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OFTALMOLOG

Special issue: diabetic retinopathy

Reflecting on the way forward:

Discussing diabetic retinopathy

Diabetic retinopathy (DR) is the most frequent complication in diabetes and a feared cause of blindness. Systematic screening for sight-threatening DR is needed to identify patients, often free of symptoms, early enough to introduce timely treatments to prevent irreversible vision loss. Given the increasing prevalence of diabetes, having multiple clinicians and researchers throughout Europe engaged in this important task is vital. The European Association for the Study of Diabetes Eye Complications Study Group (EASDec) was formed to meet these challenges. Since its formation in 1990, annual EASDec meetings have gathered dedicated ophthalmologists, diabetologists, and scientists from around the world.

Compelling arguments by EASDec President Simó

The hybrid EASDec meeting in Odense Concert Hall on the 28th–30th of October last year was the 31st consecutive annual meeting and the first hosted in the Nordics since 2006. Despite the pandemic, the 2021 conference received the highest number of attendees ever. The 215 participants came from 22 different countries, including representatives from all five Nordic countries. For many attendees, this conference was the first in-person scientific meeting since the onset of the pandemic.

As an important part of the meeting, EASDec collaborated with the 5th Conference on Screening for Diabetic Retinopathy in Europe. This symposium addressed the ongoing effort established by the 2005 St. Vincent Declaration, which asserted that European countries should “reduce the risk of visual impairment due to diabetic retinopathy... through [a] systematic programme of screening reaching at least 80% of the population with diabetes using trained professionals and personnel universal access to laser therapy.” The 2021 conference in Odense, the first since the 2016 meeting in Manchester, gave the national representatives from all European countries an opportunity to present national initiatives and discuss future landmarks to combat diabetes-induced visual loss and blindness.

Before the official opening of the meeting and the welcome reception at the brand-new Hans Christian Andersen House, the first day of the meeting contained several sponsored sessions on hot topics, including ocular and systemic treatment of diabetes, inflammation in the diabetic eye, and the potential of ultra-wide field imaging of DR.

The second day included a variety of talks based on the 88 submitted abstracts. Although most presenters attended in person, presentations were also live-streamed from the US, Japan, Iceland, and the UK. The morning sessions on experimental and translational research were followed by epidemiological presentations and the invited Eva Kohner Lecture given by Professor Simon Harding, in honor of the EASDec-founder, who, sadly, passed away shortly before the meeting.

Former EASDec President Harding

A central part of the EASDec meeting is the annual poster session, which enables students and younger scientists to engage with more senior colleagues while presenting cutting-edge research on DR. This year, 53 virtual and physical posters were presented on topics including experimental research, epidemiology, medical and surgical treatment of DR, retinal imaging, and various aspects of DR screening.

After the poster presentations was a session on clinical studies and novel treatments and two excellent keynote lectures. Professor Ryo Kawasaki from Osaka, Japan discussed the translation of epidemiological studies into clinical care, and Professor Einar Stefánsson from Reykjavik, Iceland described the importance of individualized DR-screening. These thought-provoking talks were food for conversation at the conference dinner at Restaurant Nordatlanten, which gave room for vivid scientific discussions and socializing in a relaxed atmosphere celebrating Nordic cuisine and culture.

EASDec President-Elect Peto

After three extensive days, the meeting concluded on Saturday with several talks on ocular and systemic care in diabetes, novel methods in retinal imaging, and the rapidly evolving field of artificial intelligence in DR screening. Associate Professor Patrice Fort from Michigan elegantly took the audience on a detailed journey through the world of multi-omics in DR, before a symposium presenting the results of a large Danish national registry-linkage study. The meeting concluded with the prize ceremony for best poster.

Dr. Dabbah giving a poster presentation

Professor Gardner introducing the Moonshot initiative

Hosting the 31st EASDec meeting has been a wonderful experience, and I am delighted to have been given the honor to act as the Guest Editor of this special issue of *Oftalmolog*. I hope you will enjoy the following short presentations from the meeting as much as I did during EASDec. With 88 abstracts, it was not easy to decide, but these were among the very best and include the award-winners for best abstract and best poster.

Enjoy, and I hope to see you at a future EASDec meeting!

Jakob Grauslund
Guest Editor

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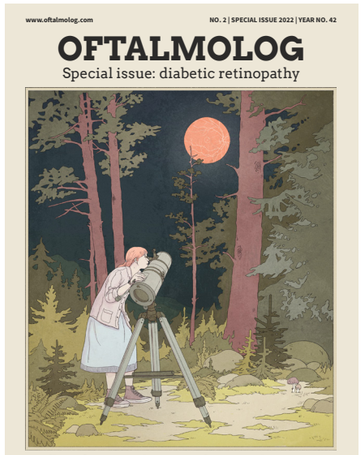

Reflecting on the way forward:
Discussing diabetic retinopathy

COVER:

The idea for this cover arose from the theme of the European Association for the Study of Diabetes Eye Complications Study Group (EASDec) 2021 conference, Odense's native son, fairy tale writer Hans Christian Andersen. Therefore, the style mirrors that of old fairy tales. The woman in the image gazes into the darkness to explore the retinal image found in the moon.

The illustrator, Agnes Guttormsgaard says this about the cover: "I liked the thought of the telescope as a metaphor for how we use technology to make the invisible visible. This is a reference to how the field of ophthalmology entails both examining the eyes and literally giving people better sight, which I thought was pretty clever. The fairytale style made the project a fun challenge!"

Congratulations

to two of our authors for winning awards at EASDec 2021

2021 **EASDec Best Poster Prize**

Lasse Jørgensen Cehofski
Department of Ophthalmology, Odense University Hospital &
Department of Clinical Research, University of Southern Denmark,
Odense, Denmark

EASDec Young Investigator Award
in honor of
Anne-Katrin Sjølie
20 21

Hanagh Winter
Wellcome-Wolfson Institute for Experimental Medicine,
Queen's University Belfast

EASDec 2021
ODENSE

Seeing the way forward:

OCT angiography and oximetry as non-invasive diagnostic tools in non-proliferative diabetic retinopathy


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Aida Simeunovic[2,3,4,5]


Martin Heier[3,5]

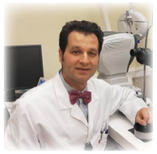
Cathrine Brunborg[6]

Naila Saddique[1]


Morten C. Moe[1,4]


Knut Dahl-Jørgensen[3,4,5]


Hanna D. Margeirsdottir[3,5]


Goran Petrovski[3,4]

Abstract

***Purpose**: To determine whether optical coherence tomography angiography (OCTA) and retinal oximetry can detect diabetic retinopathy (DR) before it is visible by ophthalmoscopy.*
***Methods**: Retinal oximetry and OCTA were performed in a cross-sectional study of 166 young individuals with type 1 diabetes (T1D) and 88 healthy controls (mean age: 24.3 years).*
***Results**: Vessel density (VD), measured by OCTA, was significantly lower in T1D patients with no DR compared to controls. This decreased significantly with increasing grade of DR. O_2 saturation inside retinal venules increased significantly with increasing DR.*
***Conclusion**: OCTA and oximetry can detect signs of DR before they are visible by ophthalmoscopy. The decrease in VD can be detected before the increase in O_2 saturation. Increased O_2 saturation in retinal vessels may indicate early microvascular disease and hypoxia.*

Introduction

Diabetic retinopathy (DR) is characterized by microvascular disease, hypoxia, and edema.[1] The oxygen (O_2) saturation in the retinal vessels is affected in DR.[2, 3] This can be measured by non-invasive oximetry, where a fundus camera takes images at two different wavelengths simultaneously.[4,5] The O_2 saturation in larger retinal vessels increases with increasing severity of DR.[2] Early DR is asymptomatic. When visual impairment is detected, chronic or progressive pathology has already developed in the retinal microvasculature. Diabetic macular edema (DME) and proliferative diabetic retinopathy (PDR) are the two advanced stages of DR and the main causes of visual loss in patients with diabetes mellitus. Diabetic macular ischemia (DMI) is due to capillary loss. Before optical coherence tomography angiography (OCTA), DMI required fluorescein angiography (FA) to diagnose. Now, OCTA can detect microvascular changes not visible by ophthalmoscopy at the early stages of DR.[6] OCTA uses the principle of "motion contrast" to detect blood flow and generate high-resolution cross-sectional images of the human retina in a non-invasive and reliable manner. OCTA enables detailed, independent, depth-resolved visualization of the superficial and deep macular capillary plexuses (SCP and DCP), without the need for dye injection. This is particularly useful for studying DMI. OCTA can measure, among others, the vessel density (VD) and foveal avascular zone (FAZ) area, parameters that may have significant functional and prognostic implications in DR.[7] It can play an increasing role in defining the individual prognosis of DR and assessment of treating options.[7–10] In our study, we wanted to confirm whether any detectable

List of abbreviations:
DR—diabetic retinopathy
O_2—oxygen
DME—diabetic macular edema
PDR—proliferative diabetic retinopathy
DMI—diabetic macular ischemia
FA—fluorescein angiography
OCTA—optical coherence tomography angiography
SCP—superficial capillary plexus
DCP—deep capillary plexus
VD—vessel density
FAZ—foveal avascular zone
T1D—type 1 diabetes
NPDR—nonproliferative diabetic retinopathy
NDR—no apparent diabetic retinopathy
TRV—total retinal volume
CMT—central macular thickness

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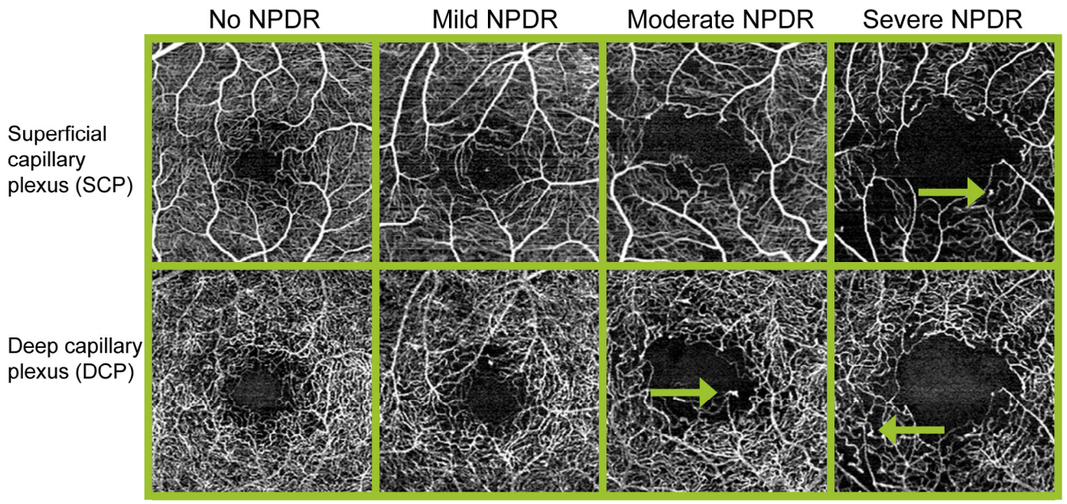


Figure 1. Example OCTA scan of the superficial and deep capillary plexus for different degrees of non-proliferative DR (NPDR) in T1D patients. The FAZ area increases while the vessel density decreases due to capillary dropouts with increasing disease severity. The arrows indicate visible microaneurysms. Adapted with permission from Veiby, N., et al. Associations between Macular OCT Angiography and Nonproliferative Diabetic Retinopathy in Young Patients with Type 1 Diabetes Mellitus. J. Diabetes Res. 2020;2020:8849116

changes in OCTA and O_2 saturation existed before DR was visible for the clinician, and whether they were associated with the development and progression of DR in young individuals with at least 10 years of type 1 diabetes (T1D).

