features. Thereafter, we used the softmax function to distinguish between arteries and veins in the segmented vascular areas. After thorough training, the model achieved automatic segmentation of retinal vessels and quantitative output of vascular parameters.

By collecting and comparing fundus photographs of normal individuals, patients with prediabetes, and patients with diabetes, and utilizing the retinal vascular parameters automatic segmentation model developed in this study, multiple potential parameter parameters were output. The results indicated significant differences in 10 retinal vascular morphological parameters, including artery caliber, fractal dimension, vein curvature, artery branching points, vein branching points, artery branching angle, vein branching angle, artery angle asymmetry,

Table 3 Basic statistical descriptions of retinal vascular parameters

Parameters	Mean	Standard deviation	Median	IQR	Kurtosis	Skewness
Artery caliber	155.254	29.535	156.872	30.658	3.575	-0.967
Vein caliber	225.883	39.981	227.681	43.245	4.498	-0.946
FD	1.522	0.071	1.537	0.055	64.852	-6.159
AVR	0.691	0.153	0.689	0.106	63.236	-2.749
Artery curvature	0.442	0.189	0.401	0.23	2.561	1.338
Vein curvature	0.556	0.188	0.53	0.231	1.908	0.969
Artery BSTD	16.126	7.3	15.206	9.955	1.17	0.542
Vein BSTD	28.642	9.906	28.295	12.858	0.212	0.253
Artery simple curvatures	1.074	0.079	1.076	0.017	174.519	-12.89
Vein simple curvatures	1.081	0.08	1.082	0.018	169.001	-12.537
Artery branching coefficient	1.378	1.396	1.26	0.458	176.868	11.838
Vein branching coefficient	1.195	1.321	1.057	0.256	269.799	14.473
Artery Num1stBa	2.863	1.654	3	2	-0.239	0.371
Vein Num1stBa	2.903	1.501	3	2	-0.092	0.322
Artery branching angle	88.488	38.491	85.641	32.189	2.898	0.408
Vein branching angle	89.58	32.947	86.098	33.665	2.259	0.147
Artery angle asymmetry	33.301	17.233	32.648	20.367	0.896	0.345
Vein angle asymmetry	35.927	16.816	36.242	18.936	1.097	0.257
Artery LDR	6.343	6.93	5.058	10.394	0.91	1.045
Vein LDR	6.528	6.483	5.943	10.951	0.145	0.813
Artery asymmetry raito	0.433	0.202	0.445	0.242	0.085	-0.226
Vein asymmetry raito	0.366	0.181	0.355	0.235	0.359	0.331
Artery JED	-0.144	0.481	-0.115	0.574	1.338	-0.46
Vein JED	0.14	0.44	0.178	0.453	8.879	-1.879
Vessel length density	0.031	0.006	0.032	0.007	2.918	-1.057
Vessel area density	0.109	0.023	0.112	0.026	3.325	-1.088

FD: Fractal dimension; AVR: Arteriovenous ratio; BSTD: Standard deviation of vessel widths; Num1stBa: Number of first branching; LDR: Length-diameter ratio; JED: Junctional exponent deviation; IQR: Interquartile range.

vessel length density, and vessel area density (P<0.05) among the normal group, prediabetes group, and diabetes group. This implies that there are significant differences in retinal vascular parameters among individuals with different glycemic states. These parameters hold the potential to be used for the early detection of diabetic retinopathy and to assist in the diagnosis of prediabetes and diabetes.

Among these, the study found significant statistical differences in arterial diameter between different groups. As prediabetes and diabetes progress, the diameter of retinal arteries gradually decreases, and this difference is statistically significant. Retinal artery diameter quantifies the diameter of retinal arteries[35] and serves as an important parameter reflecting the morphology of systemic arteries. Changes in retinal vessel diameter reflect the slow accumulation of chronic pathophysiological changes related to aging, inflammation, nitric oxide-dependent endothelial dysfunction, and other conditions[36-37]. Previous research has shown that a decrease in retinal artery diameter can be used to predict diabetes, coronary heart disease, and metabolic syndrome[33,37-39]. This study corroborates previous findings but adds a comparison of fundus photographs of individuals with prediabetes and diabetes. The results indicate that with the gradual deterioration of patients' glycemic function, the retinal artery diameter gradually decreases to the point of being visually indistinguishable. Although the specific mechanism of the impact of glycemic status on retinal artery diameter is not yet clear, this parameter holds promise as an important biomarker for predicting prediabetes and diabetes in the future.

