Photoreceptor Layer Regeneration is Detectable in the Human Retina Imaged by SD-OCT after Laser Treatment Using Subthreshold Laser Power

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Purpose. We evaluated the morphologic changes in retinal structure after laser photocoagulation using supra- and subthreshold laser fluence.

Methods. In a prospective cohort study 10 consecutive patients received scatter laser photocoagulation. Treatment was performed using a semiautomated patterned scanning laser system. In a study area adjacent to the temporal vessel arcades, 2×2 pattern laser spots were applied with halving the flux of the laser power in a stepwise manner starting from a power producing a typical grayish lesion. The study areas then were imaged on days one, three, and seven, and on months one, two, three, and six using color fundus photography, autofluorescence (AF), infrared (IR) imaging, and spectral domain optical coherence tomography (SD-OCT).

Results. The starting threshold power lesions each were visible on color fundus photography, IR, and AF in all patients, and showed characteristic changes on OCT throughout the follow-up period. The halved flux laser burns (first step) were undetectable ophthalmoscopically during the laser session, but during the follow-up always were detectable on IR and AF images, and sometimes on fundus photography. On OCT they showed changes similar to the suprathreshold laser scars, but were much smaller in diameter, and in some instances an inward migration of the photoreceptor layer was observed.

Conclusions. Subthreshold laser burns with halved energy flux produced similar morphologic changes in the retina as threshold power, but with a smaller size. They induced less collateral damage to the neuroretina, and permit a level of reorganization in the outer retina. (ClinicalTrials.gov number, NCT00682240.) (*Invest Ophthalmol Vis Sci.* 2012;53:7019–7025) DOI:10.1167/iovs.12-10196

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The authors alone are responsible for the content and writing of the paper.

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Since the results of the Diabetic Retinopathy Study (DRS) and Early Treatment Diabetic Retinopathy Study (ETDRS), threshold scatter laser photocoagulation is the standard of care for patients with proliferative diabetic retinopathy.[1–3] The hypothesis behind the effectiveness of scatter laser photocoagulation is to destroy partially the RPE and photoreceptor layer at the retinal midperiphery, thus reducing the oxygen deficit and oxidative stress of the entire retina.[4] This is less desirable for the physician since laser treatment is destructive, causing potentially permanent functional deficits, such as reduction of central vision due to macular edema, constriction of the visual field, poor dark adaptation, and reduction of accommodation.[3,5] It is important to find an alternative solution to prevent these unwanted side effects while still preserving the beneficial effects of therapy.

Recent advances in laser technology, such as short pulse lasers, have been shown to be more selective in targeting the RPE and photoreceptor layer than conventional lasers, but they still cause definite collateral damage in the inner layers of the retina.[6–8]

In two previous reports, Kriechbaum et al.[8] and Bolz et al.[9] described the typical appearance of full threshold short pulse laser burns and macular threshold grid laser burns in vivo using spectral domain OCT. In both studies full fluence laser settings were used as usual in clinical settings, causing permanent damage to the RPE and neuroretina. The aim of our study was to investigate whether reducing the laser fluence still results in detectable morphologic changes in the human retina and, if so, whether the side effects induced in the neuroretina are less in these reduced fluence laser spots than with the usual laser settings.

Patients and Methods

Inclusion Criteria

Ten consecutive patients with retinal or anterior segment neovascularization due to diabetic retinopathy (8 patients) or central retinal vein occlusion (2 patients) were enrolled in this prospective cohort study performed at the Department of Ophthalmology, Medical University of Vienna, Austria.

The study protocol adhered to the tenets of the Declaration of Helsinki agreement, and was approved by the institutional ethics committee. All participants signed an informed consent after a detailed explanation of the study design, associated investigations for scientific purposes, and adjuvant imaging procedures. The study was registered on Clinical Trials (NCT00682240).

