# Early retinal neurovascular impairment in patients with diabetes without clinically detectable retinopathy

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## ABSTRACT

**Aims** To investigate the function and the corresponding neurovascular structures in patients with diabetes without clinically detectable retinopathy.

**Methods** Sixty-six patients with type 2 diabetes without retinopathy (NDR) and 62 healthy controls were recruited. The 16 and 32 Tds flicker electroretinography (ERG) was performed using a mydriasis-free, full-field flicker ERG recording device (RETeval). The vessel density (VD) of superficial capillary plexus (SCP) and deep capillary plexus (DCP), FD300 and ganglion cell complex (GCC) thickness in the macula were quantified using optical coherence tomography angiography (OCTA). The retinal nerve fibre layer (RNFL) thickness and the radial peripapillary capillary (RPC) density in the peripapillary area were also measured with OCTA.

**Results** Parafoveal and perifoveal VD in both SCP and DCP decreased in NDR group in comparison to control group (all p<0.01). However, macular GCC thickness was comparable between the two groups (p=0.661). Peripapillary RNFL thickness and RPC density were significantly lower in NDR group (p<0.001 and p=0.009, respectively). With regard to ERG parameters, delayed implicit time and decreased amplitude were found in NDR group in comparison to the control group (all p<0.01). In the multiple linear regression analyses, delayed implicit time for 16 and 32 Tds stimuli was significantly correlated with increased HbA1c (β=0.350, p<0.001; β=0.328, p<0.001, respectively) and decreased VD of SCP in the parafoveal region (β=−0.266, p=0.013; β=−0.253, p=0.005, respectively). However, delayed implicit time for 16 and 32 Tds stimuli was not correlated with the thickness of GCC (β=−0.008, p=0.818) in multiple linear regression analyses.

**Conclusion** Functional and structural impairments have already started in diabetic retina even in the absence of visible retinal lesions. Subtle microvascular abnormalities rather than ganglion cell loss might be associated with early functional changes in NDR patients. Poor control of blood glucose was associated with delayed implicit time of flicker ERG in preclinical diabetic retinopathy.



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## INTRODUCTION

Diabetic retinopathy (DR) could be a devastating complication of diabetes mellitus (DM) and it is a leading cause of blindness worldwide.[1] Study found that early detection of type 2 DM (T2DM) and screening for retinopathy are associated with reduced prevalence and severity of retinopathy.[2] It is difficult to reverse the damage and the risk of DR progression is increased once retinal lesions become clinically visible.[3 4] The current managements for DR are focused on the late stages, when retinal structures and visual acuity have already been affected.[5] Having a clearer understanding of the pathogenesis of the neurovascular impairments might provide novel and more effective preventive strategies. Thus, it is necessary to detect and monitor the subtle neurovascular changes in patients with diabetes with subclinical DR.

Studies on the retinal structures showed that there was reduced ganglion cell complex (GCC) layer thickness, reduced retinal nerve fibre layer (RNFL) thickness and decreased capillary vessel density (VD) in patients with diabetes without DR (NDR).[6–8] Also, delayed implicit time and decreased amplitude were found in electroretinographic studies.[9] Although studies have demonstrated the changes of structural and functional abnormalities in NDR patients, the topic remains controversial. More investigations are needed to clarify the relationships among neural structures, functional changes and their corresponding microcirculation.

RETeval is a handheld, mydriasis-free, full-field electroretinography (ERG) recording device, which uses a special skin electrode to pick up ERG. The device is effective in investigation of ERG abnormalities in diabetic retina.[10 11] The cone density is highest in the fovea, and flicker ERG with a frequency of 28.3 Hz could demonstrate the electrical activity from the cone system.[12] Moreover, optical coherence tomography angiography (OCTA) is able to quantify the neural structures and their corresponding blood supply in the macular and optic disc. To the best of our knowledge, few studies demonstrated the combined results of the flicker ERG and the OCTA characteristics in NDR patients. Investigating the cone pathway function and the corresponding neurovascular structures may provide more evidence to reveal the nature of neurovascular degeneration in preclinical DR.

