Investigation of Factors Associated with Retinal Oxidative Stress and Inflammation that affect the Foveal Avascular Zone in Healthy Eyes: An Optical Coherence Tomography Angiography Study

Susan M. O'Shea (C06466176@mytudublin.ie)
Technological University Dublin
Veronica M. O'Dwyer
Technological University Dublin
John S. Butler
Technological University Dublin
Grainne Scanlon
Technological University Dublin

Research Article

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Abstract

Purpose

The size and shape of the foveal avascular zone (FAZ) can change due to retinal diseases associated with oxidative stress and inflammation, such as diabetic retinopathy, glaucoma, hypertensive retinopathy and macular degeneration. Macular pigment (MP), a powerful retinal antioxidant, may confer protection. This study aims to assess the relationship, if any, between factors that may affect the superficial FAZ (*i.e.* blood pressure (BP), vessel density, perfusion, overweight/obesity), and possible links with MP optical density (MPOD), in young, healthy subjects.

Methods

One hundred and fifty-four subjects, aged 18 to 35 years, were recruited. The superficial FAZ area, foveal vascularity and central macular thickness (CMT) were assessed using the Cirrus 5000. Health parameters including, BP, body mass index (BMI), trunk fat % and MPOD were analyzed, to determine possible associations with the FAZ.

Results

Mean FAZ area was 0.23 ± 0.08mm^2. FAZ area was positively correlated with BMI (Pearson's r = 0.189, p = 0.03) and significantly larger in participants with lower MPOD, on bivariate analysis (p = 0.04). Significant correlates of FAZ area in the multivariate model included age, sex, BP, vessel perfusion central, CMT and trunk fat %, which collectively contributed 65.2% of the overall variability.

Conclusion

These findings suggest that reduced vessel perfusion, thinner CMT, higher trunk fat % and low MPOD are plausible predictors of a larger FAZ area, in healthy eyes. Non-invasive OCTA testing, in association with these predictors, may aid in the early detection and monitoring of retinal diseases associated with oxidative stress and inflammation.

Introduction

The foveal avascular zone (FAZ) is a highly specialized region at the centre of the fovea in the retina. The fovea is tightly packed with photoreceptors to detect light, with capillaries arranged in rings around its centre, an avascular area called the FAZ. It was first imaged using fluorescein angiography (FA), which is still the gold standard in retinal vascular imaging.[1] However, FA requires injection of fluorescent dye into the blood which can cause anaphylaxis, thus restricting its frequent clinical usage.[1] FA also cannot

segment the retinal layers or measure retinal thickness.[1] Optical coherence tomography angiography (OCTA) is a newer technology which permits superior imaging of the FAZ area, its layers and retinal thickness and is comparable to FA, even in the presence of certain ocular diseases.[1]

Age, gender and some retinal diseases can affect the size and shape of the FAZ.[2]–[8] With its quick, non-invasive imaging, OCTA can be useful in the management of eye diseases including diabetic retinopathy (DR), glaucoma, hypertensive retinopathy and age-related macular degeneration (AMD).[8]–[11] More recently, OCTA has detected microvascular changes such as reduced vessel density and perfusion in subjects with Covid-19.[12] Increased FAZ size and irregular shape has diagnostic relevance in diabetes.[8], [13] Changes can result from capillary dropout as smaller blood vessels die due to decreased perfusion.[8] FAZ shape may also become more irregular with increasing severity of diabetes.[13] In eyes with primary open angle glaucoma (POAG), circularity and vascular density decreased, while FAZ perimeter was statistically larger.[14] This may relate to reduced blood perfusion, as altered perfusion has been proposed as a pathogenic factor in glaucoma, and may indicate another diagnostic use for FAZ assessment using OCTA.[15] Studies have found reduced vessel density in eyes with intermediate and advanced AMD.[16] Vascular density was lower in eyes with exudative compared with non-exudative AMD, while circularity was reduced in the presence of advanced AMD, when compared with early stages of the degeneration.[16] Lee et al found that eyes with AMD had lower vascular density than those without, although there was no difference in FAZ area between the two groups.[16] The authors suggested that alterations in FAZ area may not occur until more advanced stages of AMD.

Oxidative stress plays a prominent role in the development and acceleration of eye diseases such as DR, glaucoma, AMD and hypertensive retinopathy.[17]–[20] It is caused by an imbalance between the production and accumulation of reactive oxygen species (ROS) in cells and the ability of the biological system to detoxify them.[19] While ROS are by-products of normal chemical processes within the body, their build-up without adequate protective antioxidants for counterbalance can damage cells, proteins, lipids and deoxyribonucleic acid (DNA). Retinal tissue is particularly sensitive to oxidative stress, due to its high oxygen demand and exposure to high-energy short wavelength light.[19] Healthy retinal cells can easily inhibit pro-oxidant factors and maintain homeostasis, however, advancing age and/or disease causes decline in the efficiency of homeostatic mechanisms.[19] Excessive oxidative stress and associated inflammation may lead to blood-retinal barrier compromise and tissue damage.[19] While there is limited normative data on vessel density, perfusion and the ganglion cell layer in the FAZ region, recent studies have demonstrated changes in retinal microvasculature in the presence of early hypertension, even without visible retinopathy.[21], [22] Hua et al found an association between hypertension and decreased retinal vessel density.[21] Hypertensive retinopathy was also coincident with reduced retinal nerve fibre layer (RNFL) and ganglion cell layer thickness.[23] Evidence suggests that inflammation caused by oxidative stress is a causative factor in hypertensive retinopathy, leading to lower blood flow and increased inner retinal thinning.[17], [22] Earlier detection of these changes could aid in the treatment of hypertension.[22] The novel Covid-19 virus is another inflammatory disease which can affect the eye.[12] Inflammatory signs such as retinal haemorrhages, cotton-wool spots and venous

dilation have been identified in subjects with Covid-19, along with reduced retinal vessel density and perfusion.[24]

