MACULAR THICKNESS IN DIABETIC AND NON-DIABETIC VETERANS AS MEASURED BY OPTICAL COHERENCE TOMOGRAPHY

A thesis presented to the graduate faculty of The New England College of Optometry in partial fulfillment of the requirements for the degree of Master of Science

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ABSTRACT (CHAPTER 2): RACIAL DIFFERENCES IN MACULAR THICKNESS IN HEALTHY EYES

This abstract is based on an article in press: *Optometry and Vision Science*, October 2007.

BAHARAK ASEFZADEH, O.D.

NEW ENGLAND COLLEGE OF OPTOMETRY, 2007

Purpose: The relationship between race and macular thickness remains unknown. This relationship may be important for early and accurate diagnosis of macular disease and glaucoma, and may also provide insight into disease mechanisms. In this study, we compared macular thickness in healthy eyes of Black and White subjects using Optical Coherence Tomography (Stratus OCT).

Methods: This study used a matched, cross-sectional design. Subjects underwent OCT macular thickness map scanning in each eye, four-field, 45-degree digital retinal imaging in each eye, and blood pressure measurement. Retinal images were evaluated for absence of posterior pole disorders, including macular and optic nerve disease. Retinal thickness was evaluated in the central fovea, and in rings placed at 1 mm, 3 mm and 6 mm from fixation.

Results: Compared to Whites (n = 7), Blacks (n = 7) had significantly thinner total foveal thickness (TFT, retinal thickness in the central 1 mm diameter area; OD: $p < 0.03$; OS: $p < 0.02$; OU average: $p < 0.02$), and thinner total macular thickness (TMT, retinal thickness in 6mm diameter area excluding central foveal thickness; OS: $p < 0.02$; OU average: $p < 0.03$).

There was a trend for central foveal thickness (retinal thickness at fixation) to be thinner in Blacks than Whites (OD: $p = 0.12$; OS: $p = 0.08$). There was no significant difference in macular thickness between right and left eyes.

Conclusions: Retinal thickness as measured by Stratus OCT in the fovea and macula is significantly thinner in Blacks compared to age-matched Whites. Larger multi-racial prospective studies are needed to confirm these results and to evaluate the need for race-specific normative values.

ABSTRACT (CHAPTER 3): MACULAR THICKNESS IN NON-DIABETIC AND DIABETIC EYES WITH NO OR MILD RETINOPATHY: INFLUENCE OF RACE AND RELATIONSHIP WITH SYSTEMIC MARKERS FOR DIABETES

BAHARAK ASEFZADEH, O.D.

NEW ENGLAND COLLEGE OF OPTOMETRY, 2007

Purpose: To evaluate the relationship between macular thickness, race, and systemic markers for diabetes in individuals with no or mild diabetic retinopathy using Optical Coherence Tomography (StratusOCT).

Methods: This was a prospective, cross-sectional study. All subjects underwent digital retinal imaging and OCT macular thickness scanning OU. Retinal images were assessed for absence of posterior pole disease. Retinal thickness was evaluated at 37 points along six linear scans centered at fixation and in rings located at 1 mm, 3 mm, and 6 mm from center. Retinal thickness was assessed in retinal quadrants, rings, and hemispheres. Central foveal thickness (CFT), total foveal thickness (TFT), and total macular thickness (TMT) were also determined. Continuous data were analyzed by using two-tailed, unpaired Student's *t*-tests, linear regression or multiple regression. Differences between groups were analyzed using one-way or two-way factorial analysis of variance (ANOVA) for independent samples.

Results: A total of 92 non-diabetic control subjects, 92 subjects with diabetes and no DR, and 24 subjects with mild DR were evaluated. In subjects with diabetes, there was a significant negative correlation between retinal thickness and diabetes duration in all macular quadrants, rings, and hemispheres. CFT, TFT, and TMT were also significantly thinner with longer duration of disease (CFT: $p = 0.0025$, r = -0.28; TFT: $p = 0.0062$, r = -0.25; TMT: $p = 0.0026$, r = -0.28). In subjects with diabetes, there were no significant relationships between retinal thickness and HbA1c level (average of last 3 readings), systolic blood pressure, diastolic blood pressure, blood glucose, or triglyceride levels. Additionally, no significant differences in retinal thickness were found between control subjects, subjects with no DR, and subjects with mild DR in any retinal sector. Black subjects ($n = 26$) showed significantly thinner macular thickness compared to White subjects ($n = 179$) in the Control, No DR, and Mild DR groups (CFT: $p < 0.0001$; TFT: $p < 0.0001$; TMT: $p < 0001$; ANOVA).

Conclusions: In subjects with no or mild diabetic retinopathy, macular and foveal thickness as measured by OCT is significantly thinner with longer duration of disease. This may reflect neurodegenerative changes in the diabetic retina with loss of neuronal cell bodies and axons. Black subjects had thinner macular thickness compared to White subjects in all groups. Future studies should confirm these findings in larger populations. Also, longitudinal studies should examine the evolution of retinal thinning with OCT and its relationship to retinal functional alterations.

Acknowledgements

I would like to thank my thesis advisor, Dr. Tony Cavallerano, for his mentorship and unfaltering willingness to help in both the execution and write-up of this study. I would also like to thank the VA Optometric Research Fellowship Director, and my mentor, Dr. Barry Fisch, for his guidance and frequent intellectual challenges. Lastly, I thank my husband, Michael, for supporting this endeavor with abundant and infectious enthusiasm.

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CHAPTER 1
GENERAL OVERVIEW

Diabetes is a leading cause of severe vision loss in the United States. Approximately 4.1 million (1 in 29) US adults 40 years and older have diabetic retinopathy (DR); 1 of every 12 persons with Diabetes Mellitus (DM) in this age group has advanced, vision-threatening retinopathy (Kempen, O'Colmain, Leske, Haffner, Klein, Moss, Taylor, and Hamman, 2004). Due to the aging of the US population and increasing prevalence of diabetes, these figures are expected to increase substantially by the year 2020, thus posing an even more formidable public health problem.

Vision loss from diabetes: Severe vision loss (best-corrected visual acuity 5/200 or worse) due to diabetic eye disease largely results from proliferative diabetic retinopathy (PDR), while moderate vision loss (doubling of the visual angle) primarily results from diabetic macular edema (DME: Aiello, Cahill, and Wong, 2001). Ninety percent of diabetics in America have type 2 DM, and since DME is more common than PDR in these individuals, a greater number of people suffer vision loss from DME than PDR (Klein, Davis, Segal, Long, Harris, Haug, Magli, Syrjala, 1984a). Additionally, many diabetic persons with moderate vision loss from DME will eventually meet the criteria for legal blindness in the United States, and thus generate significant medical and governmental expenses.

Risk factors for DME: There are several risk factors for developing DME. The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) found that patients diagnosed with

DM at age 30 years or older and with greater than 5 years duration of disease have 10 times greater prevalence of DME than diabetics with less than 5 years duration (Klein, Klein, Moss, Davis, and DeMets, 1984b). The Early Treatment of Diabetic Retinopathy Study Research group reported 33% risk of developing moderate vision loss from DME after 3 years of disease duration (ETDRS, 1985). In addition to duration of diabetes, diabetic macular edema is associated with insulin use, presence of proteinuria, higher glycosylated hemoglobin levels, and higher systemic arterial blood pressure (Klein et al., 1984b: Klein, Klein, Moss, Davis, and DeMets, 1984c; Klein, Klein, Moss, and Palta, 1995a; Klein, Klein, Moss, and Cruickshanks, 1995b; Klein, Moss, Klein, and Surawicz, 1991; Klein, Klein, and Moss, 1996; Klein, Klein, Moss, and Cruickshanks, 1994; ETDRS, 1985; Lopes de Faria, Jalkh, Trempe, and McMeel, 1999; UKPDS 1998; Matthews, Stratton, Aldington, Holman, and Kohner, 2004; UKPDS, 1998a; Miljanovic, Glynn, Nathan, Manson, and Schaumberg, 2004; Kohner, Aldington, Stratton, Manley, Holman, Matthews, and Turner, 1998; Tovi, Ingemansson, and Engfeldt, 1998). Indeed, diabetic patients with concomitant hypertension have been reported to be 3.2 times more likely to develop diffuse DME (Lopes de Faria et al., 1999). DME is also associated with cardiovascular disease and higher serum lipids (Lopes de Faria et al., 1999; Miljanovich et al., 2004).

Reduction of the incidence and progression of DME and DR: Diabetic macular edema and diabetic retinopathy are end-organ complications of systemic disease. Therefore, early diagnosis and metabolic control are of critical significance for prevention or at least postponement of potential visual compromise. Large clinical trials have demonstrated the

benefits of obtaining near-normal blood glucose levels in patients with diabetes as measured by reduced progression of DR, reduced incidence of PDR and DME, and reduced systemic microvascular complications (DCCT, 1995; UKPDS, 1998a; DCCT, 1993). Control of concomitant risk factors, such as high blood pressure and high cholesterol, further reduces the risk of diabetic complications, such as diabetes-related death, progression of diabetic retinopathy, and deterioration in visual acuity (UKPDS, 1998b).

In addition to improving systemic control of diabetes and other risk factors, the major clinical trials in the area of diabetic eye disease, (ETDRS, DCCT, UKPDS), have demonstrated that laser photocoagulation also reduces the risk of visual loss. Laser photocoagulation according to ETDRS protocol is the current standard of care for patients with PDR and/or DME. Scatter laser photocoagulation significantly reduces the risk of severe vision loss from PDR (ETDRS, 1991a), and focal laser photocoagulation reduces the risk of moderate vision loss from DME by 50% or more (ETDRS, 1985).

