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Retinal oxygen saturation is associated with HbA_{1c} but not with short-term diabetes control, internal environment, smoking and mild retinopathy - ROXINEGLYD Study

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ABSTRACT

Purpose **of this prospective uncontrolled single-centre pilot study was to find an association of retinal oxygen saturation ($SatO_2$) with acid-base balance (ABB), carboxyhaemoglobin concentration, current plasma glucose concentration (PG), mean PG and PG variability over the last 72 hrs, haemoglobin A_{1c} (HbA_{1c}), and other conditions.**

Methods: **Forty-one adults (17 men) with type 1 (N=14) or type 2 (N=27) diabetes mellitus, age 48.6±13.5 years, diabetes duration 9 (0.1-36) years, BMI 29.4±6.3 kg/m^2, HbA_{1c} 52±12.7 mmol/mol completed the study.**

The 4-day study comprised two visits (Day l, Day 4) including 72 hrs of continuous glucose monitoring (CGM) by iPro®2 Professional CGM (Medtronic, MiniMed, Inc., Northridge, CA, USA). Retinal oximeter Oxymap T1 (Oxymap ehf., Reykjavik, Iceland) was used to assess $SatO_2$.

Results: **Wilcoxon signed-rank test showed no $SatO_2$ difference between eyes and visits. Spearman's correlation analysis revealed a significant correlation between arterial $SatO_2$ and PG variability in type 2 diabetes mellitus, a positive correlation of venous $SatO_2$ with HbA_{1c} and with finger pulse oximetry. However, no correlation of $SatO_2$ with ABB, carboxyhaemoglobin, current PG, mean PG over the 72 hrs, age, diabetes duration, BMI, lipoproteinaemia, body temperature, systolic and diastolic blood pressure, heart rate, central retinal thickness and retinal nerve fibre layer thickness was found.**

Conclusion: **This study confirmed the association of venous $SatO_2$ with long-term but not with short-term diabetes control, ABB, and other conditions. The increased $SatO_2$ and questionable impact of PG variability on retinal $SatO_2$ is a research challenge.**

Key words: diabetes mellitus – retinal vessel oxygen saturation – retinal oximetry – acid-base balance – carboxyhaemoglobin – haemoglobin A1c – continuous glucose monitoring

Introduction

Retinal oximetry is a relatively new method for measuring **retinal oxygen saturation ($SatO_2$)**. In the course of the last decades $SatO_2$ changes have been described in individuals with retinal vein occlusion, retinitis pigmentosa, glaucoma, cataract, eyes after pars plana vitrectomy, sclerosis multiplex, Alzheimer´s disease, Parkinson´s disease, etc. (Safi et al. 2018; Stefánsson et al. 2019; Hübnerová et al. 2020). In addition, several studies have shown that venous $SatO_2$ is elevated in diabetic retinopathy (Hammer et al. 2009; Hardarson & Stefánsson 2012; Khoobehi et al. 2013; Jørgensen et al. 2014; Šínová et al. 2016; Veiby et al. 2020). These findings became a challenge and researchers focused on a more detailed understanding of the origin and progression of diabetic retinopathy. An increase in venous $SatO_2$ was shown to be related to severity of retinopathy (Hammer et al. 2009; Jørgensen et al. 2014; Šínová et al. 2016; Veiby et al. 2020). It appears necessary to recognize all the possible pathophysiological factors that might influence the reproducibility of $SatO_2$ and, independently, progression of diabetic retinopathy. However, recent references dealing with $SatO_2$ are scant. Lower arteriovenous $SatO_2$ difference reflects reduced oxygen delivery to tissues (Jørgensen et al. 2017). The Maastricht study (without paying any attention to $SatO_2$) revealed that type 2 diabetes and HbA_{1c} are independently associated with wider retinal arterioles (Li et al. 2020). The question is whether early regular investigations of $SatO_2$ might help to assess the risk and progress of diabetic retinopathy. That is why we have suggested the following pilot study titled **ROXINEGLYD** (Contribution of **R**etinal **OX**imetry to impact the assessment of **IN**ternal **E**nvironment, **GLY**caemia, **D**iabetes control, and other parameters on retinal vessel oxygen saturation in people with diabetes mellitus). The purpose of this study was to assess whether $SatO_2$ is associated with (1) acid-base balance and carboxyhaemoglobin concentrations, (2) current plasma glucose concentration (PG), (3) mean PG in the last 72 hrs, 48 hrs, 24 hrs, and 1 hr, (4) variability of PG over the last 72 hrs and 4 hrs, (5) haemoglobin A_{1c} (HbA_{1c}) concentration, (6) other clinical conditions and/or laboratory parameters.

