

RETINOPATHY IN SUBJECTS WITH PRE-DIABETES AND ELECTROPHYSIOLOGICAL STUDIES IN DIABETES PATIENTS WITH AND WITHOUT RETINOPATHY

av

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<p>Abstract</p> <p>Diabetes is increasing worldwide and hence the risk developing complications where retinopathy is the theme of this study.</p> <p>The aim of the study was to evaluate the presence of (vascular) retinopathy in subjects with the pre-diabetes stage, impaired fasting glucose (IFG), and the development of retinopathy in these subjects during five years. In the second part of the study diabetes patients with different duration of diabetes and grade of (vascular) retinopathy was examined with electrophysiological methods to investigate if there are alterations detectable in diabetes patients.</p> <p>Results: Retinopathy was present in 10% of subjects with impaired fasting glucose. The retinopathy was associated with higher systolic and diastolic blood pressure, weight and body mass index (BMI). The 5-year incidence of developing retinopathy was 6.9%. In the second part of the study electrophysiological alterations was found in full- field ERG and multi focal ERG in newly onset type 2 diabetes patients even before any vascular retinopathy was detectable on fundus photography. Alterations were also verified before visual deterioration in patients with ischemic maculopathy and long duration of diabetes.</p> <p>Conclusion: Blood pressure appears to be a major concern in the development of retinopathy in subjects with impaired fasting glucose, IFG. The incidence of retinopathy was, however low in these subjects who had received recommendations regarding diet and lifestyle. The full-field and multifocal electroretinography demonstrate early alterations of the retinal function identified before any retinopathy is visible on fundus photography. In future electrophysiological examinations may be valuable to follow these alterations in the natural course of the disease and to evaluate effects of treatment including the neuroretina.</p>			
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Contents

List of papers.....	5
Abbreviations.....	7
Introduction.....	9
Background.....	11
Diabetes.....	11
Prediabetes.....	11
Diabetic (vascular) retinopathy.....	12
Retina - a vascularised neuronal tissue.....	14
Retinal neurodegeneration in diabetes.....	16
Aims of the study.....	17
General aims.....	17
Specific aims.....	17
Subjects and methods.....	19
Subjects.....	19
Ophthalmological examination.....	19
Fundus photographs and grading.....	19
Physical examination.....	20
Laboratory tests.....	20
Electrophysiology.....	21
Statistical methods.....	23
Results.....	25
Paper I.....	25
Paper II.....	26
Paper III.....	26
Paper IV.....	27
Paper V.....	28
Discussion.....	31
Conclusions.....	35
Svensk sammanfattning.....	37
Acknowledgement.....	41
References.....	43

List of papers

- I. Tyrberg M, Melander A, Lövestam-Adrian M, Lindblad U (2008). *Retinopathy in subjects with impaired fasting glucose, The NANSY-Eye Study, Baseline report.* Diabet Obes Metab. 2008 **10**(8):646-51.
- II. Tyrberg M, Melander A, Lövestam-Adrian M, Lindblad U. *Low 5-year incidence of retinopathy in subjects with IFG- the NANSY-Eye Study.* Submitted
- III. Tyrberg M, Ponjavic V, Lövestam-Adrian M (2005) *Multifocal Electroretinogram (mfERG) in insulin depending diabetics with or without clinically apparent retinopathy.* Doc Ophthalmol **110**:137-43.
- IV. Tyrberg M, Ponjavic Vesna, Lövestam- Adrian M (2008). *Multifocal electroretinogram (mfERG) in patients with diabetes and an enlarged foveal avascular zone (FAZ).* Doc Ophthalmol **117**(3):185-9.
- V. Tyrberg M, Lindblad U, Melander A, Lövestam-Adrian M, Ponjavic V, Andréasson S. *Electrophysiological studies in newly onset type 2 diabetes without visible vascular retinopathy.* Manuscript

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Abbreviations

BMI	body mass index
DR	diabetic retinopathy
ERG	electroretinography
FAZ	foveal avascular zone
f-fERG	full-field ERG
IFG	impaired fasting glucose
IGT	impaired glucose tolerance
NANSY	NEPI ANtidiabetes StudY
NEPI	Network for PharmacoEPIdemiology
mfERG	multifocal ERG
mfVEP	multifocal vision evoked potential
UKPDS	United Kingdom Prospective Diabetes Study
VEP	vision evoked potential
WHR	waist hip ratio

Introduction

The incidence of type 2 diabetes is increasing worldwide and hence the risk of developing complications [1, 2], of which retinopathy is the theme of this thesis. Despite the availability of laser photocoagulation, which is the primary treatment for sight-threatening retinopathy, diabetic retinopathy is still a major cause of visual impairment in middle-aged people in the western world [3]. Less destructive treatments at an earlier stage of diabetic retinopathy are now being searched for.

It is not uncommon in clinical practice that diabetic retinopathy is already present at the first visit after diagnosis of type 2 diabetes. In the United Kingdom Prospective Diabetes Study (UKPDS), 25% of patients with newly diagnosed type 2 diabetes had retinopathy, indicating that the disease started years before the diagnosis [4].

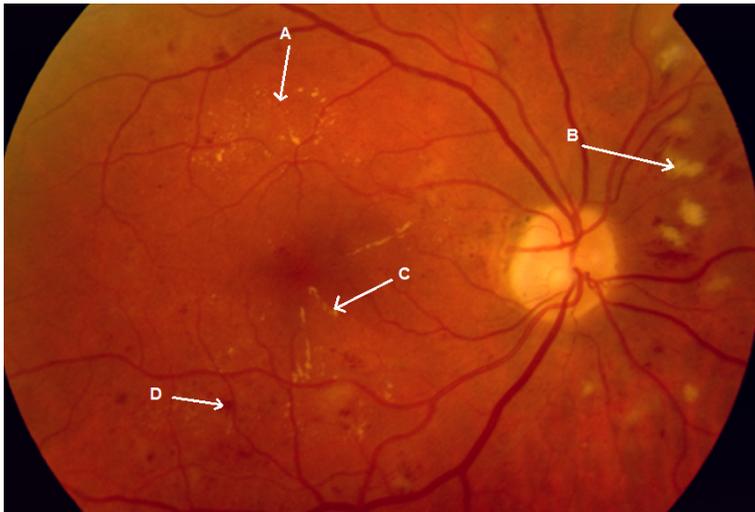


Figure 1. Diabetic retinopathy with microaneurysm (A), cotton wool spots (B), hard exudates (C) and small haemorrhages (D), already at the first visit to the eye clinic, soon after the diagnosis of type 2 diabetes.

Vascular retinopathy, microaneurysms, small haemorrhages, and exudates are also present in 9-10 % of non-diabetic people [5, 6]. In subjects with impaired glucose tolerance (IGT), the prevalence of retinopathy is 11-12 % [6, 7]. In the Hoorn

Study a correlation was found between retinopathy and elevated blood pressure, BMI and serum levels of cholesterol and triglycerides [6].

The first part of this thesis focuses on the vascular changes, and the questions asked were how early we can see these microvascular changes (microaneurysm, small haemorrhages, and exudates) and whether there are any risk factors other than hyperglycaemia for the development of diabetic retinopathy.

