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Measuring the foveal avascular zone in diabetes: A study using optical coherence tomography angiography

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ABSTRACT

Aims/Introduction: Diabetes is a global issue that currently affects 425 million people worldwide. One observable microvascular complication of this condition is a change in the foveal avascular zone (FAZ). In this study, we used optical coherence tomography angiography to investigate the effect of diabetes on the FAZ.

Materials and Methods: A total of 11 participants with diabetes and 11 participants without diabetes took part in this study. Participants in both groups were matched for age ($P = 0.217$) and sex ($P = 0.338$), and had no history of ocular disease. Macular optical coherence tomography angiography (OCT-A) scans of participants' right and left eyes were taken. Glycosylated hemoglobin (HbA_{1c}) and blood glucose levels were also measured. The FAZ area was manually segmented at the levels of the superficial capillary plexus (FAZ_{SCP}) and deep capillary plexus (FAZ_{DCP}).

Results: There was a strong relationship between the FAZ area of participants' right and left eyes ($P \leq 0.001$) in both diabetes and non-diabetes groups. In the diabetes group, the FAZ_{SCP} ($P = 0.047$) and FAZ_{DCP} ($P = 0.011$) areas was significantly larger than in the non-diabetes group. Moreover, multiple linear regression analysis predicted a 0.07-mm^2 increase in the FAZ_{SCP} and FAZ_{DCP} areas of individuals with diabetes for every 1% increase in their HbA_{1c} level.

Conclusions: Our findings show that there is enlargement of the FAZ in individuals with diabetes compared with individuals without diabetes. In the diabetes group, this enlargement appears to be correlated with HbA_{1c} level. OCT-A imaging could, therefore, be a useful tool to monitor the FAZ and identify potential early microvasculopathy in diabetes.

INTRODUCTION

Diabetes affects an estimated 425 million people worldwide. In developed countries, diabetic retinopathy (DR) is the leading cause of preventable vision loss in working-age adults aged between 20 and 65 years[1]. Indeed, the presence of DR is approximately 35% in the diabetes population[2]. Consequently, DR has a considerable economic impact[3].

The retina has high metabolic and oxygen demands; therefore, it is vulnerable to sight-threatening, microvascular complications of diabetes[4]. The central area of the macula is peculiarly susceptible in diabetes because of the foveal avascular zone (FAZ). This region of the human retina, which has the highest cone photoreceptor cell density and provides high-resolution visual acuity, is completely devoid of retinal capillaries[5]. The neurons within the FAZ, like the photoreceptor layer elsewhere in the retina, rely on the blood supply from the choriocapillaris: the superficial capillary plexus (SCP) supplies the retinal nerve fiber layer and ganglion cell layer, whereas the inner retina and outer plexiform layer are supplied by the deep capillary plexus (DCP).

The normal FAZ in a well-developed human fovea is circular when viewed *en face*[6,7]. The anatomy of the FAZ has been found to vary with age, race, and sex. Previous research has shown that there is a difference between the FAZ in diabetic and healthy eyes[6,8,9]. Furthermore, a recent review found that the diameter of the FAZ in diabetic eyes differs between the capillary plexuses[10].

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Glycosylated hemoglobin (HbA_{1c}) is the gold-standard method used to assess long-term glycemic control. Glucose is added to the hemoglobin molecules irreversibly by enzymically catalyzed glycosylation at a rate that is proportional to glucose concentration in the blood. HbA_{1c} is broken down when these erythrocytes, which have a 3-month lifespan, are destroyed in the liver and spleen. The proportion of hemoglobin that is glycosylated thus serves as a measure of plasma glucose in the preceding 3-month period. The reference range for HbA_{1c} in an adult without diabetes is 4.0–5.9%[11], with a value >6.5% being diagnostic of diabetes[12].

Measuring the blood glucose level permits assessment of instantaneous glycemic control in real time. On application of the blood sample to the glucose testing strip, the glucose oxidase enzyme present on the strip interacts with glucose in the sample, taking an electron and forming gluconic acid. The enzyme then passes the electron to water and oxygen, regenerating the enzyme and forming hydrogen peroxide. On glucose testing strips, a mediator replaces oxygen, and this mediator accepts the electron and passes it to an electrode to generate the current that is reported as the glucose concentration[13]. The reference range for blood glucose level in adults is 74–106 mg/dL[11], with a value below this range indicating hypoglycemia, and a value above this range indicating hyperglycemia. Participants' glucose levels were recorded with a view to ensuring that it was safe for them to proceed with the study.

Fundus fluorescein angiography (FFA) has been recognized as an important functional imaging technique that provides two-dimensional images of the retina, and permits assessment of blood circulation and vessel integrity. Some diabetic features can be better assessed with FFA than fundus photography. Indeed, FFA can detect primary vascular lesions, such as microaneurysms and intraretinal microvascular abnormalities, and this imaging technique can also identify areas of non-perfusion and neovascularization. However, FFA is limited when studying microvascular histopathological processes: dye-based angiography does not permit separate evaluation of the SCP and DCP, because the capillary plexuses are overlapped when viewed in two dimensions[14]. In addition, the procedure is time consuming and involves the intravenous administration of fluorescein dye with its attendant risks[15].