Materials and Methods

This **prospective non-randomized uncontrolled single-centre pilot study** was designed and performed between March 2016 and December 2017 considering the Declaration of Helsinki as revised in 2008. It was approved by the Ethics Committee of University Hospital Olomouc and carried out in accordance with good clinical practice. Written informed consent was obtained from all participants, any of whom could leave the study at any time without any explanation.

Participants

A total of 59 adults with diabetes mellitus were recruited. On screening, six of them were excluded due to high astigmatism, amblyopia, glaucoma, moderate non-proliferative diabetic retinopathy, age-related macular degeneration; nine of them refused to adhere to the scheduled procedures; three participants were excluded at the end of the study to prevent bias due to idiopathic pulmonary fibrosis, incipient diabetic macular oedema and cortical cataract causing blurred retinal oximetry images. This means that 41 adults (17 men) with type 1 (N=14) or type 2 (N=27) diabetes mellitus completed the study. All of them fulfilled the inclusion criteria: age ≥18 years, type 1 or type 2 diabetes mellitus (T1D, T2D), absence of diabetic retinopathy except very mild or mild non-proliferative diabetic retinopathy (microaneurysms and mild retinal haemorrhages only, Davis et al. 1998). Exclusion criteria were suboptimal co-operation for experimental procedures, spherical equivalent larger than ±6 dioptres, astigmatism larger than ±3 dioptres, ocular disease except for very mild or mild non-proliferative diabetic retinopathy not requiring treatment, previous retinal laser photocoagulation and intraocular surgery, the usage of any topical or systemic eye medication with the exception of artificial tears, any skin disease interfering with subcutaneous insertion of glucose sensor.

Detailed ophthalmological, clinical and laboratory investigation and education was carried out in all of them (Table 1 - Table 5).

Design of the study

The four-day study protocol consisted of two visits performed at the start (Day 1) and at the end (Day 4).

On Day 1, the following fasting venous blood and urine sampling was made and the Enlite™ Sensor was inserted into the subcutaneous tissue of an arm or the abdomen. Then, scheduled teaching of tested participants on sensor calibration, meal plan, physical activities, smoking and data registration was carried out by an experienced educator.

The ophthalmological examination included:

- best-corrected visual acuity testing using the Early Treatment Diabetic Retinopathy Study (ETDRS) charts,
- non-contact tonometry (mean intraocular pressure of 3 measurements; Canon TX-F, Canon, Inc., Tokyo, Japan),
- slit lamp biomicroscopy,

- standard automated perimetry (G Standard White/White / Dynamic; Octopus 900, Haag-Streit AG, Köniz, Switzerland; EyeSuite™ Static, V3.1.1).

The following ophthalmological investigations were performed after pupil dilation using topical 1% tropicamide and 10% phenylephrine hydrochloride:
- slit lamp ophthalmoscopy,
- retinal oximetry (Oxymap T1, Oxymap ehf., Reykjavik, Iceland),
- fundus photography (TRC-50DX, Topcon Corporation, Tokyo, Japan),
- optical coherence tomography (OCT) (Spectralis® OCT, Heidelberg Engineering GmbH, Heidelberg, Germany) (Table 4).

Just before the retinal oximetry on Day 1 (and Day 4) the following parameters were measured:
- axillary body temperature (BT) (mercury thermometer),
- blood pressure (BP) and heart rate (HR) (automatic electronic blood pressure monitor LD7, Shanghai Little Doctor Electronic Co., Ltd., Shanghai, China),
- finger pulse oximetry (PM-60, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China) (Table 1),
- capillary PG concentration (Wellion CALLA light, MED TRUST, Austria),
- acid-base balance parameters, carboxyhaemoglobin and glucose in venous blood (ABL800 FLEX, Radiometer Medical ApS, Brønshøj, Denmark) (Table 5).

On Day 4, the same measurements related to retinal oximetry (BT, BP, HR, finger pulse oximetry, capillary PG, acid-base balance parameters and carboxyhaemoglobin and glucose) as on Day 1 were carried out and at the end of the visit the Enlite™ Sensor was removed.

<u>Key investigations</u>
Haemoglobin A_{1c} (HbA_{1c}) to assess long-term (2-3 months) diabetes control.
An approved method (Hanas et al. 2010) using the automatic glycohaemoglobin analyzer Adams™ A1c HA-8180V (Arkray, Inc., Kyoto, Japan) and measuring HbA_{1c} in blood by means of reversed-phase cation exchange chromatography was applied. HbA_{1c} concentrations were reported according to International Federation of Clinical Chemistry (IFCC) standards.