The main inclusion criterion for the study was the need for scatter laser photocoagulation because of retinal neovascularization (neovascularization on the disc or elsewhere), or neovascularization on the iris

TABLE. The Mean Laser Parameters of the Titration Lesions

Patient	Threshold				1/2 Fluence				1/4 Fluence				1/8 Fluence			
	Power, mW	Spot Size, µm	Time, ms	Fluence, J/cm²	Power, mW	Spot Size, µm	Time, ms	Fluence, J/cm²	Power, mW	Spot Size, µm	Time, ms	Fluence, J/cm²	Power, mW	Spot Size, µm	Time, ms	Fluence, J/cm²
1	1025	200	20	65	500	200	20	32	250	200	20	16	125	200	20	8
2	650	200	20	41	300	200	20	19	150	200	20	10				
3	450	200	20	29	225	200	20	14	100	200	20	6				
4	1300	200	20	83	650	200	20	41	325	200	20	21	150	200	20	10
5	625	200	20	40	325	200	20	21	150	200	20	10	100	200	20	6
6	1150	200	20	73	575	200	20	37	300	200	20	19				
7	925	200	20	59	500	200	20	32	225	200	20	14	100	200	20	6
8	1900	200	20	120	950	200	20	60	475	200	20	30				
9	875	200	20	56	400	200	20	26	200	200	20	13				
10	1000	200	20	64	500	200	20	32	250	200	20	16	130	200	20	8
Mean ± SD	990 ± 409	200 ± 0	20 ± 0	63 ± 26	493 ± 207	200 ± 0	20 ± 0	31 ± 13	243 ± 107	200 ± 0	20 ± 0	16 ± 7	120 ± 21	200 ± 0	20 ± 0	8 ± 2

caused by type 1 or 2 diabetes mellitus, or retinal vascular occlusion. Further requirements were no prior laser photocoagulation, no indication for intravitreal drug injection, and clear optical media.

Examination and Documentation

Before laser treatment, each patient underwent a complete baseline evaluation, including slit-lamp examination, ophthalmoscopy, best corrected ETDRS visual acuity (BCVA) testing, fluorescein angiography (FA), color fundus photography (CFP), fundus autofluorescence (FAF), and spectral domain optical coherence tomography (SD-OCT) imaging. Follow-up visits were performed at days one, three, and seven following laser treatment, and monthly intervals thereafter until month 3 with a final visit at 6 months. The standardized examination procedures were repeated according to protocol at each follow-up visit, except FA, which was performed only at baseline.

Retinal Photocoagulation

All laser treatments were performed using the PASCAL Pattern Scan Laser System (OptiMedica Corporation, Santa Clara, CA), and the Mainster PRP 165 laser lens (laser spot magnification 1.96×; Ocular Instruments Inc., Bellevue, WA). A study area was selected beside the superior or inferior temporal vessel arcades. After titrating the laser power to produce the typical gray-white lesion, the first 2×2 pattern was applied in the study area. Afterwards the laser power was decreased to halve the laser irradiation fluence (J/cm^2) and a second 2×2 pattern was placed adjacent to the first pattern. The same process was repeated another two times to produce four groups of laser spots from threshold to 1/8 fluence. Following the completion of the study zone a standard scatter laser therapy was applied in 2 or 3 sessions using standard threshold fluence laser spots.

SD-OCT Imaging

SD-OCT evaluation was performed using the Spectralis OCT system (Heidelberg Engineering GmbH, Heidelberg, Germany). This novel fourth generation OCT system has a 7 µm transversal resolution and is equipped with a real-time eye movement tracking system (TruTrack; Heidelberg Engineering GmbH) enabling the capturing of multiple OCT scan frames of an identical retinal location. Multiple frames taken from the same site allow the system to reduce the background noise of the image significantly and to visualize retinal structures with enhanced contrast. The tracking system furthermore allows identifying exactly the same retinal location throughout follow-up for a precise evaluation of progressive changes during the healing response. At 30 minutes after laser therapy, the patients were imaged using single line scans aligned to fit at least 2 laser spots of the study area.

RESULTS

The mean age of the patients was 60.7 years (range 28–79 years), and 2 patients had type 1 and 6 type 2 diabetes mellitus. The mean duration of diabetes was 26 years. The durations of symptoms in the two patients with central retinal vein occlusion (CRVO) were 7 and 12 months. The mean visual acuity of all patients was 0.5 logMAR (SD ± 0.57) at baseline and 0.68 logMAR (SD ± 0.62) at the end of the study. Two patients had a vitreous hemorrhage and were dropped from the study (both after month 3). The laser settings of the titration lesions and the fluences for each patient are shown in the Table.

