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Central Macular Thickness in Diabetic Patients: A Sex-based Analysis

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Abstract

Significance: The pathological changes in clinically significant diabetic macular edema lead to greater retinal thickening in males than in females. Therefore, male sex should be considered a potential risk factor for identifying individuals with the most severe pathological changes. Understanding this excessive retinal thickening in males may help preserve vision.

Purpose: To investigate the sex differences in retinal thickness for diabetic patients. We tested whether males with clinically significant macular edema had even greater central macular thickness than expected from sex differences without significant pathological changes. To determine which retinal layers contribute to abnormal retinal thickness.

Methods: From 2047 underserved adult diabetics from Alameda County, CA, 142 patients with clinically significant macular edema were identified by EyePACS certified graders using color fundus images (Canon CR6-45NM). First, central macular thickness from spectral domain optical coherence tomography (iVue, Optovue) was compared for 21 males vs. 21 females without clinically significant macular edema. Then, a planned comparison contrasted the greater values of central macular thickness for males vs. females with clinically significant macular edema, as compared to those without. Mean retinal thickness and variability of central macular layers were compared for males vs. females.

Results: Males without clinically significant macular edema had a 12 μm greater central macular thickness than females, $245 \pm 21.3 \mu\text{m}$ and $233 \pm 13.4 \mu\text{m}$, respectively, $t(40) = -2.18$, $P = .04$. Males with clinically significant macular edema had a 67 μm greater central macular thickness than females, $383 \pm 48.7 \mu\text{m}$ and $316 \pm 60.4 \mu\text{m}$, $P < .001$, i.e. males had 55 μm or $> 5\times$ more, $t(20) = 2.35$, $P = .015$. In males, the outer nuclear layer thickness was more variable $F_{10,10} = 9.34$.

Conclusions: Underserved diabetic males had thicker retinas than females, exacerbated by clinically significant macular edema.

Keywords

central macular thickness; clinically significant macular edema; diabetic macular edema; sex; optical coherence tomography

Diabetic retinopathy and diabetic macular edema are the primary causes of vision loss in working age adults, with a total of 93 and 21 million people worldwide living with diabetic retinopathy and diabetic macular edema, respectively.¹⁻² Retinal vascular and neural changes are the main characteristics of these sight threatening complications of diabetes²⁻³ with diabetic macular edema affecting 20% of all individuals with diabetes of at least 20 years duration.^{1,4} The breakdown of the inner and/or outer retinal blood barriers can lead to the formation of cystoid structures, hard exudates, and subretinal fluid anywhere in the retina.^{2,5-7} The formation of these structures leads to the thickening of the retina, and clinically the presence of hard exudates is normally a sign of current or previous macular edema.⁶ Thus, diabetic macular edema is defined by the presence of retinal thickening as seen by a three-dimensional assessment performed by a dilated fundus examination using slit-lamp biomicroscopy with a condensing lens; and/or stereo fundus photography.⁶ In cases of uncertainty about the presence of macular edema, either fundus evaluation with Goldmann macular contact lens⁸ or optical coherence tomography⁹ can be used. Identifying high risk

individuals for diabetic macular edema may clinically aid in monitoring disease progression and management of these patients.

Clinically, diabetic macular edema can occur at any stage of diabetic retinopathy severity and can be classified as mild, moderate or severe depending on the location of retinal thickening in relation to the center of the macula.⁶ The relation of the proximity of the retinal thickening to the fovea and its effect on visual acuity is important for diabetic macular edema.^{5-6,10} Macular edema becomes clinically significant when any of the following is present: thickening of the retina at or within 500 μm of the center of the macular, hard exudates at or within 500 μm of the center of the macular with thickening of the adjacent retina or a zone of retinal thickening one disc area or larger which is within one disc diameter of the center of the macular.⁵⁻⁶ The formation of cystoid structures and hard exudates indicating a compromise of the inner retinal blood barrier can lead to the disorganization of the inner retinal neurons in diabetic macular edema.^{3,8,11} Photoreceptor dysfunction and potential damage can result from subretinal fluid formation,^{2,12-13} which can occur without visible inner retinal blood barrier damage.¹³ The fluid pressure from the cystoid structures can cause retinal thickening in a particular retinal layer as well as a corresponding thinning of the adjacent layers. Hence in diabetic macular edema, increased retinal thickness can occur for one or more layers throughout the retina, while the adjacent layer is thin. Furthermore, as neurodegeneration can occur early in diabetic retinas,^{3,14} one or more retinal layers can be abnormally thin.