As a parameter of vascular branching complexity, FD quantitatively summarizes the overall structure of the retinal circulation. Its primary advantage is that it is not affected by image magnification due to the refractive properties of the eye or retinal imaging. In the retina, a decrease in vascular FD suggests local hypoxia leading to vascular rarefaction and collapse[40]. Zekavat *et al*[34], using a convolutional neural network to segment retinal microvasculature from 54 813 retinal fundus images of 97 895 UK Biobank participants, found that a decreased retinal vascular FD is significantly associated with higher mortality rates, hypertension, congestive

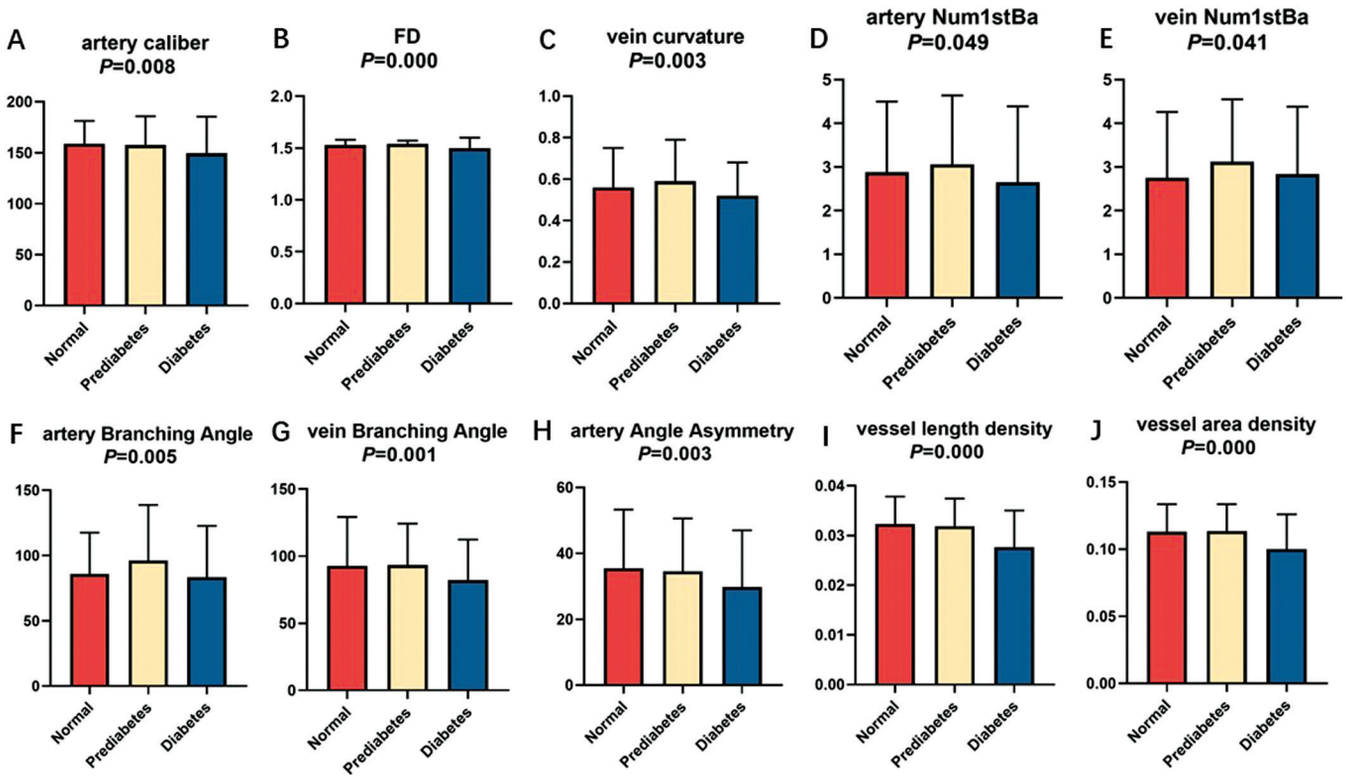

Figure 4 Statistically significant retinal vascular parameters with inter-group differences A: Artery caliber, *P*=0.008; B: FD, *P*=0.000; C: vein curvature, *P*=0.003; D: Artery Num1stBa, *P*=0.049; E: Vein Num1stBa, *P*=0.041; F: Artery branching angle, *P*=0.005: G: Vein branching angle, *P*=0.001; H: Artery angle asymmetry, *P*=0.003; I: Vessel length density, *P*=0.000; J: Vessel area density, *P*=0.000. Num1stBa: Number of first branching; FD: Fractal dimension.

heart failure, renal failure, type 2 diabetes, sleep apnea, anemia, and various eye diseases. This study also found that genetically, these biologically significant retinal vascular parameters are associated with pathways related to angiogenesis and inflammation. Wu *et al*[41] found that the superficial retinal vascular FD linearly decreases with worsening kidney function and can serve as an effective non-invasive assessment for predicting and monitoring kidney function progression. Other studies indicate correlations between retinal FD and cerebral blood flow and cerebrovascular events in patients with diabetes[40,42]. The results of this study reveal that, with the gradual progression of impaired glycemic function in patients, retinal FD gradually decreases, and this difference is statistically significant. Therefore, it appears that subtle changes in retinal FD occur when there is damage to patients' glycemic function.