Morphology of Laser Burns during the Follow-Up

One Hour. On CFP threshold laser burns were visible as light gray spots with blurry contour. Subthreshold burns with halved fluence became visible in 20% of our cases one hour

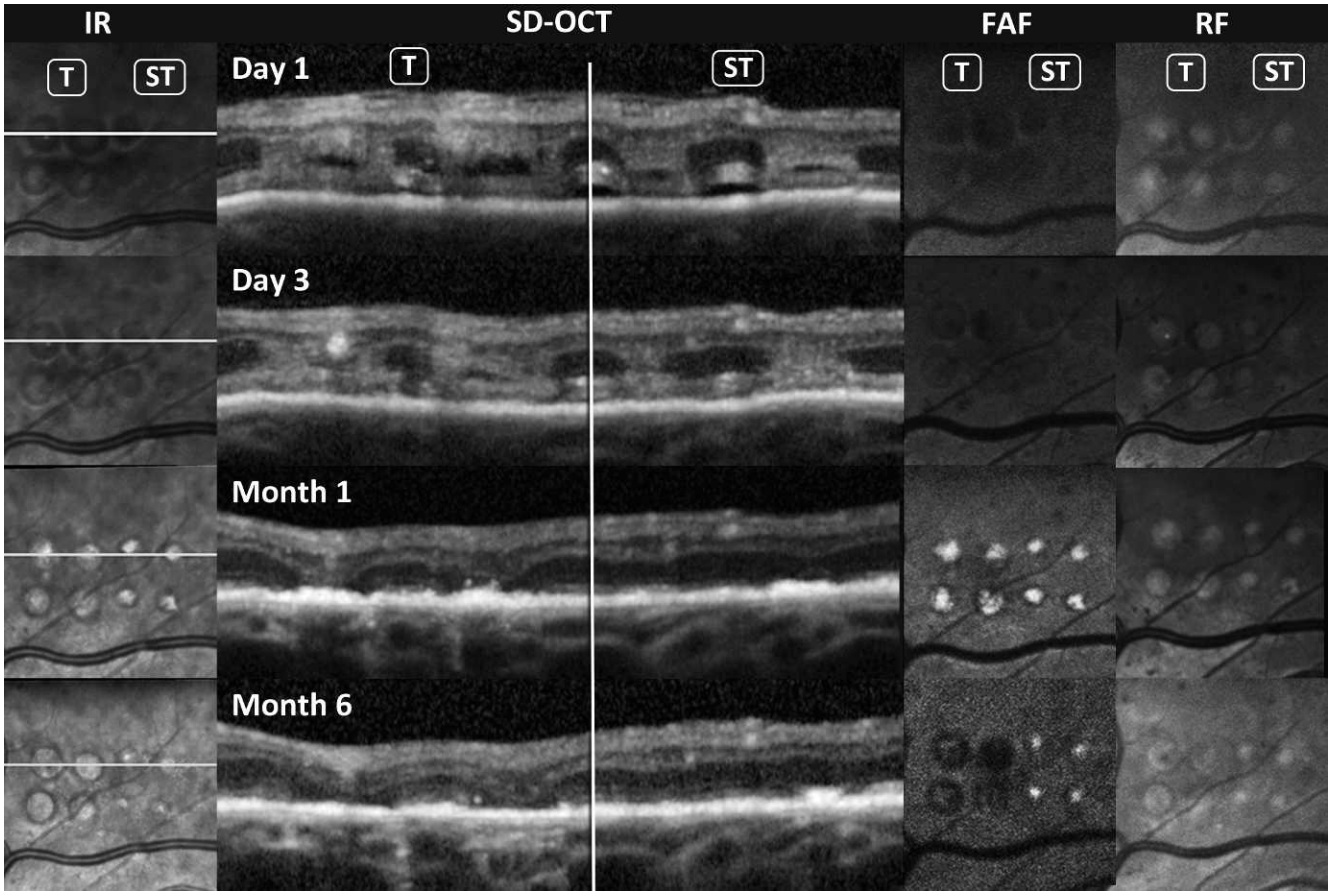

Figure 1. Images of patient 4 during the 6-month follow-up. *Left*: infrared reflection images (IR) showing the section of the OCT scan in relation to the laser lesions. The threshold (T) and halved fluence subthreshold (ST) lesions gain reflectivity during the follow-up. The difference in the size of the lesions is clearly visible. On SD-OCT images of the threshold and halved fluence lesions, at day 1 the intraretinal vacuole formation and subretinal fluid accumulation between the burns is visible, which almost completely resolves until day 3. The typical archway structure is seen in the threshold lesions at months 1 and 6, with a wide PRL defect and RPE loss. The halved fluence burn (the nasal one is not well centered on the scan) develops no ONL atrophy, and there is no sign of RPE loss around the central RPE/glial proliferation. Nevertheless, the PRL only reaches the edge of the proliferative tissue, and no reorganization is seen in the central lesion area. On fundus autofluorescence, the slowly developing central hyperautofluorescence is seen with the absence of atrophy ring around the halved fluence lesions. *Right side*: red-free (R) images of the lesions.

after treatment, and were seen as barely visible light gray spots. Otherwise, they were not visible. Laser burns with lower fluence were never detectable on CFP. FAF showed barely visible hypofluorescence of the RPE with the threshold and halved fluence burns, while on infrared (IR) and red-free (RF) images they were clearly visible. Lower fluences were not detectable with any of the mentioned imaging modalities. On SD-OCT threshold laser burns and halved fluence laser burns showed similar changes: hyperreflectivity in the outer nuclear layer (ONL) and relative hyporeflectivity in the photoreceptor layer (PRL). Irregularities in the RPE also were seen in groups. Although during the laser treatment the same spot size setting was used, the halved fluence lesions had a smaller transverse diameter (greatest linear diameter [GLD]) than threshold lesions (234 ± 52 vs. 402 ± 42 μm, mean ± SD, respectively, measured on SD-OCT). Laser burns created with quarter or lower energy flux were undetectable on OCT (Figs. 1, 2).