The quantitative measurement of central macular thickness is a widely adopted method of detecting diabetic macular edema, normally done by comparing the central macular thickness values of diabetics to that of an age-matched normative database using optical coherence tomography.^{2,9,11} Central macular thickness is defined as the average retinal thickness within the 1 mm Early Treatment Diabetic Retinopathy Study circle centered on the fovea.⁵ Optical Coherence Tomography provides a cross-sectional image (b-scan) of diabetic retinas, now with an improved axial resolution sufficient to detect tissue disruption in diabetic macular edema that is caused by the fluid pressure from the cystoid structures.^{2,11,15} Thus, retinal thickening or thinning as a result of the fluid pressure from the cystoid structures or neurodegeneration can be visualized and quantified using optical coherence tomography, for the whole retina or individual layers. Segmentation of the individual retinal layers demonstrates the relative thicknesses of these retinal layers, with inner retinal changes often thinner prior to clinically observable vascular changes, although this judgment was based on only clinical color fundus photography rather than angiography or more advanced techniques.¹⁴ The thinner inner retinal layers have been largely ganglion cell layers sampled near the optic nerve head.¹⁴ However, thinner ganglion cell layer results were found at 1 to 3 μm from the fovea.¹⁶ Measuring the inner retinal layers at the foveal center is typically inaccurate because of the formation of a fovea pit, and consequentially the lack of thinner inner retinal layers at the foveal center. Thus, we did not measure inner retina in the central 1 mm.

Male sex has been identified as an independent risk factor for diabetic macular edema severity.¹⁷⁻¹⁸ In subjects with diabetic retinopathy, males are greater than six times more likely to develop edema than females for a given local retinal location.¹⁷ In diabetic subjects

with minimal or no diabetic retinopathy, a significantly greater central subfield thickness of 16 μm has been found in males relative to females using optical coherence tomography.¹⁸ Local neuroretinal function in type 2 diabetics without retinopathy has also been found to be worse in males compared to young females using multifocal electroretinogram.¹⁹ Thus, relative to diabetic males, younger diabetic females have been suggested to have some form of neuroprotection from the neurodegeneration that occurs with diabetes. The presence of estrogen has been suggested as a possible explanation for this.¹⁹⁻²⁰ Males with diabetic macular edema therefore may have highly distorted retinal layers compared to females with diabetic macular edema who are similar in age, and HbA1c.

The purpose of this study is twofold. First, we compared central macular thickness measurements between male and female diabetic subjects with and without clinically significant macular edema, to investigate whether the typical extra thickness of male retinas explains sex differences found in diabetic subjects with clinically significant macular edema. Second, we investigated the sex difference in the individual retinal layer thicknesses in subjects with clinically significant macular edema, to determine if there were specific layers that accounted for thicker retinas in male diabetic patients.

METHODS

Subjects

The study conformed to the tenets of the Declaration of Helsinki and informed consent was obtained from the subjects after explanation of the nature and possible consequences of the study. The research was approved by the Institutional Review Boards of Indiana University, Alameda Health, and University of California Berkeley.

Image, demographic, and HbA1c data in this study were acquired from patients who participated in a prospective diabetic retinopathy photoscreening study with Aeon Imaging, EyePACS, University of California Berkeley, and Indiana University.²¹⁻²² We included 2047 underserved, adult diabetic patients, of whom over 90% self-identified as a racial/ethnic identity other than non-Hispanic white, as described previously.²¹ At the time of study, a significant proportion of these patients did not have access to routine eye care, as documented by more than 66% of these subjects reporting that their last eye exams were conducted more than 3 years ago.²

Non-mydratic color fundus images (Canon CR6-45NM) were used by EyePACS certified graders to classify subjects into the categories of the International Classification of diabetic retinopathy and diabetic macular edema severity.^{6,22} The criterion for diagnosing clinically significant macular edema was the presence of hard exudates in the central 1500 μm of the fovea using color fundus images, a method validated by a dilated clinical examination.⁸ Two of the authors led the image grading (TVL and TJG). Two additional authors (SGB and SBY) transferred the image and clinical data, located patients with clinically significant macular edema, verified that the spectral domain optical coherence tomography data were sufficient for further study grading, and prepared the data for further analysis. Before further data analysis, two additional graders (CAC, VEM) determined from the spectral domain optical coherence tomography data that the clinically significant macular edema was not

merely due to vitreoretinal traction, which was seen in some patients. Blood tests were administered routinely at the diabetic examination, and we used the closest HbA1c value to the diabetic retinopathy screening.