Continuous glucose monitoring (CGM) to assess short-term (72 hrs) diabetes control using means and/or medians of PG concentrations and PG variability in defined periods before retinal oximetry.

An iPro®2 Professional CGM (Medtronic, MiniMed, Inc., Northridge, CA, USA) is a blinded medical system monitoring glucose concentration over 144 h (Mohan et al. 2016; Bajaj et al. 2017; Baretić & Bralić Lang 2020). It consists of an Enlite™ Sensor, an iPro®2 Recorder and CareLink® Software. The Enlite™ Sensor is inserted under the skin and continuously generates an electrical current proportional to the glucose concentration. The iPro®2 Recorder connected to the Enlite™ Sensor takes readings every 5 minutes (i.e. 288 readings per day). The sensor was inserted on Day 1, calibrated 4 times a day using the glucometer system Wellion CALLA light, MED TRUST, Austria (Chlup et al. 2013) and removed on Day 4. Next, data from the iPro®2 Recorder was downloaded and processed by the CareLink® Software.

Retinal oximetry to assess present retinal vessel oxygen saturation ($SatO_2$).

The retinal oximeter Oxymap T1, (Oxymap ehf., Reykjavik, Iceland) attached to the view port of a fundus camera (Topcon DX-50, Topcon, Inc., Tokyo, Japan) was used. This device measures haemoglobin $SatO_2$ using the ratio of light absorbance at 600 nm and 570 nm (Geirsdottir et al. 2012).

"Protocol for acquisition and analysis of Oxymap T1 oximetry images" (version from November 21, 2013) was applied as a basic guideline. Investigations were performed in a darkroom to avoid influence of light. Fundus photographs in a 50° field were focused on the temporal edge of the optic disc and the light was set to flash to 50 Ws.

The following authors' ideas for the analysis of images were added to support the reliability of results and to prevent bias:

1. The analysis of all 164 images (41 tested participants, 2 eyes and 2 visits each) was performed by one specialist over the period of 10 days.
2. Using the circle tool, a circle around the optic disc was drawn by the maximal diameter of it. In the majority of cases the size of the circle was determined by the vertical diameter of the optic disc and horizontally was centred on the imaginary vertical line in the middle of the optic disc. This procedure resulted in small identical parapapillary areas nasally and temporally between the optic disc and circle around it.
3. Two images of one eye (on Day 1 and on Day 4) were analysed consecutively.

a. The strictly same diameter of the optic disc in pixels and the same centration of it were used in both images (see also item 2 above).

b. The same retinal vessel segments were analysed in both images (in the standardized area between 1.5 and 3 times the optic disc diameter circles concentric with the optic disc). In case of absence of a specific vessel in the first image (e.g. the vessel was not detected by the Oxymap Analyzer software, version 2.4.0), this vessel was omitted from the analysis in the second image and vice versa.

The rest of the image analysis was performed by the Protocol for acquisition and analysis mentioned above. In other words, we followed the protocol exactly, but in situations where the protocol does not clearly specify the sub-steps, we added new clear principles (mentioned above) in order to obtain the most comparable results between the visits (on Day 1 and on Day 4). Mean oxygen saturation was calculated by means of weighing with diameter in the fourth power.

Statistics

Standard descriptive statistics were used to summarise the data, including absolute and relative frequencies for categorical variables and median (range) for continuous non-normal variables; the mean±SD (standard deviation) was used for continuous normal distributed variables. Variability of glucose concentration was assessed as SD of concentrations measured over the defined period. To compare retinal oximetry parameters (Table 6) of the right eye (RE) and left eye (LE) during the same visit (on Day 1 or on Day 4) and retinal oximetry parameters of the same eye on Day 1 and Day 4, Wilcoxon signed-rank test was used. Spearman's correlation analysis was used to find an association of retinal oximetry parameters with other potentially relevant parameters (acid-base balance, carboxyhaemoglobin, current PG, mean PG over the 72 hrs, mean PG concentration and PG variability, age, diabetes duration, BMI, lipoproteinaemia, HbA_{1c}, fructosamine, BT, systolic BP, diastolic BP, HR, finger pulse oximetry, central retinal thickness and retinal nerve fibre layer thickness). Normal distribution was verified by the Shapiro-Wilk test. Statistical analyses were performed by use of the IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA). A significance level less than 0.05 was considered statistically significant ($P < 0.05$).

Results

Retinal oxygen saturation ($SatO_2$) parameters are listed in Table 6.

