Choroidal Thinning in Diabetes Type 1 Detected by 3-Dimensional 1060 nm Optical Coherence Tomography

Marieh Esmaeelpour,[1,2] *Simon Brunner,*[1] *Siamak Ansari-Shahrezaei,*[1] *Susanne Nemetz,*[3] *Boris Považay,*[2] *Vedran Kajic,*[2] *Wolfgang Drexler,*[2] *and Susanne Binder*[1]

Purpose. To map choroidal (ChT) and retinal thickness (RT) in patients with diabetes type 1 with and without maculopathy and retinopathy in order to compare them with healthy subjects using high speed 3-dimensional (3D) 1060 nm optical coherence tomography (OCT).

Methods. Thirty-three eyes from 33 diabetes type 1 subjects (23–57 years, 15 male) divided into groups of without pathology (NDR) and with pathology (DR; including microaneurysms, exudates, clinically significant macular-oedema and proliferative retinopathy) were compared with 20 healthy axial eye length and age-matched subjects (24–57 years, 9 male), imaged by high speed (60.000 A-scans/s) 3D 1060 nm OCT performed over 36° × 36° field of view. Ocular health status, disease duration, body mass index, haemoglobin-A1c, and blood pressure (bp) measurements were recorded. Subfoveal ChT, and 2D topographic maps between retinal pigment epithelium and the choroidal/scleral-interface, were automatically generated and statistically analyzed.

Results. Subfoveal ChT (mean ± SD, μm) for healthy eyes was 388 ± 109; significantly thicker than all diabetic groups, 291 ± 64 for NDR, and 303 ± 82 for DR (ANOVA $P < 0.004$, Tukey $P = 0.01$ for NDR and DR). Thinning did not relate to recorded factors (multi-regression analysis, $P > 0.05$). Compared with healthy eyes and the NDR, the averaged DR ChT-map demonstrated temporal thinning that extended superiorly and temporal-inferiorly (unpaired *t*-test, $P < 0.05$). Foveal RT and RT-maps showed no statistically significant difference between groups (mean SD, μm, healthy 212 ± 17, NDR 217 ± 15, DR 216 ± 27, ANOVA $P > 0.05$).

Conclusions. ChT is decreased in diabetes type 1, independent of the absence of pathology and of diabetic disease duration. In eyes with pathology, 3D 1060 nm OCT averaged maps showed an extension of the thinning area matching retinal lesions and suggesting its involvement on onset or progression of disease. (*Invest Ophthalmol Vis Sci.* 2012;53:6803–6809) DOI:10.1167/iovs.12-10314

From the [1]Ludwig Boltzmann Institute for Retinology and Biomicroscopic Laser Surgery, Department of Ophthalmology, Rudolph Foundation Hospital, Vienna, Austria; the [2]Center for Medical Physics and Biomedical Engineering, Medical University Vienna, Austria; and the [3]Optique Nemetz, Vienna, Austria.

Supported by grants in part by the DTI grant (OMICRON); AMR (AP1110); Med Uni Vienna, EU project FUN OCT (FP7 HEALTH, 201880), FWF-NF, OENB Jubiläumsfond (14294), CARL ZEISS Med Inc., Femtolasers GmbH, Christian Doppler Society.

Submitted for publication June 1, 2012; revised August 6, 2012; accepted August 26, 2012.

Disclosure: **M. Esmaeelpour**, None; **S. Brunner**, None; **S. Ansari-Shahrezaei**, None; **S. Nemetz**, None; **B. Považay**, None; **V. Kajic**, None; **W. Drexler**, Carl Zeiss Meditec (C); **S. Binder**, None

Corresponding author: Marieh Esmaeelpour, Rudolfstiftung, Department of Ophthalmology, Juchgasse 25, 1030 Vienna, Austria; marieh.esmaeelpour@meduniwien.ac.at.

Diabetes mellitus with its main subtypes 1 and 2 is a chronic disease that requires lifelong control and monitoring of the eyes, cardio-vasculature, feet, and kidneys. Type 1, resulting from β-cell destruction, is either autoimmune or idiopathic, while diabetes type 2 is due to insulin resistance, insulin secretion defects, or both.[1,2] Intensive therapy in type 1 diabetic patients has been shown to reduce complications to these organs, however, it has been shown that after 25 to 30 years disease duration, cumulative incidences of proliferative diabetic retinopathy (PDR), and clinically significant macular oedema (CSME) persist for up to 20% of patients.[3,4] Diabetic eye disease, when advanced, such as in PDR and CSME is a serious complication that can lead to visual impairment.[5,6] The presence of metabolic[7] and ocular perfusion[8,9] changes in diabetes suggests that the retina becomes hypoxic, which may lead to severe retinopathy over time.

