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COMPARISON OF 10-YEAR INCIDENCE OF RETINOPATHY IN SCREENING DETECTED AND IN USUAL CARE DETECTED TYPE 2 DIABETES MELLITUS PATIENTS: THE HOORN STUDY

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Purpose/Background. Hyperglycaemia may exist for several years before symptoms develop in type 2 diabetes mellitus (T2DM) patients. Such patients can be detected by screening. However, it is not clear whether screening-detected T2DM carries a lower risk of secondary T2DM complications such as retinopathy and ischemic heart disease to that of T2DM diagnosed in usual care. The authors studied this issue in the Hoorn Study.

Methods. In the Hoorn Study, a population-based cohort study (n=2484, age 50-75) (QUERY: Are ranges correct throughout abstract?) of diabetes and diabetes complications, 95 patients were classified as having newly diagnosed T2DM after screening at baseline. The 10-year incidence of complications of these screening-detected patients was compared to the 10-year incidence of complications, as estimated from cross-sectional annual medical examinations in symptom-detected T2DM patients in usual care (n=1001). The regression coefficient of the relation between the proportion of complications and the T2DM duration was used to compute an estimated 10-year incidence of T2DM complications in the usual care detected T2DM patients. Both groups underwent fundus photography to assess retinopathy and ECG recording to assess ischemic heart disease.

Results. Of the 95 screening detected T2DM patients, 22 (23%) had died, and 37 participated in the follow-up. The 10-year incidence of retinopathy and ECG abnormalities was 11.4% and 21.9%, respectively. For both retinopathy and ECG abnormalities no linear association of prevalence and T2DM duration was found in usual care detected T2DM patients. Thus, the 10-year incidence could not be estimated for this group and the risk difference between groups not computed.

Conclusions. In contrast to what was expected no linear association was observed between duration and progression of complications. The improvement of glucose control in a managed diabetes care system may explain these results.

OPTIMAL SCREENING INTERVALS FOR EARLY DETECTION OF SIGHT-THREATENING DIABETIC RETINOPATHY. A SIX-YEAR RETROSPECTIVE FOLLOW-UP STUDY

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Purpose. Several clinical practice recommendations for subsequent screening for retinopathy in patients with diabetes mellitus have been developed. However, these recommendations were mostly based on expert consensus opinion rather than direct evidence. This study estimated the most appropriate eye screening intervals for early detection of sight-threatening retinopathy (STDR) in patients with diabetes mellitus.

Setting. Funduscopic results of patients attending Hospital of Cruces (Vizcaya, Spain) were reviewed between 1998 and 2004.

Methods. A six-year retrospective follow-up study to review screening results of two cohorts of diabetic patients (i.e., cohort free of diabetic retinopathy and cohort with mild non-proliferative diabetic retinopathy at baseline) was conducted. Patients had been screened by means of a non-mydriatic

retinal camera for the presence of retinopathy. Baseline information related age, sex and diabetes mellitus (type, duration, treatment and metabolic control) was collected. Statistical analysis was based on life table method of risk estimation.
Results. A total of 286 diabetic patients free of retinopathy and 144 patients with mild non-proliferative retinopathy at baseline entered the study. The probability of remaining free of STDR for patients free of retinopathy at baseline was 97% at the end of the fourth year, and those with type 2 diabetes were more likely to progress to STDR ($p<0.01$). For patients with mild non-proliferative retinopathy at baseline, the probability dropped to 94% at the end of the second year and it was still high only for those patients with a glycated hemoglobin level less or equal to 7.5% at baseline.
Conclusions. The recommended interval for screening of STDR in patients free of diabetic retinopathy was 4 years in those who had type 1 diabetes and 3 years for those who had type 2 diabetes. In those who had mild non-proliferative retinopathy, the recommended screening interval was one year or less.

WHEN IS IT SAFE TO DISCHARGE PATIENTS BACK TO RETINAL SCREENING FOLLOWING A COMPLETE COURSE OF PAN-RETINAL PHOTOCOAGULATION FOR PROLIFERATIVE DIABETIC RETINOPATHY?

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Purpose. To determine when it is safe to discharge patients with treated stable proliferative diabetic retinopathy back to retinal screening.
Methods. The authors retrospectively looked at the notes of 88 patients who had pan-retinal photocoagulation [PRP] for proliferative diabetic retinopathy whose last treatment had been at least over 1 year previously. Notes were looked at one, two three and five years after PRP to determine if they had further neovascular growth and/or vitreous haemorrhage after treatment had been considered adequate.
Patients who no longer attended regular follow-up, attended other hospitals or were deceased were excluded.
Results. Of the 88 patients, 35 were stable for 3 years and only 5 were not stable for 3 years. Of the 35 patients, 24 were male and 11 were female and their age ranged from 38 to 85 years. Insulin dependent diabetics were 24 and their duration of diabetes ranged from 6 years to more than 50 years. Reasons for laser were NVD for 29, NVE for 36, vitreous hemorrhage for 21 and rubeosis for 3 patients. Duration of laser treatment ranged from one month to 10 years. Total laser which the patients had ranged from less than 1000 to a maximum of 10534, the mean of which was 3266. After 3 years 4 had NVD and 4 had NVE. However they were stable. After 5 years, 27 were stable. One had mild vitreous hemorrhage, 7 did not have follow up and one died during the period.
Conclusions. At both one year and two years follow-up, after previously completed PRP, 5 patients needed further laser for re-growth of neovascularisation and/or vitreous haemorrhage. Out of the 35 patients who were stable for 3 years or more, only one had mild vitreous hemorrhage (which did not require treatment) during the five year period of follow-up. We conclude that it appears to be safe to discharge patients back to retinal screening if, after completed PRP, they have not had neovascular re-growth or vitreous haemorrhage for at least three years.

BIENNIAL EYE SCREENING IN PATIENTS WITH DIABETES WITHOUT RETINOPATHY: 10-YEAR EXPERIENCE

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Aims. To evaluate the safety of every-other-year eye screening for patients with diabetes without retinopathy.
Methods. Since 1994, patients with diabetes without retinopathy in Iceland have received eye screening every other year. 296 patients with diabetes who had no diabetic retinopathy in 1994/95 were followed with biennial eye examinations until they had developed retinopathy. The 10-year experience of this approach is reviewed.
Results. Out of the 296 diabetic individuals, 172 did not develop diabetic retinopathy during the 10-year observation period. 96 patients developed mild non-proliferative retinopathy, six developed clinically significant diabetic macular oedema, 23 developed preproliferative retinopathy, and four developed proliferative diabetic retinopathy during the 10-year observation period. All the patients who developed macular oedema or proliferative retinopathy had already been diagnosed as having mild nonproliferative retinopathy and entered an annual screening protocol before the sight-threatening retinopathy developed. No patient had any undue delay in treatment.
Conclusions. Every other year screening for diabetic eye disease seems to be safe and effective in diabetics without retinopathy. Such an approach will reduce the number of screening visits more than 25%. This reduces health costs and strain on resources considerably and relieves the patients with diabetes from unnecessary clinic visits and examinations.

SURVEY OF COMPLICATION IN PATIENTS WITH DIABETES ATTENDING MOORFIELDS EYE CLINIC

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Purpose. The National Screening programme for diabetic retinopathy in the United Kingdom is likely to change the patient profile of diabetes eye clinics; only patients with compli-

cations are likely to attend, while others will remain in photographic screening. Patients with diabetic retinopathy requiring treatment are more likely to have other complications and these are likely to influence the resources needed to treat them. The purpose of this study was to survey the complication profile of patients attending the Moorfields Diabetes Eye Clinics before screening was introduced in the neighbourhood.

Results. Patients with diabetes were identified from clinic records during a 4 weeks period; those attending were interviewed by a team of trained interviewers. Overall, 830 patients were identified from the records, 4.3% were registered partially sighted and 4.7% registered blind. Altogether 580 interviews were conducted; the rest either did not attend (141), or could/would not be interviewed (109). Those interviewed were more likely to have had laser treatment in the past (p=0.016); there was no other significant difference between the groups. Mean duration of diabetes was 10 years (3 months to 52 years); 74.3% had Type 2 diabetes; 64.8% reported hypertension. Diabetes related complications were reported by a substantial proportion of patients, with diabetic neuropathy being the most prominent (48.9%), followed by heart disease (24%), diabetic nephropathy (13.4%) and stroke (9.7%). On questioning, 13.1% did not understand the relationship between these complications, diabetes and diabetic retinopathy. On the day of the interview 5.8% had FFA and 13.4% had laser treatment with more than half of the patients expecting it to work immediately (51.6%).

Conclusions. High percentage of our patients had diabetes related complications, even before the implementation of the National Screening Programme in the area. With patients who require no intervention and have minimal diabetic eye disease being removed from the eye clinics, this is expected to rise together with the percentage of patients requiring investigations and treatment. Multiple diabetes related co-morbidity will require special care from the eye units and the needs of the patients will need to be monitored carefully if quality of care is to remain high.

BLINDNESS AND MORTALITY IN A POPULATION-BASED COHORT OF DANISH TYPE 1 DIABETIC PATIENTS: 1973-2006

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Purpose/Background. Diabetic retinopathy remains a leading cause of blindness in the working population in Europe. The aim of this study was to examine the prevalence of blindness and to evaluate the relationship between blindness and mortality in a 33-year follow-up of a population-based cohort of Danish type 1 diabetic patients.

Methods. In the Danish County of Fyn, we have previously identified all insulin-treated diabetic patients with an onset before the age of 30 as of 1 July 1973 (n=727). In November 2006 survival status was evaluated by means of the Danish Central Office of Civil Registration and data on blindness was collected from the Danish Association of the Blind. The Danish Association of the Blind is a voluntary organization open for all patients with a best-corrected visual acuity of the best eye at or below 20/200 (0.1).

