CLINICAL SCIENCES

One-Year Follow-up of Blood-Retinal Barrier and Retinal Thickness Alterations in Patients With Type 2 Diabetes Mellitus and Mild Nonproliferative Retinopathy

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Objective: To examine the 1-year alterations of the blood-retinal barrier and changes in retinal thickness occurring in the macular region in patients with type 2 diabetes mellitus and mild nonproliferative retinopathy.

Methods: We classified 12 eyes of 12 patients with type 2 diabetes mellitus and mild nonproliferative retinopathy by 7-field stereoscopic fundus photography, levels 20 and 35 of Wisconsin grading, and examined them 3 times, at 6-month intervals, by fluorescein angiography, retinal leakage analyzer (RLA) (modified confocal scanning laser ophthalmoscope), and retinal thickness analyzer. The maps of retinal leakage and retinal thickness were aligned and integrated into one image. Data from the group of individuals with diabetes were compared with those from a healthy control population (n=14; mean age, 48 years; age range, 42-55 years) to establish reference maps for the RLA and the retinal thickness analyzer.

Results: Areas of abnormally increased fluorescein sodium leakage and increased thickness were detected in all eyes examined at baseline. The sites of increased fluorescein leakage reached values as high as 483% above normal, but in 10 of the total 36 examinations performed, fluorescein leakage returned to normal levels. A statistically significant correlation was found between changes in hemoglobin A_{1c} values and variations in percentage of abnormal fluorescein leakage between the 6- and 12-month examinations ($P<.001$). When comparing the RLA-leaking sites among the 3 examinations, a good correlation was seen among the location of these sites of maximum leakage, but there was a clear fluctuation in the percentage of increases. A correlation was noted between the location of the RLA-leaking sites and the location of areas of increased retinal thickness in subsequent examinations, either 6 or 12 months later. Microaneurysms showed relatively little leakage and leaked progressively less in successive examinations.

Conclusions: The dominant alteration in the retina of patients with type 2 diabetes mellitus and mild nonproliferative retinopathy is the presence of RLA-leaking sites, indicating spotty retinal vascular damage characterized by alteration of the blood-retinal barrier. This damage appears to be reversible and directly associated with variations in glycemic metabolic control. Retinal edema appears to develop mainly as a result of retinal vascular leakage.

Arch Ophthalmol. 2001;119:1469-1474

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RETINOPATHY IS the most frequent microvascular complication of diabetes mellitus. It is estimated to be the most common cause of new cases of blindness among adults aged 20 to 74 years.[1] The magnitude of the problem becomes apparent when one realizes the projected growth of the incidence of diabetes mellitus and the limitations of photocoagulation, the only tested form of therapy, which is performed when disease progress is already irreversible.[2]

To prevent and improve the treatment of diabetic retinopathy, it is fundamental to know the evolution of the earliest changes that occur in the diabetic retina and how they relate to the progression of the retinopathy.

The accepted methods to assess retinopathy are based on visual acuity changes and findings noted on fundus photography.[3] Both provide information on late changes but are not useful for identifying initial alterations. The only features that are visible on fundus photography are microaneurysms, hemorrhages, cotton-wool spots, hard exudates, venous abnormalities, and neovascularization. Examination of the initial alterations in diabetic retinal disease must involve other methods, such as measuring alterations of the blood-retinal barrier (BRB) and changes in retinal thickness. Methods of multimodal imaging of the ocular fundus have recently been developed by combining information obtained from different sources, such as fundus photography and fluores-

PATIENTS AND METHODS

PATIENTS

Twelve eyes from 12 patients (4 men and 8 women), aged 44 to 63 years (mean±SD, 55±5 years), with type 2 diabetes mellitus and mild nonproliferative retinopathy characterized on 7-field stereoscopic fundus photography (SFP), levels 20 and 35 of Wisconsin grading,[2] were examined 3 times by SFP, fluorescein angiography, RLA (Zeiss, Oberkochen, Germany), and RTA (Talia Technology, Ltd, Mevaseret Zion, Israel) at 6-month intervals during a 1-year period. All patients had a corrected visual acuity of 20/20. The study eye was chosen as the one fulfilling the inclusion criteria (levels 20 and 35 of Wisconsin grading). When both eyes met the inclusion criteria, the right eye was chosen.