Diabetic retinopathy (DR) is a microvascular complication in both type 1 and type 2 diabetes, but if we look at the function, the retina is a vascularised neuronal tissue and diabetic retinopathy includes both retinal neurodegeneration and microvascular complications [8].

It is well known that retinal function can be altered in diabetes, and especially after a long disease duration patients complain of a decline in colour vision and vision in the dark [9, 10]. Altered contrast sensitivity has been reported in diabetes patients even before vascular retinopathy is visible [11, 12]. Visual field alterations in the short wavelength perimetry (SWP) are most pronounced in eyes with more advanced retinopathy [13]. The tests mentioned above are all subjective; it is therefore important to assess the retinal function in diabetes patients in an objective way using electroretinography (ERG), which reflects the electrophysiological activity from the retinal neurons. Alterations have been found both in the full-field ERG [14] and in the multifocal ERG (mfERG) [15] even before any vascular retinopathy could be identified. These electrophysiological studies have mainly focused on patients with a long duration of diabetes.

The second part of this thesis focuses on this issue. We used electrophysiological examinations in diabetes patients with different durations of diabetes and different grades of retinopathy.

Background

Diabetes

Diabetes mellitus is a metabolic disorder characterised by chronic hyperglycaemia, resulting from defects in insulin secretion, insulin action, or both. In type 1 diabetes there is beta-cell destruction, usually leading to absolute insulin deficiency. In type 2 diabetes there is a relative (rather than absolute) insulin deficiency and resistance to the action of insulin [16].

	Glucose concentration mmol/l		
	Plasma	Whole blood	
	Venous	Venous	Capillary
Diabetes mellitus			
Fasting and/or	≥ 7.0	≥ 6.1	≥ 6.1
2-h post-glucose load	≥ 11.1	≥ 10.0	≥ 11.1
Impaired glucose tolerance			
Fasting concentration (if measured) and	< 7.0	< 6.1	< 6.1
2-h post-glucose load	7.8–11.0	6.7–9.9	7.8–11.0
Impaired fasting glucose			
Fasting and	6.1–6.9	5.6–6.0	5.6–6.0
2-h (if measured)	< 7.8	< 6.7	< 7.8

Table 1. Values for diagnosis of diabetes and other categories of hyperglycaemia [16].

Prediabetes

Impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) are metabolic stages intermediate between normal glucose metabolism and type 2 diabetes. Subjects with IFG have non-diabetic hyperglycaemia with a fasting plasma glucose level of 6.1 mmol/l or higher (whole blood: 5.6 mmol/l) but less than 7.0 mmol/l (whole blood 6.1 mmol/l) (table 1). Subjects with IGT have elevated postprandial plasma glucose. IFG is associated with a reduced ability to maintain adequate basal insulin secretion and lower insulin sensitivity in the liver to control hepatic glucose output. In IGT, there is a peripheral insulin resistance especially in skeletal muscles [17]. Both IFG and IGT are risk categories for future diabetes and cardiovascular disease [18, 19]. The risk of developing diabetes is dependent on the level of other risk factors such as age, body mass index (BMI), and family history of diabetes [17].

Lifestyle intervention including counseling on dietary changes, increased physical activity, and weight loss are highly effective in delaying the onset of type 2 diabetes in subjects with IGT [20-23]. The Network for PharmacoEPIdemiology (NEPI) Antidiabetes Study, NANSY, is a randomised placebo-controlled Swedish trial investigating whether treatment with sulphonylurea in addition to advice aiming at dietary regulation and increased exercise could delay the conversion to type 2 diabetes in subjects with IFG [24]. The study was planned and implemented in a primary health care context.

Diabetic (vascular) retinopathy

Diabetic retinopathy has been used to establish the levels of hyperglycemia that define the diagnosis of diabetes [25, 26], based on studies in which the prevalence of retinopathy increased above a fasting plasma glucose threshold of 7.0 mmol/l [26-28].

Diabetic retinopathy (DR) is a microvascular complication seen in both type 1 and type 2 diabetes. The earliest signs are microaneurysms (10-100 μm sized saccular capillary extensions due to pericyte loss) in the retinal capillaries and retinal haemorrhages. Microaneurysms have been shown to be important predictive lesions for the progression of diabetic retinopathy in newly diagnosed type 2 diabetes [29]. Increased capillary permeability (due to breakdown of the blood-retina barrier) and capillary closure leading to retinal ischemia precede the development of macular oedema and new vessel formation (proliferations), the two main complications that may lead to sight-threatening DR (figure 2).

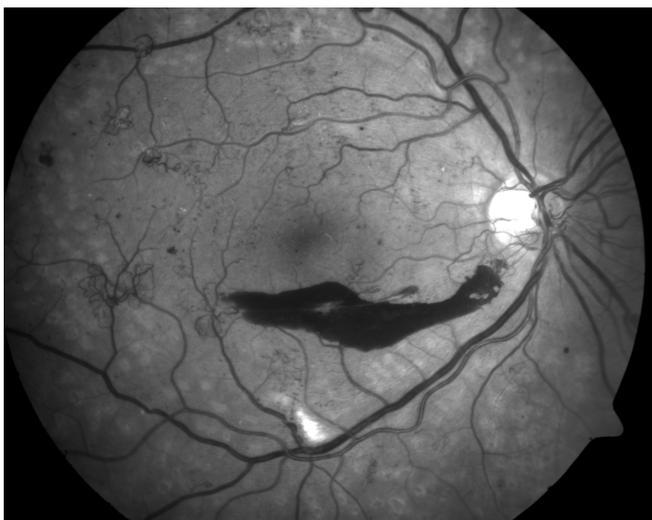


Figure 2. Red free retinal photo with proliferations on the optic disc and temporally; the new vessels bleeds easily; here is a boat-shaped bleeding under the centre.

The prevalence of DR at diagnosis in type 1 diabetes is low (about 1 %) [30, 31]. In a study from our department, the prevalence of DR was found to be 4% in type 1 diabetes patients with a disease duration of less than five years, rising to 92% after 15 years of duration [32]. In the Wisconsin epidemiological study of diabetic retinopathy (WESDR) the prevalence increased from 2% in those with a disease duration of under 2 years to 98% after 15 years [33].

In type 2 diabetes, the prevalence of DR at the first ophthalmological examination has been reported to be 11-25% [4, 7], indicating that the disease started years before diagnosis [34]. In newly diagnosed type 2 diabetes patients without retinopathy at baseline, 22% had developed retinopathy after 6 years in the UKPDS [35]. Klein et al. found a 29% prevalence of retinopathy in type 2 diabetes patients within 5 years of diagnosis [36]

In both type 1 and type 2 diabetes, glycated haemoglobin (HbA1c), an index of long-term glycaemic control, has been shown to be strongly associated with the development of retinopathy [35, 37-39].

When diabetes type 1 is accompanied by hypertension, retinopathy may become more severe [40]. In type 2 diabetes also, elevated blood pressure is associated with development of retinopathy [7, 35]. Tight blood pressure control has been shown to prevent deterioration of diabetic retinopathy in patients with type 2 diabetes who participated in the UKPDS [41, 42].

Diabetic macular oedema, which may occur at any stage of diabetic retinopathy, is characterised by increased vascular permeability resulting in leakage of plasma constituents out into the extracellular space. This results in a local or diffuse thickening of the central retina. Diabetic macular oedema is the main cause of loss of vision in diabetes patients [43].