The advent of optical coherence tomography angiography (OCT-A) has revolutionized ophthalmic clinical practice. This technology produces high-contrast images of where cells are moving, with sufficiently high resolution to show the locations of individual capillaries in the retina. It differs from FFA, which shows the lumina of retinal blood vessels by making plasma fluorescent, and which shows sites of breakdown of the inner and outer blood–retinal barriers[16]. OCT-A is able to differentiate between the SCP and DCP; therefore, it is possible to show how each plexus is affected in retinal vascular disease. OCT-A is particularly sensitive at detecting early DR[17,18], insofar as capillary dropout and early retinal neovascularization are well delineated by this method[19]. Indeed, microvascular abnormalities and areas of capillary non-perfusion can also be detected[20].

The purpose of the present study was to use OCT-A to measure the FAZ area of individuals with diabetes and compare it with that of healthy individuals without diabetes. As HbA_{1c} level is a marker of progression of diabetes, we also aimed to correlate FAZ with participants' HbA_{1c} level.

MATERIALS AND METHODS

Participants

A total of 22 participants took part in this study, and data were collected from both eyes. All participants were matriculated students at Glasgow Caledonian University or patients who attended the on-campus Vision Centre. Participants were chosen irrespective of their ethnicity and the type of diabetes with which they had been diagnosed. Appointments were arranged to avoid times of day at which participants with diabetes were at a higher risk of becoming hypoglycemic.

In the diabetes group ($n = 11$), the mean age (±standard deviation) was 37 ± 17 years, the median age (±interquartile range) was 30 ± 37 years and the male-to-female ratio was 2:9. In the diabetes group, there were six individuals with type 1 diabetes and five individuals with type 2 diabetes. In the non-diabetes group ($n = 11$), the mean age was 30 ± 14 years, the median age was 23 ± 21 years and the male-to-female ratio was 4:7. These two groups were sufficiently matched for age ($U = 42$, $z = -1.253$, $P = 0.217$) and sex (χ^2 [1, $n = 22$] = 0.917, $P = 0.338$) to permit comparison of the diabetes and non-diabetes groups.

The mean HbA_{1c} level (±standard deviation) was 7.8 ± 1.5% in the diabetes group, and the range was from 5.7% to 10.8%. In the non-diabetes group, the mean HbA_{1c} level was 5.1 ± 0.5%, and the range was 4.2–5.8%. HbA_{1c} was normally distributed in both groups (diabetes group: $W[11] = 0.948$, $P = 0.615$; non-diabetes group: $W[11] = 0.922$, $P = 0.336$), and there were no outliers. This between-group difference was statistically significant ($t[13] = 8.853$, $P < 0.0005$); as the variances were unequal ($F = 12.805$, $P = 0.001$), the degrees of freedom were adjusted from 20 to 13.

Inclusion and exclusion criteria

All participants had a best-corrected visual acuity of 0.3 logMAR or better in each eye, as measured using a logMAR chart (Thomson Test Chart; Thomson Software Solutions, Hatfield, UK), and the interocular difference in visual acuity was no greater than one line (0.1 logMAR). Participants in the diabetes group had type 1 or 2 diabetes mellitus, as diagnosed by a diabetologist. Furthermore, all participants with diabetes reported no previous diagnosis of DR or diabetic maculopathy, and participants whose fundus photographs showed such diabetic microvasculopathy were excluded. Participants with any concurrent ocular disease – for example, cataract, age-related macular degeneration or glaucoma – were also excluded from the study.

HbA_{1c}

Participants' HbA_{1c} levels were measured using the A_{1c}Now®+ System (PTS Diagnostics, Indianapolis, IN, USA), which used the principle of colorimetry. HbA_1 level was measured immediately prior to OCT-A imaging. A single 5-μL capillary blood sample was obtained using a single-use lancet. Test results were expressed as the percentage of total hemoglobin that was glycosylated in the sample. The method by which the A_{1c}Now®+ System assesses HbA_{1c} level has been described previously[21,22].

Blood glucose

Participants' instantaneous blood glucose level was measured using a glucometer (FreeStyle Freedom Lite®; Abbott Diabetes Care Inc., Alameda, CA, USA), with a view to ensuring that it was safe for them to proceed with the study. The device was approved for individuals with diabetes to self-test at home. A 0.3-μL capillary blood sample was taken at the same time as obtaining the capillary blood sample that was used for HbA_{1c} analysis. Results, in mg/dL, were made available to the investigator 5 s after collection of the sample.

