Retinal Thickness in People with Diabetes and Minimal or No Diabetic Retinopathy: Heidelberg Spectralis Optical Coherence Tomography

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Purpose. To evaluate macular thickness in people with diabetes but minimal or no retinopathy using Heidelberg Spectralis optical coherence tomography (OCT).

Methods. In a multicenter, cross-sectional study of mean retinal thickness, on Spectralis OCT in the nine standard OCT subfields, spanning a zone with 6-mm diameter, center point, and total retinal volume were evaluated. Central subfield (CSF) thickness was evaluated for association with demographic and clinical factors. Stratus OCT scans also were performed on each participant.

Results. The analysis included 122 eyes (122 participants) with diabetes and no ($n = 103$) or minimal diabetic retinopathy ($n = 19$) and no macular retinal thickening on clinical exam. Average CSF thickness was 270 ± 24 μm. Central subfield thickness was significantly greater in males relative to females (mean 278 ± 23 μm vs. 262 ± 22 μm, $P < 0.001$). After adjusting for gender, no additional factors were found to be significantly associated with CSF thickness ($P > 0.10$). Mean Stratus OCT CSF thickness was 199 ± 24 μm.

Conclusions. Mean CSF thickness is approximately 70 μm thicker when measured with Heidelberg Spectralis OCT as compared with Stratus OCT among individuals with diabetes in the absence of retinopathy or with minimal nonproliferative retinopathy and a normal macular architecture. CSF thickness values ≥320 μm for males and 305 μm for females (~2 SDs above the average for this normative cohort) are proposed as gender-specific thickness levels to have reasonable certainty that diabetic macular edema involving the CSF is present using Spectralis measurements. (*Invest Ophthalmol Vis Sci.* 2012; 53:8154–8161) DOI:10.1167/iovs.12-10290

Optical coherence tomography (OCT) has emerged as an important imaging modality in the evaluation and management of retinal diseases. Before OCT, the standard method for assessing macular thickness in the clinic or within clinical research studies was stereoscopic biomicroscopy or stereoscopic color fundus photographs.[1] Each method involves a subjective process dependent on observer skill, patient cooperation, degree of pupillary dilation, clarity of ocular media, and retinal swelling characteristics. Optical coherence tomography (OCT) has provided an objective and potentially more sensitive means of assessing macular edema, and provides data that can be collected and interpreted in a standardized fashion, which can facilitate outcome assessments in clinical studies.

Thickness measurements in normal-appearing eyes vary by patient age, sex, ethnicity, and refractive error. Older individuals, females, African Americans, and persons with myopia of −5 diopters or more, reportedly have thinner thickness measurements (Fraser-Bell S, et al. *IOVS* 2005;46:ARVO E-Abstract 1542).[2] Understanding what level of thickness will differentiate an eye in a person with diabetes mellitus but *without* diabetic macular edema (DME) from an eye *with* DME is important for clinical care and for clinical trials. A previous study conducted by the Diabetic Retinopathy Clinical Research Network (DRCR.net) indicated that patients with diabetes mellitus and no retinopathy, or minimal retinopathy, in the absence of macular thickening on clinical exam, have retinal thickness values that are similar to values from cohorts without diabetes and normal-appearing retinas.[3] However, this study used Stratus OCT3 (Carl Zeiss Meditec, Inc., Dublin, CA), a third-generation time-domain OCT (TD-OCT) model.

Spectral-domain OCT (SD-OCT), also known as Fourier-domain OCT, a relatively new imaging technique that utilizes the Fourier transformation to gather depth data from the spectra of the OCT signal, has largely replaced TD-OCT in

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[6]See the Appendix for the members of the Diabetic Retinopathy Clinical Research Network (DRCR.net) who participated in this protocol.

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clinical practice and is anticipated to do so in clinical research within the near future. Therefore, data describing normal thickness in a cohort of individuals with or without diabetes mellitus and a normal macular appearance on clinical exam are needed. There are several reasons why retinal thickness values obtained with SD-OCT are expected to be different from those obtained with TD-OCT. Increased speed of data collection by a factor of 100 (scans up to 40,000 A-scans per second) improves resolution and delineation of all retinal layers including the retinal pigment epithelium (RPE)–Bruch's membrane–choriocapillaris complex.[4,5] Various SD-OCT instruments have selected alternate specific locations within the RPE–Bruch's membrane–choriocapillaris complex to define the outer retinal boundary for computation of retinal thickness. Time-domain OCT exclusively uses the junction of the photoreceptor inner and outer segments for this boundary (Fig. 1). The Heidelberg Spectralis OCT instrument (Heidelberg Engineering, Inc., Heidelberg, Germany), one type of SD-OCT instrument, uses the posterior border of Bruch's membrane as the boundary for retinal thickness measurements.[6] Thus, retinal thickness measurements generated with the Spectralis instrument should theoretically be greater than those measured by TD-OCT. In addition, SD-OCT can obtain as many as 512 raster scans within a 6×6-mm square centered on the fovea within the same brief time span that six radial lines scan a 6-mm circle centered on the fovea with TD-OCT. Enhancing scan density decreases the need for the OCT software to extrapolate retinal thickness over wide areas of the macula. Theoretically, the precision of the measurement should be improved and the measurement would be less subject to deviations imposed by incorrect boundary line placement by the software on sporadic scans.