Methods

We examined both eyes of 166 individuals with T1D and 88 healthy controls from the Norwegian Atherosclerosis and Childhood Diabetes study, an ongoing prospective population-based study of people aged 14–30 (mean age 24.3 years) with childhood-onset T1D (mean duration of T1D 15.7 years). Slit-lamp examination with ophthalmoscopy, OCTA (NIDEK RS-3000 Advance AngioScan, NIDEK CO., LTD., Japan), and fundus photography with oximetry (Oxymap T1, Oxymap ehf., Reykjavik, Iceland) of the macula and optic disc were performed after pupil dilation. The grade of DR was classified according to the International Clinical Diabetic Retinopathy classification system,[11] and the patients with T1D were allocated into four groups:

0. no apparent diabetic retinopathy (NDR) [n=239 eyes]
1. mild DR [n=58 eyes]
2. moderate DR [n=15 eyes]
3. severe DR [n=3 eyes].

The study only comprised individuals with non-proliferative diabetic retinopathy (NPDR) without DME. Approximately 30% of the T1D patients had DR.

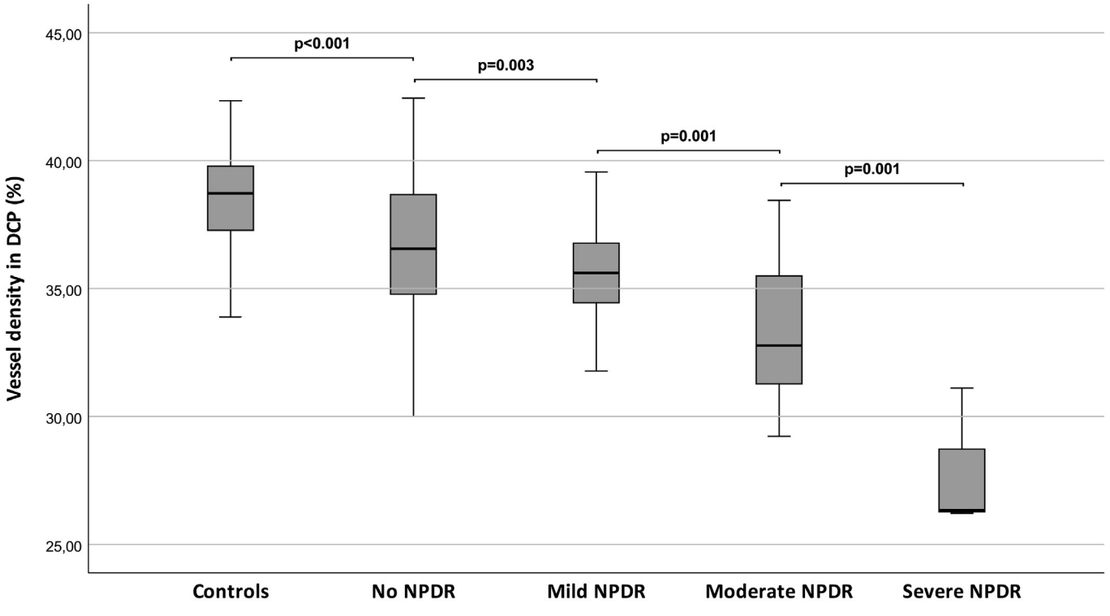


Figure 2. Vessel density significantly decreased with each increasing degree of DR.
Reprinted with permission from Veiby, N. et al. Associations between Macular OCT Angiography and Nonproliferative Diabetic Retinopathy in Young Patients with Type 1 Diabetes Mellitus. J. Diabetes Res. 2020;2020:8849116

Statistics

Clinical characteristics were presented as means with standard deviations (SD). For oximetry results, only the one eye with most DR from each patient was used. For OCTA results, both eyes were included in the analysis. A generalized estimating equation (GEE) analysis was applied to adjust for intra-individual correlation (since both eyes of each individual were included). An independent sample t-test was used to test for differences in mean OCTA parameters between NDR patients and controls. One-way ANOVA was used to test for differences in mean OCTA parameters between the four NPDR subgroups, and Tukey analysis was used as post hoc pairwise comparison after one-way ANOVA. All statistics were performed using STATA (version 15). A p-value of <0.05 was considered statistically significant.

Results

OCTA findings

VD in DCP was the only OCTA parameter that was significantly associated with the level of DR. VD in DCP was significantly lower in NDR patients than in controls, and it decreased significantly with increasing grade of DR (**Figures 1 and 2**). VD in the SCP, total retinal volume (TRV), and central macular thickness (CMT) were significantly lower in NDR patients compared to controls, but no significant change occurred with the increasing level of DR. No significant differences were found in the FAZ area when comparing NDR patients to controls. The FAZ area was not significantly associated with DR level; it was significantly higher in the severe DR group compared to the other groups (**Figure 1**), but only 3 individuals had severe DR. **Figure 3** shows an example of a small and a large FAZ area in healthy control eyes to illustrate how much the FAZ area can vary.

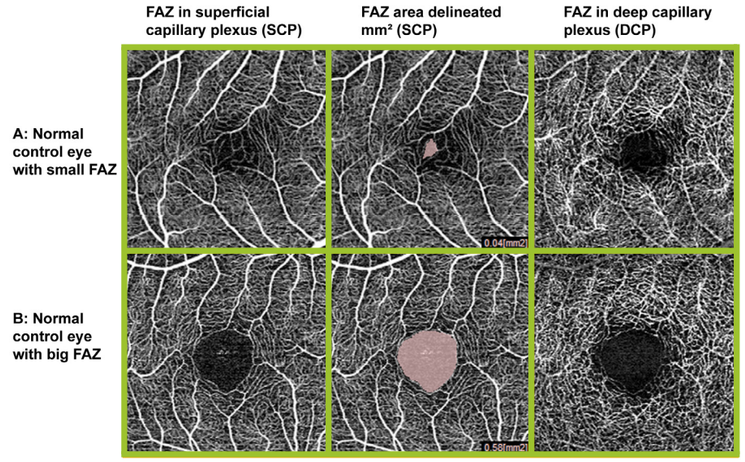


Figure 3. OCTA image showing the foveal avascular zone (FAZ) area delineated, illustrating how much it can vary between healthy eyes. Adapted and reprinted with permission from Veiby, N. et al. Associations between Macular OCT Angiography and Nonproliferative Diabetic Retinopathy in Young Patients with Type 1 Diabetes Mellitus. J. Diabetes Res. 2020;2020:8849116.

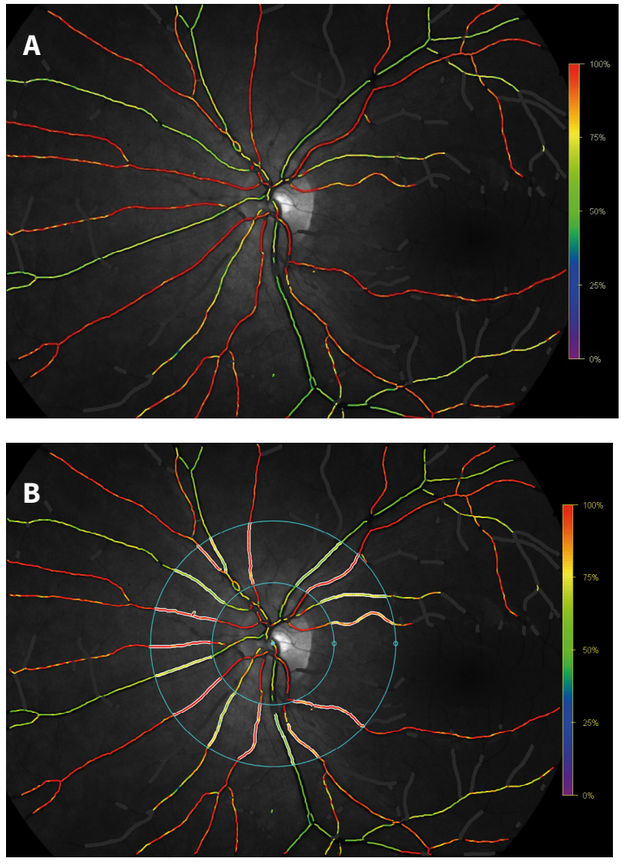
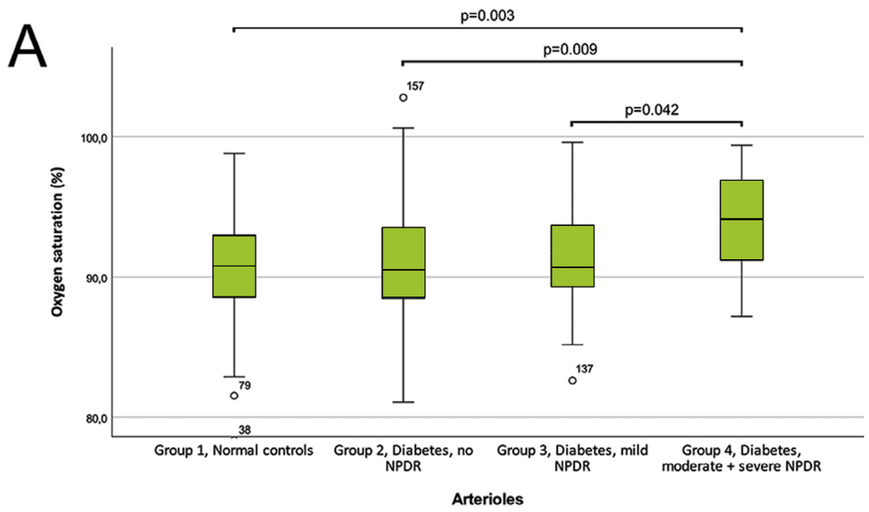


Figure 4. A: Fundus image taken by the oximeter (Oxymap T1). The colors indicate O_2 saturation in retinal vessels according to the scale on the right of the image. The red vessels are arterioles; the green vessels are venules. B: Image of the Oxymap Analyzer (software) showing the measured arterioles and venules within two circles 1.5–3.0 disc diameters from the optic disc center. The white lines indicate the delineations of the vascular segments selected for analysis. Permission to reprint from Veiby, N. et al. Venular oxygen saturation is increased in young patients with type 1 diabetes and mild nonproliferative diabetic retinopathy. Acta Ophthalmol. 2020:98:800-807.