## METHODS

### Subjects

Sixty-six patients with T2DM and 62 healthy controls were recruited for the study. The study was performed in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from all the participants.

The diagnosis of T2DM was established by endocrinologist according to the diagnostic criteria of American Diabetes Association.[13] Diagnosis and classification of DR were confirmed according to the international clinical DR and diabetic macular oedema disease severity scales.[14] One eye of the subjects was randomly selected if both were eligible in the study. The exclusion criteria were as follows: (1) patients with other ocular conditions affecting the neural and vascular structures of the eye (glaucoma, uveitis, refractive error >3 dioptres (D)); (2) history of ocular surgery, ocular trauma, amblyopia; (3) patients with DR; (4) subjects with ocular conditions that affect imaging of OCTA (scan quality <6, eg, advanced cataract); (5) intraocular pressure (IOP) >21 mm Hg; and (6) patients with history of severe systemic cardiovascular diseases.

### Clinical parameters

Subjects underwent a complete ocular examination, including best corrected visual acuity, IOP, refractive error (autorefractometry) and slit lamp fundus examination. The stage of DR was evaluated by experienced ophthalmologist according to the slit lamp fundus exam and ETDRS 35° 7-standard field colour retinal photographs (Topcon TRC; Topcon, Tokyo, Japan). The glycated haemoglobulin (HbA1c) levels and duration of diabetes of the patients with T2DM were also tested.

### Mydriasis-free, full-field flicker ERG examination

RETeval (LKC Technologies, Gaithersburg, MD) is a handheld, mydriasis-free, full-field flicker ERG recording system. The details of the device have been described in other studies.[10 15 16] The device was used according to the instructions provided by the manufacturer.

The flash stimuli were presented with a ganzfeld dome of 60 mm in diameter. White light stimuli (CIE 1931 chromaticity, x=0.33, y=0.33) were presented by combining three coloured light-emitting diodes (green 530 nm; red 622 nm; blue 470 nm). The pupil size is measured automatically in real time during the examination. The stimulus flash luminance is automatically adjusted to maintain a constant flash retinal illuminance. In our study, we chose the DR assessment protocol, which default the stimulus flash retinal illuminance as 16 and 32 photopic Tds. The protocol aims to assess the cone pathway in the inner retina. The frequency of the flicker stimuli was 28.3 Hz and the pulse duration was less than 1 ms. The electrical signals were picked up through a special skin electrode array placing on the lower eyelid. Amplitudes and implicit times were measured and two flicker ERG waveforms were displayed by the device (figure 1). The DR assessment protocol could create a numerical result with a reference range of 7.0–19.9. The cut-off value was set for the purpose of screening vision-threatening DR according to the implicit time, amplitude, age and pupil response. Detailed description could be found in the original study.[10]

### OCTA examination

OCTA examinations were conducted in a dark room after pupillary dilation by using AngioVue OCTA system (V.2017.1.0.151; RTVue-XR Avanti; Optovue, Fremont, CA, USA). We chose the macular HD 6 mm×6 mm program and HD disc 4.5 mm×4.5 mm program, which uses an 840 nm light source and provides 70 000 A scans/s. Superficial capillary plexus (SCP) and deep capillary plexus (DCP) were automatically generated by the software[17] and the segmentation of the retinal layer is demonstrated in figure 2. SCP is defined as a slab extending from internal limiting membrane (ILM) to 10 µm above inner plexiform layer (IPL). DCP is a slab extending from 10 µm above IPL to 10 µm below outer plexiform layer (OPL). Foveal density in a 300 $\mu$m region around foveal avascular zone (FD300) is a parameter demonstrating the capillary density from ILM to OPL in a 300 $\mu$m wide region around Foveal avascular zone (FAZ). The GCC layer is a slab extending from RNFL to IPL. The segmentation results of OCTA were checked manually and corrected manually if the boundary deviated from the right position.

The software automatically fits a circle (1.0 mm in diameter) centred on the fovea. The perifovea region is defined as a 2.0 mm wide round annulus around the fovea 1.0 mm circle and the perifovea defined as a 3.0 mm wide round annulus around the parafovea. The following parameters were quantified: the parafoveal and perifoveal VD in SCP and DCP, FD300 and GCC thickness.