(1) Acid-base balance parameters and carboxyhaemoglobin had no impact on the $SatO_2$ (Table 5).

(2) The $SatO_2$ (Table 6) was not influenced by current PG concentration (Table 5).

(3) No relation between $SatO_2$ (Table 6) and short-term diabetes control (mean PG concentration over the last 72 hrs, 48 hrs, 24 hrs, and 1 hr before retinal oximetry on Day 4, Table 7) was found.

(4) PG-variability both in the course of 72 hrs and in the course of the last 4 hrs before retinal oximetry is higher in persons with T1D than in persons with T2D (Table 8, Fig. 1). Evolution of PG-variability measured in 4 hourly intervals in the course of 72 hrs before retinal oximetry shows a cyclic rhythm. A difference between T1D and T2D is obvious, particularly during the last 24 hrs (Fig. 2). The PG-variability at night is less pronounced than during the day.

In the group of all T1D and T2D together (N = 36) and, independently, in a group of T1D only (n = 12), there was no significant correlation between $SatO_2$ and PG-variability either in the course of 72 hrs or the last 4 hrs before retinal oximetry.

In the group of persons with T2D (N = 24) a negative correlation between arterial $SatO_2$ and PG-variability in the course of 72 hrs in RE ($r = - 0.565$) and also during the last 4 hrs before retinal oximetry in LE ($r = - 0.437$) or RE ($r = -0.462$) was found.

(5) There was a positive correlation between the venous $SatO_2$ (Table 6) and the haemoglobin A_{1c} concentration (Table 2) in both eyes (Day 1: $r = 0.420$, $P < 0.05$ (RE), $r = 0.399$, $P < 0.05$ (LE)). The fructosamine concentration (Table 2) had no impact on the $SatO_2$.

(6) A positive correlation was found between the retinal venous $SatO_2$ (Table 6) and finger pulse oximetry (Table 1) in 3 out of 4 calculations (Day 1: $r = 0.372$, $P < 0.05$ (RE), $r = 0.339$, $P < 0.05$ (LE); Day 4: $r = 0.439$, $P < 0.05$ (RE), $r = 0.271$, NS (LE)).

Other complementary variables (ABB, carboxyhaemoglobin, current PG, mean PG over the 72 hrs, age, diabetes duration, BMI, lipoproteinaemia, body temperature, systolic and diastolic blood pressure, heart rate, central retinal thickness and retinal nerve fibre layer thickness was found (Table 1, Table 2, Table 4 - Table 6) did not show any significant influence on the $SatO_2$.

Concentration of carboxyhaemoglobin was significantly different between current non-smokers and current smokers (Day 1 and Day 4, $P < 0.0001$, Table 1), however, the respective $SatO_2$ parameters were not affected by this finding.

Discussion

This study shows that in adults with diabetes (N = 41) the $SatO_2$ was associated with a long-term indicator of metabolic control (HbA_{1c}) but not with any short-term metabolic indicators or clinical condition.

The tested persons were adults with adequate compliance and satisfactory metabolic control (HbA_{1c} 52±12.7 mmol/mol, BMI 29.4±6.3 kg/m^2, Table 1, Table 2) supported by an up to date therapeutic approach (Table 3) and education. Comprehensive laboratory tests were performed only once at the beginning of the study. However, more variable parameters (BT, BP, HR, finger pulse oximetry, acid-base balance, carboxyhaemoglobin and glucose) that could affect retinal oximetry were carried out both on Day 1 and on Day 4. Based on the 20-year history of CGM (Bode & Hirsch 2000; Šoupal et al. 2020) and on our previous experience (Mlčák et al. 2003; Mlčák et al. 2004; Peterson et al. 2009; Kudlová & Chlup 2011; Kudlova et al. 2017) we used CGM in order to investigate potential associations of its 72hr-outcomes with retinal oximetry parameters on Day 4.

We find the following 6 items worthy of mentioning:

1) Acid-base balance. We focused on the acid-base balance because its parameters affect the affinity of haemoglobin for oxygen. Decreased affinity of haemoglobin for oxygen in tissues (right shift of the oxygen dissociation curve) is associated with an increase in either body temperature, hydrogen ions (i.e. lower pH), 2,3-bisphosphoglycerate (2,3-BPG) concentration or carbon dioxide concentration. In our study, the estimated parameters (Table 5) had no significant impact on the $SatO_2$. Unfortunately, we did not find an appropriate and affordable method for 2,3-BPG measurement.