The manufacturers of the Stratus, Cirrus, and RT-Vue100 instruments have provided the DRCR.net access to data they collected on normal subjects. Using these databases the network has been able to calculate instrument-specific normative cutoff values to differentiate eyes with thickened macula from those without. However, for persons with a normal-appearing macula, particularly individuals with diabetes in the absence of significant retinopathy or clinically apparent DME, there are no published Spectralis OCT normative data that span the 6-mm diameter nine subfield grid and the manufacturer did not provide access to a normative database. As such, the network needed to collect this information to support future studies that would incorporate Spectralis OCT images when evaluating DME. Additionally, there are no assessments of possible interactions of Spectralis measured thickness with an individual's age, gender, or ethnicity. Such normative values are desirable to help in the design and interpretation of clinical trials evaluating DME. For example, when setting criteria for CSF thickness at a specific value to determine eligibility for participation in a study of DME, it is helpful to know whether instrument-specific values are needed and whether that value should differ when enrolling females compared with males.

To address these questions, the DRCR.net studied Spectralis OCT–measured retinal thickness and macular volume in diabetic subjects without retinopathy or with very mild retinopathy (a few microaneurysms and no other retinal abnormalities associated with diabetic retinopathy). In addition, Stratus OCT measurements were obtained at the same visit to report the difference between the Spectralis-generated and Stratus-derived measurements in this cohort. A comparison of this Stratus normative database with previously published Stratus OCT data from the DRCR.net in a similar cohort is also included to evaluate consistency of our data.

Methods

This study was conducted by the DRCR.net at nine clinical sites. The investigation was funded by the National Eye Institute of the National Institutes of Health, U.S. Department of Health and Human Services. The protocol and Health Insurance Portability and Accountability Act compliant-informed consent forms were approved by multiple institutional review boards. Each subject gave informed consent for participation in the study. The entire protocol is available at www.drcr.net.

Study Population

Eligible subjects were at least 18 years old with type 1 or type 2 diabetes, and no history of renal failure requiring dialysis or renal transplant. Any one of the following criteria was considered sufficient evidence that diabetes was present among the participants: current regular use of insulin for the treatment of diabetes, current regular use of oral antihyperglycemia agents for the treatment of diabetes, or documented diabetes by the American Diabetes Association and/or World Health Organization criteria. The classification of type 1 versus type 2 was determined by the clinical investigator. An eye was eligible if it met the following criteria: (1) no retinal thickening of the macula based on clinical examination; (2) no diabetic retinopathy (Early Treatment Diabetic Retinopathy Study [ETDRS] level 10) or microaneurysms only (ETDRS level 20) based on clinical exam; (3) visual acuity 20/32 or better as obtained by standard clinic method using habitual correction; (4) no prior treatment for macular edema or diabetic retinopathy; (5) no macular pathology of any kind on clinical exam; and (6) no history of major ocular surgery within the prior 6 months.

Study Procedures

Following pupil dilation, two replicate Heidelberg Spectralis OCT scans were obtained on each eye of a participant by a certified operator. The first of the two scans for each eye was used for this analysis. The following scan acquisition parameters were required: dense volume scan ($20^\circ \times 20^\circ$, roughly 6×6 mm), 49 B-scans each spaced 120 μm apart, automatic real-time mean of 16, high speed (512 A-scans/B-scan), and software version 5.1 or higher. Images obtained in version 5.1 were converted to version 5.3a by the OCT reading center (Duke Reading Center, Durham, NC) for analysis because version 5.3a calculates retinal volume over the entire 6-mm ETDRS grid (as long as at least 50% of individual subfields are scanned). Version 5.1 calculates retinal volume only in the region that the data points were acquired within the 6-mm grid. Since version 5.3a manages missing data within the 6-mm grid by extrapolating from existing data points within the grid and version 5.1 does not, differences in volume calculations would be expected in eyes with decentered scans or missing B scans. A subgroup of participants with original scan submissions, interpreted with software version 5.1, were evaluated for differences in macular volume when reevaluated with software version 5.3a. Each scan was evaluated at the reading center for evidence of morphologic abnormality, inaccurately drawn automated boundary lines affecting the CSF or macular volume computations, and for decentration of the ETDRS subfield grid relative to the fovea. The reading center submitted revised CSF thickness or volume measurements in 10 (8%) eyes in the analysis cohort following this review.

Two replicate Zeiss Stratus OCT fast macular thickness scans (six 6.0-mm radial scans consisting of 128 A-scans/B-scan), the scan protocol used in all prior DRCR.net studies involving Stratus OCT, were also obtained on each eye of each participant by a certified operator at the same visit. Central subfield thickness and macular volume were taken directly from the software output of the first scan for this analysis unless these metrics were revised by the reading center during their systematic review of a 10% sample of all Stratus scans as well as all Stratus scans in which the SD of the center point was 10% or more.

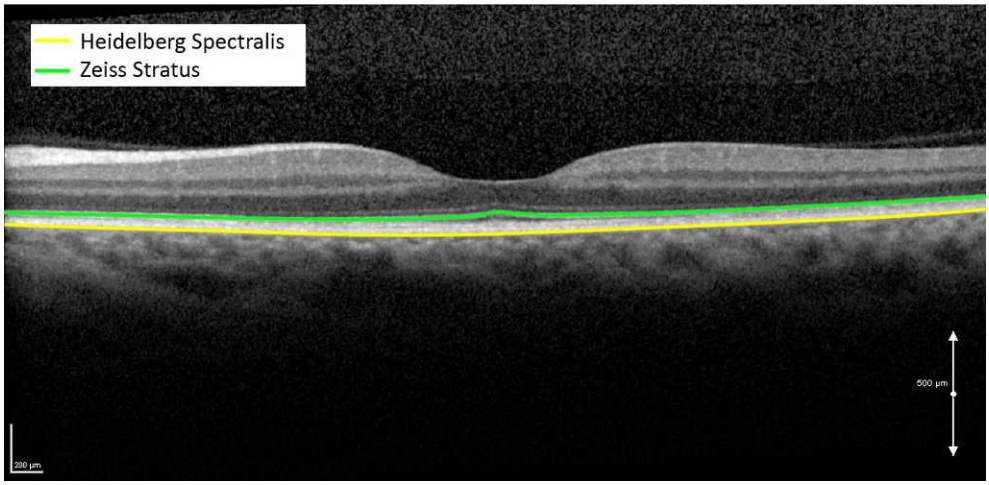

Figure 1. Outer retinal boundary lines for Spectralis and Stratus OCT. The different locations used by the instrument software to identify the outer or posterior boundary of "the retina" are indicated. Each instrument measures retinal thickness between the inner limiting membrane and the posterior boundary line.