Oxidative stress and inflammation is associated with retinal pathology while antioxidants play a protective role by restoring redox balance.[17] The carotenoids lutein, zeaxanthin and *meso*-zeaxanthin, collectively known as macular pigment (MP), are found in high concentrations in the central fovea.[25] These plant pigments protect the eyes from damage; acting as powerful antioxidants and anti-inflammatory agents.[19] This led to the hypothesis that increased levels of MP may confer protection against AMD and other ocular disorders.[26], [27] Cennamo et al examined the relationship between MP and microvascular density using OCTA in a cohort of Type 1 diabetes participants.[28] Both MP and vessel density were lower in subjects with diabetes. MP was also lower in subjects without visible DR, indicating that these developments can precede diabetic changes in the eye, suggesting that vessel density and MP optical density (MPOD) may be used as prognostic indicators of disease progression.[28] Recent studies have shown that MP is also lower in subjects with glaucoma and high blood pressure.[29], [30] MP levels, however, are variable and can become depleted by a number of factors including age, poor diet, overweight/obesity, oxidative stress and inflammation.[31], [32] To the author's knowledge, only one study has examined the distribution of MP in relation to the size of the FAZ and found that eyes with a larger FAZ area were more likely to have a secondary peak in their MPOD profile, *i.e*, a ring of higher MPOD density 0.5 to 1 degree from the centre of the fovea.[33] Further investigation is warranted to examine the relationship, if any, between total MPOD levels and size/area of the FAZ.

Given the apparent association between FAZ parameters and retinal pathology and the putative protective effects of MP against oxidative stress, this study aims to assess the relationship, if any, between factors which may affect the superficial FAZ (*i.e.*, blood pressure, vessel density, perfusion, overweight/obesity), and possible links with MP status, in a young healthy population, using the Cirrus 5000 HD-OCT (Zeiss, California). An understanding of these factors under normal conditions may assist in the earlier detection and monitoring progression of diseases associated with oxidative stress and inflammation, as alterations to the FAZ can be detected using OCTA.

Materials And Methods

Ethics and Consent

Approval was obtained from the Technological University of Dublin, (TU Dublin) ethics committee in accordance with the principles of the Declaration of Helsinki.

Subjects

One-hundred and fifty-four healthy subjects, aged 18–35 years, were recruited to this prospective, cross-sectional study. Subjects with visual acuity ≥ 6/12 were included. Those with refractive error ≥ +/-8.00DS spherical equivalent and subjects who had taken dietary supplements containing lutein or zeaxanthin during the 6 months prior to the study were excluded.

Demographics and Lifestyle Questionnaire

Subjects completed a brief questionnaire on demographic information including age, general and ocular health, and a validated food frequency questionnaire (FFQ) known as the LZ screener as shown in Table 1.[34] This FFQ assessed lutein and zeaxanthin intake from foods such as eggs, broccoli, corn and green leafy vegetables, weighted according to frequency of ingestion and bioavailability. Participants were categorized as low, medium or high intake.

Ocular and Clinical Examinations

Visual acuity was measured with appropriate correction in place and the eye with the best acuity was used.

Blood Pressure and Body Mass Index

Blood pressure (BP) was measured using an M3 Digital Sphygmomanometer (Omron, Netherlands). Participants were categorized into groups: normal (systolic BP ≤ 120mmHg, diastolic BP ≤ 80mmHg), pre-hypertensive (systolic BP = 121-139mmHg, diastolic BP = 81-89mmHg) and hypertensive (systolic BP ≥ 140mmHg, diastolic BP ≥ 90mmHg) as described by Schwartz & Sheps (1999) and shown in Table 2. Height was measured using a measuring rod to the nearest centimetre (cm). Weight was measured in kilograms (kg) using the Tanita Bioelectrical Impedance Analyzer (BIA) (Tanita Europe BV, Amsterdam, The Netherlands). Body Mass Index (BMI) was calculated from measured height and weight as: weight/height (kg/m^2) and categorized as normal (≤ 25 kg/m^2) and overweight (>25 kg/m^2), according to World Health Organization (WHO) guidelines.[36] Waist to height ratio was calculated and subjects were divided into normal (≤ 0.5) and high (> 0.5) groups.[37] The BIA also calculated trunk fat percentage (%) which is the % of weight of the body's core taken up with fat (mainly visceral fat).[38] Trunk fat % was divided around the mean for males and females and categorized as normal (males = ≤ 33, females = ≤ 30) and high (males = > 33, females = > 30).[39]

Optical Coherence Tomography Angiography

OCTA was performed using the Cirrus 5000 HD-OCT. [40] A 200 x 200mm macular cube was obtained, followed by a 3 x 3 mm OCTA scan. The Angioplex metrix identified FAZ area, perimeter, circularity, vessel density and perfusion in the superficial FAZ, which was defined from the inner limiting membrane to the inner plexiform layer.[40] The software also measured vessel density and perfusion in the region of the FAZ. For analysis, the macula was divided into rings as per the Early Treatment Diabetic Retinopathy Study (ETDRS). As shown in Fig. 1, the central 6mm of the macula is subdivided into central, inner, outer and full regions, these correspond approximately with the anatomy of the macula. The middle portion coincides with the central 1mm (the foveola and partial inner fovea), while the inner (1mm ring) is concerned with the parafovea and partially takes in the outer portion of the fovea. The 3x3mm scans used in the current study did not include the outer region.

Values for all vessel density and perfusion measurements were split into low and high groups around the median for analysis, informed by Lim et al.[41] The cut-off values chosen for vessel density central, inner and full were 14mm/mm^2, 22mm/mm^2 and 23mm/mm^2 and for vessel perfusion central, inner and full were 24%, 41% and 39%, for each group respectively.[41] The macular cube scan provided central macular thickness (CMT) and ganglion cell thickness measures. Cut-off values were chosen based on the mean CMT from the UK Biobank study.[42] A value of $\leq 264.5\mu m$ was considered normal and $> 264.5\mu m$ was high. Ganglion cell plus inner plexiform layer thicknesses (GCL + IPL) were similarly divided into groups; cut-off values for GCL + IPL were chosen as follows: mean GCL + IPL of $\leq 82.1\mu m$ was low and $> 82.1\mu m$ was high, while minimum GCL + IPL $\leq 80.4\mu m$ was considered low and $> 80.4\mu m$ was high, derived from Mwanza et al.[43]

Macular Pigment

MPOD was measured using the Macular Metrics densitometer (Macular Metrics, Rehoboth, MA, USA), which is based on the principal of heterochromatic flicker photometry and has been described in detail elsewhere.[44] In brief, a test stimulus was presented which flickers between a luminance of 460nm (peak absorption of MP), and a luminance of 540nm, (minimum absorption by MP). The difference in luminance noted by the subject is directly proportional to the amount of MP present in the eye. Five readings were taken both centrally (at 0.5 degrees eccentricity) and parafoveally (at 7 degrees eccentricity). The ratio of blue light required in the fovea to that required in the parafovea indicates the amount of pigment present. The logarithm of this ratio is the optical density (OD) of MP. A standard deviation of < 0.05 was accepted.[45] For analysis, the group was divided around the mean into low MP ($\leq$ 0.4OD) and high MP (> 0.4OD) as shown in Table 2.