The foveal region is avascular, and is usually 350-750 μm in diameter [44]. In diabetic retinopathy, the foveal avascular zone (FAZ) and the perifoveal intercapillary areas (PIAs) are often enlarged due to capillary closure (figure 4). This may contribute further to macular oedema [45].

Retinopathy in subjects with IGT has been evaluated in earlier studies, and has a prevalence of 11-12% [6, 7]. In the Hoorn study, the 9-year incidence of retinopathy in subjects with IGT was 13.6%. Risk factors associated with retinopathy were hyperglycaemia, hypertension, and abdominal obesity [19]. There have been no previous studies evaluating the prevalence or incidence of retinopathy in subjects with IFG.

Retina - a vascularised neuronal tissue

The vascular supply to the retina comes from the retinal vessels lying in the inner retina mainly in the ganglion cell layer, and from the choroidal vessels under the pigment epithelium. The neural retina is transparent and largely invisible on clinical examination. There are five classes of neurons: photoreceptors (rods and cones), bipolar cells, ganglion cells and amacrine cells (figure 3).

Full-field ERG

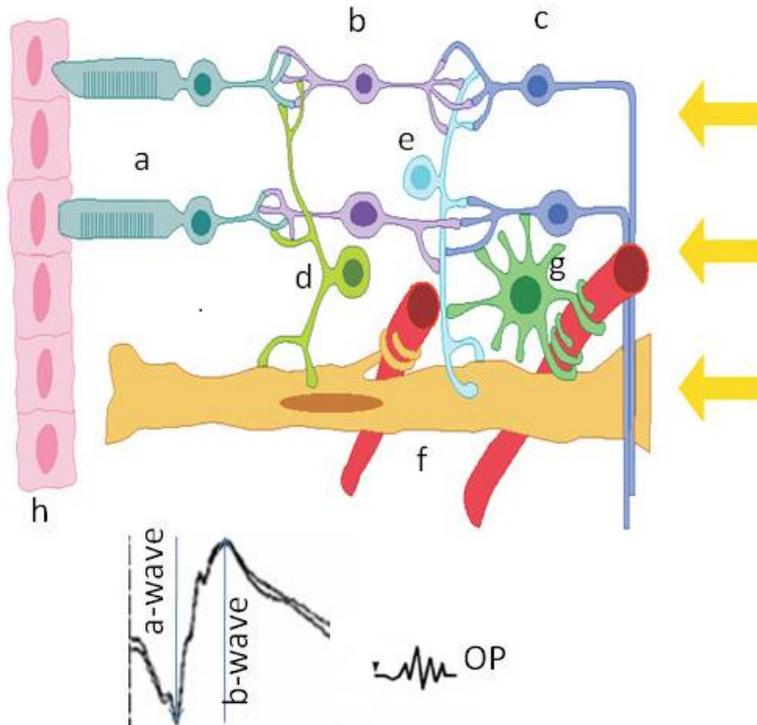


Figure 3. Retina with neuroretina and the retinal vessels. Light is coming in from the right. a: photoreceptors (rods and cones), b: bipolar cell, c: ganglion cell, d: horizontal cell, e: amacrine cell, f: müller cell, g: astrocyte, h: pigmentepithelium. Lower part of the figure shows the full-field ERG answers and their origin: a-wave from rods, b-wave from photoreceptors and bipolar cells and oscillatory potentials (OP) from amacrine cells. (original)

Absorption of light by the photoreceptor initiates a cascade of events that change the membrane potential, and thus the amount of neurotransmitter released. Three kinds of neurons: photoreceptor cells, bipolar cells, and ganglion cells (whose axons form the optic nerve) process the signal to the visual cortex in the occipital lobe of the brain. In the outer plexiform layer, where the synapses between photoreceptors and bipolar cells are located, horizontal cells have lateral interactions, which are thought to maintain the sensitivity to contrast. In the inner plexiform layer, the amacrine cells have lateral connections with bipolar cells and ganglion cells.

Astrocytes and müller cells, the two types of macroglial cells in the retina, have connections with the retinal vessels and with neurons, (figure 3). One of their functions is to regulate neuronal nutrition and metabolism. Astrocytes are situated in the ganglion cell layer and their processes wrap around blood vessels [46]. Müller cells are spindle-shaped and span the entire retina from the pigment epithelium to the inner limiting membrane, and they play an important role in maintaining the blood-retina barrier [47-49]. Increased vascular permeability due to breakdown of this barrier is one of the first signs of diabetic retinopathy visible with fluorophotometry [50].

Retinal neurodegeneration in diabetes

In 1962, Bloodworth [51] described diabetic retinopathy as a complex degenerative disease of all elements of the retina. In recent years, more attention has been given to the neurodegenerative part of diabetic retinopathy [8, 47, 52, 53]. Colour vision defects and reduced contrast sensitivity have been reported in diabetes patients with minimal or no retinopathy [11, 54]. Also, reduced contrast sensitivity has been found in subjects with IGT [55, 56]. Electrophysiological examinations, which could be used to investigate disturbance regarding neuroretinal degeneration in a more objective way, have recently been used more frequently in this group of patients.

Previous studies have verified alterations in the full-field ERG, which reflects the electrical answer from the whole retina. The most described are delayed or declined oscillatory potentials (OPs) [14, 57-61], which are thought to come from the amacrine cells in the inner retina [62] (figure 3). There was found to be a delay in the 30-Hz flicker response in type 1 diabetes patients with advanced retinopathy and a mean duration of diabetes of 16 years [63]. Holopigian found evidence of photoreceptor changes (delay in the a- and b-waves) in a group of 12 diabetes patients (4 type 1 and 8 type 2) with a duration of diabetes of more than 5 years [64].

Studies using multifocal ERG, which mainly reflects the cone-driven outer retina [65] have shown delay in the implicit time in patients with diabetes (both type 1 and type 2) with a duration of approximately 10-15 years [66-68]. In diabetes patients without retinopathy, alterations in the second order kernel of the multifocal ERG, which is thought to represent mainly the inner retina, have been shown with lower amplitude and a reduction in a specific waveform [15]. A progressive delay in the visual evoked potential (VEP), which measures the optic nerve, has been shown in diabetes patients [69]. Most of these electrophysiological studies have concentrated on patients with a long duration of diabetes, with or without (vascular) retinopathy.

Aims of the study

General aims

To study the early retinopathy by fundus photography in subjects with impaired fasting glucose (IFG), and by electrophysiological methods in patients with diabetes.

Specific aims

Paper I

To determine the prevalence of retinopathy in subjects with IFG, and to explore any associations with components of the metabolic syndrome.

Paper II

To determine the 5-year incidence of retinopathy in subjects with IFG and its relationship to metabolic risk factors, and to test the possible preventive effect of sulphonylurea.

Paper III

To evaluate differences in the multifocal ERG response in patients with type 1 diabetes with different levels of retinopathy.

Paper IV

To examine whether there is a correlation between an enlarged foveal avascular zone (FAZ) in diabetes patients with good visual acuity without macular oedema and functional alterations assessed by multifocal ERG.