Analysis of the macula via OCT: A major challenge faced by eye care professionals in the care of diabetic patients is appropriate diagnosis of eye findings that may lead to imminent or future vision loss. Clearly, earlier diagnosis is critical for timely referral for either laser treatment or tightening of systemic control. In cases of DME, diagnosis is traditionally based on ophthalmoscopically visible retinal changes, and frequently, the findings are confirmed by fluorescein angiography. Recently, however, Optical Coherence Tomography (StratusOCT^{TM}, Zeiss Meditec, USA) has revolutionized the way clinicians can assess the

retina. OCT allows non-invasive, *in-vivo* visualization of retinal anatomy that is rapid and easy to acquire, and well-tolerated by patients. Using Michelson interferometry and short coherence light (820 nm), OCT generates tomographic, cross-sectional retinal images displayed on a pseudocolor scale. Images have resolution of approximately 10 μm, far superior to slit lamp biomocroscopy and other *in-vivo* imaging techniques such as scanning laser ophthalmoscopy or B-mode ultrasound. In addition to retinal morphological information, OCT generates quantitative information regarding thickness of the retina and retinal nerve fiber layer. Objective quantification of retinal thickness is advantageous not only for improving diagnostic accuracy, but also for monitoring change over time. This is especially relevant in DME, where diagnosis is intimately tied to an increase in retinal thickness, and recovery linked to reduction in retinal thickness.

Quantitative investigation of DME with OCT: Indeed, the most sensitive parameter related to DME is precise measurement of macular thickness (Goebel and Kretzchmar-Gross, 2002). OCT has been shown to be an accurate technique for quantifying macular thickness in patients with DME (Hee, Puliafito, Duker, Reichel, Coker, Wilkins, Schuman, Swanson, and Fujimoto, 1998). In such individuals, retinal structural changes and increased retinal thickness are characteristic (Otani, Kishi, and Maruyama, 1999). Additionally, macular thickness in DME correlates with worsening visual acuity (Lattanzio, Brancato, Pierro, Bandello, Iaccher, Fiore, and Maestranzi, 2002; Yang, Cheng, Lee, Hsu, and Liu, 2001; Goebel et al., 2002) and may be a sensitive indicator of visual acuity recovery after surgical treatment.

Quantitative investigation of the diabetic macula without DME using OCT: In addition to detection of DME and quantification of retinal thickness in cases where the macula is edematous, OCT seems to be more sensitive than slit-lamp biomicroscopy to small changes in retinal thickness where slit-lamp exam appears normal (Hee, Puliafito, Wong, Duker, Reichel, Rutledge, Schuman, Swanson, and Fujimoto, 1995; Brown, Solomon, Bressler, Schachat, DiBernardo, and Bressler, 2004). This prompts the following questions: 1) can OCT detect subclinical changes in retinal thickness in individuals with diabetes and normal retinal exam by slit-lamp biomicroscopy? 2) Do diabetic individuals differ from non-diabetic counterparts in terms of retinal thickness?

Previous studies have produced conflicting results regarding whether retinal thickness in diabetics without diabetic retinopathy and without clinically visible macular edema differs from retinal thickness in non-diabetic counterparts. Some studies have shown that compared to controls, macular thickness is increased in diabetic subjects at the superior nasal foveal rim (Schaudig, Glaefke, Scholz, and Richard, 2000) and at the fovea (Lattanzio et al., 2002; Sanchez-Tocino, Alvarez-Vidal, Maldonado, Moreno-Montanes, and Garcia-Layana, 2002). In contrast, other studies have found no difference in retinal thickness in any macular sector between diabetic and non-diabetic subjects (Massin, Erginay, Haouchine, Mehidi, Paques, and Gaudric, 2002; Alkuraya, Kangave, and Abu El-Asrar, 2005).

Racial differences in diabetic eye disease: Previous studies of macular thickness in individuals with no or minimal diabetic retinopathy have not evaluated the effect of race.

This is of relevance since the prevalence of diabetic retinopathy, the rate of blindness from diabetic eye disease, and the rate of visually significant macular edema is higher in Black populations compared to White populations in the U.S. (Rabb, Gagliano, and Sweeney, 1990; Harris, Feldman, Robinson, Sherman, and Georgopoulos, 1993; Harris, Klein, Cowie, Rowland, and Byrd-Holt, 1998). The discrepancy between Blacks and Whites has been attributed to worse overall systemic control in Blacks compared to Whites, especially in regards to systemic arterial blood pressure. Any potential difference in macular thickness in these populations, adjusted for blood pressure and other risk factors, may offer novel insight into disease mechanisms. Analysis of potential differences between normal, non-diabetic eyes in Black and White populations is also warranted for comparative purposes.

Pathogenesis of DR: In all populations with diabetic retinopathy, pathogenesis clearly involves microvascular damage, as evidenced by hemorrhages, microaneurysms, venous caliber changes, and exudates. However, there appears to be a neurodegenerative component to DR development as well. Prior to onset of clinically visible retinopathy, histological studies using rat models have shown apoptosis of retinal neural cells and reduction in retinal thickness (Aizu, Oyanagi, Hu, and Nakagawa, 2002; Park, Park, Park, Kim, Chung, Chun, and Oh, 2003). Neurodegeneration in DR is further supported by reduced retinal function demonstrated on electroretinography in both humans and rats with no visible DR (Di Leo, Falsini, Caputo, Ghirlanda, Porciatti, and Greco, 1990; Caputo, Di Leo, Falsini, Ghirlanda, Porciatti, Minella, and Greco, 1990; Ghirlanda, Di Leo, Caputo, Falsini, Porciatti, Marietti, and Greco, 1991; Fortune, Schneck and Adams, 1999; Bearse, Han, Schneck and Adams

2004a; Bearse, Han, Schneck, Barez, Jacobsen, and Adams, 2004b). On OCT, microvascular changes are expected to show increased local retinal thickness, whereas neurodegenerative changes are expected to show reduced thickness. It is unclear which process occurs first, or if both evolve concurrently. It is also unclear if these processes differ between races.

Clinical Implications: The investigation of *in-vivo* macular architecture in diabetic individuals with no or very minimal retinopathy, and no macular edema, may provide novel information about neurodegeneration in the diabetic retina. This is of clinical relevance because visual acuity is typically intact at this stage and improving glycemic control may preserve visual function. This prompts another question: is there a relationship between macular thickness in diabetic individuals with no or minimal retinopathy, and no macular edema, and systemic markers for diabetes control? As stated above, incidence and progression of diabetic eye disease have been linked to worse glycemic control (as measured by HbA1c and blood glucose levels), higher systemic arterial blood pressure, and longer duration of diabetes. This effect is especially pronounced in Blacks compared to Whites. The relationship between these risk factors and macular thickness has yet to be formally explored. Elucidation of such relationships may have clinical implications and influence how eye care providers monitor their diabetic patients.

Since visual impairment from diabetic retinal disease is preventable, it is important to identify new paradigms for the detection of early retinal structural alterations. OCT allows

novel *in-vivo* examination of retinal structure and may provide valuable information regarding retinal thickness changes prior to the onset of clinically appreciable DR. Given that race, increased duration of diabetes, worse diabetes control, and higher systemic arterial blood pressure have been linked to the prevalence and progression of diabetic eye disease, it is important to evaluate macular thickness in light of these factors. In order to address these issues, the aims of this study are: 1) to investigate differences in macular thickness between normal Black and White subjects; 2) to investigate differences in macular thickness between Black and White subjects with diabetes and no or minimal DR; 3) to investigate the relationship between macular thickness in subjects with no or minimal DR and systemic markers for diabetes control, and 4) to investigate differences in macular thickness between subjects with diabetes and no or minimal DR compared to non-diabetic subjects.

CHAPTER 2

RACIAL DIFFERENCES IN MACULAR THICKNESS IN HEALTHY EYES

This chapter is based on an article in press: *Optometry and Vision Science*, October 2007.

Introduction: The relationship between race and normal macular thickness remains poorly characterized. Diagnosis of macular abnormalities by Optical Coherence Tomography often depends upon comparison to normative values. While the normative database of the Stratus OCT™ includes subjects of several racial backgrounds, the majority of subjects were Whites (per com: Zeiss Meditec). If they exist, race-dependent differences in normal retinal thickness should be taken into account for accurate diagnosis of macular disease.

Glaucoma and diabetic macular edema are more prevalent in Blacks than in Whites (Tielsch, Sommer, Katz, Royall, Quigley, and Javitt, 1991; Wong, Klein, Islam, Cotch, Folsom, Klein, Sharrett, and Shea, 2006; Racette, Wilson, Zangwill, Weinreb, and Sample, 2003) and can affect macular thickness. In glaucoma, loss of retinal nerve fibers leads to decreased total macular thickness (Lederer, Schuman, Hertzmark, Heltzer, Velazques, Fujimoto, and Mattox, 2003; Guedes, Schuman, Hertzmark, Wollstein, Correnti, Mancini, Lederer, Voskanian, Velazquez, Pakter, Pedut-Kloizman, Fujimoto, and Mattox, 2003; Kanadani, Hood, Grippo, Wangsupadilok, Harizman, Greenstein, Liebmann, and Ritch, 2006; Medeiros, Zangwill, Bowd, Vessani, Susanna, and Weinreb, 2005) while in diabetic macular edema, accumulation of intraretinal fluid causes an increase in total macular thickness (Hee et al., 1998; Hee et al.,

1995). Identification of racial differences in macular thickness in healthy eyes may be useful to predict early or sub-clinical macular edema or glaucoma, and may also provide insights into disease mechanisms. As a first step towards this goal, we compared mean macular, foveal, and central foveal thickness in healthy eyes of Blacks and Whites.