Results. Of the 727 patients identified in 1973 72 (9.9%) were at some point registered as blind. Median age at registration at the Danish Association of the Blind was 45.5 years (range 21-70 years) corresponding to a median duration of diabetes of 29.5 years (range 14-64 years). Cumulative incidence proportion of blindness was 11.9% and 8.0% (p=0.08) for patients with an onset before and after 1960, respectively. Cumulative incidence proportion of blindness was higher among women (11.8%) than men (8.5%) but this did not reach statistical significance (p=0.14).

Although statistical significance was not reached, patients who became blind had a higher mortality (62.5%) than patients who did not (52.0%), p=0.09. For patients who became blind and died, median time from blindness to death was 10.7 years.

Conclusions. Despite recent improvements in care, blindness is still a major concern for diabetic patients. In a population-based cohort of Danish type 1 diabetic patients we found a 33-year cumulative incidence proportion of blindness at 9.9%. There was a tendency toward a higher cumulative incidence proportion of blindness among women and among patients with an onset of diabetes before 1960, and a higher mortality was found in legally blind patients.

CORNEAL DIABETIC NEUROPATHY: A NEW HALLMARK OF PERIPHERAL DIABETIC NEUROPATHY

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Purpose. The cornea, the most innervated human tissue, may be affected by a specific diabetic neuropathy: corneal diabetic neuropathy (CDN), mainly involving the corneal subbasal nerve plexus.The aim of this study was to investigate, in diabetic population, the reproducibility and reliability of quantification of corneal subbasal nerve plexus parameters, using in vivo corneal confocal microscopy.

Methods. Sixty two consecutive diabetic patients (107 eyes) were investigated using corneal confocal microscopy (Con-

foscan, Nidek, Japan). A validated technique for subbasal nerve plexus detection was applied. Corneal confocal microscopy parameters for subbasal nerve plexus evaluation were: number and density of nerve fibers, nerve tortuosity, nerve branching, and number of nerve beadings. Two masked examiners evaluated and quantified corneal confocal microscopy images. Peripheral diabetic neuropathy was also assessed using the Michigan Neuropathy Screening Instrument (MNSI). Fifty three normal subjects (96 eyes) served as controls.
Results. Corneal confocal microscopy allowed in all cases a quantitative analysis of subbasal nerve plexus, and showed the presence of CDN even before peripheral diabetic neuropathy was detected by standard non invasive methods ($p<0.001$). No side effects were documented. Intra and inter–examiner agreement for confocal microscopy images were almost perfect ($k= 0.95$ and 0.92 , respectively). Significant decrease of nerve beadings ($p<0.001$) and increase of nerve tortuosity ($p<0.001$) were the most distinctive parameters of CDN. Decrease of nerves density and fibers, and decrease of nerve branching were also documented ($p< 0.001$, <0.001 and <0.005, respectively).
Conclusions. Corneal confocal microscopy is a key diagnostic technique in evaluating and monitoring corneal disorders, namely corneal diabetic neuropathy. Quantification of corneal subbasal nerve plexus parameters allows a correct, reproducible and objective, in vivo, non invasive approach to CDN, allowing to characterize and follow (untreated and treated) peripheral diabetic neuropathy, a potentially highly disabling complication of diabetes.

THE INFLUENCE OF DIABETES MELLITUS TYPE 1 ON THE THICKNESS, SHAPE AND EQUIVALENT REFRACTIVE INDEX OF THE HUMAN CRYSTALLINE LENS

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Purpose. To examine the influence of diabetes mellitus (DM) type 1 on the thickness, radius of curvature, equivalent refractive index and power of the crystalline lens.
Methods. 114 patients with DM type 1 and 75 healthy control subjects were examined by means of Scheimpflug imaging, to determine the thickness and shape of the lens, and with Hartmann-Shack aberrometry, to determine ocular refraction. The equivalent refractive index and the power of the lens were calculated from these parameters. Subjects with cataract or any other ocular pathology were excluded.
Results. The lenses of the patients with DM type 1 were significantly thicker and more convex, compared to those of the control group ($p<0.001$). Furthermore, there was a significant decrease in the equivalent refractive index of the diabetic lenses, compared to the control group. The duration of DM type 1 was an important determinant of lens biometry; the independent effect of the duration of DM per year on lens thickness, anterior radius, posterior radius and equivalent refractive index was respectively 95%, 88%, 207%, and 45% of the effect of age per year. Lens power and ocular refractive error were not affected by DM type 1.
Conclusions. The results of this study show that DM type 1 has a major impact on lens biometry. The decrease in equivalent refractive index of the lens appeared to compensate for the profound increase in lens convexity in patients with DM type 1, resulting in no significant changes in lens power or ocular refraction with the duration of DM.

HARD EXUDATES DEMONSTRATE A PROLONGED IMPLICIT TIME ASSESSED WITH MFERG

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Purpose. To evaluate the influence of hard exudates on macular function in patients with diabetic retinopathy.
Methods. 37 eyes from 27 diabetic patients, aged 14-57 years, (QUERY: Are ranges correct through abstract?) diabetes duration 12.59 years, not previously treated with photocoagulation, underwent fundus photography, multifocal electroretinography (mfERG) and optical coherent tomography (OCT).
Hard exudates were graded from fundus photography with superimposed OCT and a hexagonal pattern (mfERG) by two independent retinal specialists, masked to mfERG and OCT results. We defined three groups; A=eyes with exudates in the analyzed zone and elsewhere, B=eyes with no exudates in the analyzed zone but elsewhere, and C=eyes with no exudates (n=15), which were controls. We compared the mfERG responses from four defined areas in the macula, between the three groups. The average thickness (m) assessed with OCT was measured in the corresponding zones and compared between the groups.
Results. The implicit time was significantly prolonged in group A compared to group C in all four defined areas, 31.9msec vs. 29.1; $p=0.045$, 30.0 vs.29.1; $p= 0.029$, 33.4 vs. 30.3; $p=0.024$ and 31.3 vs. 29.3; $p=0.016$, respectively. A significantly prolonged implicit time was also seen in group B compared to group C in two areas, 33.3 vs. 29.1; $p=0.007$ and 30.7 vs. 29.3; $p=0.032$, respectively. Amplitudes differed

between group A and C only in one area, 14.2 vs. 21.1; p=0.038, and between group B and C in one area, 13.7 vs. 20.1; p=0.045. Macular thickness assessed with OCT was similar between the groups.
Conclusions. In patients with diabetic retinopathy areas with hard exudates demonstrate a significantly prolonged implicit time assessed with mfERG. Our results indicate that hard exudates have an influence on macular function, irrespective of macular thickness.

INTRAVITREAL INJECTION OF BEVACIZUMAB (AVASTIN) FOR PERSISTENT DIABETIC MACULAR EDEMA

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Purpose. To evaluate the efficacy of intravitreal injection of Bevacizumab (Avastin) for the treatment of persistent diabetic macular edema.
Methods. 75 patients with persistent, therapy resistant diabetic macular edema were treated with a 0.05-mL intravitreal injection containing 1.25 mg of bevacizumab. Visual acuity and retinal thickness measurement by optical coherence tomography (OCT) were evaluated at base line and follow-up.
Results. At baseline, mean visual acuity was 0.25±0.18 Snellen letters. In the first 6 months the mean rate of injections was 31, in the following 6 months 11 injection were administered (n= 24). After 6 months the mean visual acuity increased with 1 line Snellen to 0.36±0.21 (p=0.048). After 1 year there was no significant improvement of visual acuity in comparison to baseline visual acuity (p=0.26). Injections after 6 months did not improve or decrease the visual acuity 0.050.1 Snellen) (p=0.48)
Conclusions. In patients with persistent diabetic macular edema not responding to photocoagulation (steroid injections) a small but significant improvement of visual acuity was observed 6 months after treatment with intravitreal injections of Avastin.

TRANSCELLULAR AND PARACELLULAR PERMEABILITY ARE BOTH INVOLVED IN BLOOD-RETINA BARRIER BREAKDOWN IN STREPTOZOTOCIN-INDUCED DIABETES

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Purpose. Disruption of the blood-retina barrier (BRB) is an early phenomenon in preclinical diabetic retinopathy (PCDR). Two pathways may be affected, the paracellular pathway involving endothelial cell tight junctions and transcellular pathway mediated by endocytotic vesicles (caveolae). The relative contribution of both pathways to vascular permeability in PCDR is unknown.
Methods. Transcription levels of genes coding for proteins involved in endothelial cell tight junctions and vesicles were evaluated in intact rat retinas and cultured bovine retinal endothelial cells (BRECs) and pericytes (BRPCs). These were compared with transcription levels in rat retinas at 6 and 12 weeks after streptozotocin (STZ)-induced diabetes and in BRECs exposed to high levels of glucose or VEGF, using real-time quantitative RT-PCR. Protein expression was investigated in rat retinas by immunohistochemistry.
Results. Paracellular transport-related mRNA and protein were specifically expressed by retinal endothelial cells, whereas vesicle transport-related mRNA and proteins were present in various retinal cell types, including endothelial cells. Expression of selected endothelial cell junction-related genes and particularly that of Ocln and Cldn5 was reduced in the diabetic retina and in BRECs but not BRPCs after exposure to high levels of glucose or VEGF. Expression of the majority of vesicular transport-related genes was upregulated after STZ-induced diabetes. Cav1 immunostaining was mainly vascular in vivo, but transcription levels were not upregulated in vitro. The endothelium-specific vesicle protein PV-1 was induced in diabetic retina and in BRECs by high levels of glucose and VEGF in vitro.
Conclusions. The alterations in gene expression indicate that diabetes-induced BRB permeability occurs not only by induction of paracellular leakage, but also by vesicle-mediated transport.