The patients were followed up by the same diabetologist and remained within limits of acceptable good medical control. Their hemoglobin A_{1c} (HbA_{1c}) values ranged from 5.4% to 9.7% (given as the percentage of total hemoglobin), and their duration of diabetes ranged from 2 to 15 years (**Table**).

The tenets of the Declaration of Helsinki were followed, and approval of the institutional review board was obtained for this study. Informed consent was obtained from the patients before they were enrolled in the study.

METHODS

Stereoscopic Fundus Photography

Stereoscopic fundus photography was performed according to the Early Treatment Diabetic Retinopathy Study (ETDRS) protocol.

Stereoscopic pairs of fields were obtained using a 30° fundus camera to classify levels 20 and 35 of Wisconsin grading, but only field 2 was used to examine and correlate with other data (leakage and thickness).

Fundus Fluorescein Angiography (FFA)

Fluorescein angiography was performed using a fundus camera with a 45° field after patients received an injection of fluorescein sodium, 14 mg/kg of body weight. Early- and late-phase (5 minutes) studies were analyzed.

Retinal Leakage Analyzer

The RLA is a new method that quantifies localized fluorescein leakage in humans from the retina into the vitreous across the BRB. The instrumentation and all the image processing have been previously described.[4]

The RLA obtains images of the fundus with real 3-dimensional information. Two types of information are obtained simultaneously, one for optical imaging and one representing fluorescence measurements being scanned. Axial graphics of the fluorescein measurements obtained from the vitreous representing a volume of $75 \times 75 \times 2550$ µm were converted into retinal leakage maps. Multiple measurements of retinal leakage can be graphically assembled in a false-color retinal leakage map that represents the distribution of alterations in the BRB in any chosen area of the total area of the posterior pole under examination. Intravisit and intervisit reproducibility values of the method are ±10.2% and ±13%, respectively.[4]

Retinal Thickness Analyzer

The RTA is a quantitative and reproducible method for evaluating retinal thickness.[6] The system acquires optical images of the retina in sections by projecting the laser into the retina at an angle, allowing the reflection or scattering of the laser light from the vitreoretinal and chorioretinal interfaces to be viewed. The separation between the reflections (and scatter) from these 2 interfaces is a measure of retinal thickness. The data are analyzed, and the retinal thickness is given as a numerical value and a color-coded map.

Data Analysis

Data from the group of individuals with diabetes were compared with those from a healthy control population (N=14; mean age, 48 years; age range, 42-55 years) to establish reference maps for the RLA and RTA.[5]

Because of the differences in resolution between the 2 instruments (RLA and RTA), a value of leakage is obtained for a 75×75-µm area, while a single value of thickness covers a 200×200-µm area. Therefore, the smallest area that can be constructed that contains an integer number of leakage and thickness values is 600×600 µm, that is, 8×8 values of leakage and 3×3 values of thickness.

To compare information from the RLA and RTA and to use the more detailed data from the leakage maps, we

cein angiography, with measurements obtained with instrumentation, such as the retinal leakage analyzer (RLA) and the retinal thickness analyzer (RTA).[4-6] To our knowledge, we provide the results of the first 1-year follow-up study of changes occurring in the macular region of eyes from patients with type 2 diabetes mellitus and mild nonproliferative retinopathy.

RESULTS

The results are summarized in the Table. Areas of abnormally increased fluorescein leakage were detected in all eyes examined at baseline.

Retinal leakage analyzer–leaking sites, that is, sites of maximal fluorescein leakage for each area of abnormally increased leakage, reached values as high as 483% above normal, but in 10 of the 36 examinations performed, the fluorescein leakage returned to normal levels. This finding occurred in 5 eyes 6 months after inclusion in the study and in another 3 eyes at the 12-month examination.