Methods: This was a matched, cross-sectional study. Subjects were recruited as they presented for a routine eye examination at the Jamaica Plain Veteran's Affairs Medical Center. Sample size was determined prospectively for adequate statistical power to detect a difference between the means in Black and White samples. The study protocol followed the tenets of the Declaration of Helsinki and was approved by the VA Boston Institutional Review Board and Research and Development Committee. All subjects provided informed consent. Black and White subjects were matched for age and gender, and similar refractive error and mean arterial blood pressure. White subjects were matched to Black subjects without regard for retinal thickness data.

Selection Criteria: Subjects were excluded from the study if they met any one of the following criteria: 1) best-corrected visual acuity worse than 20/70 in either eye due to media opacity; 2) any macular disorder, including diabetic macular edema and drusen; 3) abnormal Amsler grid testing; 4) previous retinal laser treatment (e.g. focal or scatter photocoagulation); 5) central visual field defect preventing fixation on OCT; 6) poor quality fundus or OCT images; 7) diagnosis of diabetes; 8) diagnosis of impaired glucose tolerance or impaired fasting glucose; 9) intraocular inflammation; 10) diagnosis of glaucoma; 11)

previous intraocular surgery apart from cataract extraction; 12) cataract extraction within the past 12 months; 13) refractive error greater than or equal to 6 diopters, or 14) inability or unwillingness to provide informed consent.

Data Collection: We obtained self-report of race and ethnicity. Medical and ocular histories were obtained from the VA computerized patient record system (CPRS). All subjects underwent pupillary dilation. Optical Coherence Tomography (Stratus OCT, Zeiss Meditec™, USA) Macular Thickness Mapping was acquired in each eye, in addition to four-field, 45-degree digital retinal imaging (Canon, USA) in each eye obtained according to VA digital imaging standards (Cavallerano, Cavallerano, Katalinic, Blake, Rynne, Conlin, Hock, Tolson, Aiello, and Aiello, 2005). All subjects also underwent blood pressure measurement with the Omron IC Intellisense™ Digital Blood Pressure Monitor after imaging was completed. Snellen visual acuity, Amsler Grid, refractive error, and intraocular pressure were recorded from the CPRS comprehensive eye exam notes from the same day as the study.

Data Analysis: Stratus OCT: Macular thickness was determined at 37 points along six, 6mm line scans (Figure 2.1). Each data point was automatically calculated by entering pre-determined axial scan numbers along each of the six lines using the Retinal Thickness Analysis Protocol (Figure 2.2).

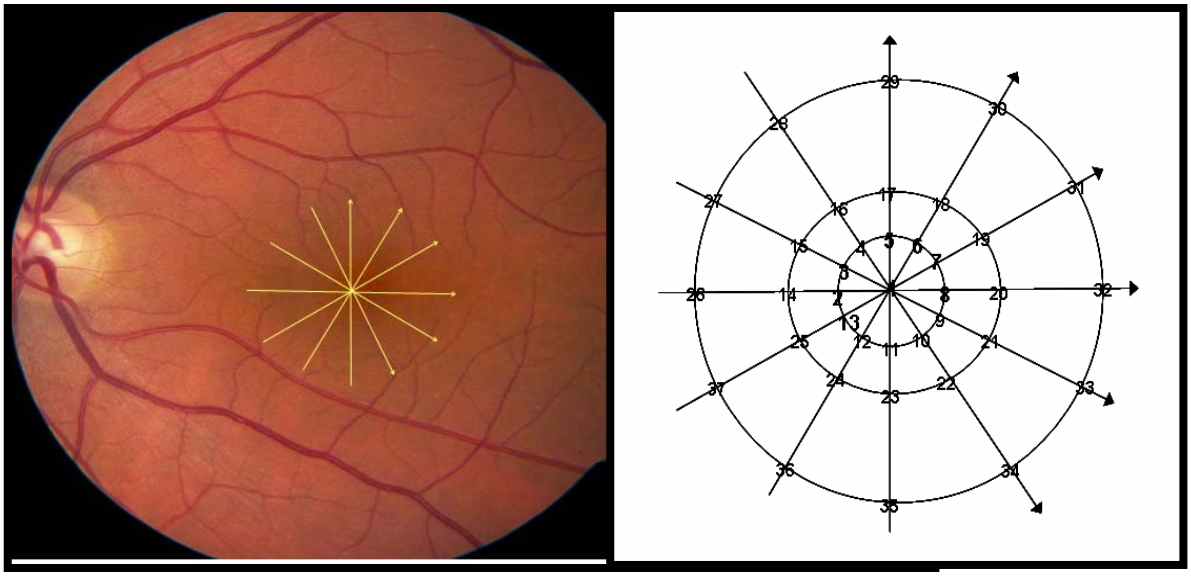


FIGURE 2.1: Retinal Thickness Map Protocol. a) Stratus OCT Macular Thickness Map of the left eye consisting of six, 6 mm line scans. b) Retinal thickness measurement for the left eye at 37 points, with inner ring diameter of 1mm, middle ring diameter of 3mm, and outer ring diameter of 6 mm.

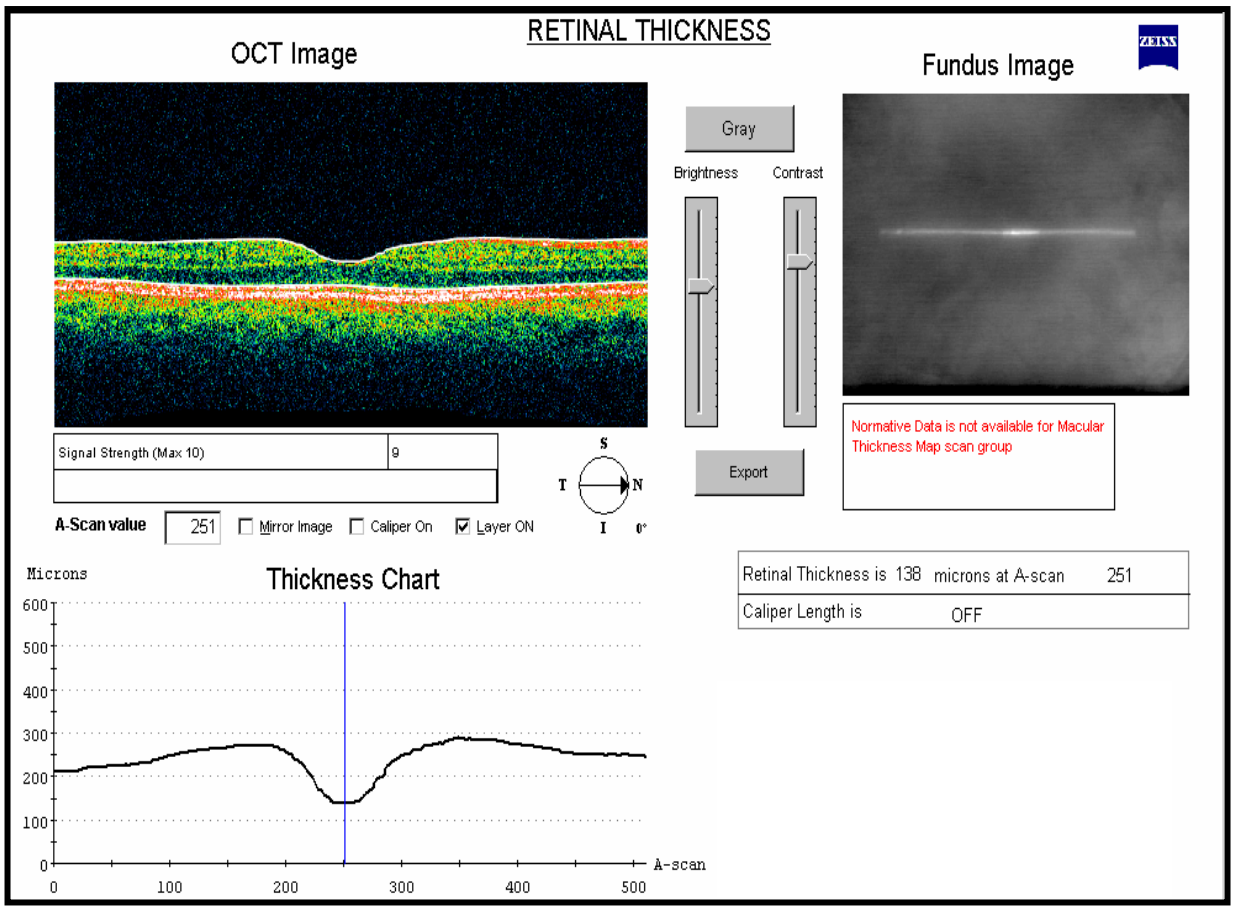


FIGURE 2.2: : Retinal Thickness Measurement Protocol: After using the "Macula Map" protocol to acquire 6 radial line scans, retinal thickness values were calculated by entering 7 pre-determined axial scan numbers along each of the six radial line scans using the "Retinal Thickness" Analysis Protocol.

