A New Model for Assessment of Change in Visual Function in Diabetes

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2014

A New Model for Assessment of Change in Visual Function in Diabetes

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LUND
UNIVERSITY

DOCTORAL DISSERTATION
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To be defended at Jubileumsaulan, Jan Waldenströms gata 5, SUS Malmö, Friday September 12 at 9:15 a.m.

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Organization	Document name
LUND UNIVERSITY	DOCTORAL DISSERTATION
Department of Clinical Sciences, Ophthalmology, Malmö	Date of issue Sept 12, 2014
Author Karl-Johan Hellgren	Sponsoring organization

Title and subtitle: A New Model for Assessment of Change in Visual Function in Diabetes

Abstract

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Two cohorts were investigated. The first cohort comprised 55 diabetic patients with various degrees of diabetic retinopathy. Using a cross-sectional design, we studied how refraction and visual acuity varied in patients under routine care (Study I), and we assessed limits for significant change in visual fields by use of standard automated perimetry (SAP) and short-wavelength automated perimetry (SWAP) based on short-term test–retest variability (Study II). The second cohort consisted of 81 diabetic subjects with no or mild/moderate diabetic retinopathy. We applied a longitudinal prospective design to explore the correlation between functional change and progression of microvascular abnormalities (Study III), and examined the usefulness of SAP with our limits of significant change for detecting early retinal dysfunction over 3–5 years (Study IV).

In Study I, we demonstrated that refraction was stable in most eyes, and assessments of visual acuity were highly reproducible despite substantial fluctuations in blood glucose levels. In Study II, we defined limits of significant change for SAP and SWAP for diabetic subjects. In Study III, we used the defined limits for change to monitor visual function in diabetes by SAP. After 18 months of follow-up, deterioration was common but improvement was rare, and deteriorated fields were reproducible despite an unchanged degree of retinopathy. In Study IV, up to five years of follow-up, confirmed visual field deterioration in eyes without any retinopathy or with stable mild/moderate retinopathy.

Standard automated perimetry with our new model for detecting change can successfully determine early retinal dysfunction over time in diabetes, which can represent early signs and progression of retinal neurodegeneration.

Key words diabetic retinopathy; visual function; perimetry

Classification system and/or index terms (if any)

Supplementary bibliographical information		Language English
ISSN and key title1652-8220		ISBN 978-91-7619-013-5
Recipient's notes	Number of pages 92	Price
	Security classification	

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ISBN 978-91-7619-013-5
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Printed in Sweden by Media-Tryck, Lund University
Lund 2014

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To Susanna

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List of papers

In this thesis, the present studies and the corresponding specific aims and papers (listed below) are designated I–IV.

I. Agardh E, Hellgren KJ, Bengtsson B. Stable refraction and visual acuity in diabetic patients with variable glucose levels under routine care. Acta Ophthalmol 2011;89:107–110

II. Bengtsson B, Hellgren KJ, Agardh E. Test–retest variability for standard automated perimetry and short-wavelength automated perimetry in diabetic patients. Acta Ophthalmol 2008;86:170–176

III. Hellgren KJ, Bengtsson B, Agardh E. Functional and structural change in diabetic eyes. Interim results from an ongoing longitudinal prospective study. Acta Ophthalmol 2013;91:672–677

IV. Hellgren KJ, Agardh E, Bengtsson B. Progression of Early Retinal Dysfunction in Diabetes over Time: Results of a Long-term Prospective Clinical Study. Diabetes 2014; published online May 21, DOI: 10.2337/db13-1628

Abbreviations

abs	apostilb
D	diopter
ERG	electroretinogram
ETDRS	Early Treatment Diabetic Retinopathy Study
FDT	frequency-doubling technology
HbA1c	glycated hemoglobin A1c
logMAR	logarithm of the minimum angle of resolution
MD	mean deviation
NGSP	National Glycohemobglobin Standardization Program
PSD	pattern standard deviation
SAP	standard automated perimetry
SITA	Swedish Interactive Threshold Algorithm
SWAP	short-wavelength automated perimetry

Introduction

This thesis demonstrates how we applied a new perimetric model for detecting change in visual function in patients with diabetes. With this model we describe visual dysfunction over time in eyes without as well as with stable mild/moderate retinopathy, as shown by data obtained in a longitudinal study comprising up to five years of follow-up.

Diabetic retinopathy

One of the most feared complications of diabetes is visual impairment caused by vascular abnormalities, i.e. diabetic retinopathy.[1] Microaneurysms constitute the first morphological sign of such retinopathy, and later signs include leakage, retinal hemorrhages, and eventually formation of new vessels (proliferative retinopathy; Figure 1), vitreous hemorrhages, fibrous tissue, and retinal detachment. Diabetic retinopathy affects vision when leakage involves the center of the fovea, or when the retinopathy has become proliferative. Microvascular abnormalities can be seen at any location in the retina but can be more common in the temporal part.[2, 3] Diabetic retinopathy is graded in predefined levels associated with increasing risk of progression to proliferative retinopathy.[4-7] According to an international severity scale used in clinical practice, diabetic retinopathy is graded as follows: not present, mild (microaneurysms only), moderate to severe non-proliferative (severe intraretinal hemorrhages in four quadrants, venous beading in at least two quadrants or intraretinal microvascular abnormalities), or proliferative.[7] The gold standard for monitoring diabetic retinopathy in clinical research is the more detailed Early Treatment Diabetic Retinopathy Study (ETDRS) final severity scale.[5]

Considering diabetic subjects throughout the world in the 21st century, the overall prevalence of diabetic retinopathy (defined as the presence of any microvascular abnormalities) has been estimated to 26.8% in Norway,[8] 28.5% in the United States,[9] 43.1% in China,[10] and 17.6% among individuals with type 2 diabetes in India.[11] The major risk factors for progression of retinopathy are long duration of diabetes and poor control of glycemia and blood pressure.[12-15] The prevalence of vision-threatening diabetic retinopathy ranges from 4.4%[9] to 6.3%.[10] Notably, the number of diabetic subjects in the world is expected to increase from 382 million

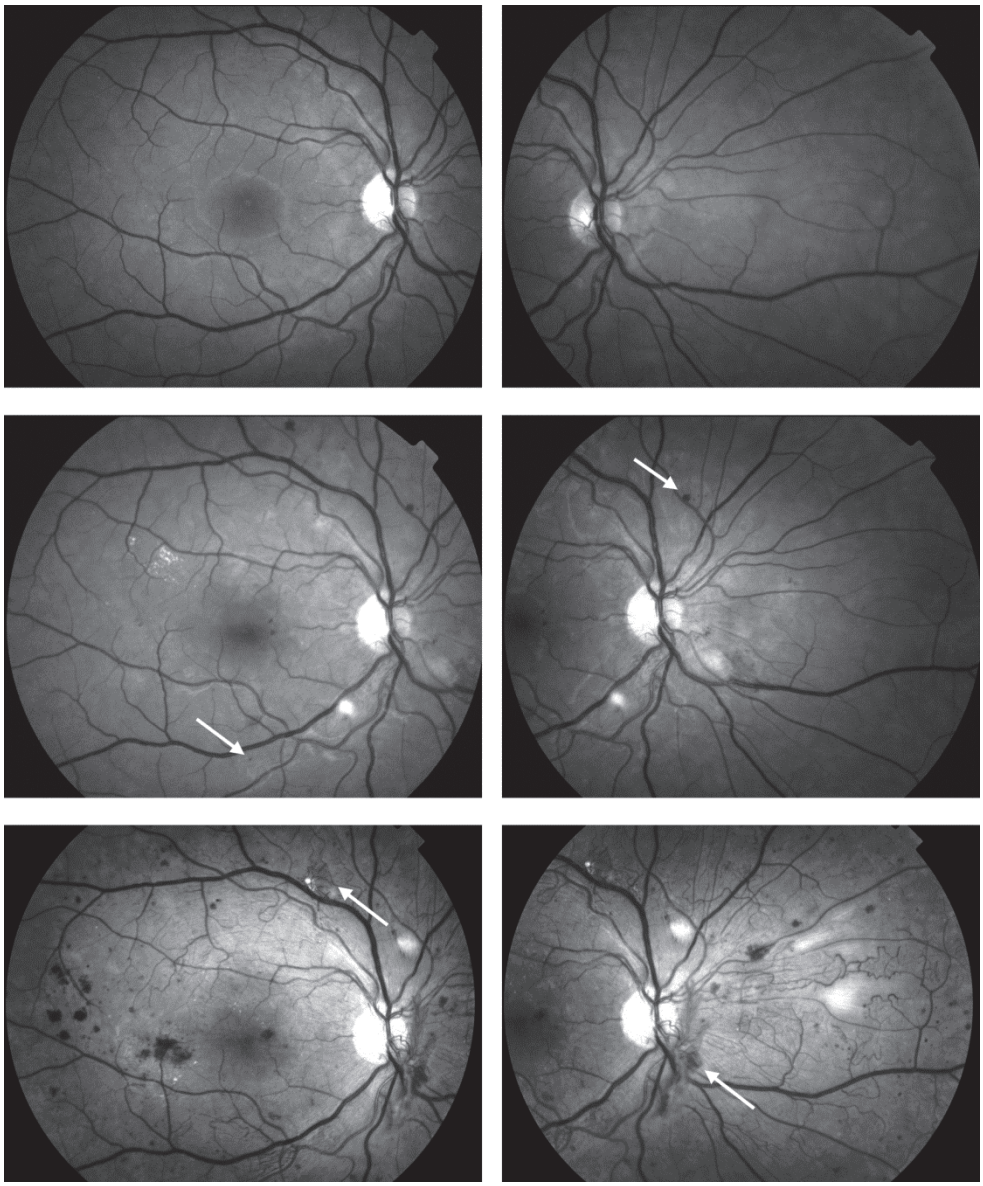

Figure 1. Fundus photographs showing the devastating course of diabetic retinopathy over four years in a young woman with type 1 diabetes. (Top) In 2009, this subject was 22 years old and had had diabetes for 13 years; her glycated hemoglobin A1c was 126 mmol/mol and blood pressure 155/105, and she showed no signs of diabetic retinopathy. (Middle) Three years later (in 2012), retinopathy was moderate, and microaneurysms (arrow in left image) and hemorrhages (arrow in right image) were present. (Bottom) One year after that (in 2013), she had developed severe proliferative diabetic retinopathy, and new vessels were present at the superior temporal vascular arcade and at the papilla (arrows in the left and right images, respectively).

in 2013 to 592 million in 2035,[16] and diabetic retinopathy is still one of the most common causes of blindness.[17] Visual functional loss in diabetes is routinely assessed by visual acuity measurements. However, visual acuity does not correlate well with the degree of retinopathy during earlier stages of this condition, and it can be normal despite severe diabetic retinopathy. A number of cross-sectional studies using functional measures other than visual acuity have proposed that visual dysfunction due to diabetic retinopathy can arise even before retinal vascular lesions are present. Nevertheless, longitudinal studies have not yet provided evidence that any particular functional test is useful for detecting early visual dysfunction in diabetes.

Functional tests potentially useful in diabetes

Perimetry

Perimetry measures light sensitivity at different locations in the visual field, and it has been proposed that this method can serve as a useful functional test in patients with diabetes. Modern perimetry performed to assess visual function entails automated static threshold tests, which are computerized tests applying a visual stimulus at pre-specified locations throughout the visual field. At each location the threshold is determined by the dimmest stimulus that is seen. Different types of perimetry defined by stimulus and background illumination have been used to evaluate visual function in diabetic subjects.

SAP

Standard automated perimetry (SAP) is the most widely used type of perimetry worldwide. This method assesses discrimination of a white stimulus on a white background, called differential light sensitivity. Thresholds reflecting retinal sensitivity are determined at predefined test locations within the central 30° on a background illumination of 31.5 apostilbs (asb). The stimulus is a Goldman size III object, representing a stimulus size of 0.43°. Perimeters to assess SAP provides a number of features to aid interpretation of the results, such as the following: threshold values corrected for deviations from age; p-values for being outside normal limits, which are displayed in maps showing the location of the visual field defect.[18, 19]

Visual dysfunction in diabetes assessed by SAP has been correlated with the presence and/or degree of diabetic retinopathy. Cross-sectional studies have shown that using SAP to evaluate visual function indicates worse dysfunction in eyes with more severe than in those with less severe diabetic retinopathy,[20-24] and this also applies to levels of severity of retinopathy according to the ETDRS final

severity scale.[25] It has been proposed that early visual dysfunction in diabetes can be assessed by SAP, but the results in the literature are inconsistent. Some cross-sectional studies have found reduced retinal sensitivities in diabetic eyes compared to healthy controls,[23, 26, 27] whereas others have not.[24, 28] One longitudinal study suggested that SAP could predict the development of diabetic retinopathy,[29], but only baseline functional data were reported by the authors. Results are lacking with regard to whether functional change over time can be assessed by SAP in diabetes.

Microperimetry

Microperimetry, also called fundus-related perimetry, has been used to test differential light sensitivity and uses eye tracking based on fundus imaging. The stimulus employed most often is a white Goldman size III object on a white background. The examination time is longer than for SAP[30].

Microperimetry in diabetic patients is used mainly in studies of macular edema.[31-35] Furthermore, it has been reported that retinal sensitivity decreases with increasing degree of retinopathy[36], but it is not yet clear whether this method is useful for detecting early retinal dysfunction. Three cross-sectional studies have compared retinal sensitivity in diabetic patients without retinal vascular lesions and non-diabetic controls: one of those investigations found retinal sensitivity to be similar in the two groups,[37] and the other two detected lower retinal sensitivity in the diabetic subjects.[36, 38] The study which found similar sensitivities,[37] included more diabetic subjects without retinopathy, n=70, compared to the other two studies,[36, 38] n=40 and n=39 respectively. The literature offers no longitudinal functional data obtained by microperimetry to evaluate its usefulness for detection of early visual dysfunction in diabetes.

Microperimetry can assess only smaller parts of the visual field, and the stimulus used has a shorter dynamic range, resulting in a ceiling effect. To some extent, this issue has been addressed in microperimeters that were introduced more recently.[39, 40] One instrument provides 0.25 asb in brightness of the dimmest stimulus on a background illumination of 4 asb. Another microperimeter offers 3.93 asb in brightness of the dimmest stimulus but with the same background illumination as used in SAP (i.e., 31.5 asb); this can be compared with the dimmest stimulus in the above-mentioned SAP assessment, which is 0.08 asb.

SWAP

Short-wavelength automated perimetry (SWAP) uses a blue Goldman size V (1.72°) stimulus on an intense yellow background with illumination of 100 asb. Presentations of thresholds and perimetric indices in SWAP are similar to those in SAP. Also, the range in brightness of SWAP stimulus is the same as for SAP but the effective dynamic range is somewhat shorter because of the brighter background illumination. The purpose of using a blue on yellow perimeter is to

test a specific subpopulation of retinal ganglion cells, which theoretically should improve sensitivity.

Visual dysfunction assessed by SWAP worsens with increasing degree of retinopathy according to the ETDRS final severity scale.[25] It is unclear whether SWAP sensitivity is lower in diabetic subjects without diabetic retinopathy than in non-diabetic subjects. Some researchers have reported a slight reduction in SWAP,[26, 28] whereas others have found SWAP sensitivity to be similar compared to controls.[41-43] It has been suggested that functional loss can be detected earlier by SWAP than by SAP.[26, 28] So far, no longitudinal studies have been performed to establish the usefulness of SWAP in diabetes.

Perimetry based on frequency-doubling technology

Frequency-doubling technology (FDT) uses a stimulus consisting of light and dark bars that flicker at a high frequency to create the illusion of there being twice as many bars. The purpose of using FDT is to test a specific subpopulation of retinal ganglion cells, which theoretically should improve sensitivity.

Reduced sensitivity assessed by FDT has been associated with diabetic retinopathy,[24, 44] but there is conflicting reports whether FDT can detect visual dysfunction in diabetic eyes before diabetic vascular lesions occur. In diabetic subjects threshold sensitivity was reduced and the pattern standard deviation (PSD), reflecting irregulatiries in the visual field, was increased using the 10° stimulus,[45, 46] but results obtained with a 5° stimulus are inconsistent.[24, 27] Thus, even though the smaller stimulus may enhance localization of visual field defects, it is not certain that it can enable early detection. Longitudinal studies to evaluate the usefulness of FDT to assess early functional change in diabetes have not yet been performed.

ERG

The electroretinogram (ERG) measures the electrical response of the retina to various visual stimuli, and a variety of parameters can be recorded. Here, visual function assessed by ERG in diabetes is presented according to the major subgroups classified by stimuli[47]: (1) full-field ERG; (2) pattern ERG; (3) focal and multifocal ERG.

Abnormal ERG responses associated with severity of diabetic retinopathy have been assessed using all three major ERG subgroups. There are several reports of reduced oscillatory potentials being correlated with severity of retinopathy when using full-field ERG,[48-52] and abnormal responses have been observed when using pattern ERG[48, 53] and multifocal ERG.[54] Also, all three ERG subgroups have been used to evaluate impaired responses in diabetic eyes without retinopathy compared with non-diabetic eyes[54-57]. Reduced responses shown by full-field ERG have been

reported to predict proliferative retinopathy[58] for up to 6–8 years[51], and onset of diabetic retinopathy within one year has been predicted by multifocal ERG.[59] Longitudinal studies using ERG are rare. Two investigations examined ERG responses in type 1 diabetic patients receiving intensified antiglycemic treatment: one of these studies revealed improved ERG responses over one year in 30 patients,[60] whereas the other found no such change over three years in 45 patients.[61] Only one longitudinal study has examined early functional change in diabetic subjects, and it showed decreased ERG response in 21 type 1 diabetic patients over three years.[62] Thus the usefulness of ERG for early detection of change in visual function has not yet been established.

Color vision tests

Color vision tests assess the ability to discriminate light with different spectral properties. Deficient color vision has been correlated with severity of retinopathy,[63-65] and it has also been observed that color vision is impaired in diabetic subjects without retinopathy compared to healthy controls.[66-68] Color vision has been reported to predict diabetic retinopathy,[69] but testing color vision for this purpose may be less useful in younger patients.[70] Longitudinal data on change in color vision have only been obtained in patients with type 1 diabetes, and the results have been inconsistent. Two longitudinal studies noted that impairment of color vision developed over time in seven and 10 subjects, respectively,[68, 71] whereas two other investigations found stable color vision in 10 and 37 subjects, respectively.[67, 72] Longitudinal studies are lacking, and thus far there is no evidence that any color vision test can be useful for evaluation of diabetic retinal damage.[73] In relation to the description of SWAP presented above, it can be mentioned that a more pronounced loss of sensitivity to short wavelengths (blue light) has been noted in assessments of color vision in diabetic eyes.[63, 69, 74-76]

Contrast sensitivity tests

Contrast sensitivity is tested by quantifying the amount of contrast that is necessary for an object to be detected at different spatial frequencies. Contrast sensitivity has been associated with severity of diabetic retinopathy,[77-80] and it has been found to be similar in diabetic patients and healthy controls,[24, 67, 79] although reduced contrast sensitivity has also been observed in individuals with diabetes and no retinopathy.[77, 80-82] One four-year longitudinal study of 37 type 1 diabetic patients detected a reduction in contrast sensitivity over time in subjects with no retinopathy[67]. Another two-year longitudinal study describe an improvement in contrast sensitivity over time in a subgroup of 30 type 1 diabetic subjects with

improved glycemic control with mild/moderate retinopathy but detected no changes in eyes with no or severe/proliferative retinopathy.[80]

Nyctometry

Nyctometry, also called macular recovery, is used to assess visual acuity over time at low background illumination after exposure to bright light. In diabetes, nyctometry has been correlated with severity of retinopathy.[83-85] Whether macular recovery is reduced in eyes without retinopathy is still a matter of debate,[78, 83, 85, 86] and it remains unclear whether this method can predict development of retinpathy.[83, 87, 88] Macular recovery over time has been reported both to be reduced[86, 87] and to be improved[87] in diabetic subjects. A new device for evaluating macular recovery has recently been introduced, but it does not seem to be suitable for detecting early retinal functional change.[89]

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Diabetic retinopathy is conventionally considered to be a microvascular disease, but inasmuch as the retina is not a vascular tissue but rather a neuronal tissue with a vascular supply, it would be interesting to investigate the effects of diabetes on the retina from a functional point of view. Until now, it has not been possible to monitor retinal function over time in diabetes.

Our work started with construction of a tailored analysis for interpretation of perimetric change in diabetic eyes and continued with a longitudinal evaluation of our model. We also investigated how visual acuity, the most widely used functional measure to date, can be reliably assessed in diabetic patients.

Aims

The general aim of the present research was to develop and test a new model for assessment of change in visual function in diabetes over time.

The specific aims of the four studies were as follows:

I. To investigate how refraction and visual acuity varies in diabetic patients under routine care.

II. To assess limits for significant improvement or deterioration of visual fields in diabetic patients based on short-term test–retest variability in subjects with different degrees of retinopathy.

III. To present a study protocol and 18-month interim results from an ongoing longitudinal study of the correlation between functional and structural change in diabetic eyes.

IV. To demonstrate the usefulness of SAP with our limits of significant change for detecting early retinal dysfunction over 3–5 years in patients with diabetes.

Methods

Study design

The present research comprised four studies on two different cohorts of diabetic subjects, and one eye per patient was assessed in both cohorts. The first and second studies (designated I and II) used a cross-sectional approach. Fifty-five diabetic subjects attending the Department of Ophthalmology, Skåne University Hospital, Malmö, Sweden, and had various degrees of retinopathy according to the ETDRS scale[5] were recruited and scheduled for five visits within one month, assuming that no important change in the diabetic retinopathy affecting visual function would occur during that period. At the first visit, all the subjects performed a perimetric training session and underwent fundus photography and blood sampling for assessment of HbA1c. At each subsequent visit, best-corrected visual acuity was measured, SAP and SWAP were performed, and sampling was done to assess blood glucose. Out of the 55 recruited patients; two were excluded because they did not complete all visits and three due to unreliable perimetry. In all, 53 patients were included in Study I evaluating refraction and visual acuity, and 50 participated in Study II concerning assessment of limits for visual field change.