Oximetry findings

The overall arteriolar O_2 saturation levels (mean ± SD) were 90.6% in controls and 91.3% in T1D patients. The venular O_2 saturation levels were 58.2% and 59.3%, respectively. **Figure 4** shows an image of the oximetry analysis. The O_2 saturation in venules increased significantly with increasing grade of DR when adjusting for age; this was not the case for arterioles (**Figure 5**). No significant difference existed in arteriolar and venular O_2 saturation between controls and NDR. The venular O_2 saturation was significantly higher in mild NPDR than in NDR. Arteriolar and venular O_2 saturation were significantly higher in moderate/severe NPDR than in all other groups (**Figure 5**).



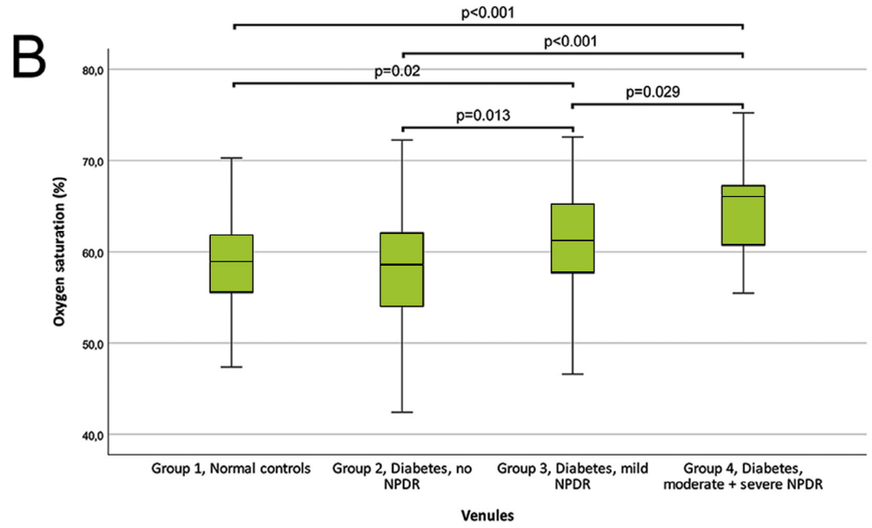


Figure 5. Oxygen saturation in retinal arterioles (A) and venules (B) in the four groups. The boxes show the median and interquartile range. P-values are only shown for the groups that are significantly different. The O_2 saturation in arterioles and venules increased with increasing grade of retinopathy. Adapted and permission to reprint from Veiby, N. et al. Venular oxygen saturation is increased in young patients with type 1 diabetes and mild nonproliferative diabetic retinopathy. Acta Ophthalmol. 2020:98:800-807.

Key points:

- Macular vessel density (VD) on OCTA was significantly lower in patients with T1D without DR compared to controls, and this decreased significantly with increasing grade of DR.
- Venular O_2 saturation increased significantly with increasing DR.
- Arteriolar and venular O_2 saturation were significantly higher in moderate/severe DR than in all other DR stages.
- The decrease in VD can be detected before the increase in O_2 saturation.
- Increased O_2 saturation inside retinal vessels may indicate early microvascular disease and hypoxia.

Discussion

In young individuals with childhood-onset T1D, the VD in DCP was lower in patients with T1D without DR (NDR) than in healthy controls. Lower VD in DCP was significantly associated with increasing severity of DR. Decreasing VD is an early process in DR and is detectable by OCTA before any visible DR by ophthalmoscopy. The O_2 saturation in arterioles and venules increased with increasing level of DR. Specifically, increased O_2 saturation in venules appeared already in mild DR, while in arterioles, the increase appeared in moderate/severe DR. Inside the larger vessels, higher O_2 saturation is a sign of hypoxia in the retinal tissue around the vessels. Although many theories exist as to why this occurs, this is beyond the scope of this paper.[12]

Conventional OCT can also measure TRV and CMT. However, because they were not associated with increasing levels of DR (without DME), OCTA was superior to conventional OCT for detecting changes associated with DR progression. Our data confirm and contribute to previously published data that VD in DCP is the most robust OCTA parameter for the differentiation of clinical stages of NPDR.[13–16] Enlargement of the FAZ area is caused by loss of capillaries in the inner vascular ring around the FAZ. However, this was not significantly associated with DR level, except in severe cases. The FAZ area appears to be an unreliable marker for DR in early stages due to the large variation in size between eyes, even without DR. A recent review concluded that most studies on DR found the FAZ area was larger in patients with diabetes compared to controls, but that this was more evident in patients with advanced levels of DR.[17]

The traditional subjective grading of fundus photos will remain clinically relevant when screening large populations for DR, but it may fail to discover early capillary pathology. Because this is important and only reliably detected by OCTA, OCTA may be included in future screening programs of patients with diabetes mellitus. Evidence suggests that vascular changes, detected by non-invasive OCTA and retinal oximetry, precede the progression to more advanced DR. This may also reflect the status of the microvasculature in other organs, only accessible by invasive biopsies. OCTA has an advantage over FA, which can only show the superficial plexus and cannot be automatically quantified. In addition to being invasive, expensive, and time-consuming, FA has side effects not seen in OCTA.[18–21] Therefore, wide-field OCTA will likely replace FA in the near future.[22]

In conclusion, VD in the DCP measured by OCTA can detect the earliest signs of DR, before they are visible by ophthalmoscopy. Further, it can discriminate between different levels of DR. O_2 saturation in arterioles and venules increases with increasing grade of DR. The decrease in VD can be detected before the increase in O2 saturation, suggesting that this increase is most likely a consequence of microvascular disease (microaneurysms and capillary dropouts). Our results show that increased venular and arteriolar O_2 saturation may indicate early microvascular disease and hypoxia in the retinal tissue, and, thus, can be used as markers for detecting early DR. OCTA and oximetry are reliable tests and may even complement programs aimed at individualizing the control interval for patients with DR in the future.

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Acknowledgments:
Geir Aksel Qvale assisted with images and graphical figures.

Conflicts of Interest:
The authors declare no conflict of interest regarding the publication of this paper. The results have been published in *Acta Ophthalmologica* and *Journal of Diabetes Research.*

This paper is a summary of the following two papers:

1. Veiby N, Simeunovic A, Heier M, Brunborg C, Saddique N, Moe MC, Dahl-Jørgensen K, Margeirsdottir HD, Petrovski G. Associations between Macular OCT Angiography and Nonproliferative Diabetic Retinopathy in Young Patients with Type 1 Diabetes Mellitus. *Journal of diabetes research.* 2020;8849116.
2. Veiby N, Simeunovic A, Heier M, Brunborg C, Saddique N, Moe MC, Dahl-Jørgensen K, Margeirsdottir HD, Petrovski G. Venular oxygen saturation is increased in young patients with type 1 diabetes and mild nonproliferative diabetic retinopathy. *Acta Ophthalmol.* 2020:98:800-807.

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Navigated retinal photocoagulation for treating proliferative diabetic retinopathy and diabetic macular edema

Findings from two randomized clinical trials

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Diabetic retinopathy (DR) is the most common complication of diabetes and a cause of vision loss.[1,2] As demonstrated by landmark studies such as the Diabetic Retinopathy Study[3] and the Early Treatment Diabetic Retinopathy Study (ETDRS),[4] treatment with retinal photocoagulation for proliferative diabetic retinopathy (PDR) and clinically significant diabetic macular edema (DME) substantially reduces the risk of irreversible vision loss.

In PDR, retinal hypoxia induces vascular endothelial growth factor (VEGF) upregulation, leading to the formation of retinal new vessels. Peripheral retinal treatment with panretinal photocoagulation (PRP) induces photoreceptor atrophy and improves choroidal oxygen diffusion.[5] As PRP lowers the retinal oxygen consumption, the treatment decreases VEGF levels, regressing PDR. Although PRP was a huge advance in the treatment of retinal diseases, concerns remain about its adverse effects, including the increased risk of night blindness and peripheral vision loss.[6]

Although not fully understood, it is believed that VEGF-induced breakdown of the inner blood–retina barrier is a vital component of the pathophysiology of DME. Focal/grid photocoagulation is thought to improve oxygenation, lowering VEGF expression, as in PDR5. Although focal/grid photocoagulation can often halt the progression of DME,[4] intravitreal VEGF inhibition has greater potential for visual acuity improvement.[7] However, direct comparison between focal/grid photocoagulation and intravitreal VEGF inhibition is often difficult.[8] In two recent clinical trials, we tested the efficacy of navigated retinal photocoagulation as a stand-alone or adjunctive treatment in previously untreated patients with PDR and DME.

Navigated photocoagulation in diabetic retinopathy

Navigated photocoagulation using the Navilas® laser system (**Figure 1**) differs substantially from other laser systems. Instead of including a slit-lamp to facilitate preoperative planning, retinal images are displayed on a monitor that uses software to integrate fluorescein angiography or optical coherence tomography thickness maps. Furthermore, an eye-tracking system optimizes the precision of the treatment. The Navilas® at our site uses a 532-nm (green) frequency-doubled, diode-pumped laser. We recently introduced a new 577-nm (yellow) laser as well. Because previous studies had found higher accuracy[9] and less pain and discomfort during treatment,[10] we wanted to evaluate the potential for treatment of PDR and DME through two randomized clinical trials.

Figure 1. Display of the Navilas® laser, which combines eye-tracking with various imaging options and a target-locked frequency-doubled laser.[20]

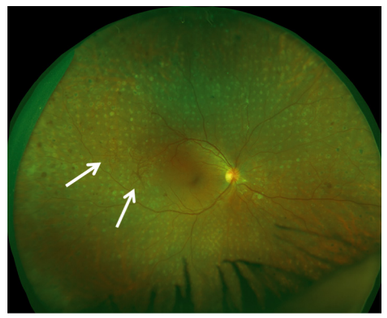

Figure 2. Wide-field retinal image of proliferative diabetic retinopathy with new vessels elsewhere (white arrows) despite full panretinal laser treatment.

Navigated treatment of PDR—lessons from the IMPETUS study

The Individually-Marked PanrEtinal laser phoTocoagUlation for PDR Study (IMPETUS 2018) was a two-fold, clinical PhD study, supported by VELUX FONDEN. The overall aim was to see whether individualized PRP would lead to a better balance between efficacy and adverse effects, compared to the one-size-fits-all PDR treatment, the standard for more than 40 years.[3]

Dr. Thomas Lee Torp performed the first part of the study (IMPETUS-DETECT). Over 6 months, PRP was used in 65 eyes of 52 patients with treatment-naïve PDR to identify potential non-invasive metabolic, structural, and functional markers of postoperative disease activity. Patients received Navilas® treatments over two independent sessions, and postoperative disease progression was identified in 25% and 37% of eyes after 3 and 6 months, respectively (**Figure 2**).