Mean central foveal thickness (CFT) for each subject was calculated as the average of measurements from the midpoint of each individual line scan (data point 1 in Figure 2.1b). Mean total foveal thickness (TFT) was calculated as the average of measurements at 12 points along a ring at 1000 μm from fixation and the average central foveal thickness from the midpoint of each individual line scan (data points 2-13 in Figure 2.1b). Mean total macular thickness (TMT) was calculated as the average of all values within the 6000 μm ring with the exclusion of central foveal measurements (data points 2-37 in Figure 2.1b). Average macular retinal nerve fiber layer (RNFL) thickness was measured using the RNFL Thickness analysis protocol.

Fundus Images: Digital fundus images were evaluated for lack of macular disease by optometrists who were trained and certified in digital retinal image reading for diabetic retinopathy.

Statistical Analysis: Continuous data were analyzed by two-tailed, unpaired Student's *t*-tests. Pearson's correlation coefficient was used to assess the relationship between macular thickness and age, and macular thickness and mean arterial blood pressure.

Results: *Subject Characteristics:* Seven non-Hispanic Black subjects and 7 age-matched non-Hispanic White subjects were evaluated. Table 2.1 summarizes subject characteristics. All subjects were male. No significant differences in mean systolic and diastolic arterial blood pressure, spherical equivalent refraction, and intraocular pressure were found between Black and White subjects.

	Whites (***n*** **= 7)**	**Blacks** (***n*** **= 7)**	***p***
Mean Age (yrs, range)	69 ± 5 (64-74)	69 ±5 (64-74)	1.00
Mean Systolic Blood Pressure (mm Hg)	136 ±18	138 ±18	0.79
Mean Diastolic Blood Pressure (mm Hg)	82 ±11	79 ±10	0.59
Mean Spherical Equivalent OD (diopters)	+0.89 ± 0.81	+0.57 ± 0.43	0.38
Mean Spherical Equivalent OS (diopters)	+0.89 ± 1.0	+0.57 ± 0.40	0.46
Mean IOP OD (mm Hg)	15 ± 2	16 ± 2	0.49
Mean IOP OS (mm Hg)	15 ± 2	16 ± 3	0.67

TABLE 2.1: Systemic and ocular characteristics for age and gender matched subjects

Table 2.2 summarizes individual subject characteristics and retinal thickness values. No subject was myopic. Spherical equivalent refractions ranged from plano to +2.75 D. Average mean arterial blood pressure (MAP) was 100 ±13 mm Hg and 99 ±10 mm Hg for Blacks and Whites, respectively (p = 0.85). Additionally, there were no significant differences in CFT, TFT, or TMT between right and left eyes in either group

Retinal Thickness Differences: Table 2.3 summarizes the retinal thickness data. Mean total foveal thickness (TFT) and mean total macular thickness (TMT) were significantly thinner in Blacks than in Whites (TFT OD: $p < 0.03$; TFT OS: $p < 0.02$; TFT OU average: $p < 0.02$; TMT OS: $p < 0.02$; TMT OU average: $p < 0.03$). There was a trend for central foveal thickness (CFT) to be thinner in Blacks compared to Whites (p = .12 OD; p = 0.08 OS). Average macular RNFL thickness in Blacks was 27 ± 2 µm OD, 26 ± 3 µm OS compared to 32 ± 6µm OD and 30 ± 6µm OS in Whites (p = .09 and p = 0.12, respectively). The numbers of subjects with detached, partially attached, and attached vitreous were similar in both groups. No correlation between average retinal thickness OU and MAP was found in either group.

TABLE 2.2: Individual subject characteristics and retinal thickness values

SN	Race	Age (yrs)	MAP (mm Hg)	VA OD	VA OS	SE OD	SE OS	CFT OD (μm)	CFT OS (μm)	TFT OD (μm)	TFT OS (μm)	TMT OD (μm)	TMT OS (μm)
1	Black	60	92	20/20	20/20	Plano	Plano	203	186	234	245	245	240
2	White	60	98	20/20	20/20	Plano	+.25	202	185	220	235	229	234
3	Black	63	86	20/20	20/20	+1.0	+1.25	162	175	197	208	238	237
4	White	63	84	20/20	20/20	+1.5	+1.75	167	145	219	234	241	245
5	Black	66	92	20/20	20/20	+.50	+.75	131	132	197	207	238	237
6	White	66	87	20/20	20/20	Plano	Plano	129	141	208	221	250	249
7	Black	68	108	20/20	20/20	+.25	+.25	151	153	203	215	231	229
8	White	68	104	20/20	20/20	+1.0	+.75	212	227	260	276	271	265
9	Black	71	113	20/20	20/20	+.50	+.50	180	177	218	237	241	236
10	White	71	117	20/20	20/20	+2.25	+2.75	208	193	234	248	254	250
11	Black	74	108	20/20	20/20	+.50	+.50	132	127	180	189	209	210
12	White	74	115	20/20	20/20	+1.0	+.75	178	192	218	232	233	228
13	Black	74	93	20/20	20/25	+1.25	+.75	160	136	200	198	211	206
14	White	74	94	20/20	20/20	+.50	Plano	192	189	234	253	248	252

SN: Subject Number; MAP: mean arterial pressure; SE: spherical equivalent; CFT: central foveal thickness; TFT: total foveal thickness; TMT: total macular thickness; OD: right eye; OS: left eye

TABLE 2.3: Mean Retinal Thickness by Race (μm)

	Whites (***n*** = 7)	**Blacks** (***n*** = 7)	***p***
Mean CFT OD	**184 ± 29**	**160 ± 26**	**0.12**
Mean CFT OS	**182 ± 29**	**155 ± 24**	**0.08**
Mean TFT OD	**228 ± 17**	**204 ± 17**	**< 0.03***
Mean TFT OS	**243 ± 18**	**214 ± 20**	**< 0.02***
Mean TMT OD	**248 ± 14**	**232 ± 13**	**0.05**
Mean TMT OS	**248 ± 13**	**229 ± 13**	**< 0.02***

CFT, central foveal thickness; TFT, total foveal thickness; TMT, total macular thickness; *****statistically significant

Discussion: As retinal quantification becomes more prevalent in clinical decision making, it is important to investigate racial differences in normative data. In the present study, we found that Blacks have significantly thinner foveal and macular thickness compared to Whites.

Retinal nerve fiber layer: Previous studies of healthy eyes demonstrate that mean peripapillary RNFL is similar in Blacks compared to Whites (Racette, Boden, Kleinhandler, Girkin, Liebmann, Zangwill, Medeiros, Bowd, Weinreb, Wilson, and Sample, 2005), while other studies show that it is significantly thinner (Tjon-Fo-Sang and Lemij, 1998; Poinoosawmy, Fontana, Wu, Fitzke, and Hitchings, 1997). A potential hypothesis regarding why total macular thickness was thinner in Blacks compared to Whites in our study is thinner RNFL. Peripapillary RNFL correlates with total macular thickness (Greenfield, Bagga, and Knighton, 2003; Wollstein, Schuman, Price, Aydin, Beaton, Stark, Fujimoto, and Ishikawa, 2004). Retinal thickness in the macula as measured by OCT includes the RNFL. Mean macular RNFL thickness has been reported as 30.44 ± 4.11 μm in normal Chinese eyes (Leung, Chan, Yung, Ng, Woo, Tsang, and Tse, 2005) and 44.8 ±14.8 μm in a normal Latino population (Varma, Bazzaz, and Lai, 2003). In the present study, there was a trend for average macular RNFL thickness to be thinner in Blacks compared to Whites (p = .09 OD and p = 0.12 OS). However, this only corresponded to an approximately 5 μm difference. It therefore does not seem likely that thinner RNFL alone fully accounts for the approximately 20-30 μm difference we observed in TFT and TMT in Blacks compared to Whites, especially since no subject had glaucoma or other disease affecting the RNFL. Furthermore, we found

a trend for thinner retinal thickness at fixation (CFT), a measurement that does not include the RNFL, in Blacks compared to Whites (p = .12 OD; p = 0.08 OS). While these approximately 24-27 μm differences approached but did not meet statistical significance, they may suggest race-specific outer retinal thickness variation. Further studies with a larger sample size are required to address these issues.

Axial length and refractive error: Axial length and refractive error may cause differences in OCT macular thickness measurements. Increasing myopia is associated with reduced macular thickness (Huynh, Wang, Rochtchina, and Mitchell, 2006). However, there is no correlation between retinal thickness and amount of myopia when high myopes are excluded (Mrugacz, Bakunowicz-Lazarczyk, and Sredzinska-Kita, 2004). We did not measure axial length in our study, but did record refractive error for each subject. Important features of our sample are that no eyes were myopic and all spherical equivalent refractions ranged from plano to +2.75 diopters. There was no significant difference in refractive error between Black and White groups. Therefore, it is unlikely that refractive error played a role in the differences we observed between groups.

Systemic arterial blood pressure: It is not known if systemic blood pressure affects macular thickness in normal eyes. Our sample had a relatively narrow range of blood pressure measurement, since all subjects with systemic arterial hypertension were well-controlled medically. We found no correlation between mean arterial pressure (MAP) and macular thickness in Black and White groups.

Study Limitations: Further study of larger samples will help to confirm the observations of this study. It will also be important to study these phenomena in women and children. While a previous study found significantly greater average macular thickness in the central 1000 micron diameter area in men compared to women (Massin et al., 2002) more recent studies demonstrate no difference in macular thickness between men and women (Chan, Duker, Ko, Fujimoto, and Schuman, 2006; Lattanzio et al., 2002). Although we were able to evaluate Blacks and Whites, our population did not include any Asian, Native American, Mexican, or Indian subjects. Examination of macular thickness in normal eyes of these populations may also be of great value due to their higher risk for diabetes (CDC, 2005) and subsequent diabetic eye complications (Harris et al., 1998).