Days One and Three. On CFP threshold burns became more prominent. In 3 eyes subthreshold burns were barely visible. On FAF threshold and subthreshold burns became less hyporeflective. On SD-OCT a discrete retinal thickening was observed on day 1, which lessened by day 3 and was more prominent at the site of threshold burns. The hyperreflectivity of the ONL was unchanged, but intraretinal "vacuoles" developed between the ONL and PRL in 4 of the eyes. Furthermore subretinal fluid (SRF) was observed in three eyes between the laser burns. In these cases an irregular thickening of the choroid around the laser burns also was seen (Figs. 1, 3). All of these signs decreased by day 3.

Week One. On CFP and biomicroscopy the acute whitening of the laser burns had begun to diminish and the burns were seen best in indirect light. The subthreshold burns became invisible at this time. On FAF a distinct hyperfluorescence surrounded by a hypofluorescent ring had begun to develop. On SD-OCT the intraretinal fluid (if previously present) together with subretinal fluid had disappeared, and the RPE had flattened out. Together with this, the retinal thickness had decreased. The hyperreflectivity of the ONL was replaced with a downward shift of the inner layers forming the typical archway figure described in our previous report.[8] This archway structure either was not present or only moderately visible in the halved fluence burns.

Month One. On ophthalmoscopy, the threshold laser burns had started to pigment and were surrounded by an atrophy ring. The subthreshold burns either were invisible or seen as small pigment irregularities. On FAF, threshold and subthreshold burns showed hyperfluorescence with surrounding hypofluorescence. The only difference was the size of the lesions, the subthreshold burns being smaller and often irregular. On SD-