2) Current PG concentration (glycaemia). In our study, the $SatO_2$ was not influenced by current PG concentration measured in venous plasma using point-of-care testing (POCT). Our findings are different from Klefter et al. 2015. Differences could be caused by variable metabolic state of the tested participants. Whereas the participants in our study were stable, Klefter investigated changes in $SatO_2$ in the course of acute hyperglycaemia due to a discontinuation of diabetes therapy (except insulin) and during an oral glucose tolerance test.

3) Mean PG concentration in the last 72 hrs, 48 hrs, 24 hrs, and 1 hr. No association of the retinal $SatO_2$ and short-term diabetes control in the respective period of the last 72 hrs was found. We sought correlations of the $SatO_2$ with mean PG over the last 72 hrs, 48 hrs, 24 hrs, and 1 hr preceding the oximetric investigation on Day 4. The application of the CGM system

appears to be a new approach to this problem. We have not found any similar references in literature.

4) PG variability. In 5 persons, failures of CGM system made the measurement of variability impossible. A difference between T1D (N=12) and T2D (N=24) is significant (Table 8, Fig. 2). Small number of tested persons and a non-parametric distribution of data made the use of linear regression analysis impossible. That´s why Spearman analysis was applied to assess the correlations. However, we are unable to explain why the correlation between arterial $SatO_2$ and PG-variability could be confirmed in persons with T2D only.
Continuation of this study and increasing the number of tested persons might help to find a correct answer.

5) Haemoglobin A_{1c} (HbA_{1c}) concentration in blood is determined by chronic hyperglycaemia but not glucose variability (Kohnert et al. 2007). There are three options on how to measure HbA_{1c} (Hanas et al. 2010). In our study, the high-performance liquid chromatography (HPLC) was used. We found a positive correlation between the venous $SatO_2$ in either eye and the HbA_{1c}. Bek et al. 2019 showed that diabetes duration, HbA_{1c}, central retinal thickness and venous $SatO_2$ were independent risk factors for the severity of retinopathy on scales with both diabetic macular oedema and proliferative diabetic retinopathy. Although HbA_{1c} has increased affinity for oxygen (Ditzel 1976; Bek et al. 2019) the concentration of HbA_{1c} strongly correlates with the presence of microvascular complications which are related to diabetes control. The question is whether $SatO_2$ is dependent especially on microvascular retinal vessel involvement or on HbA_{1c}.

6) Other complementary parameters.

Recent metaanalyses have demonstrated the complex and versatile pathophysiology of diabetic retinopathy making any assessment of pivotal risk factors difficult (van der Heijden et al. 2020). Our results indicate that the retinal venous $SatO_2$ in adults with diabetes was significantly higher (65.6±6.0%, RE, Table 6) than in healthy individuals published by Geirsdottir et al. 2012 (55.6±6.3%), Our finding also corresponds with previous observations of others where people with diabetes had higher retinal venous $SatO_2$ (Hammer et al. 2009; Hardarson & Stefánsson 2012; Khoobehi et al. 2013; Jørgensen et al. 2014; Šínová et al. 2016; Veiby et al. 2020).

In addition, longer diabetes duration was associated with higher retinal arterial $SatO_2$ (Klefter et al. 2016). This is also consistent with the results of our study in which retinal arterial oxygen saturation was only slightly higher than in healthy individuals (Geirsdottir et al. 2012).

Nevertheless, we could not confirm any significant correlation between $SatO_2$ (Table 6) and diabetes duration (Table 1). In addition, diabetes duration is often better defined in T1D, whereas persons with T2D are sometimes only diagnosed after having had the disease for 5 years or more. All these controversies appear to be worthy of complex research based on systematic routine implementation of retinal oximetry.

In our study, a positive correlation between the venous $SatO_2$ (Table 6) and finger pulse oximetry (Table 1) was found. Another study did not confirm any association of finger pulse oximetry with $SatO_2$ (Geirsdottir et al. 2012).

The strengths of our study include the design of the single centre protocol with one experienced ophthalmologist investigating all the tested participants and analysing retinal images according to his own defined approach based on the generally recommended schedule; retinal oximetry of both eyes in two consequent sessions confirming identical values of the respective retinal oximetry parameters; application of the iPro®2 Professional CGM to identify a missing association of short-term diabetes control with $SatO_2$, and using the HbA_{1c} to confirm the impact of long-term control on $SatO_2$; the broad array of additional analyses, which gave deeper insight into the potential associations of $SatO_2$ with various conditions.