Future Directions: In conclusion, this study found that foveal thickness and macular thickness are significantly thinner in normal eyes of non-Hispanic Blacks compared to non-Hispanic Whites. Race-specific differences in macular thickness are of relevance in the assessment of a variety of disorders affecting retinal thickness, ranging from diabetic macular edema to glaucoma. Thinner baseline macular thickness must be taken into consideration when interpreting Stratus OCT macular scans in Black patients, especially in cases of early macular edema where subtle thickening may be masked. Additional multi-racial studies with larger sample sizes are needed to confirm these results and to evaluate the need for race-specific normative values.

CHAPTER 3

MACULAR THICKNESS IN NON-DIABETIC AND DIABETIC EYES WITH NO OR MILD RETINOPATHY: INFLUENCE OF RACE AND RELATIONSHIP WITH SYSTEMIC MARKERS FOR DIABETES

Introduction: Systemic risk factors for diabetic macular edema (DME) include longer duration of diabetes, worse glycemic control, increased systolic blood pressure, and increased serum lipids (Klein et al., 1984b: Klein et al., 1984c; Klein et al., 1995a; Klein et al., 1995b; Klein et al., 1991; Klein et al., 1996; Klein et al., 1994; ETDRS, 1985; Lopes de Faria et al., 1999; UKPDS, 1998a; UKPDS, 1998b; Matthews et al., 2004; Miljanovic et al., 2004; Kohner et al., 1998; Tovi et al., 1998). Large clinical trials have demonstrated the value of obtaining near-normal blood glucose levels in patients with diabetes as measured by reduced progression of diabetic retinopathy (DR), reduced incidence of proliferative diabetic retinopathy (PDR) and DME, and reduced systemic microvascular complications (DCCT, Ophthalmology, 1995; UKPDS, 1998a). Control of concomitant risk factors, such as high blood pressure and high cholesterol further reduces the risk of micro- and macrovascular complications (UKPDS, 1998b; DCCT, 1993).

Optical Coherence Tomography (OCT) has won clinical acceptance as a useful method for in-vivo quantification of retinal thickness at the macula, especially in cases of DME. Compared to slit-lamp biomicroscopy, OCT can measure small changes in retinal thickness with vastly better resolution (10 μm) (Hee et al., 1995; Brown et al., 2004). While it is well known that retinal thickness as measured by OCT is increased in clinically detectable DME

(Otani et al., 1999), studies of macular thickness in diabetic individuals *without* clinically evident macular edema are inconsistent. Retinal thickness in diabetic eyes with no DR and without clinically detectable DME has been previously reported as thicker compared to control eyes at the fovea (Lattanzio et al., 2002, Sanchez-Tocino et al., 2002), and at the superonasal foveal rim (Schaudig et al., 2000). In contrast, others have reported retinal thickness as no different from control eyes in any macular sector (Massin et al., 2002, Alkuraya et al., 2005). Furthermore, the relationship between diabetic risk factors and macular thickness in individuals with no or minimal retinopathy, and without clinically detectable DME, has yet to be formally explored. Elucidation of these relationships may provide novel *in-vivo* markers early in the course of diabetic eye disease.

The evolution of diabetic retinopathy involves both microvascular and neurodegenerative changes. However, it remains unclear which process occurs first, or if both processes evolve concurrently. Vascular insults are readily observable as hemorrhages or microaneurysms (h/ma's), exudates, and alterations in venous caliber. In contrast, neurodegenerative changes are not ophthalmoscopically visible *in-vivo*, but are detectable by functional tests such as multifocal electroretinography and visual evoked potentials. Altered retinal function has been demonstrated not only in subjects with diabetic retinopathy, but also in those with diabetes and without clinically discernable diabetic eye disease (Di Leo et al., 1990, Caputo et al., 1990; Ghirlanda et al., 1991; Fortune et al., 1999, Bearse et al., 2004a; Bearse et al., 2004b). Likewise, rat models of early diabetes show loss of neural cells prior to the onset of visible retinopathy (Aizu et al., 2002; Park et al., 2003). Since OCT can detect loss of retinal

neurons, as evidenced by reduction in overall retinal thickness, it may provide useful and novel information about *in-vivo* retinal structural alterations in human eyes with no or minimal retinopathy.

The effect of race has not been previously examined in studies of macular thickness in diabetic individuals. Racial differences are of clinical value since the prevalence of diabetic retinopathy differs amongst races and has been shown to be higher in Black individuals compared to White individuals (Rabb et al., 1990; Harris et al., 1993; Harris et al., 1998). When corrected for concomitant factors, such as diabetes control and systemic arterial blood pressure, any potential differences in macular thickness between these two populations may provide clues as to the pathogenesis of diabetic eye disease.

Undoubtedly, there is considerable interest in modalities to study and risk-stratify individuals with no or minimal retinopathy since visual acuity and fundus appearance are typically unaffected. Such a modality may not only represent a new tool in preventative vision care, but may also provide a novel means by which diabetes control can be monitored. As a first step towards this goal, we assessed whether OCT measures of macular thickness correlated with race, diabetes control, or duration of disease in subjects with no or minimal retinopathy. We also investigated whether early changes in macular thickness were detectable in this subset of diabetic eyes compared to non-diabetic control eyes.

Methods: This was a prospective, cross-sectional study involving 92 non-diabetic control subjects, 92 diabetic subjects with no DR (DM No DR), and 24 diabetic subjects with mild

nonproliferative diabetic retinopathy (DM Mild DR). Subjects were recruited as they presented for a routine eye examination at the Jamaica Plain Veteran's Affairs (VA) Medical Center. The study protocol followed the tenets of the Declaration of Helsinki and was approved by the VA Boston Institutional Review Board and Research and Development Committee.

Inclusion Criteria: All subjects were between the ages of 60-75 years. Subjects were equally distributed in four age cohorts: 60-63, 64-67, 68-71, and 72-75 years. Diabetic subjects carried a diagnosis of Type II Diabetes Mellitus, as indicated in their official medical record, CPRS, and received diabetic care and eye care through the VA Boston Healthcare System.

Exclusion Criteria: Diabetic subjects were excluded if they met any of the following: 1) best-corrected visual acuity worse than 20/70 in either eye; 2) any macular disorder, including diabetic macular edema and macular drusen; 3) abnormal Amsler grid testing; 4) previous retinal laser treatment (e.g. focal or scatter photocoagulation); 5) central visual field defect preventing fixation on OCT; 6) poor quality retinal imaging with OCT; 7) diagnosis of moderate or severe retinopathy at last eye examination according to ETDRS guidelines (ETDRS, 1991b); 8) intraocular inflammation; 9) diagnosis of glaucoma; 10) cataract extraction within the past 12 months; 11) previous intraocular surgery apart from cataract extraction; 12) refractive error greater than or equal to 6 diopters, or; 13) inability or unwillingness to provide informed consent.

Control subjects were excluded from the study if they met any of the criteria listed above or carried a diagnosis of impaired glucose tolerance.

Data Collection: Subjects provided informed consent. Duration of diabetes was marked from the self-reported date of diagnosis or the first date of diabetic medical therapy (as indicated in CPRS), whichever came first. Pertinent medical information (HbA1c level, serum triglycerides, HDL, and LDL) was gathered from CPRS. Hemoglobin A1c level (HbA1c) was determined by calculating the average of the last three HbA1c values within 1 year of the study visit. Fasting blood glucose information was gathered by self-report for the same day as the study. If same-day results were unavailable, the most recent (within 2 weeks) blood glucose value was used. All subjects underwent pupillary dilation. Optical Coherence Tomography (Stratus OCT, Zeiss Meditec, USA) Macular Thickness Mapping was acquired OU, in addition to four-field, 45-degree digital retinal imaging OU (Canon, USA) obtained according to VA digital imaging standards (Cavallerano et al., 2005). Systemic arterial blood pressure was measured for all subjects with the Omron IC Intellisense™ Digital Blood Pressure Monitor on the right arm (unless contraindicated) with the subject in an upright, seated position. Snellen visual acuity, Amsler Grid, refractive error, and intraocular pressure were recorded from the CPRS comprehensive eye exam notes from the same day as the study.

Stratus OCT: Using the retinal thickness map analysis protocol, macular thickness was determined at 37 points along six line scans of 6 mm length (Figure 2.1). Each data point

was automatically calculated by entering pre-determined axial scan numbers along each of the six lines using the Retinal Thickness Analysis Protocol (Figure 2.2). Mean central foveal thickness (CFT) for each subject was calculated as the average of measurements from the midpoint of each individual line scan. Mean total foveal thickness (TFT) was calculated as the average of measurements at 12 points along a ring at 1000 μm from fixation and the average central foveal thickness from the midpoint of each individual line scan. Mean total macular thickness (TMT) was calculated as the average of all values within the 6000 μm ring with the exclusion of central foveal measurements. Retinal thickness was also calculated as the average of individual retinal thickness measurements in 23 additional retinal sectors, divided into quadrants, concentric rings, and hemispheres. The status of the vitreo-retinal interface on OCT was indicated as either "attached vitreous" (no visualization of the posterior hyaloid), "detached vitreous" (visualization of the posterior hyaloid with no points of retinal insertion), or "partially detached vitreous" (one or two points of insertion of the posterior hyaloid onto the macular surface).