The third and fourth studies (designated III and IV) used a longitudinal and prospective approach. Eighty-one diabetic subjects attending the Department of Ophthalmology, County Council of Värmland, Karlstad, Sweden, were consecutively recruited regardless of degree of retinopathy. All these subjects performed a training session to avoid perimetric learning and were subsequently scheduled for a baseline visit. Thereafter, they were scheduled for visits every six months for three years and then annually until five years from the baseline visit. Visual acuity, SAP, fundus photography, and HbA1c levels were assessed at each visit. Follow-up was completed for 18 months by 76 patients (94%; Study III) and for at least 36 months by 74 (91%; Study IV).

In all four studies, patients with any of the following were excluded: (a) any disease other than diabetes likely to affect the visual field; (b) non-gradable photographs; (c) previous laser treatment or any other local treatment for diabetic retinopathy; (d) inability to perform a reliable visual field, which was defined as

having > 15% false-positive answers. Patients in need of laser treatment were also excluded from Studies III and IV.

Characteristics of the patients included in the cross-sectional and longitudinal studies are presented in Table 1, ETDRS levels are given in Figure 2, and reasons for drop-out from the longitudinal studies are shown in Figure 3. The studies were approved by the Regional Ethical Review Board of Lund University, Sweden, and were performed in accordance with the tenets of the Declaration of Helsinki. All participants gave written informed consent.

Table 1. Patient characteristics

Characteristic	Studies I and II (cross-sectional)	Studies III and IV (longitudinal)
Female, n (%)	23 (42%)	30 (37%)
Male, n (%)	32 (58%)	51 (63%)
Age (years)	54 ± 12	57 ± 11
Age at onset of diabetes (years)	36 ± 17	44 ± 15
Diabetes duration (years)	18 ± 11	13 ± 12
Glycated hemoglobin, HbA1c (mmol/mol)	66 ± 16	60 ± 12
Insulin treatment only, n (%)	30 (54%)	33 (41%)
Insulin and oral hypoglycemic agent, n (%)	8 (15%)	15 (18%)
Oral hypoglycemic agent only, n (%)	16 (29%)	24 (30%)
No pharmacological treatment, n (%)	1 (2%)	9 (11%)
Antihypertensive medication, n (%)	31 (56%)	42 (52%)
Systolic blood pressure (mmHg)	145 ± 24	133 ± 16
Diastolic blood pressure (mmHg)	83 ± 11	78 ± 11

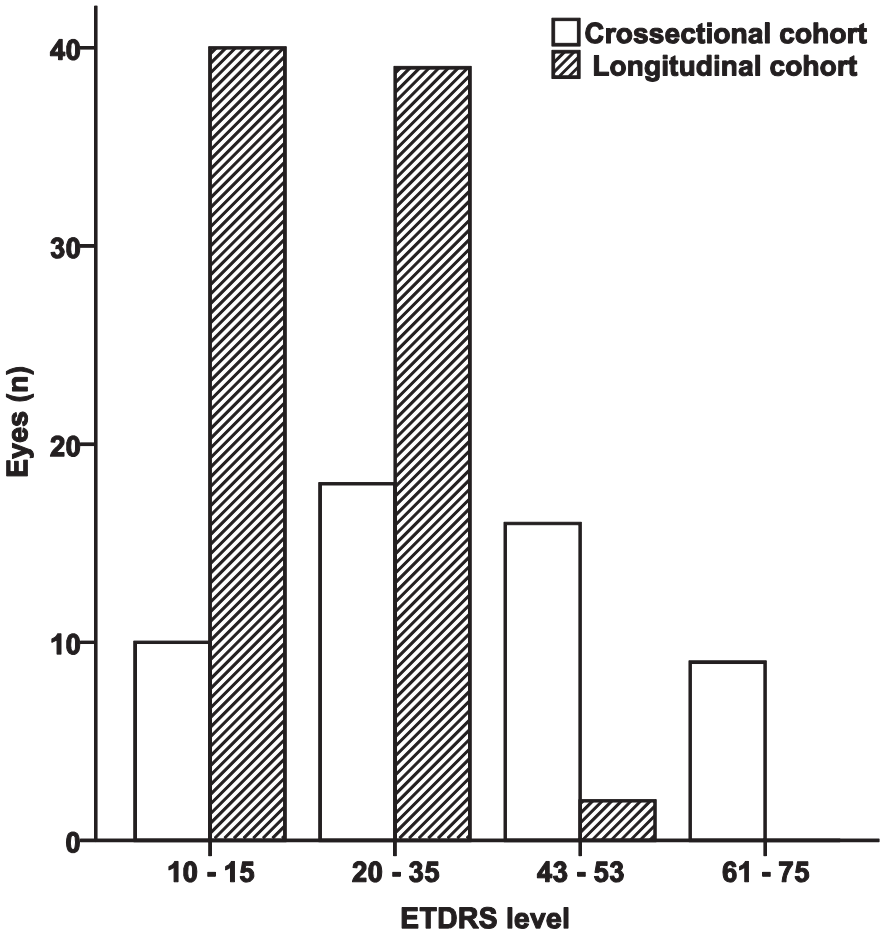

Figure 2. Degree of diabetic retinopathy according to the Early Treatment Diabetic Retinopathy Study ETDRS final severity scale. The cohort in the cross-sectional studies (I and II) represented various levels of diabetic retinopathy ranging from no (level 10) to proliferative (≥ level 61). The cohort in the longitudinal studies (III and IV) represented diabetic retinopathy ranging from no (level 10) to moderate (levels 35 and 43).

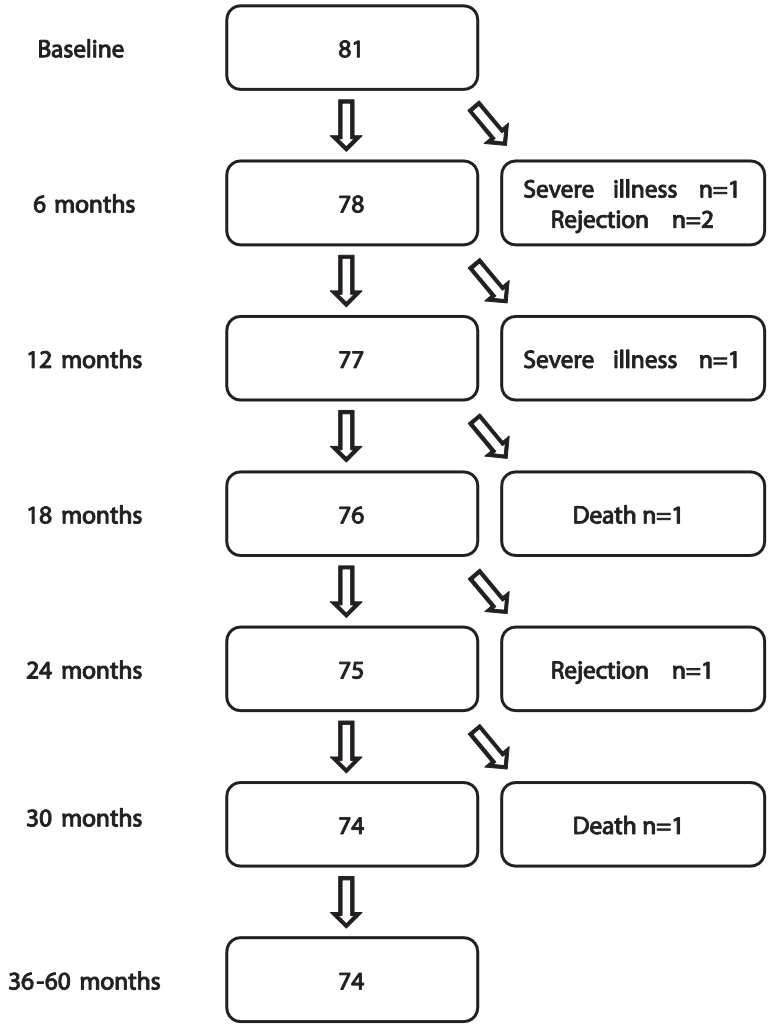

Figure 3. Numbers of subjects and reasons for drop-out in the longitudinal studies (III and IV).

Visual acuity

Best-corrected visual acuity was tested using ETDRS charts[90] after refraction measured by an autorefractor (Automatic Refractor 595, Carl Zeiss Meditec Inc., Dublin, CA, USA in Studies I and II; KR-8100P Topcon, Tokyo, Japan, in Studies III and IV) followed by manual adjustment. At follow-up visits, manual refraction

was performed only if the autorefractor revealed any change in sphere or cylinder > 0.25 D. Visual acuity was expressed in logMAR scores based on the number of correctly read letters.

Perimetry

Visual fields were tested by SAP and SWAP using a Humphrey Field Analyzer model 750 (Carl Zeiss Meditec Inc., Dublin, CA, USA) and the SITA Standard 24-2 and SITA SWAP 24-2 test strategies. The 24-2 test pattern includes 54 test locations, two of which are in the area of the blind spot within the central 24° of the visual field. The test locations are fixed in a grid covering the central retina, with a distance of 6° between test points (Figure 4).

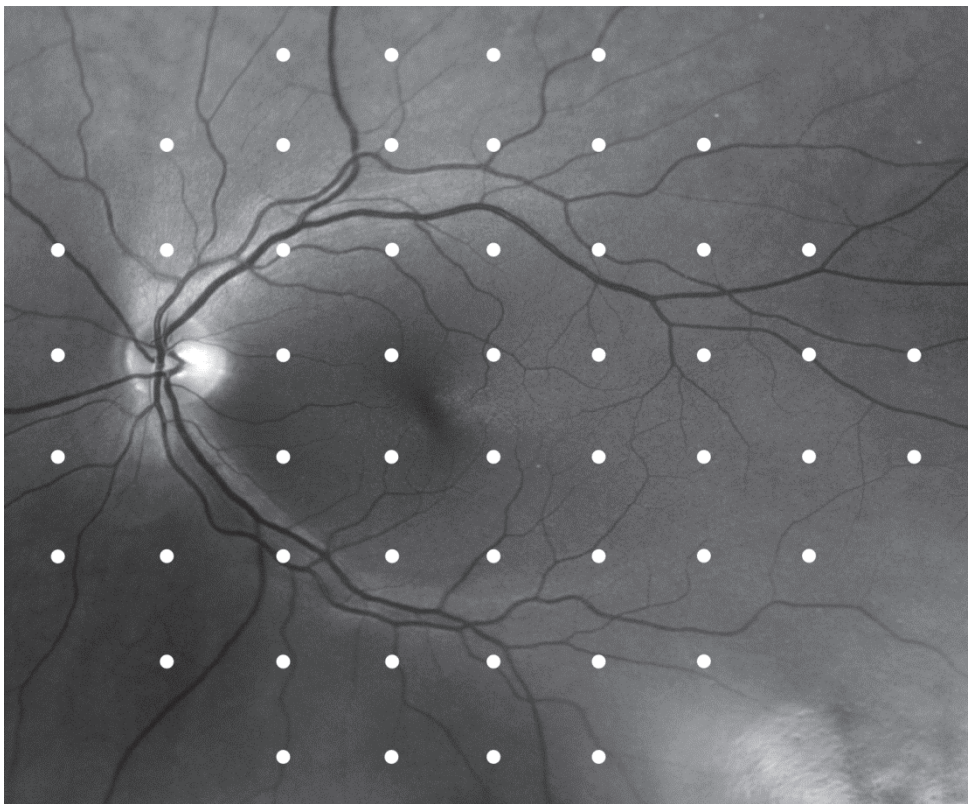

Figure 4. Stimulus location of the 24-2 pattern used in standard automated perimetry (SAP) superimposed on a fundus image.

The following conventional perimetric indices were used: (a) mean deviation (MD), which is the summary index of the visual field and shows the global deviation from the age-corrected normal field in dB, and an MD value of approximately 0 dB represents a normal visual field and approximately –30 dB a perimetrically blind eye; (b) deviation from age-corrected threshold values at each test point that displays defect depth in dB.[91]

Fundus photography and grading of diabetic retinopathy

After dilatation of the pupil, stereo fundus color slides of seven photographed 35° fields[92] (TRC 50 IX; Topcon, Tokyo, Japan) were obtained. Diabetic retinopathy was graded according to the ETDRS final severity scale[5], and progression of diabetic retinopathy was defined as a two-step change in the study eye. The grading procedure was blinded to patient characteristics and outcome of functional tests, and in Studies III and IV also to visit number.

Blood measurements

Capillary blood glucose levels were determined using a Hemocue Glucose 201+ Analyzer (Hemocue AB, Ängelholm, Sweden) and expressed as mmol/l. Glycated hemoglobin A1c (HbA1c) was analyzed by high-performance liquid chromatography (HPLC) using the Variant II Hemoglobin A1c program (BioRad, Hercules, California, USA) or a TOSOH G8 analyzer (Medinor, Tokyo, Japan), normal range 27–42 and 31–46 mmol/mol for individuals <50 and ≥50 years, respectively. The HbA1c values were presented as follows: according to the Mono-S method (%) in Studies I and II; as Mono-S (%) and International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) units (mmol/mol)[93] in Study III; as IFCC units (mmol/mol) and according to the National Glycohemoglobin Standardization Program (NGSP) (%) in Study IV. Mono-S values were converted to IFCC units using the converter provided by the Swedish Society for Clinical Chemistry (www.HbA1c.nu) and from IFCC to NGSP using the converter available from the NGSP (www.ngsp.org/convert1.asp).

Analyses

Studies I and II

Individual variations in spherical equivalent, visual acuity, and blood glucose fluctuations were expressed as the range between the highest and lowest value. In addition, variability of visual acuity was expressed as individual standard deviation. Visual field random test–retest variability for all test points except the two located in the blind spot area was calculated as the mean of all possible differences between the four measurements. We also analyzed how test–retest variability was affected by the global MD value, test point location, deviation from age-corrected threshold values, and blood glucose fluctuations. By taking into account the factors that were found to have a significant impact on the variability, we were able to define empirical significance for evaluating change.

Studies III and IV

In each follow-up visual field, test points exceeding the change limits defined in Study II were flagged as deteriorated or improved compared to the baseline visual field. In Study III, the first visual field obtained after the training session formed the baseline. In Study IV, the baseline consisted of the mean of the first and the second tests performed after the training session. A probable visual field change was defined as ≥ 5 deteriorated or improved test points, and a likely change was designated as ≥ 5 deteriorated or improved test points in at least the two latest consecutive tests. To confirm the results provided by the empirically derived limits for change, we applied a second approach in Study IV: pointwise linear regression analysis of the age-corrected threshold values over time. Eyes were considered to have changed if ≥ 5 test points showed significant regression slopes. Kappa statistics was applied to compare visual field change identified by our change maps and by linear regression analysis.

Results

Refraction and visual acuity

In Study I, we found that refraction was stable in most eyes, and assessments of visual acuity were highly reproducible despite substantial blood glucose fluctuations.

Mean spherical equivalent and visual acuity were –0.10 D (Min; Max: -4.13; 4.75) and –0.03 logMAR (0.50; –0.22), respectively. Refraction was completely stable in 43 of 53 eyes, and the mean range was 0.40 D (0.13; 0.87) in the remaining ten eyes. Visual acuity was completely stable in two eyes. The mean range of visual acuity was 0.08 logMAR (0.00; 0.22), equivalent to four letters (0; 11). Variability of visual acuity expressed as standard deviations was mean 0.04 logMAR. The median blood glucose fluctuation was 6.3 mmol/l (max. 18.1 mmol/l), and there was no association between change in refraction or visual acuity and blood glucose fluctuations.

Limits for visual field change

In study II, we defined limits for SAP and SWAP change for diabetic subjects based on the random test–retest variability results. SAP showed less variability than SWAP. Three factors affected the test–retest variability: the global MD value, the test point location, and the defect depth (i.e., the deviation from the normal age-corrected threshold value).

The MD values decreased with a higher degree of retinopathy, and the number of points with significant defect depth increased. Mean Deviation for SAP and SWAP were median –0.90 dB (–11.97; 1.78) and –2.46 dB (–18.47; 3.00), respectively. Less test–retest variability in MD was noted for SAP than for SWAP, with median variability of 0.71 and 1.21 dB, respectively ($p < 0.0001$). Random variability in test points with mild or no depression was slightly lower for SAP than for SWAP.

The test–retest variability was less at paracentrally located test points than at more peripherally located points (Figure 5). Less pointwise variability was also noted

for fields with normal or only slightly depressed MD values, but the defect depth had a greater impact than global MD on the variability at both paracentral and peripheral locations. The variability was less at normal or close to normal test points and increased with a more pronounced defect depth (Figure 6). Blood glucose fluctuations had no effect on threshold variability at paracentral test points and only negligible effects at peripheral test points.

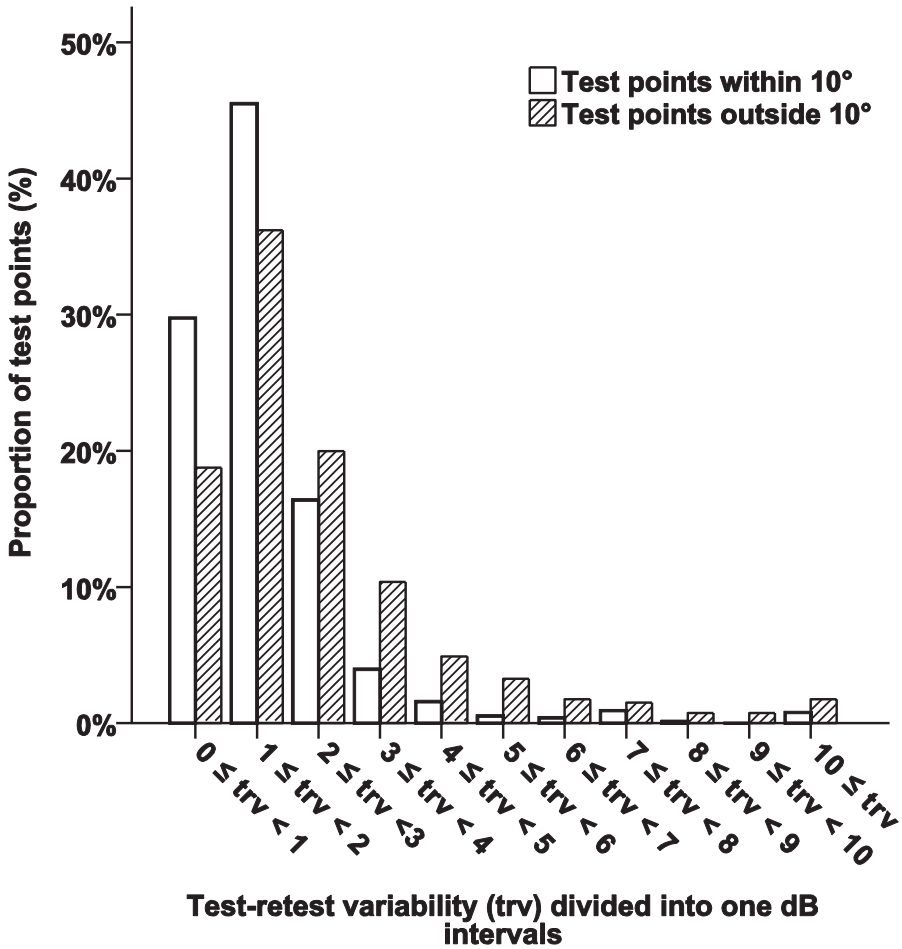

Figure 5. Test–retest variability of test points within and outside the central 10°. Test points within the central 10° showed less variability than those outside that area.

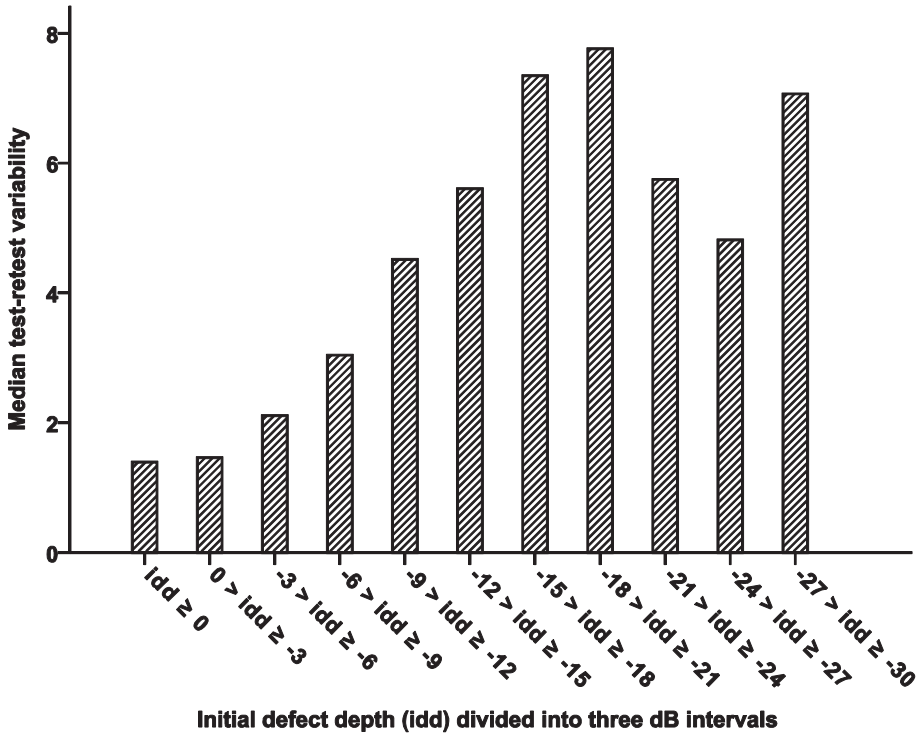

Figure 6. Test–retest variability increased with greater initial defect depth until the thresholds were equivalent to a brightness close to the strongest stimulus the perimeter could present.

Limits for significant change at each test point (i.e., deterioration or improvement) were defined separately for test points within and outside the central 10° of the visual field. In both zones, limits were defined for the full range of defect depths, from positive values indicating threshold values better than the age corrected normal values to the lowest values with no visible stimuli, divided into 3 dB intervals. Thus, the limit for change depends on test point location and defect depth.

Functional and structural change, 18 months interim-report

In study III, we used our previously defined limits for change when monitoring visual function by SAP in a new cohort of diabetic subjects. After 18 months of follow-up, deteriorations were common whereas improvements were rare, and deteriorated fields were reproducible despite unchanged degree of retinopathy.