Statistical Methods

Central subfield thickness was the primary OCT parameter used in the analysis. Separate unadjusted least-squares regression models were used to evaluate the relationship of demographic and clinical characteristics with CSF thickness. Factors with a value of $P < 0.10$ in univariate models were included in multivariate models, with a final model consisting of factors with a value of $P < 0.01$ using a backward-selection process. Retinal volume, a secondary OCT parameter of interest, was evaluated similarly. All P values reported are two-tailed. Statistical analyses were performed using commercial software (SAS software, version 9.2; SAS Institute, Inc., Cary, NC).

Results

Of 318 eyes of 174 participants meeting eligibility criteria to be enrolled into the study, 194 were eligible for the analyses. In all, 124 eyes were excluded due to lost or damaged OCT scans (6), incorrect OCT scan acquisition parameters (16), or abnormal macular morphology noted on the OCT scans at the time of reading center assessment (102 eyes, including one or more of vitreomacular traction/vitreomacular adhesion [53], epiretinal membrane [14], cystoid edema [21], subretinal fluid [3], or retinal pigment epithelial [RPE] detachment/sub-RPE fluid [29]). A repeat analysis of the Spectralis OCT parameters of interest (mean + SD of the six subfields, center point, and volume) that included eyes with reading center identified morphologic abnormalities produced results similar to those of our analysis cohort (data not shown). Since retinal thickness was highly correlated between the right and left eyes among the 72 participants with 2 eligible eyes (0.95 CSF thickness [$n = 66$] and 0.97 for volume [$n = 65$]) if CSF thickness and volume data were available from both eyes, one eye was selected at random for analyses; otherwise, the eye with the available data was used. This left a total of 122 eyes of 122 participants in the analysis cohort. Forty-eight eyes in the analysis cohort had scans that were interpreted with both 5.1 and 5.3a software (Duke Reading Center).

Median participant age was 59 years (range: 22–88 years), 67 (55%) were females, and 37 (30%) were not Caucasian. Type 1 diabetes was present in 14 (11%) subjects and type 2 in 99 (81%), whereas 9 subjects could not be classified. Median duration of diabetes was 8 years (range: <1–48 years). Visual acuity ranged from 20/12 to 20/32, and was 20/20 or better in 85 eyes (70%). No diabetic retinopathy was present in 103 (84%) eyes and microaneurysms were present in 19 (16%).

Figure 2 displays the mean and SD Spectralis OCT thickness measurements for the center point, CSF, 4 inner subfields, 4 outer subfields, and retinal volume. On average, the CSF thickness was 270 ± 24 µm and volume was 8.4 ± 0.4 mm^3. The inner subfields were thicker than the outer subfields, the nasal subfields were thicker than the temporal subfields, and the superior subfields were thicker than the inferior subfields. The SD of the thickness was similar in the inner and outer subfields (ranging from 15–19) and slightly larger in the CSF and center point, (24 and 25 µm, respectively). The CSF for the 122 eyes in this study ranged from 213 to 346 µm, whereas the center point thickness ranged from 176 to 324 µm. In the subgroup with volume measurements on both 5.1 and 5.3a software, the mean and median difference was 0.18 and 0.14 mm^3, respectively, in favor of larger volume measurements with 5.3a software. Signal strength varied from 14 to 34 with a median of 26.

Central subfield thickness was significantly greater in males than that in females ($P < 0.001$, Table 2), with a mean of 278 ± 23 µm in males and 263 ± 22 µm in females. These gender differences were noted consistently across other clinical characteristics (Table 1). The shift in distribution of the mean CSF thickness of males compared with that of females is illustrated in Figure 3. The difference in thickness by gender was less pronounced in the outer subfields (Table 2). After adjusting for gender, no additional factors (i.e., those listed in Table 1), were found to be significantly associated with the retinal thickness of the CSF ($P > 0.01$ in multivariate model using a backward-selection process of factors, where $P < 0.01$).

Retinal volume also was greater in the macula of males when compared with that of females ($P = 0.04$, unadjusted; $P = 0.003$, adjusted for age). After adjusting for gender, increasing age also was found to be significantly associated with decreasing volume ($P < 0.001$, adjusted).

Figure 4 displays the mean and SD of measurements from the Stratus OCT for center point, CSF, 4 inner subfields, 4 outer subfields, and retinal volume. The mean measurements for each subfield were within 4 µm of the mean measurements made within each subfield on 97 eyes participating in an earlier DRCR.net study in which all scans had been reviewed by an independent reading center.[3] The earlier study initially reported Stratus OCT thickness of the macula in a similar cohort of individuals with diabetes and minimal or no retinopathy and no central retinal thickening on clinical exam.