Our study also has limitations. First, evaluation of the influence of the examined parameters on retinal oximetry may be affected by the small size of our group and its relative heterogeneity (e.g. T1D vs T2D, different smoking status). Second, we were unable to measure 2,3-BPG concentration in erythrocytes. Third, although investigational procedures were standardised, participants were allowed to continue doing usual activities (including smoking) between Day 1 and Day 4 and to take a light meal before ophthalmological investigation which may influence individual homeostatic parameters, retinal microvascular diameters, etc. So, there is still a possibility of residual confounding by variables that were not included in the analyses. Fourth, ABB parameters could be affected by the method used. Although we chose the measurement from venous blood, blood collection was often difficult and stretched. We did not choose capillary blood due to the risk of sample degradation and the consequent impossibility of its analysis. We evaluated the use of arterial blood for ABB examination as too invasive in clinically stable participants. Fifth, the presence of hypertension as well as the use of vasoactive medications could also influence the $SatO_2$. In our study group participants were stable and therefore change of antihypertensive therapy was not indicated.

In conclusion, retinal oxygen saturation ($SatO_2$) appears to be independent of short-term PG concentration, internal environment, carboxyhaemoglobin concentration, and other complementary parameters. The association of the venous $SatO_2$ with the HbA_{1c} supports potential benefits of long-lasting diabetes control for the prevention of diabetic retinopathy. The impact of glucose variability remains questionable. The increased $SatO_2$ in our tested group becomes a challenge for future research. HbA1c, diabetes duration, glucose variability, hypertension etc. have already been shown to be strong predictors of diabetic retinopathy and maculopathy development. Hence, oximetry might be viewed as a tool to add information to the known predictors of vision-threatening diabetic retinopathy. If it reveals information about long-term and/or short-term glycemia, it may hold the potential to predict long-term diabetic retinopathy development. However, the perspectives of retinal oximetry in the field of routine prevention, diagnosis and progress of diabetic retinopathy are still obscure.

Conflict of Interest

The authors have no financial interest in any product or method mentioned in the text.

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Figure 1. PG-variability in the course of 72 hrs before retinal oximetry in respective groups of individuals with T1D (N=12) and T2D (N=24). In two individuals with T1D, grey spots are hidden under black spots of individuals with T2D (No 2 and No 12).

Figure 2. Medians of 4-hr-PG-variability in 18 consequent 4 hourly intervals in the course of 72 hrs before retinal oximetry in individuals with T1D (N=12) and with T2D (N=24).

Table 1. Clinical characteristics and comorbidities of 41 study participants.

Parameter	Unit	Value
Gender (M/F)	N/N	17/24
Age	years	48.6±13.5
Height	cm	175±9.9
Body mass	kg	90.3±22.0
Body mass index	kg/m^2	29.4±6.3
Diabetes mellitus (T1D/T2D)	N/N	14/27
Diabetes duration	years	9 (0.1-36)
Insulin pump (yes/no)	N/N	14/27
Smoker (yes/no)	N/N	13/28
Systolic arterial blood pressure*	mmHg	125.6±12.2
Diastolic arterial blood pressure*	mmHg	79.7±9.2
Heart rate*	beats/min	74.3±11.2
Axillary body temperature *	°C	36.6±0.3
Finger pulse oximetry*	%	97.6±1.0
Dyslipidaemia	N	24
Hypertension	N	21
Hepatopathy	N	11
Hypothyroidism	N	10
Diabetic neuropathy	N	10
Diabetic retinopathy (very mild and mild only)	N	8
Rare comorbidities: asthma (3), sleep apnoea syndrome (3), adrenal gland adenoma (3), lower extremity varices (3), reflux esophagitis (2), pregnancy (2), hyperuricaemia (2), benign prostatic hyperplasia (2), diabetic nephropathy (1), prostatitis (1), sarcoidosis (1), colorectal adenoma (1), monoclonal gammopathy (1), Hodgkin´s lymphoma (1), varices cruris (1), erectile dysfunction (1), coxarthrosis (1), gonarthrosis (1), bipolar affective disorder (1).		

Results are presented as mean±SD, median (range) or numbers.

*The values were calculated as the mean of two measurements from both visits (one from Day 1 and one from Day 4).

Table 2. Metabolic parameters of 41 study participants.