THIAMINE AND BENFOTIAMINE NORMALIZE APOPTOSIS OF HUMAN RETINAL PERICYTES ON HIGH-GLUCOSE CONDITIONED MATRIX

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Purpose/Background. Thickening of basement membrane and pericyte loss are well-know early events in the pathogenesis of diabetic retinopathy. We have demonstrated that human retinal pericyte (HRP) apoptosis is strongly affected by matrix produced in high glucose (Beltramo E et al, 2007). The aim of this study was to verify if thiamine and benfotiamine are able to reverse this phenomenon, when added to high glucose in producing extracellular matrices (ECMs).
Methods. Conditioned ECMs were obtained by growing human endothelial cells (HUVECs) for 7 days in culture media containing physiological (5.6 mmol/L) or high (28 mmol/L) D-glucose with or without 50 and 100 mol/L thiamine and benfotiamine. Cells were then lysed and ECM fixed by NH4OH. HRP were cultured in physiological glucose on these condi-

tioned ECMs. Pericyte apoptosis was evaluated measuring DNA fragmentation by ELISA; the expression of two molecules known to be involved in glucose-mediated apoptosis, Bax with a pro-apoptotic and Bcl-2 with a pro-proliferating function, was measured by RT-PCR and their concentration by ELISA.

Results. Apoptosis in HRP was greatly enhanced by high glucose-conditioned matrix in comparison with physiological glucose ECMs (1.230.48 abs units vs 0.060.02, p=0.002) and this was confirmed by Bax and Bcl-2 mRNA expression (Bax: 193.843.0% of physiological glucose, p=0.008; Bcl-2: 50.313.0%, p=0.001) and protein concentration (Bax: 36.189.8 vs 17.6613.00 ng/mg total proteins, p=0.001; Bcl-2: 0.660.22 vs 1.290.31 ng/mg total proteins, p=0.002). Both thiamine and benfotiamine were able to reverse this effect: DNA fragmentation in HRP cultured on ECMs produced by HUVECs in high glucose plus thiamine and benfotiamine at both concentrations was fully normalized, as well as Bax and Bcl-2 expression and concentration.

Conclusions. Thiamine and benfotiamine are able to counteract the damaging effects of high glucose, probably reducing the glycation of matrix proteins, which, in turn, can lead to damaging interactions between pericytes and their surrounding microenvironment and, finally, to the onset of diabetic retinopathy.

References. Beltramo E, Nizheradze K, Berrone E, Tarallo S, Porta M. High glucose conditioned matrix enhances apoptosis of human retinal pericytes. Eur J Ophthalmol 2007; 17: 466 (abstr).

ESTABLISHMENT OF A HUMAN RETINAL PERICYTE CELL LINE AND COMPARISON OF ITS RESPONSE TO HIGH GLUCOSE WITH WILD-TYPE CELLS

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Purpose/Background. To investigate the pathophysiological roles of pericytes, animal cells have been used so far for in vitro studies. We have previously demonstrated that human retinal pericyte (WT-HRP) are more vulnerable to fluctuating glucose concentrations than bovine retinal pericytes (Beltramo E et al, 2006). Our aims were to establish an immortalized human retinal pericyte cell line and to compare its behaviour with that of wild-type human pericytes, after pulsed exposure to high glucose concentrations.

Methods. WT-HRP were immortalized by electroporation with a plasmid vector containing the Bmi-1 oncogene, that induces telomerase activity. These cells (Bmi-HRP) were characterized for typical pericyte morphology and marker expression, and evaluated for senescence-associated-galactosidase activity. Subsequently, Bmi-HRP were grown in physiological/high glucose for 7 days, and then in physiological glucose for another 24, 48 or 72h. HRP (wild-type and immortalized) were also kept intermittently at 48h intervals in high/normal glucose for 8 days. DNA fragmentation, Bcl-2 and Bax mRNA expression and protein concentration, as markers of glucose-induced apoptosis, were determined.

Results. The oncogene Bmi-1 transfection resulted in the establishment of a permanent pericyte cell line (Bmi-HRP) which showed telomerase activity and facilitated propagation, maintaining the same morphology and typical pericyte markers. Apoptosis in Bmi-HRP increased within 24h of physiological glucose re-entry, but not with continuous exposure to high glucose, as already shown in WT-HRP. Bcl-2/Bax expression/concentration results were consistent with DNA fragmentation in HRP. Intermittent exposure to high glucose increased apoptosis in both type of HRP.

Conclusions. Bmi-HRP have the structural and functional properties characteristic of WT-HRP, suggesting a role for this cell line in studying retinal pathophysiology. Moreover, Bmi-HRP behave like WT-HRP when exposed to fluctuating glucose levels, reinforcing the hypothesis that daily blood glucose fluctuations play a major role in the development of diabetic retinopathy.

DEFECTIVE RETINAL VASCULAR REACTIVITY TO A CHANGE IN BLOOD PRESSURE AS A RISK MARKER FOR DIABETIC RETINOPATHY

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Purpose. We seek to identify early retinal abnormalities that can function as risk markers for diabetic retinal microangiopathy, and thus as informative surrogate endpoints to test drugs for prevention.

Methods. To focus on the abnormalities that are not prevented by the best efforts with current means of controlling hyperglycemia, we studied patients with type 1 diabetes (age 31±9 years, 16F/11M, diabetes duration 12±6 years) on intensive insulin treatment and free of retinopathy. We evaluated retinal hemodynamics (diameter, blood speed, and blood flow in the superior temporal retinal artery) by laser Doppler, foveal thickness by optical coherence tomography, and macular sensitivity by microperimetry (i) at steady-state in comparison to matched nondiabetic controls; (ii) at steady-state in longitudinal observations, and (iii) in response to a stimulus –a postural change from sitting to reclining, which produces an increased blood pressure at the entrance to the retinal circulation.

Results. The retinal measurements at steady-state were similar in the diabetic and control group. Diabetic patients showed over 12 months a decrease in HbA1c from 7.6 1.0 to 7.0 0.7% (p=0.002), and an increase in foveal thickness (from

217±22 to 221±20 m, p=0.01). In response to reclining, only 9 of the 15 diabetic patients tested to date showed a normal decrease in arterial diameter (-6.4±3.8%); the remaining 6 showed no change or an increase. All patterns were repeatable. The two subgroups of diabetic patients differed only with respect to the longitudinal behavior of retinal arterial diameter: unchanged over 12 months in the subgroup with normal vasoconstriction but increased (from 119±8 to 125±11m, p=0.05) in the subgroup without vasoconstriction.
Conclusions. Defective retinal vasoconstriction in response to reclining precedes clinical microangiopathy in a subgroup of well-controlled type 1 diabetic patients. Insofar as the abnormality is qualitative, reproducible, and occurs in response to a vessel-intrinsic stimulus and in association with the widening of arterial diameter, it is a candidate risk marker for retinal microangiopathy. Studies of mechanisms and reversibility are in progress to determine suitability as a surrogate endpoint.

RETINAL FUNCTIONAL ABNORMALITIES IN DIABETIC PATIENTS WITHOUT RETINOPATHY

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Purpose. The pathogenesis of diabetic retinopathy is complex and still remains incompletely understood. The diabetic retinopathy is usually considered as a retinal microangiopathy. However, an increasing number of studies suggest that the impairment of retinal neural cells may precede the earliest signs of retinal vascular impairment. The aim of the study was to assess in a controlled study the retinal functional abnormalities in diabetic patients without clinical appearance of retinopathy.
Methods. Twenty-eight patients with diabetes (11 patients with type 1 diabetes, 17 patients with type 2 diabetes) without diabetic retinopathy on fundus examination, and 28 age- and sex-matched healthy volunteers were included. Each subject underwent a complete ophthalmologic examination, a colour vision test (Lanthony D-15 desatured colour test), a contrast sensitivity test (Peli-Robson test), a pattern-ERG, a full-field ERG, and a multifocal ERG, according to the ISCEV guidelines. The comparison of the results between the 2 groups were assessed using the Student-t test. $p< 0.05$ were considered as the level of significance.
Results. In diabetic patients, the total colour difference score at the D-15 desatured colour test was significantly higher than in healthy volunteers. The implicit times of the oscillatory potentials were significantly longer and the oscillatory potential amplitudes were significantly lower in diabetic patients as compared with the healthy volunteers. The implicit times of the N35 and N95 waves at the pattern-ERG were significantly longer, and the amplitudes of the P50 and N95 waves were significantly lower; the b-wave implicit time at the rod ERG and the a and b- wave implicit times at the single-flash cone ERG were significantly longer in diabetic patients as compared with the healthy volunteers.
Conclusions. Retinal functional abnormalities occurred in diabetic patients before the clinical appearance of diabetic retinopathy. The most early impaired retinal cells seem to be the amacrine and bipolar cells, the ganglion cells and cone photoreceptors cells. These results suggest that an early neurodegenerative process, which could precede the vascular impairment, may occur in diabetic retinopathy

LOSS OF PERICENTRAL INNER RETINAL LAYER IN SEGMENTED OPTICAL COHERENCE TOMOGRAPHY DERIVED TOPOGRAPHIC MAPS OF DIABETIC PATIENTS WITH NO OR MINIMAL RETINOPATHY

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Purpose. A comparison of thickness measurements of segmented optical coherence tomography (OCT) derived topographic maps in patients with diabetes mellitus (DM) and no or minimal diabetic retinopathy (DR) versus healthy controls.
Methods. Ninety nine patients, 59 with type 1 DM, and 40 with type 2 DM, with no or minimal DR underwent full ophthalmic examination, fundus photography and OCT (StratusOCT, Model 3000, Carl Zeiss Meditec, Dublin, CA, USA, software version 4.0.1) using the fast macular thickness protocol. Following automated segmentation mean thickness was calculated for 5 layers: A/ Retinal Nerve Fibre Layer (RNFL), B/ Ganglioncell layer(GCL) + Inner Plexiform Layer (IPL), C/ Inner Nuclear Layer + Outer Plexiform Layer, D/ Outer Nuclear Layer + Inner Segments (photoreceptor), E/ Outer Segments (photoreceptor) in the ETDRS defined regions of the macula and compared to 100 age and sex matched normal controls.
Results. In diabetic patients both the RNFL and the GCL + IPL were significantly thinner in the pericentral area (area 2 to 5 ETDRS region) compared to the controls. The largest averaged difference occurred at 20 A-scans from the fovea, with a mean of 6 micron ($p < .05$). All other areas, and all other layers did not show a significant difference.
Conclusions. The previously described decreased total retinal thickness in the pericentral area in diabetic patients with no or minimal retinopathy is due to a selective loss of thickness in the inner retinal layer (RNFL and the GCL + IPL), and supports the concept of early DR as a neurodegenerative disease.