Of the 12 patients included in the study, 8 improved in metabolic control during the second period of follow-up, that is, between the 6- and 12-month examinations, and the correlation between changes in HbA_{1c} values and decreases or increases in percentage of abnormal fluorescein leakage was statistically significant ($P<.001$). A direct correlation between these 2 variables was present in 10 of the 12 eyes. No correlations were found between systolic or diastolic blood pressure

chose 300 × 300-μm areas. The maps are, therefore, composed of 63 (9 × 7) values, each representing a 300 × 300-μm area, for a total of 2700 × 2100 μm.

For each 300 × 300-μm area, the value of leakage is the mean and SD of 16 (4 × 4) values.

To obtain a value of thickness for an equivalent 300 × 300-μm area, we performed a 2-step procedure. First, values of thickness were computed as the mean and SD of 9 (3 × 3) values, corresponding to a 600 × 600-μm area. Afterward, this area was split into 4 adjacent 300 × 300-μm areas, and the mean and SD values obtained for the 600 × 600-μm area were given to each of these 4 adjacent areas.

In the healthy control population, the mean values of the 63 mean values of leakage and thickness are 21.29×10^{-7} cm/s and 168.13 μm, respectively; for the SD, the mean values are 6.19×10^{-7} cm/s and 22.56 μm, respectively. The relatively large SD for leakage, registered after averaging 14 normal retinal leakage maps, is expected considering the high resolution of the system associated with regional variations in vascular distribution.

By comparing values from the patients with those from the controls, we can compute maps of increased leakage or increased thickness as percentages.

All of the increases are computed considering the reference mean plus 2 SDs of the healthy control population.

An extended description of the data analysis methods is available elsewhere.[4,5]

Correlations between percentages of increases in RLA and RTA values occurring between examinations and HbA_{1c} values and systolic and diastolic blood pressure measurements were performed using the χ^2 test to compare proportions.

Characterization of the Fundus Lesions

The fundus lesions are first characterized by grading each eye based on images from 7-field SFP and using ETDRS protocols.

Only eyes showing levels 20 and 35 of Wisconsin grading were included in this study. Combined information is obtained only from field 2, using color SFP; FFA, early and late phases; scanning laser ophthalmoscope angiography; RLA; and RTA.

The alterations registered in field 2 with the different methods used were as follows.

Stereoscopic Fundus Photography. Red dots and yellowish white deposits.

Fundus Fluorescein Angiography. Early phase: outline of the foveal avascular zone, hyperfluorescent (bright) spots, and hypofluorescent (dark) spots; and late phase: leaking spots (spots surrounded by diffuse hyperfluorescence).

Scanning Laser Ophthalmoscope Fluorescein Angiography (Only Late Phase). Leaking spots.

Retinal Leakage Analyzer. Areas of increased fluorescein leakage (300 × 300 μm) are areas where fluorescein leakage is more than a reference made equal to the mean plus 2 SDs of a healthy control population. The increase is expressed as a percentage over the reference. Retinal leakage analyzer–leaking sites are identified as the central sites (300 × 300 μm) of maximum percentage of increased leakage occurring in each area. In many cases, there is continuity between areas of increased fluorescein leakage. Still, it is possible to determine these RLA-leaking sites, checking for the first- and second-order derivatives of the leakage map.

Retinal Thickness Analyzer. Localized measurements of retinal thickness are obtained from a 200 × 200-μm area. The findings on fundus photographs and fluorescein angiograms were registered by 3 different observers (C.L.L., J.R.F.A., and J.G.C.-V.) in an independent manner. Disagreements were resolved after joint examination. Definitions for the lesions used in the text followed ETDRS definitions.

Only eyes that showed no capillary closure during the early phase of the fluorescein angiogram were included in the study analysis. Absence of capillary closure was accepted when a smooth and symmetrical outline of the foveal avascular zone was observed, with defects no larger than 10% of the entire outline.