Recently, a study by our group demonstrated wide areas of thinning in the choroid in patients with diabetes type 2, even in the absence of retinopathy.[10] The choroid is an important source of nutrition and oxygen for the outer retina and the macular region of the eye and this choroidal alteration may support adverse retinal oxygenation. However, in diabetes type 2 it is difficult to determine the onset of diabetes as it can take years before it is detected,[1,2] and it is not possible to determine if choroidal thinning is related to the duration of diabetes. Systemic pathologies, such as arteriosclerosis, that often co-exist in diabetes type 2 may have also contributed to the thickness change. In contrast, diabetes type 1 patients are, on average, younger at the onset of diabetes, and while type 2 diabetes may be preceded by a long period of asymptomatic hyperglycemia,[1] the onset of diabetes type 1 with its acute complications is well known. In order to investigate possible choroidal change and being able to relate it to the onset and the glycemic component of the diabetic disease, this study aimed to measure choroidal thickness (ChT) in patients with diabetes type 1 and compare ChT with healthy age and axial eye length (AL) matched eyes. To enable the analysis of choroidal change over a large field of view, retinal and choroidal imaging was performed by optical coherence tomography (OCT) at 1060 nm wavelength and images were used for measurements with the newly developed automatic mapping of ChT.[11]

Methods

Subjects

Thirty-three subjects with type 1 diabetes mellitus and 20 healthy subjects were recruited. Ethical approval was obtained from the Ethical Commission of Vienna. The studies followed the Declaration of Helsinki, and informed consent was obtained from all subjects prior to participation. Exclusion criteria were diseases other than diabetes,

Table 1. Demographic and Statistical Data of the Cohort

	Age, y	AL, mm	Hemoglobin A1c, %	BMI, kg/m²	LogMAR	Systemic BP, mm Hg	Diastolic BP, mm Hg	Duration*, y
NDR, $n = 15$, males = 7, BP medication = 2								
Mean ± SD	37 ± 10	24.19 ± 0.82	7.3 ± 0.7	24.7 ± 2.9	−0.01 ± 0.05	125 ± 18	74 ± 11	16 ± 8
Range	23 to 57	22.76 to 25.77	5.8 to 8.8	21 to 30	−0.2 to 0.02	92 to 149	60 to 93	6 to 34
DR, $n = 18$, males = 8, BP medication = 2								
Mean ± SD	39 ± 9	23.37 ± 1	7.7 ± 0.9	26.4 ± 5	0.01 ± 0.16	124 ± 10	79 ± 12	23 ± 8
Range	25 to 54	21.03 to 25.3	6.5 to 10	20 to 36	−0.1 to 0.26	108 to 137	60 to 102	10 to 38
Healthy, $n = 20$, males = 9, BP medication = 0								
Mean ± SD	40 ± 9	23.7 ± 1.1		24.4 ± 3.3	−0.03 ± 0.07	117 ± 9	75 ± 6	
Range	24 to 57	22.12 to 25.15		20 to 32	−0.2 to 0.1	100 to 136	60 to 84	

Unpaired *t*-test.
* $P < 0.03$.

history of retinal surgery, retinal pathology, or physiological variation from normal, such as glaucoma or retinal scarring. Inclusion criteria were a previous diagnosis of diabetes type 1. All subjects were diagnosed prior to the age of 30 years. Demographic data of the subject cohort can be found in Table 1. Retinal status of diabetic eyes was evaluated after pupil dilation by an experienced ophthalmologist with slit lamp biomicroscopy with a Volk lens (Volk Optical, Inc., Mentor, OH). All lesions and their location on the retina were recorded and eyes were graded according to Early Treatment Diabetic Retinopathy Study (ETDRS).[12] For the study grouping, all subjects with eyes with no clinically visible diabetic abnormalities, such as microaneurysms, were grouped together to ensure a homogenous selection. This resulted in 15 left eyes from 15 subjects with no diabetic retinopathy (NDR). The second group consisted of all remaining eyes presenting with diabetic retinopathy (DR) and the eye with the more advanced retinopathy was chosen. The DR group consisted of 10 eyes with microaneurysms, two eyes with additional exudates, two eyes with PDR (one eye with neovascularization at the disk and temporal retina and one eye with within 1-disc diameter superior retinal oedema and temporal neovascularization) and four eyes with CSME and macular cysts.

Healthy control subjects were included only if they had fewer than three of the following risk factors for diabetes: family history of diabetes, age greater than 50 years, ethnic background, diagnosed high blood pressure, heart attack, or stroke Only non smokers were included. Only one eye was included per healthy subject. Healthy subjects and eyes were chosen to match age and AL according to the mean and SD of the diabetic groups (ANOVA, $P > 0.05$).

Study Protocol

Monocular visual acuity was determined with ETDRS Original Series Charts (Precision Vision, LaSalle, IL). Five AL measurements were averaged from each eye using optical biometry (IOL Master Zeiss, Jena, Germany). All subjects had blood pressure (BP) measured after at least 20 minutes resting in a sitting position. All diabetic subjects had their glycosylated hemoglobin A1c (an estimate of the average blood glucose over the past 1–3 months) recorded if the measurement was not older than 3 months or else it was tested on the day of the recruitment. Fasting blood glucose was not obtained, as this would have altered homeostatic mechanisms, and not allowed a representative comparison to be made between normal and diabetic eyes. Information about diabetes duration, weight, and height were recorded.