List of abbreviations:

DR—diabetic retinopathy
ETDRS—Early Treatment Diabetic Retinopathy Study
PDR — proliferative diabetic retinopathy
DME—diabetic macular edema
VEGF—vascular endothelial growth factor
PRP—panretinal photocoagulation
IMPETUS—Individually-Marked PanrEtinal laser phoTocoagUlation for PDR Study
DRCRnet—Diabetic Retinopathy Clinical Research Network
ADDENDUM—Aflibercept anD navigateD vErsus coNventional laser in Diabetic macUlar edeMa

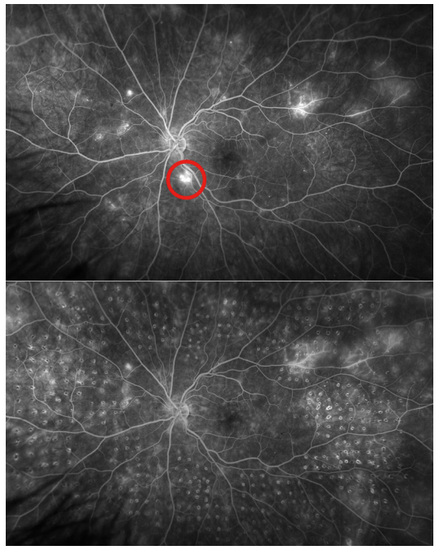

Figure 3. Wide-field fluorescein angiography of patient with proliferative diabetic retinopathy before (upper image) and after (lower image) panretinal photocoagulation. New vessels elsewhere identified by late-stage fluorescein leakage (red circle) before treatment, but in regression after treatment.

We discovered that wide-field fluorescein angiography was often surprisingly difficult to use to assess disease activity after PRP (**Figure 3**). Instead, we found two alternative markers to assess post-operative progression. Retinal oximetry was identified as a potential marker of postoperative disease activity (**Figure 4**). Patients with worse DR have higher retinal venular oxygen saturations[11] because oxygen extraction is impaired in DR. In IMPETUS-DETECT, we demonstrated that each percentage-point increase in retinal venular oxygen saturation from baseline to follow-up was independently associated with a 30% higher risk of associated PDR progression.[12] On the other hand, patients with decreasing retinal venular oxygen saturation were more likely to stabilize clinically after PRP. Peripheral capillary non-perfusion was the second marker identified because patients with subsequent disease progression already had larger areas before navigated PRP.[13]

Based on the finding of IMPETUS-DETECT, Dr. Anna Stage Vergmann tested the effect of individualized navigated PRP in IMPETUS-TREAT. In a 6-month randomized trial, the eyes of patients with treatment-naïve PDR received full navigated PRP (n=27) or individualized navigated PRP (n=26) that only targeted retinal quadrants with active proliferations. The principal endpoints were treatment efficacy and adverse effects, given that full PRP could lead to loss of the peripheral visual field.[6]

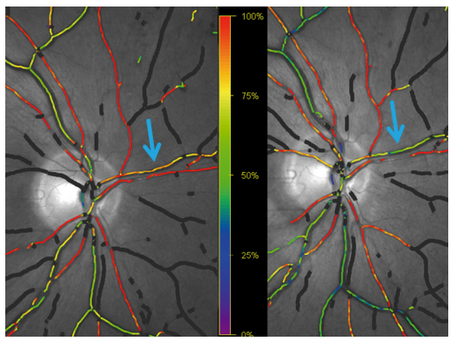

Figure 4. Retinal oximetry as a marker of successful panretinal photocoagulation in proliferative diabetic retinopathy. Decreased retinal venular oxygen saturation was measured as indicated by the color saturation charts with color saturation decrement in specific retinal venular segment (blue arrow).

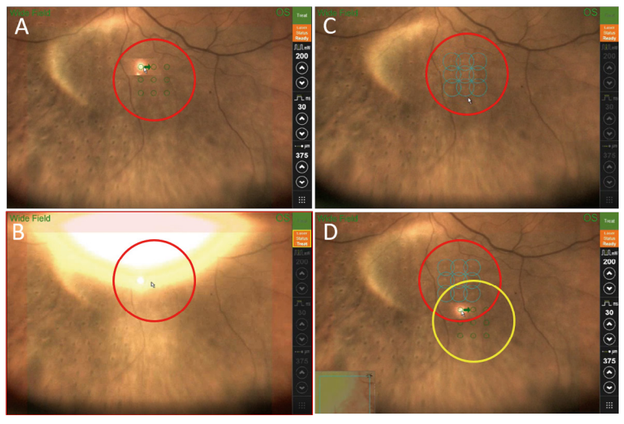

Figure 6. (Above) Navigated panretinal photocoagulation. A: Three by three position pattern planned on the touchscreen (red circle). B: Treatment performed by foot pedal. C: Post-treatment photocoagulation marks can be identified (blue circles in red circle). D: A new position pattern can be identified on the touchscreen (yellow circle).

Figure 5. Navigated panretinal photocoagulation performed by Anna Stage Vergmann, PhD. Image courtesy of VELUX FONDEN.

Key points:

- Navigated retinal lasers have high efficacy and negligible adverse effects in the treatment of sight-threatening complications in diabetic retinopathy.
- Non-invasive retinal markers can be used to predict treatment-outcome in proliferative DR.
- Timely application of focal/grid laser therapy in diabetic macular edema is likely to reduce the treatment burden of intravitreal therapy.

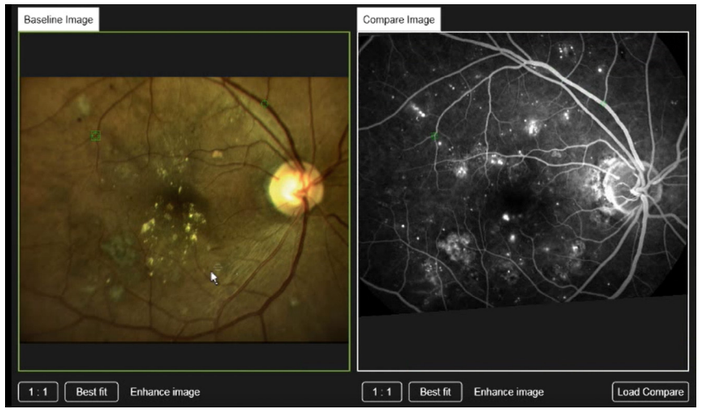

Figure 7. (Left) Navigated focal/grid treatment in diabetic macular edema. Macular image (left side) is captured by Navilas® camera and aligned with previously performed fluorescein angiography (right side) by comparable retinal vascular structures (e.g., branching angles as identified by small green circles and squares).

Hence, we speculated that a more gentle, individualized treatment would lead to fewer adverse effects with the same efficacy.

Six months after treatment, progression of PDR did not differ between patients who received the full or individualized treatment (59.3% vs. 48.0%, p=0.27).[14] Intriguingly, neither group had any measurable adverse effects concerning loss of peripheral vision, dark adaptation, incident DME, or quality of life.[14] The fact that neither full nor individualized navigated PRP induced loss of visual fields was particularly encouraging and in stark contrast to the Protocol S study by the Diabetic Retinopathy Clinical Research Network (DRCRnet), which demonstrated a mean loss of 422 dB for peripheral visual field sensitivity in PDR patients treated with traditional PRP.[15]

ADDENDUM: navigated focal/grid photocoagulation in diabetic macular edema

The Aflibercept anD navigateD vErsus coNventional laser in Diabetic macUlar edeMa (ADDENDUM) study was Søren Leer Blindbæk's PhD research, comparing navigated and traditional focal/grid photocoagulation in addition to intravitreal aflibercept in 48 eyes of 37 patients with treatment-naïve DME. Our earlier review demonstrated that, even in intravitreal angiostatic monotherapy studies of DME, focal/grid laser treatment was often included (e.g., as rescue treatment) in as many as 20–50% of patients, but the exact combination regimen was seldom described in study protocols.[8]

Because we speculated that focal/grid photocoagulation would have the optimal efficacy after aflibercept loading, the ADDENDUM study was designed as a randomized clinical trial with two arms. We compared navigated and conventional focal/grid treatment in center-involved DME-patients, who received loading with three monthly aflibercept injections followed by focal/grid photocoagulation (navigated or traditional PASCAL® laser) and intravitreal aflibercept pro re nata.

In navigated focal/grid treatment, fluorescein angiography is imported and aligned with a real-time macular image (**Figure 7**), allowing treatment to be targeted towards leaking retinal microaneurysms and areas of diffuse leakage, according to ETDRS-protocol (**Figure 8**). The treatment is then performed with assistance from the eye-tracking system and without the need of a contact lens.

At month 12, patients in ADDENDUM

had an average best corrected visual acuity improvement of 8.4 ETDRS letters and a central retinal thickness reduction of 97.4 µm.[16] Even though the principal outcome did not differ statistically significantly between groups, there was a trend toward a better functional outcome in patients treated with navigated photocoagulation (+9.4 vs. +7.1 ETDRS letters, p=0.17).

Most interestingly, after the loading phase, patients in both groups only had a limited need for additional intravitreal therapy after adjunct focal/grid photocoagulation. In fact, during the 12 months of the study, patients received on average only 1.4 intravitreal injections after three loading injections and focal/grid photocoagulation.[16] In other 12-month DME studies such as the DRCRnet Protocol I,[17] RESOLVE,[18] and Da Vinci,[19] similar visual gains were only achieved at the expense of 9.0–10.8 injections within the first year. Therefore, while we did not demonstrate a better effect of navigated laser, the initial results indicate that timely focal/grid photocoagulation after intravitreal aflibercept loading has a beneficial clinical effect and may substantially reduce the need for intravitreal therapy. Long-term studies are important to explore whether these findings can be extended beyond 12 months.

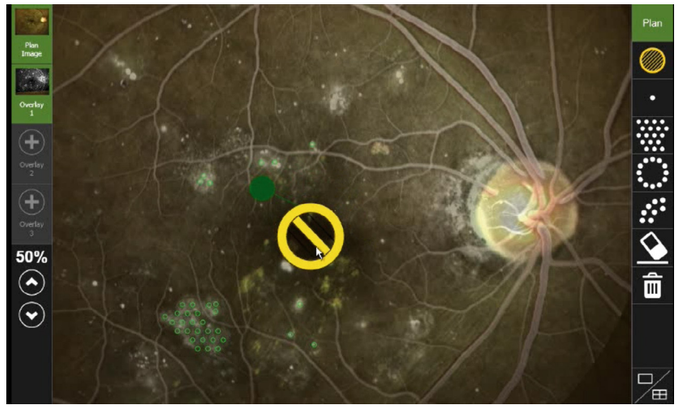

Figure 8. Following Figure 7, an overlap macular image is created, and focal/grid treatment can be planned in accordance with areas of focal and diffuser retinal leakage (green circles). Specific zones that should not be treated (e.g., foveal avascular zone and optic disc) can be identified (yellow circle) before treatment.

Conclusions and perspectives

In IMPETUS and ADDENDUM, we tested the potential of navigated photocoagulation in randomized trials of PDR and DME. In PDR, we demonstrated similar efficacy of individualized navigated PRP compared to full treatment. We were also encouraged by the fact that neither led to any measurable adverse effects.

For DME, the ADDENDUM study indicated that timely focal/grid treatment (regardless of the type) could substantially lower the need for additional intravitreal treatment, if the former was performed after aflibercept loading, which reduced the macular edema sufficiently to optimize the effect of the retinal photocoagulation. We are currently expanding these findings by comparing them with real-life results from our clinical department to implement a better treatment protocol. By combining the visual acuity improvement from intravitreal angiostatic treatment with the lasting effect of focal/grid photocoagulation, we aim to maximize the effect, while lowering the burden of treatment.

The research group:

This article summarizes some of the recent work done by our research group, including studies included in three recent PhD dissertations. I want to recognize and thank the three hardworking PhD students who collaborated with me on these projects.