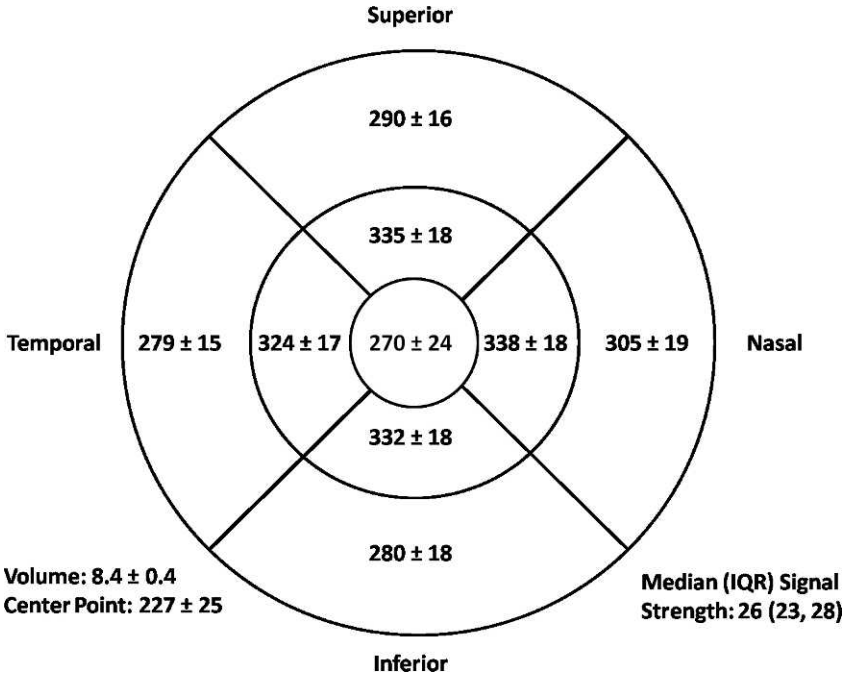

Figure 2. Heidelberg Spectralis OCT retinal thickness in diabetic participants with no or very mild retinopathy (mean + SD). Based on $n = 122$ eyes; number missing due to nongradable: 0 Central Subfield, 1 Inner Superior, 1 Inner Nasal, 1 Inner Inferior, 1 Inner Temporal, 0 Outer Superior, 3 Outer Nasal, 3 Outer Inferior, 2 Outer Temporal, 6 Volume, 0 Center Point.

The mean CSF thickness and volume in our present cohort were 199 ± 24 μm (71 μm less than Spectralis) and 6.7 ± 0.4 mm^3 (1.7 mm^3 less than Spectralis), respectively. The inner subfields were 66 to 71 μm less than Spectralis, whereas the outer subfields were 55 to 62 μm less (Fig. 5).

Discussion

In this study we show that SD-OCT measured thickness of the retina in individuals with diabetes without retinopathy or with very minimal retinopathy, as generated by the Heidelberg Spectralis instrument at multiple clinical sites within the DRCR Network, is comparable to thickness data obtained with spectral-domain or time-domain instruments in healthy individuals without diabetes.[3,7–10] For example, retinal thickness was thinnest within the central 1-mm circle, increased in thickness in the parafoveal area (inner subfields 0.5–1.5 mm from the fovea) and subsequently showed a slight decrease in the perifoveal area (outer subfields, 1.5–3.0 mm from the fovea). These observations are consistent with the normal histologic macular contours. In addition, the order of greater retinal thickness measurements in the inner and outer zones were nasal > superior > inferior > temporal as has been true in previous investigations with time-domain and spectral-domain OCT.

Macular thickness, as measured by OCT, is one of the key variables used for eligibility and outcome measures in most clinical trials of macular disease and to guide clinical applications of study results. Because Stratus OCT had been the most common commercially available OCT instrument during the past decade, most trials were designed to incorporate retinal thickness parameters as obtained from Stratus instruments. Trials for diabetic retinopathy have frequently defined CSF thickness values ≥250 μm as indicative of macular edema based on the observation that this value is more than approximately 2 SDs beyond the mean value of CSF thickness among cohorts of subjects with diabetes and no or minimal retinopathy and no apparent central retinal thickening on clinical exam. In fact, the Stratus values obtained in this cohort of subjects without any apparent macular thickening are within 4 μm of the measurements made within each subfield of an earlier DRCR.net cohort of subjects with diabetes and no clinically apparent macular thickening.[3]

The apparent similarity in these numbers provides further validation that the network's current practice to limit reading center oversight to Stratus scans with an SD of at least 10% from the center point and a random 10% sample are satisfactory. In addition, this observation provides further validation that the values we typically use to define "normal" for Stratus measurements are correct. In this study, the mean CSF thickness with the Heidelberg Spectralis instrument was 270 + 24 μm, a measurement that is remarkably similar to the measurement of 270 + 23 μm as determined by Grover et al.[7] in 50 eyes of 50 individuals without any retinal disease. A value of 318 μm would reflect 2 SDs beyond the mean in our cohort of persons with diabetes and no retinopathy or minimal retinopathy. Therefore, 320 μm may be used as a cutoff point to presume macular edema is present when using this instrument in trials of diabetic eye disease, if gender differences are not taken into account. It is recognized that when proposing any cutoff point some eyes with thickness values below the cutoff point may also be edematous (potentially due to cell loss within the neurosensory retina), and that some eyes above the cutoff point will not be edematous, but the goal in suggesting a cutoff point is to maximize the odds that individuals with this measurement or higher measurements truly have abnormal macular thickness.