Poster Presentations

VARYING RETINOPATHY LEVELS WITHIN A NATIONAL SCREENING PROGRAMME. HOW MUCH IS ENOUGH ? A FIRST AUDIT

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Background. The English National Screening Programme for Diabetic Retinopathy is currently provided by 96 locally commissioned programmes. The regulation of these programmes is output based to a series of nationally applied quality standards. External quality assurance is now being put into place and annual report data for the year 2006 2007 from 67 programmes has so far been analysed. Whilst natural variation in retinopathy levels is to be expected in programmes with differing age and ethnicity mixes, it was considered unlikely that the reported levels could be explained on that basis. The range of diabetic retinopathy levels has been reported in a continuous distribution from 14 65% and within this distribution the levels of reported referable retinopathy also varied from 1% to 21%.
Results/Conclusions. This study reports early audit of grading results within external quality assurance visits, for six programmes. It is concluded that despite careful drafting of grading criteria there is still variation between programmes on how the criteria are being interpreted and applied. This has been compounded in some instances by the way that services have configured reporting of retinopathy within their IT systems.

ARBITRATION GRADING AND REFERRAL IN DIABETIC RETINOPATHY SCREENING

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Purpose. In the English National Screening Programme for Diabetic Retinopathy, following first and second full disease grading, differences of outcome and grades may occur. Arbitration grading is assessed across both grade and referral outcome to evaluate primary and secondary grading accuracy.
Methods. Grading disagreements from Screeners, Graders and Optometrists over a 9 month period were reviewed by Arbitrators (KHW, RR, PMD, MC) based within Heart of England Diabetic Retinal Screening Centre (HEDRSC), South, Central and West Birmingham; data was compiled prior to and after the refresher grading training for Optometrists provided by HEDRSC. Results are analysed for grading consistency, and standards achieved. False positive diagnosis and missed sight threatening retinopathy is documented.
Results. 525 arbitration episodes were analysed. 371 were due to first full disease and 127 second full disease grade error; with 27 complete disagreement on both grades by arbitration. 61 encounters (11 %) failed to identify referable sight threatening retinopathy (26 at first disease grade, 35 at secondary). 91 false referrals were identified (85 at first full disease grading). R1 (background retinopathy present) was falsely recorded on 173 first full disease and 28 second full disease grade encounters. Data from Heart of Birmingham and Solihull optometrists compiled 1 month prior to and post additional grading training resulted in a 51% reduction in arbitration grading confirming improved grading accuracy at all levels.
Conclusions. Arbitration grading across both clinical outcome and grade is necessary as a valuable and robust method of ensuring accuracy and detection of retinopathy, ensuring safety and detection of sight threatening retinopathy, whilst reducing unnecessary ophthalmology referrals.

A STUDY OF THE PATTERNS OF EARLY MAGNIFIED MACULA LESIONS TO ASSESS CLINICAL SIGNIFICANCE

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Purpose/Background. Observations from macular digital images of diabetic subjects have shown a number of subtle changes, the significance of which is unknown. Tiny microaneurysms are common but on magnification, a number demonstrate a pale circular rim (MAPCR) which could represent early lipid leakage which could lead to sight threatening clinical significant macular oedema (CSMO). In order to elucidate the nature and significance of these lesions, the authors investigate changes of early lesions in the macula with a six month follow up to identify how these lesions might change.

Methods. Retinal images from patients with diabetes are used to identify spatial distribution of macula lesions, by means of a macula grid. All data including retinopathy grades, visual acuity and outcomes are recorded.
Results. Out of 162 patients being monitored in the Ophthalmic Photographic Diabetic Review (OPDR) clinic, 75 have been re-photographed after six months, 17/75 (23%) have been referred to ophthalmology of which 2 have CSMO, 25/75 (33%) regressed and have been put back to annual recall and 33/75 (44%) continued in OPDR. The lesions were more commonly distributed in the inner region of the macula, compared to the outer region.
Conclusions. The data suggests significance of these macula lesions, with over 60% of leakage and exudation (ophthalmology referral or continued to be re-photographed in OPDR) compared to less than 35% being put on annual review. This lesion of a MAPCR in many cases represents early exudative maculopathy.

IS RE-PHOTOGRAPHING PATIENTS A COST EFFECTIVE METHOD FOR MANAGING EARLY DIABETIC RETINOPATHY?

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Purpose/Background. Digital diabetic retinopathy screening programmes increase the cases of early diabetic retinopathy discovered that fit the national protocol for referral to ophthalmology. Re-photographing outside the screening pathway in an Ophthalmic Photographic Diabetic Review (OPDR) clinic, at a designated time period, is an alternative method of managing these cases. The purpose is to show the proportion of patients monitored in OPDR returning to annual screening or continuing in OPDR, is safe and cost effective as a method of reducing referrals to the hospital eye service.
Methods. Patients with early diabetic retinopathy are re-photographed at a suitable period from 2-6 months. The images are compared to the initial photographs and graded as improved, unchanged or deteriorated. A consultant then decides whether to return the patient to annual screening, continue OPDR or refer to ophthalmology.
Results. From January 1st 2007 to the end of March 2007, 120 referrals were made to ophthalmology, of which 54/120 (45%) images with significant early retinopathy were assessed and followed up in OPDR. 34/54 (63%) have been reviewed so far. 13/34(38%) were returned to annual screening, 19/34 (56%) continued in OPDR and 2/34 (6%) were referred to ophthalmology. Without OPDR all 34 would have been seen in hospital eye clinics costing approximately 3,298. With OPDR, the total cost was in the order of £1,214, making the total saving in the order of £2,084. 32 ophthalmology appointment slots were also made free.
Conclusions. OPDR clinics are a safe, cost effective method of reducing the referral burden in ophthalmology from implementing a new retinopathy screening programme.

DETECTION OF EARLY STAGES OF DIABETIC MACULAR EDEMA

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Purpose. Currently, DME is treated when is fully developed and sight-threatening; and interventions are seldom curative. Seeking to identify preclinical stages of DME might yield useful markers, new insights into pathogenesis, and eventually preventative approaches. Aim of this study was to identify early abnormalities predictive of DME as detected by Spectral-OCT.
Methods. During the period October/December 2007, we evaluated 28 consecutive diabetic patients with levels 20-35 in the ETDRS retinopathy scale but neither symptoms nor signs of DME. For each patient we recorded age, gender, diabetes type, duration and treatment, recent HbA1c value and history of hypertension. All patients underwent complete ophthalmological examination and Spectral-OCT. A library of macular images obtained by Spectral-OCT in age-matched individuals without diabetes or retinal diseases was evaluated in parallel.
Results. The type 1 diabetes group included 17 patients (31 eyes), 5F/12M. Age range was 11-38 years, (QUERY: Are ranges correct throughout abstract?) diabetes duration 9.7-17.3 years, HbA1c 81.5%; hypertension in 23.5%, ETDRS-20: 6 eyes. OCT showed foveal thickness 223.9±29.7 (QUERY: is ± correct here and throughout abstract?) microns, and in 6/25 eyes with ETDRS-35(24%) small iporeflective zones in the inner nuclear layer appearing as microcysts. In the type 2 diabetes group (11 patients, 19 eyes; 7F/4M; age 61.79, diabetes duration 10.7±6.5, HbA1c 7.61.6%, hypertension in 63.6%, ETDRS-20: 2 eyes) foveal thickness was 280.2±87.3 microns, and microcysts were present in 16/17 eyes with ETDRS-35(94.1%). No such images were detected in the controls.
Conclusions. Retinal abnormalities with the appearance of microcysts are detectable in eyes with retinopathy level ETDRS-35 and no clinical DME, with prevalence higher in type 2 than in type 1 diabetes. Further studies and longitudinal follow up will clarify if the abnormalities are part of the natural history of DME.