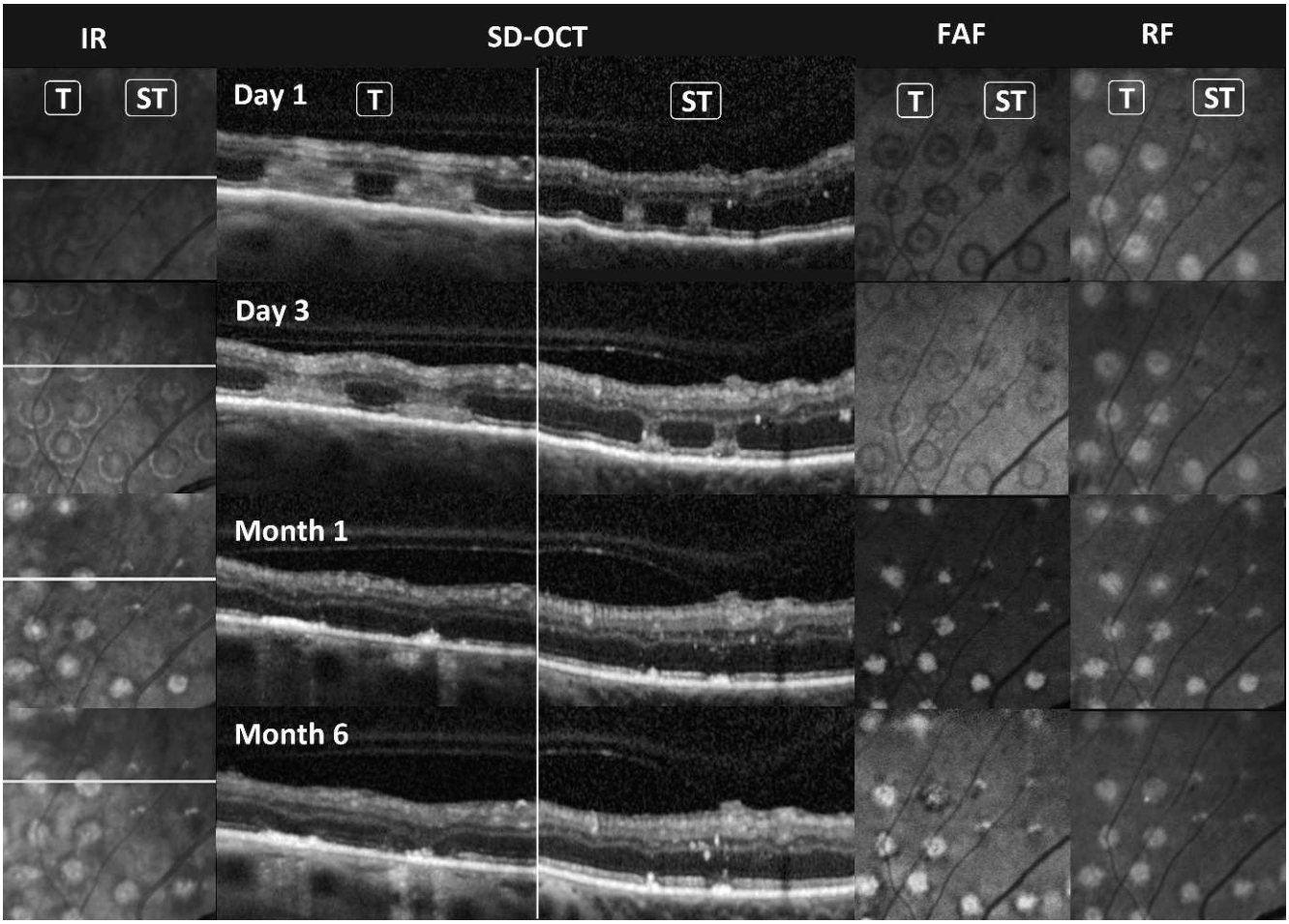

Figure 2. IR and separate SD-OCT images of the threshold and subthreshold laser spots accompanied by the FAF and RF images of a patient during the follow-up. At months 1 and 6 there is a centripetal reorganization of the PRL and ELM in the ST laser burns.

OCT, in the threshold burns the archway configurations had stabilized or, in some cases, had lessened. In the PRL/ RPE layers a central hyperplasia of the RPE was seen surrounded by a ring of atrophy (window defects seen on OCT). The PRL was destroyed in the whole region of the laser burn. In case of the subthreshold burns only a minimal thinning of the ONL layer was observed, and no archway configuration was present. The RPE had a similar hyperplasia in the center of the burn, but the surrounding atrophy was much more discrete than for threshold burns. In some cases an impending closure of the defect of the PRL was seen as the PRL band together with the external limiting membrane (ELM) started to reappear at the edges of the lesions as seen in Figure 2.