Statistical Analysis

The Kolmogorov-Smirnov test was used to assess normality of data. As FAZ area data were not normally distributed, a square root transformation of these data was performed. The derived data were normally distributed and used as the dependent variable for subsequent statistical analysis of the FAZ. Mean and standard deviation of FAZ data is presented as the non-transformed original measure for ease of interpretation. An independent samples t-test and/or one-way ANOVA was used to test for differences in normally distributed data. The Kruskal-Wallis H test was used to test for differences in group medians in non-normally distributed data (age, vessel perfusion inner and full and mean GCL + IPL thickness). Pearson's product-moment correlation was used to assess the relationship between normalized FAZ area and other study variables where appropriate. All values were expressed as mean ± standard deviation throughout. A *p*-value of < 0.05 was considered significant.

Results

Demographic, Health and Lifestyle factors:

Fifteen subjects were excluded due to underlying health conditions. Demographic information for the remaining 139 subjects is shown in Table 1.

Table 1
Demographic characteristics of the study group (n = 139)

Characteristic	n	%
Age (years)		
18–26	96	69.1
27–34	43	30.9
Sex		
Male	44	31.7
Female	95	68.3
Race		
White	113	81.3
Mixed	6	4.3
Asian	8	5.8
Pakistani	3	2.2
African American	6	4.3
Hispanic	1	0.7
Indian	2	1.4
Blood Pressure		
Non-hypertension	63	45.3
Pre-hypertension	65	46.8
Hypertension	11	7.9
BMI		
Normal	95	68.3
Overweight	44	31.7
Waist to Height Ratio		
Normal	89	64.1

Non-hypertension: Systolic blood pressure ≤ 120mmHg, diastolic blood pressure ≤ 80mmHg), Pre-hypertension: Systolic blood pressure = 121-139mmHg, diastolic blood pressure = 81-89mmHg, Hypertension: Systolic blood pressure ≥ 140mmHg and/or diastolic blood pressure ≥ 90mmHg, BMI; Body Mass index: Normal = ≤ 25 Overweight = > 25, Waist to height ratio: ≤ 0.5 = normal, > 0.5 = high, Trunk fat %: normal = ≤ 33% (men), ≤ 30% (women), high = > 33% (men), > 30% (women); MPOD category: low = ≤ 0.40, high = > 0.40, LZ; Lutein-Zeaxanthin category.

Characteristic	n	%
High	50	35.9
Trunk Fat %		
Normal	60	43.2
High	79	56.8
Smoking Status		
Non-smoker	123	88.5
Current or past smoker	16	11.6
MPOD Category		
Low	75	54
High	64	46
LZ Category		
Low	39	28.1
Medium	46	33.1
High	54	38.8

Non-hypertension: Systolic blood pressure ≤ 120mmHg, diastolic blood pressure ≤ 80mmHg), Pre-hypertension: Systolic blood pressure = 121-139mmHg, diastolic blood pressure = 81-89mmHg, Hypertension: Systolic blood pressure ≥ 140mmHg and/or diastolic blood pressure ≥ 90mmHg, BMI; Body Mass index: Normal = ≤ 25 Overweight = > 25, Waist to height ratio: ≤ 0.5 = normal, > 0.5 = high, Trunk fat %: normal = ≤ 33% (men), ≤ 30% (women), high = > 33% (men), > 30% (women); MPOD category: low = ≤ 0.40, high = > 0.40, LZ; Lutein-Zeaxanthin category.

FAZ area by demographic, health and ocular variables are shown in Table 2. The mean age of the full group was 23.80 ± 4.9 years and ranged from 18 to 34 years; mean age of males was (25.68 ± 5.2 years); females (22.93 ± 4.5 years), and this difference was significant ($p < 0.001$). Participants were divided into two age groups: 18 to 26 years (mean: 20.89 ± 2.33) and 27 to 34 years (mean: 30.30 ± 1.98). Age was not significantly associated with FAZ area in the bivariate model ($p = 0.461$), (see Table 2) and there was no significant correlation between age and FAZ area (Pearson's r = -0.157, $p = 0.064$).

Gender

Mean FAZ area for the study group was 0.23 ± 0.08mm^2. Females had a larger FAZ area (0.25 ± 0.08mm^2) compared with males (0.20 ± 0.08mm^2), and this difference was significant, ($p < 0.001$); (see Table 2).

Body Mass Index, Waist to Height Ratio and Trunk Fat Percentage

Mean BMI was 23.63 ± 3.37 and ranged from 17.22 to 31.62. FAZ area was not significantly different between the “normal” and “high” groups, (p = 0.074); (see, Table 2). There was, however, a small significant positive correlation, between FAZ area and BMI (Pearson’s r = 0.189, p = 0.026), as shown in Fig. 2. FAZ area was not significantly different between both “normal” and “high” groups for waist to height ratio, (p = 0.079). Mean trunk fat % was 33.15 ± 14.69% in males and 30.29 ± 6.13% in females. There was no significant difference in FAZ area in the “normal” or “high” group for either males or females, (p = 0.451, 0.323 respectively). There was no significant correlation between trunk fat % and FAZ area, (Spearman’s rho = 0.031, p = 0.713).