The software automatically fits a circle (2 mm in diameter) centred on the optic disc and the peripapillary region is defined as a 1.0 mm wide round annulus around the optic disc 2.0 mm circle. The peripapillary VD and RNFL thickness were quantified in the radial peripapillary capillary segment, which was defined as a slab extending from the inner limiting membrane (ILM) to RNFL. The VD was quantified using split-spectrum amplitude

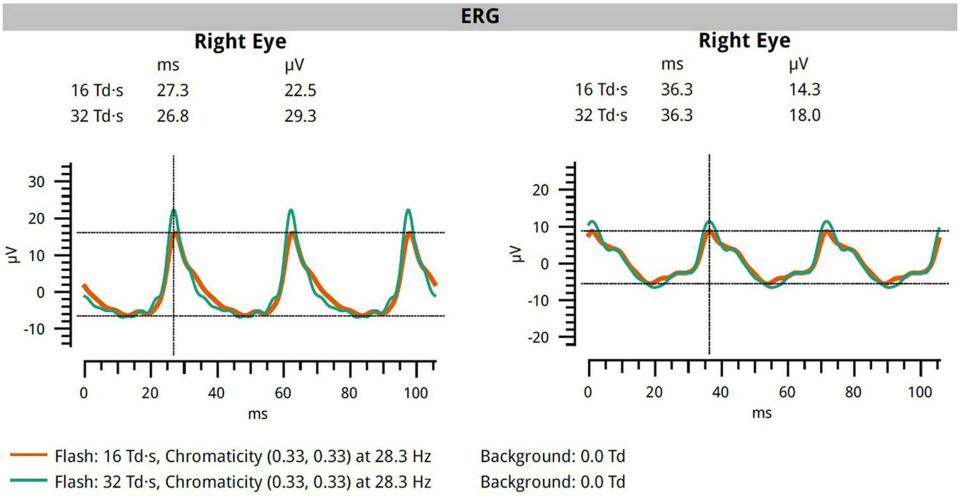


**Figure 1** The right eye electroretinography (ERG) waveforms of a healthy control (left) and NDR patient (right) for 16 and 32 Tds.

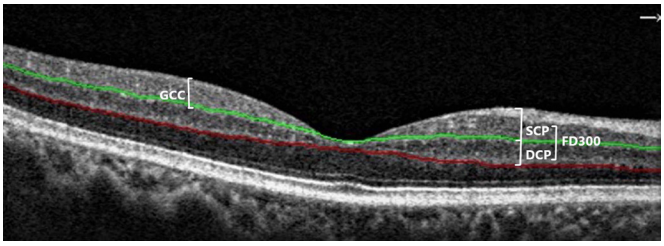


**Figure 2** Optical coherence tomography angiography segmentation boundaries. Optical coherence tomography (OCT) of the human retina showing the segmentation boundaries for ganglion cell complex (GCC) is retinal nerve fibre layer (RNFL) to inner plexiform layer (IPL). The capillary plexuses can be grouped into superficial and deep capillary complexes (SCP and DCP, as shown on the right). The segmentation boundaries for FD300 are from internal limiting membrane (ILM) to 10 µm below outer plexiform layer. FD300, foveal density in a 300 µm region around foveal avascular zone.

decorrelation angiography software algorithm. VD is defined as the percentage of signal positive pixels per total pixels.

### Statistical analysis

Data analyses were performed using SPSS software V.19.0 (SPSS). Shapiro-Wilk test was used to test the normality of the data. The differences between NDR group and the control group were compared using the Student's t-test. Pearson's correlation coefficient was used to evaluate the linear correlation between functional, structural and clinical parameters, and we used Bonferroni correction to adjust the p values for multiple analyses. Multiple linear regression analysis was performed to investigate the correlation of implicit time and other parameters. P values <0.05 were considered as statistically significant.