Thomas Lee Torp, PhD- The Individually-Marked PanrEtinal laser phoTocoagUlation for proliferative diabetic retinopathy Study (IMPETUS-DETECT), 2017

Søren Leer Blindbæk, PhD- Aflibercept anD navigateD vErsus coNventional laser in Diabetic macUlar edeMa (ADDENDUM), 2019

Anna Stage Vergmann, PhD - The Individually-Marked PanrEtinal laser phoTtocoagUlation for proliferative diabetic retinopathy Study (IMPETUS-TREAT), 2020

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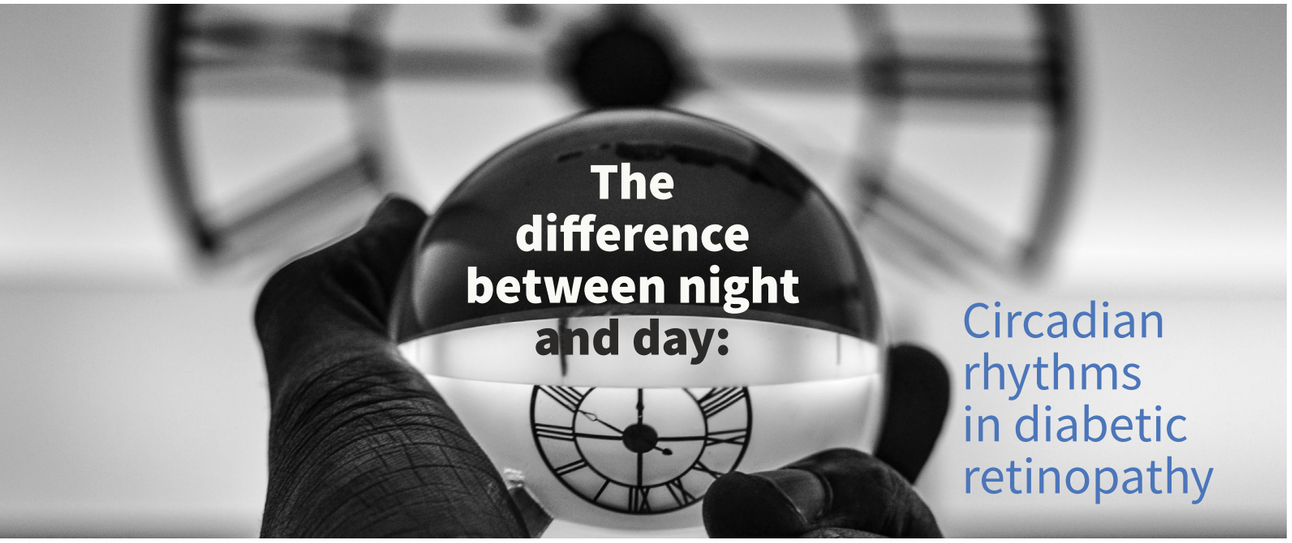

The difference between night and day: Circadian rhythms in diabetic retinopathy

Abstract

Mounting evidence suggests that the circadian system is integral to the development and management of diabetic complications. Circadian disruption increases the risk of diabetes, which, in turn, causes desynchrony in the circadian system and the molecular clock that exists in the cell. Despite several of the underlying pathologies being either controlled by or linked to the circadian clock, little is known about the impact of a dysfunctional circadian system in the most common complication of diabetes, diabetic retinopathy (DR). For example, hypoxia, a major therapeutic target for DR, alters the expression of the core genes in the molecular clock in endothelial cells. This presents a novel mechanistic hypothesis for the role of a disrupted circadian clock in DR.

Introduction

The rotation of the Earth imposes daily environmental cycles on the organisms on its surface. The circadian system has evolved to anticipate these patterns to help organisms survive and thrive. This system integrates the rhythms of the environment, especially light, to modulate our physiology between day and night. Unfortunately, in modern society, we often inadvertently confuse the circadian system. Longer time spent indoors, artificially extended days, and more extreme changes, such as shift work and jet lag, all challenge the synchrony of the circadian system. The immediate symptom of this disruption is poor sleep and groggy waking hours, but the actual impact on the body is much more widespread. Circadian rhythms exist in diverse processes across all organs in the body. Their centrality to human health was highlighted with the 2017 Nobel Prize in Physiology or Medicine awarded to Dr. Jeffrey C. Hall, Dr. Michael Rosbash, and Dr. Michael W. Young for their discoveries of molecular mechanisms controlling circadian rhythms. It makes sense then that circadian disruption has been implicated in a multitude of diseases, with diabetes as a prominent example. Circadian disruption increases the risk of developing diabetes; diabetes, in turn, diminishes the robustness of circadian rhythms. Many crucial functions of the retina are regulated by the circadian system,[1] so understanding the roles of circadian rhythms in diabetic retinopathy (DR) is greatly important.

The circadian system

The retina is the sole source of light to the circadian master clock, the suprachiasmatic nucleus in the hypothalamus. Light is the most important timing cue for this master clock. As such, the retina is uniquely important, and blinding diseases like DR might have implications far beyond the eye. The master clock uses light information from the retina to synchronize every other clock in the body except the retina's own circadian clock since it receives its own light information (**Figure 1**). This is one reason why the retina clock is less studied than others, with current research mostly exploring the master clock or larger tissue clocks. The retina clock is also very complex, with distinct clocks on each retinal layer including the inner nuclear and vascular layers,[2] further complicating the elucidation of its clock system.

The smallest level of the circadian system is a molecular clock, found in almost all cells and acting in concert to drive daily rhythmic processes. The molecular clock is a set of interlocked loops of genes that interact to control their own expression. The main loop in this molecular clock has two pairs of genes. The first pair form the positive arm, so called because together they create a positive transcription factor that initiates the expression of other genes in the nucleus. The second set of genes is initiated by this positive arm,[4] and once they are expressed, they inhibit further action of the positive arm. Therefore, they are called the negative arm. In this way the molecular clock regulates its own expression in an ongoing cycle, where one turn takes around 24 hours, depending on genetics, age, and environmental cues (**Figure 2**). This clock is important because the positive arm does not just target other clock genes, it also triggers transcription

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Key points:

- General clock gene expression is altered in induced, pluripotent stem cell-derived endothelial cells from diabetes patients.
- Hypoxia, but not hyperglycemia, acutely changes the amplitude and patterns of expression of core circadian clock genes in retinal endothelial cells.
- A core circadian transcription factor is upregulated and peaks earlier in hypoxia.
- Conversely, an important negative regulator in the molecular clock is downregulated in hypoxia.

Social media links

LinkedIn
www.linkedin.com/in/hanagh-winter

Twitter
@HanaghWinter

Website
https://pure.qub.ac.uk/en/persons/eleni-beli

Twitter
@belielen

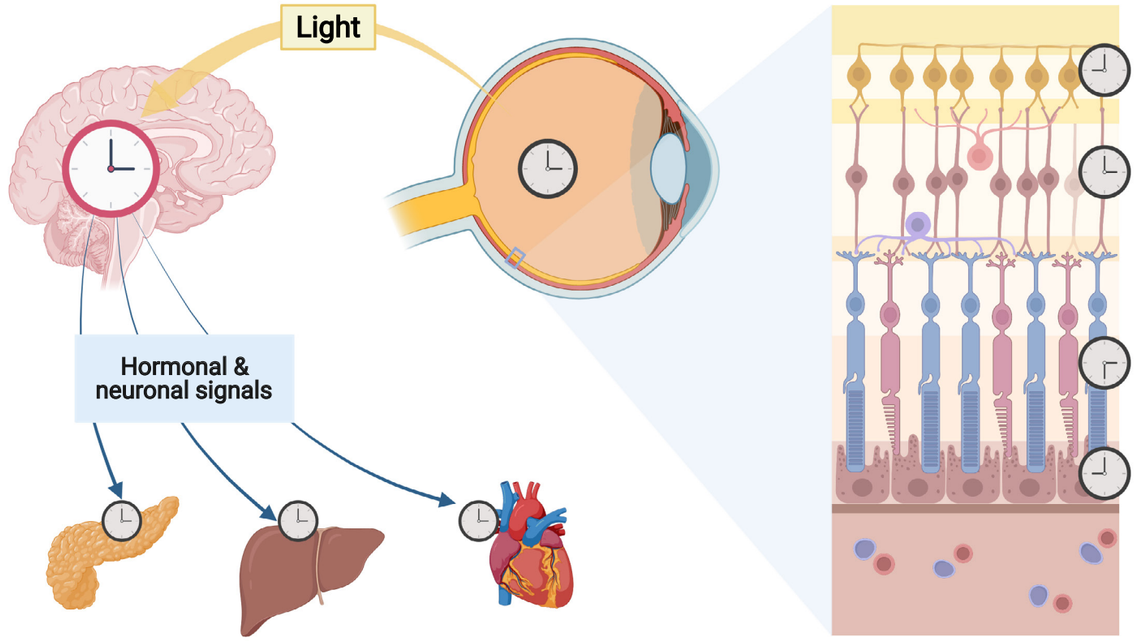

Figure 1. The master clock in the hypothalamus of the brain is the topmost layer of the circadian system, synchronizing all the peripheral clocks that exist in organs around the body so that they act in concert with each other. The most important timing cue for the circadian system is light signaling, which comes via non-visual photoreceptor cells in the retina called intrinsically photosensitive retina ganglion cells.[3] One of the reasons that the retina clock is unique is that it is made up of many different clocks on the different retinal layers that oscillate in different phases. Created by the authors using BioRender.com.