Blood Pressure, Vessel Density and Vessel Perfusion

A one-way ANOVA showed no significant difference in FAZ area between participants with and without high BP (p = 0.342), as shown in Table 2. Additionally, no significant correlation was found between BP, (systolic or diastolic) and FAZ area (Pearson’s r = 0.040, p = 0.638; r = 0.070, p = 0.413, respectively). Mean values for vessel density central, inner and full were 13.66 ± 2.93mm/mm^2, 23.18 ± 0.92mm/mm^2 and 22.11 ± 1.01mm/mm^2, respectively. The mean FAZ area was significantly larger in participants with lower vessel density central and full (p <0.001 for both); (see Table 2). FAZ area was negatively correlated with vessel density central and full (Pearson’s r = -0.793, -0.400 respectively, p < 0.001 for both), see Figure 3. Vessel density inner was not, however, associated with FAZ area on bivariate analysis (p = 0.278), and there was no correlation between vessel density inner and FAZ area, (Pearson’s r = -0.166, p = 0.050). Mean values for vessel perfusion central, inner and full were 23.87 ± 5.26%, 41.44 ± 1.48% and 39.46 ± 1.68%, respectively. A significantly larger FAZ area was found in participants with lower vessel perfusion central and full (p < 0.001 for both). While participants with lower vessel perfusion inner had a larger FAZ area (0.25mm^2) than those with higher perfusion inner (0.22mm^2), this difference was not significant, (p = 0.053). FAZ area correlated negatively with both vessel perfusion central and full (Pearson’s r = -0.777, -0.391 respectively, p < 0.001 for both), see Figure 3, however, there was no correlation with vessel perfusion inner, (Spearman’s rho = -0.140, p = 0.100).

Central Macular Thickness, Ganglion Cell + Inner Plexiform Layer Thickness

Mean CMT for the group was 258.96 ± 20.82μm. FAZ area was significantly larger in the group with thinner CMT, and this finding was significant (p < 0.001); (see Table 2). There was also a significantly strong negative correlation between FAZ area and CMT (Pearson’s r = -0.679, p < 0.001) as shown in Figure 4.

Mean values for average and minimum GCL + IPL thicknesses were 83.22 ± 5.23μm and 81.89 ± 5.31μm, respectively. There was no significant difference in FAZ area between the low and high groups for average and minimum GCL + IPL ($p > 0.05$ for both); (see Table 2). There was no correlation between FAZ area and either average (Spearman's rho = 0.136, $p = 0.110$) or minimum GCL + IPL thickness (Pearson's r = 0.095, $p = 0.268$).

MPOD and LZ Category

Mean MPOD was 0.41 ± 0.18 and it ranged from 0.33 to 1.03 OD. FAZ area was significantly larger in participants with lower MPOD compared with those with higher MPOD ($p = 0.038$); (see Table 2). However, no correlation was found between FAZ area and MPOD (Pearson's r = -0.153, $p = 0.073$). While there was no correlation between MPOD and BMI (r = -0.043, p = 0.617) and no difference in MPOD between the waist to height groups, ($p = 0.883$), there was a negative correlation between MPOD and trunk fat % (Spearman's rho = -0.181, $p = 0.033$). A one-way ANOVA showed no significant difference in FAZ area between low, medium and high LZ categories, although this was a borderline finding.

Table 2

FAZ area by demographic, health and ocular variables.

	n	Mean Percentile	SD	25th	50th	75th	sig
Age							
18-26	96	0.24	0.09	0.17	0.23	0.29	0.461†
27-34	43	0.22	0.08	0.17	0.24	0.27	
Gender							
Male	44	0.20	0.08	0.14	0.20	0.25	**<0.001‡**
Female	95	0.25	0.08	0.19	0.24	0.30	
BMI (kg/m^2)							
Normal	95	0.22	0.08	0.16	0.22	0.27	0.074
Overweight	44	0.25	0.09	0.19	0.24	0.29	
Waist to Height Ratio							
Normal	89	0.22	0.09	0.16	0.22	0.27	0.079
High	50	0.25	0.08	0.19	0.25	0.29	
Blood Pressure (mmHg)							
Normal	63	0.23	0.09	0.18	0.23	0.27	0.342
Pre-hypertensive	65	0.22	0.09	0.16	0.23	0.29	
Hypertensive	11	0.26	0.04	0.23	0.27	0.29	
Vessel Density Central (mm/mm^2)							
≤14.00	81	0.28	0.07	0.23	0.26	0.31	**<0.001‡**
>14.00	58	0.17	0.06	0.13	0.16	0.20	
Vessel Density Full (mm/mm^2)							
≤22.00	64	0.26	0.09	0.20	0.25	0.31	**<0.001‡**
>22.00	75	0.21	0.08	0.15	0.20	0.25	
Vessel Perfusion Central (%)							

≤24.00	74	0.28	0.07	0.23	0.26	0.31	**<0.001‖**
>24.00	65	0.17	0.06	0.13	0.17	0.20	
Vessel Perfusion Full (%)							
≤39.00	52	0.27	0.09	0.21	0.25	0.32	**<0.001†**
>39.00	87	0.21	0.08	0.15	0.21	0.26	
CMT (µm)							
≤264.5	87	0.26	0.08	0.22	0.25	0.31	**<0.001‖**
>264.5	52	0.18	0.07	0.12	0.17	0.23	
Mean GCIPL (µm)							
≤82.1	69	0.22	0.08	0.18	0.23	0.27	0.345†
>82.1	70	0.24	0.09	0.17	0.23	0.29	
Min GCIPL (µm)							
≤80.4	57	0.21	0.08	0.15	0.22	0.25	0.062
>80.4	82	0.24	0.08	0.19	0.23	0.29	
MPOD (OD)							
≤0.4	75	0.24	0.09	0.19	0.23	0.29	**0.038‖**
>0.4	64	0.21	0.08	0.14	0.22	0.26	
LZ category							
Low	39	0.23	0.08	0.17	0.22	0.16	**0.047‡**
Medium	46	0.21	0.08	0.14	0.23	0.23	
High	54	0.25	0.09	0.19	0.25	0.25	

Independent Samples t-test; †Kruskal-Wallis test of Medians; ‡One-Way ANOVA. Significant differences highlighted in bold. Abbreviations: SD; standard deviation, BMI; body mass index, CMT; central macular thickness, GCIPL; ganglion cell layer plus inner plexiform layer thickness, MPOD; macular pigment optical density, LZ; Lutein-Zeaxanthin category.