Parameter	Unit	Value	Note
Fasting plasma glucose	mmol/l	6.7 (3.0-18.1)	
Blood haemoglobin A_{1c} (IFCC)	mmol/mmol	52±12.7	
Serum fructosamine	umol/l	287 (199-454)	
Serum insuline	mIU/l	17.5 (6.5-212.5)	
Serum C-peptide	pmol/l	686 (13-1789)	N = 33
		Undetectable	N = 8
Serum albumin	g/l	46.1±2.9	
Serum C-reactive protein	mg/l	1.5 (0.6-18.0)	
Serum total cholesterol	mmol/l	4.34 (3.14-7.04)	
Serum triacylglycerols	mmol/l	1.37±0.65	
Serum high-density lipoprotein	mmol/l	1.27 (0.69-2.61)	
Serum low-density lipoprotein	mmol/l	2.29 (1.30-4.87)	
Serum apolipoprotein A-I	g/l	1.64±0.31	
Serum apolipoprotein B	g/l	0.86 (0.61-1.53)	
Serum lipoprotein (a)	nmol/l	17 (7-233)	

Results are presented as mean±SD or median (range).

Table 3. Medical therapy (drugs) in 41 study participants.

Medication	N
Insulin, incretin, SGLT 2 inhibitors, metformin	
Short-acting insulins	29
Long-acting insulins	14
Liraglutide	6
Metformin	23
Dapagliflozin	8
Gliptins	6
Gliclazide	1
Cholesterol-lowering drugs	
Statins	22
Fibrates	2
Ezetimib	1
Antihypertensives	
Calcium channel blockers	13
ACE inhibitors	12
Angiotensin II antagonists	7
Diuretics	9
Beta-blockers	8
Imidazoline receptor agonists	4
Spironolacton	1
Other drugs: Magnesium (13), Levothyroxine (10), Antiepileptics, antidepressives, antipsychotics (10); B Vitamin group (7); Inhaled glucocorticoids and/or beta2-agonists (6); Proton pump inhibitors (5), Acetylsalicylic acid (4), Hesperidin (4), Hepatoprotectives (4), Analgetics and/or non-steroidal antiinflammatory drugs (4), Levocetirizine or cetirizine (3), Allopurinol (2), Amylase + lipase + protease (1), Ferrum + ascorbic acid (1), Itopride (1), Pentoxifyllin (1), Sildenafil (1), Sulodexid (1), Sumatriptan (1), Tamsulosin (1), Tolperisone (1). Zolpidem (1)	

Table 4. Ocular characteristics of 41 study participants.

Eye		Right (RE)	Left (LE)
N		41	41
Spherical equivalent refraction*	Diopters	0 (-4.75 to 2.63)	0 (-4.75 to 4.00)
Best corrected visual acuity*	LogMAR†	-0.05±0.07	-0.06±0.07
Intraocular pressure*	mmHg	15.1±2.5	15.3±2.6
Diabetic retinopathy (absence/very mild/mild)	N	34/3/4	33/2/6
Optical coherence tomography (OCT)			
Average retinal nerve fibre layer thickness	μm	104±10	103±10
Central retinal thickness	μm	276±24	276±23

Results are presented as mean±SD or median (range).
*The values were calculated as the mean of two measurements from both visits (one from Day 1 and one from Day 4).
†Logarithm of the Minimum Angle of Resolution

Table 5. Acid-base balance parameters, carboxyhaemoglobin and glucose in venous blood collected within 5 min before retinal oximetry in 41 study participants; analysed by point-of-care testing (POCT): ABL800 FLEX, Radiometer. Medical ApS, Brønshøj, Denmark.

Parameter	Unit	Day 1	Day 4
pH		7.39±0.03	7.39±0.03
Partial pressure of carbon dioxide (PCO_2), kPa	kPa	5.96±0.81	5.88±0.72
Partial pressure of oxygen (PO_2)	kPa	4.15±1.59	4.21±1.02
Actual bicarbonate	mmol/l	26.7±2.7	26.4±2.2
Standard bicarbonate	mmol/l	24.8±1.5	24.7±1.4
Base excess (BE)	mmol/l	1.68±1.97	1.44±1.77
Haemoglobin	g/l	144.9±13.7	141.1±16.3
Haematocrit	%	44.5±4.1	43.4±5.0
Carboxyhaemoglobin	%	0.8 (0.2-5.3)	0.9 (0.3-6.2)
Glucose	mmol/l	10.5±4.2	7.7±2.7

Results are presented as mean±SD or median (range).

Table 6. Retinal oximetry parameters in both eyes on Day 1 and Day 4.

Eye		Right (RE)		Left (LE)		P
Visit		Day 1	Day 4	Day 1	Day 4	
N		41	41	41	41	
Retinal arterial $SatO_2$	%	95.4±3.3	95.3±3.3	95.5±3.5	95.3±3.4	NS
Retinal venous $SatO_2$	%	65.6±6.0	65.2±5.7	66.1±4.5	65.5±5.6	NS
Arteriovenous $SatO_2$ difference	%	29.9±5.9	30.1±5.5	29.4±4.2	29.9±5.1	NS
Retinal arterial diameter	pixel	12.3±1.2	12.3±1.2	12.4±1.0	12.4±1.1	NS
Retinal venous diameter	pixel	16.6±1.6	16.5±1.6	16.8±1.4	16.7±1.5	NS

Results are presented as mean±SD.