OCT Imaging and Thickness Maps

High speed, 60.000 A-scans/s 3D OCT–imaging at 1060 nm was performed with less than 2.5 mW at the cornea, well below the maximum power limit for 10 second exposure.[13,14] 3D OCT volumes were acquired at 1060 nm with 15- to 20-μm transverse resolution, approximately 7-μm axial resolution and 512 voxels per depth-scan (A-scan). Raster scans across a 36 × 36° field were centered on the fovea and resulted in up to 120 frames/second. Automatic retinal and choroidal segmentation, automatic measurement of ChT from preprocessed images with ImageJ software (available at http://rsb.info.nih.gov/ij/index.html; National Institutes of Health, Bethesda, MD)[8] and the generation and statistical analysis of thickness maps is described elsewhere.[11,15] Briefly, axial retinal thickness was defined as the distance between internal limiting membrane and the center of the peaks originating from the retinal pigment epithelium/Bruch's membrane/choriocapillaris (RBC) complex and axial ChT as the distance between RBC complex and the choroidal-scleral interface (Fig. 1 demonstrates segmentation lines at each of the interfaces). For the investigation of the thickness variation throughout the entire field of view, thickness maps were generated based on automatic segmentation.[11] This method uses training data from manual segmentations in healthy and diseased eyes of previous publications to build a statistical model. Its advantage is that it can actively learn and determine the segmentation line in a low signal, noisy environment such as in OCT tomograms in the region of the choroid without having to rely on boundary edge information. The resulting pixel distance was converted into optical distance using the depth sampling calibration for the 1060 nm OCT system and further to the anatomical distance. This resulted in thickness maps for individual eyes. Reported variation for automatic segmentation in eyes with pathology is 13%,[11] comparable to values seen by retinal automated segmentation.[16] Interobserver variability, measured by re-imaging seven subjects (two with pathology) and comparing automatic segmentation of subfoveal ChT, ranged between 0% and 10% with a median of 1% difference between the first and the second image.

For the investigation of a correlation between the location of retinal change and choroidal thickness alterations, individual ChT-maps were

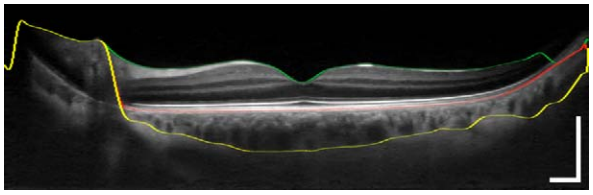

Figure 1. Example of segmentation lines in an OCT-tomogram: retinal thickness as the distance between internal limiting membrane (*green line*), and the center of the peaks originating from the retinal pigment epithelium/Bruch's membrane/choriocapillaris (RBC) complex (*red line*), and axial ChT as the distance between RBC complex and the choroidal-scleral interface (*yellow line*). *White bars* represent *scale bars* of 0.5-mm length.

viewed at the location of recorded microaneurysms, exudates and focal edema, or proliferation for obvious changes in thickness.

For the statistical analysis of mean and variation, all the individual ChT-maps and RT-maps were aligned to each other in respect to the macula and optic nerve position with Matlab software (The MathWorks, Inc., Natick, MA). Unreliable portions of the images with five or less than five measurements at one location were excluded. Before statistical analysis the maps were median filtered by a 30×30 kernel to suppress the influence of local fluctuations of the individual maps (e.g., vessels) or positioning errors. To judge the clinical significance of possible thickness alteration in this study, subfoveal ChT was measured after automatic segmentation and smoothing for compound maps and controlled for manually by an experienced observer. The average difference was below 1% for each group of subjects with an SD of 9% (9% for NDR, 7% for DR, and 10% for healthy, $P > 0.05$, ANOVA).

To create a compound map of average thickness, mean and SD was obtained for these three groups of eyes with color-coded thickness maps. The coefficient of variation was used to map contour lines of 45%, 30%, and 15% for the variation within each group. Difference maps were generated to investigate the change in ChT and RT by subtracting each diabetic category from the healthy eyes group. A further statistical analysis of the difference between the healthy and each diabetic group was generated by conducting *t*-tests over the field of view. To show areas of statistically significant difference between the compound maps, contour lines for *P* values smaller than 0.05 were drawn on the difference maps. The statistics software IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY) was used for conducting ANOVA testing and a multiregression analysis of the contribution of groups and their characterizing factors to ChT. Therefore, central ChT measurement was located beneath the foveola.

Results

All 1060 nm OCT tomograms were of sufficient quality to allow retinal and choroidal mapping. Examples of the 3D 1060 nm OCT images viewed in tomograms, enface views of the choroid and related RT- and ChT-maps are given in Figure 2 for healthy, and several diabetic eyes at various disease stages.