At baseline, mean visual acuity and visual field MD were –0.13 logMAR (0.10; –0.28) and –0.67 dB (–5.69; 2.33), respectively. At the 18-month visit, retinopathy had progressed in only two eyes. Considering the cohort, visual acuity had declined by 0.04 logMAR (two letters), and MD values were similar to baseline.

Change maps were created for each follow-up field (Figure 7). In these maps, the local differences between follow-up and baseline field were flagged if they were outside the limits defined in Study II, and the number of deteriorated and improved test points were counted. No changes were found over time in the conventional perimetric indices (i.e., in MD values or the number of points with significant defect depths), although the change maps revealed significant deterioration in several eyes. The number of deteriorated test points observed at 12 months was sustained at 18 months in most cases. Considering all 52 test points for all 76 participants at 18 months, representing a total of 3,962 points, we found that 450 points had deteriorated and only 35 had improved.

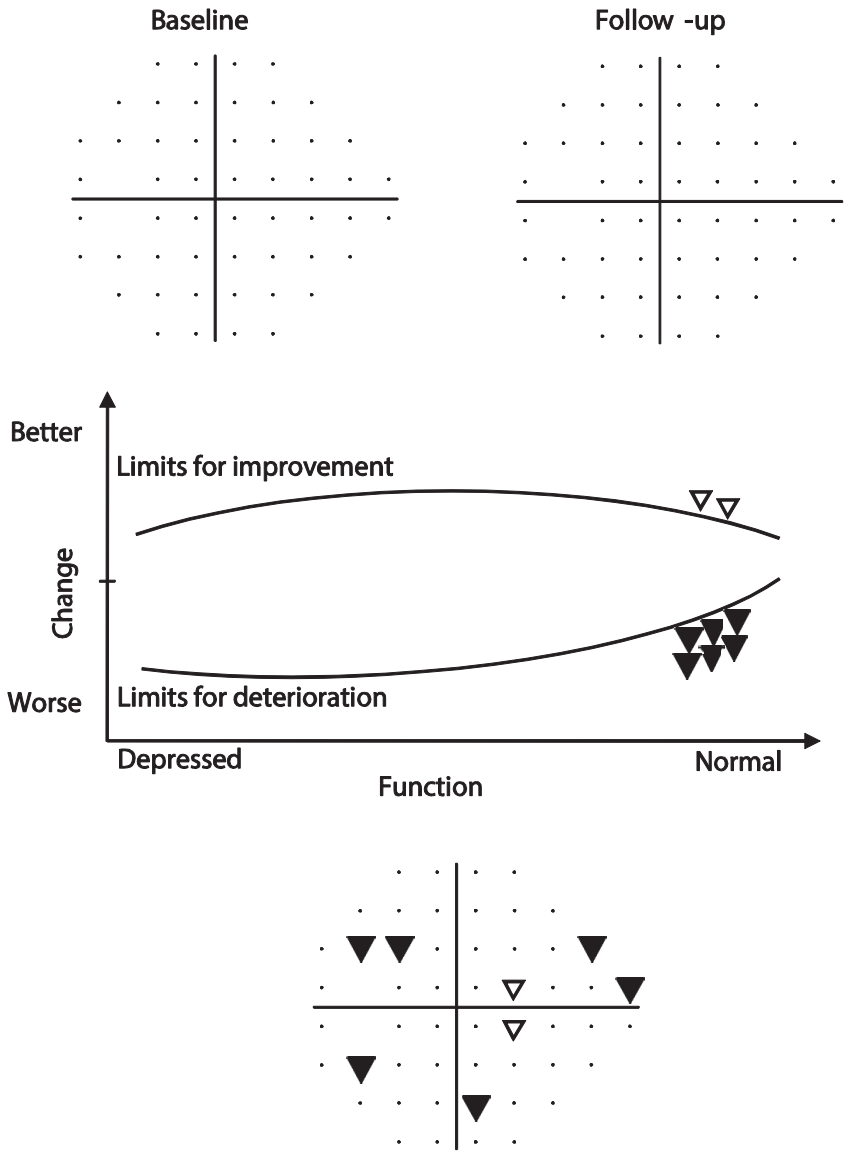

Figure 7. (Top) The baseline and 18-month follow-up visual fields of a patient included in the longitudinal study. All 52 test points in the 24-2 test point pattern were within normal limits, both at baseline and at follow-up, as shown in the single field analysis probability maps. (Middle) Limits for significant change were considerably narrower at test points with normal sensitivity than at test points with reduced sensitivity. (Bottom) A change probability map of the follow-up field showing the locations of the deteriorated and improved test points. A black triangle indicates significant deterioration at the $p < 5\%$ level, and an open triangle represents an improvement at the $p < 5\%$ level compared to baseline. Despite a clean single field analysis probability map of the follow-up field (top), the illustrated change probability map (bottom) flags 6 points as significantly deteriorated and 2 points as significantly improved.

Visual field change over time

In Study IV, we detected visual field deterioration over 3–5 years in eyes without progression of retinopathy (Figure 8). Furthermore, both in eyes with diabetic retinopathy and those without such disease, we found that deterioration was not associated with age, diabetes duration, HbA1c, or blood pressure levels.

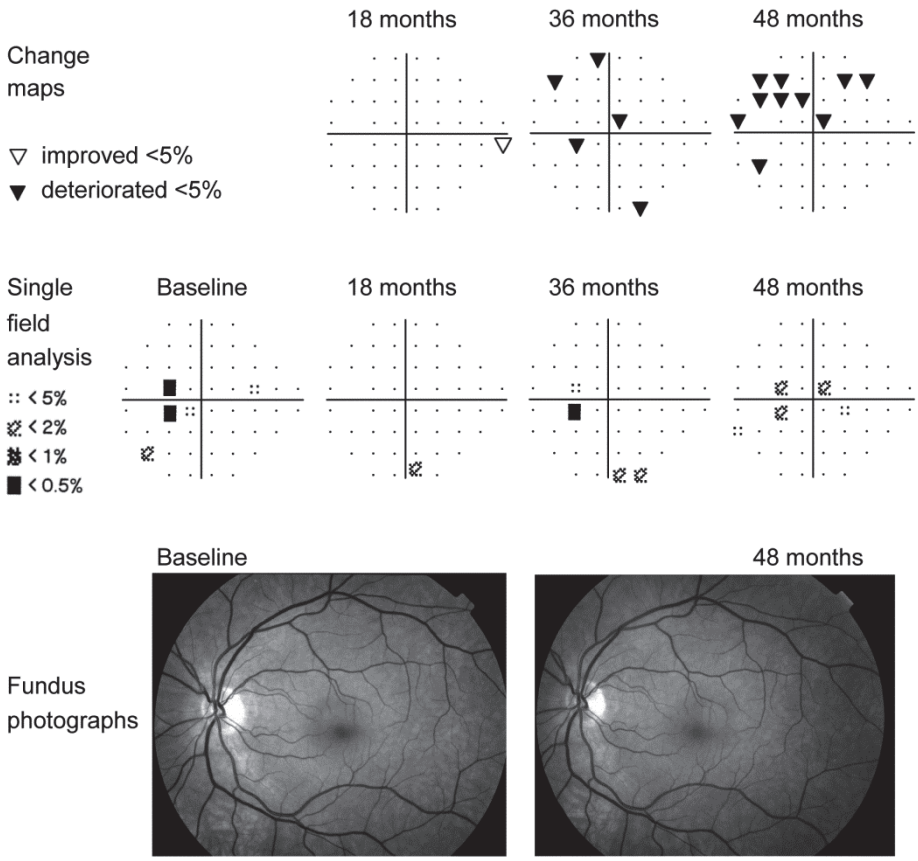

Figure 8. Visual fields from baseline to 48 months of follow-up and fundus photographs at baseline (left) and at the last follow-up visit (right) of a 63-year-old subject with type 2 diabetes and no retinopathy throughout the study. The single field analysis probability maps were mainly unchanged (middle), whereas a likely deterioration appeared in the change maps (top). Visual acuity was > 6/6 at each follow-up visit.

At the last visit, progression of retinopathy had occurred in only two eyes and regression in four. Considering the entire cohort, visual acuity had declined by 0.04 logMAR ($p < 0.001$), MD values were unchanged, and the number of test

points with significant defect depths had increased from two to three per visual field ($p = 0.04$). Thus the conventional perimetric indices were stable or showed very little change.

The change maps revealed a possible deterioration in the visual fields of 27 eyes (36%) and a likely deterioration in the visual fields of 16 eyes (22%), whereas only three eyes showed a possible improvement and one eye a likely improvement. In eyes with likely visual field deterioration, the number of deteriorated test points increased gradually over time. The deteriorated test points displayed no specific patterns but were instead evenly distributed throughout the visual field. Equal numbers of deteriorated test points were found in the upper and lower hemifield, as well as inside and outside the central 10° of the visual field. There were no associations between the number of deteriorated test points and age, diabetes duration, HbA1c levels, or blood pressure.

Our method using the limits for significant change based on random test–retest variability showed good agreement with the pointwise linear regression approach ($\kappa = 0.60$), although our method was more sensitive. Using the linear regression method for defining change ≥ 5 significantly deteriorated test points were found in 11 eyes compared to a likely change in 16 eyes using our method.

Progression or regression of retinopathy occurred in too few eyes to enable analysis of any correlation with change in visual function. At the final visit, eyes with ($n = 50$) and without ($n = 24$) any signs of vascular lesions showed nearly the same number of deteriorated test points: 3 and 2.5, respectively.

Discussion

The purpose of this thesis was to investigate whether perimetry could be a useful method when monitoring diabetic subjects with the specific aim of detecting early diabetic retinal dysfunction. Testing visual acuity is valuable for describing late visual dysfunction associated with severe diabetic retinopathy.[13, 14, 94, 95] However, visual acuity is not correlated with the degree of diabetic retinopathy during earlier stages of this disease, and it is still a matter of debate whether other functional measures, such as perimetry, can reflect retinopathy at those stages. Investigations have been performed to examine the associations between diabetic retinopathy and visual field loss measured by standard automated perimetry,[20-25, 29] and a number of other functional measures have also been suggested.[24, 25, 36, 44, 48-54, 63-65, 77-80, 83-85] Despite that, longitudinal studies in humans have not yet provided clear evidence of the applicability of any of the evaluated tests. In the present research, we applied a new approach to detect early change in visual function in diabetes, using perimetry with pre-defined limits for significant change and longitudinal follow-up. Our results suggest that measureable deterioration occurs both in eyes with no vascular lesions and in those with stable vascular retinopathy. Furthermore, our findings demonstrate that visual acuity which to date represents the most accepted and most widely used functional test in diabetic retinopathy can be performed with high reproducibility in diabetic patients under routine care.

We used ETDRS charts[96] and standardized procedures to score visual acuity as number of letters read correctly,[90] an approach that is generally recommended as an end point in clinical studies. Test–retest variability is lower when visual acuity is scored as individual letters read correctly rather than as the last line read correctly[97-99]. Visual acuity is also strongly dependent on refraction and refractive changes have been reported in diabetic patients with poor glycemic control, and during intensified anti-glycemic treatment.[100 -108] Notably, refractive changes have not previously been described in diabetic patients under routine care, even though such individuals undergo regular ophthalmological examinations and visual acuity measurements. Variability of refraction in diabetic patients can also have a number of other implications, for example, when prescribing glasses or performing refractive surgery**.**

In Study I, we observed stable refraction over a period of one month in the eyes of subjects under routine care, despite substantial blood glucose fluctuations. Refraction was performed carefully to an accuracy of 0.25 D in sphere and

cylinder, a level that is below any significant refractive change.[109] Also, our highly reproducible visual acuity measurements indicated accurate refraction in diabetic subjects. These results agree with two studies showing stable refraction in diabetic subjects attending outpatient clinics.[110,111] In one of the cited investigations,[110] examinations were performed over a longer time span than in our study (on average 51 days) in subjects who initially had symptoms of blurred vision. In the other study,[111] the examinations were repeated during a single day. Our results provide no evidence that refraction varies with fluctuations in blood glucose in diabetic subjects, and conflicting results have been reported with regard to experimentally induced hyperglycemia and refractive changes. Wiemer et al.[112] found that only one of five healthy subjects had any refractive change, which in that case was a hyperopic shift, whereas other researchers observed a myopic shift in six diabetic subjects following intravenous administration of 50 ml of 50% glucose solution.[105]

The visual acuity measurements in Study I were highly reproducible. The standard deviation of repeated measurements was 0.04 logMAR, which is comparable to the previous lowest values of 0.04[98] and 0.05 logMAR[97, 99] found in the literature. Reproducibility has been demonstrated to be similar in healthy subjects and patients with various retinal diseases.[99] In a multicentre study of diabetic patients,[113] variability increased with deterioration of visual acuity. In our subjects, the lowest visual acuity was 0.50 logMAR. Accordingly, by using a standardized test protocol and ETDRS charts, and scoring visual acuity by counting correctly read letters, reliable and highly reproducible visual acuity measurements can be achieved in diabetic patients under routine care. Poorer compliance with testing procedures will lead to greater variability.[114]

Previous cross-sectional studies have indicated that the visual field defects seen in diabetes are initially subtle and progress slightly with increasing degree of retinopathy. Before the occurrence of vascular lesions, diabetic eyes were found to have MD values that were normal or close to normal in SAP[23, 24, 26-28] and SWAP.[26, 28] Moreover, with increasing degree of retinopathy, MD values were observed to decrease by 0.44 and 0.40 dB/ETDRS step for SAP and SWAP, respectively.[25] Therefore, before beginning an expensive and time-consuming longitudinal study to identify potentially elusive change in visual function, it was important to create a sensitive model that could reveal such change in patients with diabetes. This was achieved by determining random perimetric test–retest variability and then defining limits for local perimetric change.

We chose to use SAP and SWAP to investigate random perimetric test–retest variability. SAP is the most common type of perimetry and is the standard method for follow-up of visual function in eye diseases such as glaucoma, and SWAP has been reported to be sensitive to early retinal dysfunction[26, 28] (Study II). Other types of perimetry, such as microperimetry[36-38] and frequency-doubling perimetry,[24, 27, 45] have been used to assess visual function in diabetes, but so far

these methods have only been applied in cross-sectional studies. Although microperimetry has been reported to provide results that correlate with an increasing degree of retinopathy according to the ETDRS scale[36], it seems less likely that this technique can be useful for measuring the earliest visual dysfunction over time in diabetes, because it has a short dynamic range. This is exemplified by the finding that healthy subjects can usually discern the dimmest stimulus.[115-117]

We applied a new approach to detect visual field deterioration/improvement in diabetic subjects, using limits for change analogous to the glaucoma change probability maps[118] implemented in the Humphrey Field Analyzer (Carl Zeiss Meditec Inc., Dublin, CA, USA) (Studies III and IV). In the first step in our work, those limits were to be defined in eyes with a spectrum of retinopathy (i.e., various levels of the disease; Study II). Therefore, we recruited diabetic subjects with retinopathy ranging from not present to proliferative. An increasing degree of retinopathy was correlated with decreasing retinal sensitivity assessed by SAP and SWAP, which confirmed previously published results.[25] The decreases in the global visual field index MD and local defect depths were found to be at least as pronounced when evaluated by SAP as by SWAP. Random test–retest variability was less for SAP than for SWAP. Consequently limits for change were slightly narrower and thereby more sensitive with SAP compared to SWAP. SWAP is more affected than SAP by developing cataract,[119] which increases the risk of false-positive deterioration being identified in such eyes. Thus it seems that, to identify change exceeding random variability, SAP is preferable to SWAP in longitudinal follow-up studies. We found that the pointwise test–retest variability was affected by three features of the visual field: test point eccentricity, defect depth, and general visual field status. We took these factors into account to define limits for significant local change in diabetes. Visual fields deteriorate with increasing degree of retinopathy, and variability is less for normal or close to normal test points, and together these observations indicate that small changes in function can indeed be detected at early stages. To test our new approach for analyzing visual field change in diabetic subjects, we compared our results with those obtained using another method based on pointwise linear regression.[120, 121] There was good agreement between the two techniques, although our change analysis identified a greater number of deteriorated eyes than the regression analysis did. Thus our approach is in analogy with change analysis based on random test–retest variability in glaucoma, which is more efficient than linear regression at detecting deterioration.[122, 123]

Using the limits of significant change specified for diabetic patients in Study II, we conducted Studies III and IV to explore the usefulness of SAP to detect early retinal dysfunction over time in a representative cohort of diabetic subjects. These patients were consecutively recruited from the screening program for early detection of sight-threatening retinopathy at the Department of Ophthalmology in

Karlstad. After only 18 months of follow-up, deterioration was common and improvement rare in the patients' change maps, despite stable diabetic retinopathy and no changes in either the conventional perimetric indices MD or the number of points with significant defect depth. We believe our limits for change were appropriate. If the limits had been too narrow, a number of improved test points would have been found as well, and the reproducibility of the deteriorations also suggests reliable limits. We were able to confirm these results in the longer follow-up investigation covering up to five years (Study IV). Visual field deterioration still occurred without any progression of diabetic retinopathy.

We do not believe that increasing cataract had any significant influence on our results. Cataract causes a general depression of the visual field, perhaps somewhat more pronounced in the center,[124] but this was not seen in our patients. Cataract is also strongly associated with older age,[125] but the patients with likely visual field deterioration in our study were not older than those without such deterioration. Furthermore, there was a poor correlation between visual field deterioration and loss of visual acuity. Three patients had cataract surgery during follow-up. Only one of these individuals had a deteriorated visual field before the surgery, and this subject's visual field was still deteriorated after cataract removal.

Drop-out from research studies is a common problem. In our investigations, adherence to complete all visits was good, with low drop-out of 4%, 4%, 6%, and 9% in Studies I, II, III, and IV, respectively. The low migration rates in Skåne and Värmland Counties might be one reason for this fortunate situation.[126] More males than females were included in our studies, which might be explained by the fact that more males are diagnosed with diabetes in Sweden in general, as well as in both of the mentioned counties.[127]

Early detection of visual dysfunction in diabetes has been suggested by a large number of cross-sectional studies[23, 26-28, 36, 38, 45, 46, 54-57, 66, 68, 77, 81, 82, 86] but only a few longitudinal studies. Functional change in eyes without diabetic retinopathy was reported by Di Leo et al.[62], who found reduced ERG responses over three years in 21 diabetic subjects, and by North et al.[67], who observed reduced contrast sensitivity over four years in 37 type 1 diabetic subjects. By comparison, we collected more longitudinal functional data, and our results demonstrated a consistent deterioration in 22% of the eyes that we analyzed. Two studies have suggested that visual dysfunction can predict the onset of local[59] and global[29] diabetic retinopathy after one and eight years using ERG and SAP, respectively. However, the practicability of predictive values is limited to rare events, in this case incidence of diabetic retinopathy. We used a different approach, namely, assessment of functional change over time. In our cohort, there was nearly no incidence, nor progression, of retinopathy, and therefore it was not possible to correlate visual dysfunction with progression of retinopathy over the study period. Fortunately, the rates of progression of diabetic retinopathy are slowing down.[128]

Longer follow-up studies including more subjects are needed to further clarify the correlation between visual dysfunction and microvascular abnormalities.

Defining progression and regression of diabetic retinopathy as a two-step change according to the final ETDRS severity scale may seem to be too crude a method for measuring subtle progression. Quantitative assessment of various vascular lesions has been proposed,[129-133] but, in our patient cohort, the number of microaneurysms and hemorrhages was small and the extension of exudates very limited. Furthermore, it would not be entirely appropriate to compare functional change determined by our novel method with morphological change assessed using a non-validated morphological scale. For those reasons, we chose to apply the ETDRS final severity scale, which is a more qualitative type of grading and is the acknowledged gold standard in this context.[5]

We found that deterioration was not associated with diabetes duration or HbA1c or blood pressure levels, and these results agree with some studies in the literature[29, 41, 62, 78] but not with others. This observation suggested that worsening of contrast sensitivity and macular recovery over time is associated with higher HbA1c levels.[67, 87] These risk factors are correlated with visual function at late stages of retinopathy, but they are not necessarily associated with a change in visual function at early stages.

As previously mentioned, the retina is not a vascular tissue but rather a neuronal tissue with a vascular supply. Neurodegeneration, such as apoptosis and glial activation, has been observed without signs of vascular lesions in post mortem retinas from humans with diabetes,[134, 135] and similar neurodegenerative patterns have been demonstrated in various animal models. [135-145] Streptozotocin-induced diabetes in rats represents the most frequently used animal model, and studies have shown early neuronal dysfunction[136, 137, 146, 147] along with neurodegeneration[136, 146, 147] in such experimental animals. Aung et al.[137] conducted a longitudinal investigation of diabetic rats and found reduced visual acuity as well as delayed ERG responses before any signs of microvascular abnormalities appeared. In humans, retinal thinning has been proposed to be an indirect sign of neurodegeneration in individuals with diabetes, was first reported in 1993 by Chihara et al.[148]. Investigators using the modern technique of spectral domain optical coherence tomography have shown that thinning of specific retinal layers occurs before the appearance of diabetic retinopathy.[37, 38, 149-151] In our research, SAP showed deterioration both in diabetic subjects with and in those without retinopathy, and since the level of retinopathy was stable over time, we suggest that the detected visual field deterioration reflects neuronal dysfunction, possibly caused by primary neurodegeneration.

It is plausible that SAP change analysis using significance limits for change based on random test–retest variability can enable monitoring of diabetic retinal damage from a functional perspective and also serve as a complement to morphological

measurement of potential interactions between neuronal dysfunction and microvascular abnormalities. Moreover, a functional test may provide a useful endpoint in clinical trials aimed at early intervention for diabetic retinal damage. Evaluation of new functional methods is a pressing issue. It can be mentioned that, two trials that have addressed early retinal damage in diabetes have used non-validated functional measures of outcome,[40, 152] and two additional trials are planned (NCT01726075, NCT01646047; available at http://clinicaltrials.gov).