Attention to the software version used by the OCT instrument to generate the metrics from the OCT scan is recommended to confirm that all studies within a cohort, or that all studies that belong to an individual over time, are interpreted with the same version, since important differences in the metrics generated may occur with software upgrades. Given the inherent differences in 5.1 and 5.3a software we anticipated that macular volume would be the same or larger with 5.3a software, as was observed in our data. Of note, when a discrepancy existed the difference was small, but when

Table 1. Heidelberg Spectralis Optical Coherence Tomography Central Subfield Thickness Stratified by Demographic and Clinical Characteristics

	Overall				By Gender			
					Females		Males	
Factor	*n*	Mean ± SD	*P* Value Unadjusted	*P* Value from Full Multivariate Model*	*n*	Mean ± SD	*n*	Mean ± SD
Overall	122	270 ± 24						
Gender								
Female	67	262 ± 22	<0.001	0.01				
Male	55	278 ± 23						
Age, y†								
<60	68	268 ± 22	0.95		40	262 ± 20	28	275 ± 24
≥60	54	272 ± 25			27	263 ± 24	27	281 ± 22
Duration of diabetes (y)†								
<10	69	271 ± 24	0.09	0.10	40	262 ± 21	29	283 ± 23
≥10	53	268 ± 23			27	263 ± 24	26	273 ± 23
Diabetes type								
Type 1	14	272 ± 32	0.94		9	261 ± 29	5	290 ± 32
Type 2	99	271 ± 22			51	264 ± 20	48	278 ± 22
Uncertain‡	9	250 ± 15			7	250 ± 16	2	250 ± 18
Race/Ethnicity								
Asian‡	3	256 ± 13	0.001	0.08	2	253 ± 16	1	262
Black/African American	19	254 ± 18			14	250 ± 15	5	266 ± 21
Hispanic or Latino	15	264 ± 17			9	256 ± 13	6	277 ± 13
White	85	274 ± 24			42	269 ± 23	43	280 ± 24
Retinopathy severity								
None	103	269 ± 24	0.51		57	262 ± 21	46	278 ± 25
Microaneurysms only	19	273 ± 20			10	268 ± 22	9	279 ± 15
Visual acuity†								
20/12	2	330 ± 23	0.30		1	314	1	346
20/16	10	268 ± 17			4	264 ± 22	6	270 ± 14
20/20	73	268 ± 22			42	261 ± 21	31	278 ± 20
20/25	25	272 ± 24			15	263 ± 18	10	284 ± 27
20/35	12	268 ± 26			5	260 ± 30	7	270 ± 24
Clinical site								
1	21	257 ± 20	0.04	0.15	13	248 ± 18	8	270 ± 17
2	23	272 ± 33			11	265 ± 27	12	280 ± 37
3‡	2	266 ± 28			1	246	1	285
4‡	2	288 ± 11			—	—	2	288 ± 11
5	18	268 ± 18			13	265 ± 19	5	274 ± 19
6‡	1	307			—	—	1	307
7	21	270 ± 18			8	262 ± 11	13	275 ± 21
8	10	264 ± 28			6	252 ± 29	4	282 ± 15
9	24	279 ± 17			15	276 ± 17	9	284 ± 17

* The full multivariate model includes all factors with $P < 0.10$ in the univariate models; after a backward-selection process using $P < 0.01$ to stay in the model, gender was the only factor that remained.

† Factor was treated as a continuous variable in the model. (Visual acuity scores were converted to logMAR in the model.)

‡ Categories where $n < 10$ were excluded from the model.

following eyes longitudinally, version control is needed to look for true differences in volume over time.

The Spectralis-generated scans enabled identification of morphologic changes in the area of the retina scanned that were not suspected by the clinician. Nearly one-third of the eyes submitted for inclusion in this trial were eliminated from the analysis due to the reading center identification of abnormalities such as vitreomacular traction, epiretinal membrane, and cystoid macular edema. Future studies that incorporate SD-OCT may have increased sensitivity to detect macular conditions at baseline that preclude study participation, resulting in more pure cohorts of the pathology of interest. In the present study of thickness parameters in eyes with normal anatomy we elected to exclude eyes with these OCT abnormalities to concentrate on a cohort for whom any suspicion of pathology was minimized.

The mean difference between the macular thickness on Spectralis and Stratus OCT was 71 μm in the CSF, remarkably similar to the 69 μm difference identified by Grover in subjects without diabetes and without any retinal pathology.[4] One of the major differences between the Stratus and Spectralis OCT is the location the instrument software has been programmed to define as the outer retinal boundary for retinal thickness measurements. The instrument thickness differences vary across subfields with the greater differences in the central and inner subfields than those in the outer subfields. This is consistent

Table 2. Heidelberg Spectralis Optical Coherence Tomography Retinal Thickness Stratified by Gender

Factor	Females ($n = 67$) Mean ± SD	Males ($n = 55$) Mean ± SD	*P* Value
Center Point	222 ± 21	233 ± 27	0.02
Central Subfield	262 ± 22	278 ± 23	<0.001
Inner Zone			
Superior	330 ± 20	340 ± 15	0.004
Nasal	333 ± 19	343 ± 15	0.001
Inferior	327 ± 19	338 ± 15	0.001
Temporal	319 ± 19	329 ± 13	<0.001
Outer Zone			
Superior	289 ± 17	292 ± 15	0.31
Nasal	301 ± 19	309 ± 19	0.03
Inferior	279 ± 19	281 ± 16	0.55
Temporal	276 ± 17	281 ± 13	0.09
Volume	8.3 ± 0.5	8.5 ± 0.4	0.04

Number missing due to nongradable:

Females: 0 Center Point, 0 Central Subfield, 1 Inner Superior, 1 Inner Nasal, 1 Inner Inferior, 1 Inner Temporal, 0 Outer Superior, 3 Outer Nasal, 2 Outer Inferior, 2 Outer Temporal, 6 Volume.

Males: 0 Center Point, 0 Central Subfield, 0 Inner Superior, 0 Inner Nasal, 0 Inner Inferior, 0 Inner Temporal, 0 Outer Superior, 0 Outer Nasal, 1 Outer Inferior, 2 Outer Temporal, 6 Volume.

with the histologic findings that both photoreceptors and the retinal pigment epithelium are taller in the fovea and parafoveal areas than those in the surrounding perifoveal macula.