Months Two through Six. During months 2 through 6, the changes seen on CFP and FAF at month 1 remained unchanged, and on SD-OCT the diameter of threshold and halved fluence burns had shrunk further 295 ± 63 μm (73.5% of original GLD) and 147 ± 81 μm (61.7% of original GLD), respectively. Both changes were statistically significant ($P < 0.0001$). No changes were seen in the threshold laser burn

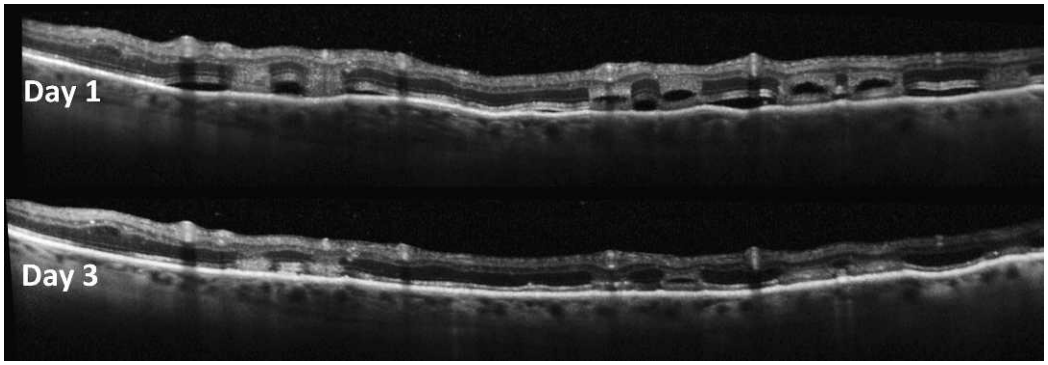

Figure 3. SD-OCT image of subretinal fluid accumulation and intraretinal vacuole formation 1 day following laser surgery.