To interpret the data, the following definitions, coinciding largely with ETDRS definitions, were used: *microaneurysms*, isolated red dots in SFP images that coincide with hyperfluorescent spots in the early FFA; *hemorrhages*, round or linear red spots in SFP images that do not fluoresce on FFA or that appear as dark spots in FFA images; *drusen*, deeply located yellowish white deposits in the SFP image that became hyperfluorescent in early FFA; and *hard exudates*, white or yellow-white deposits that do not fluoresce or that appear as dark spots in FFA images.

values and changes in percentage of abnormal fluorescein leakage, either between baseline and the 6- and 12-month examinations or between the 6- and 12-month examinations.

When comparing the RLA-leaking sites among the 3 examinations, there was good correlation between the location of these sites of maximum leakage but a clear fluctuation in the percentage increases (**Figure 1** and **Figure 2**). In total, the eyes examined showed 51 different RLA-leaking sites unrelated to microaneurysms or other apparently identifiable vascular abnormalities. These RLA-leaking sites showed the larger percentage increases in fluorescein leakage. Of 51 RLA-leaking sites, 34 were identified in the same location in the retina in subsequent examinations. Clear fluctuation in the percentages of abnormal fluorescein leakage between the subsequent examinations was observed in 8 of the 12 eyes examined. The other 4 eyes showed a progressive decrease in fluorescein leakage in the subsequent examinations.

There were also 29 microaneurysms in field 2 of the 12 eyes examined. They remained in the same location during the 1-year follow-up period. Although associated with some degree of abnormal fluorescein leakage, the percentage increases in fluorescein leakage were relatively low (Figures 1 and 2). Furthermore, they were found to leak progressively less in successive examinations. Only 5 of the 29 microaneurysms showed some increase in fluorescein leakage at the 12-month examination.

Patient Characteristics*

Patient No./ Eye/Age, y	Duration of Diabetes, y	HbA_{1c}, %†	BP, mm Hg	Grading Level	Field 2 Lesions, MA	Leakage, %		Thickness, %	
						Area‡	Maximum§	Area‡	Maximum§
1/Right/60	3	6.7	160/90	35	4	84	67	79	20
		7.0	160/85	35	4	8	30	0	0
		6.2	155/80	35	4	3	10	13	11
2/Left/44	6	7.9	160/90	35	3	100	483	14	2
		7.4	160/90	35	3	66	62	0	0
		7.5	160/90	35	3	70	90	41	11
3/Right/53	7	9.4	140/80	35	4	57	72	10	4
		9.5	150/90	35	4	70	61	0	0
		8.5	150/85	35	4	0	0	33	13
4/Left/54	10	6.3	110/70	35	1	33	50	29	12
		7.8	120/65	20	1	5	30	93	23
		6.7	120/70	20	1	4	15	86	25
5/Left/55	10	6.9	160/85	20	0	5	3	43	14
		7.4	160/90	20	0	0	0	27	11
		7.5	160/85	20	0	14	40	25	3
6/Right/60	9	8.6	130/70	20	1	20	27	0	0
		9.7	135/75	20	1	0	0	44	10
		8.5	120/60	20	1	0	0	44	9
7/Left/50	10	7.6	125/80	20	2	100	175	48	13
		8.3	155/90	35	2	21	29	4	1
		9.3	140/90	20	2	100	250	0	0
8/Right/54	2	5.4	130/80	20	3	31	27	0	0
		5.4	120/80	20	3	0	0	17	9
		5.6	130/80	20	3	0	0	0	0
9/Left/63	4	6.6	140/75	20	4	9	29	7	1
		6.3	140/60	20	4	2	10	63	21
		5.9	130/70	20	4	0	0	46	27
10/Right/51	15	8.4	145/90	20	3	67	72	48	14
		7.8	155/85	20	3	0	0	62	18
		8.0	130/80	20	3	83	83	68	34
11/Left/56	9	6.9	120/70	20	1	76	120	16	10
		7.3	160/90	20	1	100	129	86	31
		7.0	120/70	20	1	0	0	100	24
12/Left/56	12	9.4	150/90	20	3	41	47	93	26
		9.0	160/90	20	3	0	0	100	29
		9.5	170/95	20	3	30	41	64	21
Minimum / . . . / 44	2	5.4	. . .	. . .	. . .	0	0	0	0
Mean / . . . / 55	8	7.6	. . .	. . .	. . .	33	57	39	12
Maximum / . . . / 63	15	9.7	. . .	. . .	. . .	100	483	100	34
SD / . . . / 5	4	1.2	. . .	. . .	. . .	37	91	33	10