## RESULTS

Sixty-six patients in the NDR group and 62 healthy controls were included in the study. Table 1 shows the demographic and clinical data of the control group and NDR group. The age, gender and eyes selected were comparable between the two groups (all p>0.05).

VD of peripapillary region and SCP and DCP in both parafoveal and perifoveal regions were decreased significantly in NDR group compared with the control group, while the results for neural structures were variable. Table 2 shows the structural parameters and the comparisons in the control group and NDR group. The VD of macular SCP and DCP was decreased significantly in NDR group compared with the control group (all p<0.01). FD300 was also decreased significantly in the NDR group (p<0.001). GCC thickness was not significantly different between two groups (p=0.661).

**Table 1** Demographic and clinical data of the control group and NDR group

| Characteristics | Control group (n=62) | NDR group (n=66) | P value |
|---|---|---|---|
| Age (years) | 55.16±12.50 | 58.77±12.13 | 0.100 |
| Gender (male/female) | 31/31 | 38/28 | 0.390 |
| Eye (right /left) | 30/32 | 35/31 | 0.599 |
| Duration of diabetes (years) | NA | 8.65±6.50 | NA |
| HbA1c (%) | NA | 9.21±2.54 | NA |

HbA1c, glycated haemoglobulin; NA, not applicable; NDR, patients with diabetes without clinically detectable retinopathy.

**Table 2** Comparison of structural parameters in the control group and NDR group

| Parameters | Control group (n=62) | NDR group (n=66) | P value |
|---|---|---|---|
| VD of SCP (parafovea, %) | 52.47±4.31 | 49.97±4.45 | **0.002**** |
| VD of SCP (perifovea, %) | 50.42±3.73 | 48.12±4.01 | **0.001**** |
| VD of DCP (parafovea, %) | 55.99±4.09 | 52.70±4.51 | **<0.001**** |
| VD of DCP (perifovea, %) | 52.71±6.56 | 48.62±6.39 | **0.001**** |
| FD300 (%) | 55.49±4.28 | 51.13±5.55 | **<0.001**** |
| GCC thickness (µm) | 98.26±6.46 | 98.80±7.50 | 0.661 |
| Peripapillary VD (%) | 52.48±2.73 | 50.27±4.10 | **<0.001**** |
| RNFL thickness (µm) | 115.06±10.61 | 109.85±11.50 | **0.009**** |

*P<0.05; **P<0.01.
DCP, deep capillary plexus; FD300, foveal density in a 300 µm wide region around foveal avascular zone; GCC, ganglion cell complex; NDR, patients with diabetes without clinically detectable retinopathy; RNFL, retinal nerve fibre layer; SCP, superficial capillary plexus; VD, vessel density.

However, the RNFL thickness in NDR group was significantly lower than the control group (p=0.009). Also, peripapillary VD was significantly decreased in NDR group in comparison to the control group.

The comparisons of the DR assessment protocol results between the groups were demonstrated in table 3. Delayed implicit time and decreased amplitude were found in NDR group in comparison to the control group (all p<0.01). The DR score was significantly higher in the NDR group, while the ratio of pupil area was significantly lower in the NDR group (all p<0.01).

Pearson's correlation coefficients between the diabetic parameters and ocular structural and functional parameters were shown in table 4. After Bonferroni correction for the multiple analysis, implicit time for 16 and 32 Tds stimuli was positively associated with the level of HbA1c (r=0.434, corrected p=0.024; r=0.448, corrected p<0.001, respectively). Longer duration of DM was associated with lower VD of DCP in the parafoveal region (corrected p=0.048) (table 4).