Duration of diabetes was not included as a variable in this study because most of these subjects did not have health insurance, had not been seeking regular medical care, and did not remember the date of their diabetes diagnosis. Further evidence for the lack of reliability of self-report of duration of diabetes is shown for those who have not had regular urine or blood glucose testing, i.e. unknown values precede the first diagnosis of the disease or screening for diabetic retinopathy.²³

Macular Thickness Measurement

Retinal thickness and visualization of retinal pathology were obtained from spectral domain optical coherence tomography data (iVue, Optovue Inc, Fremont, CA). The iVue spectral domain optical coherence tomography used 840 ± 10 nm light with a power at the pupil of 750 μ W. The depth of resolution in tissue is 5 μ m, and the transverse resolution is 15 μ m. Each image covered a 6 $\times$ 6 mm area centered on the fovea, acquired at 26,000 A-scan/second and composed of 256 to 1024 A-scan/frame. A pseudo color topographic image is then constructed displaying average retinal thickness from the inner limiting membrane to the retinal pigment epithelium, with the average given numerically. The macular thickness map is divided into 9 regions with 3 concentric circles centered on the fovea, having diameters of 1 mm (innermost ring/fovea) defined as central macular thickness, 3 mm (inner ring), and 6 mm (outer ring) (Figure 1).^{2,5} While the thickness maps are computed from optical coherence tomography data consisting of half resolution volumes from a macular grid, the OptoVue stores only 7 full density b-scans for each eye. We reviewed the b-scans to make sure the Early Treatment Diabetic Retinopathy Study maps were accurately centered on the fovea ensuring each subject in this study had proper fixation. To ensure independence of samples, one eye per subject was selected for analysis. The optical coherence tomography b-scans were graded for fixation and the presence of macular edema versus which ones were false positives due to vitreo-retinal traction. However, in selecting the fovea, images of either eye of the subjects was selected based on proper fixation and good scan quality.

Central macular thickness measurements were compared for male and female diabetic subjects without clinically significant macular edema for a group of 21 male and 21 female diabetic subjects, who had good fixation and no other ocular complications and were matched with respect to age and HbA1c. The mean age of males (54 ± 9 yr, range = 38-70 yr) did not differ from females (57 ± 8 yr, range = 34-70 yr), $t(40) = 1.34$, $P = .19$. The mean HbA1c of males (7.71 ± 1.80 %, range = 5.3-11.4 %) did not differ from females (7.08 ± 1.52 %, range = 5.7-12.7 %), $t(40) = -1.22$, $P = .23$.

Next, to investigate whether the measured difference in retinal thickness between males and females without clinically significant macular edema accounted for any sex difference in central macular thickness when clinically significant macular edema was present, central macular thickness measurements were compared for the male and female subjects with the thickest retinas from the 142 subjects with clinically significant macular edema. Our initial observation as the Phase II data were collected was that some males had excessively thick

retinas. To test whether this finding was the case for the entire sample, we selected the 11 males with good fixation and no other ocular complications having the greatest central macular thickness, which were all $> 300 \mu\text{m}$. We excluded from this sample a male with the greatest central macular thickness because of the presence of subretinal fluid, leaving 11 and not 12 males. We compared these data to those of 11 females with the same criteria. The mean age of males (59 ± 11 yr, range = 33-74 yr) did not differ from females (60 ± 3 yr, range = 57-65 yr), $t(20) = 0.16$, $P = .88$. The mean HbA1c of males (8.52 ± 1.55 %, range = 6.6-10.8 %) did not differ from females (8.76 ± 2.12 %, range = 6.1-13.1 %), $t(17) = 0.28$, $P = .78$. HbA1c values were not available for two females and one male.

For subjects with the severe clinically significant macular edema, manual segmentation of the individual retinal layers in the b-scan centered on the fovea was performed using custom software (Matlab, Mathworks, Natick, MA) (Figure 2), as reported previously.²⁴ Manual segmentation, rather than automatic segmentation, was performed because most of these subjects had distorted retinal layers: the boundaries of these layers could not be automatically segmented (Figure 2). The location of the fovea was determined by the lowest point on the flattened b-scan, or the middle of the lowest points. The eccentricity was measured from this value, not the middle of the b-scan. The following retinal layers were segmented: nerve fiber layer, ganglion cell layer-inner plexiform layer complex, inner nuclear layer, outer plexiform layer, outer nuclear layer, inner segment, outer segment, and retinal pigment epithelium. The ganglion cell layer and inner plexiform layer were combined as one-layer complex because it was difficult to clearly segment these two layers. Individual retinal layer thickness measurements for regions 1 degree – 2 degrees outside the fovea center in the nasal and temporal retina were compared for males and females with clinically significant macular edema (Figure 3A). This region was chosen because the inner retinal layers are laterally displaced in the central fovea and hence there are no inner retinal layers in the center of the fovea. The central 1 mm region of the fovea was also analyzed for the outer retinal layers (outer nuclear layer, inner segment, outer segment, and retinal pigment epithelium) and compared between males and females with clinically significant macular edema (Figure 3B).