*Data are given as values at baseline and at the 6- and 12-month examinations, respectively, unless otherwise indicated. HbA_{1c} indicates hemoglobin A_{1c}; BP, blood pressure; MA, number of microaneurysms; and ellipses, data not applicable.

†Values are given as the percentage of total hemoglobin.

‡Values indicate the percentage of altered area (any increase of leakage or thickness over the reference) of leakage or thickness relative to the total scanned area for that eye.

§Data indicate the maximum value of increase over the reference that occurred in that eye.

Increases in retinal thickness were also detected in all eyes examined at 1 of the 3 examinations performed in each eye. The sites of maximum percentage thickness were generally lower than 30% above normal values, with the sole exception of the last examination of patient 10.

Changes were found between the location of sites of maximum percentage increase in retinal thickness when comparing examinations performed in the same eye in 7 of the 12 eyes. The 5 eyes that had a more stable abnormal increase in retinal thickness also showed involvement of the fovea and had higher maximum percentage increases in retinal thickness. The changes in retinal thickness showed no statistical correlation with changes in HbA_{1c} values or with systolic or diastolic blood pressure values. Finally, a correlation was observed between the location of the RLA-leaking sites represented by sites of maximum percentage of increased fluorescein leakage at one examination and the location of sites of maximum percentage increase in retinal thickness in subsequent examinations, either 6 or 12 months later (Figures 1 and 2).

The coincidence between the location of sites of increased thickness and RLA-leaking sites in previous examinations was 61% for the 6-month examination and 71% for the 12-month examination.

COMMENT

Our method of multimodal imaging of the ocular fundus allows correlations to be established between fundus lesions, localized alterations of the BRB identified by