The present study also showed a significant difference in mean CSF thickness and macular volume between males and females as has been previously reported with Stratus OCT,[1,2,4] other SD-OCT instruments (Fraser-Bell S, et al. *IOVS* 2005;46:ARVO E-Abstract 1542), and other time-domain instruments.[7–9] With the Spectralis instrument, a similar trend among 50 eyes was noted by Grover and colleagues[7] (CSF thickness 274 μm in males vs. 266 μm in females). The observed gender differences were noted across clinical characteristics as was found previously with Stratus measurements.[3] This report adds to the consistency of this observation strengthening our previous recommendation to consider separating norms by gender when designing clinical trials for DME based on OCT and determining "normal" upper limits for assessing eligibility and outcome measures. As such, a CSF thickness of 320 μm for males and 305 μm for females (~2 SDs above the average for this normative cohort) are proposed as the gender-specific minimum thickness criteria for trials including eyes with central or center involved DME using Spectralis measurements.

Although the mean CSF thickness was higher in Caucasians compared with that of other racial groups, the number of non-Caucasians was too small for a meaningful statistical evaluation, and the observed difference was not statistically significant in our multivariate analysis after adjusting for gender. Others have found racial differences when measuring CSF with the Stratus instrument (Fraser-Bell S, et al. *IOVS* 2005;46:ARVO E-Abstract 1542).[8] Wagner-Schuman et al evaluated race differences in retinal thickness on SD-OCT and concluded that the variation in thickness observed between the races appears to be driven by differences in foveal pit morphology.[2] It has also been hypothesized that this is due to attenuation of incident optical radiation by the increased pigment in the apical portion of the RPE cells, leading to a decreased signal of posterior retinal segments and concomitant underassessment of retinal thickness in darkly pigmented persons.[7]

Our cohort had a median age of 59 years and older subjects had very slightly thinner macular volume measurements on average than those of younger subjects. This finding affirms, as previously reported by Fraser-Bell et al., that separating norms by age is a reasonable consideration when the primary area of interest is volume rather than CSF (Fraser-Bell S, et al. *IOVS* 2005;46:ARVO E-Abstract 1542).

In conclusion, this study reports the nine standard ETDRS grid subfield mean retinal thickness values in a cohort of individuals with diabetes and no retinopathy, or minimal retinopathy and a normal-appearing macular architecture, as

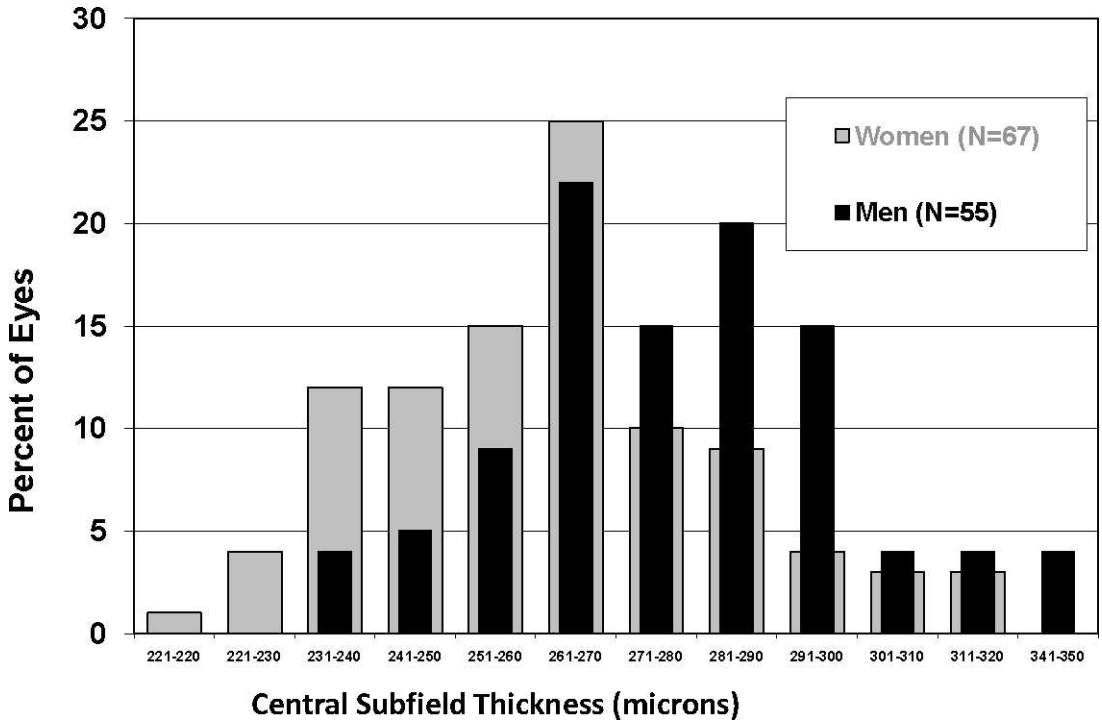

Figure 3. Distribution of central subfield thickness by gender.