Statistical Analysis

All statistical analysis was performed using IBM SPSS Statistics for Windows, Version 24.0 (IBM Corporation, Armonk, NY, USA) and Microsoft Excel 2013 (Microsoft Corp., Redmond, WA, USA). All values are presented as mean $\pm$ standard deviation. An independent t test was used to compare central macular thickness separately for males and females without clinically significant macular edema and with clinically significant macular edema. An unpaired t-test compared males vs. females with clinically significant macular edema with the critical distance not zero, but rather the difference between mean retinal thickness for males and females without clinically significant macular edema. Thus, we determined whether males with clinically significant macular edema had extra thick retinas, and not merely thicker in the same amount as without clinically significant macular edema. To test proportions of males vs. females with clinically significant macular edema or with central macular thickness $> 300 \mu\text{m}$, we formed contingency tables and computed Chi

Square or Fisher's Exact Test, as appropriate for sample size. A p value < 0.05 was considered statistically significant.

For subjects with clinically significant macular edema, we compared the relation of retinal thickness to sex (male and female) and meridian (nasal and temporal) by performing a series of 2×2 mixed model ANOVA with sex and meridian as independent variables and each of the individual retinal layer thicknesses as the dependent variable. Inspection of the b-scans led us to perform a one-way random model intraclass correlation to investigate the association between the outer nuclear layer thickness versus outer plexiform layer thickness in subjects with clinically significant macular edema. A simple linear regression of outer nuclear layer thickness versus age was done separately for males and females with clinically significant macular edema. Similarly, an F-test for two sample variances was used to compare the variability between the outer nuclear layer thickness of males and females with clinically significant macular edema.

RESULTS

For subjects without clinically significant macular edema, males ($245 \pm 21.3 \mu\text{m}$) had a $12 \mu\text{m}$ significantly greater central macular thickness than females ($233 \pm 13.4 \mu\text{m}$), $t(40) = -2.18$, $P = .04$ (Figure 4A). However, for subjects with clinically significant macular edema, males ($383 \pm 48.7 \mu\text{m}$) had a $67 \mu\text{m}$ significantly greater central macular thickness than females ($316 \pm 60.4 \mu\text{m}$). When using the expected difference of the $12 \mu\text{m}$ difference between males and females without clinically significant macular edema, males had even thicker than expected retinas, $t(20) = 2.35$, $P = .015$ (Figure 4B). Thus, the sex difference in central macular thickness when clinically significant macular edema was present ($67 \mu\text{m}$) could not be explained by the sex difference observed when clinically significant macular edema was not present ($12 \mu\text{m}$). This sex difference of $55 \mu\text{m}$ for the most severe clinically significant macular edema was 5 – 6 X that of the diabetic subjects without clinically significant macular edema.

To understand the prevalence of clinically significant macular edema in our sample, we used conditional probabilities to remove sampling bias. Of the total number of underserved diabetic subjects (2048) screened with color fundus photographs and/or SD-OCT images, 941 (45.9%) were males and 1107 (54.1%) females. For subjects with gradable spectral domain optical coherence tomography images, 743 were males and 943 were females. Of the 142 subjects with clinically significant macular edema, 75 (52.8%) were males and 67 (47.2%) were females. By using the proportion of each sex that was included in the grading, the proportion of males graded as having clinically significant macular edema was 75 of 743 = 0.10 and that of females was 67 of 943 = 0.071, with the proportion of males significantly greater, $\chi^2(1) = 4.41$, $P = 0.036$. Despite no difference in age or HbA1c, 12 of 75 (16.0%) males graded with clinically significant macular edema had central macular thicknesses $> 300 \mu\text{m}$, but only 4 of 67 (5.97%) females. This difference in proportion did not reach statistical significance when the whole group with clinically significant macular edema was included in the analysis, $\chi^2(1) = 3.16$, $P = 0.075$. However, when only the 11 males and 11 females with the most extreme retinal thickening were classified by whether their central macular thickness $> 300 \mu\text{m}$, there was a significantly larger proportion of males (100%)

than females (36%), Fisher's exact test statistic = 0.0039. Thus, the males with the worst macular edema not only had thicker retinas on average than females, but males were overrepresented among those subjects with excessively thick retinas.

The distorted retinas of the 22 patients with the most severe clinically significant macular edema led to large standard deviations of the individually segmented retinal layers (Tables 1, 2). Nevertheless, certain individual layers between 1 – 2 degrees were thicker in males than in females with clinically significant macular edema. The nerve fiber layer was thicker in males ($13.3 \pm 2.85 \mu\text{m}$) than females ($10.1 \pm 6.13 \mu\text{m}$), $F_{1,40} = 4.89$, $P = .03$ (Figure 5A). For these samples near the foveal center of patients with retinal exudates, and cysts, and more general thickening, there was no significant difference between the nasal ($12.6 \pm 4.51 \mu\text{m}$) and temporal ($10.8 \pm 5.38 \mu\text{m}$) retina with respect to nerve fiber layer thickness, $F_{1,40} = 1.60$, $P = .21$ (Figure 5A). There was also no significant interaction of sex and meridian on nerve fiber layer thickness, $F_{1,40} = 1.10$, $P = .30$ (Figure 5A).