Paper V

To investigate whether early alterations of retinal function (as assessed by electrophysiology) could be identified in newly diagnosed type 2 diabetes patients with normal visual acuity and no vascular retinopathy detectable on fundus photography.

Subjects and methods

Subjects

In paper I, 154 subjects with impaired fasting glucose (B-glucose 5.6-6.0 mmol/l) participated while in the 5-year follow-up (paper II), 67 subjects remained.

In paper III we examined 31 patients with type 1 diabetes (mean duration of 23 ± 9 years) and normal visual acuity.

In paper IV, twenty-five (14 type 1 and 11 type 2) diabetes patients with an enlarged foveal avascular zone (FAZ) participated. The visual acuity was 0.6 or better, and the duration of diabetes was 22 ± 7 years.

In paper V we examined 17 consecutive patients with new-onset type 2 diabetes with a mean duration of 7 ± 3 months, from the screening at the Department of Ophthalmology, Helsingborg Hospital. The visual acuity was 0.8 or better and no-one had any diabetic retinopathy detectable on fundus photography.

Controls for the electrophysiological studies (paper III-V) were non-diabetes subjects, of the same age and without any eye disease.

Ophthalmological examination

Visual acuity was assessed using Snellen charts (papers III-V) and with Early Treatment Diabetic Retinopathy Study (ETDRS) charts [70] (paper V). The ocular pressure was measured to exclude patients with glaucoma (paper V) using Topcon CT 80.

Fundus photographs and grading

Fundus photography was taken (after dilation of the pupil) using 35-mm diapositive film (papers I and II) or digital images (Imagenet 2000 system version 2.55; Topcon) (papers III-V). The alternative classification of the Wisconsin Epidemiological Study of Diabetic Retinopathy was used to classify the level of retinopathy where level 10 represents no retinopathy, 21 very mild retinopathy and level 31 mild retinopathy. Grade 41 and higher represents more advanced retinopathy [71, 72].

Fluorescein (25%) angiography was performed after dilation of the pupil (paper IV). The foveal avascular zone and the perifoveal intercapillary areas (PIAs, which are adjacent to the avascular zone) were measured as described by Sleightholm [73] (figure 4). The Image Net software was used.

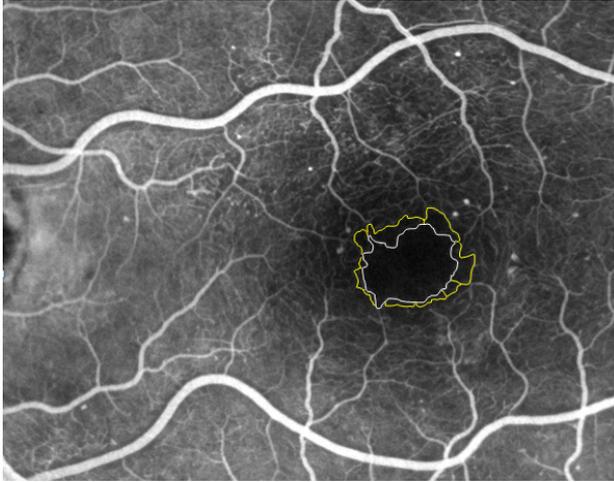


Figure 4. The foveal avascular zone (FAZ) marked in the centre and the adjacent perifoveal intercapillary areas (PIAs) outside.

Physical examination

The baseline physical examination in NANSY (papers I and II) included blood pressure in supine position after 5 minutes of rest, using the Tricuff (with automatic adjustment of cuff size to arm circumference) [74]. Hypertension was defined as blood pressure $\geq 160/90$ mmHg (one or both) [75], or ongoing pharmacological treatment for hypertension.

Laboratory tests

In NANSY (papers I and II), blood specimens were drawn in the morning after a 10-h overnight fast. Serum samples were immediately frozen at -20 °C and stored for later analysis. Routine tests included fasting blood glucose (Hemocue), S-triglycerides, S-total cholesterol, S-HDL cholesterol, and S-LDL cholesterol. Haemoglobin A1c (HbA1c) was analysed by ion exchange chromatography, normal range 3.7-5.5% [76]. In paper V, the HbA1c analysis method was immunoassay (normal range 3.6-5.3%) [77]. Actual plasma glucose was analysed (Hemocue 201+) (paper V).

Electrophysiology

Full-field electroretinography (ERG) (paper V)

The full-field electroretinogram (ERG) was recorded in a Nicolet analyzing system (Nicolet Biomedical Instruments, Madison, WI, USA). After dilation of the pupil with topical cyclopentolate (1%) and phenylephrine hydrochloride, the subject was dark-adapted for 40 min. A Burian Allen bipolar contact lens was applied to the anaesthetised cornea (oxibuprocain) and a ground electrode was attached to the forehead. Responses were obtained with a wide band filter (-3 dB at 1 Hz and 500 Hz), stimulating with brief (30 μ s) single, full-field flashes of white light 0.81 cd.s/m² and white light 3.93 cd.s/m². Cone responses were obtained with 30-Hz flickering white light (0.81 cd.s/m²) averaged from 20 sweeps with and without background illumination. Recording procedures were according to the ISCEV standardised protocol [78] with slight modifications. Cone responses were obtained with 30 Hz flickering white light obtained without any light adaptation. The average oscillatory potential (OP) amplitude was calculated as the arithmetic mean of the first three major peak-to-peak values (OP1, OP2 and OP3), and the OP2 implicit time was measured [59].

Multifocal electroretinography (mfERG), papers III-V

MfERG was recorded using the Visual Evoked Response Imaging System (VERIS) and according to the ISCEV guidelines [79, 80]. The pupil was dilated with tropicamide (studies III and IV) or cyklopentolate 1% (study V), and phenylephrine hydrochloride. A ground electrode was attached to the forehead. Retinal activity was recorded with a bipolar contact lens (gold in papers III and IV), which was placed on the anesthetised (oxibuprocain) cornea. The contralateral eye was occluded with an eye patch. The stimulus matrix consisted of 103 hexagonal elements displayed on a screen in an infrared (IR) camera. A small black fixation object was placed at the centre of the stimulus matrix driven at 75 Hz frame rate. The luminance of each hexagon was independently alternated between black and white according to a pseudo-random binary m-sequence at 75 Hz. The fundus was visualised by using infrared light from the recording electrode. The first-order component of the mfERG was analysed regarding implicit times of P1 (first positive peak) and amplitudes (N1-P1) within the six concentric rings registered by the mfERG, (figure 5). In papers III and V, the second-order component was also analysed.