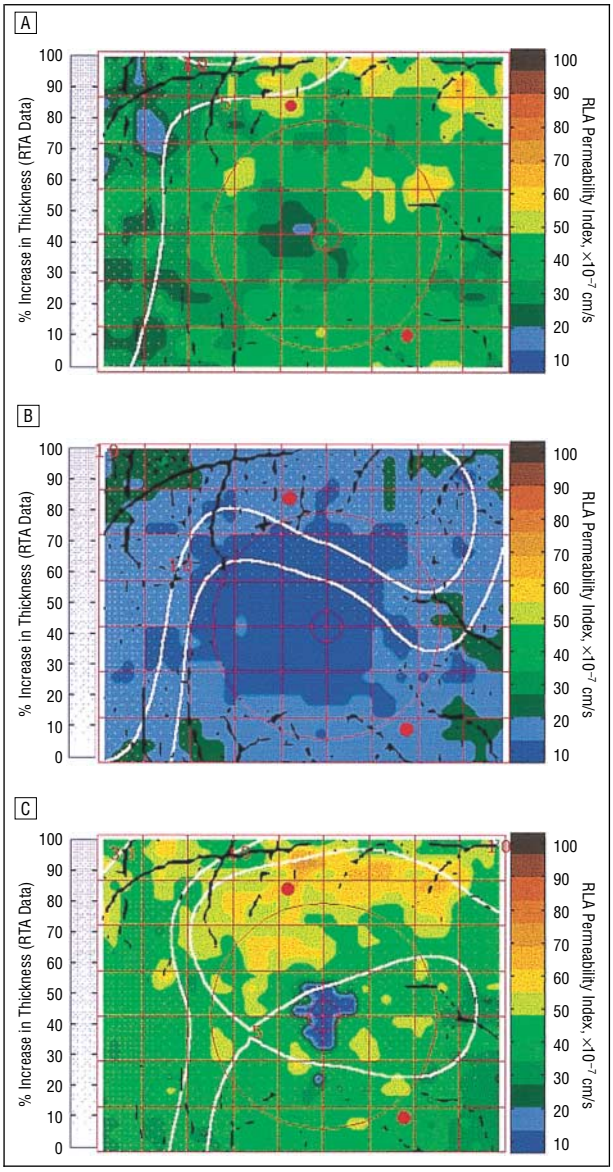

Figure 1. Integrated maps of patient 10, including data from stereoscopic fundus photography (black lines and red dots), the retinal leakage analyzer (RLA) (color-coded maps of blood-retinal barrier permeability indexes), and the retinal thickness analyzer (RTA) (white dot density maps of percentage increases in retinal thickness) obtained from visits at baseline (A), 6 months (B), and 12 months (C).

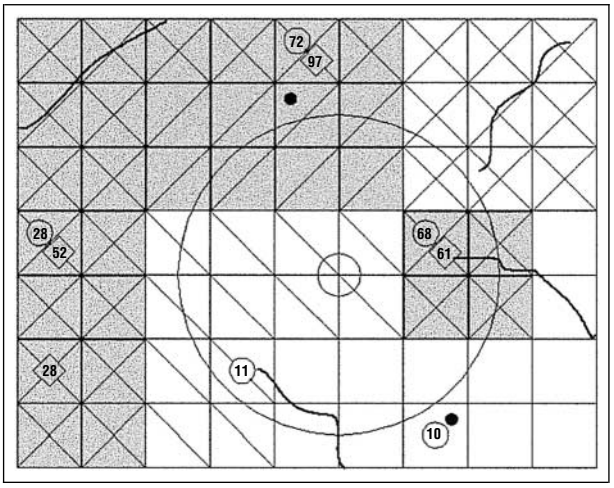

Figure 2. Integrated information for patient 10 for the 3 visits (at 0, 3, and 6 months). Each square represents data from a 300×300-µm area in the fundus. The 2 black dots show the location of 2 microaneurysms that were present at the 3 visits. An abnormal increase in thickness at the first visit is shown as shaded squares; at the second visit, as 45° lines (/); and at the third visit, as 135° lines (\). For an abnormal increase in leakage, only the central retinal leakage analyzer (RLA)–leaking sites are shown. The numbers inside the circles represent the percentage of increase over the reference map for the first visit. For the second and third visits, the value is shown inside squares (none for this patient) and inside diamonds, respectively. Note the location of the RLA-leaking sites and their fluctuation during the 1-year period. There are 3 leaking sites common to the first and third visits. All of them decreased to within normal values at the second visit and showed important increases at the third visit, thus clearly demonstrating fluctuation in the percentage of fluorescein sodium leakage at these leaking sites. The small circle in the center of each map denotes the location of the center of the fovea; the large circle, centered on the fovea, denotes a circle of 1500 µm in diameter; and the black curvy lines, retinal vessels.

mapping fluorescein leakage into the vitreous, and changes in retinal thickness. Previous studies[5,7] have shown in the macular region of patients with type 2 diabetes mellitus and nonproliferative retinopathy, in most eyes examined, that localized sites of increased fluorescein leakage did not show good correlation with the zones of increased retinal thickness. To explain this apparent lack of correlation between sites of alteration of the BRB and zones of retinal edema in the diabetic macula, the presence of 2 types of edema, vasogenic and cytotoxic, was put forward.[5,7] There is a need for studies of the natural history of these alterations occurring in the early stages of diabetic retinal disease using these new methods and performed in a prospective fashion.

In the present study, a 1-year follow-up of patients with type 2 diabetes mellitus and mild nonproliferative retinopathy, we performed 3 examinations, at baseline and 6 and 12 months later.

The most frequent alterations observed were, in decreasing order of frequency, RLA-leaking sites, areas of increased retinal thickness, microaneurysms, and drusen. Hemorrhages and hard exudates were relatively rare in the macular region (photographic field 2 of the ETDRS) in these eyes with mild nonproliferative diabetic retinopathy (levels 20 and 35 of Wisconsin grading).