Males also had thicker ganglion cell layer-inner plexiform layer complex ($62.5 \pm 21.2 \mu\text{m}$) than females ($48.1 \pm 25.9 \mu\text{m}$), $F_{1,40} = 4.12$, $P = .049$ (Figure 5B). Nasal ($54.9 \pm 23.7 \mu\text{m}$) and temporal ($55.7 \pm 25.8 \mu\text{m}$) retina did not significantly differ with respect to ganglion cell layer-inner plexiform layer complex thickness, $F_{1,40} = .011$, $P = .92$ (Figure 5B). There was no significant interaction between sex and meridian on ganglion cell layer-inner plexiform layer complex thickness, $F_{1,40} = 2.04$, $P = .16$ (Figure 5B).

There was no significant effect of sex, meridian, or interaction of these variables on the remaining retinal layer thicknesses (inner nuclear layer, outer plexiform layer, outer nuclear layer, inner segment, outer segment, and retinal pigment epithelium) (Table 1), $P > .05$. The average values for thickness for males as compared to females were not greater or were similar to values for all the remaining retinal layers (Table 1). Results have been summarized in Table 1 to provide mean, standard deviation, and p-values of the different retinal layer thicknesses with respect to sex and meridian.

There were no significant differences between males and females for the layers of the outer retina in the central 1 mm foveal region (Table 2), $P > .05$. Outer nuclear layer thickness was significantly more variable in males than females, $F_{10,10} = 9.34$, $P < 0.001$ (Figure 6A). The outer nuclear layer thicknesses in males were highly variable, consistent with being more distorted by cysts (Figures 6B, C, and 7).

For subjects with the most severe clinically significant macular edema, outer nuclear layer thickness did not significantly depend on age for males, $F_{1,9} = 0.33$, $P = .58$ with an $R^2 = 0.035$, and females, $F_{1,9} = 0.11$, $P = .75$ with an $R^2 = 0.012$. There was a negative association between outer nuclear layer and outer plexiform layer thickness, but this association was not statistically significant, intraclass correlation coefficient = -0.46 , 95 % confidence interval (-0.73 , -0.056), $P = 0.99$. The negative trend in the association between outer nuclear layer and outer plexiform layer thickness is suggestive that as the outer nuclear layer thickens as a result of cystoid structures, the fluid pressure in the layer pushes against the outer plexiform layer and neighboring structures and flattens those layers and vice versa.

DISCUSSION

Diabetic macular edema is characterized by a compromise of the inner retinal blood barrier leading to the formation of intraretinal cysts, hard exudates, and disorganization of the inner retinal layers^{2-3,6-11,22-25} and also a compromised outer retinal blood barrier.^{2-3,12-13} In our subjects, evidence of subretinal fluid was found, causing us to omit from quantitative analysis the male patient with the thickest retina (Figure 7) due to the presence of subretinal fluid. As this subject had an exceptionally thick retina (819 μm), the greater central macular thickness of males as compared to females would have been even larger. There is a relationship between damage to the inner retinal neurons and/or photoreceptors and worse visual acuity in subjects with diabetic macular edema.^{11,15,25-27} Male sex has been identified as an independent risk factor for diabetic macular edema severity.¹⁷⁻¹⁸ Clinically, for diabetic patients with or without clinically significant macular edema, a more intensive treatment for males compared to females who are similar in age, and HbA1c may reduce the extent of neurodegeneration and preserve visual function.

Our results showed a 12- μm greater central macular thickness for male than female diabetic patients without clinically significant macular edema, who were matched with respect to age and HbA1c (Figure 4A). This is consistent with a previous study that found a 16- μm greater central subfield thickness in male than female diabetics with minimal or no diabetic retinopathy and normal macular morphology using optical coherence tomography.¹⁸ These similar results were found despite using a different subject population and different instrumentation. Thus, for diabetic subjects without clinically significant macular edema as well as for subjects with normal macular morphology, males have thicker retinas compared to females. For male and female diabetic subjects with clinically significant macular edema, despite being matched with respect to age and HbA1c, the retinal thickness and thus the pathology in males is greater than in females: our results showed a 67 μm greater central macular thickness for male than female diabetic subjects with clinically significant macular edema. Thus, for diabetic patients with clinically significant macular edema, the greater central macular thickness found for males compared with females could not be explained by routine differences in thickness with sex when clinically significant macular edema was not present, nor by age or HbA1c.