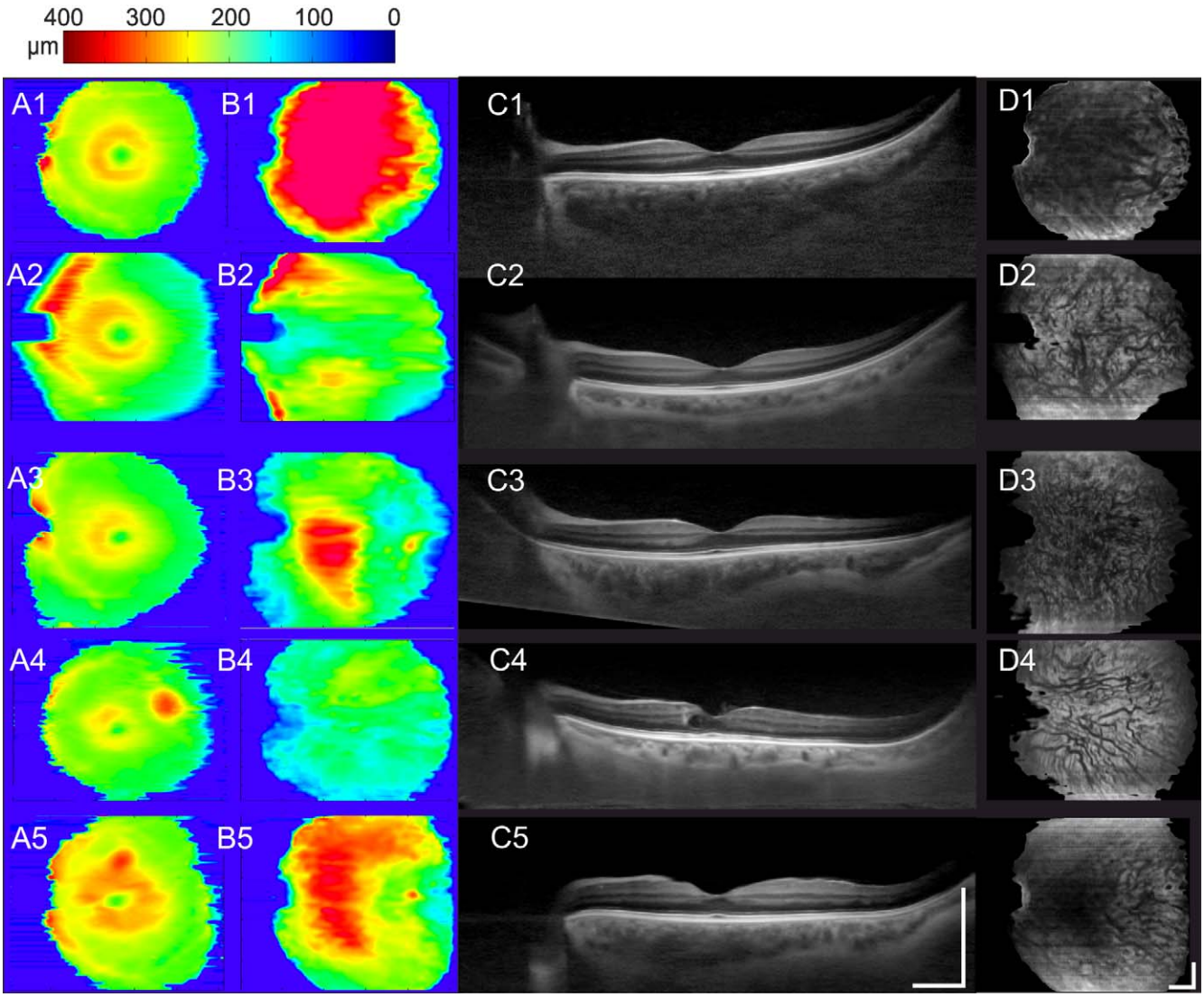

Figure 2. Examples in healthy (*row 1*) and diabetic subjects (*rows 2–5*) of retinal thickness maps (**A**), choroidal thickness maps (**B**), horizontal tomograms through the fovea, and optic nerve (**C**), and enface tomograms through the Haller's and Sattler's layer of the choroid (**D**). Subjects with diabetes had different stages of retinopathy: NDR (2), microaneurysms (3), cyst underneath the fovea and edema within one disk diameter (4), and proliferative diabetic retinopathy (5). All subjects had short axial eye length (1: 23.01 mm, 41 years; 2: 23.31 mm, 44 years; 3: 22, 65 mm, 36 years; 4: 23.03 mm, 49 years, 5: 22.736 mm, 28 years). *White bars* represent *scale bars* of 1-mm length.

Table 2. Retinal Foveal and Choroidal Subfoveal Thickness Measurements (μm) from Healthy Eyes and Eyes of Subjects with Diabetes

	NDR	DR	Healthy Eyes
Retina			
Mean ± SD	217 ± 15	216 ± 27	212 ± 17
Range	192 to 237	173 to 280	183 to 248
Choroid			
Mean ± SD	291 ± 64	303 ± 82	388 ± 109*
Range	206 to 457	185 to 396	212 to 570

* Significantly different from the choroidal thickness in the diabetic groups (ANOVA $P = 0.004$, Tukey $P < 0.01$ for the difference between healthy and each diabetic group).

In the healthy eyes, mean subfoveal ChT was thickest (Table 2) when compared with diabetic groups (ANOVA, $P = 0.004$, Tukey, $P = 0.01$ for comparison with each diabetic eye group). There was no difference between the diseased groups (Tukey $P > 0.05$). Mean foveal retinal thickness did not show any difference neither between the healthy and diabetic eyes nor among the two diabetic groups (ANOVA, $P > 0.05$). When comparing factors characterizing the three study groups (Table 1) there was no difference between their BP, both systolic and diastolic, logMAR vision and BMI index (ANOVA, $P > 0.05$). Between the NDR and DR group the mean hemoglobin A1c was not significantly different (*t*-test, $P > 0.05$). The DR group had a significantly longer disease duration than the NDR group (unpaired *t*-test, $P = 0.03$). Multi-regression analysis including age, AL, hemoglobin A1c, BP, BMI, and disease duration showed no significant effect of any of these factors on ChT of diabetic eyes ($R^2 = 0.19$, $P > 0.05$). In healthy eyes the only factor with a trend to contributing to ChT was AL ($R^2 = 0.44$, $P > 0.05$, Beta = –0.58, $P = 0.07$).

When examining the compound RT-maps, contour lines delineating the coefficient of variation (Fig. 3) show large areas of the retina to have 15% or less variation, although this area is slightly smaller in the DR group. The difference maps show a relative thinning of the overall retina in the NDR group in comparison with the healthy eyes; however, this difference is not statistically significant. In the DR group there is a ring form increase of retinal thickness that is incomplete in the temporal macula. However, the thickening reaches statistical significance in only a very small superior-temporal area.