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Change in visual function represents an early event in humans with diabetes. Retinal sensitivity assessed by SAP shows deterioration that coincides with the degree of retinopathy, and our longitudinal and prospective studies have demonstrated that early visual dysfunction can be detected in mild retinopathy, even before visual retinal vascular lesions appear. A future challenge is to characterize the interaction between visual dysfunction and microvascular impairment. We hope that future work in this area will be inspired both by our tailored perimetric change analysis for interpretation of early retinal dysfunction in diabetic eyes, and by the evaluation of this model we performed in our longitudinal studies.

Populärvetenskaplig sammanfattning

Denna avhandling visar att synfältsundersökning är en metod som kan påvisa försämrad synfunktion vid diabetes, redan innan man kan se några förändringar med konventionell ögonbottenfotografering. Med kunskap om synfältets normala och slumpmässiga variationer har vi konstruerat en modell för att tolka en förändring i synfälten och har följt patienter under upp till fem års tid. Modellen kan få betydelse för att utvärdera samspelet mellan skador på näthinnans nervvävnad och blodkärl och för att utvärdera nya behandlingsmetoder för att bromsa ögonskador vid diabetes. Diabetes är en vanlig orsak till blindhet idag.

Näthinnan, retina, är den del av ögat som omvandlar ljus till nervsignaler som kopplas vidare till hjärnan och ger oss synintryck. Retinas nervvävnad är beroende av syre och näring för att fungera och försörjs av talrika blodkärl som genomkorsar vävnaden. Personer med diabetes utvecklar ofta skador på dessa kärl vilket man kan se vid ögonbottenfotografering. Skadorna utvecklas ofta gradvis under många år och så småningom kan nervvävnaden påverkas med en synförsämring som följd. Näthinneskador vid diabetes kallas diabetesretinopati. Om diabetesretinopatin orsakar en svullnad i gula fläcken eller om det börjar utvecklas nya kärl, så kallad proliferativ retinopati, är ofta en kraftig synförsämring nära förestående. Laserbehandling av näthinnan kan i detta skede ofta bevara synen. Personer med diabetes erbjuds därför att ingå i ett kontrollprogram/screening där ögonbotten regelbundet fotograferas och man graderar de eventuella skador som hunnit uppkomma. Ett vanligt sätt att gradera skadorna på blodkärlen är: ingen, mild, måttlig, allvarlig och proliferativ diabetesretinopati. Genom god blodsockerkontroll och blodtryckssänkande behandling kan man fördröja utvecklingen av kärlskadorna. Trots denna kunskap och en god tillgång på sjukvård i västvärlden är diabetesretinopati fortfarande en av de vanligaste orsakerna till synnedsättning. Diabetes är en sjukdom som ökar, i världen finns idag totalt 382 miljoner människor med diabetes. Denna siffra beräknas stiga till 592 miljoner år 2035. Cirka 30 % av alla personer med diabetes har någon form av skada på näthinnans blodkärl och ca 4 % har synhotande förändringar.

Näthinnan är mycket tunn och själva nervvävnaden som fångar upp ljuset är nästan helt genomskinlig och knappt synlig vid ögonbottenundersökning. För att undersöka hur näthinnan fungerar används olika synfunktionstest, vanligen synskärpa. När man mäter synskärpa provar man först ut vilken glasögonstyrka

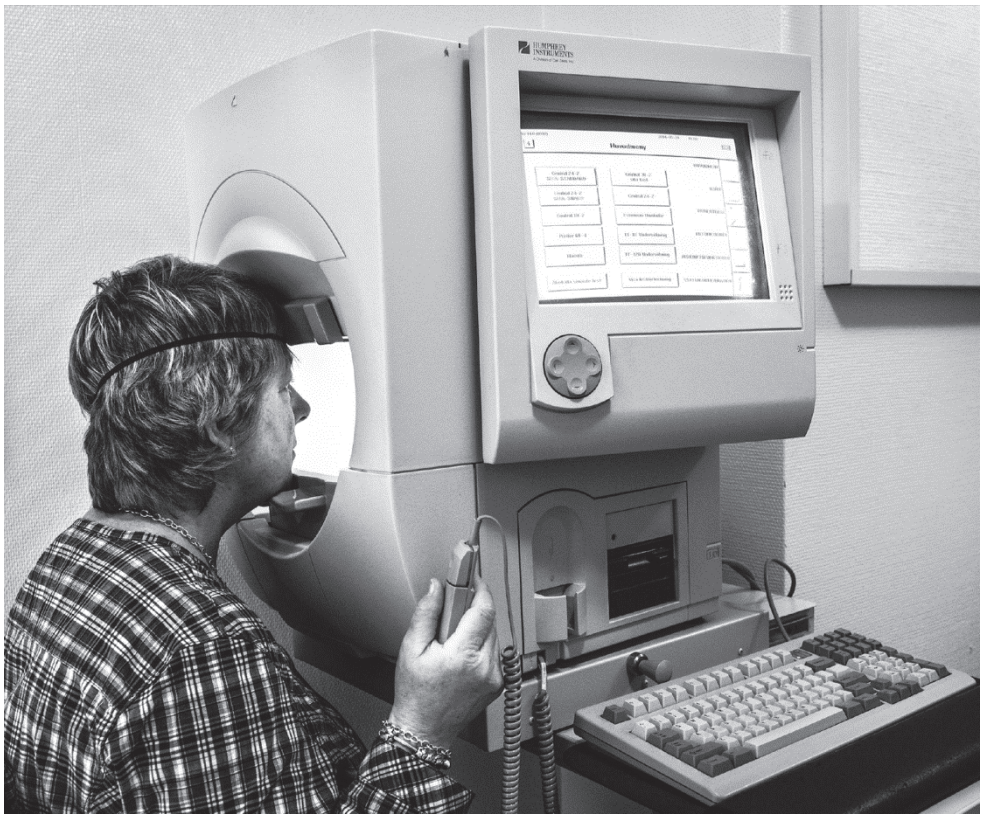
eller så kallad glaskorrektion som ger bäst skärpa. Vid mycket högt blodsocker och vid intensifierad behandling för att sänka blodsockret kan linsen påverkas så att ögats brytningsförmåga ändras. För att då mäta synskärpan behövs en annan glasögonstyrka än den som patienten brukar ha. Trots att patienter med diabetes regelbundet besöker ögonsjukvård och att synskärpa är det vanligaste funktionstestet så har det inte varit känt i vilken utsträckning förändringar av ögats brytningsförmåga förekommer hos personer med diabetes i allmänhet.

I studie I fick patienter med diabetes komma på upprepade undersökningar av glaskorrektion och synskärpa under en månad. Undersökningarna utfördes vid ögonkliniken i Malmö, Skånes universitetssjukhus. Vid varje besök mättes också blodsocker. Studien visade att de flesta hade stabil glaskorrektion och att synskärpan kunde uppmätas med hög precision. Blodsockernivåer eller förändring av blodsockernivån påverkade varken glasögonstyrkan eller synskärpan.

Synskärpan påverkas inte av diabetesretinopatin i tidigare stadier och ett känsligare synfunktionstest än synskärpa skulle kunna komplettera ögonbottenundersökningar, om det kunde beskriva hur diabetes tidigt påverkar näthinnan. Flera olika synfunktionstest har föreslagits, men än så länge har inget test visat sig användbart och studier där man utvärderat förmågan hos sådana test att mäta en förändring av synfunktionen över tid är sällsynta.

Synfältsundersökning är ett av testen som föreslagits, även för tidig funktionsnedsättning. Denna undersökning återspeglar hur näthinnan fungerar i många olika mätpunkter till skillnad från undersökning av synskärpa, som bara testar funktionen i en central testpunkt. I de studier som redovisas i denna avhandling utfördes en synfältsundersökning som går till på följande sätt: Patienten fixerar en central punkt och runt denna presenteras testobjekt i ett förutbestämt mönster, testobjektet som är en upplyst rund fläck projiceras på en jämnt upplyst bakgrund, det svagaste testobjektet som kan uppfattas i varje testpunkt bestäms. Mätningen utförs kontrollerat och automatiserat av en dator och tar bara några få minuter, Figur 9.

I Studie II undersöktes hur synfältet varierade för personer med diabetes som hade olika allvarlighetsgrad av diabetesretinopati från ingen till proliferativ diabetesretinopati. Undersökningarna utfördes vid ögonkliniken i Malmö, Skånes universitetssjukhus. Upprepade synfältsundersökningar gjordes under en månad och vid varje besök mättes också blodsocker. Synfältsundersökning gjordes både med vitt testobjekt på vit bakgrund, s.k. standard automated perimetry (SAP), och med blått testobjekt på gul bakgrund, short wavelength automated perimetry (SWAP). Den vit-vita metoden, SAP, är världens vanligaste. Den blå-gula, SWAP, har ibland framhållits som känsligare. Syftet var att så långt som möjligt kunna beskriva slumpmässiga variationer i synfälten för att visa hur stor en sann förändring statistiskt behöver vara, det vill säga bestämma gränsvärden för när en verklig förändring i synfunktionen uppstått. Om man hittar faktorer, som påverkar



Figur 9. En patient redo för synfältsundersökning. Hon fixerar blicken rakt fram och när hon uppfattar ett objekt, som är en liten rund ljus fläck någonstans i synfältet, trycker hon på knappen. Testpunkterna där objekten presenteras ter sig slumpmässiga för patienten, men utgör ett förutbestämt antal testpunkter i ett mönster. När synfältsapparaten avgjort det svagaste synliga objektet i varje testpunkt är undersökningen klar.

variationerna, kan man ta hänsyn till dessa och anpassa sina gränsvärden så att de så långt som möjligt enbart representerar slumpmässiga variationer. Vi fann tre faktorer som påverkade variationerna i synfältets testpunkter. 1) Testpunkter hade större variationer om synfältet generellt var sämre 2) Testpunkter hade större variationer om det vid första mätningen var ett lokalt sämre värde i den testpunkten. 3) Perifera testpunkter hade större variationer än mer centralt belägna testpunkter. Med hänsyn till dessa faktorer bestämde vi gränsvärden för förbättring och försämring i synfältet. Med sådana gränsvärdena kan man på ett känsligt sätt upptäcka en förändring över tid. Vi fann också att SAP hade mindre variationer än SWAP vilket talar för att det är lättare att upptäcka en förändring med SAP. Blodsockernivåer eller förändring av blodsockernivån påverkade inte synfälten på något betydande sätt.

I studie III och IV undersöktes hur synfältet förändrades med hjälp av gränsvärdena från studie II. Undersökningarna utfördes vid ögonkliniken, Centralsjukhuset Karlstad. Eftersom förändringar i diabetesretinopatin uppträder gradvis under flera års tid var det nödvändigt att utvärdera synfunktions-mätningarna över en längre tidsperiod. Patienterna rekryterades i löpande turordning från screening-programmet för diabetesretinopati. Deltagarna undersöktes var sjätte månad i tre år och sedan en gång årligen, tills det gått maximalt fem år från utgångsbesöket. Vid varje besök utfördes ögonbottenfoto-grafering och synfältsundersökning med SAP. Vid studiestart hade hälften av patienterna ingen och hälften hade mild eller måttlig diabetesretinopati. Det förekom överlag ingen ökning av skadorna på näthinnans blodkärl under studietiden. Redan vid 18 månaders uppföljning, studie III, visade synfälten, med hjälp av våra gränsvärden för förändring, många fler försämrade testpunkter än förbättrade. Efter i genomsnitt fyra års uppföljning bekräftades detta resultat och hos 16 av 74 patienter kunde försämringen bekräftas genom att det återfanns minst fem försämrade testpunkter i de två senaste synfältsundersökningarna. Synfältsförsämringarna var utspridda i hela synfältet och återfanns hos patienter med och utan kärlskador.

Att en synfunktionsförsämring uppmättes hos patienter även utan skador på näthinnans talar för att funktionsförändringen kan återspegla en påverkan på näthinnans nervvävnad. I näthinnor från döda djur och människor med diabetes har andra forskare visat att förlust av nervceller sker redan innan några tecken på blodkärlsskador är synliga. Skadade och dåligt fungerande nervceller framhålls allt oftare som det tidigaste tecknet av diabetessjukdomen i näthinnan. Nya behandlingsstrategier för att tidigt skydda nervvävnaden har föreslagits, men mer forskning behövs. En förutsättning för sådan forskning är ett känsligt och pålitligt funktionstest. Vår modell erbjuder en möjlighet att på ett känsligt sätt kunna följa näthinnans funktion vid diabetes med synfältsundersökning. Modellen kan bli ett viktigt verktyg för fortsatt forskning om hur näthinnan påverkas vid diabetes och för att utvärdera nya behandlingar som syftar till att förebygga skador i näthinnan redan i ett tidigt skede.

Acknowledgements

I wish to thank everyone who has contributed to this thesis through the past ten years. First of all, I want to express my sincere gratitude towards the patients who have participated in the studies. Second, I wish to thank

Elisabet Agardh – my main supervisor, PhD, professor at the Department of Clinical Sciences, Malmö, for lessons on science and in life, for believing in me, for encouragement, for structure and for sharing with me the joy within this field of research of diabetic retinopathy.

Boel Bengtsson – my second supervisor, PhD, associate professor at the Department of Clinical Sciences, Malmö, for inspiration, for teaching me a constructive attitude, for the many instructive assignments and for your patience with my entry into the world of visual fields and statistics.

Anders Heijl – PhD, professor at the Department of Clinical Sciences, Malmö, for your appreciated suggestions and inspiration along the way – filled with both science and humor.

Anders Hallberg - PhD, associate professor, head of Department of Clinical Research, County Council of Värmland, for your encouragement concerning my research.

Lennart Sjöbom – head of Department of Ophthalmology, Karlstad, for your never-ending encouragement and valuable advice.

Mikael Hasselgren – PhD, former head of Department of Clinical Research, County Council of Värmland, for support and encouragement concerning my education and the longitudinal study in Karlstad.

Kerstin Edlund – former head of the Department of Ophthalmology, Karlstad, for believing in the longitudinal study in Karlstad.

Johnny Ring – photographer at the Department of Clinical Sciences, Malmö, for your solid support concerning both software and hardware. Thank you for supporting crafting sensible graphs.

Christina Wenklo-Byléhn & Anne Lundh – ophthalmic nurses at the Department of Ophthalmology in Karlstad, for the high-quality fundus photographs.

Fredrik Lundin – PhD, statistician at the Department of Clinical Research, County Council of Värmland, for helping out with statistics and programming useful for the analysis of the visual field data to obtain the change maps.

Elisabeth Pranter –ophthalmic nurse at the Department of Ophthalmology, Skåne University Hospital, Malmö, for high quality fundus photographs.

Jari Appelgren & Dan Larsson – Karlstad Business School, Karlstad University, for technical support in performing the pointwise linear regression analysis.

Karin Ohlsson – ophthalmic nurse at the Department of Ophthalmology in Karlstad for technical support concerning software and hardware.

Johan Jendle – PhD, associate professor, head of the Endocrine and Diabetes Center, County Council of Värmland, Karlstad, for support and encouragement during my research in Karlstad.

Leif Johansson – photographer, County Council of Värmland, for helping out with posters and fundus photographs.

Helena Sandberg – graphic designer at Sandberg design & kommunikation, for visualization and a beautiful design.

Pia Tekin – secretary at the Department of Clinical Research, County Council of Värmland, for your helpful supervision of available research grants.

Britt Beding Barnekow – PhD, my former clinical tutor at the Department of Ophthalmology, Skåne University Hospital, Malmö, for introducing me to the Department of Clinical Sciences and for teaching me integrity and giving me confidence.

Sabina Andersson – PhD at the Department of Clinical Sciences, Malmö, for reaching out a helping hand when I needed to restructure a data file or just have another piece of chocolate.

Carin Gustavsson – PhD at the Department of Clinical Sciences, Malmö for pointing to the essence of research; it is fun.

Poya T-Kahni – PhD-student at the Department of Clinical Sciences, Malmö for peaceful work, for your friendship, and for your generosity in sharing desk space.

Ulrika Bergqvist – my secretary at the Department of Ophthalmology, Karlstad, for carful support concerning my patients.

Birgitta Svensson – ophthalmic nurse at the Department of Ophthalmology, Karlstad, for flexibility regarding my schedule.

Johanna Molinder – secretary at the Department of Ophthalmology, Karlstad, for help with my travels and accounting.

Sigbritt Johnsson – supporter, for your generous and friendly shelter during my many visits in Malmö.

Additionally, I would like to thank the following company:

Hemocue AB, Sweden – for lending me a glucose meter.

Finally, I want to express my deepest gratitude towards:

My family and friends – for your support and unconditional love throughout my thesis.

My collegues at the Department of Ophthalmology, Malmö, Skåne University Hospital – for your support and friendship, as well as for your interest in my thesis.

My collegues at the Department of Ophthalmology, Karlstad, County Council of Värmland - for your support and friendship, as well as for your interest in my thesis.

The work in this thesis was supported by: the Department of Ophthalmology, Karlstad, and Centre for Clinical Research, County Council of Värmland, Lund University along with Skåne County Council, the Malmö University Hospital Foundation, the Järnhardt Foundation, the Foundation for the Visually Impaired in Former Malmöhus län, the Crown Princess Margareta Foundation for the Visually Handicapped, the Swedish Medical Society, the Swedish Eye Foundation, Skåne University Hospital Foundation, the Cronqvist Foundation.

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Paper I

Acta Ophthalmologica

Stable refraction and visual acuity in diabetic patients with variable glucose levels under routine care

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ABSTRACT.

Purpose: **To investigate how refraction and visual acuity may vary in patients with diabetes under routine care.**

Methods: **Fifty-three eyes of 53 patients with various degrees of diabetic retinopathy were examined prospectively on four different occasions within a month. Refraction, best-corrected visual acuity (expressed as logMAR score) and blood glucose were measured on each occasion. Intraindividual variability was calculated as the range between the highest and lowest measurements. Associations between blood glucose levels and each of the other variables were tested by linear regression analysis for each patient.**

Results: **Refraction was completely stable in 43 patients and changed only slightly in 10, in whom the mean intraindividual variability of the spherical equivalent was 0.4 dioptres. Visual acuity test results were also highly reproducible. Mean intraindividual variability in visual acuity was 0.08 logMAR. Mean haemoglobin A1c (HbA1c) was 7.3 ± 1.5% but individual blood glucose levels ranged from 2.8 to > 22.2 mmol/l. Intraindividual variability ranged from 0.5 to 18.1 mmol/l, with a median of 6.0 mmol/l for the entire group. There were no associations between refraction or visual acuity and blood glucose levels or inter- or intraindividual glucose variations.**

Conclusion: **Refraction and visual acuity test results were highly reproducible and stable in patients with reasonably well controlled diabetes but variable blood glucose levels under routine care.**

Key words: blood glucose – diabetes – refraction – visual acuity

Acta Ophthalmol. 2011: 89: 107–110

doi: 10.1111/j.1755-3768.2009.01664.x

Introduction

Reliable tests on visual function are important when evaluating the progression of diabetic retinopathy and the benefits of treatment. Previously, we have suggested visual fields to be a useful tool for monitoring visual function in patients with diabetes (Bengtsson et al. 2005; Agardh et al. 2006) and characterized limits for significant change at various degrees of diabetic retinopathy (Bengtsson et al. 2008). However, those perimetric methods need further evaluation in longitudinal studies before they can be used in clinical practice.

Presently, visual acuity (VA) is the most common and widely used test on visual function in patients with diabetic retinopathy under routine care. VA is highly dependent on refraction, but the refractive error of the eye is a multifactorial condition involving not only the length of the eye but also the cornea and the lens (Olsen et al. 2007). In diabetic patients, VA variations have been ascribed to changes within the lens and subsequent altered refraction associated with changes in blood or serum glucose levels. Various refractive changes have been documented in patients with poor glycaemic control and during intensified glycaemic treatment, but the results are conflicting and the type of refractive errors reported are inconsistent (Planten 1975; Gwinup & Villarreal 1976; Eva et al. 1982; Fledelius 1987; Saito et al. 1993; Okamoto et al. 2000; Giustu 2003; Sonmez et al. 2005; Tai et al. 2006). However, little is known about the extent to which refraction changes also occur in patients with diabetes under routine care in out-patient clinics. Therefore, the aim of the present study was to examine if and how refraction and VA may vary in patients with diabetes under routine care with different degrees of diabetic retinopathy attending an out-patient eye clinic.

Materials and Methods

Patients and clinical setting

Fifty-three patients with diabetes mellitus and various degrees of retinopathy attending the Department of Ophthalmology, Malmö University Hospital, were recruited from October 2004 to December 2005. Patients older than 70 years or with previous laser treatment for diabetic retinopathy, or any other eye disease likely to affect the visual function, were excluded.

One eye per patient was selected according to the worst degree of retinopathy or randomly if there was no difference between the eyes. Stereographic colour slide (Kodachrome 64) fundus photographs of seven 35° fields were taken after dilation of the pupil (fundus camera TRC 50 IX; Topcon, Tokyo, Japan). Retinopathy grading was performed according to the Early Treatment Diabetic Retinopathy Study (ETDRS) severity scale (1991). Recognition of macular oedema was confirmed by optical coherence tomography (Stratus OCT; Carl Zeiss Meditec, Dublin, California, USA) using the fast macular mapping protocol, scan length 6 mm. Retinal thickness was assessed as the mean thickness of the most central area (1 mm) (Hee et al. 1998).

Patients were scheduled to come for five visits. The first visit included photography and collection of medical history; the four subsequent visits measured refraction, VA and blood glucose measurements, and were completed within 1 month presuming that no progression of diabetic retinopathy affecting foveal function would occur during that time period. The examination procedure was the same on all occasions and refraction and VA testing were followed immediately by measuring the blood glucose level.

The Research Ethical Committee of Lund University approved the test protocol and the tenets of the Declaration of Helsinki were followed. Informed consent was obtained from all participating patients.

Refraction and VA

Refraction was based on a once-repeatable value, i.e. two consecutive measurements with the same results in sphere and cylinder, using Humphrey Instrument Automatic Refractor 595 (Carl Zeiss Meditec Inc.). Spherical equivalent was calculated as cylinder value divided by two plus spherical value, expressed as dioptres (D). Best-corrected VA was obtained by manual adjustment in sphere and cylinder to 0.25 D accuracy. At subsequent visits, manual adjustment was performed only if the sphere or cylinder differed by more than 0.25 D from the first value. VA was tested according to the procedures recommended by Ferris & Bailey (1996) using the ETDRS charts at 4 m and expressed as the logarithm of the minimal angle of resolution (logMAR).