These structural changes are consistent with functional changes found in a previous study where local neuroretinal function in type 2 diabetics without retinopathy is shown to be worse in males compared to young females using multifocal electrogram.¹⁹ This suggests a mechanism of neuroprotection from the neurodegeneration that occurs with diabetes, such as the presence of estrogen.¹⁹⁻²⁰ Perhaps in the clinic, a more intensive management of diabetic males without clinically significant macular edema compared to diabetic females without clinically significant macular edema who are similar in age and HbA1c may reduce the progression of the neurodegeneration in these subjects and preserve visual function.

This indicates that for diabetic subjects with clinically significant macular edema, pathological changes and neurodegeneration in males are much worse than females. When comparing the thickness of retinal layers and variability of the outer nuclear layer of males versus females, we found differences were for thickened retinas for males, as well as more

variability of the outer nuclear layer for males. These results are consistent with that of a previous study that found that for diabetic subjects with retinopathy, males are more than six times likely to develop edema for a given local retinal location compared to females.¹⁷ This is consistent with our findings that males are more likely to have thicker and more distorted retinas than females. The greater distorted thicker retinas in the central 1 mm macular of males than females with clinically significant macular edema may also impact visual function in these subjects and therefore, a more intensive management and/or follow up of these subjects may be beneficial to control the edema and preserve visual function.

We investigated which retinal layers in regions at 1-2 deg from the fovea in the nasal and temporal retina were significantly thicker in male than female diabetics with clinically significant macular edema. The nerve fiber layer and ganglion cell layer-inner plexiform layer complex were found to be significantly thicker (Figure 5). Different layers other than the nerve fiber layer and ganglion cell layer-inner plexiform layer complex could be significantly thicker for regions of the retina other than 1-2 degrees nasal and temporal meridian. These differences in the structural changes of the nerve fiber layer and ganglion cell layer-inner plexiform layer complex, and indicated by the variable outer nuclear layer in the central fovea, may lead to differences in visual functions.

In the central 1 mm foveal region, the outer nuclear layer thickness was highly variable in male than female diabetics with clinically significant macular edema (Figure 6). This is suggestive that the outer nuclear layer in the central 1 mm foveal region of the male subjects with clinically significant macular edema was highly distorted by cystoid structures. Thus, the fluid pressure from the cystoid structures in the outer nuclear layer that causes retinal thickening presses against the neighboring retinal layers, along with deposition of materials leaked from blood vessels, resulting in thinning of those layers (Figure 6B). Also, when cystoid structures are present in neighboring layers such as the outer plexiform layer, the fluid pressure of the cystoid structures causes thickening of those layers and presses against the outer nuclear layer to cause thinning (Figure 6C). This is evident in the large variability in the outer nuclear layer thickness in male than female diabetics with clinically significant macular edema (Figure 6) and also in the negative trend of the association between outer nuclear layer vs. outer plexiform layer thickness even though the association was not statistically significant.

Outer nuclear layer thickness did not significantly depend on age for male and female diabetics with clinically significant macular edema. For our sample, we cannot support or disprove that outer nuclear layer thickness in diabetic subjects with clinically significant macular edema changes with advancing age. These associations may be different for a larger sample size, as a reduction in parafoveal thickness in diabetic patients with increasing age has been previously reported.²⁸

The results of this study should be interpreted in light of the following limitations. The subjects in this current study are from our Phase 2 SBIR study, and did not have regular eye examinations, but often had extremely high values of HbA1c. Our subjects were mainly of ethnic categories other than non-Hispanic Caucasians. Our subjects were mostly of working age, since older patients can have other options for eyecare. Our quantitative comparison of

mean central retinal thicknesses was for males vs. females with no difference for age or HbA1c, so as to minimize the noise in the analysis due to these factors. Particularly important, the typical ages of our subjects with the most severe changes are greater than the young ages at which males have been shown to have earlier or more severe differences.¹⁷⁻²⁰ Our subjects did not have visual acuity measures that would have helped quantify the impact of these structural changes on visual function. However, fixation stability, which has been shown to be associated with retinal thickness parameters in diabetic patients²⁷ is available from the experimental portion of the study, including for patients who did not have measurable optical coherence tomography values due to poor fixation.²⁹ Also, we did not include duration of diabetes as an independent variable in this study since these subjects did not have health insurance, had no long term data for blood glucose or lipids, and often did not recollect when they were diagnosed with diabetes. For most of our patients, it is likely that age is highly correlated with duration, and we reported analyses with age.