Compound ChT-maps show that the minimum of variation between the eyes in each group is between 15% to 30% (Fig. 4). Healthy eyes have the thickest choroid distributed over a large area including the fovea and extending furthest temporally and superiorly. In the NDR group, identified by their deep

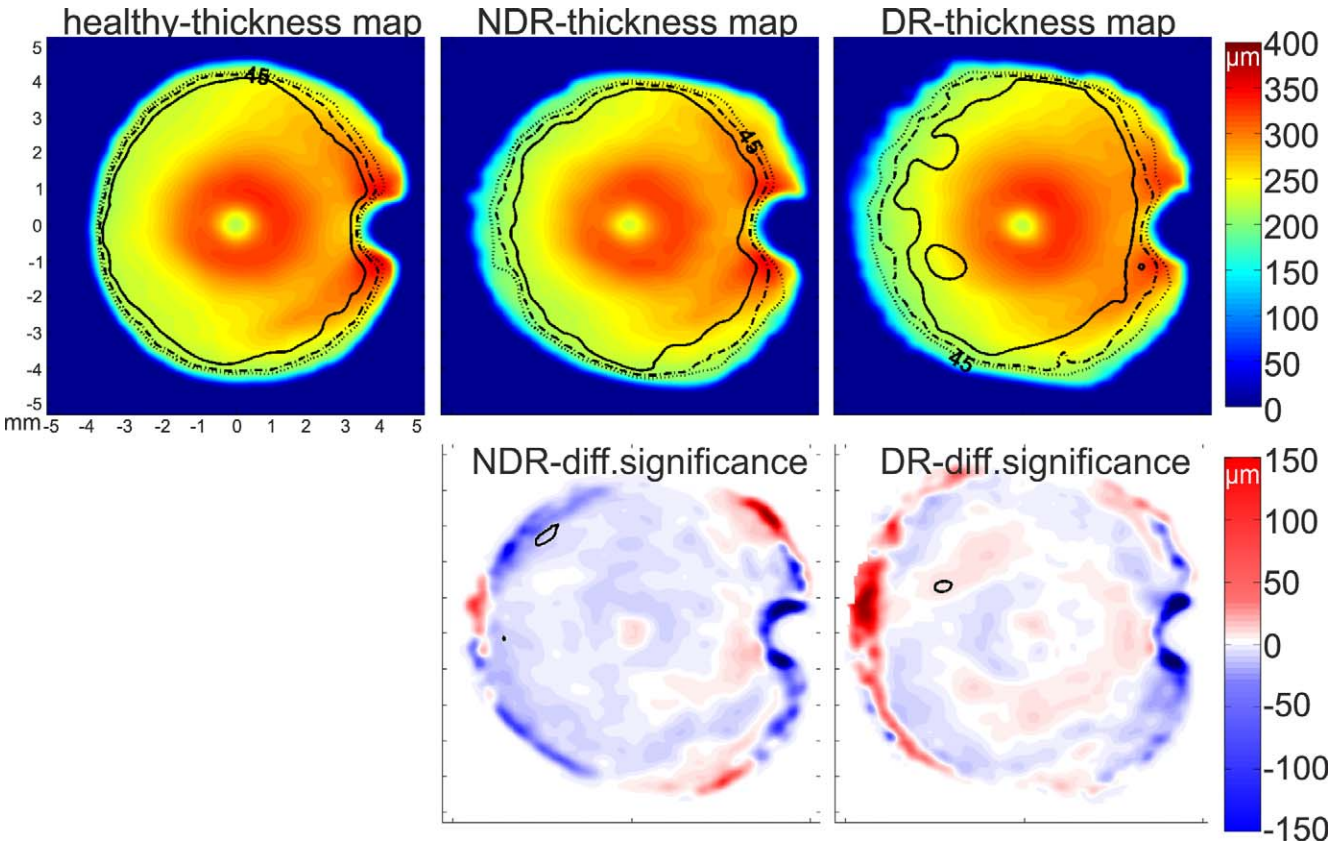

Figure 3. Retinal thickness maps averaged for each study group (*upper row*) and a comparison of thickness change mapped with significant difference *contour lines* (*lower row*). In the *upper row dotted, broken, and solid lines* represent 45%, 30%, and 15% variation, respectively. *Solid contour lines* in the *lower row* represent a statistical significance *P* value of 0.05.