INDIVIDUALISATION OF SCREENING INTERVALS REQUIRES CONSIDERATION OF RISK FACTORS OCCURRING OVER TIME AND DIFFERENCES IN RISK RELATED TO SEX

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Background. Vision threatening diabetic retinopathy is discovered by fundus photographic screening. The screening interval is defined on the basis of diabetes type, duration of the disease and retinopathy so that patients with aggressive disease progression are detected. However, having standardized screening intervals implies that patients with slow disease progression will experience superfluous examinations. This may be optimized by individualizing screening intervals on the basis of the patients' individual risk factors. The authors therefore set out to build a screening model for optimizing intervals for screening for diabetic retinopathy by including other risk factors, such as HbA1c and blood pressure, in the decision making.
Methods. Data from 31,590 screening examinations, 81.228 measurements of HbA1c and 16.189 blood pressure measurements in 10.039 diabetic patients (2274 type 1 and 7765 type 2) followed since 1994 were included in a decision model based on methods from survival analysis and cox-regression. The present study reports the input data to the model.
Results. Mean artery pressure decreased 0.85mmHg/year from baseline = 103.8mmHg for women and 0.97mmHg/year from baseline = 106.5mmHg for men ($p<0.001$) for both trends. HbA1c decreased in type 1 diabetic patients: from 8.4% to 8.2% in women and from 8.9% to 8.5% men, and in type 2 diabetes: from 8.9% to 8.0% in women and from 8.5% to 7.9% in men ($p<0.001$ for all comparisons). Using a modified ETDRS grading scale there was no significant difference in the retinopathy grade between the two sexes. (Pearson correlation, p-value = 0.22.). There was a significant overrepresentation of men among type 2 diabetic patients in the data set (4449 men vs 3397 women, $p< 0.01$), but no difference among the type 1 diabetic patients (1174 vs 1082, $p=0.06$). Furthermore, after 30 years duration of type 1 diabetes men had been treated more frequently than women (55% of men vs 65% of women, Kaplan-Meyer plot).
Conclusions. It is possible to develop an individualized screening system including other variables than diabetes type, duration and retinopathy grade. These models should take into account changes in risk factors occurring over time and differences in risk of developing retinopathy related to sex.

PREVALENCE OF VASCULAR RISK FACTORS IN PATIENTS ATTENDING A DIABETIC EYE SCREENING PROGRAMME: PRIMARY VERSUS SECONDARY CARE

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Purpose/Background. Control of glucose, blood pressure (BP) and cholesterol can slow progression of retinopathy. The authors aimed to assess the control of vascular risk factors for morbidity in diabetic patients attending a retinopathy screening programme, under either primary (PC, general practitioner and practice nurse) or secondary (SC, hospital endocrinologist) care.
Methods. Cross-sectional observational study on patients screening positive for sight-threatening diabetic retinopathy (STDR, defined as moderate pre-proliferative retinopathy or greater and/or ciricinate maculopathy or exudates within one disc diameter of centre of the fovea), ungradable images or other significant eye disease in either eye and consequently attending for slit-lamp biomicroscopy. Data were collected on demography, glycaemic control (HbA1c), blood pressure (BP) and dyslipidaemia and compared to national targets. Poor glycaemic control was defined as HbA1c >8%. Undiagnosed hypertension was defined as systolic >140mmHg and/or diastolic >90mmHg. Targets in known hypertension: type 1 130/80mmHg, type 2 140/80mmHg. Total random cholesterol (TC) target was <5mmol/L.
Results. 1447 patients attended between June 6, 2006 and September 1, 2008: mean age 66.5 (21-93) yrs; mean duration of diabetes 8.9 (<1-50) yrs; 131 type 1 (3.4% PC: 25.8% SC) and 1263 type 2 (96.6% PC: 74.2% SC). HbA1c, BP and TC results were available for 823, 1385 and 706 patients respectively.

	Primary care mean ± SD		Secondary care mean ± SD	
	Type 1	**Type 2**	**Type 1**	**Type 2**
HbA1c (%)	7.6±1.7	7.2±1.6	7.6±1.7	7.5±1.6
Systolic BP (mmHg)	151±20	145±22	145±20	145±21
Diastolic BP (mmHg)	81±19	78±31	81±13	79±13
Total cholesterol (mmol/L)	4.3±0.84	4.0±1.1	4.2±1.3	3.9±1.1

	Primary care targets failed (%)		Secondary care targets failed (%)	
	Type 1	**Type 2**	**Type 1**	**Type 2**
HbA1c	18.8	20.1	29.7	27.7
BP	42.9	51.0	65.2	68.8
Total cholesterol	21.4	12.7	18.2	11.7

Conclusions. This study provides useful current estimates of prevalence during a time of changing diabetes management. Risk factors appear to continue to be poorly. **(QUERY: Please complete sentence)**

PREVALENCE OF VASCULAR RISK FACTORS IN SIGHT THREATENING DIABETIC RETINOPATHY IDENTIFIED IN A RETINOPATHY SCREENING PROGRAMME

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Purpose/Background. To assess vascular risk factors in patients with sight threatening diabetic retinopathy (STDR) identified from a primary care based retinopathy screening programme.
Methods. Cross-sectional observational study on patients screening positive for STDR (defined as moderate pre-proliferative retinopathy or worse and/or circinate maculopathy or exudates within one disc diameter of centre of fovea), ungradable images or other significant eye disease in either eye and consequently attending for slit-lamp biomicroscopy by retinal specialists. Data were collected on demography, glycaemic control (HbA1c), blood pressure (BP) and dyslipidaemia and compared to national targets. Poor glycaemic control was defined as HbA1c >8. Undiagnosed hypertension was defined as systolic >140mmHg and/or diastolic >90mmHg. Targets in known hypertension: type 1 130/80mmHg; type 2 140/80mmHg. Total random cholesterol (TC) target set <5mmol/L.
Results. 1447 patients attended between June 6, 2006 and August 1, 2008. 21% of patients had STDR, of whom 21% had type 1 and 78% type 2 diabetes. Mean age was 66.5 (21-93) yrs; mean duration of diabetes 8.9 (<1-50) yrs.

Risk Factor	STDR		non-STDR	
	mean ± SD	% Target failure	mean ± SD	% Target failure
HbA1c (%)	8.35 ± 2.10	50	7.13 ± 1.50	17
Diastolic BP (mmHg)	79 ± 12	14	78 ± 29	14
Systolic BP (mmHg)	144 ± 23	52	145 ± 23	56
Total cholesterol (mmol/L)	4.3 ± 1.3	27	4.0 ± 0.9	16

Statistical analysis using the Kolmogorov-Smirnov test (α<0.05, one-sided) showed STDR to be related (H0=1) to HbA1c (strongly), and to cholesterol and diastolic BP, but not related (H0=0) to systolic BP.
Conclusions. Vascular risk factors appear poorly controlled in patients with STDR, with the mean HbA1c being significantly higher than current targets. Uncontrolled glycaemia seems to be most strongly correlated with the presence of STDR and requires particular attention in pathways of patient care.

DUTCH PATIENTS' INCENTIVES AND BARRIERS TO PARTICIPATING IN DIABETIC RETINOPATHY SCREENING

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Background. In the Western world diabetic retinopathy is one of the most important causes of acquired visual impairment and blindness among adults up to 65 years. Because 80% of this can be prevented with laser-coagulation, early detection of diabetic retinopathy by means of screening is necessary. Nevertheless, optimizing compliance to this retinopathy screening is shown to be difficult. The authors assessed the prevalence of participation in diabetic retinopathy screening among patients with diabetes in Dutch general practices and investigated patients incentives and barriers to participating.
Methods. A questionnaire was sent to all patients (aged 18 years and over) with a diagnosis of diabetes enlisted in 20 general practices (n=3241). This questionnaire asked for participation in retinopathy screening in the last 3 years and for the relevance of incentives and barriers to participating.
An inventory of these incentives and barriers was made by analysis of five focus-group interviews with patients with diabetes. These were attended by both participants and nonparticipants in screening, both urban and rural living patients, including immigrants and active members of the Dutch Diabetes Association.
Results. In total 2051 patients (response 63.3%) participated in our study, of which 1688 (82.3%) had attended retinopathy screening in the last 3 years and 363 (17.7%) had not. No differences in participation were found between men and women (86.5% vs. 86.8%, p=0.822). Participation did not increase with age (p=0.913). Participation was higher among high educated patients with diabetes compared to low educated patients (87.5% vs. 78.9%, p=0.003).
Focus-group results showed motivational factors to consist of 3 domains, being sense of duty, medical considerations and fear. Lack of knowledge, doubting medical necessity, practical inconveniences (e.g. immobility and long waiting time), fear and religious grounds were reasons not to attend screening. Quantitative analyses of these incentives and barriers are currently performed.
Conclusions. 17.7% of Dutch patients with diabetes in general practices did not participate in retinopathy screening in the

last 3 years. Clues to raise compliance are both emphasizing incentives and intervening in barriers. Quantitative data regarding the relevance of several incentives and barriers will be presented at the congress.

IMPACT OF PATIENTS' AND PHYSICIANS' ATTITUDES ON DIABETIC RETINOPATHY IN TYPE 1 DIABETES: 3 CASE REPORTS

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Purpose. Evaluation of patients and physicians attitudes to diabetic retinopathy in type 1 diabetes.
Methods. Distinct attitudes are shown in three case reports. The authors study the history, ophthalmological examination, treatment and outcome.
Results. Case 1: female, born 1978, diabetes since 1982, regularly monitored at our department since 1986. First symptoms of diabetic retinopathy (DR) in 2004, visual acuity (VA) 5/10, 5/7.5, proliferative bilateral DR found, panretinal photocoagulation carried out. Got pregnant in 2006 after a consultation, has an insulin administration device. Photocoagulation spots continuously supplemented. Confinement in February 2007. Autumn 2007 VA 5/5, 5/5 with a correction, findings stabilized.
Case 2: male, born 1957, diabetes since 1970, regularly monitored in his home. In September 2007, visits due to deteriorating visual acuity on the left eye. VA 5/7.5, 1/50. Bilateral proliferative diabetic retinopathy with a total traction detachment found on the left. A panretinal photocoagulation performed on the right and a pars plana vitrectomy on the left. Autumn 2007 VA 5/10, 1/50, on the left the finding stabilized, the retina is lying, but the VA does not improve.
Case 3: male, born 1987, diabetes since 1991, regularly monitored by his physician, first symptoms of DR in 2004. Focal photocoagulation begun in July 2006. VA then 5/10 and 5/7.5. Inappropriate job as a cook. Does not observe the diet, diabetes subcompensated, refuses an insulin administration device. In January 2007 renal functions decompensated and secondarily ophthalmologic findings to proliferative DR bilaterally, panretinal photocoagulation launched. In August a hemophthalmus on the left, 2 months later a PPV carried out, VA 5/15 and 1/30.
Discussion. Patient 1, perfect cooperation of the patient with her physician. Patient 2, what has the physician been studying during the examinations? Patient 3, one can not help those who will not follow advice.
Conclusions. Optimal cooperation between physician and patient helps avoid serious complications. On the other hand, lack of cooperation leads to substantially faster progress of complications. The worst situation occurs when the patient attends the examinations but the physician fails to notice the progress of the finding.