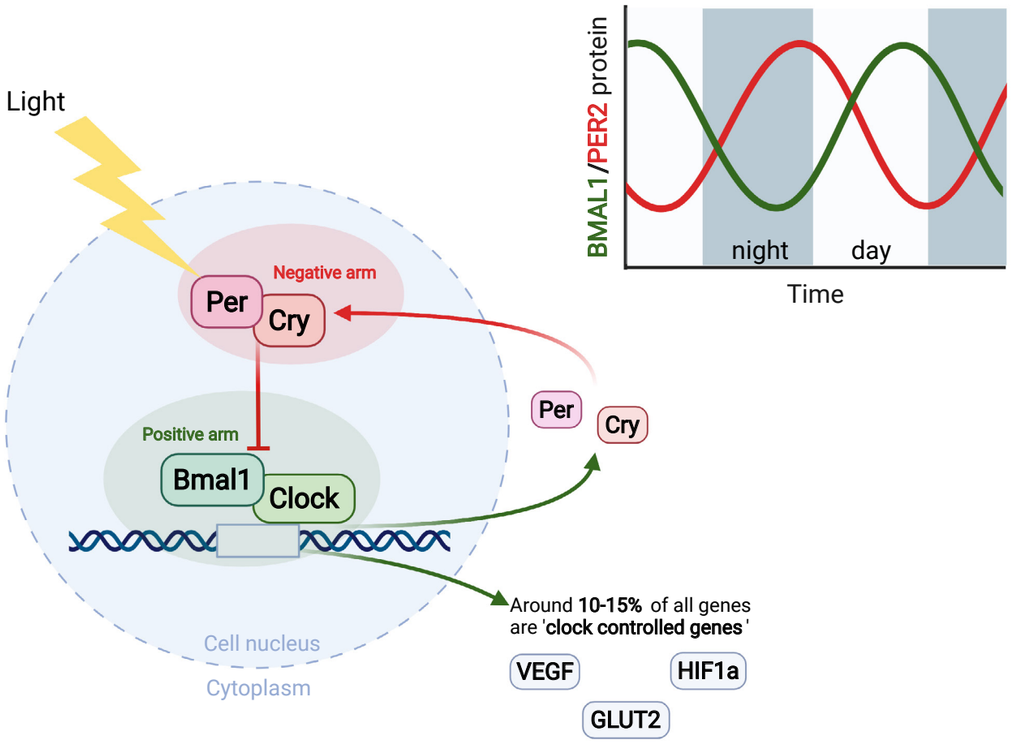

*Figure 2. The main loop of the molecular circadian clock completes a cycle once approximately every 24 hours. The positive arm of the clock is made up of two positive transcription factors, BMAL1 and CLOCK. Over the circadian day, BMAL1 and CLOCK drive the transcription of PER and CRY proteins. When PER and CRY reach a high enough concentration in the cytoplasm, they move back into the nucleus to inhibit any further action of the positive arm, including their own transcription. PER and CRY are degraded overnight until the cycle can start again the next morning. This loop is the pathway by which environmental light cues are integrated into the circadian system. Light causes induction of the PER proteins to change the molecular clock each day and keep it in line with the external environment.[6] Positive arm proteins = BMAL1 and CLOCK; negative arm proteins = PER and CRY; clock-controlled gene examples = vascular endothelial growth factor (*VEGF*), hypoxia-inducible factor 1-alpha (*HIF1a*), and glucose transporter 2 (*GLUT2*). Created with BioRender.com.*

of a multitude of 'clock-controlled genes,' and so the effect of the clock ripples each day to affect diverse processes in tissue-specific ways. In fact, between 10 and 15% of all gene transcription is rhythmic, with as many as 50% of genes in mammals predicted to be rhythmically expressed in at least one tissue.[5]

Circadian rhythms were first described by the French astronomer Jean-Jacques d'Ortous de Mairan in the mid-1700s, based on his observations of how the mimosa plant continued to move its leaves up and down each day, even in constant darkness. However, disagreements as to whether circadian rhythms were truly self-sustained and endogenous and not due to some external cue persisted until at least the 1950s. This was addressed in a seminal Cold Spring Harbor symposium in 1960, where Pittendrigh and Aschoff laid out their 'generalizations'[7] and 'rules.'[8] These comprehensive lists, collating the work in the field to date, placed them among a list of founding 'fathers of chronobiology.'

The master clock in the mammalian hypothalamus and the retinohypothamic tract that leads to it from the eye became the subject of intense study upon their discovery in 1972,[9] but it was the retina that was the first tissue outside of the central circadian pacemaker to be described as having a self-contained circadian function.[10] Shortly after, in the 1980s, the molecular basis of the clock was explicated by various groups, with the discovery of the negative clock arm gene *period* by Hall, Rosbash, and Young leading to a shift of the field from a behavioral to a molecular basis.[11] A study decades later, using new opportunities afforded by genetic knockout models, revealed the extent of rhythmicity in the retina. This study used a novel retina-only genetic knockout of the clock positive arm transcription factor BMAL1 to reveal that hundreds of genes in the mammalian eye are controlled by the circadian clock[12] (**Figure 3**).

In the eye specifically, counts of rhythmicity in gene expression range from just over 5% in primate retinas[13] to almost 7% in whole eyes of mice.[12] More recently, the Beli lab has detected just over 9% rhythmicity in the mouse retina. Perhaps surprisingly, slightly more genes appear to be expressed rhythmically in the diabetic retina. Among these are genes involved in processes under the direct control of the clock, including angiogenesis in the retina,[14] the hypoxia response,[15] and inflammation.[16] The potential relevance of the clock to DR is, therefore, immediately obvious. Rhythmicity in diverse gene expression allows the function of the retina to change dramatically between day and night, and this change even manifests in the ways that we clinically assess the retina, such as those to investigate vessel permeability, retina thickness,[17] or retina function with electroretinography, depending on the time of measurement. In mice, electroretinograms change from day to night since the sensitivity of the retina to light is under circadian control,[12] showing that at least some of the retina's functions

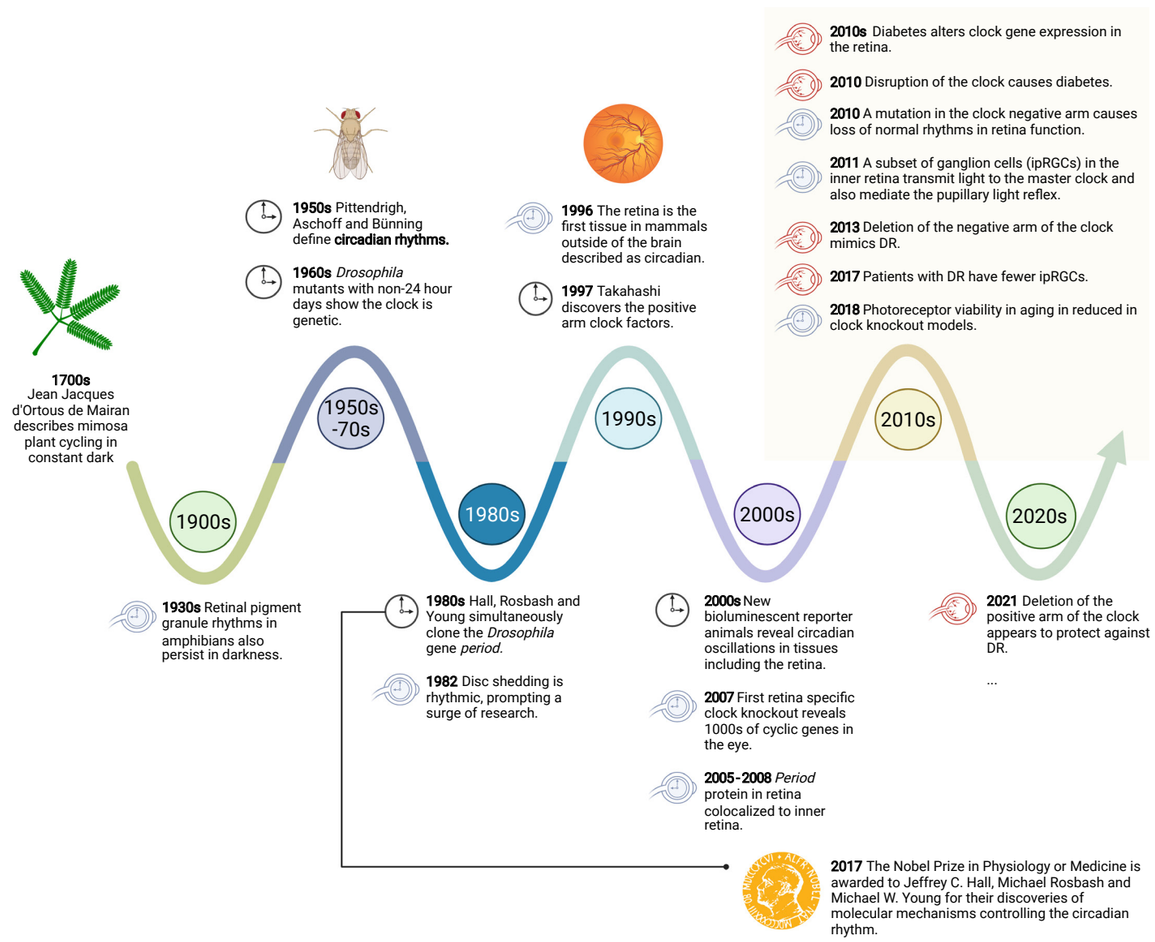

Figure 3. The first description of circadian rhythms appeared in the 1700s. Since then, the field of chronobiology has expanded rapidly with a string of technological and molecular discoveries in the 1900s. Epidemiological studies first implicated the circadian clock as a factor in human disease in 1996, specifically in breast cancer incidence in shift workers,[18] and since then, the list of diseases linked to circadian disruption has grown, including diabetes. Since 2009,[19] the evidence surrounding the role of circadian disruption specifically in DR has grown, promising compelling new opportunities for understanding DR progression and in the future, perhaps even targets for intervention and treatment. Created with BioRender.com.

that can be detected in the clinic are also under the control of the circadian clock. We are at the forefront of discovering functions of the retina controlled by the clock that could be translated to clinical settings as this research is still in its infancy.

The molecular clock in diabetes

In animal models of diabetes,[19,20] clock gene expression is altered in the peripheral organ clocks such as the liver and kidney, including the retina clock.[21] Endothelial cells have a robust circadian clock with rhythms in processes like the expression of adhesion molecules.[22] Thus, we asked whether diabetes alters the expression of circadian genes in endothelial cells. In endothelial cells derived from induced pluripotent stem-cell[23] of diabetic patients and healthy controls, we observed changes in clock gene expression.

Our next question was what processes in diabetes were responsible for these changes. To answer this, we measured the expression of the core clock genes in primary human retinal endothelial cells, mimicking specific elements of the diabetic microenvironment to see which drive the largest clock changes. Hypoxia, rather than hyperglycemia, has so far proven to stimulate more changes in the circadian clock in these cells, at least more acutely, as presented at the EASDec 2021 meeting.

Hypoxia is a major underlying driver for the pathogenesis of DR; thus, understanding how it disrupts the clock may give better insight into the role of the clock in disease progression. Early reductions of oxygen in the diabetic retina are suggested in patient studies, where inhalation of pure oxygen improves visual functions in early diabetes,[24] and supported by various animal model studies.[25] Early hypoxia in diabetic retinopathy appears to be caused by blood-flow abnormalities in the diabetic vasculature that reduce tissue oxygen in the retina. Later in the disease, progressive vascular degeneration leads to areas of non-perfusion (ischaemia) that results in more profound hypoxia and end-stage complications.[25] Later, cells respond to this early hypoxia by increasing their expression of angiogenic factors such as VEGF in an attempt to increase oxygen, which in turn

results in neovascularization that typifies the proliferative disease. Retinal hypoxia in DR is thought to be exacerbated at night by the normal rise in oxygen demand of the photoreceptor cells when dark-adapted. Increased photoreceptor oxygen consumption, therefore, limits the amount of oxygen reaching the inner retina from the choroid at this time (**Figure 4**).[26] It is this hypothesis that has provided the rationale for studies seeking to modify nightly dark current activity.[27] An example is the CLEOPATRA trial in the UK, which asked patients to wear light masks overnight to reduce dark adaptation and, by extension, hypoxia. In this case, the result appeared not to be therapeutically beneficial.[28] Therapies using light have also been explored for reasons related directly to the clock, although not concerning DR.

