Retinal oxygen saturation is altered in diabetic retinopathy

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ABSTRACT

Aim Retinal oxygen metabolism is thought to be affected in diabetic retinopathy. The aim of this study was to test whether retinal vessel oxygen saturation is different in patients with diabetic retinopathy from that in healthy controls.

Methods The retinal oximeter is based on a fundus camera. It estimates retinal vessel oxygen saturation from light absorbance at 586 nm and 605 nm. Retinal vessel oxygen saturation was measured in one major temporal retinal arteriole and venule in healthy volunteers and in patients with diabetic retinopathy.

Results Oxygen saturation in the retinal arterioles of healthy volunteers was 93±4% and 58±6% in venules (mean±SD, n=31). The corresponding values for all diabetic patients (n=20) were 101±5% and 68±7%. The difference between healthy volunteers and diabetic patients was statistically significant ($p<0.001$ for arterioles and venules). Three subgroups of diabetic patients (background retinopathy, macular oedema and pre-proliferative/proliferative retinopathy) all had higher saturation values than the healthy volunteers ($p<0.05$ for arterioles and venules).

Conclusion Retinal vessel oxygen saturation is higher in patients with diabetic retinopathy than in healthy controls. Possible explanations include shunting of blood through preferential channels, bypassing non-perfused capillaries in the capillary network. Parts of the retinal tissue may be hypoxic while blood in larger vessels has high oxygen saturation.

INTRODUCTION

Diabetic retinopathy damages retinal capillaries[1 2] and may therefore disturb the distribution of oxygen to retinal cells and cause tissue hypoxia. Several studies have been performed on retinal oxygenation in diabetes. Most of them were performed on animal models with oxygen-sensitive probes, which measure oxygen tension (partial pressure of oxygen). The oxygen tension measurements show the amount of free oxygen in the retina or in the vitreous cavity, depending on the location of the probe. The oxygen tension in the vitreous cavity is believed to reflect mostly the inner retinal oxygenation. Linsenmeier *et al*[3] measured oxygen tension with intra-retinal probes and demonstrated inner retinal hypoxia in long-term diabetic cats. Animal experiments in which oxygen tension in the vitreous has been measured have not shown hypoxia in models of diabetes.[4–7] However, Holekamp *et al*[8] measured oxygen tension in patients undergoing vitrectomy and found oxygen tension in the vitreous cavity to be lower in the diabetic retinopathy eyes compared with non-diabetic eyes.

Since most of the oxygen in blood is bound to haemoglobin, oxygen saturation in retinal vessels can give valuable information on retinal oxygenation, particularly if combined with knowledge on blood flow. Earlier studies with a spectrophotometric technique have shown a decrease in retinal venular saturation during hyperglycaemia in diabetic patients without diabetic retinopathy[9] (see also the discussion section).

Evidence for the role of oxygenation in diabetic retinopathy has also been found by studying the effect of supplemental oxygen. Drasdo *et al*[10] found decreased oscillatory potentials in the electroretinogram of diabetic patients without retinopathy. The oscillatory potentials, which reflect inner retinal activity, were normalised by supplemental oxygen. Similarly, Harris *et al*[11] found improved contrast sensitivity during hyperoxia in diabetic patients with minimal retinopathy and Nguyen *et al*[12] found that diabetic macular oedema was improved by breathing pure oxygen.

Although direct evidence for retinal hypoxia in diabetic retinopathy has only emerged recently, retinal hypoxia has been implicated in the pathogenesis of diabetic retinopathy for more than 50 years.[13 14] Retinal hypoxia is believed to stimulate both neovascularisation and retinal oedema through various mechanisms. Furthermore, treatment of diabetic retinopathy with laser or vitrectomy may function by alleviating retinal hypoxia (for review, see Stefansson[15]).

In this study a non-invasive retinal oximeter was used to test whether retinal vessel oxygen saturation of haemoglobin in blood is different in patients with diabetic retinopathy from that of healthy controls.

MATERIALS AND METHODS

The retinal oximeter (Oxymap ehf., Reykjavik, Iceland) is an investigational device that has been described previously.[16–18] It is based on a fundus camera (Canon CR6-45NM; Canon Inc., Tokyo, Japan) that is coupled with a beam splitter (MultiSpec Patho-Imager; Optical Insights, Tucson, Arizona, USA) and a digital camera (SBIG ST-7E; Santa Barbara Instrument Group, Santa Barbara, California, USA). It yields fundus images with four wavelengths of light simultaneously. A dark image is subtracted from each image to reduce the effect of sensor noise. Specialised software automatically selects measurement points on the oximetry images and calculates the optical density (absorbance) of retinal vessels at two wavelengths, 605 nm and 586 nm. Optical density is sensitive to oxygen saturation at 605 nm but not at the reference wavelength, 586 nm. The ratio of these optical

densities is approximately linearly related to haemoglobin oxygen saturation[19 20] and the oximeter yields relative oxygen saturation values. The calibration was performed by matching the optical density values from healthy individuals with saturation measurements, which were performed with a calibrated device[21] in manner similar to that described previously.[17]

Infrared light was used to align the fundus camera (oximeter) and the images were taken in a dark room. The time between images (flashes) of the same eye was on average about 1 min. Pupils were dilated with 1% tropicamide (Mydriacyl; S.A. Alcon-Couvreur N.V., Puurs, Belgium), which was in some cases supplemented with 10% phenylephrine hydrochloride (AK-Dilate; Akorn Inc., Lake Forest, Illinois, USA).