Fundus Images: Stereoscopic evaluation of color digital retinal images using the ScreenVu Stereoscope stereo viewer (Eye Supply USA, Inc.) was carried out by optometrists trained and certified in digital retinal image review. Diagnosis of "no retinopathy" or "mild nonproliferative diabetic retinopathy" was made according to the International Classification of Diabetic Retinopathy Severity Grading Scale (Wilkinson CP, Ferris FL 3rd, Klein RE, Lee PP, Agardh CD, Davis M, Dills D, Kampik A, Pararajasegaram R, Verdaguer JT, 2003).

Statistical Analysis: Continuous data were analyzed by using two-tailed, unpaired Student's *t*-test, linear regression, or multiple regression. Differences between groups were analyzed using one-way or two-way factorial analysis of variance (ANOVA) for independent samples.

Results: *Subject Characteristics:* A total of 92 non-diabetic control subjects, 92 subjects with diabetes and no DR, and 24 subjects with mild DR were evaluated (Table 3.1). Retinal thickness values were analyzed for right eyes; in cases where right eye values were unreliable, left eye values were used. There was no significant difference in any retinal thickness parameter between right and left eyes. All subjects with mild retinopathy had less than 5 retinal h/ma's. Subjects with mild DR had a significantly longer duration of diabetes compared to subjects with no DR (12.8 ± 6.9 vs. 9.0 ± 7.4 yrs; $p = 0.021$). Subjects with mild DR had a 2.3 higher percentage of insulin use compared to those with no DR. There were no significant differences in blood pressure or average HbA1c between groups. There were no significant differences in refractive error, intraocular pressure, or visual acuity among subjects in the Control, No DR, and Mild DR groups (Table 3.2).

Duration of Diabetes: In subjects with diabetes, there was a significant inverse correlation between retinal thickness and diabetes duration in all macular quadrants, rings, and hemispheres (Table 3.3). CFT, TFT, and TMT were also significantly thinner with longer duration of disease (Figure 3.1).

Table 3.1: Subject Characteristics

	Control (***n*** **= 92)**	**No DR** (***n*** **= 92)**	**Mild DR** (***n*** **= 24)**
% Male	98	98	92
Mean Age (yrs) (range)	68 ± 5 (60-75)	67 ± 5 (60-75)	67 ± 5 (60-75)
Mean Systolic Blood Pressure (mm Hg)	142 ± 16	145 ± 18	142 ± 19
Mean Diastolic Blood Pressure (mm Hg)	81 ± 11	82 ± 12	78 ± 12
Mean Triglyceride	135 ± 64	167 ± 98	146 ± 80
Mean Duration of DM	-	9.0 ± 7.4	12.8 ± 6.9
Mean HbA1c	-	7.0 ± 1.2	7.4 ± 0.9
% of Subjects on Insulin	-	25	54

HbA1c: average of last 3 readings within previous year

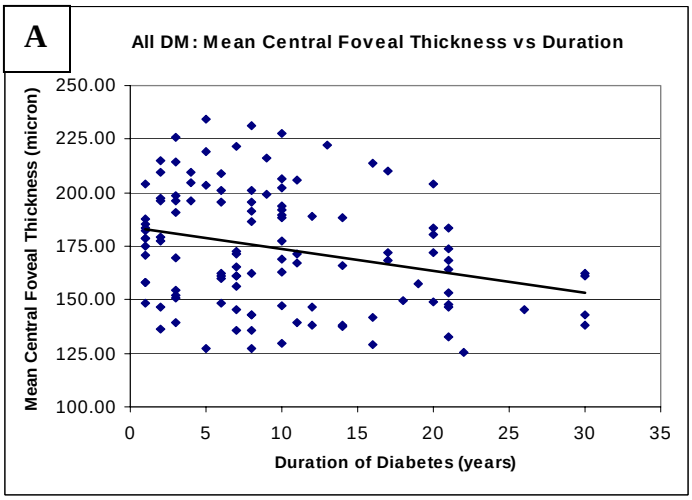
Table 3.2: Subject Ocular Characteristics

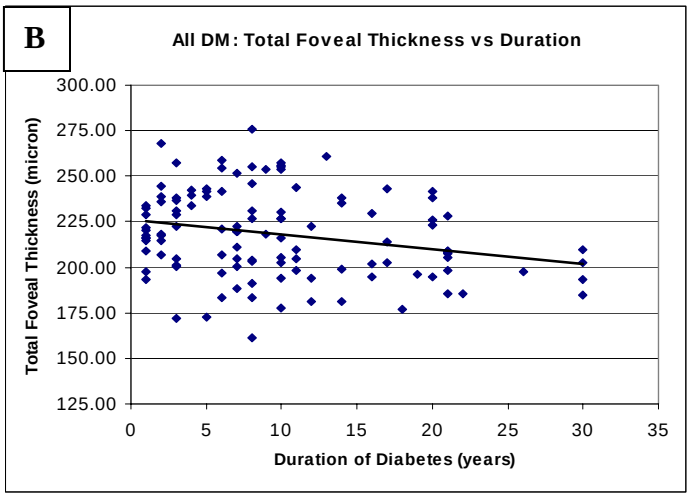
	Control (***n*** **= 92)**	**No DR** (***n*** **= 92)**	**Mild DR** (***n*** **= 24)**
Mean Spherical Equivalent (diopters)	+0.82 ± 1.61	+0.48 ± 1.77	+0.34 ± 1.33
Mean IOP (mm Hg)	15 ± 3	15 ± 3	15 ± 3
Mean Visual Acuity (logmar)	0.02 ± 0.05	0.02 ± 0.05	0.02 ± 0.07
% Vitreous Attached	72.8	70.6	58.3
% Vitreous Detached	7.6	12.0	12.5
% Vitreous Partially Attached	19.6	17.4	29.2

Table 3.3: Mean retinal thickness vs. duration of diabetes (linear regression)

Retinal Thickness Location	All DR (n = 116)		No DR (n = 92)		Mild DR (n = 24)	
	p	**r**	***p***	**r**	***p***	**r**
Central Foveal	0.003*	-0.28	0.003*	-0.30	0.101	-0.34
Total Foveal	0.006*	-0.25	0.012*	-0.26	0.095	-0.35
Total Macular	0.003*	-0.28	0.013*	-0.26	0.029*	-0.44
ST Quadrant	0.009*	-0.24	0.061	-0.19	0.011*	-0.51
IT Quadrant	0.006*	-0.25	0.040*	-0.21	0.024*	-0.46
SN Quadrant	0.002*	-0.28	0.010*	-0.27	0.041*	-0.42
IN Quadrant	0.014*	-0.23	0.057	-0.20	0.059	-0.39
Ring 1	0.020*	-0.21	0.046*	-0.21	0.119	-0.32
Ring 2	0.025*	-0.21	0.107	-0.17	0.029*	-0.45
Ring 3	0.004*	-0.27	0.053	-0.20	0.008*	-0.53
Superior Hemisphere	0.004*	-0.26	0.026*	-0.23	0.021*	-0.47
Inferior Hemisphere	0.011*	-0.24	0.046*	-0.21	0.055	-0.40
Nasal Hemisphere	0.004*	-0.27	0.022*	-0.24	0.034*	-0.43
Temporal Hemisphere	0.006*	-0.25	0.053	-0.20	0.015*	-0.49

ST: superotemporal; IT: inferotemporal; SN: superonasal; IN: inferonasal
*statistically significant to $p<0.05$, two-tailed t-test





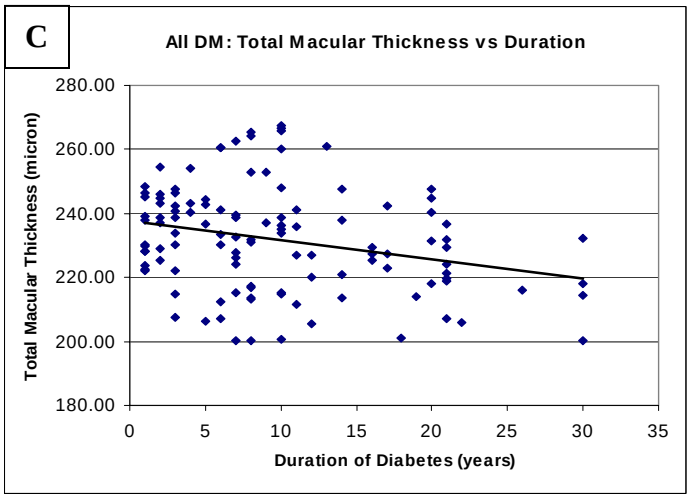


FIGURE 3.1: **Retinal Thickness vs Duration of Diabetes (Linear Regression).**
A: CFT: $p = 0.0025$, r = -0.28; **B**: TFT: $p = 0.0062$, r = -0.25; **C**: TMT: $p = 0.0026$, r = -0.28

Separate analyses of the No DR and Mild DR groups showed a significant negative correlation between TMT and diabetes duration in both groups. CFT and TFT were significantly correlated with diabetes duration in the No DR group only. Multivariate analysis showed no interaction effect with age.

Systemic Diabetes Control: There was no significant relationship between retinal thickness in any sector and average HbA1c level in subjects with diabetes (CFT: $p = 0.485$, r = -0.07; TFT: $p = 0.461$, r = -0.07; TMT: $p = 0.47$, r = -0.07). Furthermore, no significant relationship was found on multivariate analysis between retinal thickness in any sector and systolic blood pressure, diastolic blood pressure, blood glucose, or triglyceride levels.