OCT-A

The scanning protocol comprised a 4.5 × 4.5-mm macular OCT-A scan (DRI OCT Triton™; Topcon, Tokyo, Japan) of participants' right and left eyes. This instrument had a scanning speed of 100,000 A-scans/s and used a wavelength-sweeping laser with a central wavelength of 1,050 nm. An in-built eye-tracking system was applied during image acquisition (SMART-Track™; Topcon), and proprietary ratio analysis software (OCTARA™; Topcon) was used for angiographic processing. *En face* images were generated of the SCP and DCP (Figure 1), based on automated layer segmentation carried out by the in-built digital software (IMAGEnet6®; Topcon Medical Systems, Oakland, NJ, USA).

These *en face* macular images were then exported to an image processing and analysis software package (ImageJ; National Institutes of Health, Bethesda, MD, USA). In essence, each file was converted to an 8-bit image, and a scale was set such that 320 pixels represented 4.5 mm. The Phansalkar method, with a 15-pixel radius, was used for binarization, as previously reported[23]. The FAZ was manually outlined using

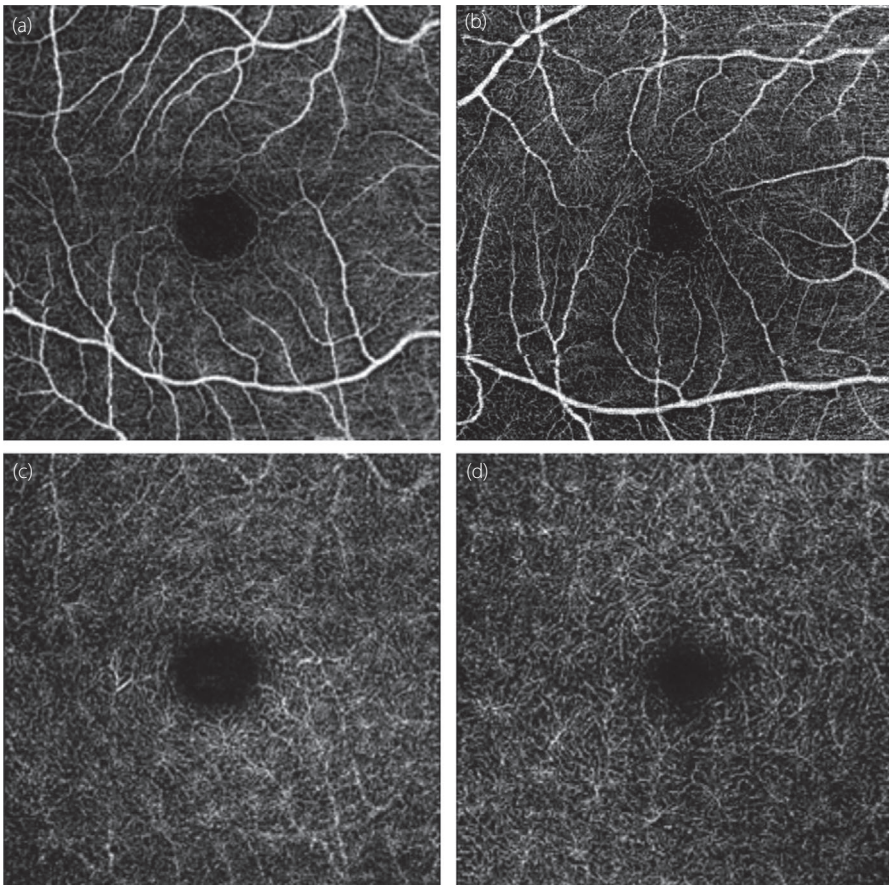

Figure 1 | Right *en face* macular images of the foveal avascular zone of a typical participant with diabetes and a typical participant without diabetes (pre-processed). (a) Superficial capillary plexus of a participant with diabetes. (b) Superficial capillary plexus of a participant without diabetes. (c) Deep capillary plexus of a participant with diabetes. (d) Deep capillary plexus of a participant without diabetes.