Hypoxia and the circadian clock

While the impact of hypoxia on the clock in the diabetic retina is not yet clear, the emerging picture in the wider literature is of a dichotomy in how the positive and negative clock arms steer the hypoxic response and vice versa. It is already clear that there is a strong interaction between these two processes (**Figure 5**). For example, the magnitude and type of response induced by hypoxia are controlled by the molecular clock, meaning that hypoxia at different times of day creates different outcomes in different tissues.[30] The transcription factors that make up the positive arm of the clock and that drive the transcription of all of the clock target genes share a very close structural similarity with the major proteins in the hypoxia response. Models where the positive clock arm is knocked out have lower expression of hypoxia outputs,[15] and the clock positive arm has even been shown to target the same gene promoter regions that stimulate the hypoxic response.[15]

How the negative arm of the clock, which represses the positive clock transcription factors, responds in hypoxia is more complex. Cells manipulated to remove the negative arm of the clock have much stronger reactions to hypoxia, suffering more cell death.[30] As with the positive arm, though, the negative arm gene *PER2* and a central hypoxia gene share a similar structure and belong to the same superfamily of signal sensors, allowing the clock to sense light and the hypoxia pathway to sense oxygen. In ischemia, the clock gene *PER2* even stabilizes hypoxia drivers[31] but protects against injury by enhancing glycolytic capacity.[31] In the heart, this *PER2*- mediated protection comes from the endothelial cells.[32]

Because of these interactions, using light therapeutically appears promising for reducing hypoxia damage, such as in myocardial ischemia.[30,32] However, light treatment in DR has proven more complicated. As mentioned above, a study asking patients to wear light masks overnight to reduce dark adaptation, and

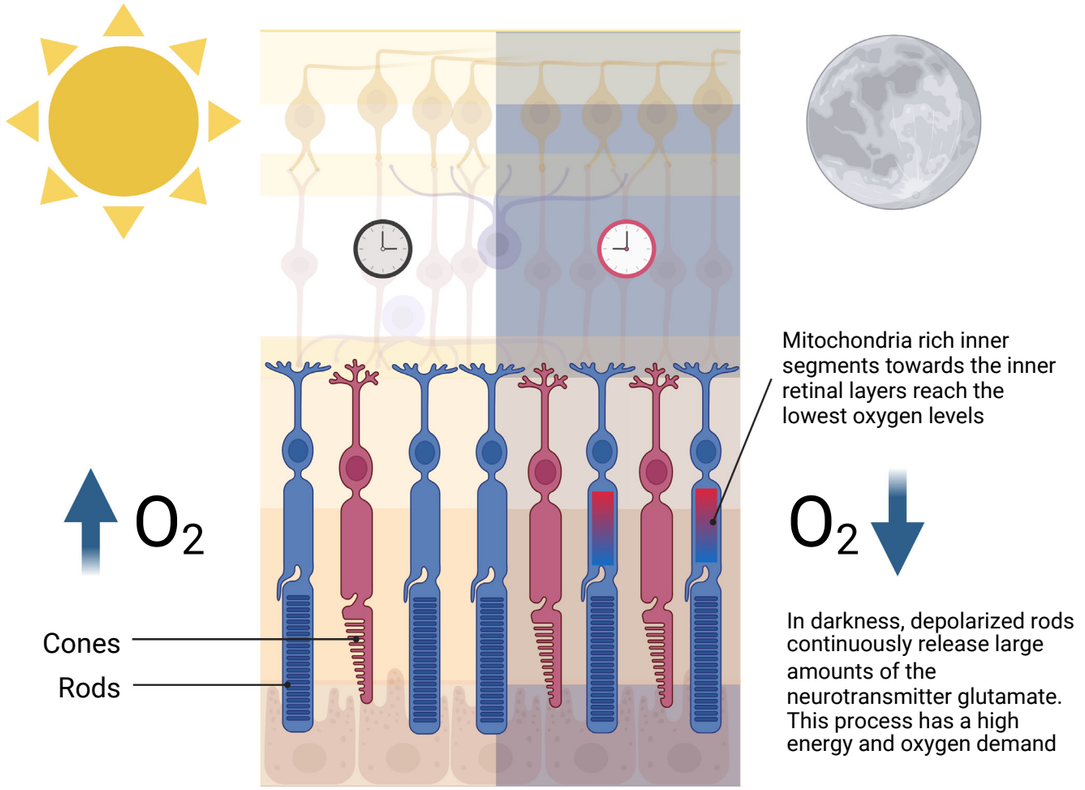

Figure 4. Nightly dark adaptation of the photoreceptors creates an energy and oxygen demand that cannot be met in the diabetic retina. This results in nightly low oxygen that triggers hypoxia response in the cells and possibly also alters the circadian molecular clock. Since this hypoxia is not experienced equally across the retinal layers,[29] desynchrony between the layers might also be triggered. Created with BioRender.com.

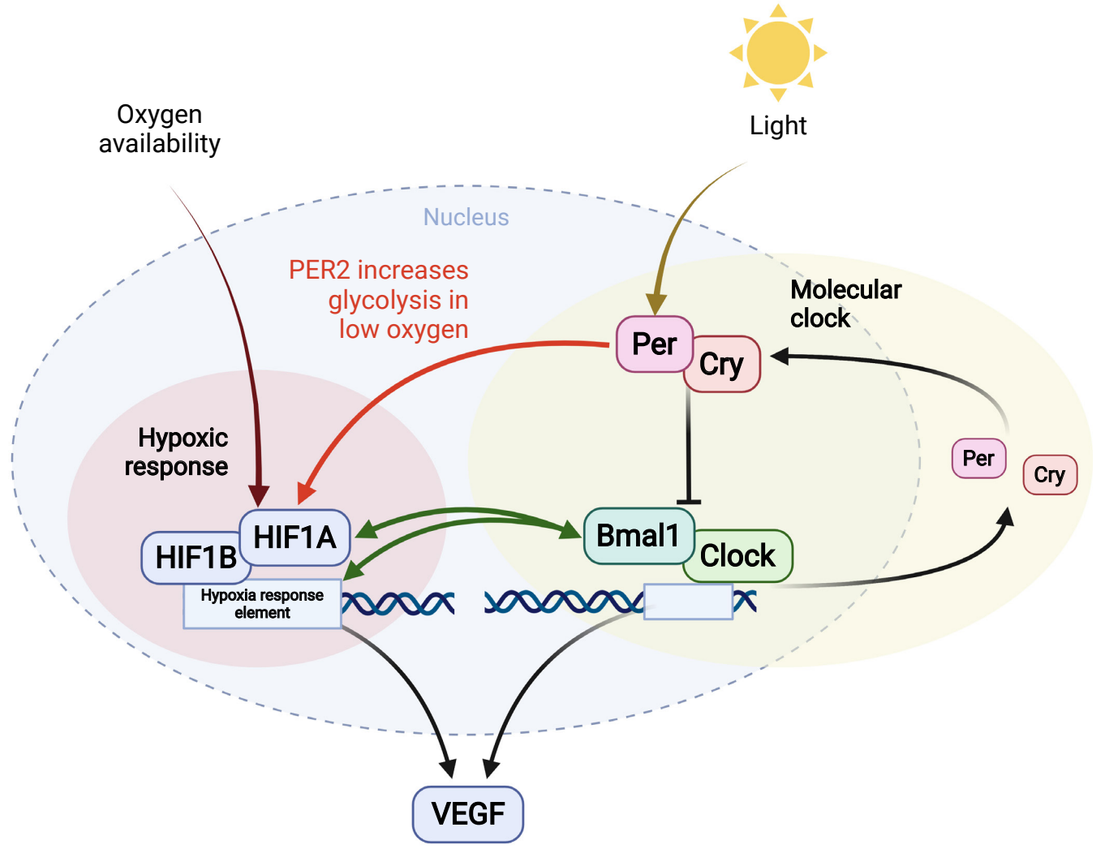

Figure 5. There are several parallels and links between the molecular clock and the hypoxia response pathway. Negative clock factor PER2 and key hypoxia effector gene HIF1A even belong to the same family of signal sensor proteins, the first sensing light and the second oxygen. Positive arm factor BMAL1 also interacts with the same HIF1A gene. In health, these interactions should result in an appropriate and time-dependent response to environmental changes, but, in disease, these measured responses are often lost. Positive arm molecular clock proteins = BMAL1 and CLOCK; negative arm molecular clock proteins = PER and CRY; hypoxia response proteins = HIF1A and HIF1B.

therefore hypoxia, found no therapeutic benefit. Conversely and counter-intuitively, a study in diabetic mice found that light deprivation was actually protective against DR.[33] Since the retina is critical to providing light timing to the overall clock system, these approaches must be considered carefully. Although the CLEOPATRA trial used light levels lower than those known to cause melatonin changes in humans, constant light has the effect of weakening circadian rhythm strength across the circadian system.[34] An intervention designed to target the molecular clock rather than just reduce hypoxia might, instead, use an intense but short light application, which has the effect of strengthening the circadian system. If hypoxia, such as that created by the photoreceptor cells during the night, can change the molecular clock in the retina as suggested by our observations in retinal endothelial cells, an intense application of light during the day may aid in retrieving normal clock timing by restoring the negative arm of the clock.

The changes that we observe in retinal endothelial cells suggest that hypoxia bolsters the positive arm of the clock by increasing its expression while its regulator, PER2, is reduced at all time points (**Figure 6**). The peak expression for these genes comes earlier after synchronization than in cells maintained at normal oxygen levels. While the period of a single oscillation of the positive arm is reduced in hypoxia, the opposite happens for the negative arm. These changes together suggest that hypoxia causes a desynchrony of this central clock in the diabetic retina. Targeting the clock might be a tool to fine-tune the hypoxic response.

Conclusion

Hypoxia, which is widespread in the diabetic retina, causes dysregulation of the cellular circadian machinery in retinal endothelial cells by favoring the positive arm so that its transcription factors are more abundant in the endothelium. What might the impact of this be for diabetic retinopathy? Ramifications are potentially as far-reaching as the targets of these positive arm transcription factors, which notably include VEGF,[14] a major growth factor implicated in DR. The next steps will be to see how outcomes like metabolism and barrier function are impacted as a result of this manipulation of the circadian clock. As we start to build a clearer picture of the circadian clock in the diabetic retina, we may even be able to target the clock itself to manage the pathologies that underlie diabetic retinopathy.