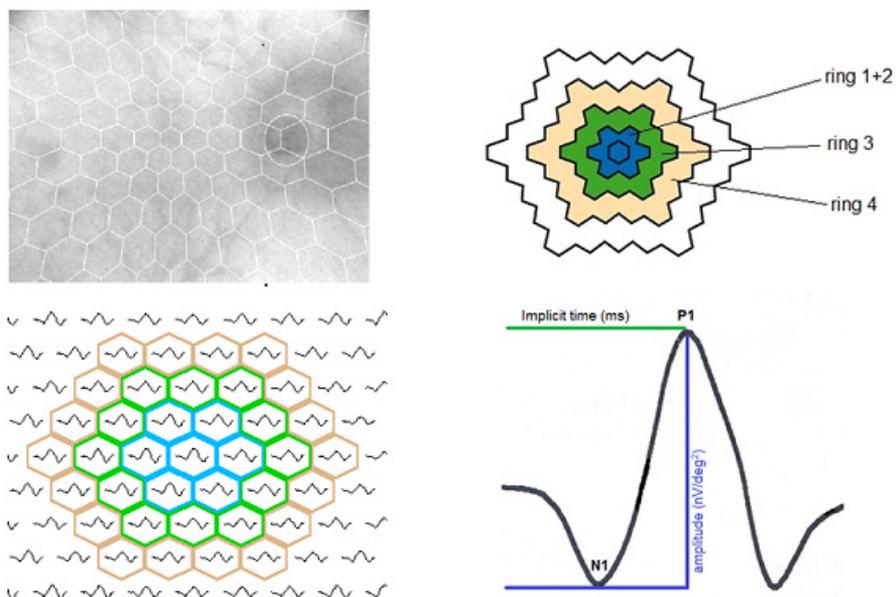


Figure 5. Multifocal ERG, Upper left panel: the IR image during recording, to control stable fixation. Lower left panel: in every hexagon, a summed response is presented with a curve. Upper right panel: summed answers in ring areas marked with different colours. Lower right panel: the first-order mfERG is analysed regarding implicit time to the first positive peak P1 and amplitude. (original)

Multifocal visual evoked potential (mfVEP), paper V

MfVEP was recorded using the Visual Evoked Response Imaging System (VERIS, EDI, San Mateo, CA, USA) [81]. The stimulation was monocular, without pupil dilation. A visual stimulus with the appearance of a dartboard, containing 60 cortically scaled segments, was displayed on a cathode-ray tube (CRT) screen in an infrared (IR) camera. Each segment contained a dartboard pattern composed of 16 checks, 8 white and 8 black reversing in contrast in a pseudo-random binary m-sequence at 75Hz. The signals were amplified 100 000 times and passed through a band-pass filter between 3 Hz and 100 Hz. At a viewing distance of 5 cm, the radius of the stimulation array was approximately 20-25 degrees. To control fixation, the camera part of the IR camera was used. Three pairs of electrodes were used; one pair was placed on the inion and 4 cm above and the other two pairs 4 cm to the left and 4 cm to the right of the midline electrode position. Each run contained 16 segments at 27 seconds each, giving a total recording time of 7.2 minutes. The two components in the responses, probably similar to the P70 and N100 in the classic VEPs [82], were summerised and analysed from the midline electrodes (inion). The amplitude was measured

from the first positive peak at about 70 ms and the first negative peak at about 100 ms. The implicit time was analysed for the first negative peak at about 100 ms. Dim room light was used as background illumination. To minimise muscle disturbances, the subject was seated comfortably during the recording.

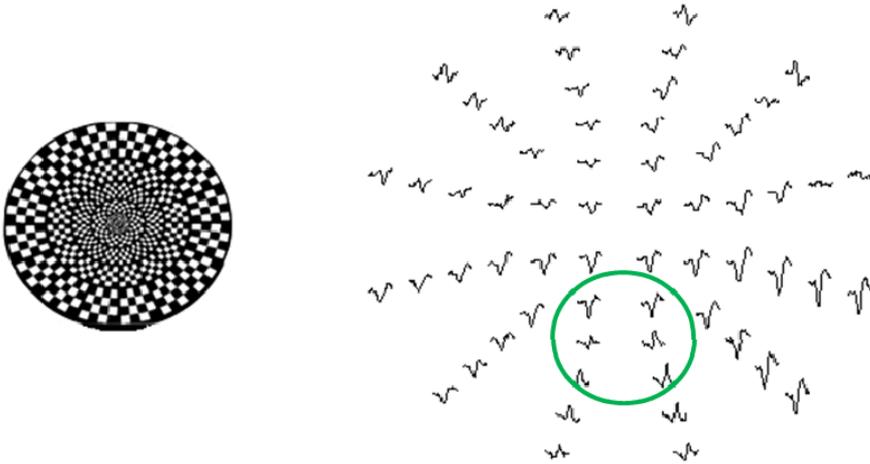


Figure 6. To the left the stimulation picture. To the right the registered traces, marked with green: the region in the mfVEP analysed.

Statistical methods

In papers I and II, general linear models and t-test were used to compare differences in means in continuous variables between groups. Confounding was accounted for by multivariate analyses and by stratification.

In paper III, Student's t-test was used to compare age and chi-squared test was used for testing categorical variables. Repeated measure analysis of variance was used for multiple comparisons.

In papers IV and V, the Mann-Whitney test was used for comparison of amplitude values and implicit times in different groups and correlation between variables was tested with Spearman's rank test.

SPSS version 11.0-16.0 was used.

Results

Paper I

NEPI ANtidiabetes StudY, NANSY-Eye baseline report

Subjects in the age of 40-70 years were surveyed in primary care with repeated fasting blood glucose measurements. Those with a mean of two consecutive values ≥ 5.6 and < 6.1 mmol/l were invited to participate if both tests were ≥ 5.6 mmol/l. Hypertension was defined as blood pressure $\geq 160/90$ (one or both) or ongoing pharmacological treatment for hypertension.

At baseline, 90 men and 64 women underwent fundus photography within the first 6 month after inclusion in the main study. Of these, 16 subjects (10%) had retinopathy, none had more than mild retinopathy (level 31) in both eyes.

Subjects with retinopathy had a higher systolic blood pressure (154 vs. 141 mmHg, $p = 0.013$) and diastolic blood pressure (86 vs. 81 mmHg, $p = 0.008$) than those without retinopathy, also when adjusted for differences in age, sex and known hypertension. There was a corresponding difference in BMI, being greater in subjects with retinopathy than in those without (32.4 vs. 29.2 kg/m², $p = 0.013$). Neither HbA1c nor fasting blood glucose levels were associated with retinopathy (table 2).

	Retinopathy n=16	no retinopathy n=138	p-value
Age	63 (7.0)	62 (6.5)	0.731
fB-glucose (mmol/l)	5.9 (0.13)	5.9 (0.11)	0.211
HbA1c (%)	4.6 (1.4)	4.8 (0.7)	0.325
Weight (kg)	91 (14)	85 (13)	0.015
BMI	32.4 (4.0)	29.2 (3.9)	0.013
Systolic blood pressure (mmHg)	154 (22)	141 (18)	0.013
Diastolic blood pressure (mmHg)	86 (12)	81 (9)	0.008

Table 2. Metabolic characteristics of those with retinopathy compared with those without, mean (SD), paper I.

Paper II

The 5-year follow up of the NANSY-Eye Study

Participants were followed with repeated fasting B-glucose measurement and those who developed diabetes were excluded from the main study. All subjects got dietary and exercise advice during the study. Fundus photos were taken after 5 years, using 35 mm diafilm, as at baseline.

Sixty-seven subjects participated in the 5-year follow up. At study term those with retinopathy had higher diastolic (89 vs 77 mm Hg $p = 0.047$) blood pressure than those without. Systolic blood pressure, BMI and WHR were non-significantly higher in those with retinopathy.

The retinopathy was dynamic during the 5-years, in 7 subjects the retinopathy regressed, in these subjects both the systolic and the diastolic blood pressure was lower at study term compared with baseline, while the blood pressure increased in those 4 who developed retinopathy.