Glucose levels and haemoglobin A1c

Glucose was analysed in capillary blood using Hemocue Glucose 201 + (Hemocue AB, Ängelholm, Sweden) and expressed as mmol/l. Glycated haemoglobin A1c (HbA1c) was analysed using high-performance liquid chromatography (HPLC) (Varian II Haemoglobin A1c program; BioRad, Hercules, California, USA), normal range 3.6–5.0% or 4.0–5.3% below and above 50 years of age, respectively.

Analyses

Glucose levels from the four measurements were analysed for all patients together as well as for subgroups according to type of diabetes [type 1 (n = 19), type 2 on insulin treatment (n = 18) and type 2 without insulin (n = 16)] and retinopathy level according to the ETDRS severity scale [stages 10–15 no retinopathy (n = 10), stages 20–35 mild retinopathy (n = 18), stages 43–53 moderate to severe non-proliferative retinopathy (n = 16) and stages 61–75 proliferative retinopathy (n = 9)]. Glucose variability was calculated as the range between the highest and lowest measurement for each individual. Means of intraindividual blood glucose variability were compared between subgroups using analysis of variance (ANOVA).

Test results are presented as mean or median depending on the type of distribution. The variability of refraction and VA are given as the individual range between the maximum and minimum measurements and as standard deviations. Associations between blood glucose levels on the one hand and refraction and VA on the other were tested by linear regression analyses calculating slopes for each individual. Distributions of regression slopes for each parameter were plotted and tested for a hypothesized mean of zero using one-sample t-tests. Macular oedema involving the foveola was present in six patients only, a number too small to allow separate analyses.

Results

Patient characteristics and distribution of retinopathy levels are shown in Table 1. Refraction and VA varied considerably between patients (Table 2), but intrapatient variability was very small (Fig. 1).

Refraction was completely stable in 43 of the 53 patients. In the entire group, mean intraindividual variability based on range was 0.08 D, with a maximum of 0.87 D. In nine patients with less stable refraction, the mean variability was 0.40 D. When using standard deviation instead of range as a measurement for intraindividual variability, the mean was 0.04 D for the entire group and 0.20 D for the nine patients with less stable refraction. The variation in refraction was associ-

Table 1. Patient characteristics at baseline.

Type 1*/Type 2 (n)	19/34
Male/female (n)	31/22
Age (years, mean ± SD)	54 ± 12
Age at onset (years, mean ± SD)	36 ± 16
Diabetes treatment: insulin/no insulin (n)	37/16
Known duration (years)	18 ± 11
HbA1c (%)	7.3 ± 1.5
Blood glucose (mmol/l)	10.2 ± 4.4
Retinopathy level† (n): 10–15/20–35/43–53/61–75	10/18/16/9

SD, standard deviation; HbA1c, haemoglobin A1c.

* Type 1 diabetes: insulin treatment within 1 year of diagnosis.

† Retinopathy levels according to the Early Treatment Diabetic Retinopathy Study severity scale: 10–15, no retinopathy; 20–35, mild non-proliferative retinopathy; 43–53, moderate to severe non-proliferative retinopathy; 61–75, proliferative retinopathy.

Table 2. Refraction and visual acuity in all patients.

Refraction (spherical equivalent in dioptres): mean (minimum, maximum)	−0.10 (−4.13, +4.75)
Visual acuity (logMAR): mean (minimum, maximum)	−0.03 (0.50, −0.22)

logMAR, logarithm of the minimal angle of resolution.

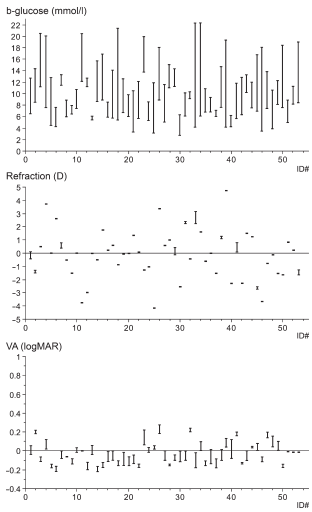

Fig. 1. The intraindividual variability of blood glucose levels, refraction and visual acuity. Despite substantial blood glucose variations, refraction and visual acuity remained stable. b-glucose, blood glucose; D, spherical equivalent; VA, visual acuity; ID#, patient number.

ated with neither higher blood glucose levels nor retinopathy stage.

Intraindividual variability in VA was also small. The mean was 0.08 logMAR, which is equivalent to four letters, with no variation between the retinopathy groups. The mean variability based on standard deviations was 0.05 logMar. The variation in VA was associated with neither higher blood glucose levels nor retinopathy stage.

The median blood glucose level for the entire group was 9.2 mmol/l, ranging from 2.8 to > 22.2 mmol/l (22.2 mmol/l being the highest measurable value). The intraindividual variability is shown in Fig. 1. Two patients had values higher than 22.2 mmol/l on four occasions; these values were set to > 22.2 mmol/l. The median intraindividual variability of blood glucose was 6.3 mmol/l, ranging from 0.5 (minimum) to 18.1 (maximum) mmol/l. For the entire group, mean standard deviation was 2.8 mmol/l.

There were no significant differences in intraindividual blood glucose variability among the diabetic subgroups ($p = 0.11$). In patients with type 1 diabetes, the mean variability was 7.7 mmol/l, in type 2 on insulin 6.5 mmol/l and in patients with type 2 diabetes without insulin treatment the mean variability was 4.4 mmol/l. Corresponding mean standard deviations were 3.6, 2.9 and 1.9 mmol/l, respectively. The highest blood glucose variability was seen in eyes with ETDRS retinopathy stages 43–53 (mean of 8.3 mmol/l), followed by ETDRS stages 61–75 (mean of 6.5) and stages 20–35 and 10–15 (average fluctuations of 5.7 and 4.7 mmol/l, respectively). The differences were of borderline significance ($p = 0.063$).

Blood glucose levels were associated with neither refraction nor VA. The individual regression slope coefficients for refraction were centred around zero and the mean slope was −0.001 D/mmol/l (Fig. 2). The mean variability in patients with type 1 diabetes was a 0.07 D; in patients with type 2 diabetes with and without insulin treatment values were 0.12 D and 0.03 D, respectively. The 10 patients with less stable refraction did not differ from the others regarding either blood glucose levels or variations. Individual regression slopes for VA were also centred around zero with a mean slope of −0.0007 logMAR/mmol/l (Fig. 3). There were no associations between blood glucose variations and any of the visual parameters.

Discussion

The present study describes diabetic patients under routine care who were recruited on the basis of various degrees of retinopathy ranging from no retinopathy to proliferative retinopathy. Our patient cohort was a mixture of patients with type 1 (36%) and type 2 (64%) diabetes, of whom 53% were on insulin treatment. Mean HbA1c was relatively good, suggesting that the patients were metabolically controlled reasonably well.

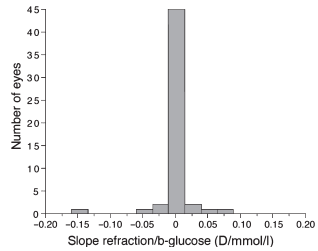

Fig. 2. The distribution of individual regression slopes of change in spherical equivalent (dioptres, D) per mmol/l change in blood glucose. A negative slope indicates a myopic change with increasing blood glucose level and a positive slope indicates a hyperopic change. Most slopes are flat, i.e. close to zero and the number of negative slopes is similar to the number of positive slopes, indicating no association between blood glucose levels and refraction. b-glucose, blood glucose.

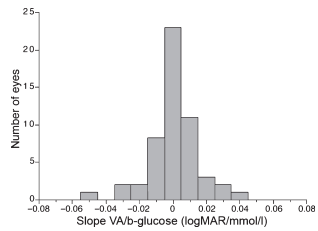

Fig. 3. The distribution of individual regression slopes of change in visual acuity (VA) (logarithm of the minimal angle of resolution, logMAR) per mmol/l change in blood glucose. A negative slope indicates improved VA with increasing blood glucose level and a positive slope indicates deterioration. The slopes are centred around zero, indicating no association between blood glucose levels and VA. b-glucose, blood glucose.

Despite substantial blood glucose variations, refraction hardly changed during the 1-month period. There was no association between blood glucose levels and refraction: the mean slope was −0.001 D/mmol/l. VA was tested by the same person (K.-J.H.) meticulously and according to the principles introduced by Bailey & Lovie (1976). This letter-by-letter method has lower test–retest variability than the line-assignment method in both healthy individuals (Arditi & Cagenello 1993; Vanden Bosch & Wall 1997) and those with macular disease (Vanden Bosch & Wall 1997). We could demonstrate low test–retest variability similar to that reported by Vanden Bosch & Wall

(1997), i.e. a standard deviation of 0.05 logMAR (95% CI 0.00–0.08). Others have demonstrated slightly higher variations of between ± 0.1 and ± 0.15 logMAR units (Arditi & Cagenello 1993; Siderov & Tiu 1999; Rosser et al. 2003). Our low test–retest variability shows that with the use of logMAR charts, reliable measurements of VA can be obtained in patients with diabetes mellitus, those without any retinopathy and those with mild, moderate or even proliferative retinopathy.

In this case series, we found no evidence for associations between blood glucose levels or blood glucose variations and refraction or VA, which is in accordance with a recent study by Wiemer et al. (2009). Using a blood glucose meter can result in some inaccuracy of both high and low readings; therefore, blood glucose levels and blood glucose variations could have been more pronounced than reported but not underestimated. Because metabolic control was relatively good in our patients, elevations in blood glucose were probably transient. Measurements recorded before dinner have been shown to contribute to glucose variability the most (Moberg et al. 1993). In our study, testing and blood glucose measurements took place within various time spans after a meal, and some of the recordings might have reflected a short transient hypo- or hyperglycaemic condition. However, our study results should be applicable in clinical practice because the study design corresponds to conditions in routine ophthalmological care, in which diabetic patients are seen at any time during the day.

In the present study on variations in refraction and VA in patients with reasonably well controlled diabetes under routine care, analyses of a cause relationship between blood glucose levels and/or variations and the vision test parameters was not possible because the study design included neither controlled intervention of blood glucose regulation and assessment of consequent changes nor intensified anti-hyperglycaemic treatment during the examination period. Therefore, our results are not applicable to patients with diabetes subjected to intensified treatment. A cause relation between hypoglycaemia and refraction has been reported previously. By increasing anti-hyperglycaemic treatment, Gwinup & Villareal (1976) studied how a reduction in serum glucose concentration of at least 3 mmol/l during 1–4 weeks influenced refraction, i.e. approximately the same time frame as in our study. In their study, refraction changed by 0.09 D/mmol/l. The improved blood glucose regulation resulted in less myopia or increased hyperopia.

In conclusion, our results suggest that refraction changes are minimal and VA assessments highly reproducible, provided a letter-by-letter method is used for testing, within a wide range of blood glucose levels in diabetic patients under routine care with different degrees of retinopathy. The use of ETDRS charts (Ferris & Bailey 1996) may well be recommended, not only in studies but also in clinical practice.

Acknowledgements

This study was supported by grants from Lund University, Skåne County Council, the Malmö University Hospital Foundation, the Järnhardt Foundation, the Centre for Clinical Research at the County Hospital of Värmland and the Foundation of Visually Impaired in Former Malmöhus län, Sweden. Hemocue AB, Sweden, is acknowledged for lending us the glucose meter.

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Received on September 19th, 2008.
Accepted on April 13th, 2009.

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Paper II

Test–retest variability for standard automated perimetry and short-wavelength automated perimetry in diabetic patients

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ABSTRACT.

Purpose: **To assess limits for significant improvement or deterioration of visual fields in diabetic patients based on short-term test–retest variability in subjects with different degrees of retinopathy.**

Methods: **Fifty patients with diabetic retinopathy ranging from level 10 to 75 [according to the Early Treatment Diabetic Retinopathy Study (ETDRS) severity scale] were tested repeatedly with both standard automated perimetry (SAP) and short-wavelength automated perimetry (SWAP) with short intervals. The association between visual field loss and degree of retinopathy outside fovea was analysed. Test–retest variability of global and local visual field indices and prediction limits for significant change were calculated.**

Results: **The amount of visual field loss was significantly associated to the degree of retinopathy, with a correlation coefficient of −0.51 for SAP ($P = 0.0003$) and −0.45 for SWAP ($P = 0.002$). Global test–retest variability was smaller with SAP than with SWAP ($P < 0.0001$). For both SAP and SWAP, local test–retest variability was considerably smaller at test points with normal sensitivity than at test points with reduced sensitivity ($P < 0.0001$). Paracentral test points within 10° of eccentricity had less variability than peripheral points ($P < 0.0001$), implying that smaller change is required to reach statistically significant improvement or deterioration at initially normal and paracentral points than at depressed points and peripherally located test points.**

Conclusion: **Our results propose that SAP, as well as SWAP, can be useful for monitoring visual function outside fovea in diabetic patients with various degrees of retinopathy. We report a preference for SAP because of less variability generally. Limits for significant improvement or deterioration have been assessed but need future validation in a longitudinal study.**

Key words: diabetes – function – perimetry – progression – regression – retinopathy – SWAP – visual field

Acta Ophthalmol. 2008: 86: 170–176

doi: 10.1111/j.1600-0420.2007.01019.x

Introduction

Diabetic retinopathy is still the most common cause of severe visual impairment and blindness in the Western world among people below 65 years of age (Taylor & Keeffe 2001). The development of drugs regulating hyperglycaemic and related metabolic disturbances is a pressing issue. Effects of drug therapy are typically monitored by photographic documentation of morphological diabetic vascular changes of the fundus. In addition to documentation of morphology, reliable functional tests would provide important information, particularly at early stages of diabetic retinopathy when morphological signs are scarce and visual acuity is preserved.

Visual acuity (VA) is commonly used for measuring visual function, but assesses foveal function only. Quantification of visual function outside fovea would add important information to retinal morphology in patients with diabetes. Function in paracentral and peripheral parts of the visual field is examined by perimetry, widely used in ophthalmologic practice for other eye diseases, like glaucoma. Association between visual field defects – using standard automated perimetry (SAP) – and diabetic retinopathy has been studied (Federman & Lloyd 1984; Trick et al. 1990; Henricsson & Heijl 1994; Verrotti et al. 2001, Pahor 2003); however, so

far the results are not conclusive regarding the usefulness of perimetry at early stages of retinopathy. A few studies have reported reduced retinal sensitivity with short-wavelength automated perimetry (SWAP) and normal sensitivity with SAP in diabetic patients with no retinopathy (Lobefalo et al. 1998; Afrashi et al. 2003). Non-conventional perimetry as flicker (Stavrou & Wood 2005) and SWAP (Hudson et al. 1998) has also been suggested to be more sensitive than SAP to changes close to fovea, not only in macula oedema but also in damage to the perifoveal capillary network (Remky et al. 2000; Agardh et al. 2006). Thus, several studies have described SWAP and SAP loss in diabetic patients, but there is not yet sufficient data to ensure valid interpretation of visual field defects in correlation to changes in diabetic retinopathy.

We recently described the correlation between peripheral retinopathy, excluding the fovea, and perimetry using both SAP and SWAP (Bengtsson et al. 2005). We showed that perimetric threshold sensitivities decreased with increasing severity of retinopathy as documented by stereo fundus photographs, graded according to the Early Treatment Diabetic Retinopathy Study (ETDRS) severity scale (Early Treatment Diabetic Retinopathy Study Research Group 1991). This correlation was significant for both SAP and SWAP, suggesting that perimetry can be useful for monitoring visual function in patients with diabetes. However, visual fields may well vary from time to time, also in healthy eyes (Lewis et al. 1986; Heijl et al. 1987a). Knowledge about such variability is important when monitoring visual field status over extended periods of time to determine whether it is stable or significant change has occurred. Less perimetric variability would increase the ability to detect smaller steps of change compared to larger variability. Identification and correction of factors that may influence the perimetric test results can reduce variability. To that end, the aim of the current study was to investigate the short-term individual test–retest variability of both SAP and SWAP in patients with different degrees of retinopathy and to define empirical limits for the detection of 'true' change of visual field loss in diabetic subjects.

Materials and Methods

Subjects

Patients with diabetes mellitus and different degrees of retinopathy examined at the Department of Ophthalmology, Malmö University Hospital were invited from October 2004 to December 2005. We aimed to collect data from patients representing retinopathy stages 10–75 according to the ETDRS severity scale (Early Treatment Diabetic Retinopathy Study Research Group 1991). Patients older than 70 years, or with previous laser treatment for diabetic retinopathy, or with any other eye disease likely to affect the visual field (except mild cataract), were considered ineligible. Informed consent was obtained from all participating patients. The Committee for Research Ethics at Lund University approved the test protocol and the tenets of the Declaration of Helsinki were followed.

Visits

Patients were scheduled to come for five visits within a month assuming that no true visual field changes would occur during that period. At the first visit, medical history – including treatment, onset and duration of diabetes, as well as medication for other diseases – was recorded, visual field was tested (training session) and retinal fundus photographs were taken. Each of the four following visits included visual fields, refraction, VA and blood glucose (b-glucose) measurements using Hemocue Glucose 201+ (Hemocue AB, Ängelholm, Sweden).

Degree of retinopathy

One eye per patient was selected according to the worst degree of retinopathy. If both eyes had the same ETDRS level, one eye was randomly selected. Stereographic fundus photographs (35°) of seven fields were taken at the first visit (TRC 50 IX; Topcon, Tokyo, Japan) using colour slide film (Kodachrome 64). The final grading of degree of retinopathy was performed according to the ETDRS severity scale based on these fundus photographs and masked for the visual field test results.

Refraction and visual acuity

Refraction was determined by Humphrey Automatic Refractor 595 (Carl Zeiss Meditec Inc., Dublin, California, USA) and confirmed or adjusted by manual refraction to obtain best VA with 0.25 D precision in sphere and cylinder. Automated refraction was repeated at each visit, and manual adjustment was performed only if the automatic refractor values differed more than 0.25 D from the previous one. VA was tested at each visit using the ETDRS charts and was expressed as logMAR (minimal angle of resolution) scores (Ferris et al. 1982).

Visual fields

Visual fields were assessed by the 24-2 SITA Standard SAP program (Bengtsson et al. 1997) and the SITA SWAP 24-2 program (Bengtsson 2003) of the Humphrey Field Analyzer model 750 (Carl Zeiss Meditec Inc.). The 24–2 test pattern covers the central 24° of the central visual field including 52 points, corresponding to a considerable part of the fundus area covered by the standard photographs. All patients were corrected for refractive error and near. Fifty per cent of the patients started with SAP followed by SWAP. The same test order was kept for each patient and visit. The subjects rested for 15 min between the visual field tests to minimize fatigue effects. Unreliable visual field tests, defined as a frequency of false-positive answers $> 15\%$, were excluded and only patients with a full series of reliable field tests were included in the analyses. Visual fields assessed at the first visit were not included in any analyses to avoid perimetric learning effects (Heijl et al. 1989a). Thus, all patients included in the analyses contributed with four tests each.

Analyses and statistics

Visual field parameters chosen for analyses were the total deviation value describing the deviation from the age-corrected normal threshold value at each test location (defect depth) in the visual field, and the global mean deviation (MD) value, a weighted mean of all total deviation values. The statistical significance of depressed sensitivities

as measured by total deviation is displayed in the probability maps (Fig. 1). Total deviation values, probability maps and MD values are parts of the Statpac interpretation tool (Heijl et al. 1987b) implemented in the Humphrey Field Analyzer, for both SAP and SWAP.

The association between degree of retinopathy according to the 11 steps of the ETDRS severity scale and the amount of visual field loss as determined by the MD value, as well as the number of significantly ($P < 5\%$) depressed test points, were examined by Spearman's rank correlation and linear regression analyses assuming equal steps in the ETDRS scale.

Visual field test–retest variability was calculated for each eye as the mean of all differences between MD values, as well as the mean of all pointwise intra-eye differences between the total deviation values. Test–retest variability for SAP and SWAP was compared using Wilcoxon signed rank test. B-glucose fluctuation was calculated as the mean of all possible intra-patient differences.

With the intention of isolating random test–retest variability effects from a possible influence of b-glucose fluctuation, global visual field status (MD), local defect depth (total deviation value) and test point eccentricity on visual field test–retest variability, we applied multivariate linear regression analyses.

In order to estimate limits for significant local change, all possible intra-individual differences in total deviation values were calculated for each test location, i.e. first test versus second test and first test versus third test, etc., for SAP and SWAP. The first test after the training session was regarded as a baseline examination to which subsequent tests were compared. All calculated differences were then pooled and divided into groups with 3 dB intervals according to the total deviation values of the baseline test. Test points were also divided into two zones according to eccentricity, one paracentral covering the central 10° and one peripheral including all points outside the 10° circle (Fig. 2). Distributions of pointwise individual differences for each 3 dB interval and eccentricity zone were created, empirical limits were calculated for each distribution at the 5th and 95th percentile, and smoothened. The 90% prediction intervals where then compared using paired sign tests.

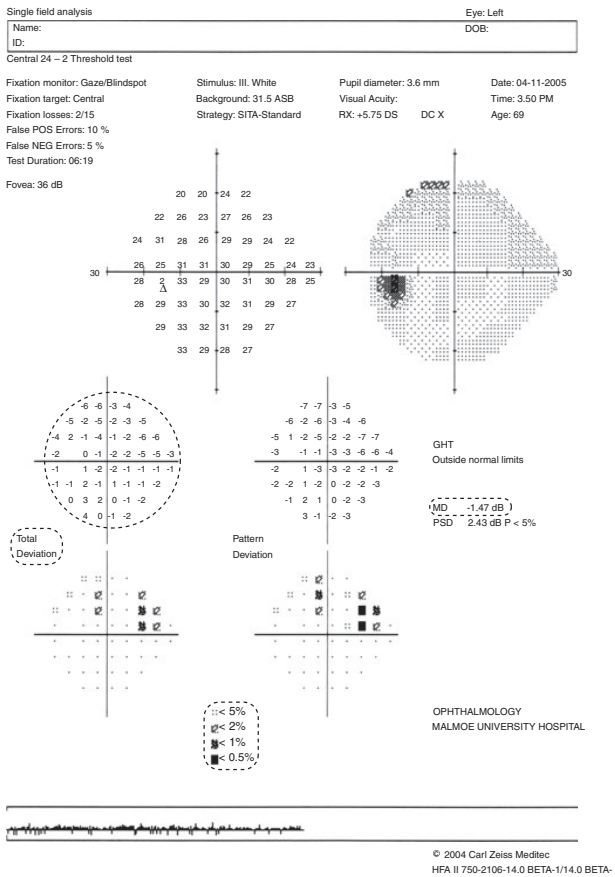

Fig. 1. Visual field of a patient with type 2 diabetes and retinopathy level 35 according to the ETDRS severity scale. Encircled are total deviation (TD) values expressing age-corrected threshold values and their probability symbols, and mean deviation (MD, a weighted mean of numerical TD values). A total deviation value of zero represents the estimated normal threshold value; negative values represent depressed threshold sensitivities. The significance of a depression is expressed in the probability maps located below the numerical deviation maps. This field shows some test point locations with significantly depressed threshold sensitivities in the upper hemifield.