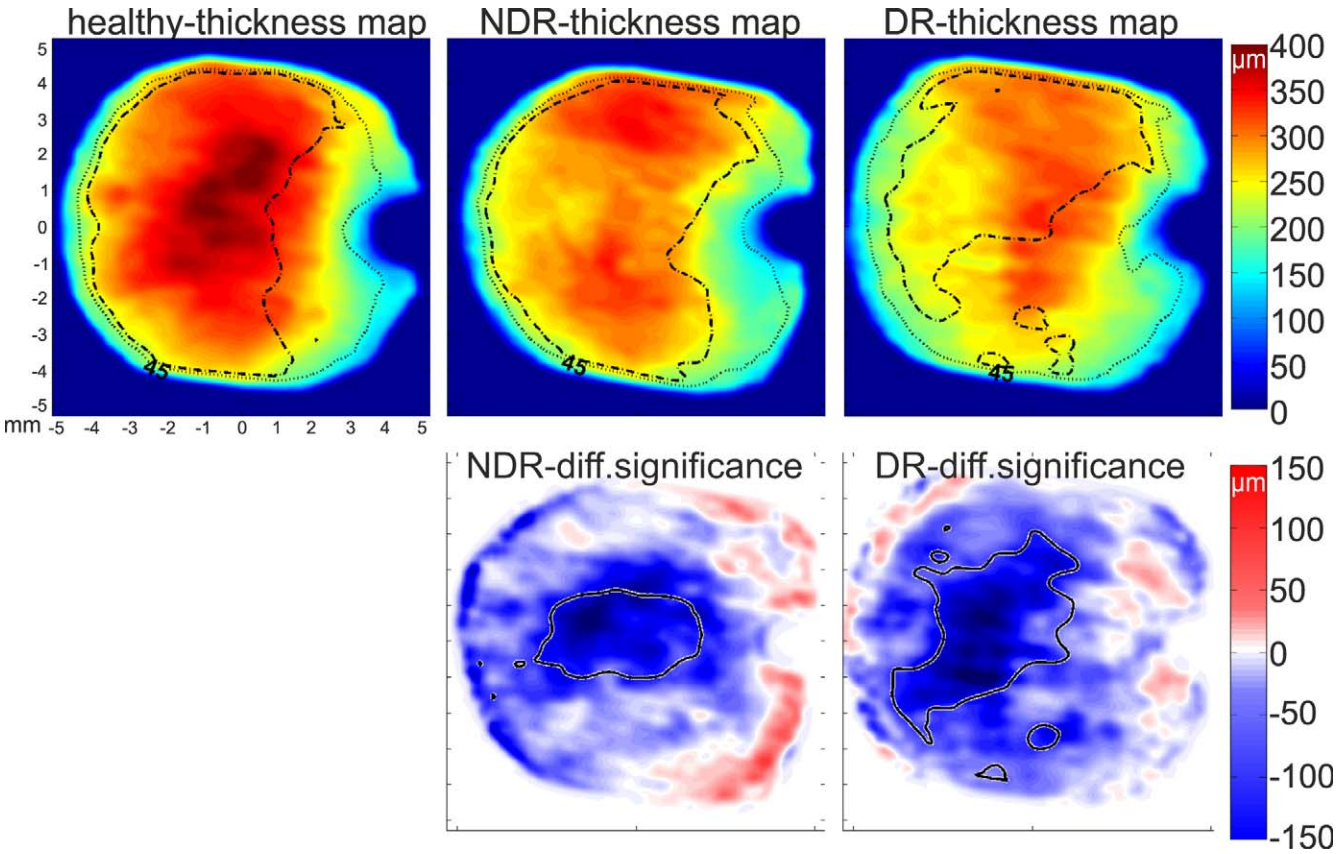

Figure 4. Choroidal thickness maps averaged for each study group (*upper row*) and a comparison of thickness change mapped with significant difference *contour lines* (*lower row*). In the *upper row dotted and broken lines* represent 45% and 30% variation, respectively. *Solid contour lines* in the *lower row* represent a statistical significance *P* value of 0.05.

red color, there are two areas of similarly thick choroid as in the healthy eye group, the larger area is superiorly and the other smaller area is inferior to the fovea. This group has a slightly larger area of least amount of variation of below 30%, reaching farthest in the superior, inferior, and temporal retina. ChT in the DR group is thickest between the macula and optic nerve, but the map also shows the highest variation area distributed from around the optic nerve to the inferior choroid. In contrast to retinal thickness maps, despite the larger variation within the ChT-maps, there are large joint areas of statistically significant thinning in the diabetic eyes. In the NDR group this area is covering the parafovea and reaching farther into the temporal retina. In the DR group the area of significant thinning corresponds to the areas of least variation in this group. The largest area of significant thinning is distributed from the superior choroid to the inferior-temporal side including the fovea.

To investigate a possible relationship between the retinal lesions and choroidal thickness change, 3D 1060 nm OCT images and maps were viewed individually for the distribution of vessels in Haller's and Sattler's layer. Overall, microaneurysms were reported by the examining ophthalmologist in various locations and showed no preference for a specific location on the retina. In the two eyes with exudates, the exudates were located in one eye temporally and in the other temporally and nasally within 1 disk-diameter to the fovea. More severe lesions such as increased retinal thickening and proliferative changes were found temporally or superiorly to the macular region and in between these two areas (Fig. 2, rows 4 and 5). Examining individual ChT-maps in both diabetic groups an overall thin choroid could be seen in some eyes (Fig. 2, rows 2 and 4), while others seemed to have most thinning in the temporal retina (Fig. 2, rows 3 and 5). Both cases were observed independent of the presence of a lesion. This structural distribution of ChT, explains the higher ChT variation in the nasal retina in comparison with the temporal area.

Discussion

Recent advances in understanding the mechanisms of retinal vessel occlusion and leakage increase leading to edema, angiogenesis, and in the proliferative phase to new vessel formation, has resulted in a wider choice of treatment options, such as intensive glycemic and lipid control,[17] fenofibrate treatment,[18] and the use of ANTI-VEGF agents[19] in addition to the classical retinal laser treatment. However, adverse effects, such as an increase of cataract and glaucoma incidence,[20] and unwarranted treatment success, are seen with each choice.[18,21] Recent interest has been focused on the choroid as an important structure involved in the pathophysiology of diabetic eye disease. The choroid supplies the outer retina with glucose and oxygen and in diabetic eyes with advanced disease capillary dropout has been visualized with histology.[22] But as it was shown in diabetes type 2 eyes,[10] the amount of ChT difference between healthy control eyes and diabetes type 1 eyes in this study is not explained by choroidal capillary loss alone. Vascular sclerosis and connective tissue changes as described in the ageing choroid[23] may explain the amount of choroid thinning in diabetes type 1 and type 2. Choroidal thinning seen in this study is also well above the variability

caused by automatic segmentation and filtering that are needed for map generation.