The 5-year incidence of developing retinopathy was 6.9%.

Paper III

Multifocal ERG in diabetes patients with different grades of retinopathy

Thirty-one patients with type-1 diabetes (11 without retinopathy, 12 with mild retinopathy and 8 with moderate retinopathy) were examined with multifocal electroretinogram (mfERG). The duration of diabetes was 23 ± 9 years. Sixteen non-diabetes subjects without any eye disease were used as controls, all patients and controls had a visual acuity of 1.0.

In the first order kernel there were significantly higher amplitudes in the diabetes patients compared to non diabetic controls ($p = 0.001$).

In the second-order kernel, the patients with retinopathy had a prolonged implicit time compared to those without retinopathy ($p = 0.026$).

The third positive waveform at 40-50 ms in the second order kernel were absent in 15/31 diabetes patients, but were easily distinguished in all the controls ($p = 0.001$).

Paper IV

Multifocal ERG in diabetes patients with enlarged foveal avascular zone (FAZ)

Twenty-five diabetes patients (14 with type-1 diabetes and 11 with type 2) who had an enlarged FAZ (largest diameter $>650\mu\text{m}$) measured in fluoresceinangiograms were examined with mfERG. The visual acuity was over 0.6, the retinopathy level was mild to preproliferative and no one had macular oedema. The largest FAZ diameter, the FAZ area and the adjacent perifoveal intercapillary area (PIA), was calculated from the fluorescein angiogram. Sixteen healthy subjects with no evidence or history of any ocular or systemic disease were controls for the mfERG data.

There was a correlation between increasing FAZ diameter and a prolonged implicit time in the innermost concentric rings and in the third concentric ring in the first order kernel of the mf ERG ($p = 0.003$ and $p = 0.008$). An increasing summed area (FAZ and PIA) was correlated to increasing implicit time in the same areas of the first order mfERG ($p = 0.005$ and $p = 0.026$). No correlation was seen between the ischemic areas and the mf ERG amplitudes.

In the diabetes patients, the implicit times were longer in the four innermost rings compared to the controls. The amplitudes in the corresponding ring areas were lower than in non-diabetes controls (table 3).

	Diabetes (n=25)	Control (n=16)	P value
Amp 1+2 (nV/deg ²)	31.0 (14.5)	47.3 (19.4)	0.005
Amp 3	22.8 (11.9)	34.2 (14.9)	0.014
Amp 4	19.2 (11.4)	29.7 (14.3)	0.019
It 1+2 (ms)	28.5 (3.8)	27.3 (1.8)	0.006
It 3	28.4 (1.6)	26.3 (1.5)	<0.001
It 4	27.9 (1.4)	25.8 (1.2)	<0.001

Table 3. Amplitudes (amp) and implicit times (it) in diabetes patients compared with non diabetes controls (paper IV).

Paper V

Electrophysiological studies in new-onset type 2 diabetes patients without any visible vascular retinopathy

Seventeen new onset type 2 diabetes patients were examined with full-field ERG, multifocal ERG, and multifocal visual evoked potential (VEP). Controls for the electrophysiological studies were non-diabetes subjects, of the same age and without any eye disease

In the dark adapted full-field ERG, the white light (0.81 cd/m^2) a-wave implicit time was delayed in the diabetes patients compared with non diabetes controls ($p = 0.001$). No difference was seen in the a-wave amplitudes. Also, in the dark-adapted 30-Hz flicker the diabetes patients had delayed implicit time ($p = 0.020$) and the amplitudes were higher in the diabetes patients ($p = 0.041$) (table 4).

Multifocal ERG

In the first-order kernel, the implicit time was delayed in the diabetes patients in the separate ring areas measured, and also when the entire area was analysed. The amplitudes were marginally higher in the diabetes group, but not statistically significantly so (table 5).

In the second-order kernel, no differences were seen regarding the presence of the third waveform (at 40-50ms).

Multifocal VEP

The amplitudes were marginally lower in the diabetes group but not statistically significantly so, and, no significant differences were found in the implicit times.

	Diabetes (n=17)	Control (n=18)	p- value
DA white (0.81) b-wave amp (μV)	285.2 (51.6)	263.3 (108.6)	0.083
DA white (0.81) a-wave amp	86.0 (30.7)	66.1 (29.4)	0.062
DA white 0.81) a-wave it (ms)	25.3 (1.0)	23.8 (1.5)	0.001
DA white (3.93)b-wave amp	310.9 (45.9)	282.5 (125.5)	0.232
DA white (3.93) a-wave amp	148.2 (29.1)	129 (53.3)	0.126
DA white (3.93) a-wave it	18.4 (2.8)	18.8 (2.8)	0.613
DA 30Hz flicker amp	51.9 (11.4)	43.5 (21.5)	0.041
DA 30 Hz flicker it	31.4 (1.6)	30.2 (1.4)	0.020
LA 30Hz flicker amp	32.1 (8.2)	29.3 (15.8)	0.287
LA 30Hz flicker it	27.6 (1.0)	27.2 (1.4)	0.351
OP total amp	17.9 (4.9)	15.3 (10.1)	0.163
OP2 it	23.9 (2.7)	24.3 (0.7)	0.287

Table 4. Full-field ERG results, paper V. DA: dark adapted, amp: amplitude, it: implicit time, LA: light adapted, OP: oscillatory potential. The age in the study group was 63.1 and in non diabetic controls 62.0 years, $p = 0.287$.

	diabetes (n=17)	control (n=15)	p-value
amp 1+2 (nV/deg^2)	41.4 (11.6)	35.9 (14.4)	0.082
it 1+2 (ms)	30.3 (1.4)	28.5 (1.8)	0.002
amp 3	29.2 (7.1)	25.4 (10.9)	0.089
it 3	29.6 (1.2)	27.7 (1.6)	0.001
amp 4	23.6 (6.1)	22.1 (9.8)	0.411
it 4	29.4 (1.1)	26.9 (1.4)	<0.001
sum amp 1-4	21.7 (6.2)	20.1 (9.2)	0.331
sum it 1-4	29.7 (1.1)	27.4 (1.3)	<0.001

Table 5. Multifocal ERG results, paper V. Amp: amplitude, it: implicit time. The age in the study group was 63.1 and in non diabetic controls 63.0, $p = 0.682$.

Discussion

High blood pressure promotes retinopathy in subjects with impaired fasting glucose (IFG) (papers I and II)

Retinopathy was present in 10% of subjects with IFG, and it was positively associated with both blood pressure and body mass index (paper I). After 5 years (paper II) the diastolic blood pressure was still associated with retinopathy. The prevalence found at baseline was at the same level as previously reported in subjects with impaired glucose tolerance (IGT) (8-12%) [6, 7, 25]. In our study population, there may have been some subjects with both IFG and IGT, as there was no possibility of doing the oral glucose tolerance test (OGTT). In a recent report the prevalence of retinopathy was found to be 14.6% in subjects with IGT and 10.3 % in IFG subjects, and only IGT was associated with retinopathy. [83].