Table 5 shows the Pearson's correlation coefficients between the flicker ERG parameters and the neurovascular structures. After Bonferroni correction, increased implicit time of 16 and 32 Tds light stimuli was significantly correlated with decreased VD of SCP in both parafovea and perifovea regions, and increased implicit time of 32 Tds light stimuli was significantly correlated with VD of DCP in perifovea area. The thickness of GCC was negatively correlated with implicit time for 16

**Table 3** Comparison of flicker ERG parameters in control group and NDR group

| Parameters | Control group (n=62) | NDR group (n=66) | P value |
|---|---|---|---|
| 16 Tds | | | |
| Implicit time (ms) | 28.70±1.64 | 29.98±1.87 | <0.001** |
| Amplitude (µV) | 20.09±6.08 | 17.36±5.35 | 0.008** |
| 32 Tds | | | |
| Implicit time (ms) | 27.79±1.51 | 29.03±1.71 | <0.001** |
| Amplitude (µV) | 24.20±6.75 | 20.89±5.75 | 0.003** |
| Pupil area ratio | 2.03±0.38 | 1.85±0.28 | 0.003** |
| DR score | 17.89±2.96 | 19.25±2.19 | 0.004** |

*P<0.05; **P<0.01.
DR, diabetic retinopathy; ERG, electroretinography; NDR, patients with diabetes without clinically detectable retinopathy.

**Table 4** Pearson's correlation coefficients of diabetic parameters and structural ERG parameters

| Parameters | HbA1c | Duration of DM |
|---|---|---|
| VD of SCP (parafovea) | (−0.009, 0.944) | (−0.332, 0.007) |
| VD of SCP (perifovea) | (−0.131, 0.318) | (−0.280, 0.023) |
| VD of DCP (parafovea) | (0.197, 0.132) | (−0.372, 0.002)* |
| VD of DCP (perifovea) | (0.016, 0.902) | (−0.263, 0.033) |
| FD300 | (−0.026, 0.844) | (−0.206, 0.098) |
| GCC thickness | (−0.201, 0.123) | (−0.121, 0.332) |
| Peripapillary VD | (−0.71, 0.590) | (0.021, 0.866) |
| RNFL thickness | (0.125, 0.342) | (−0.111, 0.373) |
| 16 Tds implicit time | (0.434, 0.001)* | (−0.066, 0.600) |
| 16 Tds amplitude | (0.044, 0.739) | (0.027, 0.829) |
| 32 Tds implicit time | (0.448, <0.001)* | (−0.060, 0.635) |
| 32 Tds amplitude | (0.051, 0.700) | (−0.020, 0.874) |

Pearson's correlation coefficients and the original p values (in brackets).
*Statistically significant after Bonferroni correction.
DCP, deep capillary plexus; DM, diabetes mellitus; ERG, electroretinography; FD300, foveal density in a 300 µm wide region around foveal avascular zone; GCC, ganglion cell complex; RNFL, retinal nerve fibre layer; SCP, superficial capillary plexus; VD, vessel density.

**Table 6** Multiple regression models of (A) 16 Tds implicit time and (B) 32 Tds implicit time (dependent variables) with variables that showed significant associations in univariate analyses (independent variables)

| | β | 95% CI | Standardised β | P value |
|---|---|---|---|---|
| (A) | | | | |
| VD of SCP (parafovea) | −0.266 | −0.472 to 0.059 | −0.635 | 0.013* |
| VD of SCP (perifovea) | 0.131 | −0.122 to 0.385 | 0.276 | 0.303 |
| VD of DCP (perifovea) | −0.039 | −0.128 to 0.050 | −0.135 | 0.381 |
| FD300 | −0.012 | −0.086 to 0.111 | 0.037 | 0.801 |
| GCC thickness | −0.008 | −0.076 to 0.060 | −0.030 | 0.818 |
| HbA1c | 0.350 | 0.184 to 0.516 | 0.461 | <0.001** |
| Adjusted $R^2$=0.360 | | | | |
| (B) | | | | |
| VD of SCP (parafovea) | −0.253 | −0.428 to 0.078 | −0.664 | 0.005** |
| VD of SCP (perifovea) | 0.076 | −0.139 to 0.291 | 0.175 | 0.483 |
| VD of DCP (perifovea) | −0.033 | −0.109 to 0.042 | −0.126 | 0.382 |
| FD300 | 0.023 | −0.061 to 0.106 | 0.074 | 0.588 |
| GCC thickness | 0.006 | −0.051 to 0.064 | 0.027 | 0.824 |
| HbA1c | 0.328 | 0.187 to 0.469 | 0.474 | <0.001** |
| Adjusted $R^2$=0.444 | | | | |

*P<0.05; **P<0.01.
DCP, deep capillary plexus; FD300, foveal density in a 300 µm wide region around foveal avascular zone; GCC, ganglion cell complex; SCP, superficial capillary plexus; VD, vessel density.