For building potential grading schemes or automatic classification methods, further work is needed to quantify focal changes and cover a wider area of retina. Our samples of retinal thickness are global averages over a region of interest, and we discussed between subject variability, not within subject variability, as we did not measure the focal change over cysts. Thus, while the inverse relation of outer nuclear layer and outer plexiform layer thicknesses, and variability of layer thickness was evident in the images of individual retinas, these were not demonstrated quantitatively with our method. We did not have a sufficiently large sample size to fully study the interaction of meridian and sex on clinically significant macular edema, retinal thickness, or the thickness of individual retinal layers. We did not measure individual layers outside the central macula, and these may better show thinning due to neurodegeneration.¹⁶

In conclusion, our results show that the central macular thickness in diabetic subjects with clinically significant macular edema is significantly greater in males than females. This difference is not explained by the difference in central macular thickness with sex when clinically significant macular edema is not present, nor by age or HbA1c. The nerve fiber layer and ganglion cell layer-inner plexiform layer complex were significantly thicker in the central macula for diabetic males with clinically significant macular edema compared to diabetic females with clinically significant macular edema. In diabetic subjects with clinically significant macular edema, the outer nuclear layer was highly variable and distorted by cystoid structures in males than females. Clinically, a more intensive management and/or follow up of diabetic males with or without clinically significant macular edema compared to diabetic females with or without clinically significant macular edema may be advisable in order to reduce the progression of neural damage or resolve the edema, and preserve visual function in these subjects.

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Figure 1.

Color fundus image, Early Treatment Diabetic Retinopathy Study map, and foveal b-scan of the left eye of a 53 yr old male with clinically significant macular edema. **(A)** Color fundus image showing areas of retinal thickening by hard exudates (thin arrows) in the fovea and superior-temporal to the fovea as well as a dot hemorrhage inferior-temporal to the fovea (thick arrow). **(B)** A 6×6 mm pseudo color topographic image centered on the fovea showing average retinal thickness from the inner limiting membrane to retinal pigment epithelium within the 9 regions of the Early Treatment Diabetic Retinopathy Study map. The Early Treatment Diabetic Retinopathy Study map consists of 3 concentric circles of diameters 1, 2, and 3 mm respectively (long, thin arrows). Central Macular Thickness measurements refer to the average retinal thickness within the 1 mm concentric circle (398 μm). **(C)** Foveal b-scan corresponding to the red line in panel B, showing cystoid structures (thin arrows) and distorted retinal layers.

Figure 2.

Foveal b-scan and corresponding Early Treatment Diabetic Retinopathy Study map of a 67 yr old male with clinically significant macular edema. **(A)** Manual segmentation of distorted retinal layers in the foveal b-scan. Foveal b-scan corresponds to the red line in panel B. Manual segmentation, instead of automatic segmentation, was performed because the retinal layers were so distorted that automatic detection of the retinal layer boundaries was inaccurate. **(B)** Corresponding Early Treatment Diabetic Retinopathy Study map showing average retinal thickness in the 9 regions and a central macular thickness of 444 μm .

Figure 3.

Left eye foveal b-scan of a 57 yr old female with clinically significant macular edema, and central macular thickness of 293 μm , showing the regions where the average individual retinal layer thickness measurements were computed. **(A)** Foveal b-scan showing regions 1 degree – 2 degrees outside the fovea center in the nasal and temporal retina (blue arrows). Average thickness of the following retinal layers was computed in these regions: Nerve Fiber Layer, Ganglion Cell Layer-Inner Plexiform Layer, Inner Nuclear Layer, Outer Plexiform Layer, Outer Nuclear Layer, Inner Segment, Outer Segment, and Retinal Pigment Epithelium. **(B)** Foveal b-scan showing the central 1 mm foveal region (blue arrow). Average thickness of the outer retinal layers (Outer Nuclear Layer, Inner Segment, Outer Segment, and Retinal Pigment Epithelium) was computed in this region. White star denotes the center of the fovea. Scale bar = 200 μm .

Figure 4.

Mean plot with standard deviation error bars comparing central macular thickness between males and females with clinically significant macular edema and without clinically significant macular edema. **(A)** Mean plot showing a significant 12 µm greater central macular thickness in males than females without clinically significant macular edema. **(B)** Mean plot showing a significant 67 µm greater central macular thickness in males than females with clinically significant macular edema. The sex difference in central macular thickness when clinically significant macular edema was present could not be explained by the sex difference observed when clinically significant macular edema was not present.

Figure 5.

The effect of sex and meridian on nerve fiber layer and ganglion cell layer-inner plexiform layer thickness. (A) Nerve fiber layer plotted for temporal and nasal meridians, showing a significantly thicker nerve fiber layer in males than females, $P = .03$. There is no effect of meridian and no significant interaction between sex and meridian on nerve fiber layer thickness, $P > .05$. (B) Ganglion cell layer-inner plexiform layer plotted for nasal and temporal meridians, showing a significantly thicker ganglion cell layer-inner plexiform layer in males than females, $P = .049$. There is no effect of meridian and no significant interaction between sex and meridian on ganglion cell layer-inner plexiform layer thickness, $P > .05$. Error bars represent standard deviations.