Recently choroidal thickness change was found in type 2 diabetic eyes, irrelevant of the presence of retinopathy.[10] Type 2 diabetes is often accompanied by systemic pathologies such as high BP and high lipid levels which are known to have adverse effects on the retina. Hence, the thinning found in type 2 diabetic eyes could represent a cumulative effect of the glycemic disease component and other accompanying factors. In this study, the ChT decrease in comparison with healthy eyes was found in eyes where no clinical signs of retinopathy were present. Subjects were asked about their medical history and their medication at recruitment. All subjects were treated with insulin and only four subjects (two in NDR and two in the DR group) were on BP control medications with no other medications prescribed. This does not exclude accompanying systemic diseases in type 1 diabetics entirely; however, this diabetic study cohort presented with relatively good general health.

It is known that diabetic disease duration has a cumulative effect on the posterior eye and that the incidence of retinopathy and maculopathy in diabetes increases with time.[3,4] This was reflected in the statistically significant difference in disease duration between the NDR group and DR group. Although this range of disease duration was present in this study, 'duration' did not prove to be a factor contributing to the subfoveal ChT decrease.

When observing the individual ChT-maps in this study two thinning patterns were seen: one with the thinning area sparing the region nasally to the fovea, and one where the overall choroidal thickness was decreased, both patterns independent of the retinopathy stage. It is unclear if these patterns are related to each other or to the retinopathy location. Literature has described the distribution of diabetic retinal lesions to be lateral (including superior and inferior-temporal) of the macula for clusters of microaneurysms[24] and temporal of the paramacular region for vessel leakage.[25] It has also been demonstrated that foveal hemorrhages and PDR can occur without an increase in the foveal avascular zone.[26] Beside location and number of retinal leakages, their dynamic needs to be considered since leakages may not always be associated with blood–retinal breakdown.[27] In this study, retinal thickness showed some change however with little overall statistical significance and advanced edematous and proliferative lesions were found also close and temporal to the fovea (extending to superior and inferior-temporal regions) correlating to the area of the overall ChT thinning. Further study of type 1 diabetic eyes with a comparison of choroidal thickness with the retinopathy stages and advanced lesion location is necessary to gain more insight into the thinning pattern and if it is related to the pathogenesis of diabetes type 1 retinal changes. Possibly also a better understanding of the overall choroidal blood vessel structure in diabetic type 1 eyes could explain changes seen in this study.

Clinically the first visible signs of diabetic retinal pathology are associated with retinal vascular pathology such as microaneurysms. Examinations exist to show subclinical signs of change such as measurement of vessel capillary diameter and functional electroretinograms.[28,29] These examinations are commonly used for research and less often in a screening or a clinical setting. In contrast, OCT is widely used to assess the retina in diabetes, particularly to screen for or view details of edema or before and after intervention.[30,31] Using a 3D 1060 nm OCT for choroidal thickness would add another clinically applicable examination of diabetic eyes in addition to examining retina alterations with OCT. However, there is a need to establish if thinning areas in NDR have a predictive value for future lesions.

In conclusion, this study is a proof of concept that 3D 1060 nm OCT imaging and mapping can show a decrease in choroidal thickness, likely attributed to the glycemic disease itself. This decrease is already present before the clinical retinal signs of microvasculopathy in diabetes can be observed. The choroid offers a future biomarker for diabetic retinal disease and the pathophysiolocal effect of the choroid on hypoxia and ischemia driven maculopathy and retinopathy needs to be further investigated.

Acknowledgments

The authors thank Leopold Schmetterer, Michael Lasta, May-Van Che, Aneesh Alex, Richu Cottacal, and the staff of the Macula Clinic at the Rudolf Foundation Clinic for their valuable support throughout the study.