NS: No significant difference of retinal oximetry parameters between the right and left eyes either on Day 1 or on Day 4, or between the same eye on Day 1 vs Day 4 was found.

Table 7. PG concentration measured by CGM system in the respective periods of time before retinal oximetry on Day 4 in 36 out of 41 participants. Only participants with minimum 845 of 864 (98%) PG values/72 hrs were considered.

Period before retinal oximetry	Mean±SD [mmol/l]	Median (range) [mmol/l]
Last 72 hrs	7.7±2.0	6.8 (5.6-13.1)
Last 48 hrs	7,6±2.0	6.7 (5.4-14.0)
Last 24 hrs	7.7±2.4	6.7 (4.9-15.5)
Last 1 hr	7.3±2.3	6.6 (3.7-14.4)

Table 8. PG-variability [mmol/l] in the course of 72 hrs and 4 hrs before retinal oximetry in the group of T1D and in the group of T2D.

	T1D (N=12)		T2D (N=24)	
Parameter	72-hr-PG-variability	4-hr-PG-variability	72-hr-PG-variability	4-hr-PG-variability
Mean	3.125	2.102	1.539	1.214
SD	1.155	0.806	0.440	0.774
Median	**3.110**[a]	**2.178**[b]	**1.527**[a]	**0.963**[b]
Minimum	1.463	0.903	0.751	0.245
Maximum	4.722	3.407	2.146	3.057

[a] Significant difference in 72-hr-PG-variability in T1D vs T2D (P=0.001).

[b] Significant difference in the last 4-hr-PG-variability before retinal oximetry in T1D vs T2D (P=0.002).

Figure 1. PG-variability in the course of 72 hrs before retinal oximetry in respective groups of individuals with T1D (N=12) and T2D (N=24). In two individuals with T1D, grey spots are hidden under black spots of individuals with T2D (No 2 and No 12).

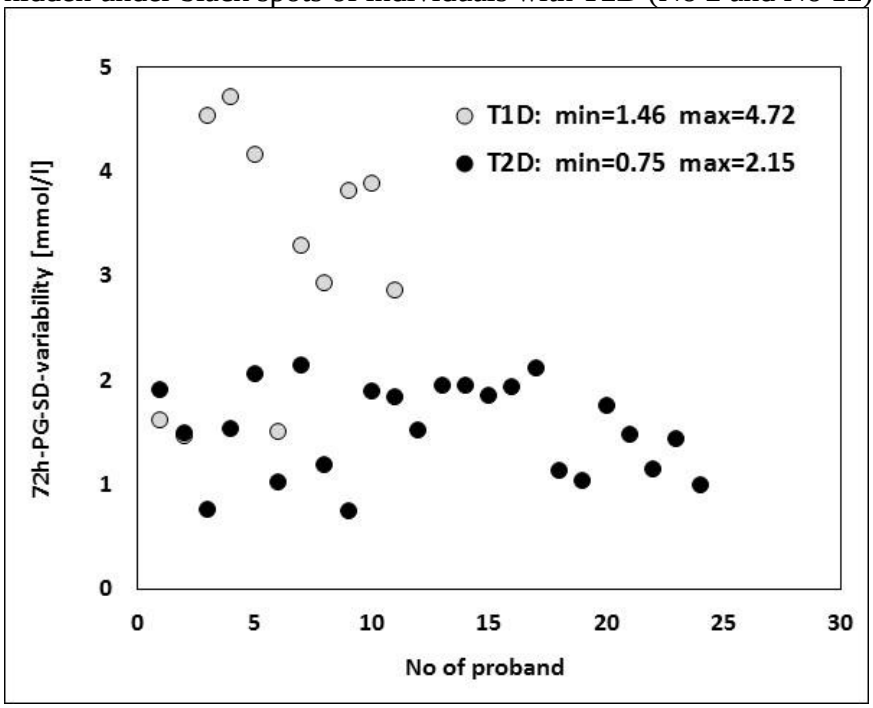

Figure 2. Medians of 4-hr-PG-variability in 18 consequent 4 hourly intervals in the course of 72 hrs before retinal oximetry in individuals with T1D (N=12) and with T2D (N=24).