Results

Fifty-five patients participated in this study. Fifty-three patients completed all tests. Out of those 53, 18 had been on insulin treatment within 1 year of diagnosis and were considered as having type 1 diabetes; the remaining 32 were diagnosed as having type 2 diabetes. Mean age was 54 years, ranging from 23 to 69 years, and mean diabetes duration was 18 years, ranging from 2 to 52 years. Three patients were excluded because of unreliable visual field tests, leaving 50 patients to be included in the analyses. Forty-nine of these had all visual fields tested within 1 month; only one exceeded the 1 month limit (by 5 days).

All stages of retinopathy between 10 and 75 were represented. The

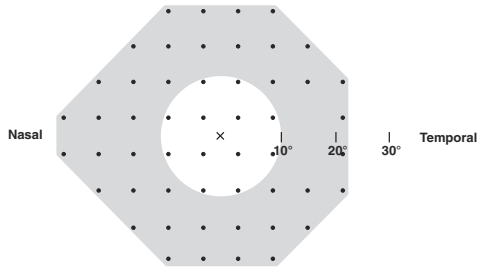

Fig. 2. The 24–2 test point pattern (right eye) of the Humphrey Field Analyzer. Eccentricity had a significant influence on test–retest variability. For that reason, the 52 test point locations were divided into two zones of eccentricity, one including paracentral points within 10° and another covering the points outside 10°.

Table 1. Distribution of patients across the Early Treatment Diabetic Retinopathy Study (ETDRS) retinopathy severity scale.

ETDRS severity scale	10–15	20–35	43–53	61–75
Number of patients	10	17	15	8

distribution across the ETDRS retinopathy severity scale is shown in Table 1.

Blood glucose, VA and visual fields

The median b-glucose level was 9.46 mmol/l. Three patients exceeded the upper limit of the b-glucose meter (22 mmol/l) at at least one visit; the lowest value analysed was 2.8 mmol/l. Median individual b-glucose fluctuation was 3.28 mmol/l, ranging from 0.25 to 9.88 mmol/l.

Median VA was −0.045 logMAR, ranging from 0.39 to −0.19 logMAR. These values correspond approximately to 1.0 (median), and from 0.4 to 1.6 (minimum and maximum), respectively, using the decimal scale.

The perimetric MD median value was −0.90 dB for SAP (ranging from −11.97 to 1.78 dB) and −2.46 dB for SWAP (ranging from −18.47 to 3.00 dB). A MD value of approximately −30 (SAP) to −25 dB (SWAP) represents a blind or almost blind eye, whereas a MD value around 0 dB represents an eye with a normal field. The median number of significantly depressed points was nine for SAP and eight for SWAP, ranging from 0 to 52 for both test modalities.

Correlation between visual field and ETDRS levels

Degree of retinopathy according to the 11 steps of the ETDRS severity scale correlated significantly with MD values (Fig. 3). The Spearman's correlation coefficient was −0.51 for SAP ($P = 0.0003$) and −0.45 for SWAP ($P = 0.002$). The average visual field threshold sensitivity decreased to 0.46 dB per ETDRS step using SAP ($P = 0.001$) and 0.72 dB per ETDRS step using SWAP ($P = 0.011$). The number of significantly depressed points also correlated significantly to the ETDRS steps. The Spearman's correlation coefficient was 0.54 for SAP ($P = 0.0001$) and 0.44 for SWAP ($P = 0.002$). For each ETDRS step the number of significantly depressed points increased to 2.4 on average for SAP ($P = 0.002$) and 2.3 for SWAP ($P = 0.02$).

Test–retest variability of MD

MD test–retest variability was smaller with SAP compared to SWAP ($P < 0.0001$). Median variability was 0.71 dB for SAP (ranging from 0.14 to 3.05 dB) and 1.34 dB for SWAP (ranging from 0.28 to 3.05 dB). In multivariate analyses, the field status affected the test–retest variability, i.e. more depressed fields had larger test–retest variability than fields closer to normality. The effects were small, but significant. The variability increased, with 0.06 dB per dB worsening of MD for both SAP ($P = 0.04$) and SWAP ($P = 0.003$). A normal SAP field with a MD value of 0 dB had an estimated test–retest variability of 0.59 dB, while a field with a MD of −20 dB had an estimated variability of 1.79 dB. Corresponding estimated variability for SWAP was 1.19 dB for a normal field and 2.39 dB for a field with MD at −20 dB. There was no significant association between MD test–retest variability and ETDRS level, neither for SAP ($P = 0.19$) nor for SWAP ($P = 0.81$).

B-glucose fluctuation did not affect the test–retest variability of MD, either for SAP ($P = 0.20$) or for SWAP ($P = 0.68$).

Test–retest variability of total deviation

The median local test–retest variability for all points was 2.07 dB with SAP and 2.67 with SWAP ($P = 0.83$). The test–retest variability was slightly smaller with SAP (1.50 dB) than with SWAP (2.17 dB) at test points with initially normal sensitivity or mild reduction of sensitivity (defined as total deviation better than or equal to −5 dB). On the other hand, SWAP test–retest variability was non-significantly smaller than SAP at points with total deviation values worse than −5 dB, 3.44 dB and 3.62 dB, respectively.

Eccentricity of test point location significantly affected the test–retest variability ($P < 0.0001$) for both SAP and SWAP. The variability increased with increasing eccentricity. A primary analysis performed at each of five rings of eccentricity revealed similar magnitude of variability within the central 10° of the visual field, and larger and similar magnitude of variability outside the 10°. Therefore, the visual field test point pattern was divided into two zones of eccentricity (Fig. 2). More and narrower zones of eccentricity did not further improve the regression model.

Local defect depth, as defined by the total deviation value in the baseline field, was the most important factor explaining test–retest variability (Table 2).

MD also had a significant effect on local test–retest variability, except at paracentral locations using SWAP (Table 2). Although significant, the MD coefficients were considerably smaller than those for local defect depth.

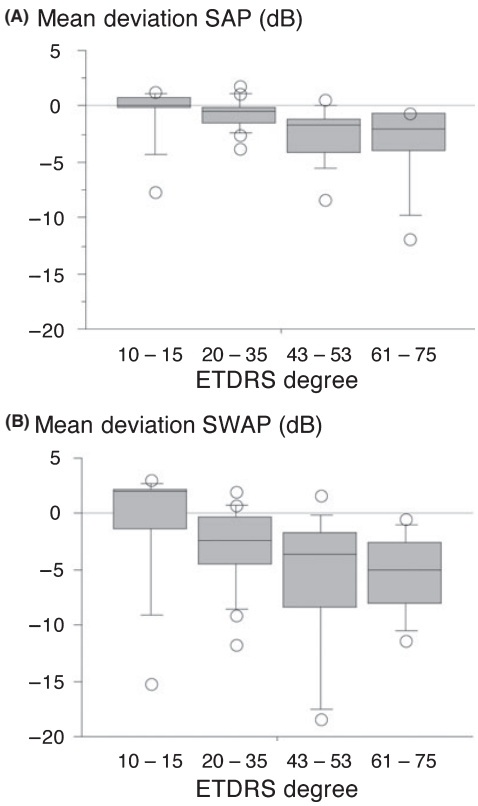

Fig. 3. Perimetric mean deviation (MD) in each of the four groups of patients with different degrees of retinopathy according to the ETDRS severity scale. The association between severity of retinopathy and MD is clear: for both SAP (A) and SWAP (B), MD worsens gradually along the course of increasing retinopathy. The inter-subject variability is considerably large with SWAP compared to SAP.

B-glucose fluctuations did not significantly affect local test–retest variability at paracentral test locations, but had a divergent effect at peripheral locations (Table 2). The effect was of borderline significance ($P = 0.06$) for SAP, with a positive coefficient suggesting that an increase in b-glucose fluctuation would increase the local test–retest variability. For SWAP, the effect was in the opposite direction: a decrease in b-glucose fluctuation would increase test–retest variability. However, the coefficients were very small (0.03 dB per mmol/l for SAP and −0.05 dB per mmol/l for SWAP).

Prediction limits for change

Generally, prediction intervals were considerably narrower at test points located in normal areas or in areas with shallow depression than at points located in areas with severe depression, and prediction limits were slightly narrower at paracentral locations compared to peripheral locations (Fig. 4). Thus, less change is required to reach significant deterioration or improvement at test points with normal or near-normal sensitivity than at test points with reduced sensitivity. Prediction intervals were somewhat, but not significantly, narrower with SAP than with SWAP at points with no or mild depression.

Discussion

Our results suggest that perimetry can be useful for monitoring visual function outside fovea in patients with diabetes. Conventional SAP, which is currently used at most eye clinics, performed just as well as the less commonly used SWAP. The association between visual field loss and degree of retinopathy confirms our earlier results (Bengtsson et al. 2005). Previous studies have reported short-wavelength sensitivity to be affected earlier than achromatic sensitivity early in the course of diabetic retinopathy (Greenstein et al. 1989; Lobefalo et al. 1998; Nomura et al. 2000; Afrashi et al. 2003). Although our study was not designed to detect temporal differences between SAP and SWAP in individual patients, we found that SAP identified at least as much local significant loss as SWAP.

Knowledge about random test–retest variability is essential when separating 'true' change from noise. Significant change has to be bigger than random variability and variability caused by unknown factors. Variability caused by known factors (e.g. age in the perimetric Statpac program) can be corrected. In diabetic subjects, b-glucose could be a possible factor of variability, but the significance of the very small effect at peripheral test point only might be explained by the large number of test points located in this area yielding significance of as weak trend of no practical importance.

We identified and quantified three factors affecting test–retest variability of threshold values in diabetic patients with different degrees of retinopathy: the eccentricity of the test point and the global and local defect status (as represented by the MD value and the total deviation value). By taking these factors into account we defined limits for significant change, analogous to that used for identifying glaucoma change (Heijl et al. 1989b). The smaller variability obtained at points with normal or close to normal threshold values compared to variability measured at points with reduced threshold sensitivities also suggests that small changes at early stages can be detected. The non-significantly smaller test–retest variability and narrower prediction

Table 2. Factors associated with local test–retest variability.

Factors	Coefficient	*P*-value	R^2
SAP – Paracentral zone			
Defect depth (dB)	−0.34	< 0.0001	0.33
MD (dB)	0.13	< 0.0001	
b-glucose fluctuation (mmol/l)	0.01	0.524	
SAP – Peripheral zone			
Defect depth (dB)	−0.40	< 0.0001	0.43
MD (dB)	0.18	< 0.0001	
b-glucose fluctuation (mmol/l)	0.03	0.0592	
SWAP – Paracentral zone			
Defect depth (dB)	−0.16	< 0.0001	0.26
MD (dB)	0.02	0.378	
b-glucose fluctuation (mmol/l)	−0.003	0.898	
SWAP – Peripheral zone			
Defect depth (dB)	−0.22	< 0.0001	0.19
MD (dB)	0.10	< 0.0001	
b-glucose fluctuation (mmol/l)	−0.05	0.012	

R^2, coefficient of determination.
SAP, standard automated perimetry; SWAP, short-wavelength automated perimetry; MD, global mean deviation value.

limits for SWAP compared to SAP at locations with more severely depressed sensitivity might be explained by the decreased dynamic range of the SWAP stimulus compared to the SAP stimulus.

We chose to model functional change by testing the visual fields of patients with different degrees of diabetic retinopathy repeatedly with short intervals assuming no true change would occur during the test period. An ideal model for functional change would be based on longitudinally collected data from diabetic patients with stable as well as progressing and regressing retinopathy. That kind of model would require a large number of patients to be followed for several years, and then to be evaluated in separate longitudinally

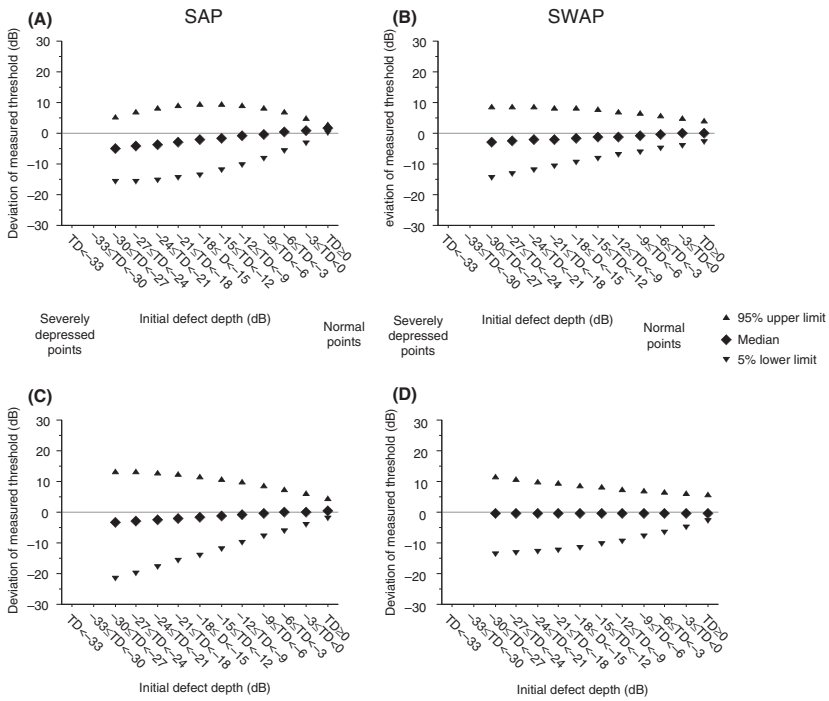

Fig. 4. Median and 5% and 95% limits of test–retest variability as a function of defect depth [total deviation (TD)]. A and B display limits at paracentral test points, and C and D at peripheral test points. A and C show SAP results, and B and D SWAP results. Changes must be outside these limits to be considered significant. Limits were generally slightly narrower at paracentral test points than at peripheral test points. Points with normal threshold sensitivity, i.e. TD close to zero, required considerably less change to reach significance than points with depressed sensitivity.

collected patient material. Instead, we applied a cross-sectional study design to assess limits for significant change, which await validation in an already ongoing longitudinal study.

In conclusion, our results suggest that change in diabetic retinopathy can be monitored using conventional SAP, as well as SWAP, thus adding useful information to the conventionally used photographic documentation, particularly at early stages. Prediction limits for 'true' visual field change caused by progression or regression of diabetic retinopathy were based on knowledge about random visual field variability and identification of factors affecting that variability in patients with different degrees of retinopathy.

Acknowledgements

This study was supported by the Järnhardt foundation, Malmö University Hospital Foundation, Foundation of Visually Impaired in former Malmöhus län, Sweden, and by the Crown Princess Maragreta Foundation for the Visually Handicapped (Stiftelsen Kronpinsessans Margaretas arbetsnämnd för synskadade).

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Received on March 7th, 2007.
Accepted on June 29th, 2007.

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Paper III

Acta Ophthalmologica

Acta Ophthalmologica 2013

Functional and structural change in diabetic eyes. Interim results from an ongoing longitudinal prospective study

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ABSTRACT.

Purpose: **To presents results after 18 months of follow-up of a longitudinal study aiming at exploring the correlation between diabetic retinal vascular lesions and functional change.**

Methods: **Patients were consecutively recruited from attendees to the screening program for diabetic retinopathy. Subjects are followed every sixth month for the first 3 years and thereafter annually up to 5 years. Progression of diabetic retinopathy is evaluated using the Early Treatment Diabetic Retinopathy Study (ETDRS) scale and improvement/deterioration in visual fields by predefined significance limits for change.**

Results: **Of 81 subjects, with no/mild/moderate diabetic retinopathy included, 76 have passed the 18-month visit. At that time, retinal progression by two steps according to the ETDRS scale had occurred in two subjects. Visual acuity was −0.14 logMAR and had decreased with two letters (0.04 logMAR) ($p < 0.001$) from baseline. The global visual field index mean deviation was almost unchanged with a negligible improvement of 0.03 dB ($p = 0.79$). In 21 subjects, repeated significant deterioration was seen in ≥10% of all points tested in the field, while almost no improved points were noted. The two subjects with retinal progression were not among those 21 with indication of perimetric progression.**

Conclusions: **This is, to our knowledge, the first longitudinal study evaluating change of visual fields in a representative diabetic cohort with no or mild/moderate retinopathy. In this interim report, we demonstrate deteriorated perimetric sensitivity in subjects already at 18 months of follow-up. The results will have implications for evaluating change in visual function in future clinical trials.**

Key words: diabetic retinopathy – longitudinal study design – perimetry – visual function

Acta Ophthalmol. 2013: 91: 672–677

doi: 10.1111/j.1755-3768.2012.02508.x

Introduction

The prevalence of diabetes is rapidly increasing worldwide (Wild et al. 2004) and so is diabetic retinopathy. This microvascular complication with subsequent visual impairment is indeed feared by people with diabetes (Luckie et al. 2007). Although more than 25 years have passed since treatment with laser was established for proliferative retinopathy (Diabetic Retinopathy Study research group 1981) and diabetic macular oedema (Early Treatment Diabetic Retinopathy Study research group 1985), diabetic retinopathy is still the most common cause of blindness among working age adults in the United States (Centers for Disease Control and Prevention 2011). Good metabolic and blood pressure control can postpone development of sight-threatening vascular lesions, but photocoagulation remains the treatment of choice once those lesions are established (Mohamed et al. 2007). Recently, new treatment approaches (PKC-DRS Study Group 2005; Chaturvedi et al. 2008; Sjolie et al. 2008; Mauer et al. 2009) have been explored, but those studies have not given consistent evidence for the benefit of medical intervention. One possible explanation could be that evaluation of medical treatment strategies hitherto has relied on progression of diabetic retinopathy as verified by fundus photography only and not by sensitive functional tests. To that end, National Institute of Health and Food and Drug Administration arranged a symposium, in which they recommended to include change in visual function as one primary outcome in clinical studies (Csaky et al. 2008).

Visual acuity (VA) is the most commonly used functional test but not particularly useful as a functional outcome measure in diabetes because it is not affected at early stages of diabetic retinopathy. It reflects the function of the fovea only, and even so, VA is usually not impaired until oedema involves the centre of the macula (Agardh et al. 2006).

Computerized threshold perimetry is a psychophysical test using multiple stimuli to measure differential light sensitivity reflecting the retinal function in a predefined pattern, typically covering the central 20–30° of the visual field. The most common type of perimetry is Standard Automated Perimetry (SAP) using a white stimulus on an evenly illuminated white background.

Several previous studies, all but one with a cross-sectional design, have reported results of computerized threshold perimetry in patients with diabetes. The results have been somewhat conflicting, describing reduced SAP sensitivity, either at no (Mastropasqua et al. 1995; Verrotti et al. 2001) or early stages of diabetic retinopathy (Trick et al. 1990) or not until at more advanced stages (Henricsson & Heijl 1994). A different type of perimetry, Short Wavelength Automated Perimetry (SWAP), using a blue stimulus on an intense yellow background has also been reported to be affected before any detectable diabetic retinopathy (Lobefalo et al. 1998; Afrashi et al. 2003), a finding that our group was unable to confirm (Bengtsson et al. 2005).

One advantage with longitudinal studies is that change from baseline, modelled by random test–retest variability of threshold values in diabetic patients, can be applied. This approach may be more sensitive to subtle changes than deviations from age-corrected normal threshold values. Knowledge about random test–retest variability is essential when estimating worsening or improvement of perimetric test results. Based on repeated measurements in patients with different degrees of retinopathy, we calculated limits for significant change for all test points included in the central 24° SAP visual field, except for the two located in the area for the blind spot (Bengtsson et al. 2008). This approach is similar to the one applied for detecting changes in the SAP visual field in glaucoma subjects (Heijl et al. 1989a). The limits for detecting change in subjects with diabetes were, similar those for glaucoma, wider at peripheral than at paracentral test locations and also wider at locations with depressed sensitivity than at locations with normal sensitivity. Wider limits require larger change to reach significance, which usually means less sensitivity to detect significant change. To that end, we created graphical maps, similar to the glaucoma change probability maps suggested by Heijl et al. 1989a; indicating test point locations with significant change compared to baseline fields for patients with diabetes (Bengtsson et al. 2008; Fig. 1). These graphical maps are compared to change in retinopathy levels according to the Early Treatment Diabetic Retinopathy (ETDRS) final severity scale (Early Treatment Diabetic Retinopathy Study research group 1991).

The aim of this report is to present results at 18 months of follow-up in a longitudinal ongoing study on morphological and functional change in diabetic retinopathy as assessed by conventional fundus photography, VA and SAP.

Methods

Objectives

The longitudinal study design enables temporal comparison between functional change and progression of diabetic retinopathy. The primary aim is to explore whether early functional change assessed by SAP may occur and progress in parallel to the development of vascular diabetic lesions verified by fundus photography by monitoring a cohort of subjects up to 5 years from baseline. The study was approved by the Regional Ethical Review Board at Lund University, Sweden, and all patients gave written informed consent.

Eligible were subjects with diabetes between 18 and 75 years of age attending the screening program for diabetic retinopathy at the Department of Ophthalmology, County Hospital of Värmland. The screening is open for all subjects with diabetes older than 10 years of age and includes fundus photography. Patients were consecutively recruited from September 2006 to May 2009.