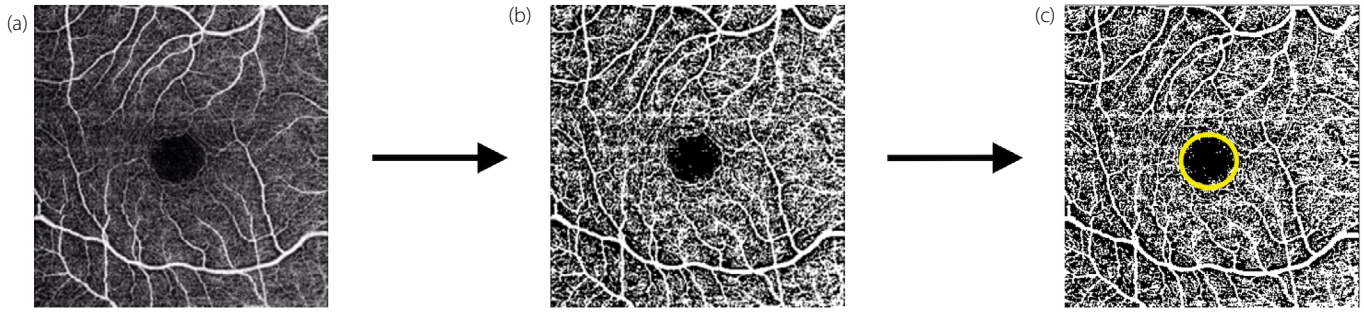

Figure 2 | Image processing and analysis of foveal avascular zone (FAZ) area, for each *en face* macular image. (a) Pre-processed FAZ image; (b) *en face* FAZ image after Phansalkar binarization; (c) best-fit oval (in yellow) of the FAZ area after binarization and manual outlining using the polygon tool.

the polygon tool, and a best-fit oval was generated. FAZ area, in mm^2, was then automatically calculated by the software at either the level of the SCP (FAZ_{SCP} area) or that of the DCP (FAZ_{DCP} area), depending on the particular image that was being processed (Figure 2).

Statistical analysis

Statistical analyses were carried out using SPSS Statistics 26 (IBM Corp., Armonk, NY, USA). Intraclass correlation analysis was used to assess the strength of any interocular relationship that might have existed between the FAZ area of the right eye and that of the left eye. Two-way mixed analysis of variance (ANOVA) was then used to assess for a difference in the FAZ area, measured in the two distinct capillary plexuses, between the diabetes and non-diabetes groups. A simultaneous method of multiple linear regression was used to examine the effects of HbA_{1c} level, age, and sex on FAZ area in the diabetes and non-diabetes participants. For all statistical tests, normality of distribution was assessed using Shapiro–Wilk *W*-tests, and outliers were assessed by inspection of boxplots for values >1.5-fold the interquartile range. Furthermore, all parametric assumptions were met, and the alpha-level (α) was set at 0.05.

RESULTS

Interocular relationship

Intraclass correlation analysis showed that there was a strong, statistically significant relationship between the FAZ area of participants' right and left eyes (Table 1). This was the case for measures obtained at the levels of the SCP and DCP in both groups. A two-way random effects model with single measures and absolute agreement was used (intraclass correlation [A,1])[24]. In accordance with the statistical guidelines for data obtained from two eyes[25,26], because there was strong interocular concordance for FAZ area, we used the mean value of the right and left eyes for each participant. In other words, subsequent analysis considered each participant to have two values: (i) the mean FAZ area of right and left eyes, measured at the level of the SCP (FAZ_{SCP} area); and (ii) the mean FAZ area of right and left eyes, measured at the level of the DCP (FAZ_{DCP} area).

Table 1 | Intraclass correlation analysis of the foveal avascular zone (FAZ) area between participants' right and left eyes

	Diabetes group	Non-diabetes group
FAZ_{SCP} area	$\rho = 0.901$***	$\rho = 0.957$***
FAZ_{DCP} area	$\rho = 0.840$***	$\rho = 0.908$***

The intraclass correlation coefficient is denoted by ρ. FAZ_{SCP}, foveal avascular zone area manually segmented at the level of the superficial capillary plexus; FAZ_{DCP}, foveal avascular zone area manually segmented at the level of the deep capillary plexus. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$.

Effect of diabetes on FAZ area

A two-way mixed ANOVA was run to examine the effect of diabetes on the FAZ area. The within-subjects' factor was capillary plexus (FAZ_{SCP} area or FAZ_{DCP} area), and the between-subjects' factor was group (diabetes or non-diabetes). Both groups were matched for age ($U = 42$, $z = -1.253$, $P = 0.217$) and sex (χ^2 [1, $n = 22$] = 0.917, $P = 0.338$). Measures of FAZ_{SCP} area were normally distributed in both groups (diabetes group: W [11] = 0.941, $P = 0.532$; non-diabetes group: W[11] = 0.980, $P = 0.966$). Likewise, measures of FAZ_{DCP} area were normally distributed (diabetes group: W[11] = 0.960, $P = 0.768$; non-diabetes group: W[11] = 0.936, $P = 0.479$). For measures of both FAZ_{SCP} area and FAZ_{DCP} area, there were no outliers present in the diabetes and non-diabetes groups.