Multivariate Model

Significant correlates of FAZ area in the multivariate regression model included age, sex, BP, vessel perfusion central, CMT and trunk fat %, which collectively contributed 65.2% of the overall variability. Age,

vessel perfusion central and CMT were negative predictors of FAZ area, after adjusting for all other covariates, (p = 0.022; < 0.001 and = 0.028 respectively). Larger FAZ area was associated with female sex on both univariate (p < 0.001) and multivariate analysis (p = 0.032). While FAZ area was not associated with BP or trunk fat % on univariate analysis (see Table 2), diastolic BP and trunk fat % were positive predictors of FAZ area in the multivariate model, (p = 0.042, 0.035 respectively). Although lower MPOD was associated with larger FAZ area on univariate analysis, (p = 0.038), no association was found in the multivariate model, (see Table 3).

Table 3
Multivariate regression analysis between demographic, health and ocular variables and FAZ area in a young, healthy population (n = 139).

Independent variable	Unstandardised β coefficient	Standard error	t	*p*
Constant	0.846	0.092	9.172	< 0.000
Age	-0.002	0.001	-2.310	**0.022**
Sex* (female)	0.010	0.011	0.939	**0.032**
Mean Diastolic BP	0.001	0.001	2.055	**0.042**
MPOD	-0.016	0.028	-0.562	0.575
Vessel Perfusion Central	-0.011	0.001	8.421	**< 0.001**
CMT	-0.001	0.000	-2.218	**0.028**
Trunk Fat %	0.001	0.000	2.128	**0.035**
Adjusted r^2 = 0.652; F = 37.87; p = < 0.001. Dependent variable = FAZ area square root (normalised FAZ area); Std. Error, Standard Error. *Male = control group.				
Abbreviations: *BP; blood pressure, MPOD; macular pigment optical density, CMT; central macular thickness.*				

Discussion

In this study, reduced vessel perfusion central (≤ 24%), low MPOD (≤ 0.4OD) and high BMI (> 25kg/m^2) were associated with larger FAZ area, which, to the author's knowledge, are novel findings. FAZ area was significantly larger in participants with lower vascular density/perfusion, findings which concur with previous analysis.[2] Age, vessel perfusion central and CMT were negative predictors of FAZ area in the multivariate model, while female sex, diastolic BP and trunk fat % remained positive predictors, after adjusting for all other covariates, collectively explaining 65.2% of the variability.

Blood pressure, Vessel Density and Vessel Perfusion

The current study findings suggest that increased BP, reduced retinal vessel density and perfusion are associated with larger FAZ area, in this young healthy group. Studies have found reduced vascular

density (full) in hypertensive subjects aged 60-70 years compared with non-hypertensive controls, which was particularly marked in uncontrolled hypertension.[22], [46] Donati et al found that FAZ area was significantly larger among hypertensive subjects, particularly in the deep FAZ.[47] Sun et al found a significant increase in the size and perimeter of the deep FAZ of eyes of subjects with hypertension, but not in the superficial plexus, suggesting that the superficial FAZ is affected later in the disease process, if at all.[11] Macular blood flow and thickness were also reduced in hypertensive eyes.[11] While diastolic BP was a positive predictor of FAZ area in the current study, there was no correlation between BP and FAZ area on univariate analysis. A plausible reason for these findings is that the subjects were young and healthy with only a small percentage (7.9%) of hypertension detected. Traditionally, hypertensive retinopathy is assessed and graded using fundoscopy or retinal photography, however, these methods are problematic as early changes are difficult to detect.[48] While the current study found no significant difference between participants with and without high BP (p = 0.342), further studies with more hypertensive subjects versus controls are warranted to investigate if diagnostic indicators, such as vessel density and/or perfusion (central and full) can be used in the early detection of hypertension. As expected, no correlation was found between vessel density or perfusion inner and FAZ area, as this part of the retina is not avascular. A simple non-invasive, objective test such as OCTA could prove useful as a diagnostic indicator for hypertension by monitoring vascular changes in the FAZ area.

Recently, OCTA has found reduced vessel density and perfusion, particularly in the deep plexus, of subjects who have recovered from Covid-19, while another study found that macular vascular flow was further reduced at 6-month follow up.[12], [49] Early identification of Covid-19 changes within the eye, using a low-contact test such as OCTA, may lead to more efficient treatment. Covid-19 causes an inflammatory response by binding to angiotensin converting enzyme 2 which damages endothelial cells in all areas of the body, including the eye.[50] Retinal vascular changes consistent with inflammation/oxidative stress such as cotton wool spots, haemorrhages and venous dilation have been reported in subjects with Covid-19.[49] Longitudinal studies, however, are necessary to fully elucidate these findings. The non-invasive nature of OCTA, as an investigative test over conventional FA, is a major advantage of this technology, given that Covid-19 is highly transmissible.

Body Mass Index, Waist to Height Ratio and Trunk Fat Percentage

The study findings that BMI correlated positively with FAZ area (r = 0.189, p = 0.026) and that trunk fat % was a positive predictor of FAZ area in the multivariate model, (p = 0.035) are important given that overweight/obesity is a significant risk factor for many health conditions, including diabetes, high cholesterol, hypertension and AMD. These findings are significant, as only 32% of our subjects were overweight, with an average BMI of 24 kg/m^2. Furthermore, mean trunk fat % was within the normal range for both males (≈33%) and females (≈30%), as this was a young, healthy group. Overweight/obesity is prevalent among European adults.[51] To date there has been very little research on FAZ area and overweight/obesity. However, one small study, (n = 65) found a larger FAZ area in obese

subjects.[52] Obesity is associated with oxidative stress, which in turn; is linked with capillary dropout and damage to the retinal vasculature.[53] Overweight/obesity is also considered an important factor in the aetiology of Type 2 diabetes.[54] Higher body fat, visceral fat in particular, is associated with increased insulin resistance and inflammation.[55], [56] In one study, FAZ area was larger in subjects with Type 2 diabetes compared with Type 1 diabetes and controls, possibly due to differences in glycaemic control and/or duration of disease.[57] While measurement of BMI can identify overweight/obesity, it does not isolate where adipose tissue is accumulating in the body, nor can it differentiate between fat or muscle mass.[37] The use of specific analysis, such as BIA, may provide more in-depth evaluation of body fat distribution, considering trunk fat % (*i.e.* visceral fat) was a positive predictor of FAZ area in the current study.