Insulin Use: Compared to those not taking insulin, subjects using insulin in the Mild DR group had significantly longer duration of diabetes (8 ± 6 yrs and 17 ± 5 yrs, respectively; $p < 0.001$), and higher HbA1c levels (6.9 ± 0.9 and 7.8 ± 0.7, respectively; $p = 0.015$). Similar results were found in the No DR group for duration of diabetes (no insulin: 7.9 ± 6.8 compared to insulin: 12.1 ± 8.4; $p = 0.04$) and HbA1c (no insulin: 6.8 ± 1.0 compared to insulin: 7.7 ± 1.4; $p = 0.011$). There was no significant difference in age between insulin-requiring diabetic subjects and those on oral or diet control only (66 ± 5 yrs, and 68 ± 4 yrs, respectively; $p = 0.165$). There were no significant differences in any thickness parameter between the No DR and Mild DR groups when insulin use was taken into account.

Diabetic versus Control Eyes: No significant differences were found between control subjects, subjects with no DR and subjects with mild DR for any of the 26 retinal sectors analyzed. Table 3.4 summarizes the retinal thickness data.

Status of the Vitreous: Subjects with partial attachment of the vitreous to the macula had significantly greater retinal thickness compared to those with attached or detached vitreous in the Control, No DR, and Mild DR groups (CFT: $p = 0.027$; TFT: $p = 0.033$; TMT: $p = 0.036$). However, there was no significant difference in retinal thickness between Controls, No DR, and Mild DR groups when status of the vitreous was taken into account (CFT: $p = 0.416$; TFT: $p = 0.534$; TMT: $p = 0.698$).

Racial Differences: Control vs. diabetic eyes: There was no significant difference in CFT, TFT, or TMT between the No DR, Mild DR, and Control groups when race was taken into account (CFT: $p = 0.39$; TFT: $p = 0.43$; TMT $p = 0.56$; ANOVA). However, retinal thickness was significantly thinner in Blacks compared to Whites in each group (CFT: $p < 0.0001$; TFT: $p < 0.0001$; TMT: $p < 0001$; ANOVA). There were no significant interaction effects between race and systemic diabetes control indicators. Table 3.5 summarizes the retinal thickness data in the Black and White groups.

Table 3.4: Retinal thickness values for all groups

Retinal Thickness Location	Retinal Thickness (micron)		
	Control (n = 92)	**No DR (n = 92)**	**Mild DR (n = 24)**
Central Foveal	176 ± 28	173 ± 28	176 ± 27
Total Foveal	219 ± 21	217 ± 23	218 ± 29
Total Macular	232 ± 14	231 ± 16	231 ± 20
ST Quadrant	237 ± 16	236 ± 15	237 ± 19
IT Quadrant	229 ± 14	228 ± 15	226 ± 19
SN Quadrant	249 ± 15	249 ± 17	247 ± 21
IN Quadrant	243 ± 16	244 ± 16	242 ± 21
ST Ring 1	242 ± 25	240 ± 26	242 ± 30
ST Ring 2	258 ± 18	258 ± 15	260 ± 17
ST Ring 3	211 ± 15	211 ± 14	209 ± 16
IT Ring 1	241 ± 25	237 ± 24	231 ± 40
IT Ring 2	259 ± 17	257 ± 18	257 ± 19
IT Ring 3	205 ± 13	204 ± 14	201 ± 14
SN Ring 1	242 ± 26	239 ± 28	242 ± 33
SN Ring 2	271 ± 18	274 ± 16	270 ± 19
SN Ring 3	234 ± 15	233 ± 17	229 ± 16
IN Ring 1	239 ± 24	237 ± 25	238 ± 32
IN Ring 2	268 ± 19	271 ± 19	271 ± 17
IN Ring 3	224 ± 16	222 ± 18	216 ± 23
Ring 1	241 ± 21	239 ± 23	238 ± 31
Ring 2	265 ± 16	265 ± 16	265 ± 17
Ring 3	219 ± 13	219 ± 13	216 ± 15
Superior Hemisphere	243 ± 14	242 ± 15	242 ± 19
Inferior Hemisphere	239 ± 14	239 ± 15	237 ± 20
Nasal Hemisphere	246 ± 14	246 ± 16	244 ± 20
Temporal Hemisphere	237 ± 14	236 ± 14	234 ± 20

ST: superotemporal; IT: inferotemporal; SN: superonasal; IN: inferonasal

Table 3.5: Retinal Thickness by Race

	Control		No DR		Mild DR	
Race	Whites	Blacks	Whites	Blacks	Whites	Blacks
n	83	8	79	13	17	5
Mean CFT	179 ± 28	159 ± 24	175 ± 27	155 ± 25	189 ± 19	148 ± 24
Mean TFT	222 ± 21	204 ± 19	220 ± 22	201 ± 20	231 ± 22	195 ± 23
Mean TMT	234 ± 14	223 ± 13	233 ± 15	220 ± 13	240 ± 18	219 ± 16

CFT: central foveal thickness; TFT: total foveal thickness; TMT: total macular thickness

Racial Differences: Retinal thickness vs. diabetes duration: White subjects in the No DR group showed significant negative correlations between CFT and TFT and diabetes duration (CFT: p = .006, r = -0.31; TFT: p = 0.021; r = -0.26). There was a trend for TMT to be thinner with longer duration (p = 0.059, r = -0.21) in White subjects. In Black subjects with no DR, retinal thickness was not significantly correlated with diabetes duration in any of the three global sectors (CFT: p = .498, r = -0.21; TFT: p = 0.567; r = -0.17; TMT: p = 0.192; r = - 0.39).

White subjects in the Mild DR group showed significant negative correlations between CFT and TMT and diabetes duration (CFT: p = .034, r = -0.52; TMT: p = 0.029; r = -0.53). There was a trend for TFT to be thinner with longer duration (p = 0.07, r = -0.45). There were no significant correlations between retinal thickness and diabetes duration in Black subjects with mild DR for any of the three global sectors (CFT: p = .780, r = 0.18; TFT: p = 0.904; r = 0.08; TMT: p = 0.860; r = - 0.11).

Racial Differences: Blood pressure: In the No DR group, average systolic blood pressure in Whites was 145 ± 18 mm Hg compared to 148 ± 19 in Blacks (p = 0.58). Average diastolic blood pressure was 82 ± 12 in Whites and 82 ± 12 in Blacks (p = 0.97). In the Mild DR groups, average systolic blood pressure was 142 ± 20 in Whites and 141 ± 18 in Blacks (p = 0.94). Mean diastolic blood pressure was 78 ± 14 in Whites compared to 79 ± 7 in Blacks (p = 0.84). Multivariate analysis showed no interaction effect between blood pressure and retinal thickness when other diabetic status indicators and age were taken into account.

Discussion: The most sensitive parameter related to diabetic macular edema (DME) is precise measurement of macular thickness (Goebel et al., 2002). OCT is an accurate technique for quantifying macular thickness in patients with DME (Hee et al., 1998). OCT studies have consistently revealed retinal structural changes and increased retinal thickness in patients with DME (Otani et al., 1999, Yang et al., 2001). However, it is unclear whether alterations in macular thickness exist in diabetics with no macular edema and very minimal retinopathy, and if macular thickness is related to systemic markers of diabetic health in these individuals.

In this study, we found 1) there was a significant inverse relationship between duration of diabetes and macular thickness in subjects with no diabetic retinopathy or mild retinopathy; 2) there were no significant relationships between macular thickness and level of glycemic control (as measured by average of the three most recent HbA1c levels), most recent blood glucose level, or systemic arterial blood pressure; 3) there were no significant differences in macular thickness in any retinal sector between non-diabetic controls, subjects with diabetes and no retinopathy, and subjects with mild retinopathy, and 4) Black subjects with diabetes had thinner macular thickness when compared to White subjects.

Microvascular theory of diabetic retinopathy: The most commonly accepted pathophysiological model for diabetic retinopathy development involves microvascular dysfunction. Abnormalities in glucose metabolism lead to alterations in retinal capillaries and

breakdown of the blood/retinal barrier, resulting in microaneuryms, hemorrhages, and retinal exudates. These retinal alterations lead to retinal thickening detectable by OCT.

Neurodegenerative theory of diabetic retinopathy: functional loss: In addition to microvascular changes, diabetic retinopathy seems to involve neurodegenerative changes. Electroretinographic and psychophysical studies have demonstrated that retinal function is altered in diabetic eyes and is related to duration of disease. Electroretinography (ERG) is able to detect retinal dysfunction prior to the onset of visible retinopathy (Han, Bearse, Schneck, Barez, Jacobsen, and Adams, 2004). Average pattern ERG amplitude is reduced at low and medium spatial frequencies in subjects with type 1 DM and no or mild retinopathy, and is negatively correlated with disease duration (Di Leo et al., 1990, Caputo et al., 1990; Ghirlanda et al., 1991). More recently, abnormalities on multifocal ERG (MfERG) have been noted in subjects with diabetes and no retinopathy (Fortune et al., 1999; Bearse et al., 2004a; Bearse et al., 2004b) although it is unclear whether these changes correlate with duration of diabetes. In subjects with diabetes mellitus and no retinopathy, visual evoked potential (VEP) latencies are delayed with longer duration of disease (Yaltkaya, Balkan, and Baysal, 1988; Mariani, Moreo and Colucci, 1990). Contrast sensitivity is also diminished prior to onset of retinopathy in individuals with type 2 DM and shows greater reduction with longer duration of disease (Dosso, Bonvin, Morel, Golay, Assal, and Leuenberger, 1996; Trick, Burde, Gordon, Santiago, and Kilo, 1988).