This study found a significant statistical difference in retinal vein curvature among the three patient groups. Notably, compared to healthy individuals, the retinal vein curvature increased in patients with prediabetes, while it significantly decreased in patients with diabetes. Previous research suggests that an increase in vascular tortuosity is indicative of vascular wall dysfunction and damage to the blood-retinal barrier. Evaluating retinal vascular tortuosity may aid in the early detection of retinal vascular diseases[30,43]. In a quantitative analysis of retinal vascular features related to retinopathy of prematurity, images of "aggressive posterior retinopathy of prematurity" had higher vein curvature than those of "plus disease", suggesting that retinal vein curvature may be a differentiating feature between aggressive posterior retinopathy of prematurity and plus disease[44]. In the diagnosis of systemic chronic diseases, retinal vascular tortuosity has been identified as an early predictive parameter for diabetes, cerebrovascular diseases, stroke, and ischemic heart disease[45]. However, research on this parameter's predictive role in prediabetes is currently lacking. The results of this study suggest that, during the process of glycemic dysfunction, retinal vein curvature exhibits a trend of first increasing and then decreasing.

Additionally, this study found that four other vascular parameters (including artery Num1stBa, vein Num1stBa, artery branching angle, and vein branching angle) also exhibited a trend of first increasing and then decreasing during the progression of glycemic dysfunction. Similar to FD parameter parameters, these four parameters represent the complexity of retinal vascular branching. In a cross-sectional study evaluating the impact of myopia on retinal vascular branching, the results showed that the angle of retinal small artery branching, the angle of small artery branching in highly myopic patients, and the asymmetry of the vein angle were significantly lower than those in low to moderately myopic

Table 4 ANOVA results among different groups mean±SD

Parameter name	Normal group (*n*=200)	Prediabetes group (*n*=200)	Diabetes group (*n*=200)	*F*/Welch *F*	*P*
Artery caliber	158.82±22.35	157.37±28.28	149.37±35.94	4.936	0.008
Vein caliber	229.70±31.70	227.67±34.71	220.07±50.87	2.518	0.082
FD	1.53±0.05	1.54±0.03	1.50±0.10	15.231	0.000
AVR	0.70±0.08	0.69±0.10	0.68±0.23	0.389	0.678
Artery curvature	0.42±0.17	0.45±0.20	0.45±0.19	1.308	0.271
Vein curvature	0.56±0.19	0.59±0.20	0.52±0.16	5.754	0.003
Artery BSTD	16.00±6.69	15.79±7.56	16.61±7.65	0.656	0.519
Vein BSTD	29.21±10.29	28.83±8.49	27.84±10.82	0.875	0.418
Artery simple curvatures	1.08±0.02	1.08±0.02	1.06±0.14	1.802	0.167
Vein simple curvatures	1.09±0.02	1.09±0.02	1.07±0.14	2.266	0.105
Artery branching coefficient	1.11±0.85	1.37±2.03	1.10±0.58	2.626	0.073
Vein branching coefficient	1.11±0.85	1.37±2.03	1.10±0.58	1.623	0.199
Artery Num1stBa	2.88±1.62	3.06±1.58	2.65±1.74	3.027	0.049
Vein Num1stBa	2.75±1.51	3.12±1.43	2.84±1.54	3.208	0.041
Artery branching angle	85.87±31.74	96.19±42.54	83.28±39.45	5.471	0.005
Vein branching angle	92.73±36.48	93.47±30.76	82.29±30.10	7.142	0.001
Artery angle asymmetry	35.42±17.91	34.56±16.08	29.79±17.21	5.982	0.003
Vein angle asymmetry	35.87±18.34	36.84±15.46	35.05±16.54	0.555	0.574
Artery LDR	6.32±7.37	7.01±6.87	5.68±6.47	1.793	0.167
Vein LDR	6.00±6.76	7.21±6.42	6.37±6.22	1.809	0.165
Artery asymmetry raito	0.42±0.19	0.46±0.18	0.42±0.23	2.969	0.053
Vein asymmetry raito	0.35±0.18	0.38±0.16	0.37±0.20	1.853	0.158
Artery JED	-0.13±0.50	-0.13±0.49	-0.17±0.44	0.533	0.587
Vein JED	0.18±0.46	0.12±0.46	0.12±0.40	1.173	0.31
Vessel length density	0.0323±0.0055	0.0319±0.0055	0.0277±0.0073	28.031	0.000
Vessel area density	0.1130±0.0205	0.1134±0.0201	0.1002±0.0257	22.213	0.000