Retinal leakage analyzer–leaking sites reached permeability indexes of the BRB as high as 483% above normal. These sites of alteration of the BRB, well identified in the RLA maps, varied little in their location on successive examinations at 6-month intervals, but their BRB permeability indexes fluctuated greatly when comparing subsequent examinations. In 10 of the 36 examinations, RLA maps returned to normal levels. Most of the RLA-leaking sites were not associated with any visible morphologic alteration of the retina, such as microaneurysms, hemorrhages, or other vascular abnormalities.

The intensity of fluorescein leakage, represented by the BRB permeability indexes in these RLA-leaking sites, correlated well with the changes in HbA_{1c} levels, when comparing the 6- and 12-month examinations. Decreases in HbA_{1c} levels, indicating improved metabolic control, were associated with decreasing fluorescein leakage, and increases in HbA_{1c} levels with increasing fluorescein leakage. No correlation was found, however, between variations in fluorescein leakage and blood pressure changes, either systolic or diastolic, but this may be be-

cause of the small number of patients involved and the fact that the blood pressure levels remained relatively stable during the study, in each patient. These findings confirmed and expanded previous reports using slit-lamp vitreous fluorometry[8] and vitreous fluorometry,[9] showing that an increase in permeability of the BRB to fluorescein is an early finding in patients with diabetic retinal disease.[10,11] The RLA, based on scanning laser ophthalmoscope methods, goes a step further by identifying the actual sites of increased fluorescein leakage.

A particularly interesting observation in this study is the widespread presence of increased retinal thickness, ie, retinal edema. Our findings offer some insight into the mechanisms of development of retinal edema in the initial stages of diabetic retinopathy. The location of RLA-leaking sites appears to be predictive of the later development in the same location of areas of increased retinal thickness. An alteration of the BRB appears, therefore, to be associated with the development of diabetic retinal edema, at least in this stage of retinopathy.

Another interesting observation was the variation found between subsequent examinations in the location and extent of the areas of increased retinal thickness. These variations give support to the view that the retinal edema observed in these eyes is extracellular and not intracellular. We have, therefore, retinal edema that fulfills the criteria for being classified as *vasogenic edema*, that is, it is associated with an alteration of the BRB and appears to be predominantly extracellular. This follow-up study, allowing comparisons to be made between 3 subsequent examinations, performed at 6-month intervals, indicates that vasogenic edema is the predominant type of retinal edema occurring in these initial stages of nonproliferative diabetic retinopathy.

The extent and percentage increases in retinal thickness did not show any clear correlation with HbA_{1c} levels or blood pressure variations. They appeared, however, to be a late result of an alteration of the BRB identified in previous examinations.

Longer follow-up studies are, therefore, needed to examine relationships between the occurrence of retinal edema and changes in metabolic control or blood pressure levels.

During the 1-year follow-up period, microaneuryms leaked progressively less at successive examinations. This observation suggests that these vascular lesions are in the process of progressive occlusion and gives support to the view that they are sites of localized thrombosis.[12]

In conclusion, our observations suggest that the dominant alteration in the retina of patients with type 2 diabetes mellitus and mild nonproliferative retinopathy is the presence of RLA-leaking sites, indicating spotty retinal vascular damage characterized by alteration of the BRB. This spotty retinal vascular damage appears to be reversible and directly associated with variations in metabolic glycemic control. These findings offer particularly exciting perspectives for medical therapy of diabetic retinopathy.

The methods of examination we used could not show, however, if these leaking sites are associated with or preceded by dynamic changes in the retinal vasculature, such as localized vasodilation or vasoconstriction.

Retinal edema, also a frequent finding, appears to develop as a result of retinal vascular leakage.

Longer follow-up studies, using new methods of examination, such as the RLA and the RTA, ideally combined with blood flow studies at the capillary level, integrated in multimodal imaging of the ocular fundus, are expected to clarify many of the remaining questions.

Accepted for publication February 23, 2001.

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