WHITE SPOTS IN THE MACULA OF PATIENTS WITH DIABETES MELLITUS TYPE 1, WITHOUT OR WITH MINIMAL DIABETIC RETINOPATHY, EVALUATED WITH SPECTRAL DOMAIN OPTICAL COHERENCE TOMOGRAPHY

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Purpose/Background. To evaluate white spots seen in the macula of patients with diabetes mellitus (DM) type 1, without or with minimal diabetic retinopathy (DR) with spectral domain optical coherence tomography (SD-OCT).
Methods. Forty-five patients with DM type 1, without or with minimal DR on biomicroscopy, underwent a full ophthalmologic examination, including best-corrected VA, slitlamp examination, funduscopy, (red-free) fundus photography and OCT scanning of the macula. At the same day, HbA1c, blood pressure and total cholesterol were measured. Small white spots (diameter 10 micron) seen on (red-free) fundus photography scattered around the fovea were examined on corresponding B-scan images using SD-OCT (3D OCT-1000, Topcon).
Results. Ninety eyes of 45 patients were included in the study. Fundus photography showed white spots in the central fundus in 39 eyes (28 patients), the median number of spots per eye was 5, range 1 to 50. High definition b-scan images corresponding with the localization of the white spots showed hyperreflective lesions at the level of the retinal nerve fiber layer (RNFL). There was no correlation found between the presence of white spots and the presence of DR, nor with high blood pressure, HbA1c or total cholesterol.
Conclusions. White spots seem to be a common occurrence in the retina of patients with DM type 1, without or with minimal DR. Previous studies located these white spots at the level of the retinal pigment epithelium, based on stereoscopic slitlamp examination. In contrast, the authors localized the white spots at the level of the RNFL using the SD-OCT. This implicates that these white spots are not small depigmented lesions of the RPE. Their true nature is still unclear.

CENTRAL SEROUS CHORIORETINOPATHY AND DIABETES: THE MISSING LINK

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Purpose. This consecutive case series aimed to establish the etiopathological role of diabetes in the development of central serous chorioretinopathy (CSCR) and to aid early visual recovery.
Methods. Four consecutive patients presenting with CSCR and found to have diabetes were included in this 12-month

study at a major teaching hospital. They underwent thorough ophthalmic assessment, optical coherence tomography (OCT) and fundus fluorescein angiography (FFA) which showed the classic leakage within the neurosensory detachment.
Results. Subretinal fluid of varying degree and pigmentary disturbances in the macula were noted in all cases and CSCR was confirmed by OCT and FFA. Three of the four patients had recent onset of diabetes. One of these had pregnancy induced diabetes at the onset of CSCR. The fourth patient was a known diabetic with recently uncontrolled sugar levels which coincided with the onset of his visual symptoms of CSCR. None of the patients had diabetic retinopathy with the onset of the CSCR.
Conclusions. CSCR was thought to be idiopathic in aetiology and run an unpredictable course. It has been associated with the young, stressed male prototype. It has also been associated with higher levels of cortisol which influence the retinal pigment epithelium junctions, allowing breaks and the development of CSCR. Higher levels of cortisol are found in stress, diabetes and pregnancy, which could be the missing aetiological link for the development of CSCR. To our knowledge this is one of the few series correlating the aetiopathology of CSCR with diabetes. CSCR could be an early manifestation of diabetes emphasizing the importance of early detection and treatment of diabetes and hence the early resolution of CSCR.

CHANGES IN THE ANTERIOR CHAMBER ANGLE IN DIABETIC PATIENTS WITH SECONDARY NEOVASCULAR GLAUCOMA USING OCT VISANTE

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Purpose. To document the changes in the anterior chamber angle in diabetic patients with secondary neovascular glaucoma (SNVG) using OCT Visante.
Methods. OCT Visante (anterior segment optical coherence tomography) is an imaging technology enabling anterior segment evaluation including corneal thickness and anterior chamber angle anatomy. The clinical sign of SNVG is rubeosis iridis with elevated intraocular pressure (IOP). The authors examined the anterior segment in diabetic patients with SNVG using OCT Visante and, subsequently, assessed corneal thickness, anterior chamber depth, and potential angle adhesion. The authors compared findings with the other eye of the patient. The examination is noninvasive, noncontact, and without mydriasis.
Results. The thickness of the cornea was bigger in the eyes with SNVG than in the other eyes. The worst finding proved 976 microns thickness of the cornea in its centre (nearly twice the thickness in comparison with the other eye). The depth of the anterior chamber firstly depends on whether the patient is phakic or pseudophakic, and secondly on the presence of adhesions in a chamber angle. In the eyes with SNVG and with the adhesions in an angle, the anterior chamber was in all cases shallower than in the other eye. One of the patients from the group, who has SNVG on both eyes, showed the following: the depth of his right anterior chamber was 1,92, the left 2,12. In the right eye, where the anterior chamber was shallower, the clinical finding was worse, the atrophy of the iris was bigger and there were more adhesions in the angle.
Conclusions. OCT Visante is very helpful in diagnostics and in monitoring the changes of the angle in diabetic patients with SNVG. The authors can measure the angle, document in all sections and evaluate in the course of time. The authors always compare the finding in an angle with gonioscopy. During gonioscopy, a problem may arise when a physician presses the cornea and this pressure opens an angle. This is why gonioscopy with the use of OCT Visante is revolutionary it can never be distorted.

THE DIFFERENCE IN FOCAL PHOTOCOAGULATION TREATMENT OF CLINICALLY SIGNIFICANT MACULAR EDEMA DIAGNOSED WITH OCT VERSUS SLITLAMP BIOMICROSCOPY.

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Purpose. To evaluate the difference in focal photocoagulation treatment of clinically significant macular edema (CSME) diagnosed with StratusOCT versus stereo slitlamp biomicroscopy.
Methods. Twenty-five eyes of 16 diabetic patients with a clinical suspicion of CSME were examined by stereo slitlamp biomicroscopy and StratusOCT to determine the exact location and extent of the possible CSME. Results of these observations were drawn into a set of FA images (early/mid/late phase) and color fundus photographs. Based on these images, observers, masked for the method of assessment of CSME, marked the position, pattern, and number of laserspots on the corresponding color photograph.
Results. The authors found a difference in location and extent of CSME in 11 eyes (44%) comparing StratusOCT with stereo slit-lamp biomicroscopy. Treatment decisions based on these different observations differed substantially with respect to the amount and localization of the laserspots.
Conclusions. The focal photocoagulation treatment for CSME based on StratusOCT differs from the focal photocoagulation

treatment based on stereo slitlamp biomicroscopy with respect to the amount and localization of the laser spots. Future studies need to address whether this difference has clinical relevance.

ANTI-VEGF THERAPY IN NEOVASCULAR GLAUCOMA

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Purpose. To describe the management of neovascular glaucoma by intravitreal injection of anti-VEGF molecules.
Patients and Methods. Nine eyes of 9 patients presenting with neovascular glaucoma secondary to proliferative diabetic retinopathy (6 eyes) or retinal vein occlusion (3 eyes) were treated by intravitreal injection of bevacizumab (6 eyes) or ranibizumab (3 eyes).
Results. Initial average IOP was 42.9 mmHg (ranging from 25 to 66 mmHg), and at 1 month it decreased to an average of 23.8 mmHg (ranging from 10 to 50). Fluorescein angiography of the iris showed decrease of leakage.
Three cases were unsuccessful due to secondary obstructive glaucoma with irreversible fibrovascular tissue present in the iridocorneal angle. They were subsequently treated by diode laser cyclophotodestruction (2 eyes) or trabeculectomy (1 eye).
Discussions. VEGF is implicated in development of prepapillary, preretinal and iris neovascularization. In this study, in spite of short follow-up, anti-VEGF therapy showed promise in treating iris neovascularization. A larger, randomized trial may provide guidance on precise timing and frequency of injections.

INTRAVITREAL TRIAMCINOLONE INJECTION FOR DIABETIC MACULAR OEDEMA

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Purpose/Background. Treatment of diabetic macular oedema with laser is not always possible or successful. The authors describe results of intravitreal triamcinolone injection in these patients.
Methods. Prospective trial, in which patients were given 4 mg of triamcinolone by intravitreal injection, with minimum follow-up of 3 months.
Results. In 22 eyes (18 patients) with diabetic macular oedema (OCT >250 micron) and visual acuity <0.5, in which laser therapy was unsuccessful, or impossible, mean visual acuity was 0.25 (Snellen) before injection. Three months after the injection, mean visual acuity was 0.31.
Mean central macular thickness was 506 micron before injection, 333 micron after 3 months.
In 43% of eyes intraocular pressure was >22 mmHg after the injection; in only one eye intraocular pressure was > 30 mmHg. Medical therapy was sufficient to lower intraocular pressure. There were no other complications.
Conclusions. Intravitreal triamcinolone injection is a successful treatment for diabetic macular oedema in the short term.