MPOD and LZ Category

The current study found a larger FAZ area in subjects with lower MPOD, to the author's knowledge a novel finding. MP is a powerful antioxidant and anti-inflammatory agent which putatively protects the macula against oxidative stress. MP, however, can become depleted for many reasons, including advancing age or poor diet.[27], [58] Higher MPOD levels from an early age may offer greater retinal protection over one's lifespan. Mean MPOD was 0.41 ± 0.18OD in the current study. This is similar to Liew et al's findings that mean MPOD in a group of healthy adults was 0.44OD (range 0.06 – 1.25), albeit this group included subjects aged 17-50 years.[59] While higher levels of MPOD is preferable, there is no consensus on what constitutes a normal amount, given that many studies include a wide age-range and employ different measurement methods.[59]–[61] While no correlation was found between FAZ area and MPOD and no association between FAZ area and dietary intake of LZ in the current study, there was a significant negative correlation between MPOD and trunk fat %, a finding supported by the literature.[39] Adipose tissue, visceral fat in particular, competes with the retina to store MP carotenoids.[39] Hammond et al found lower MPOD in subjects with BMI >29kg/m^2.[31] In the current study, FAZ area correlated positively with BMI and was larger in subjects with lower MPOD (*i.e.* ≤ 0,40OD) and in those with higher trunk fat % (*i.e.* females > 30%, males > 33%). Additionally, MPOD was lower in subjects with higher trunk fat %. While MPOD is not routinely measured in clinical practice, the finding that a larger FAZ area is associated with higher trunk fat %, in participants free of ocular pathology, may indicate a lower antioxidant status in these subjects. Such individuals may benefit from dietary and lifestyle advice. There is value in counselling ophthalmic patients from a young age on the importance of a healthy diet rich in fruit and vegetables, *i.e.* carotenoids lutein and zeaxanthin, to help maintain MPOD levels into older age, in addition to regularizing BMI and reducing visceral/trunk fat % levels for their protective properties against diseases such as AMD and diabetes.[27]

Central Macular Thickness, Ganglion Cell + Inner Plexiform Layer Thickness

In the current study, CMT was a strong negative predictor of FAZ area (p = 0.028). CMT also significantly negatively correlated with FAZ area, a finding supported by the literature.[4], [62] Reduced CMT has been found in individuals with genetic risk factors for AMD.[63] Therefore, subjects with a thinner CMT may be more likely to have larger FAZ area and thereby be more vulnerable to the effects of oxidative stress.

While there was no association between FAZ area and GCL + IPL thickness in the current study, on young, healthy subjects, there was a positive correlation between average GCL + IPL thickness and vessel density and perfusion full, and a positive correlation was found between minimum GCL + IPL and vessel density full. Enlarged FAZ area has shown an association with glaucoma in disease studies.[14], [64] Assessment of the FAZ area and foveal vascularity may aid in early identification of glaucomatous changes within the eye, which can prove difficult to assess visually.[48] Current best practice involves a combination of monitoring optic disc and ganglion cell changes, visual fields, intraocular pressure and the presence of risk factors such as family history. Choi et al found that reduced vessel density, decreased FAZ circularity and increased FAZ perimeter were all significant in distinguishing POAG from normal controls, however, segmentation of the FAZ regions differed as additional software was used.[10] Zivkovic et al found larger FAZ area and reduced vessel density in normal tension glaucoma subjects.[64] The authors posited that with further study of the FAZ, it may be possible to identify a value for FAZ area, which can potentially detect a glaucomatous suspect.[64] The participants in the current study were free of pathology. Furthermore, intraocular pressure was not measured which is a limitation as the relationship between intraocular pressure and FAZ parameters could not be analysed.[65] Future studies incorporating intraocular pressure measurements, in subjects with glaucoma versus controls, would be useful to further investigate if OCTA biomarkers for the early detection of glaucoma, such as vessel density/perfusion full, could be identified.

Study strengths and limitations

There were many strengths to the current analysis. For example, to the author's knowledge, this is the first study of its kind to investigate the association between MPOD and visceral fat, and FAZ area/vascularity using the Cirrus HD-OCTA. In particular, the link between FAZ area and MP has rarely been examined in a healthy population, although it has been investigated in relation to disease.[28], [33] The results from the current study provide a reference from a normal, healthy perspective for comparison with disease studies. Specifically, this study has shown that OCTA analysis ought to concentrate on vascular density and perfusion central/full, as opposed to inner values which do not include the FAZ.

There were some important limitations in the current study which should be acknowledged. The current study contained more females than males and males were statistically significantly older.[2] This may have impacted the data; we found that age was a negative predictor of FAZ area, a finding which should be interpreted cautiously.[62] Regarding the examination of GCL + IPL thickness, the findings of positive correlations between GCL + IPL and vessel density and perfusion are inconclusive without intraocular pressure measurement.

Conclusion

The current study findings suggest that lower vessel density and perfusion (central and full), thinner CMT and higher trunk fat % are plausible predictors of a larger FAZ area in healthy eyes. Suggested normal values for young healthy subjects, from the current study are 14.00mm/mm^2 for vessel density central, 24% for perfusion central, 264.5µm for CMT and MPOD ≥ 0.4 OD. Reduced vessel density and perfusion, lower CMT and high BMI/ trunk fat % are all associated with inflammatory conditions and with a larger FAZ area.[21], [52], [64], [66], [67] Inflammation is associated with oxidative stress, which can cause retinal damage over time. Furthermore, in the current study, larger FAZ area was associated with lower MP status and, therefore, antioxidant status in a young, healthy population. The presence of MP within the retina has a protective effect against damage from oxidative stress. OCTA can non-invasively monitor vascular density and perfusion, CMT and FAZ area for deviations from the proposed normal values. The current study findings suggest that OCTA, in association with knowledge of these predictors, may have prognostic potential in the early detection of and monitoring progression of retinal diseases associated with oxidative stress and inflammation such as DR, glaucoma, hypertension, AMD and Covid-19. Further study is needed to evaluate the current study findings.