The retinopathy was dynamic during the 5-year period. In 7 subjects in which the retinopathy regressed, both the systolic blood pressure and the diastolic blood pressure was lower at the end of the study compared to baseline, while the blood pressure increased in those 4 who developed retinopathy. Also, in the Arteriosclerosis Risk in Communities study (ARIC) regression of retinopathy was seen in a subgroup of participants in a 3-year follow-up, which was related to lower levels of cardiovascular risk factors (absence of diabetes, never/past smoking, lower waist hip ratio (WHR), and higher level of physical activity) [84]. As our study population got dietary advice and advice about exercise this may have influenced the development and the regression of retinopathy.

The presence of retinopathy at baseline did not appear to predict development of diabetes. This is in agreement with the results of the ARIC study where retinopathy in non-diabetic individuals was not found to be predictive of development of diabetes after 3 years, except if there was a family history of diabetes [85]. On the other hand, in another study a twofold higher risk of developing diabetes was found after 5 years in non-diabetic subjects with retinopathy [86].

The low 5-year incidence of retinopathy (6.9%) is at the same level as has earlier been reported in non-diabetic individuals from the Beaver Dam study (with a 5-year incidence of 6.0%) [87], and recently from the ARIC study (with a 3-year incidence of 2.9%) [84]

The number of subjects was too small to be able to detect any conclusive effect of sulphonylurea treatment on the development of retinopathy.

The incidence of retinopathy was more akin to that in reports on subjects with normal glucose. Perhaps the lifestyle interventions have had a preventive effect. That would be in accordance with the decreased blood pressure seen in the lifestyle intervention arm of the Finnish diabetes prevention study in subjects with IGT [21]. General strategies to improve public health may thus also prevent the development of retinopathy.

The retinopathy in this prediabetes state appears to have a multifactorial aetiology, resembling the metabolic syndrome (high blood pressure, overweight and hyperglycaemia). As the retinopathy at this early stage does not seem to progress, it may not be relevant to follow them with repeated fundus photographs, but still recommend those with a family history of diabetes to have their blood glucose regularly checked since in other studies such individuals have been shown to be at risk of developing diabetes [85, 86].

Alterations in neuronal function assessed with multifocal ERG before vascular retinopathy is detectable and vision deteriorates in ischaemic maculopathy (papers III-V)

In previous studies on diabetes patients, the amplitudes in the multifocal ERG have been reported to be unaffected [88] or lower [89] than in controls. In contrast to these findings, one of our recent studies (paper III) has demonstrated higher amplitudes in the first-order kernel compared to non-diabetes controls. Higher amplitudes may be due to increased retinal blood flow due to abnormal auto regulation [90, 91]. Elevated glucose levels have been shown to give increased amplitudes due to a higher rate of retinal metabolism [92], however, in paper V where plasma glucose was actually measured, no correlation with amplitude levels was found. Lower amplitudes compared to the controls were present in paper IV, which may have been due to the more severe level of retinopathy in these patients. This is in accordance with a previous study where lower amplitudes were found only in patients with severe diabetic retinopathy [89].

A delay in the implicit time of the first-order kernel mfERG in diabetes patients has been reported previously [66, 67, 88]. In paper IV, the implicit time was delayed in the first-order kernel in diabetes patients relative to the non-diabetes controls. Also, in paper III where the patients studied had type 1 diabetes and a milder grade of retinopathy, there was a longer (but not significantly prolonged) implicit time in the diabetes patients. Surprisingly, also the new-onset type 2 diabetes patients had delayed implicit time (paper V), which is in accordance with a recent study involving adolescent type 2 diabetes patients without any diabetic retinopathy [93]. It will therefore be of interest to follow these patients, to investigate whether the alterations are reversible at this early stage of the disease.

A prolonged implicit time due to ischaemia has been reported in retinal vein occlusion [94]. In paper IV, a correlation was found between an enlarged foveal avascular zone FAZ, as a sign of ischaemia, and a prolonged implicit time in the first-order kernel of the mfERG. A declined cone function in diabetes patients has earlier been measured with sensitivity loss in short wavelength perimeter [13]. Also in microperimetry ischemic areas outside the fovea have as a sign of functional loss shown loss of sensitivity [95] A correlation between the ischaemic areas and prolonged implicit time in the mfERG indicates that alterations in neuronal macular function due to ischaemia might precede the deterioration of visual acuity.

The only difference found between diabetes patients and non diabetes controls in paper III, in which type 1 diabetes patients with a mean duration of 23 years participated, was the absence of the third waveform in the second kernel. This waveform is thought to represent the ganglion cell layer [96], and its absence indicates that inner retinal dysfunction may precede visual deterioration. The patients in the study had a long duration of type 1 diabetes, and in a similar group of patients the nerve fiber layer have been shown to be thinner (as measured by scanning laser polarimetry) [97]. Comparable results have been found in animal studies, where streptozotocin-treated rats, showed alteration in the inner retina with increased apoptosis [47]. In paper V, where new-onset type 2 diabetes patients were examined, there were no changes in the third waveform. Perhaps these alterations develop later in the disease, or are related to the type of diabetes.

The abnormalities found in papers III-V may reflect both vascular changes in the retina and -probably simultaneously - alterations in retinal function.

Delayed a-wave in full-field ERG in new-onset type 2 diabetes patients indicates early photoreceptor alteration (paper V)

In recent years, the a-wave in the dark-adapted full-field ERG has been analysed further and according to several studies it mainly reflects the rod photoreceptor activity [98], but may also have postreceptoral components [99]. In paper V, the dark-adapted white light (0.81 cd s/m^2) response implicit time was delayed, indicating an early alteration in photoreceptor function in patients with new-onset type 2 diabetes. To our knowledge, this is the first report on a-wave delay in new-onset type 2 diabetes patients without visible retinopathy on fundus photography. This is in accordance with a previous study that showed rod photoreceptor dysfunction rather than cone dysfunction in diabetes [100]. This may be due to the higher metabolic rate in rods compared to cones, and to the fact that hypoxia in the retina is most pronounced in the dark adapted state [101].

We also found a delay in the dark adapted 30-Hz flicker in the full-field ERG, which has previously been reported in type 1 diabetes patients with advanced retinopathy and a mean disease duration of 16 years [63].

Future implications

Electrophysiological measurements with full-field ERG and multifocal ERG are as compared with fluorescein angiography noninvasive. Our studies indicate that neurofunctional alterations may develop parallel or even before clinically detectable microvascular alterations, therefore it is of importance to follow the development of diabetic retinopathy with both electrophysiological examinations and fundus photographs in studies and when possible in clinical practice. Neuroprotective treatments are now in search for to treat glaucoma and other neurodegenerative eye diseases. In the future when the cause of neuroretinal alterations in diabetes are revealed we hopefully may have some neuroprotective treatment to prevent development or treat diabetic retinopathy.

Conclusions

Blood pressure appears to be a major concern in the development of retinopathy in subjects with impaired fasting glucose, IFG. The incidence of retinopathy was, however low in such subjects who had received recommendations regarding diet and lifestyle.

The electrophysiological studies show alterations in the outer retina (photoreceptor and bipolar cells) and the inner retina (ganglion cell layer), both early in type 2 diabetes patients and after a long duration of both type 1 and type 2 diabetes. Some of the alterations are present before any vascular retinopathy is detectable on fundus photography, indicating that neuronal damage may develop parallel with vascular changes. In the future, electroretinography may be helpful in monitoring patients with diabetic retinopathy.