INTRAVITREAL TRIAMCINOLONE ACETONIDE AND BEVACIZUMAB IN DIABETIC MACULAR EDEMA TREATMENT

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Purpose. To assess the effect of intravitreal injections of Triamcinolone Acetonide and Bevacizumab combined with laser coagulation on the diabetic macular edema (DME).
Methods. 137 eyes of 103 patients with DME were enrolled into two groups: 1st- 75 patients (96 eyes) who received 4 mg of intravitreal triamcinolone acetonide (IVT group) and 2nd - 28 patients (41 eyes) who received 1.25 mg of intravitreal bevacizumab (IVB group). All patients underwent modified grid laser coagulation after injections. Central macular thickness (CMT), total macular volume (TMV), measured by optical coherence tomography, and visual acuity (VA) were assessed. The follow-up time was 9-12 months for IVT group and 6 months for IVB group.
Results. Maximum VA improvement was in 3 months for IVT group and in 1 month for IVB group (IVT group: VA at baseline 0.27, at 1 month 0.32, at 3 months 0.33, at 6 months 0.29, at 9 months 0.20, at 12 months 0.20; IVB group: VA at baseline 0.28, at 1 month 0.35, at 3 months 0.31, at 6 months 0.29). In both groups decrease of CMT and TMV was registered by OCT during the follow-up period, the most evident one being at 1 month after injections: IVT group: mean CMT (microns)/TMV (mm^3) at baseline 519.3/10.8, at 1 month 343.6/ 8.9, at 3 months 353.3/ 8.8, at 6 months 416.9/ 9.6, at 9-12-months 399.1/ 9.6, correspondingly; IVB group: mean CMT (microns)/TMV (mm^3) at baseline 497.6/9.8, at 1 month 360.1/8.4, at 3 months 381.1/8.4, at 6 months 387.4/ 8.6, correspondingly. IOP elevation was noticed in 37.6% of the IVT group.
Conclusions. Intravitreal injections of triamcinolone acetonide or bevacizumab in combination with retinal laser coagulation may be an effective treatment of DME.

LANREOTIDE AUTOGEL FOR PERSISTENT DIABETIC MACULAR EDEMA: A PROSPECTIVE STUDY OF EFFECTIVENESS AND QUALITY OF LIFE

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Purpose. To evaluate the effectiveness of the somatostatin analogue lanreotide Autogel in patients with persistent diabetic cystoid macular edema (CME) and their quality of life.
Methods. Case series, prospective study. Two patients treated with a deep subcutaneous injection of lanreotide Autogel of 90 mg every 4 weeks were monitored at baseline and at 3, 6 and 12 months. Each patient had a complete medical examination including an upper abdominal ultrasound screening for gall bladder disease, best visual acuity (VA) measured by the logMAR chart, and foveal thickness that was documented using Optical Coherence Tomography (OCT). Data on age, sex, diabetes mellitus, and health-related quality of life (SF-12 and VF-14 questionnaires) were also collected.
Results. One year later stabilization of VA was observed in all eyes, and foveal thickness decreased by 38.2% on average by OCT. The SF-12 mental health domain increased in both patients getting closer to population norms, and visual functioning improved by VF-14. The adverse reactions were mild and subsided after 3 months of treatment.
Conclusions. Monthly subcutaneous injections of lanreotide Autogel offered an effective treatment alternative in patients with persistent diabetic CME and poor glycemic control.

VITRECTOMY IN THE TREATMENT OF DIABETIC MACULAR OEDEMA

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Purpose. To evaluate the efficiency of vitrectomy with peeling of the internal limiting membrane (ILM) in eyes with chronic diffuse and/or cystoid type of diabetic macular oedema and to perform ultrastructural histopathologic and morphometric analysis of the ILM.
Methods. The prospective study involved 56 eyes of 52 diabetic patients mean age 63±7.6 years. These patients were operated between January 2006 and June 2007. Vitrectomy with trypan blue associated peeling of the ILM was performed in a standard way. Mean period of observation of patients after vitrectomy was 8.7 months. The ILM was fixed immediately after peeling in 2.5% glutaraldehyde and submitted for electron microscopic evaluation. The ILM was photographed in standard magnification (x 5 000) with the scale of 1 m in the shot.
Results. Statistical analysis proved general improvement of the post-operative visual acuity (VA) with the prevalence of the resulting VA in intervals 0.1, 0.2 and 0.5±1.0 (related to ETDRS table). Morphometric analysis demonstrated a significant thickening of the ILM in all eyes with a mean thickness of the ILM 3.61±0.91 m.
Conclusions. Vitrectomy with peeling of the ILM in eyes with chronic diabetic macular oedema mildly improves the VA and extends hope for its stabilization. Morphometric and histopathologic analysis of the ILM contributes to more objective evaluation of ultrastructure of the vitreomacular interface.

25-GAUGE VITRECTOMY AS THE GOLD STANDARD FOR SEVERE PDR AND MACULAR EDEMA

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Purpose. To examine whether 25-gauge vitrectomy might be a good approach for treatment of severe proliferative diabetic retinopathy (PDR) and macular edema.
Methods. 31 patients with severe PDR and macular edema were randomized to either 20 or 25-gauge vitrectomy. The 25-gauge vitrectomy was performed with bimanual technique, 27-gauge chandelier illumination, Tano diamond dusted scraper. Sixteen patients underwent 25-gauge vitrectomy.
Results. Incidence of complications like iatrogenic retinal tears and bleeding were significantly less in the 25-gauge vitrectomy group when compared to the 20-gauge vitrectomy group. The efficacy between the two techniques was similar. The 25-gauge vitrector was used to cut fibrinous tissue with high accuracy and was found to replace well curved scissors usually used with the 20-gauge technique.
Conclusions. 25-gauge vitrectomy might represent the gold standard for treatment of severe pdr and macular edema.

THE POTENTIAL ROLE OF URIC ACID IN PATHOGENESIS OF DIABETIC RETINOPATHY

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Purpose. To evaluate the influence of impaired vascular permeability in diabetic retinopathy (DR) on vitreous levels of biochemical analytes.

METHODS. During pars plana vitrectomy undiluted vitreous samples were obtained from 55 patients with nonproliferative DR (NPDR), 24 patients with proliferative DR (PDR) and 48 controls with nondiabetic ocular disease. Simultaneously venous blood samples were obtained. Biochemical analytes levels (HbA1c, uric acid, glucose, electrolytes) were estimated by standard clinical chemistry methods.
RESULTS. Uric acid (UA) levels both in serum and vitreous were significantly higher in diabetic patients than in controls ($p<0.001$ serum; $p=0.024$ vitreous). Type of DR correlated significantly with UA vitreous levels, which was higher in PDR ($p=0.012$). The authors observed that UA vitreous level is not related to HbA1c, duration of diabetes, type of diabetes, or insulin therapy. Leakage rate of UA through blood-retinal barrier (BRB) is 1.876 times higher in patients with DR than without DR ($p<0.001$) and 1.168 times higher in patients with PDR than with NPDR ($p=0.03$).
CONCLUSIONS. The results of our study suggest that increased level of uric acid present in serum and vitreous of diabetic patients may contribute to the pathogenesis and progression of DR. UA may influence vascular endothelial dysfunction and increase permeability at PDR. These findings present opportunities for novel modalities in prevention of DR.

SHORT TERM DYNAMIC CHANGES IN THE RETINAL MICROCIRCULATION ARE INVOLVED IN THE PATHOGENESIS OF DIABETIC RETINOPATHY

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BACKGROUND. The pathophysiology of diabetic retinopathy is unknown, but it is assumed that disturbances in retinal blood flow including reduced autoregulation are a central part of the disease pathogenesis. However, diabetic retinopathy is characterized by a predominance of lesions corresponding to the microcirculation far away from the blood pressure trauma, and a fast turn-over of retinal lesions which may be a central phenomenon in the disease pathogenesis. Therefore, there is a need for studying dynamic changes in retinal blood flow and the resulting dynamic changes in retinopathy lesions.
METHODS. Six diabetic patients were scheduled every other week during 6 months for measurement of visual acuity, fundus photography, and measurement of the risk factors blood pressure, blood glucose and HbA1c. The fundus images were aligned, and changes in retinal morphology were related to known risk factors for developing diabetic retinopathy.
RESULTS. There was no relation between the short-term dynamic changes in retinal lesions occurring within weeks and known risk factors for developing diabetic retinopathy that only changed minimally within the study period ($p>0.05$ for all comparisons). The results and possible explanations for the findings are demonstrated by playing movies of the observed dynamics of vascular lesions in diabetic retinopathy.
CONCLUSIONS. Other factors than disturbed autoregulation and blood glucose control are co-involved in the pathogenesis of diabetic retinopathy. There is a need for elucidating short-term dynamic phenomena in the retinal blood flow responsible for the dynamic turn-over of diabetic retinopathy lesions.

N-METHYL-D-ASPARTIC ACID CAUSING RELAXATION OF RETINAL ARTERIOLES THROUGH AN ADENOSINE RECEPTER DEPENDENT MECHANISM: A POSSIBLE MECHANISM OF VASODILATION IN DIABETIC RETINOPATHY

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BACKGROUND. Disturbances in retinal perfusion due to impaired regulation of vascular tone are believed to be involved in the pathogenesis of diabetic retinopathy. These changes may be related to changes in the metabolism of glutamate and adenosine, but it remains to be elucidated in in vitro studies whether the relaxing actions of the two substances are separate or coupled.
METHODS. Porcine retinal arterioles with preserved perivascular retinal tissue were mounted in a myograph for isometric tone measurements. Changes in tone were induced by increasing concentrations of NMDA in the presence of blockers of adenosine receptors and ATP hydrolysis. Additionally, changes in tone were induced by increasing concentrations of adenosine in the presence of the NMDA receptor blocker, DL-APV. All concentration-response experiments were repeated after the perivascular tissue had been removed.
RESULTS. NMDA produced a concentration-dependent relaxing effect on retinal vessels with preserved perivascular retinal tissue ($p<0.001$) which disappeared after removal of this tissue. Blocking of the NMDA receptor, adenosine receptors, and hydrolysis of ATP significantly reduced the vasorelaxing effect of NMDA in the presence of perivascular retinal tissue ($p<0.05$ for all three comparisons). On the other hand, adenosine produced a concentration-dependent relaxation that was not significantly affected by blocking the NMDA receptor with DL-APV, either on isolated arterioles ($p=0.08$) or on arterioles with preserved perivascular tissue ($p=0.22$).
CONCLUSIONS. The findings suggest that the vasorelaxing effect of NMDA in porcine retinal arterioles in vitro is mediated by hydrolysis of ATP to adenosine in the perivascular retinal tissue. This may be a potential mechanism for producing the vasodilation leading to hyperperfusion in diabetic retinopathy.