Tds (r=−0.243, p=0.049), but they were not correlated after Bonferroni correction. In the multiple linear regression analyses (table 6), delayed implicit time for 16 and 32 Tds stimuli was significantly correlated with increased HbA1c (β=0.350, p<0.001; β=0.328, p<0.001, respectively). Besides, with regard to structural parameters, delayed implicit time for 16 and 32 Tds light stimuli was only significantly correlated with decreased VD of SCP in the parafovea (β=−0.266, p=0.013; β=−0.253, p=0.005, respectively).

## DISCUSSION

In the current study, we found that functional and structural impairments have already started in the absence of visible retinal lesions. Impairments in both neural and vascular structures were associated with delayed implicit time in univariable linear analyses, while only the VD of SCP in the parafoveal region was correlated in the multivariable regression analyses. With regard to systemic factors in the study, only the level of HbA1c was positively associated with implicit time.

More and more evidence has addressed the occurrence of neurovascular damage in patients with diabetes without clinically visible lesions. With the advance of OCTA, the reduction of VD in NDR patients has been found in previous studies.[8 18 19] Altered VD may be due to the disruption of retinal neurovascular autoregulation, which could dynamically regulate blood flow in response to metabolic demands.[20] Besides, endothelial cell injury is a central pathogenic response to chronic hyperglycaemia and initiates the progressive ischaemic characteristic of DR.[5] The retinal capillary becomes acellular once the dropout of the endothelial cells begins, which may lead to reduced VD in the retina.

With regard to neural structures, peripapillary RNFL thickness was significantly reduced in patients with preclinical DR compared with healthy controls.[21] Vujosevic and colleagues found that the average peripapillary RNFL thickness was significantly decreased in patients with DR, while only the inferior quadrant of peripapillary region in NDR group was significantly decreased in comparison to the controls.[22] Interestingly, previous study found the macular GCC thickness was significantly thinner in NDR patients.[23] However, Demir *et al* found that there was a non-significant loss of RNFL and GCC in patients with T2DM with or without DR.[24] The change of the GCC thickness was

**Table 5** Pearson's correlation coefficients of structural parameter and ERG parameters

| | 16 Tds flicker ERG | | 32 Tds flicker ERG | |
|---|---|---|---|---|
| Parameters | Implicit time | Amplitude | Implicit time | Amplitude |
| VD of SCP (parafovea) | **(−0.470, <0.001)*** | (0.085, 0.448) | **(−0.525, <0.001)*** | (0.145, 0.247) |
| VD of SCP (perifovea) | **(−0.438, <0.001)*** | (0.185, 0.137) | **(−0.503, <0.001)*** | (0.226, 0.068) |
| VD of DCP (parafovea) | (−0.142, 0.257) | (0.061, 0.629) | (−0.157, 0.208) | (0.098, 0.432) |
| VD of DCP (perifovea) | **(−0.348, 0.004)** | (0.119, 0.340) | **(−0.395, 0.001)*** | (0.156, 0.212) |
| FD300 | **(−0.341, 0.005)** | (0.090, 0.475) | **(−0.357, 0.003)** | (0.128, 0.307) |
| GCC | (−0.243, 0.049) | (0.009, 0.944) | (−0.241,<0.052) | (0.019, 0.878) |
| RNFL thickness | (−0.154, 0.217) | (0.177, 0.154) | (−0.232, <0.061) | (0.136, 0.275) |

Pearson's correlation coefficients and the original p values (in brackets).
*Statistically significant after Bonferroni correction.
DCP, deep capillary plexus; ERG, electroretinography; FD300, foveal density in a 300 µm wide region around foveal avascular zone; GCC, ganglion cell complex; RNFL, retinal nerve fibre layer; SCP, superficial capillary plexus; VD, vessel density.