Svensk sammanfattning

Vår syn är ett fantastiskt sinne. Ljuset träffar våra synceller (tappar och stavar), längst bak i ögats näthinna, där ljuset omvandlas till en elektrisk signal som via två omkopplingar lämnar ögat via synnerven och förs bak till syncentrum längst bak i hjärnan där bilden tolkas. Näthinnan behöver syre för att fungera och försörjs med det via blodkärlen dels i nähinnans plan, dels i hinnan bakom (åderhinnan).

Vid diabetes drabbas kroppen av olika komplikationer, bland annat drabbas de små blodkärlen. I ögonen är det framförallt retinas (nähinnans) kärl som drabbas och man får olika grad av diabetesretinopati. Förr, innan man kunde behandla diabetesretinopati med laser, blev dessa patienter gravt synskadade. Trots laserbehandling, som är den enda vedertagna behandlingsmetoden, blir en del patienter även idag synsvaga av diabetesförändringarna.

Antalet individer med typ 2 diabetes ökar kraftigt i hela världen och man talar om en diabetesepidemi som delvis beror på ändrade kost- och motionsvanor. Troligen kommer också antalet individer med komplikationer av sjukdomen att öka.

Man vet att andra faktorer än blodsockernivån påverkar ögonförändringarna vid diabetes, till exempel högt blodtryck och höga blodfetter. De förändringar som man upptäcker och behandlar vid diabetesretinopati är kärlförändringar, vilka man därför följer med regelbundna ögonbottenfotografier för att i tid upptäcka de förändringar som kan behandlas. Många patienter har redan vid sitt första ögonbottenfoto efter diagnos av typ 2 diabetes diabetesförändringar med mikroaneurysm (utvidgningar av små kärl), hårda exsudat (äggviteämnen som läckt ut från kärl) och blödningar, vilket talar för att diabetessjukdomen startat innan diagnos ställts.

Stiftelsen Nätverk för läkemedelsepidemiologi, NEPI initierade studien NANSY (NEPI ANTidiabetes StudY). Studiens syfte var att undersöka om man kan hindra eller fördröja insjuknandet i typ 2 diabetes genom att till individer med hög risk att utveckla diabetes (individer med IFG, dvs. blod-glukos 5,6-6,0mmol/l) ge sulfonylurea (ett insulinfrisättande läkemedel). Detta gjordes parallellt med att individerna fick kost och motionsråd, eftersom man vet att detta kan fördröja insjuknandet i typ 2 diabetes.

De två första studierna i avhandlingen är kopplade till NANSY. Vi har i NANSY-Eye basrapport (studie I) studerat prevalensen (förekomsten) av retinopati hos dessa individer som alltså ännu inte har diabetes. Vi fann att ca 10% har retinopati och att den har samband med högre systoliskt och diastoliskt blodtryck samt högre

kroppsmassa, BMI (=body mass index: vikten (kg)/längden²(m²). Vi har sedan följt dessa individer under 5 år och fann då i 5-årsuppföljningen (studie II) att retinopatin gick tillbaka hos en del medan andra som inte hade retinopati 5 år tidigare hade utvecklat det. Även här fanns det ett samband med blodtryck så att de som utvecklade retinopati hade högre blodtryck vid studiens slut än fem år tidigare och vice versa.

Sammanfattningsvis visar NANSY-studien att retinopati som vid diabetes finns redan innan diabetessjukdomen har brutit ut och att den framförallt har samband med högre blodtryck.

I ögonbottenscreeningundersökningar av diabetespatienter vid våra ögonkliniker är det som tidigare nämnts kärlförändringarna i retina som man följer. Man vet sedan tidigare att retina också påverkas funktionellt vid diabetes med till exempel försämrat kontrast-, mörker- och färgseende. Objektivt kan man mäta retinas funktion med hjälp av elektrofysiologiska undersökningar, man kan likna det vid ”EKG-undersökning av ögat”. Vi har i studierna använt tre olika metoder:

1. Full-fälts elektroretinografi (ERG), där man efter 40 minuter i mörker, stimulerar ögat med ljus av olika styrka och våglängd. Med hjälp av en elektrod i en kontaktlins på ögats yta kan nervcellernas elektriska svar registreras. Man börjar stimulera med svagare ljus, då registreras i huvudsak stavarnas svar, i det ljusadapterade senare svaret är det framför allt tapparnas svar som ses. Full-fälts ERG registrerar hela näthinnans svar.
2. Multifokalt ERG (mfERG) är en utveckling av full-fälts ERG där man registrerar svar från den centrala delen av näthinnan, undersökningen är därmed ett viktigt komplement eftersom man inte kan avläsa funktionen i lokaliserade delar av centrala näthinnan i full-fälts ERG svaret.
3. Multifocal vision evoked potential (mfVEP) mäter om de impulser som når ögat går vidare till syncentrum baktill i hjärnan. Vid den undersökningen fäster man därför elektroder i hårbotten i nacken. Undersökningen är ett mått på hur synnerven och synbanorna fungerar.

Vi har undersökt olika diabetespatienter, i studie III typ 1 diabetespatienter som haft diabetes länge. Där fann vi vid jämförelse med kontrollmaterial en påverkan hos de med diabetes som tyder på en skada i inre retina.

I studie IV undersöktes patienter (både typ 1 och 2) som haft diabetes länge och som hade en förstorad kapillärfri zon i centrala näthinnan, men en bevarad god syn. Den kapillärfria zonen vet man ökar vid diabetes hos en del patienter och blir den tillräckligt stor försämrar synskärpan. I studien fann vi att det fanns ett samband mellan storleken på den kapillärfria zonen och påverkan på multifokal ERG- svaren där vi såg en förlängd överledningstid.

I den femte och sista studien deltog nydebuterade typ 2 diabetes patienter, som inte hade någon synlig diabetesretinopati vid ögonbottenfotograferingen. De undersöktes med full-fälts ERG, multifokalt ERG och multifokalt VEP. Vi fann i full-fälts ERG en påverkan på den första vågen, a-vågen i det mörkeradapterade ögat. I multifokal ERG var överledningstiden längre hos studiepatienterna. Påverkan på a-vågen talar för en påverkan på fotoreceptorerna (stavar) och multifokal ERG svaret tyder på att retina även är påverkad längre in.

Sammanfattningsvis talar studierna för att den vaskulära (kärlrelaterade) retinopatin finns redan innan typ 2 diabetes har blivit manifest och den har då samband med högre blodtryck. Funktionellt påverkas näthinnan vid diabetes innan vi med våra screeningmetoder (ögonbottenfoto) kan se någon vaskulär retinopati (kärlpåverkan) och innan synen försämrats.

De delar av näthinnan som i våra studier visats sig vara påverkade vid diabetes är dels fotoreceptorerna och dels inre retina, troligen är det ett intimt samband mellan nervdelen och kärlet som ger det vi idag kallar diabetesretinopati. På sikt, när vidare forskning förhoppningsvis kan kartlägga vilka skador som kommer först, kan nervskyddande behandling bli aktuell för att så tidigt som möjligt bromsa utvecklingen av den allvarliga komplikation som diabetesretinopati är.

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