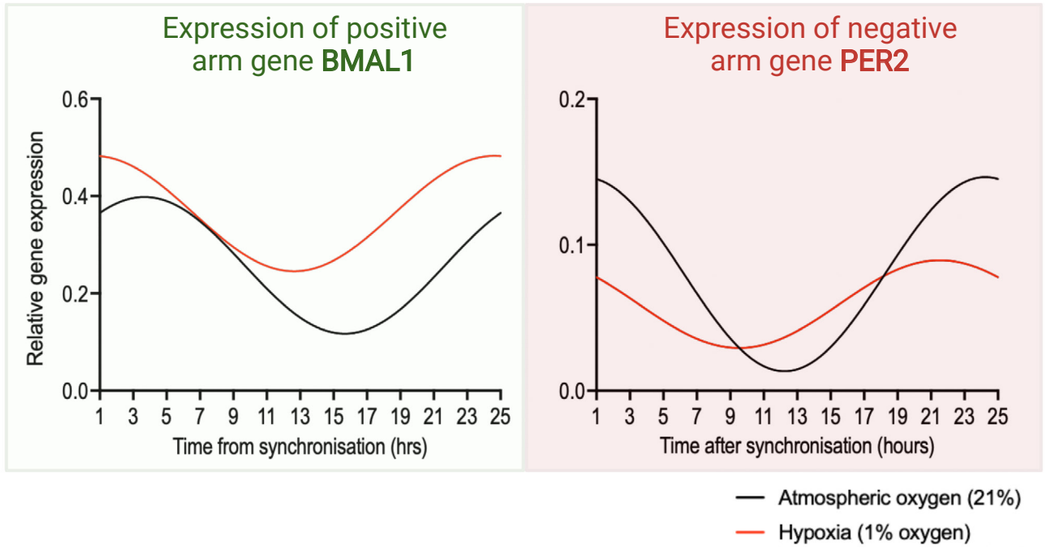

Figure 5. Expression of core clock genes BMAL1 and PER2 in endothelial cells in the hours after the cells were synchronized, cultured in either atmospheric oxygen or hypoxia (1% oxygen). The cell cultures were synchronized by treating them with a high serum content (50%) media for two hours before starting to collect samples. This ensures that all cells within the culture are at the same phase of expression. In hypoxia, BMAL1 expression is increased, and the phase shifted. Its negative regulator PER2 is downregulated. Peak transcription happens earlier for both. Created with BioRender.com.

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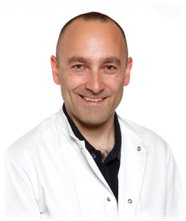

Exploring the proteome of diabetic macular edema

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Diabetic macular edema (DME) is a sight-threatening complication with a complex multifactorial pathophysiology. Clinicians continue to face challenges in managing DME, including the recurrence of edema, insufficient improvement of visual acuity, and limited absorption of edema despite relevant treatment. Patients with diabetes require continual follow-up with their health care providers, and the development of clinically significant DME is associated with even more visits. Understanding the basic science of DME is a cornerstone for successful management and the development of personalized treatment. At EASDec 2021, we presented our novel approach to better understand DME through advanced proteome analyses.

The proteome refers to the entire set of proteins in a cell, tissue, or body fluid. A proteomic analysis aims at quantifying all proteins in a sample, specially focusing on which proteins change with the disease being studied. The proteome analysis is not limited to a small number of "usual suspects" that are already known to be involved in the formation of DME. On the contrary, proteome analysis can provide the big picture of molecular mechanisms contributing to the development of DME through the identification of more than 1,000 different proteins in ocular fluids.

We have previously shown that the aqueous proteome humor widely reflects pathological changes in retinal vascular disease.[1] A unique donation from our collaborators at the Department of Ophthalmology, Kyoto Prefectural University of Medicine, recently allowed us to perform proteomic analysis for DME. The samples consisted of aqueous humor from treatment-naïve patients with DME and an age-matched control group.

Proteomic analysis of the samples is a multi-step workflow centered around the mass spectrometer (**Figure 1**). Successful proteomic experiments require careful sample preparation prior to mass spectrometric analysis. A crucial step in the sample preparation is enzymatic digestion of proteins into peptides that are unique for a given protein. The peptides are first separated on a liquid chromatography system to achieve better coverage of the proteome. Next, they are loaded onto the mass spectrometer, which measures the mass to charge ratio (m/z), and a number of peptides are specifically selected for protein quantification. To identify the proteins in the samples, the data from the mass spectrometer are searched against large protein databases. Proteins identified through this analysis that are significantly changed in DME can be correlated with clinical parameters, such as best corrected visual acuity and severity of macular edema (**Figure 2**). Key proteins identified with proteomics can be confirmed with other quantitative techniques and further tested in disease models (**Figure 2**).

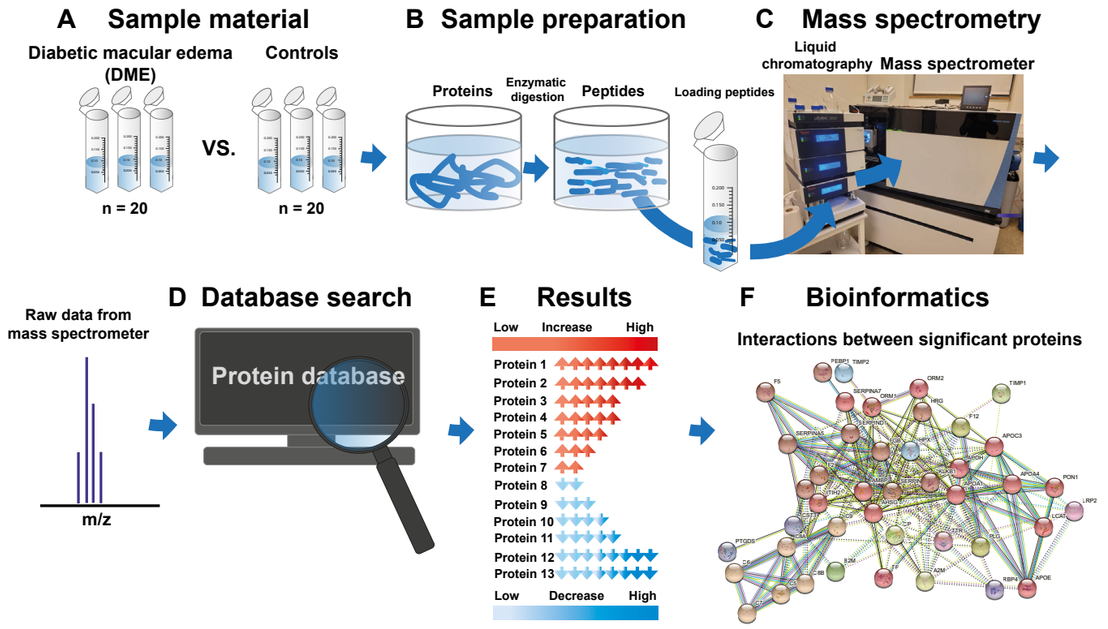

Figure 1. The successful analysis of the proteome requires a multi-step workflow. Proteomic analysis is centered around the mass spectrometer. (A) A proteome study requires a suitable sample material that allows for comparison between the disease under study and a relevant set of control samples. (B) Careful sample preparation is required. An important step is the digestion of proteins into peptides, which are used for protein quantification and identification on the mass spectrometer. (C) The peptides are loaded onto a liquid chromatography system, which separates them to allow for the identification of a greater number of proteins. After separation, peptides are passed on to the mass spectrometer. (D) Raw data are searched against existing databases to identify proteins present in the samples. (E) The output is a long list of proteins, which are either increased or decreased in the disease under study. (F) Significantly changed proteins can be grouped according to their functions and interactions to provide insights into biological processes that change in the disease under study.

At the current stage, proteomic analyses of aqueous samples from patients with DME and age-matched controls are performed in the proteomics laboratory headed by Professor Henrik Vorum. Our preliminary data show an in-depth coverage of the intraocular protein profile of DME, identifying more than 1,000 proteins with different biological functions. The identified proteins suggest that numerous biological processes contribute to the formation of DME including acute-phase response, complement activation, blood coagulation, and cholesterol metabolism. The intraocular protein profile also reflects the compromised function of glycolysis and gluconeogenesis in patients with DME.

In our next project, we are studying the aqueous proteome during anti-VEGF treatment of DME. In the first sets of samples, we predominantly observed changes in the intraocular protein profile after three or more anti-VEGF injections. Thus, the proteome analysis may indicate that at least three injections are needed to reverse the molecular mechanisms which lead to the formation of DME.

In summary, proteome analysis of DME is bringing new insights into the pathological processes underlying DME. We hope that our results can be used to improve the management of DME and contribute to the development of personalized treatment approaches.

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Key points:

- Proteomic analysis can bring novel insights into disease mechanisms leading to diabetic macular edema (DME).
- With proteomics, we identified more than 1000 proteins in aqueous humor samples from patients with DME.
- Our results suggest that acute-phase response, complement activation, coagulative changes, and cholesterol metabolism contribute to the formation of DME.
- Preliminary data indicate that at least three anti-VEGF injections are needed to reverse protein changes that lead to DME.

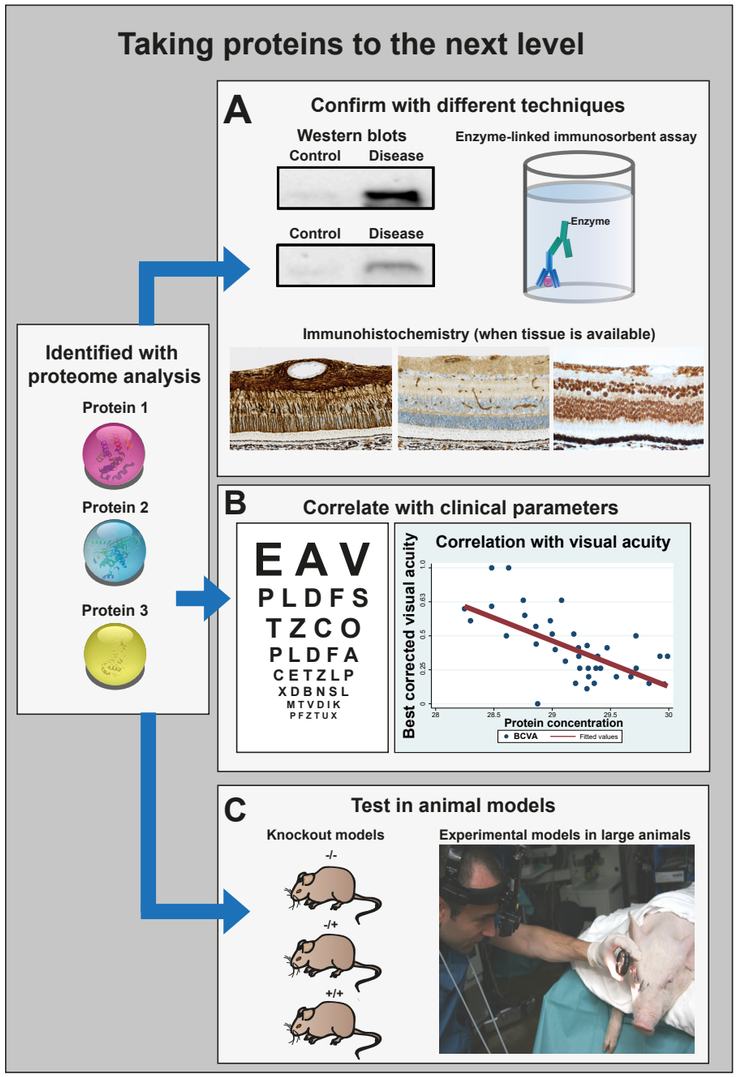

Figure 2. Proteins discovered in proteomic studies can be confirmed and further elucidated in several ways. (A) Key proteins that are significantly changed can be confirmed with other quantitative techniques, such as Western blot and enzyme-linked immunosorbent assay (ELISA). If tissue samples are available from biobanks or animal models, immunohistochemistry can be used to detect the anatomical location of the proteins. In the images provided, high concentrations of the proteins are indicated by intense brown color. (B) Significantly changed proteins can be correlated with clinical parameters such as visual acuity or severity of macular edema. (C) Identified proteins can be further explored using knockout animal models. Some disease models of ocular conditions are available in large animals.


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