Declarations

Declaration of Interest

The authors report no known financial interest or personal relationship that could appear to have influenced the work reported in this paper.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author, [SOS], upon reasonable request.

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Figures

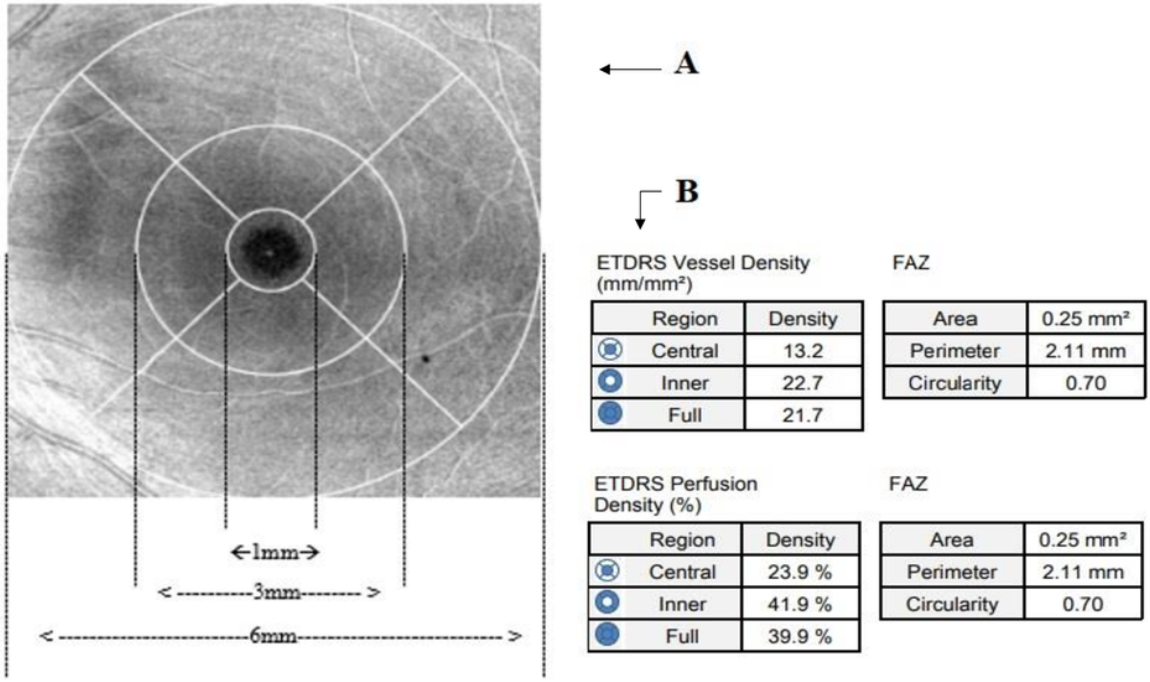

Figure 1

A. ETDRS grid centred on the macula. B. Angioplex Metrix displays the vessel density and perfusion at the central (fovea), inner (parafovea) and full sections of the macula according to the ETDRS. ETDRS; Early Treatment Diabetic Retinopathy Study.

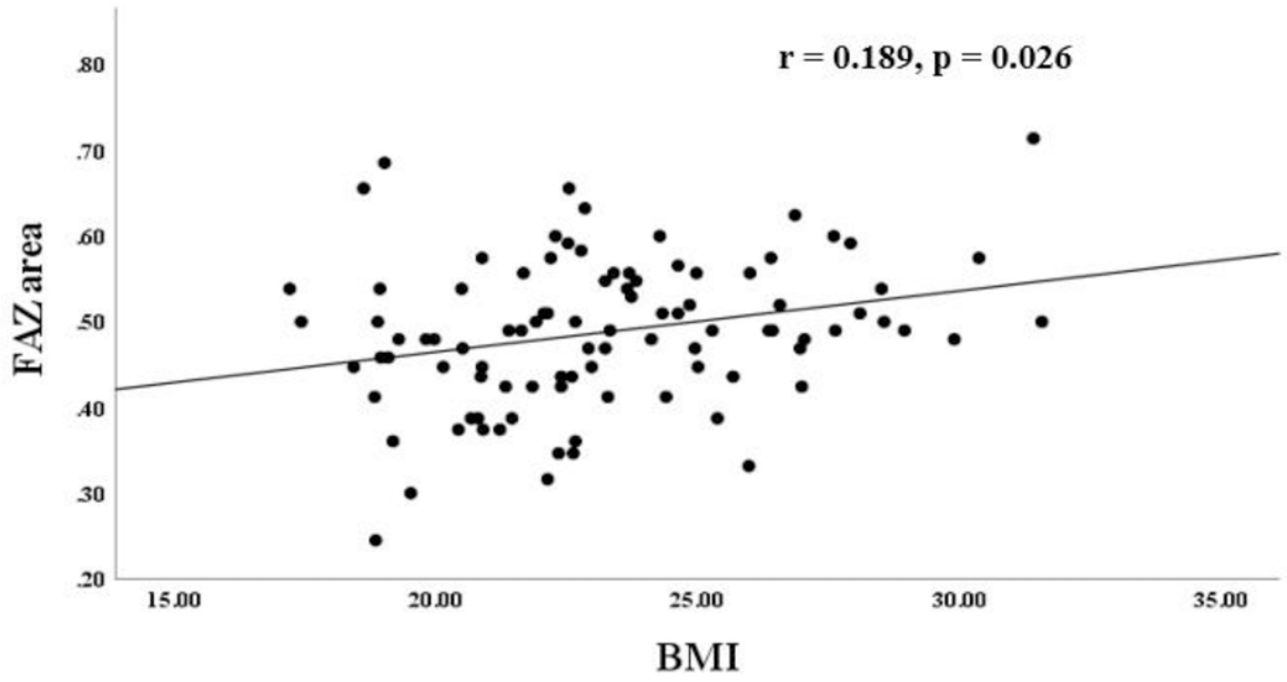

Figure 2

Scatterplot showing a significant positive relationship between normalised FAZ area and BMI.