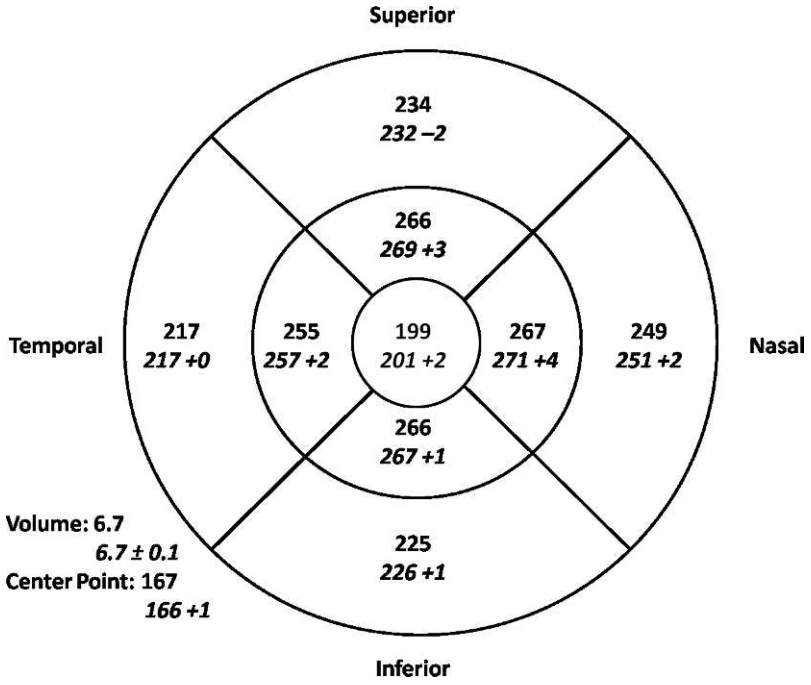

Figure 4. Differences between Zeiss Stratus OCT retinal thickness measurements in current study ($n = 122$) and a prior Diabetic Retinopathy Clinical Research Network study ($n = 97$),[3] which initially reported Zeiss Stratus OCT thicknesses in a similar cohort of individuals with diabetes and minimal or no retinopathy and no central retinal thickening on clinical exam. *Top number* represents mean on current study, *bottom number* (in *italics*) represents mean on prior study, and number to the *right* (in *italics*) represents the difference between the means (prior − current).

obtained with the Heidelberg Spectralis instrument. Spectralis measurements share similarities to those obtained with TD-OCT instruments, such as the hierarchy of greater thickness locations with respect to the fovea and gender and age differences within those values. As anticipated, and based on retinal anatomy and histology and the major differences between placement of outer retinal boundary lines with Spectralis OCT as compared with Stratus OCT, average Spectralis thickness measurements are uniformly larger, with an average difference of 71 μm in the center subfield. A CSF thickness of 320 μm for males and 305

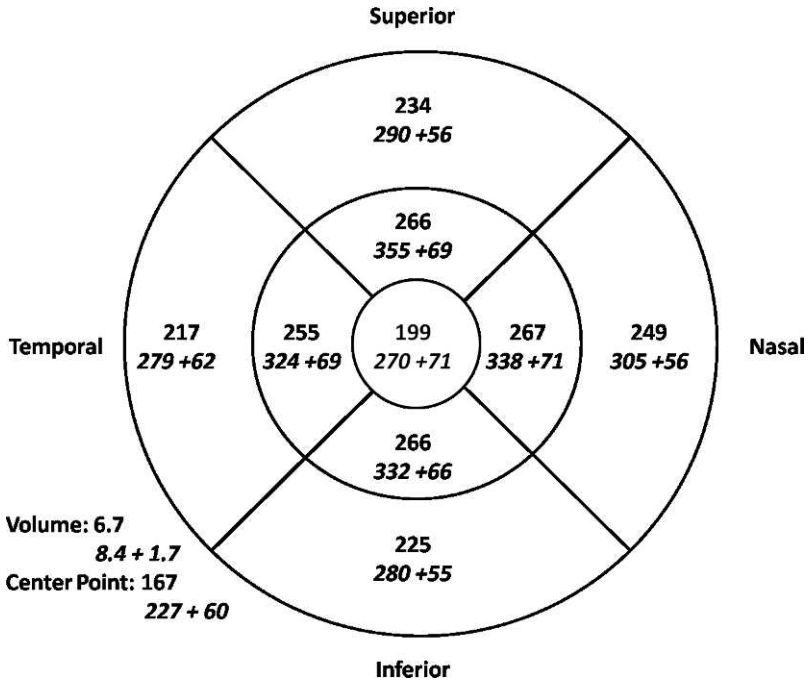

Figure 5. Differences between Heidelberg Spectralis OCT and Zeiss Stratus OCT retinal thickness measurements. *Top number* represents mean on Stratus, *bottom number* (in *italics*) represents mean on Spectralis, and number to the *right* (in *italics*) represents the difference between the means (Spectralis − Stratus). Based on $n = 122$ eyes; Spectralis number missing due to nongradable: 0 Central Subfield, 1 Inner Superior, 1 Inner Nasal, 1 Inner Inferior, 1 Inner Temporal, 0 Outer Superior, 3 Outer Nasal, 3 Outer Inferior, 2 Outer Temporal, 6 Volume, 0 Center Point; Stratus number missing due to nongradable: 1 central subfield, 1 center point, 5 for each inner and outer subfield, 6 volume.

μm for females (~2 SDs above the gender-specific average for this normative cohort) are proposed as the minimum thickness criteria for defining the presence of DME in trials on DME using Spectralis measurements.

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Appendix

Diabetic Retinopathy Clinical Research Network Clinical Sites That Participated in This Protocol

Sites are listed in order by number of subjects enrolled into the study. The number of subjects enrolled is noted in parentheses preceded by the site location and the site name. Personnel are listed as (I) for Study Investigator, (C) for Coordinator, and (P) for Photographer.