not concomitant with that of RNFL thickness in NDR patients in our cohort, and previous study also found that the changes in GCC and RNFL thickness were not correlated.[25] The exact mechanisms for reduced GCC thickness were not clear. The possible causes for the neurodegenerative changes in DR may include increased apoptosis, glial cell reactivity and altered glutamate metabolism.[26] Previous study found that perifoveal capillary loss in the SCP was associated with reduced GCC thickness in NDR patients with T1DM and associated with lower RNFL thickness in NDR patients with T2DM.[19] The inner retina has higher metabolic demands and relatively lower perfusion, which makes it more vulnerable to the metabolic stress induced by diabetes.[27] The study found that retinal ganglion cells and amacrine cells may be the first neurons in which diabetes-induced apoptosis is detected.[5] However, in the current study, the GCC thickness was comparable between NDR patients and healthy controls, which indicates that the GCC in the recruited diabetic population is relatively intact and it might not be the cause of functional impairments. Nevertheless, more evidence is needed to clarify the changes in neurovascular structures.

The results of flicker ERG in our study suggested the early onset of functional impairment in patients with diabetes without retinopathy. ERG parameters are considered as sensitive markers of early neuronal abnormalities.[28] Previous study used ERG to predict the probable relationships between neural dysfunction and vascular abnormalities.[29 30] The flicker response under 28.3 Hz light stimuli is produced by the ON and OFF pathway activity and postreceptoral ON and OFF components contribute substantially to the sine-wave flicker ERG at higher stimulus frequencies.[31] In other words, the cone pathway testing by flicker ERG mainly reflects the function of GCC. As we mentioned above, the GCC is supplied by the capillary distributing from ILM to OPL, which could be detected by OCTA. The VD detected by OCTA perfectly reveals the perfusion of the postreceptoral ON and OFF components. The cone pathway function relies on the integrity of both neural and vascular structures. In our study, the GCC thickness in NDR patients was not statistically different from the controls, while the VD has already shown signs of damage. Moreover, in the multivariable linear regression model, only the VD of SCP in parafovea was correlated with the delayed implicit time, and the thicknesses of GCC and peripapillary RNFL were not correlated with the functional changes. Soliman *et al* found that the parafovea cone cell density in patients with diabetes without DR was comparable with healthy controls.[32] Taken together, we presumed that the functional changes detected by flicker ERG in NDR patients might result from the impaired macular blood supply.

Previous study found that cone-mediated 30 Hz flicker and cone-isolated oscillatory potentials were not correlated with blood glucose or glycated haemoglobin level.[33 34] However, our study showed that a higher level of HbA1c was correlated with delayed implicit time, and longer diabetes duration was associated with decreased macular VD. High level of HbA1c is a good indicator for poor blood glucose control in around 3 months.[35] Long-term hyperglycaemia contributes to hypoxia and induces inflammation in the retina.[36 37] These factors may contribute to the impairments of structures and neural function in the diabetic retina. Thus, good control of blood glucose is crucial to prevent and delay the progression of DR.

There are several limitations in the study. First, the OCTA examination can only quantify the VD and GCC thickness in the posterior pole, while the cells of cone pathway are distributed throughout the whole retina. Although a large part of the cone pathway locates in the macular, the structural changes in the peripheral retina are unknown. The tested area in optic disc was also limited to a small region. Second, this is a cross-sectional study, further studies are needed to investigate the longitudinal development of neural function and structural impairments.

In conclusion, functional and structural impairments might precede the presence of visible retinal lesions in preclinical DR. In accordance with the delayed implicit time and decreased amplitude, the macular microcirculation in patients with diabetes without DR has shown signs of damage. Poor control of blood glucose, indicated by high HbA1c level, is associated with delayed implicit time of flicker ERG in NDR eyes.

**Contributors** LZ, YZ and DC: conception and design. YZ, HY, JL, JY, DY, XZ, QW and BL: data collection, analysis and/or interpretation. YZ and DC: drafting the article. LZ and YZ: final approval of the version to be published. All authors revised the article critically for important intellectual content.All authors read and approved the final manuscript.

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