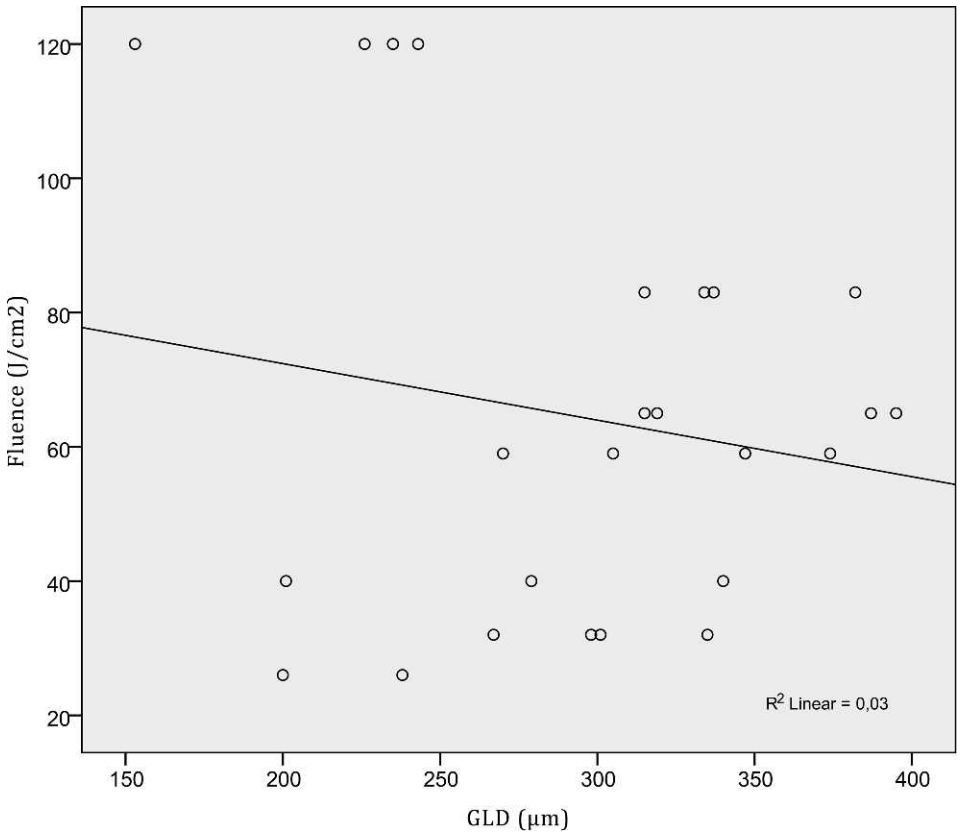

Figure 4. Scatter plot diagram of the GLD measured at month 6 of the threshold laser burns in relation to the fluence used to create them.

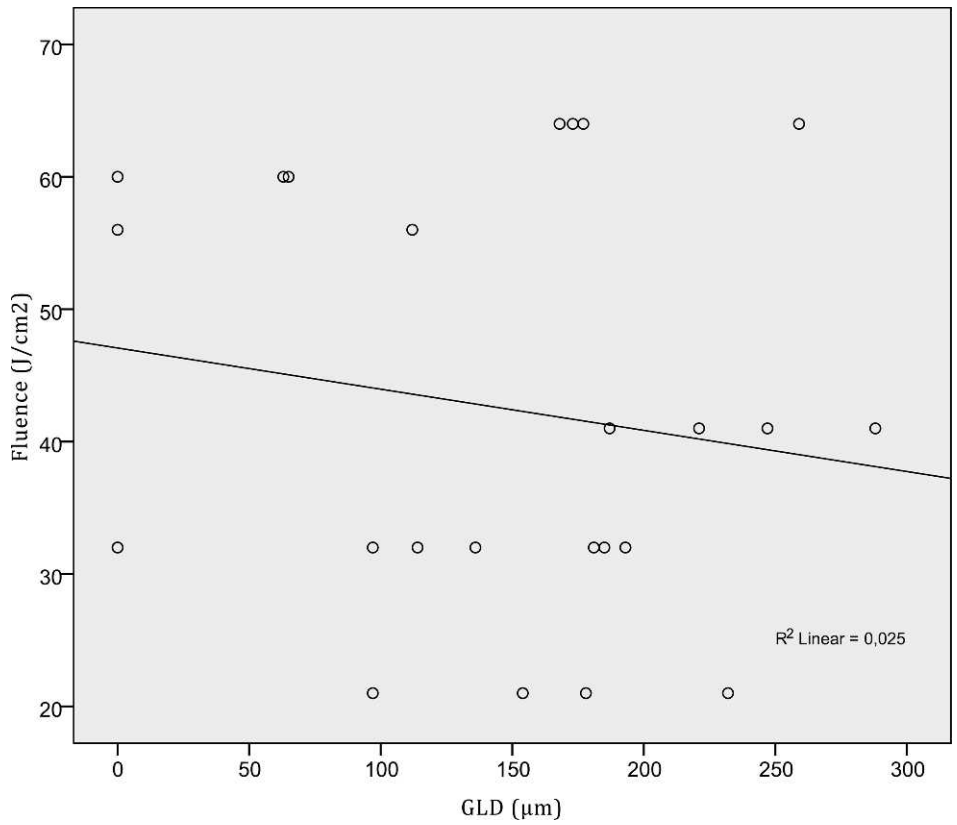

Figure 5. Scatter plot diagram of the GLD measured at month 6 of the halved fluence laser burns in relation to the fluence used to create them.

morphology, but in 40% of the patients a further centripetal reappearance of the PRL and ELM was observed in the subthreshold laser burns, as the PRL band reached and in some cases overlapped the central RPE hyperplasia/glial proliferation (Fig. 2).

Furthermore, as shown in Figures 4 and 5, there was no significant correlation between the laser fluence used and the GLD of the lesions at month 6, neither in the threshold nor in the halved fluence lesion groups ($r^2 = 0.03$, $P = 0.405$ and $r^2 = 0.025$, $P = 0.458$, respectively).