West Des Moines, IA Wolfe Eye Clinic (33) David D. Saggau (I); Jared S. Nielsen (I); Kyle J. Alliman (I); Marianne Parker (C); Jay Rostvold (P) **Jacksonville, FL University of Florida College of Medicine, Department of Ophthalmology, Jacksonville Health Science Center (29)** Kakarla V. Chalam (I); Shailesh K. Gupta (I); Sandeep Grover (I); Tamil M. Singh (C,P) **Austin, TX Retina Research Center (26)** Brian B. Berger (I); Kristen Davis (C); Ben Ostrander (P); Yong Ren (P) **Boston, MA Joslin Diabetes Center (25)** Lloyd P. Aiello (I); Deborah K. Schlossman (I); George S. Sharuk (I); Jennifer K. Sun (I); Paul G. Arrigg (I); Sabera T. Shah (I); Timothy J. Murtha (I); Hanna Kwak (C); Margaret E. Stockman (C); Ann Kopple (C); Elizabeth S. Weimann (P); Leila Bestourous (P); Rita Kirby (P); Robert W. Cavicchi (P); Deborah K. Schlossman (I); George S. Sharuk (I); Jennifer K. Sun (I); Paul G. Arrigg (I); Sabera T. Shah (I); Timothy J. Murtha (I); Hanna Kwak (C); Margaret E. Stockman (C); Elizabeth S. Weimann (P); Leila Bestourous (P); Robert W. Cavicchi (P) **Lakeland, FL Florida Retina Consultants (24)** Scott M. Friedman (I); Jessica Maldonado (C,P); Karen Sjoblom (C,P) **Charlotte, NC Charlotte Eye, Ear, Nose and Throat Association, PA (13)** Andrew N. Antoszyk (I); David Browning (I); Ashley A. McClain (C); Angela K. Price (C); Donna McClain (P); Loraine M. Clark (P); Michael D. McOwen (P); Pearl A. Leotaud (P); Susannah J. Held (P); Uma M. Balasubramaniam (P) **New York, NY Mount Sinai School of Medicine, Department of Ophthalmology (13)** Patricia J. Pahk (I); Robin Nina Ginsburg (I); Natalie Cheung (C); Barbara A. Galati (P); Eneil Simpson (P) **Lubbock, TX Texas Retina Associates (3)** Michel Shami (I);Yolanda Saldivar (C); Brenda Arrington (P); **Indianapolis, IN Raj K. Maturi, MD, PC (2)** Raj K. Maturi (I); Laura A. Bleau (C,P); Ashley Harless (P) **Philadelphia, PA University of Pennsylvania Scheie Eye Institute (2)** Alexander J. Brucker (I); Sheri Drossner (C); Jim M. Berger (P); Joan DuPont (C); Elizabeth Windsor (P); Laurel Weeney (P); William Nyberg (P); Cheryl Devine (P) **Portland, OR Retina Northwest, PC (2)** Mark A. Peters (I); Stephen Hobbs (C,P); **Augusta, GA Southeast Retina Center, PC (2)** Dennis M. Marcus (I); Harinderjit Singh (I); Graciela R. Zapata (C); Ken Ivey (P)

DRCR.net Coordinating Center: Jaeb Center for Health Research, Tampa, FL (staff as of 11/29/2011): Adam R. Glassman (Director and Principal Investigator), Roy W. Beck, Talat Almukhtar, Bambi J. Arnold, Eureca J. Battle, Caitlin Beran, Brian B. Dale, Alyssa M. Baptista, Sharon R. Constantine, Simone S. Dupre, Allison R. Edwards, Meagan L. Huggins, Paula A. Johnson, Brenda L. Loggins, Emily B. Malka, Shannon L. McClellan, Michele Melia, Pamela S. Moke, Haijing Qin, Rosa Pritchard, Cynthia R. Stockdale, Karisse Torres, Angella Wujcik.

OCT Reading Center at Duke, Durham, NC: Brannon Balsley, Adam Brooks, Russell Burns, Cynthia Heydary, Glenn J. Jaffe, MD, Beth Oakley, Kelly Shields, Garrett Thompson. Cynthia A. Toth, MD, Katrina Winter.

DRCR.net Operations Center: Johns Hopkins University School of Medicine, Baltimore, MD (staff as of 6/8/2011): Neil M. Bressler (Network Chair and Principal Investigator), Connie Lawson, Peggy R. Orr, Beth Wellman.

DRCR.net Vice Chairs: Carl W. Baker (2011–current), Scott Friedman (2009–current), Jennifer K. Sun (2012–current). Prior Chairs: Susan B. Bressler (2009–2011), Ingrid U. Scott (2009–2010).

National Eye Institute: Eleanor Schron (2009–current), Donald F. Everett (2003–2006, 2007–2009).

Executive Committee: Michael J. Elman (2006–present; Chair 2009, 2012), Lloyd Paul Aiello (2002–present; Chair 2002–2005), Carl W. Baker (2009–present), Roy W. Beck (2002–present), Abdhish Bhavsar (2007–2008; 2010–present; Chair 2011), Neil M. Bressler (2006–present; Chair 2006–2008), Ronald P. Danis (2004–present), Matthew D. Davis (2002–present), Frederick L. Ferris III (2002–present), Scott Friedman (2007–present), Adam R. Glassman (2005–present), Jeffrey G. Gross (2012–present), Diana Holcomb (2011–present), Glenn J. Jaffe (2012–present), Lee M. Jampol (2012–present), Dennis Marcus (2011–present), Eleanor Schron (2009–present), Jennifer K. Sun (2009–present), John A. Wells Jr (2012–present), Susan B. Bressler (2009–present), Prior Members: Andrew N. Antoszyk (2009), Alexander J. Brucker (2009–2010), Kakarla V. Chalam (2009–2011), Donald F. Everett (2002–2009), Joan Fish (2009), Joseph Googe Jr (2009–2010), Raj K. Maturi (2009–2011; Chair 2010), Ingrid U. Scott (2009–2010), JoAnn Starr (2010).