Exclusion criteria were the following:

(1) nondiabetic eye disease or medical disease likely to affect the visual field
(2) nongradable photographs
(3) previous laser treatment or any other previous local treatment for diabetic retinopathy
(4) need for laser treatment or any other local treatment for diabetic retinopathy

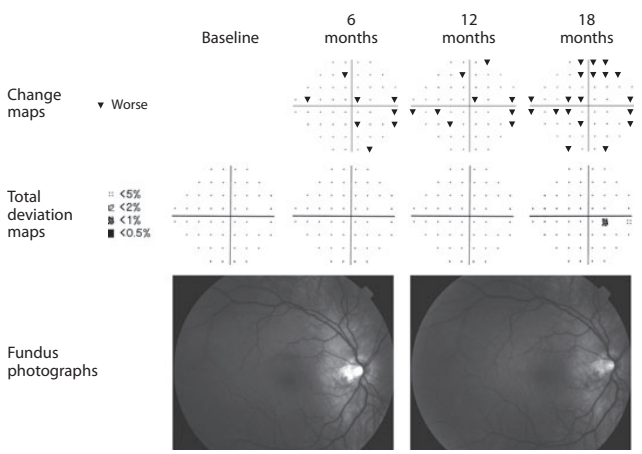

Fig. 1. Visual fields and fundus photographs in one subject with diabetes. Several reproducible deteriorated test points were present (top). Total deviation maps were normal (middle), and the level of diabetic retinopathy was unchanged (bottom).

(5) inability to perform reliable visual field, that is, false positive answers >15%.

A prebaseline visit including visual field testing was performed to avoid perimetric learning effects (Heijl et al. 1989b). A battery of examinations including fundus photography, VA and SAP was performed at baseline. Blood pressure was taken as the mean of two tests in a sitting position using an aneroid sphygmomanometer. HbA1c was analysed with high-performance liquid chromatography (Variant II Haemoglobin A1c program, Bio-Rad, Hercules, CA, USA), normal range 3.6–5.0% and 3.9–5.3% below and above 50 years of age, respectively. Information about age at onset, duration and treatment of diabetes was obtained as well as information about systemic vascular or other disease and treatment for hypertension or other medical treatment.

The participants are scheduled for examinations every sixth month until all participants have completed the minimum of 3 years of follow-up and thereafter annually until a maximum of 5 years of follow-up.

Morphological measures

After dilatation of the pupil, stereo fundus colour photographs of seven 35° fields are taken using a retinal camera (TRC 50 IX; Topcon, Tokyo, Japan) with slide film (Fujichrome Sensia 100; Fujifilm, Tokyo, Japan). Diabetic retinopathy is graded in a masked procedure, according to the ETDRS final severity scale (Early Treatment Diabetic Retinopathy Study research group 1991). We define progression of diabetic retinopathy as a two-step change according to the ETDRS final severity scale in one study eye per patient.

Functional measures

Visual acuity is tested using ETDRS charts according to the recommended procedures for clinical research (Ferris & Bailey 1996). Best corrected VA is measured after refraction with manual adjustment from auto refractometer values (KR-8100P, Topcon, Tokyo, Japan) in sphere and cylinder to 0.25 dioptres (D) accuracy. After the first measurement, manual adjustment is performed only when the refractometer value has changed >0.25 D in sphere or cylinder.

Visual fields are tested with the Humphrey Field Analyzer model 750 (Carl Zeiss Meditec Inc., Dublin, CA, USA) using the SITA Standard 24-2 including 54 test point locations, two located in the area for the blind spot, within the central 24° visual field. The perimetric measures are the commercially available Statpac indices (Heijl et al. 1986): the global visual field mean deviation (MD), which shows how much the field departs from normal where 0 dB represents a normal value, and number of test points with age-corrected sensitivity depressed below normal limits at the $p < 0.05$ level. Based on our new limits for significant change of age-corrected threshold sensitivities (Bengtsson et al. 2008), we counted the number of significantly deteriorated or improved test points at the $p < 0.05$ level.

Analysis

One eye per subject was analysed, and the study eye was chosen by flipping coin. Statistical analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA) software. Follow-up data concerning patient characteristics were compared to baseline using paired *t*-test and McNemar's test depending on type of data. Eighteen-month follow-up differences in VA, perimetric MD and number of test points depressed below normal limits were analysed using one-sample *t*-test. Number of significantly deteriorated test points in SAP was counted, and subjects having ≥5 significantly deteriorated test points at repeated examinations at the 12- and 18-month visits were considered likely to have changed from baseline. Assuming a Poisson distribution, the risk for having at least five test points falsely flagged as deteriorated or improved at the $p < 0.05$ level would be just slightly more than 10% in a single test, and by requiring at least the same number in the consecutive test, the risk for false change diminishes considerably.

Results

At the end of the recruitment period, 93 subjects had received study information and performed perimetry for training purposes. One was found to have proliferative retinopathy, eight subjects declined to continue, and three were unable to perform reliable perimetry. Thus, a total of 81 patients were included in the study. Subjects with age at onset of diabetes <30 years and insulin treatment within 1 year of diagnosis were considered as having type 1 diabetes. Of the 81 recruited subjects, 13 had type 1 and 68 type 2 diabetes. During follow-up, one subject had died, two were severely ill, and two did not continue in the study, leaving 76 patients available for analyses using data collected up to the 18-month visit.

HbA1c levels and systolic blood pressure were similar to baseline, whereas the diastolic blood pressure had improved. Patient characteristics are presented in Table 1.

Table 1. Patient characteristics at baseline and at 18 months of follow-up.

Characteristics	Baseline	18-month follow-up	p
Female	30 (37%)	29 (38%)	
Male, *n* (%)	51 (63%)	47 (62%)	
Age at onset (years)	44 ± 15		
Diabetes duration (years)	13 ± 12	15 ± 12	
Glycated haemoglobin, HbA1c (%)	6.8 ± 1.2	6.8 ± 1.2	0.92
Insulin treatment only, *n* (%)	33 (41%)	34 (45%)	1.0
Insulin and oral hypoglycaemic agent, *n* (%)	15 (18%)	13 (17%)	1.0
Oral hypoglycaemic agent only, *n* (%)	24 (30%)	22 (29%)	1.0
No pharmacological treatment, *n* (%)	9 (11%)	7 (9%)	0.5
Antihypertensive medication, *n* (%)	42 (52%)	44 (58%)	0.12
Systolic blood pressure (mmHg)	133 ± 16	132 ± 16	0.70
Diastolic blood pressure (mmHg)	78 ± 11	76 ± 11	0.01

Values are expresses as mean ± SD. Differences in glycated haemoglobin and blood pressure analysed using paired samples *t*-test, glycaemic and antihypertensive medication using McNemar's test.

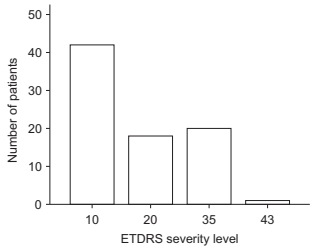

Fig. 2. Retinopathy degree according to the Early Treatment Diabetic Retinopathy Study (ETDRS) severity scale. Forty-two subjects were without and 39 subjects had mild/moderate diabetic retinopathy.

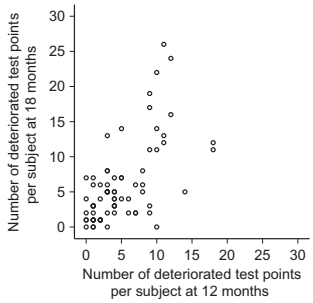

Fig. 3. Deteriorated test points at 18 months compared to 12 months of follow-up. The correlation between deteriorated test points at the 12-month and the 18-month visits was good, $r = 0.62$ with slightly more deteriorated points at the 18-month visit, average difference was 1.1 ($p = 0.052$). Seventy-six eyes completed 18-month follow-up, and 52 circles are visible in the scatter plot because of overlapping.

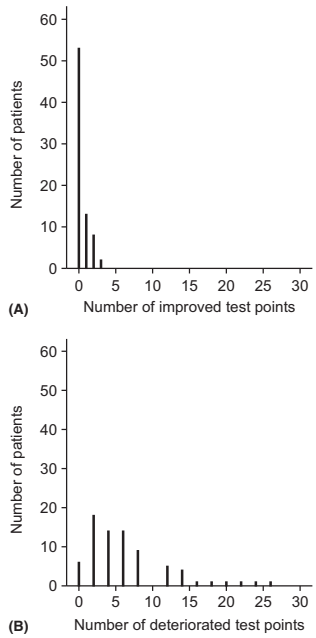

Fig. 4. Improved (A) and deteriorated (B) perimetric test points at 18 months of follow-up. Thirty-eight of 76 eyes completing 18-month follow-up had five or more deteriorated test points, but only 23 eyes had improved test points and none had more than three. Differential light thresholds measured in the 52 test points of the visual field of all 76 eyes at 18-month follow-up in comparison with baseline, in total 3962 test points, were analysed, and 450 of these were found deteriorated, whereas only 35 improved. Thus, deteriorated test points were found almost 13 times more often than improved ones.

Morphological parameters

At baseline, 42 eyes had no retinopathy (ETDRS level 10) and the remaining 39 mild or moderately severe changes (Fig. 2). At 18 months of follow-up, the degree of retinopathy was unchanged in 61 eyes progression by one step had occurred in seven eyes, but the predefined progression by two steps had occurred in two eyes only. Regression by one step occurred in six eyes.

Functional parameters

The median VA was −0.14 logMAR at baseline, ranging from −0.28 to 0.10, and after 18 months, the difference in VA had decreased by 0.04 logMAR or by two letters ($p < 0.001$). Although statistically significant, this decline in VA does not describe any meaningful change.

At baseline, perimetric MD was median −0.54 dB, ranging from −5.69 to 2.33. The median number of test points depressed below normal limits was three, ranging from 0 to 43. At the 18-month visit, MD had improved slightly with 0.03 dB ($p = 0.79$), and the number of depressed test points increased slightly by 0.3 ($p = 0.74$).

Thirty eyes had at least five significantly deteriorated test points at the 12-month visit, and 21 eyes showed repeated significant deterioration, both at the 12- and at the 18-month visits (Fig. 3). At 18 months, significant deterioration in ≥5 test points was detected in 50% (38/76) of the eyes, while almost no improved test points were seen, median 0 and never more than three for any eye (Fig. 4).

The correlation between change in retinopathy and function was poor. VA improved with three letters in one of the two eyes showing progression by two steps according to the ETDRS scale and decreased by one letter in the other eye. The visual field in the same two eyes had only four and three significantly deteriorated test points, respectively, after 18 months of follow-up.

Discussion

This report presents preliminary results of a prospective longitudinal study, in which we compare functional loss, measured as perimetric change over time, to progression of vascular lesions, measured as change in the ETDRS retinopathy level. To our knowledge, this is the first prospective longitudinal study to explore functional change over time using perimetry and compare it with progression of morphological vascular lesions according to the ETDRS scale, today's gold standard monitoring diabetic retinopathy.

The cohort can be regarded as representative for the Swedish diabetic population. The subjects were consecutively recruited; the majority of cases have fairly mild diabetes as indicated by good metabolic and blood pressure control, and 16% have type 1 diabetes. At 18 months, the predefined two-step progression had occurred in two eyes only and thus longer follow-up is needed when comparing visual field measures to morphological changes. The low migration rate in the population of the county makes the county hospital of Värmland particularly suitable for a longitudinal prospective study as designed.

In this patient cohort, the visual fields were normal or close to normal, as indicated by the Statpac indices. However, our results suggest that deterioration of retinal sensitivity, as indicated by our change limits (Bengtsson et al. 2008), occurs frequently, while improvement (Fig. 4) was almost non-

existent. The deteriorated test points appeared after a relative short follow-up period as shown in Fig. 1. That could possibly be explained by too narrow limits for change resulting in many falsely deteriorated points, but if the limits had been too narrow, a similar number of improved points would have been expected. Further, the number of deteriorated test points seemed to be well reproducible from the 12-month visit to the 18-month visit (Fig. 3), suggesting that the limits for change are reliable. However, to be able to evaluate the validity of our change analysis, longer follow-up is mandatory. Another explanation for the deterioration of test points could be development of cataract. Age-corrected threshold values are sensitive to cataract (Bengtsson et al. 1997), but increasing cataract would also affect the number of test points with sensitivities depressed below normal limits. The proportion of such depressed points was similar at baseline and the 18-month visit. It is also known that cataract affects all parts of the field equally and thus results in a general depression at all test point locations (Lam et al. 1991; Heuer et al. 1988), while our findings may suggest deterioration in a smaller portion of test points with somewhat varying locations from time to time (Fig. 1). Thus, we do not believe that our finding can be explained by increasing cataract.

The pathophysiology behind the deterioration of test points despite stable none to mild/moderate diabetic retinopathy can only be speculated upon. The retinal vasculature constitutes a minor part of the retina, whereas the main part consists of neural tissue. Experimental data suggest that impaired glucose metabolism affects the neural retina and that neuronal retinal defects are among the earliest detectable changes in diabetes (Villarroel et al. 2010). Ganglion cells are the earliest cells affected and have the highest rate of apoptosis (Kern & Barber 2008), resulting in a loss of ganglion cells and a reduction in the thickness of the nerve fibre layer in rodents as well as in humans, that is, progressive loss of neural structures in the inner retina. Hence, functional tests may be more sensitive indicators of retinal integrity than fundus photography. Reduction and deterioration of functional capacity would give valuable measures of direct early neuronal damage but also indirect later neuronal damage secondary to leakage and impaired vascular perfusion.

Diabetic retinopathy still remains one of the most frequent causes of blindness among adults in developed countries. New treatment strategies to prevent disease progression at early stages of diabetic retinopathy would be of great interest. The evaluation of new drugs should be based on reproducible outcome measures and preferably noninvasive as well as cost-effective techniques (Fishman et al. 2005). Functional outcome measures are indeed desired (Csaky et al. 2008), but so far, it has not been possible to monitor early diabetic retinal changes with such measures. Conventional perimetry may fulfil the demands as outcome functional measures for progression of diabetes-induced retinal changes. This study with its promising interim results will evaluate the usefulness of perimetry for the detection of early retinal damage because of diabetes. The results will be relevant for adopting perimetry as one possible measure of change in visual function, which should be a primary end-point in randomized clinical trials. To define an amount of individual perimetric change that can be used as a cut point in clinical trials would be desired, but we do believe our method needs to be validated before that can be done.

Funding

Department of Ophthalmology, County Hospital of Värmland, Karlstad, Sweden, Centre for Clinical Research, County Hospital of Värmland, the Järnhardt Foundation, the Foundation for Visually Impaired in Former Malmöhus län, the Swedish Eye Foundation, the Malmö Hospital Foundation, the Cronqvist Foundation, the Skåne County Council, and Lund University.

Competing interests

Boel Bengtsson has received research funding from Carl Zeiss Meditec Inc. and is a consultant of Carl Zeiss Meditec Inc. The Department of Clinical Sciences, Ophthalmology, in Malmö, Lund University, has received research funding from Carl Zeiss Meditec Inc. Karl-Johan Hellgren and Elisabet Agardh have no commercial associations connected to the submitted article.

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Received on February 14th, 2012.
Accepted on June 18th, 2012.

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Paper IV

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Progression of Early Retinal Dysfunction in Diabetes Over Time: Results of a Long-term Prospective Clinical Study

DOI: 10.2337/db13-1628

We explored signs of retinal dysfunction over time in diabetic subjects before or early in the course of retinopathy. Patients with no, mild, or moderate retinopathy were consecutively recruited and underwent standard automated perimetry, visual acuity measurement, and fundus photography. These examinations and measurements of HbA_{1c} and blood pressure were repeated for up to 5 years from baseline. Visual field improvement/deterioration in diabetic subjects was evaluated using significance limits for change. Progression or regression of retinopathy was defined as a two-step change on the Early Treatment Diabetic Retinopathy Study final severity scale. Seventy-four subjects completed at least 3 years of follow-up, and 22% showed visual field worsening, defined as repeated significant deterioration at ≥10% of the test points, whereas only 1% showed field improvement. Worsening occurred in subjects both with and without vascular lesions. The degree of retinopathy was stable throughout the observation period in 68 of 74 eyes, improved in 4, and worsened in 2. Visual field deterioration was not correlated with a change in retinopathy. By using perimetry with an analysis tailored for monitoring diabetic subjects, we were able to demonstrate progression of retinal dysfunction over time, which may represent early signs of retinal neurodegeneration.

Diabetic retinopathy has classically been considered to be a microvascular complication caused by elevated blood glucose levels and metabolic pathways triggered by hyperglycemia. Vascular lesions have been characterized in detail, and guidelines for treatment based on various grading scales have been developed to help preserve visual acuity. However, the retina is not primarily a vascular tissue, but rather a neuronal tissue with a vascular supply in which the retinal neurons, glia, and retinal vasculature are interconnected to form a functional neurovascular unit with intricate molecular interactions (1). Hyperglycemia likely affects not only the vasculature per se but also the neuroretina, resulting in dysfunctions other than impairment and loss of visual acuity.

There is increasing evidence of early retinal neurodegeneration in diabetes, which may even precede the vascular changes (2). Neuronal degeneration patterns have been observed in various animal models of diabetes, and early retinal dysfunction has been demonstrated (3,4). Postmortem studies in humans have revealed neuronal degeneration and apoptosis in retinas, mainly in the ganglion cell layer (5,6). Furthermore, use of the modern technique of spectral domain optical coherence tomography for retinal imaging (7) has shown thinning of the retinal nerve fiber (8,9) and photoreceptor layers (10) in diabetic subjects in vivo before any visible vascular lesions could be detected. In addition, thinning of the ganglion cell layer and inner plexiform layers has been reported in patients with mild diabetic retinopathy (11).

The most commonly used methods for detecting retinal dysfunction in humans are psychophysical (e.g., perimetry) or electrophysiological (e.g., electroretinogram [ERG]). Several of those techniques have been applied to evaluate retinal dysfunction in diabetic subjects, but so far, very few long-term longitudinal studies have been performed to establish the usefulness of those methods.

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Received 21 October 2013 and accepted 31 March 2014.

The most widely used test of retinal dysfunction is standard automated perimetry (SAP), which has rendered results indicating a reduction of retinal sensitivity in diabetic subjects without retinopathy (12,13) as well as in those with mild/moderate (14) or moderate/severe retinopathy (15,16). Moreover, reduction of retinal sensitivity revealed by SAP was found to correlate with stepwise increases in the severity of retinopathy (17,18). That SAP can be used to predict the development of diabetic retinopathy has also been suggested (19).

Microperimetry is another perimetric method in which a small area of the central field is tested using white stimuli but on a darker background than in SAP. This test was recently reported to show reduced sensitivity in subjects who did or did not have various types of retinopathy (20,21). A drawback of microperimetry is that the short dynamic range of the stimulus presented results in truncation of threshold sensitivities (22), and hence, normal or nearly normal function cannot be accurately measured.

Frequency doubling technology and short wavelength automated perimetry (SWAP) are two examples of selective perimetry, the latter designed to expose blue stimuli on an intense yellow background. The stimuli used in these methods aim at testing specific subpopulations of retinal ganglion cells, which increases the sensitivity of the tests. Reduced SWAP sensitivity has been reported in diabetic subjects without retinopathy (12,23) as well as in individuals with mild, moderate, or more severe diabetic retinopathy (17,18,24). SWAP is not optimal for analysis of longitudinal data. Compared with SAP, SWAP entails considerably larger test–retest variability (18), making it difficult to detect subtle changes, and SWAP is also much more sensitive to cataract development. Perimetry using frequency doubling technology stimuli has reported reduced retinal sensitivity in diabetic subjects without as well as with retinopathy (13,16,25), but so far this has only been noted in a limited number of cross-sectional studies.

The ERG is an electrophysiological test that can provide objective and quantitative information on retinal dysfunction in diabetic subjects (26). There is evidence that ERG abnormalities can be detected very early in the course of retinopathy and that special techniques can be used to demonstrate local responses. To our knowledge, changes in ERG responses over time have only been investigated in insulin-dependent diabetic patients and have yielded contradictory results (27,28), but it has been reported that a delayed ERG response can predict the onset of diabetic retinopathy (29). Those results could indicate that diabetes may affect the retinal neurons ahead of the retinal vascular network.

The purpose of our study was to demonstrate the usefulness of SAP for detecting early retinal dysfunction over time in patients with type 1 and type 2 diabetes. In our interim report published after 18 months of follow-up of subjects with and without mild/moderate diabetic retinopathy (30), we described how our previously defined limits of significant change for SAP in diabetes (18) provided promising results regarding the monitoring of perimetric change. Here, we confirm those findings in an extended longitudinal study with an average follow-up time of 4 years.

RESEARCH DESIGN AND METHODS

The subjects and study design have previously been described in detail (30) and are summarized here. From September 2006 to May 2009, patients who had type 1 (13 of 81) or type 2 (68 of 81) diabetes and were between the ages of 18 and 75 years were consecutively recruited from the screening program for diabetic retinopathy at the Department of Ophthalmology, Karlstad, County Council of Värmland. This program uses fundus photography to grade vascular lesions and is open to all individuals with diabetes who are older than 10 years of age. Type 1 diabetes was defined as having a diagnosis of diabetes at age $<$30 years and receiving insulin treatment within 1 year of diagnosis. One randomly selected eye per subject was examined and included in the study. Patients were not included if they had previously received laser treatment or any other local treatment for diabetic retinopathy, were in need of such treatment, or had other conditions that were likely to affect the visual field. Intraocular pressure measurements ranging between 11 and 22 mmHg and normal optic discs on baseline photographs ruled out the presence of undiagnosed glaucoma. To be included, patients had to be able to perform reliable visual fields defined as $\leq$15% false-positive responses. The study was approved by the regional ethical review board of Lund University, Sweden, and all patients gave written informed consent.

Patients were scheduled for follow-up visits every 6 months for the first 3 years and thereafter annually until 5 years from baseline. All visits included an examination of visual acuity, SAP, fundus photography, and measurement of HbA_{1c} and blood pressure.

Visual acuity was tested using Early Treatment of Diabetic Retinopathy Study (ETDRS) charts (31) and was expressed as the number of correctly read letters. Visual acuity was measured after refraction with manual adjustment of autorefractor (KR-8100P; Topcon, Tokyo, Japan) values in sphere and cylinder to 0.25-diopter accuracy. We considered a difference of five or more letters as a change in visual acuity based on our earlier results of measurements of short-term variation in visual acuity in diabetic patients (32).