References

1. Laakso M. Epidemiology of type 2 diabetes. In: Goldstein BJ, Mueller-Wieland D, eds. *Type 2 Diabetes: Principles and Practice*. 2nd ed. New York, NY: Country Informa Healthcare; 2007:1–12.
2. American Diabetes Association. Standards of Medical Care in Diabetes–2011. *Diabetes Care*. 2011;34(Suppl 1):S11–S61.
3. Nathan DM, Zinman B, Cleary PA, et al. Modern-day clinical course of type 1 diabetes mellitus after 30 years' duration: the diabetes control and complications trial/epidemiology of diabetes interventions and complications and Pittsburgh epidemiology of diabetes complications experience (1983-2005). *Arch. Intern. Med*. 2009;169:1307–1316.
4. Klein R, Knudtson MD, Lee KE, Gangnon R, Klein BEK. The Wisconsin Epidemiologic Study of Diabetic Retinopathy XXIII: the twenty-five-year incidence of macular edema in persons with type 1 diabetes. *Ophthalmology*. 2009;116:497–503.
5. Bresnick GH. Diabetic macular edema. A review. *Ophthalmology*. 1986;93:989–997.
6. Aiello LP, Gardner TW, King GL, et al. Diabetic retinopathy. *Diabetes Care*. 1998;21:143–156.
7. Trudeau K, Molina AJA, Guo W, Roy S. High glucose disrupts mitochondrial morphology in retinal endothelial cells: implications for diabetic retinopathy. *Am. J. Pathol*. 2010;177:447–455.
8. Ciulla TA, Harris A, Latkany P, et al. Ocular perfusion abnormalities in diabetes. *Acta Ophthalmol Scand*. 2002;80:468–477.
9. Schocket LS, Brucker AJ, Niknam RM, et al. Foveolar choroidal hemodynamics in proliferative diabetic retinopathy. *Int Ophthalmol*. 2004;25:89–94.
10. Esmaeelpour M, Povazay B, Hermann B, et al. Mapping choroidal and retinal thickness variation in type 2 diabetes using 3-dimensional 1060-nm optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2011;52:5311–5316.
11. Kajić V, Esmaeelpour M, Považay B, et al. Automated choroidal segmentation of 1060 nm OCT in healthy and pathologic eyes using a statistical model. *Biomed Opt Express*. 2011;3:86–103.
12. Anon. Grading diabetic retinopathy from stereoscopic color fundus photographs–an extension of the modified Airlie House classification. ETDRS report number 10. Early Treatment Diabetic Retinopathy Study Research Group. *Ophthalmology*. 1991;98:786–806.
13. *Safe Use of Lasers & Safe Use of Optical Fiber Communications*. New York, NY: American National Standard Institute - Z136 Committee; 2000.
14. *Revision of the Guidelines on Limits of Exposure to Laser Radiation of Wavelengths between 400nm and 1.4μm*. Munich, Germany: International Commission on Non-Ionizing Radiation Protection; 2000.

15. Esmaeelpour M, Povazay B, Hermann B, et al. Three-dimensional 1060nm OCT: Choroidal thickness maps in normals and improved posterior segment visualization in cataract patients. *Invest. Ophthalmol. Vis. Sci*. 2010;51:5260–5266.
16. Kajić V, Povazay B, Hermann B, et al. Robust segmentation of intraretinal layers in the normal human fovea using a novel statistical model based on texture and shape analysis. *Opt Express*. 2010;18:14730–14744.
17. ACCCORD Study Group. Effects of Medical Therapies on Retinopathy Progression in Type 2 Diabetes. *N Engl J Med*. 2010;363:233–244.
18. Keech AC, Mitchell P, Summanen PA, et al. Effect of fenofibrate on the need for laser treatment for diabetic retinopathy (FIELD study): a randomised controlled trial. *Lancet*. 2007;370:1687–1697.
19. Nguyen QD, Shah SM, Khwaja AA, et al. Two-year outcomes of the ranibizumab for edema of the macula in diabetes (READ-2) study. *Ophthalmology*. 2010;117:2146–2151.
20. Pearson PA, Comstock TL, Ip M, et al. Fluocinolone acetonide intravitreal implant for diabetic macular edema: a 3-year multicenter, randomized, controlled clinical trial. *Ophthalmology*. 2011;118:1580–1587.
21. Anon. Treatment techniques and clinical guidelines for photocoagulation of diabetic macular edema. Early Treatment Diabetic Retinopathy Study Report Number 2. Early Treatment Diabetic Retinopathy Study Research Group. *Ophthalmology*. 1987;94:761–774.
22. McLeod DS, Lutty GA. High-resolution histologic analysis of the human choroidal vasculature. *Invest Ophthalmol Vis Sci* 1994;35:3799–3811.
23. Cavallotti C, Cerulli L. *Age-Related Changes Of The Human Eye*. Totowa, NJ: Humana Press; 2008:217.
24. Dobree JH. Simple diabetic retinopathy. Evolution of the lesions and therapeutic considerations. *Br J Ophthalmol*. 1970;54:1–10.
25. Gass JM. Fluorescein angiographie study of macular dysfunction secondary to retinal vascular disease. IV. Diabetic retinal angiopathy. *Arch Ophthalmol*. 1968;80:581–591.
26. Lahrmann C, Bek T. Foveal haemorrhages in diabetic retinopathy. Clinical characteristics and visual outcome. *Acta Ophthalmologica Scandinavica*. 2001;78:169–172.
27. Lobo CL, Bernardes RC, Cunha-Vaz JG. Alterations of the blood–retinal barrier and retinal thickness in preclinical retinopathy in subjects with type 2 diabetes. *Arch Ophthalmol*. 2000;118:1364–1369.
28. Bronson-Castain KW, Bearse MA Jr, Neuville J, et al. Early neural and vascular changes in the adolescent type 1 and type 2 diabetic retina. *Retina (Philadelphia, Pa.)*. 2012;32:92–102.
29. Klein R, Klein BEK, Moss SE, et al. The relation of retinal vessel caliber to the incidence and progression of diabetic retinopathy: XIX: the Wisconsin Epidemiologic Study of Diabetic Retinopathy. *Arch. Ophthalmol*. 2004;122:76–83.
30. Soliman W, Sander B, Soliman KA, et al. The predictive value of optical coherence tomography after grid laser photocoagulation for diffuse diabetic macular oedema. *Acta Ophthalmol*. 2008;86:284–291.
31. Baskin DE. Optical coherence tomography in diabetic macular edema. *Curr Opin Ophthalmol*. 2010;21:172–177.