INTRACELLULAR CA2+ SPIKES IN RETINAL VASCULAR SMOOTH MUSCLE CELLS CAN BE MODIFIED TO POTENTIALLY IMPROVE MICROCIRCULATION IN RETINAL DISEASE

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Purpose. Disturbances in the regulation of the retinal blood flow are involved in the pathophysiology of a variety of sight-threatening diseases, including diabetic retinopathy. Therapeutic intervention on these diseases on a rational basis requires a detailed knowledge of the mechanisms involved in the regulation of the tone in retinal resistance arterioles. This tone depends on recruitment of intracellular calcium in the vascular smooth muscle cells.
Methods. Porcine retinal arterioles with a diameter of approximately 150 m were mounted in a wire myograph (DMT) and placed in a Zeiss LSM 5 Exciter confocal microscope allowing for simultaneous recording of vascular tone and calcium activity. The vessels were loaded with the calcium sensitive fluorophore Oregon Green and were pre-contracted with the prostaglandin analogue U46619. The vascular tone and the concentration of free calcium was studied after application of Nifedipine 10-10M – 10-6M (blocking of L-type Ca2+-channels in the plasma membrane), and cyclo piazonic acid 10-10M – 10-6M and ryanodine 10-10M – 10-6M (Ca2+-channels in the sarcoplasmic reticulum).
Results. Ryanodine did not affect the rate of spontaneous calcium spikes nor the vessel tone (p=0.8749 and p=0.98 one-way ANOVA n=6, respectively). Cyclo piazonic acid reduced the rate of intracellular calcium spikes significantly; EC50 $2.5*10^{-8}$ [$2.34*10^{-7}$; $3*10^{-9}$] (p< 0.0022, n = 5, repeated measures ANOVA). However, vessel tone was not affected (p=0.059 n=6, repeated measures ANOVA). Preliminary experiments showed that nifedipine induced an increase in the rate of spontaneous intracellular calcium in parallel with a marked decrease in vascular tone.
Conclusions. The recruitment of intracellular calcium in porcine vascular smooth muscle cells does not depend on ryanodine channels in the sarcoplasmic reticulum, but rather the IP3 channels. This feature is different from observations in other tissues. The intracellular calcium spikes can be modified using known pharmacologic drugs, including those used for the treatment of systemic cardiovascular disease in diabetic patients. This knowledge may serve as a way to intervene in diseases where the regulation of the blood flow is disturbed, such as diabetic retinopathy.

EFFECTS OF HIGH GLUCOSE AND THIAMINE ON THE BALANCE OF MATRIX METALLOPROTEINASES/TISSUE INHIBITORS IN PERICYTES AND ENDOTHELIAL CELLS

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Purpose/Background. In diabetic retinopathy, pericyte survival is dependent, among other factors, on interactions with extracellular matrix (ECM) proteins, which are susceptible to degradation by matrix metalloproteinases (MMP). Elevated glucose concentrations can influence ECM synthesis and degradation, acting on the expression of MMP and their tissue inhibitors, TIMP. The authors reported previously on reduced adhesion of pericytes cultured on ECM produced by endothelium in high glucose and its correction by thiamine. In this article, the authors aimed at verifying the effects of thiamine and benfotiamine on MMP-2, MMP-9 and TIMP-1 expression and activity in human vascular cells in high glucose concentrations.
Methods. Human retinal pericytes (HRP) and umbilical vein endothelial cells (HUVEC) were cultured in 5.6mmol/L or 28mmol/L glucose, with or without thiamine (T) or benfotiamine (BT). MMP-2, MMP-9 and TIMP-1 mRNA expression was determined by RT-PCR and their activity by gelatin zymography; TIMP-1 concentrations were measured by ELISA.
Results. In HRP, MMP-2 activity, though not expression, increased in high glucose (123.7 11.8% of G5.6, p<0.05) (QUERY: Please insert symbols as needed throughout abstract? and was reduced by thiamine (94.5 11.7% of G5.6, p<0.05) and benfotiamine (78.4 26.9% of G5.6, p<0.05); TIMP-1 expression increased in high glucose plus thiamine (118.7 30.0% of G5.6, p<0.05) and benfotiamine (118.2 30.2% of G5.6); MMP-9 was not expressed. In HUVEC, MMP-9 and MMP-2 expression did not change in high glucose, while their activity increased (MMP-2: 130.2 24.9% of G5.6, p<0.05; MMP-9: 120.0 19.1% of G5.6, p<0.05); thiamine and benfotiamine had no effects. TIMP-1 expression did not change in HUVEC. No effects on TIMP-1 concentration were found in either HRP or HUVEC.
Conclusions. High glucose may induce an imbalance in the MMP/TIMP regulation, leading to increased turnover of ECM. Thiamine and benfotiamine may correct the increase in MMP-2 activity due to high glucose in human pericytes, while increasing TIMP-1.

CONNECTIVE TISSUE GROWTH FACTOR (CTGF) INDUCES RETINAL CAPILLARY BASAL LAMINA THICKENING IN DIABETIC MICE

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Background. Diabetic retinopathy (DR) is the leading cause of blindness in the western world. Vascular basal lamina (BL) thickening is a prominent feature of early DR. Experimental prevention of BL thickening reduced early retinal vascular changes due to diabetes. This indicates that BL thickening is not an epiphenomenon of diabetes but may be instrumental in DR and blindness. Modulation of BL thickening may have a preventive effect on DR. CTGF is a pro-fibrotic factor expressed in the retina under experimental diabetic conditions and in the diabetic patient. Therefore, the authors hypothesized that CTGF plays a role in early pathogenesis of DR by inducing BL thickening.
Methods. This was tested in control and diabetic wild type mice (CTGF+/+) and mice lacking one functional allele (CTGF+/-). CTGF-/- mice die after birth and conditional CTGF-/- mice are not available yet. Diabetes was induced by streptozotocin treatment. At 16 weeks after induction of diabetes, blood glucose and CTGF levels, retinal mRNA levels of CTGF and TGF, and thickness of BL of retinal capillaries were measured at the EM level using dedicated image analysis software. Blood glucose levels confirmed diabetes in the treated mice.
Results. Blood CTGF levels were half the levels in CTGF+/- mice than in wild type mice. Retinal CTGF mRNA levels were 3-4-fold higher in diabetic CTGF+/+ mice than in control CTGF+/+ and CTGF+/- mice and diabetic CTGF+/- mice. TGF mRNA was elevated in diabetic retinas irrespective the CTGF genotype. BL thickening was significant in diabetic CTGF+/+ mice compared to control CTGF+/+ mice. Diabetes did not induce significant BL thickening in CTGF+/- mice.
Conclusions. CTGF is necessary for diabetic retinal BL thickening and prevention of BL thickening may delay subsequent blindness due to DR.

DECREASE OF HYPOXIA-INDUCED NEOVASCULARIZATION IN ANGIOPOIETIN-2 DEFICIENCY BY REDUCED MMP ACTIVITY

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Background. Proliferative diabetic retinopathy is characterized by the formation of pre-retinal neovascularization in response to intraretinal hypoxia. Previous data suggest that Angiopoietin-2 (Ang-2) plays a critical role in pericyte loss and vascular regression. Ang-2 expression is modified not only by glucose but also by hypoxia. Retinal overexpression of Ang-2 promotes intra- and pre-retinal neovascularization under hypoxia. Ang-2 stimulates matrix metalloprotease (MMP) expression in cultured tumor and endothelial cells. Furthermore, MMP expression in neovascularizations is inhibited by blocking the Ang-Tie system. However, the cellular expression pattern of Ang-2 in the vasculature under hypoxia and the association of Ang-2 to MMP activity have not been elucidated. In this study, the authors investigated the response of Ang-2 deficient retinas (Ang2LacZ mouse) to hypoxia and its correlation to activity of MMPs in a model of oxygen-induced retinopathy (OIR).
Methods. Pre-retinal neovascularizations were quantitated in vertical sections and intra-retinal angiogenesis was assessed by whole mount retinal immunofluorescence staining. MMP activity was detected by whole mount retinal in situ zymography.
Results. Ang2LacZ retinas subjected to the OIR model showed significantly reduced neovascularization and increased avascular zone at postnatal day 17 compared to wild type retinas, while the avascular zone at p12, an initial time point for hypoxia-induced neovascularization, did not differ between the groups. In the OIR, Ang-2 was weakly detected in preretinal neovascularizations and venules, but strongly in arterioles and capillary sprouts towards the deep capillary layer in Ang2LacZ mice. Retinas of Ang2LacZ mice showed a substantially decreased activity of MMP in arterioles and capillary sprouts growing towards the deep capillary layer.
Conclusions. These data suggest that the response of retinal vasculature to hypoxia is modulated by Ang-2. Ang-2 is essentially required at the fronts of pathological neovascularization/vascular repair, and MMPs modify vascular invasion in cooperation with Ang-2.