Visual fields were tested using a Humphrey Field Analyzer 750 (Carl Zeiss Meditec Inc., Dublin, CA) with the SITA Standard 24-2 program, including 54 test point locations within the central 24° visual field. Normal limits are based on a multicenter collection of perimetric data from 330 healthy subjects between 19 and 84 years of age (33). Before the initial visit during the study period, all subjects performed one visual field test to avoid

perimetric learning effects during follow-up. The perimetric interpretation tool "single field analysis" provides probability maps that flag test locations with sensitivities that are significantly depressed compared with age-corrected normal values (34) (Fig. 1). The global perimetric index mean deviation (MD) describes the global status of the visual field; a value of 0 dB corresponds to a normal field, and approximately –30 dB corresponds to a blind field.

Longitudinal visual field change was assessed by comparing each follow-up field with the baseline field representing an average of the two tests performed at the first two visits during the study period. Differences in age-corrected threshold values were calculated for each test point, except for the two located in the blind spot area, and thereafter were compared with the limits for significant improvement and deterioration that we had previously defined for diabetic subjects (18). This change analysis was developed in analogy with the glaucoma change probability maps used to assess glaucomatous progression (35). Test locations with significant change (deterioration or improvement) at the $P < 0.05$ level (Fig. 1) were counted. As in our previous study (30), fields with five or more significantly improved or deteriorated test locations were considered as showing a possible change from baseline, and repeated improvement or deterioration at five or more test locations in two or more consecutive field tests was regarded as indicating likely change. The risk for having at least five test points falsely flagged as deteriorated or improved at the $P < 0.05$ level is about 10% in a single test, and by requiring at least the same number in the consecutive test, the risk for false change diminishes considerably to 1%, assuming the tests are independent.

To confirm the results obtained by our method, we applied the established pointwise linear regression analysis (36,37) but used age-corrected threshold values over time to detect significant changes in visual fields. Eyes were considered deteriorated if five or more test points showed significantly negative regression slopes at the $P < 0.05$ level.

Stereo fundus photography of seven 35° fields was performed using a TRC 50IX retinal camera (Topcon) and Fujichrome Sensia 100 slide film (Fujifilm, Tokyo, Japan). The degree of retinopathy was graded according to the ETDRS final severity scale (38). The grader (E.A.) was masked to patient characteristics, visit number, and outcome of the functional tests. Progression or regression of retinopathy was defined as a two-step change according to the ETDRS final severity scale.

HbA_{1c} was analyzed by high-performance liquid chromatography initially using a Variant II Hemoglobin A1c Program (Bio-Rad, Hercules, CA) and from 2 February 2011 using a TOSOH G8 analyzer (Medinor, Tokyo, Japan): normal ranges are 27–42 and 31–46 mmol/mol

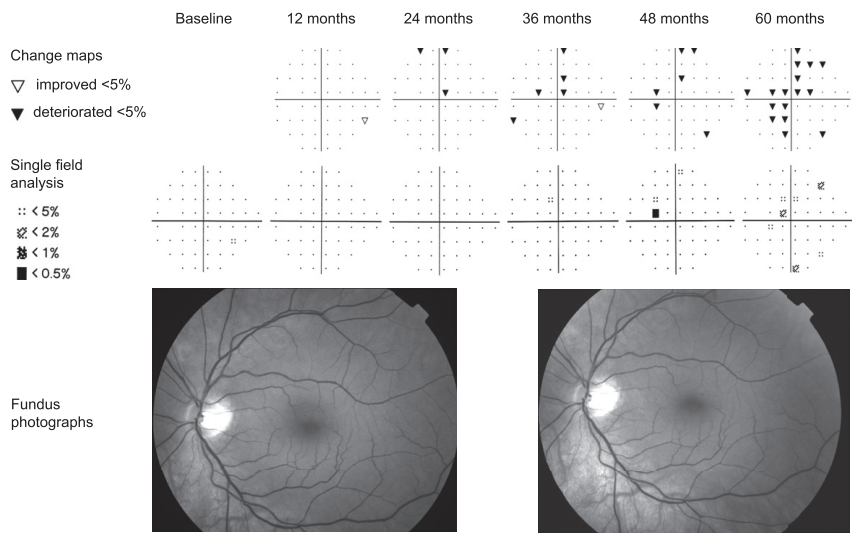

Figure 1—Visual fields from baseline to 60 months of follow-up demonstrate significantly improved and deteriorated test points (*top*) in a 59-year-old subject with type 2 diabetes and mild retinopathy (i.e., microaneurysms only). The fundus photographs were taken at baseline (*left*) and at the last follow-up visit (*right*). The single-field analysis revealed no meaningful deviation from normal age-corrected threshold values (*middle*), whereas visual field deterioration was apparent in the change maps (*top*). Visual acuity was >6/6 throughout the entire study period.

(4.6–6.0% and 5.0–6.3% according to the National Glycohemoglobin Standardization Program) for individuals aged <50 and ≥50 years, respectively. Blood pressure was expressed as the mean of two measurements performed using an aneroid sphygmomanometer with the patient sitting.

Statistics

Depending on the type of data to be analyzed, parametric (paired *t* test) or nonparametric (McNemar, Wilcoxon sign rank test, and Mann-Whitney) tests were used to compare changes in patient characteristics and perimetric MD values between baseline and the last follow-up visit. We also used κ statistics to analyze agreement between the two methods applied to assess changes in visual fields.

RESULTS

Ninety-three diabetic subjects received information on the study and participated in the training session, and 12 were excluded due to proliferative retinopathy (n = 1), unreliable perimetry (n = 3), or individual choice (n = 8). Of the remaining 81 subjects who met the inclusion criteria, 74 completed the minimum follow-up of 3 years. The reasons for dropout were death (n = 2), severe illness (n = 2), and unwillingness to continue (n = 3). The median follow-up time was 4 years, and 22, 18, and 34 of the subjects completed 5, 4, and 3 years of follow-up, respectively. Compliance with the visit schedule was high: only three of all possible visits were missed by three subjects who did not attend the 12-month visit.

Of the 74 subjects who completed follow-up, 12 had type 1 diabetes. Subject characteristics at baseline are summarized in Table 1. Very few changes occurred in their characteristics during follow-up: at the last study visit, fewer subjects were being treated by diet alone, and diastolic blood pressure was slightly lower.

After 3 to 5 years of follow-up, visual acuity had decreased five or more letters compared with baseline in 20 of the 74 patients, in 2 of 12 type 1 and in 18 of 62 type 2 diabetic subjects. In the worst case, 15 letters were lost due to development of clinically significant macular edema, and another patient with cataract at the last visit lost 11 letters. We have no explanation for the decrease in visual acuity in the remaining 18 patients. Visual acuity improved by five or more letters in six patients, one of whom underwent cataract surgery during the follow-up period.

Subjective assessment of "single field analysis" and its probability maps revealed no obvious field loss at baseline or in follow-up tests. For the entire cohort, the global MD value improved slightly, from an average of –0.55 dB at baseline to –0.39 dB at the last visit (P = 0.31).

Our change analysis revealed considerably more test locations with significant deterioration than with significant improvement at the last follow-up visit. The number of test locations showing deterioration increased over time, whereas the number exhibiting improvement remained at essentially the same level (Fig. 2). At the last visit, 27 eyes showed significant deterioration at five or more test locations, and only 3 eyes had significant improvement at five or more locations. Only 1 subject had five or more test points showing significant improvement in the last two tests, whereas 16 subjects had five or more test points with significant deterioration at least at the last two visits, in 2 of 12 with type 1 and in 14 of 62 with type 2 diabetes. The global MD value for those 16 individuals decreased from –0.19 dB to –1.26 dB (P = 0.003). Test points with repeated deterioration appeared in all parts of the visual field without any typical pattern during the study period. The proportions of deteriorated test points detected within the central 10° of the field and

Table 1—Subject characteristics at baseline and at the last follow-up visit (median 4 years, range 3–5 years)

Characteristics	Baseline	Last visit	*P*
Sex			
Female	30 (37)	29 (39)	
Male	51 (63)	45 (61)	
Age (years)	57 ± 11	61 ± 11	
Age at onset (years)	44 ± 15		
Diabetes duration (years)	13 ± 12	17 ± 12	
HbA_{1c} (% [mmol/mol])	7.6 ± 1.1 [60 ± 12]	7.6 ± 1.1 [60 ± 12]	0.46
Treatment			
Insulin only	33 (41)	33 (45)	1.00
Insulin and oral hypoglycemic agent	15 (18)	13 (18)	1.00
Oral hypoglycemic agent only	24 (30)	25 (34)	0.12
Diet only	9 (11)	3 (4)	0.03
Antihypertensive medication	42 (52)	43 (58)	0.29
Blood pressure (mmHg)			
Systolic	133 ± 16	132 ± 16	0.35
Diastolic	78 ± 11	75 ± 11	0.02

Data are presented as n (%) or mean ± SD.

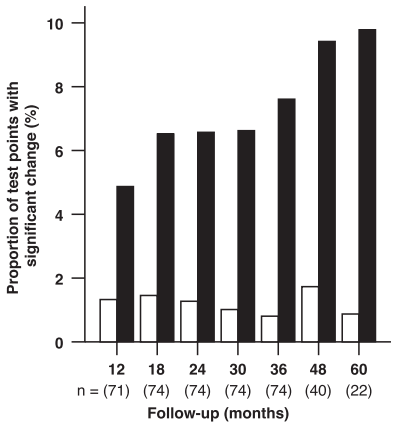

Figure 2—The proportion of deteriorated test points (■) increased over time, whereas the proportion of improved test points (□) remained stable.

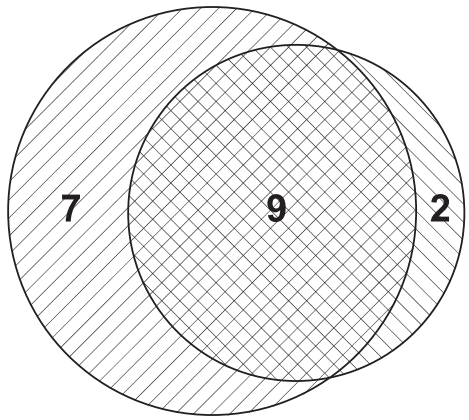

Figure 3—Venn diagram shows the number of eyes with deterioration indicated by our test–retest method (n = 16) and the pointwise linear regression analysis (n = 11). Nine eyes were identified by both methods.

outside that area were similar, at 21% and 18%, respectively. The corresponding proportions in the upper and lower hemifields were also similar. According to the pointwise linear regression method, 9 of the 16 eyes showed deterioration. There was moderate agreement between the results provided by our method and those obtained using the linear regression method (κ = 0.60; Fig. 3). Individual fluctuation of significantly deteriorated test points over time is shown in Fig. 4.

Loss of visual acuity by five or more letters was noted in 20 eyes at the final test, and 6 of these were among the 16 eyes with repeated deterioration of the visual field indicated by our method.

The number of test point locations showing deterioration varied considerably in patients older than 40 years of age, whereas almost no deteriorated points were seen in the few patients who were younger than 40 (Fig. 5*A*). The difference in age between those with and those without likely deterioration of visual fields (median ages 66 and 63 years, respectively) was not significant (P = 0.23). At the last visit, deterioration of visual fields was not explained by diabetes duration (Fig. 5*B*), mean HbA_{1c} levels during the study period (Fig. 5*C*), or diastolic blood pressure (Fig. 5*D*). HbA_{1c} levels at baseline did not correlate with visual field change at any follow-up assessment. On a group basis, HbA_{1c} values were the same at baseline as at the last visit.

Thirty-five eyes (49%) had no or questionable (n = 1) signs of vascular lesions (ETDRS level 10–15) at baseline, and the remaining 38 eyes had mild to moderate lesions (ETDRS level 20–43). Twenty-four eyes were graded as level 10 at all visits. The level of retinopathy was stable in 68 eyes, whereas the predefined two-step change occurred in only 6 eyes, progression in two of them and regression in the other four (from level 10 to 20, or conversely). The occurrence or disappearance of one or a few microaneurysms represented the only sign of structural vascular change.

No association was found between progression of retinopathy and likely deterioration of the visual field. In the last visual field test, the two patients with progression had, respectively, only one test point and two test points with significant deterioration. No test points showed significant improvement in the four patients with regression of retinopathy, but two of them had five or more test points exhibiting deterioration. Furthermore, the number of deteriorated test locations noted at the last visit did not differ between eyes without signs of vascular lesions and eyes with an ETDRS level >10 at any visit (median 2.5 [range 0–21] vs. 3 [range 0–37]).

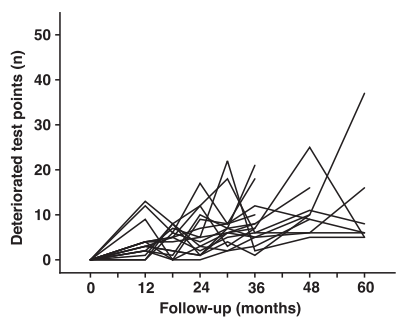

Figure 4—Individual fluctuations of significantly deteriorated test points over time are shown in the 16 eyes with deterioration at least at the last two visits. The fluctuations in most eyes were reasonably small.

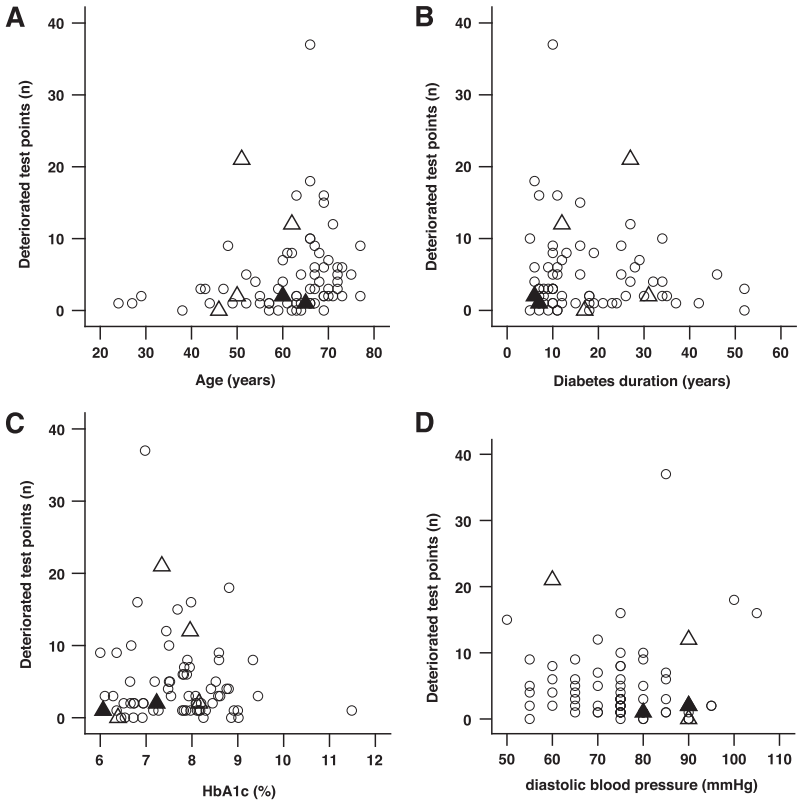

Figure 5—Number of test points showing deterioration at the last follow-up visit in relation to age (*A*), diabetes duration (*B*), HbA_{1c} level (*C*), and diastolic blood pressure (*D*). The test points with deterioration were not significantly correlated with any of the measured variables. Data represent eyes with stable retinopathy (○), progression (▲), and regression (△) of retinopathy.

DISCUSSION

To our knowledge, this is the first prospective longitudinal study to use SAP to evaluate diabetic subjects with no or mild/moderate diabetic retinopathy. This approach detected progression of early retinal dysfunction, even though the ETDRS final severity scale indicated stable retinopathy. Because visual field deterioration did not differ between subjects without and those with any signs of microvascular abnormalities and because the ETDRS level of retinopathy was stable over time, we propose that the neuronal dysfunction could represent an early feature of diabetic retinopathy due to primary neurodegeneration. A limitation of the study is that the number of subjects with progression of retinopathy during the 4 years of follow-up was insufficient to explore whether SAP is able to predict the development of microvascular impairment.

We used empirically derived limits for change based on test–retest variability previously measured in an independent sample of diabetic patients (18). Knowledge of such variability, together with longitudinal comparisons within individuals, allows more sensitive monitoring of visual function than is provided by cross-sectional comparisons of individuals. Had the limits been too narrow, a substantial proportion of significantly changed—both deteriorated and improved—test points would have been found. Our analysis yielded a substantial number of test locations showing significant deterioration but very few locations exhibiting improvement. In 27 eyes (36%), at least five test points showed deterioration in the last test. Furthermore, deterioration of at least five test points was noted in 16 eyes (22%) in the two last tests, which indicated a likely change, and those 16 eyes also had decreased MD values. Agreement was good between our method and the pointwise linear regression technique, although the latter identified fewer eyes with deterioration, indicating that our method is more sensitive. Our approach has long been applied to assess the eyes of patients with

glaucoma (35), and in that disease has been reported to identify significant deterioration earlier than is possible with the regression method (39–41). Therefore, as expected, in the current study we found the regression method revealed fewer deteriorated eyes than our method.

To evaluate sensitive methods for detecting change over time, it is necessary to use a longitudinal study design. However, such an approach always entails the risk of dropouts, which can weaken the interpretation of the results. Fortunately, the dropout rate in our study was low: more than 90% of the subjects completed the 3 years of follow-up.

Several cross-sectional investigations using various perimetric and electrophysiological methods have suggested slight dysfunction in eyes with no diabetic retinopathy (12,13,16,20,21,23–26), but few longitudinal studies have considered this issue. One longitudinal investigation was conducted by Di Leo et al. (28) in 1994, who found that patients with type 1 diabetes, but without retinopathy, exhibited significant changes in ERG responses 3 years after baseline, and two studies demonstrated that slight depression of SAP (19) and delayed ERG response (29) predicted later onset of diabetic retinopathy. The strength of our study is that we performed a longitudinal collection of more functional data. By using SAP to test the visual field every 6 months for up to 3 years and thereafter annually, we were able to identify consistent deterioration in 22% of the eyes that were evaluated.

Factors other than diabetes-induced retinal dysfunction (e.g., cataract) can explain decreased perimetric sensitivity. Although cataract is known to increase with age (42), we found no significant difference in age between patients with and those without a likely visual field change. Cataract typically leads to general depression of the visual field and might also have a more pronounced effect on paracentral than on peripheral test points (43), but such a pattern was not discerned in our 16 subjects with likely deterioration. Although we did not specifically grade lens changes, that the functional changes detected by our method were caused by cataract is unlikely. Furthermore, we did not find any difference in change in perimetric foveal (central) threshold values between the 16 eyes with repeated deterioration and those without. The mean change in foveal threshold was −0.6 dB among the 16 eyes and −1.1 dB among the other 58 eyes. Thus, cataract development is unlikely to explain our finding of repeated deterioration in some eyes.

There was a poor correlation between likely visual field deterioration and visual acuity change, and most eyes with a likely visual field deterioration did not loose visual acuity. A limitation of the study is the lack of explanation for vision loss in a subset of subjects. The present five-letter cutoff for visual acuity change can be discussed, but test–retest variability is lower in eyes with good visual acuity, as was the case for the eyes in our study. Had the cutoff been set at nine letters, which has been reported as the overall coefficient of repeatability for diabetic subjects with various visual acuity (44), six subjects would have had a worsening of visual acuity at follow-up, and merely one of those had a likely visual field deterioration.

We found no association between SAP deterioration and duration of diabetes or HbA_{1c} levels in our cohort of diabetic subjects with stable metabolic control, which agrees with results obtained by Nitta et al. (45). Di Leo et al. (28) and Verrotti et al. (19) have also reported a lack of correlation between ERG and SAP, respectively, and metabolic control. We previously demonstrated that quite extensive intraindividual blood glucose fluctuations on a 1-month basis had no or little influence on SAP deterioration in a different cohort of diabetic subjects (18). Whether fluctuating HbA_{1c} levels on a long-term basis may affect visual function, including SAP, can only be speculated about.

This prospective longitudinal study is the first of its kind, and our results demonstrate that SAP with an analysis tailored for monitoring the eyes of patients with diabetes over time can reveal repeated deterioration of retinal neuronal function in this disease. We believe our method provides reliable assessments of visual function early in the course of diabetic retinopathy. Moreover, it can serve as an alternative end point for investigation of early visual disturbances, which can be useful when evaluating new treatment strategies for diabetic retinal disease, including neuroprotection.

Acknowledgments. The authors thank Dr. F. Lundin, Centre for Clinical Research, County Council of Värmland, for technical support in obtaining the change maps; J. Appelgren and D. Larsson, Karlstad University, for technical support in performing the pointwise linear regression analysis; and C. Byhlén and A. Lundh, Department of Ophthalmology, Karlstad, County Council of Värmland, for assessment of the high-quality fundus photographs.

Funding. This study was funded by the Department of Ophthalmology, Karlstad and Centre for Clinical Research, County Council of Värmland, the Järnhardt Foundation, the Foundation for Visually Impaired in Former Malmöhus län, the Swedish Medical Society, the Swedish Eye Foundation, Skåne University Hospital Foundation, the Cronqvist Foundation, and Lund University.

Duality of Interest. B.B. has received research funding from Carl Zeiss Meditec Inc. and is a consultant of Carl Zeiss Meditec Inc. The Department of Clinical Sciences, Ophthalmology at Lund University in Malmö, has received research funding from Carl Zeiss Meditec Inc. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. K.-J.H. organized the project, recruited the subjects, performed all measurements, analyzed the data, wrote a manuscript draft, edited the manuscript, and approved the final draft. E.A. and B.B. originated the project, edited the manuscript, and approved the final draft. K.-J.H. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Prior Presentation. Parts of this work were presented at the 20th Meeting of the European Association for the Study of Diabetes Eye Complication Study Group, Paris, France, 21–22 May 2010; at the Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO), Fort Lauderdale, FL, 6–10 May 2012; and at the Annual Meeting of the Swedish Ophthalmological Society, Malmö, Sweden, 29–31 August 2013.

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