There was a statistically significant two-way interaction between capillary plexus and group (F[1,20] = 5.392, $P = 0.031$, $\eta_p^2 = 0.212$). The main effect of capillary plexus was statistically significant in both the diabetes (F[1,10] = 35.026, $P < 0.0005$, $\eta_p^2 = 0.778$) and non-diabetes groups (F[1,10] = 44.893, $P < 0.0005$, $\eta_p^2 = 0.818$). There was a statistically significant difference in FAZ_{SCP} area between the diabetes and non-diabetes groups (F[1,20] = 4.478, $P = 0.047$, $\eta_p^2 = 0.183$). The mean (±standard error of the mean [SEM]) FAZ_{SCP} area was 0.34 ± 0.03 mm^2 in the diabetes group and 0.25 ± 0.03 mm^2 in the non-diabetes group. Similar statistical significance was

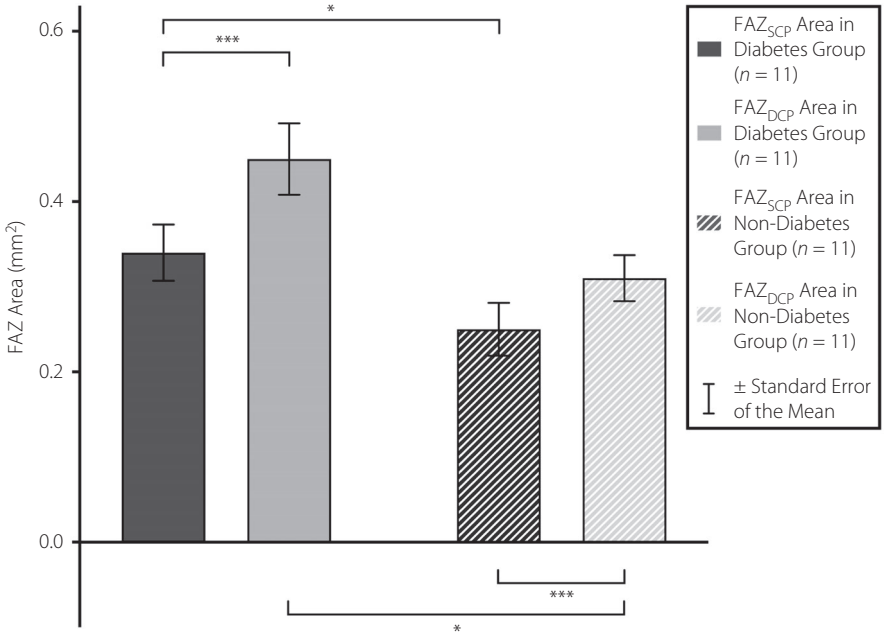

Figure 3 | Foveal avascular zone area manually segmented at the levels of the superficial capillary plexus in diabetes (dark gray solid) and non-diabetes groups (dark gray striped), and foveal avascular zone area manually segmented at the levels of the deep capillary plexus in diabetes (light gray solid) and non-diabetes groups (light gray solid). $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

found in the FAZ_{DCP} area between groups ($F[1,20] = 7.920$, $P = 0.011$, $\eta_p^2 = 0.284$). The mean (±SEM) FAZ_{DCP} area was 0.45 ± 0.04 mm^2 in the diabetes group and 0.31 ± 0.04 mm^2 in the non-diabetes group (Figure 3).

Effect of HbA_{1c} level on FAZ area

Multiple linear regression analysis was used to assess the effects of HbA_{1c} level, sex, and age on FAZ area. In all four of the models described below, a simultaneous method of multiple regression was used.

The first model examined the effect of HbA_{1c} level, sex and age on the FAZ_{SCP} area of participants with diabetes (Table 2), and the second model examined the effect of HbA_{1c} level, sex and age on the FAZ_{SCP} area of participants without diabetes (Table 3). In the diabetes group, HbA_{1c} level significantly predicted FAZ_{SCP} area ($\beta = 0.949$, $t[7] = 2.828$, $P = 0.025$): there was a 0.07-mm^2 increase in FAZ_{SCP} area for every 1% increase in HbA_{1c} level (Figure 4). There was no significant relationship between HbA_{1c} level and FAZ_{SCP} area in the non-diabetes group ($\beta = 0.192$, $t[7] = 0.598$, $P = 0.569$). Participants' sex differed between groups: in the diabetes group, male participants had a larger FAZ_{SCP} area compared with female participants ($\beta = 0.152$, $t[7] = 1.282$, $P = 0.545$), whereas the opposite was found in our non-diabetes group ($\beta = -0.502$, $t[7] = -1.573$, $P = 0.169$). In the diabetes group, male participants had, on average, a FAZ_{SCP} area that was 0.04 mm^2 larger than that of

Table 2 | Multiple linear regression analysis of foveal avascular zone area manually segmented at the level of the superficial capillary plexus in participants with diabetes

FAZ_{SCP} area	*B*	95% Confidence interval		β	*t*	R^2	ΔR^2
Diabetes group		Lower limit	Upper limit				
Model						0.544	0.349
Constant	−0.330	−0.912	0.252		−1.340		
HbA_{1c}	0.069*	0.011	0.126	0.949**	2.828		
Age	0.003	−0.001	0.008	0.530	1.695		
Sex (male−female)	0.041	−0.137	0.219	0.152	0.545		