Abbreviations: FAZ; foveal avascular zone (mm2), BMI; body mass index (kg/m2).

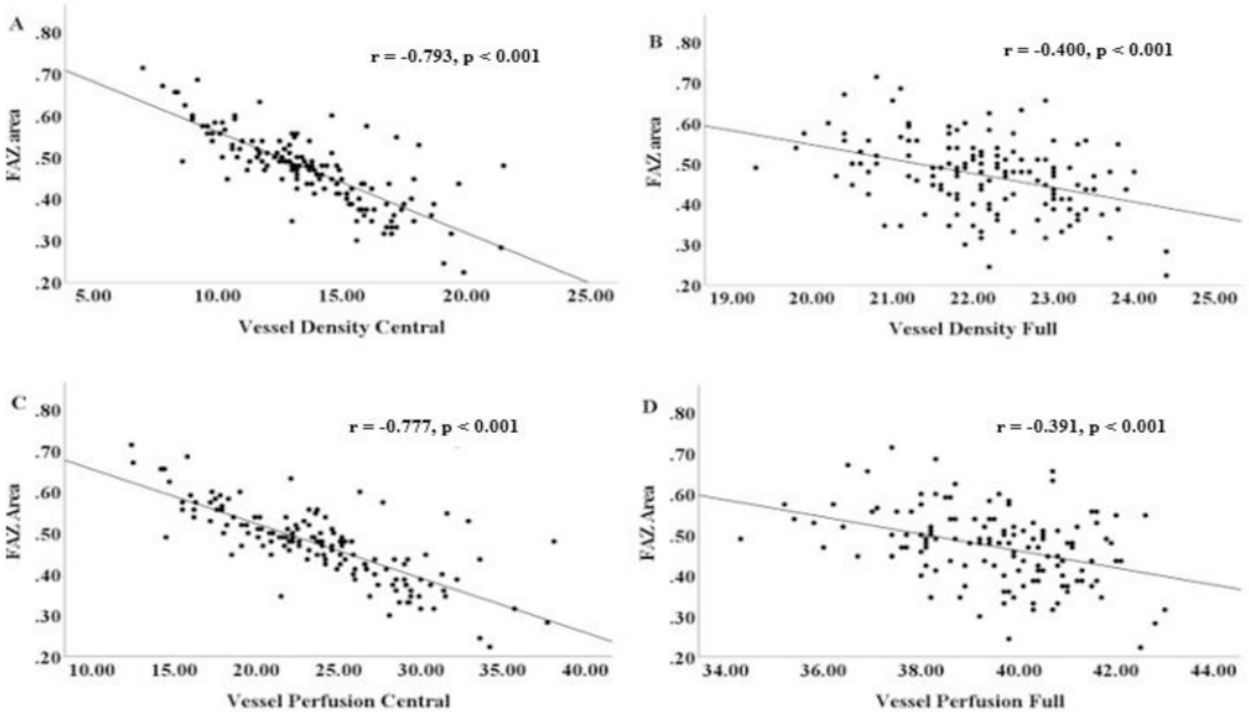

Figure 3

Scatter plots showing negative correlations between normalised FAZ area (mm2) and vessel density (mm/mm2) and perfusion (%) central and full.

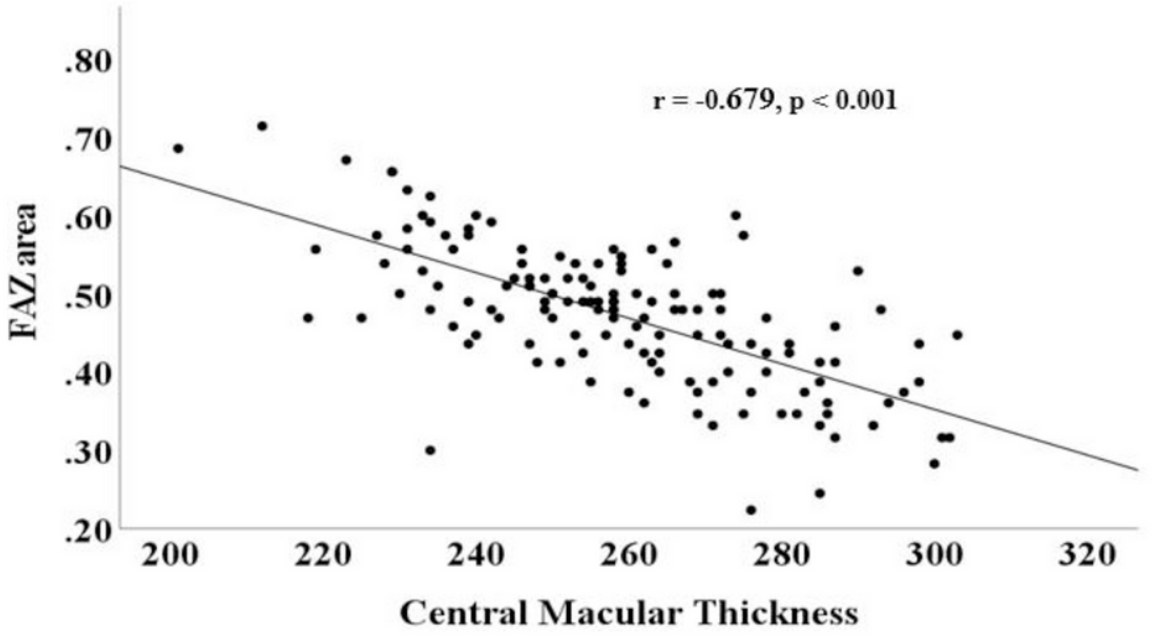

Figure 4

Scatterplot showing a significant negative relationship between normalised FAZ area and CMT.

Abbreviations: FAZ; Foveal Avascular Zone (mm2), CMT; Central Macular Thickness (µm).