Discussion

In our study we demonstrated that laser fluence levels that were half of the level used to produce threshold laser burns produced distinct morphologic changes in the retina as imaged with SD-OCT, FAF, and color fundus photography. The laser burns created with halved fluence showed characteristics similar to threshold burns, but were smaller in extent, and importantly showed less collateral damage to the surrounding neuroretina. Halved fluence subthreshold burns had a smaller ring of RPE atrophy, seen as window defects surrounding the central pigment proliferation on SD-OCT, and as a hypofluorescent ring on fundus autofluorescence. Most importantly there was a tendency for photoreceptor layer reorganization (seen as centripetal reappearance of the inner segment/outer segment [IS/OS] and ELM lines in OCT) at the edge of the halved fluence laser spots, which was not observed in the threshold burns. Lower laser fluence settings did not produce detectable changes in the retina at any point during the follow-up. This PRL reorganization may have several explanations. It may be caused by sublethal thermal irradiation of the RPE/ PRL at the periphery of the lesions. This can be explained due to the Gaussian distribution of the temperature profile during laser treatment, which is present even if the laser energy is delivered homogeneously throughout the laser lesion.[10,11] A different explanation of the reappearance of the PRL line in the periphery of the lesions may be shrinkage of the laser lesion pulling the PRL toward the glial proliferation in the center of the lesions.

In their recent study, Muqit et al. described the morphology of subthreshold, threshold, and suprathreshold laser burns with 20 to 200 ms irradiation times.[12] Their results are in agreement with our findings, but in our patients we demonstrated similar retinal changes with even lower fluence values than Muqit et al. used. Furthermore, we could demonstrate the photoreceptors' tendency to shift into the direction of the lesion center, which may be interpreted as a healing response. Inagaki et al. also described similar morphologic changes following short pulse pattern scanning laser therapy in their study, in which they compare different laser systems by macular grid laser.[13]

Subthreshold micropulse laser has been examined in several studies in diseases, such as diabetic macular edema, central serous chorioretinopathy, proliferative diabetic retinopathy, or branch retinal vein occlusion, and has been found to be effective.[14–17] There still is some debate whether micropulse delivery has an advantage over continuous laser.[18]

When the DRS and ETDRS first developed the recommended settings for scatter laser photocoagulation in patients with proliferative diabetic retinopathy, the aim was to produce "hot" white lesions. These standards were revised by several workgroups to reduce the intensity of the laser burns to the light gray lesions we use today.[19] These lesions still are visible clearly in the retina during the treatment and afterwards, and leave atrophic scars. The fact that the gray laser lesions are visible during and after laser surgery means that the heat produced by the light absorption in the RPE layer reached the neuroretina through thermal diffusion, and was high enough to change its optical quality. This thermal diffusion is not directed just toward the neuroretina, but also to the surrounding RPE and choroid causing late laser scar expansion.

Recent studies suggest that the beneficial effect of laser is not due solely to the reduction of ischemia, but also by up and down regulation of cytokines in sublethally injured RPE cells.[20–22] This may mean that the endpoint of our current laser treatment strategy potentially is too intense, since destruction of the RPE cells may not be necessary to achieve our treatment goals.

In conclusion, we showed for the first time to our knowledge that the subthreshold laser spots result in definitive changes in the outer retina, but with much less retinal pigment epithelium atrophy that enables a reparative mechanism in the photoreceptor layer. This suggests that treatment with a therapeutic effect and lesser collateral damage may be possible by reducing the used laser fluence by half. With the recent development of pattern scanning laser technology and fundus tracking imaging, it is possible to deliver complete scatter laser treatments even if the surgeon doesn't actually see the laser lesions during the treatment session, since the fundus tracking system records where the treatment was done. Recently introduced software algorithms allow the physician to set the level of subthreshold fluence easily after the titration of the threshold power, and then adjust laser parameters automatically according to the desired level. This way, the subthreshold lesions described in the our study can be delivered quickly and consistently during a panretinal scatter laser session. Limitations of our study are the relative low patient number, and that we cannot draw conclusions whether a PRP session done with halved fluence lesions is as effective as is threshold laser. Although these results are promising, further studies are necessary to assess the efficacy of these subthreshold scatter laser treatments in reducing the risk of development of severe visual acuity decrease comparable to standard threshold laser.

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Appendix

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