The unstandardized regression coefficient is denoted by *B*; the standardized coefficient is denoted by β; the standardized coefficient divided by its standard error is denoted by *t*; the coefficient of determination is denoted by R^2; the adjusted coefficient of determination is denoted by ΔR^2. FAZ_{SCP}, foveal avascular zone area manually segmented at the level of the superficial capillary plexus; HbA_{1c}, glycosylated hemoglobin. $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

Table 3 | Multiple linear regression analysis of the foveal avascular zone area manually segmented at the level of the superficial capillary plexus in participants without diabetes

FAZ_{SCP} area	*B*	95% Confidence Interval		β	*t*	R^2	ΔR^2
Non-diabetes group		Lower limit	Upper limit				
Model						0.289	−0.016
Constant	0.115	−0.567	0.796		0.398		
HbA_{1c}	0.034	−0.099	0.167	0.192	0.598		
Age	0.000	−0.005	0.005	−0.025	−0.078		
Sex (M−F)	−0.095	−0.237	0.048	−0.502	−1.573		

The unstandardized regression coefficient is denoted by *B*; the standardized coefficient is denoted by β; the standardized coefficient divided by its standard error (SE) is denoted by *t*; the co-efficient of determination is denoted by R^2; the adjusted coefficient of determination is denoted by ΔR^2. FAZ_{SCP}, foveal avascular zone area manually segmented at the level of the superficial capillary plexus; HbA_{1c}, glycosylated hemoglobin. $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

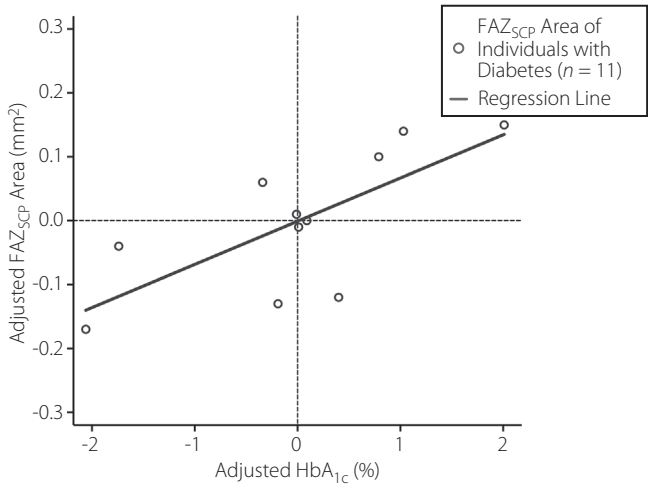

Figure 4 | Partial regression plot of the foveal avascular zone area manually segmented at the level of the superficial capillary plexus against glycosylated hemoglobin in participants with diabetes, when controlling for participants' age and sex.

their female counterparts; in the non-diabetes group, male participants' FAZ_{SCP} area was 0.10 mm^2 *smaller* than that of their female counterparts. It is important to note that these sex differences did not reach statistical significance in either group. Furthermore, there was no relationship between participants' age and FAZ_{SCP} area in either group (diabetes group: $\beta = 0.530$, $t[7] = 1.695$, $P = 0.134$; non-diabetes group: $\beta = -0.025$, $t[7] = -0.078$, $P = 0.940$).

The third multiple linear regression model that was run examined the effects of HbA_{1c} level, sex and age on the FAZ_{DCP} area of participants with diabetes (Table 4), and the fourth model examined the effects of HbA_{1c} level, sex and age on the FAZ_{DCP} area of participants without diabetes (Table 5). In the diabetes group, the model predicted a 0.07-mm^2 increase in FAZ_{DCP} area for every 1% increase in HbA_{1c} level (Figure 5); however, this relationship did not reach statistical significance ($\beta = 0.757$, $t[7] = 1.885$, $P = 0.101$). There was no relationship between FAZ_{DCP} in the non-diabetes group ($\beta = 0.198$, $t[7] = 0.622$, $P = 0.545$). Likewise, FAZ_{DCP} was not significantly associated with participants' age in either group (diabetes group: $\beta = 0.502$, $t[7] = 1.342$, $P = 0.221$; non-diabetes group: $\beta = -0.045$, $t[7] = -0.142$, $P = 0.891$). The effect of sex showed similar findings to those of the FAZ_{SCP} area regression models, but it is of note that these differences did not reach statistical significance in either group: in the diabetes group ($\beta = 0.309$, $t[21] = 0.928$, $P = 0.384$), male participants had, on average, a FAZ_{DCP} area that was 0.11 mm^2 larger than that of their female counterparts, whereas in the non-diabetes group ($\beta = -0.507$, $t[23] = -1.601$, $P = 0.153$), male participants had, on average, a FAZ_{DCP} area that was 0.09 mm^2 smaller than that of female participants.