ANOVA: Analysis of variance; FD: Fractal dimension; AVR: Arteriovenous ratio; BSTD: Standard deviation of vessel widths; Num1stBa: Number of first branching; LDR: Length-diameter ratio; JED: Junctional exponent deviation.

and non-myopic groups. This study's results trend similarly to those of our study[12]. This suggests that in prediabetes, the complexity of retinal vascular branching increases, possibly as a compensatory pathophysiological change in response to early vascular wall dysfunction. With further impairment of glycemic function, this compensatory mechanism fails, leading to a significant decrease in the complexity of retinal vascular branching. Therefore, further research is needed to explore the developmental mechanisms and diagnostic significance of these parameters in prediabetes and diabetes populations.

In Sun *et al*'s[12] study, a decrease in the asymmetry of the vein angle was related to high myopia, while the asymmetry of the artery angle was not associated with high myopia. In contrast to results associated with high myopia, this study found that the asymmetry of retinal arterial branching angles gradually decreased with the development of prediabetes and diabetes, with significant statistical differences. Although it is unknown how the asymmetry of these vascular angles affects the pressure in the vascular system and what impact these changes may have on the retina of patients with diabetes, past research suggests that an increased asymmetry of retinal vessel angles may be related to microvascular damage[46]. Therefore, changes in this parameter may indicate functional disturbances in the microvascular system of patients.

As prediabetes and diabetes progress in patients, vessel length density and vessel area density gradually decrease, and the differences have significant statistical significance. Vessel length density and vessel area density indicate the perfusion level of the inner layer tissue structure of the retina[17]. It suggests that before the onset of DR, there is already a decline in retinal perfusion, albeit these subtle changes being challenging to identify with the naked eye. The measurement of vascular parameters assisted by AI aids in the early detection of such changes.

However, this study has some limitations. First, it is a cross-sectional study, and therefore, causal relationships cannot be determined. Second, the sample size of the study is relatively small, limiting its ability to represent populations from different regions and ethnicities. Finally, although the study identified differences in retinal vascular parameters under different glycemic states, the specific biological mechanisms underlying these parameters remain unclear. Future research could further validate the effectiveness of these parameters as predictive factors, potentially making them useful tools for early screening and monitoring of prediabetic and patients with diabetes.

In conclusion, while this study provides initial insights into the differences in retinal vascular parameters under different glycemic states, there are still many questions that need further exploration and resolution. By addressing these limitations and continuing in-depth exploration, we can better understand the role of these parameters in the development of prediabetes and diabetes, providing more valuable information for clinical practice.

This study developed and trained an automatic quantification model for retinal vascular parameters based on artificial intelligence image segmentation technology. The model was used to compare and analyze retinal vascular parameters under different glucose metabolism states. The final results revealed significant differences in multiple retinal vascular parameters among normal individuals, pre-patients with diabetes, and patients with diabetes. These differences encompassed 10 specific parameters, including artery caliber, FD, vein curvature, artery Num1stBa, vein Num1stBa, artery branching angle, vein branching angle, artery angle asymmetry, vessel length density, and vessel area density. The identification of these parameters through artificial intelligence image segmentation technology holds promise as a useful tool for early screening and monitoring of pre-diabetes, diabetes, and DR.

ACKNOWLEDGEMENTS

Authors' contributions: Jiang Y and Gong D: topic selection, retrieval, methodology, writing-original draft preparation. Chen XH and Cai YJ: Data collection, visualization, assistance in writing and writing-reviewing. Yang L and Chen BB: assistance in writing and writing-reviewing. Xu JJ and Wei QJ: Development the deep learning model. Xi WQ and Zhang Z: Supervision, writing- reviewing and editing, guidance.

Foundation: Supported by Shenzhen Science and Technology Program (No.JCYJ20220530153604010).

Conflicts of Interest: Jiang Y, None; **Gong D,** None; **Chen XH,** None; **Yang L,** None; **Xu JJ,** None; **Wei QJ,** None; **Chen BB,** None; **Cai YJ,** None; **Xi WQ,** None; **Zhang Z,** None.

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