Figure 6.

Mean plot with standard deviation error bars and segmented b-scans of male subjects with clinically significant macular edema showing variability in the outer nuclear layer thickness in males. (A) Mean of outer nuclear layer, showing large variability for the outer nuclear layer thickness, especially for males. Outer nuclear layer was not on average thicker for males, $P = .32$, but was more variable $P < .001$. (B) Segmented B-scan of a 67 yr old diabetic male with clinically significant macular edema showing outer nuclear layer thickness of 211 μm in the central 1 mm foveal region. (C) Segmented B-scan of a 74 yr old diabetic male with clinically significant macular edema showing outer nuclear layer thickness of 19.5 μm in the central 1 mm foveal region, indicating the large variability in the outer nuclear layer thickness for males. The thinner outer nuclear layer observed at this location is because of cystoid structures in the outer plexiform layer which presses and flattens the neighboring outer nuclear layer. The central 1 mm retinal layers in males are highly distorted by cysts.

Figure 7.

Color fundus image, Early Treatment Diabetic Retinopathy Study map, and foveal b-scan of the left eye of a 51 yr old male Caucasian with clinically significant macular edema and subretinal fluid. **(A)** Color fundus image showing areas of retinal thickening, indicated by hard exudates (thin arrows) in the fovea, and a hemorrhage superior to the fovea (thick arrow). **(B)** A 6×6 mm pseudo color topographic image centered on the fovea showing extremely high average retinal thickness from the inner limiting membrane to retinal pigment epithelium within the 9 regions of the Early Treatment Diabetic Retinopathy Study map, central macular thickness 819 μm. **(C)** Foveal b-scan corresponding to the red line in panel B, showing cystoid structures and distorted retinal layers, hyperreflective foci, along with the subretinal fluid (thin arrows). This subject was omitted from quantitative analysis due to not being able to rule out other potential causes of the subretinal fluid, but this is a finding consistent with outer retinal blood barrier damage.

Table 1.Mean $\pm$ SD retinal layer thickness for gender and meridian.

Retinal Layers	Gender		<i>P</i> -value	Meridian		<i>P</i> -value
	M (μ m)	F (μ m)		N (μ m)	T (μ m)	
Nerve Fiber Layer	13.3 $\pm$ 2.85	10.1 $\pm$ 6.13	.03	12.6 $\pm$ 4.51	10.8 $\pm$ 5.38	.21
Ganglion Cell Layer-Inner Plexiform Layer Complex	62.5 $\pm$ 21.2	48.1 $\pm$ 25.9	.049	54.9 $\pm$ 23.7	55.7 $\pm$ 25.8	.92
Inner Nuclear Layer	50.1 $\pm$ 51.7	32.9 $\pm$ 26.7	.18	38.9 $\pm$ 25.9	44.1 $\pm$ 53.5	.68
Outer Plexiform Layer	35.9 $\pm$ 28.8	26.6 $\pm$ 8.77	.16	29.5 $\pm$ 10.5	33.0 $\pm$ 28.9	.59
Outer Nuclear Layer	104.5 $\pm$ 64.5	82.7 $\pm$ 34.4	.18	91.6 $\pm$ 50.8	95.6 $\pm$ 54.8	.80
Inner Segment	34.0 $\pm$ 6.04	32.6 $\pm$ 7.41	.53	33.4 $\pm$ 5.94	33.2 $\pm$ 7.56	.93
Outer Segment	22.4 $\pm$ 4.85	20.0 $\pm$ 4.88	.13	21.2 $\pm$ 5.13	21.2 $\pm$ 4.89	.95
Retinal Pigment Epithelium	23.9 $\pm$ 5.84	25.7 $\pm$ 4.24	.26	25.4 $\pm$ 4.27	24.2 $\pm$ 5.89	.43

Individual retinal layer thickness for male (M), female (F), 1-degree nasal (N), and 1-degree temporal (T) retina.

Table 2.

Mean $\pm$ SD outer retinal layer thickness for gender in the central 1 mm foveal region.

Retinal Layers	Gender		<i>P</i> -value
	Male (μm)	Female (μm)	
Outer Nuclear Layer	107 $\pm$ 72.2	84.3 $\pm$ 23.6	.32
Inner Segment	34.6 $\pm$ 4.65	33.4 $\pm$ 6.95	.63
Outer Segment	21.9 $\pm$ 5.61	22.4 $\pm$ 6.93	.88
Retinal Pigment Epithelium	24.4 $\pm$ 3.81	26.7 $\pm$ 4.38	.19

Central 1 mm outer retinal layer thickness for males and females