DISCUSSION

The purpose of this study was to measure the FAZ area in individuals with and without diabetes using OCT-A, and relate this measure to participants' HbA_{1c} level. Our group analysis found that individuals with diabetes had significantly larger FAZ_{SCP} and FAZ_{DCP} areas compared with age- and sex-matched individuals without diabetes. Moreover, our study revealed a positive correlation between HbA_{1c} level and FAZ area and in individuals with diabetes: for every 1% increase in HbA_{1c} level, there was a 0.07-mm^2 increase in both FAZ_{SCP} and FAZ_{DCP} areas. This relationship was not present in our cohort without diabetes.

Enlargement of the FAZ is associated with DR, and this finding has been well documented using FFA[6,27,28]. The pathological mechanisms that underlie this enlargement in diabetes are multifactorial: previous evidence has found that capillary closure/dropout[6], dysfunction of the capillary endothelium[29], and an increased level of vascular endothelial growth factor[30] might be involved in the pathogenesis. Although many FFA studies have found that diabetic eyes with established DR have an increased FAZ area compared with non-diabetic eyes, only a

Table 4 | Multiple linear regression analysis of the foveal avascular zone area manually segmented at the level of the deep capillary plexus in participants with diabetes

FAZ_{DCP} area	*B*	95% Confidence interval		β	*t*	R^2	ΔR^2
Diabetes group		Lower limit	Upper limit				
Model						0.347	0.067
Constant	−0.268	−1.157	0.521		−0.713		
HbA_{1c}	0.070	−0.018	0.158	0.757	1.885		
Age	0.004	−0.003	0.012	0.502	1.342		
Sex (M–F)	0.107	−0.165	0.379	0.309	0.928		

The unstandardized regression coefficient is denoted by *B*; the standardized coefficient is denoted by β; the standardized coefficient divided by its standard error is denoted by *t*; the coefficient of determination is denoted by R^2; the adjusted coefficient of determination is denoted by ΔR^2. FAZ_{DCP}, foveal avascular zone area manually segmented at the level of the deep capillary plexus; HbA_{1c}, glycosylated hemoglobin. $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

Table 5 | Multiple linear regression analysis of the foveal avascular zone area manually segmented at the level of the deep capillary plexus in participants without diabetes

FAZ_{DCP} area	*B*	95% Confidence interval		β	*t*	R^2	ΔR^2
Non-diabetes group		Lower limit	Upper Limit				
Model						0.297	−0.004
Constant	0.184	−0.451	0.819		0.684		
HbA_{1c}	0.033	−0.091	0.157	0.198	0.622		
Age	0.000	−0.005	0.004	−0.045	−0.142		
Sex (M − F)	−0.090	−0.223	0.043	−0.507	−1.601		

The unstandardized regression coefficient is denoted by *B*; the standardized coefficient is denoted by β; the standardized coefficient divided by its standard error is denoted by *t*; the coefficient of determination is denoted by R^2; the adjusted coefficient of determination is denoted by ΔR^2. FAZ_{DCP}, foveal avascular zone area manually segmented at the level of the deep capillary plexus; HbA_{1c}, glycosylated hemoglobin. $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

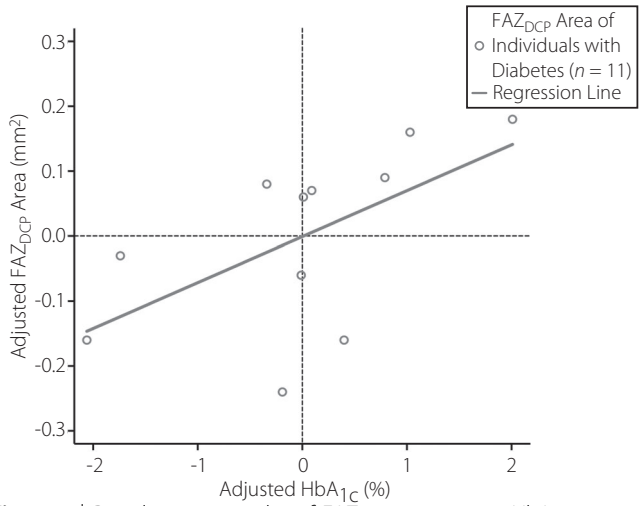

Figure 5 | Partial regression plot of FAZ_{DCP} area against HbA_{1c} in diabetic participants when controlling for participants' age and sex.

few have included patients with diabetes whose eyes have no or background DR[28,31]. One possibility for this selection bias is that such individuals rarely undergo FFA because of the attendant risks of the procedure. Recent OCT-A studies have also found an enlargement of the FAZ in diabetes[9,32]. The diabetes groups in these studies comprised eyes that had varying degrees of DR. Di *et al*[32]. found that the FAZ_{SCP} area in diabetic eyes was larger than that in non-diabetic eyes. A similar study used OCT-A to measure the largest diameter of the FAZ in diabetic eyes with DR and in healthy, non-diabetic eyes[9]; the authors found that the FAZ_{SCP} diameter in diabetic eyes was significantly larger than that in non-diabetic eyes ($P = 0.029$), with a more pronounced between-group difference being observed in the FAZ_{DCP} diameter ($P = 0.001$). The OCT-A instrument used in the present study (DRI OCT Triton™; Topcon) permitted assessment of the FAZ at the levels of the SCP and DCP, and the sample sizes of our diabetes and non-diabetes groups were equal.