Neurodegenerative theory of diabetic retinopathy: morphological changes: Alterations in retinal function have previously been linked to retinal morphological changes in rat models of early diabetes. Since retinal neurons require glucose for proper function, alterations in glucose metabolism could lead to impaired neural function and potentially, neuronal cell death. A previous study using streptazotocin-induced diabetic rats showed not only reduction of ERG a- and b-wave amplitudes early in the course of the disease, but also a reduction in thickness of the inner plexiform and photoreceptor layers on electron microscopy (Aizu et al., 2002). Furthermore, another study using a similar rat model showed that retinal thinning progressed as duration of disease increased, with apoptosis of several neuronal cells, including ganglion, amacrine, horizontal, Muller, and photoreceptor cells that preceded microvascular complications (Park et al., 2003). Studies of post-mortem human eyes also demonstrate retinal neuron cell apoptosis in eyes with diabetes (with and without retinopathy) compared to non-diabetic control eyes (Barber, Lieth, Khin, Antonetti, Buchanan, and Gardner, 1998; Barber, 2003; Lieth, Gardner, Barber, and Antonetti, 2000). In our study, we found that *in-vivo* macular thickness in Type 2 diabetics was significantly reduced as duration of diabetes increased. It is possible that this negative correlation is a result of neurodegenerative processes in the retina. In the No DR group, the correlation was significant at the central fovea and therefore may imply photoreceptoral cell loss. The reduction in total macular thickness in all diabetic eyes as duration of disease increased could be the result of ganglion cell or glial cell structural alterations as well.

Retinal thickness and duration of diabetes: Duration of diabetes is a clinically accepted marker for overall diabetic health status. In the eye, longer duration of diabetes is a risk factor for the development of diabetic retinopathy and diabetic macular edema (Klein et al., 1984c; Klein et al., 1995b). While subjects in the Mild DR group had retinopathy, it was very mild (less than 5 h/ma's) and therefore implied very early vascular dysfunction. It is unclear whether neuronal dysfunction precedes vasculopathy in diabetic retinopathy. In the subset of subjects we studied with mild DR, it is plausible that neurodegenerative processes occurred prior to the onset of vascular changes leading to decreased thickness with longer duration. Longitudinal data are required to explore this possibility. Future studies should also examine the longitudinal relationship between *in-vivo* macular structure and functional abnormalities on electrophysiological and psychophysical testing.

Retinal thickness and systemic markers for diabetes control: In addition to duration of disease, other risk factors for development and progression of diabetic retinopathy and maculopathy include higher hemoglobin A1c level, higher systolic blood pressure, and higher serum lipids (Klein et al., 1984b; Klein et al., 1984c; Klein et al., 1995a; Klein et al., 1995b; Klein et al., 1991; Klein et al., 1996; Klein et al., 1994; ETDRS, 1985; Lopes de Faria et al., 1999; UKPDS, 1998a; UKPDS, 1998b; Matthews et al., 2004; Miljanovic et al., 2004; Kohner et al., 1998). Our study did not find a significant correlation between any of these factors and macular thickness. We also did not find a significant correlation between macular thickness and blood glucose level. Since blood pressure and blood glucose readings were used from the day of the study, they represented short-term approximations and may

not have been indicative of long term diabetic status. HbA1c was calculated as the average of the last three readings within one year of the study visit and therefore characterized diabetic status better. Nevertheless, the lack of significance between HbA1c and macular thickness suggests that more long-term measures of diabetes, such as disease duration, may be necessary to understand changes in retinal architecture.

Differences between control and diabetic eyes: Previous studies have noted significantly increased macular thickness in type 2 diabetic subjects with no or mild retinopathy compared to non-diabetic controls at the superior nasal foveal rim (Schaudig et al., 2000), in the superior quadrant (Sugimoto, Sasoh, Ido, Wakitani, Takahashi, and Uji, 2005), and at the central fovea (Sanchez-Tocino et al., 2002, Lattanzio et al., 2002). In contrast, other studies comparing macular thickness in control eyes and diabetic eyes with no or mild retinopathy (Massin et al., 2002, Alkuraya et al., 2005) have demonstrated no differences. In the present study, we also found no significant differences in retinal thickness in any macular sector between Control, No DR, and Mild DR groups. Compared to previous studies, our study involved a greater number of subjects and a greater number of sampled retinal thickness points. Our inclusion of only those eyes with no or minimal retinopathy may explain the lack of thickness differences between these eyes and control eyes. From our results, it does not seem likely that Stratus OCT is able to detect subclinical changes in macular thickness in diabetic eyes with no or minimally visible retinopathy when compared to non-diabetic control eyes. It is possible that future studies using OCT instrumentation with higher

resolution may be able to detect subtle differences in macular thickness in this subset of diabetic eyes.

Racial differences in macular thickness: No previous study of macular thickness in diabetic individuals with no or minimal retinopathy has evaluated the effect of race. Racial differences are of clinical value, since the prevalence of diabetic eye disease differs amongst races. Population studies in the United States show that the prevalence of diabetic retinopathy, the rate of visual impairment from diabetes, and the rate of visually significant macular edema is higher in Blacks compared to Whites (Rabb et al., 1990; Harris et al., 1993; Harris et al., 1998). It seems that poorer systemic control of diabetes, especially higher systemic arterial blood pressure in Blacks accounts for this disparity. In our study, neither systolic nor diastolic blood pressure differed significantly between Whites and Blacks. In both the No DR and the Mild DR groups, Black subjects had significantly thinner macular thickness when compared to White counterparts. This finding may imply differences in the pathogenesis of retinal architectural changes in the diabetic retina between these two groups, with heavier emphasis on neurodegeneration in Blacks. Future studies of larger populations are necessary to confirm these results. Functional testing, such as multifocal electroretinography, is also warranted to assess the correlation between retinal structure and any alterations in retinal function.

In addition to exhibiting lower retinal thickness, Black subjects in our study also differed from Whites in regards to retinal thickness versus diabetes duration. When corrected for

blood pressure, retinal thickness was negatively correlated with diabetes duration in the Whites, but not in Blacks. The lack of significance in the Black group may have been due to small sample size. Conversely, these cross-sectional differences may imply differences in the development of diabetic retinal changes in Blacks compared to Whites. This poses an interesting question for future longitudinal studies to explore.

Study limitations: This study involved a large number of subjects, but was limited in number of female participants in a veteran's hospital setting. A comprehensive range of subject race was also lacking, as most of our subjects were White. Further studies should involve a greater number of female subjects and include subjects with a wider range of race as well as age.

Future Directions: In summary, i*n-vivo* macular thickness as measured by OCT in subjects with diabetes and no or minimal retinopathy is reduced with longer duration of disease. Black subjects with diabetes and no or minimal diabetic retinopathy have thinner macular thickness than White counterparts. Future studies should explore racial differences in macular thickness in larger cohorts and with more racially diverse populations. Longitudinal studies should assess the role of OCT as a novel way to prospectively assess anatomical changes in the neural retina as duration of diabetes increases and possibly identify retinal markers for diabetic macular disease before it becomes clinically evident or visually significant.

CHAPTER 4

GENERAL CONCLUSION

In-vivo examination of the retina with Optical Coherence Tomography in individuals with diabetes represents a novel way to assess pathophysiological mechanisms of disease. Individuals with no or mild DR are of particular interest since visual acuity is typically intact. In this study, we found that thinner macular thickness was significantly associated with longer duration of diabetes. Since microvascular damage was not clinically detectable or very mild, it is possible that neurodegeneration was the dominant pathophysiological mechanism leading to reduced retinal thickness in our subjects. Future studies should examine retinal function in these groups of subjects in relation to structural changes.

We did not find any relationship between macular thickness and systemic markers for diabetes, such as hemoglobin A1c, blood glucose level, or systemic arterial blood pressure. A limitation of this study was that blood pressure and blood glucose were single measurements. Although HbA1c was calculated as the average of the last three readings within one year, it is feasible that more long-term indicators of diabetic health, such as duration, are required to illustrate a relationship with retinal thickness. We also found no difference in macular thickness between diabetic subjects and age-matched non-diabetic control subjects. This is most likely due to the fact that our subjects had no or very mild retinopathy. Future studies using OCT with higher resolution should evaluate these findings.

Racial differences in macular thickness have yet to be formally explored and may indicate differences in retinal disease mechanisms in diabetes. In this study, we found that non-diabetic Black subjects had thinner macular thickness than White subjects. Black subjects with diabetes also had reduced macular thickness when compared to Whites in both groups without retinopathy and very mild retinopathy. Identification of racial differences is important in the accurate assessment of retinal abnormalities and may indicate differences in the pathophysiology of retinal alterations due to diabetes. This issue deserves attention in future studies of larger samples.

The importance of early identification of retinal abnormalities in diabetes cannot be underestimated, since visual impairment from diabetic retinopathy is largely avoidable with proper care, either systemic or ocular. This study raises some important questions regarding neurodegeneration in diabetic subjects with no or minimal clinically evident retinopathy. Future longitudinal studies should assess the role of OCT as a novel way to prospectively assess anatomical changes in the neural retina in order to possibly identify retinal markers for diabetic macular disease before it becomes clinically evident or visually significant.

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