Although there is no accepted method to assess the FAZ, we used manual segmentation because of its high repeatability and lower measurement error. The FAZ in all OCT-A images was manually outlined by a single clinician, with a view to eliminating interobserver variability. Furthermore, all images were graded in a randomized order at two points in time, to account for intra-observer variability; the mean value of the two FAZ

areas was used. Other commercially available OCT-A instruments – for example, RTVue XR (Avanti; Optovue Inc., Fremont, CA, USA) and Cirrus 5000 HD-OCT (Carl Zeiss Meditec, Inc., Dublin, CA, USA) – are semi-automated and use in-built algorithms. Recent evidence has found that these semi-automated instruments tend to overestimate the FAZ area compared with manual segmentation[33].

The size of FAZ is multifactorial and, even in healthy individuals, there is considerable variation in its size. This can pose a challenge when assessing, and making clinical evaluations on, possible pathological enlargement of the FAZ in retinal disease. The FAZ_{SCP} area is smaller than that of the FAZ_{DCP}; this difference is likely a result of the anatomical differences in the retinal vasculature between the two capillary plexuses. A recent study of FAZ size in a healthy cohort without diabetes found that the mean (±SEM) FAZ_{SCP} area was 0.24 ± 0.08 mm^2 and the mean FAZ_{DCP} area was 0.38 ± 0.12 mm^2 [30]. In another study of healthy individuals, the FAZ_{DCP} area was, on average, 0.08 mm^2 larger than the FAZ_{SCP} area[34]. The findings of Tan *et al.*[34] and Ghassemi *et al.*[35] mirror those of the present study: in our non-diabetes group, the mean (±SEM) FAZ_{SCP} area was 0.25 ± 0.03 mm^2 and the mean FAZ_{DCP} area was 0.31 ± 0.04 mm^2, with the difference in FAZ between the two capillary plexuses being statistically significant.

We recognize that the present study had some limitations. As the intraclass correlation coefficients between right and left FAZ measures showed a significant interocular relationship, the mean value of FAZ area from both eyes was used[35]. OCT-A studies of healthy eyes have found that interocular measures of FAZ area are similar[25,26]. As this was an explorative study with a relatively small sample size, participants with diabetes were recruited irrespective of the type of diabetes with which they had been diagnosed and irrespective of the duration since their diagnosis. The effect of duration of diabetes on the FAZ remains unclear: an FFA study found that the size of the FAZ increased with stage of DR[36], whereas a more recent OCT-A study with a similar sample size found that there was no correlation between FAZ size and duration of diabetes[31]. In order to establish the relationship between FAZ area and HbA_{1c} level, a larger sample size would be required; this would not only increase the statistical power, but it would also allow for the results to be generalized to the diabetes population. The field of view of the OCT-A images captured in the present study was 4.5×4.5 mm (approximately 15°), whereas the field of view using FFA is typically ≥30°. It is this difference in field of view that has slowed down the clinical acceptance of OCT-A. Commercially available OCT-A instruments can now produce individual 12×12-mm scans; moreover, these scans can be composited using image montage software to produce images with a field of view in the region of 90°[32].

The potential for clinical application of the present findings will depend on further longitudinal studies with serial measurement of HbA_{1c} level and FAZ area in individuals with diabetes. These additional studies would allow us to establish whether poor glycemic control leads to an increase in the FAZ area, and they would also allow us to understand the effect that disease duration might have on the FAZ area. Additional research would be required to establish whether tight glycemic control could arrest or reverse FAZ enlargement. Adaptive optics methods, which have a higher resolution than OCT-A, offer the potential for better understanding of the pathological mechanisms that are involved in FAZ changes in diabetes[37].

Although FFA is still considered to be the gold standard in imaging of the retinal vasculature, OCT-A is an evolving technology that can be carried out quickly and non-invasively alongside OCT and digital retinal imaging. As the prevalence of diabetes is projected to rise due to an aging population, early detection of the disease will play a pivotal role, enabling clinicians to better manage and monitor their patients. OCT-A imaging could, therefore, be a useful tool to monitor the FAZ and one that has the potential to identify early microvasculopathy in individuals with diabetes.

DISCLOSURE

The authors declare no conflict of interest.

Approval of the research protocol: The protocol for this research project has been approved by a suitably constituted Ethics Committee of the institution (Ethics Committee of the School of Health and Life Sciences, Glasgow Caledonian University, UK; Approval No. HLS/LS/A15/030), and the project conforms to the provisions of the Declaration of Helsinki (as revised in Fortaleza, Brazil, October 2013).

Informed consent: Informed consent was obtained from the participants for their inclusion in this research project.

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Animal studies: N/A.

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