Oxygen saturation was measured in one major temporal retinal arteriole and venule in one eye in each subject.

Statistical analysis was performed with Prism, version 5 (GraphPad Software Inc., La Jolla, California, USA). Unpaired t test, one-way ANOVA and Dunnett's test were used for comparisons of means.

Patients were included if they had diabetic retinopathy of the categories shown in table 1 and excluded if they had other eye diseases. Healthy individuals were excluded if they had ocular diseases. No subject had severe cardiovascular or respiratory problems.

Table 1 describes the groups compared in the study.

RESULTS

Oxygen saturation in healthy volunteers was 93±4% in arterioles and 58±6% in venules, and the arteriovenous difference was 35±7% (mean±SD, n=31). The corresponding values for all diabetic patients (n=20) were 101±5% and 68±7%, and 34±7%. The difference between healthy volunteers and diabetic patients was statistically significant for arterioles and venules ($p<0.001$, unpaired t test), but not for the arteriovenous difference ($p=0.53$). Retinal vessel oxygen saturation in subgroups is shown in figure 1 and in table 2.

DISCUSSION

The present results show that retinal oxygen metabolism is affected in diabetic retinopathy. Retinal vessel oxygen saturation is higher in all categories of diabetic retinopathy compared with healthy subjects. This agrees with the results of Hammer *et al*,[22] who also found higher venular saturation in patients with diabetic retinopathy and that the saturation increased with severity of retinopathy. The same group, using another technique,[23] did not find a statistically significant difference in mean saturation values between healthy individuals and patients with mild to moderate non-proliferative diabetic retinopathy. Thus, three different instruments and two groups have shown normal or elevated oxygen saturation in the larger retinal vessels in diabetic retinopathy.

There are several possible explanations for the elevated retinal vessel oxygen saturation in diabetic retinopathy. This may involve the distribution of blood flow and oxygen to the retina. Change in total supply and demand for oxygen in the retina may also play a role.

Distribution of oxygen may be disturbed by at least three mechanisms: (1) capillary non-perfusion and shunting; (2) thickening of capillary vessel walls; and (3) greater affinity of haemoglobin for oxygen in diabetic patients.

Shunting refers to blood flow through dilated preferential channels, bypassing the retinal capillary network. Cogan and Kuwabara[1] reported that in diabetic retinopathy, some capillaries close while others dilate and enlarge. Fluorescein angiography also shows that enlarged capillaries shunt the blood from the retinal arterioles to the retinal venules, bypassing areas of capillary non-perfusion. This means that a large amount of blood is transported quickly through dilated capillaries without giving much oxygen to the tissue. The capillary non-perfusion and shunting represents maldistribution of oxygen. Oxygen is not delivered to the retinal cells in the non-perfused, ischaemic areas and stays in the blood, making the venular blood relatively hyperoxic and the tissue hypoxic in the non-perfused areas.

Another reason for decreased delivery of oxygen from blood to tissue is thickening of capillary walls, which is well established in diabetic retinopathy (for reviews see Ashton[24] and Roy *et al*[25]). This increases the diffusion distance from the vessels to the

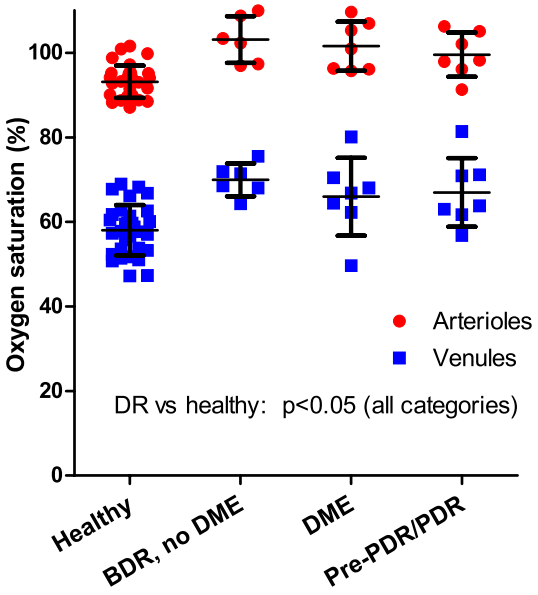

Figure 1 Retinal vessel oxygen saturation (%). Each point denotes a measurement in one major temporal vessel. One arteriole and one venule were measured in each eye. The bars denote means and standard deviations. BDR, background diabetic retinopathy; DME, diabetic macular oedema; PDR, proliferative diabetic retinopathy.

Table 1 Clinical and demographic data for the groups studied

Group	Value
Healthy volunteers (n=31)	
Age (years)	32±15
Sex	19 men, 12 women
Background DR, no macular oedema (n=6)	
Age (years)	57±16
Sex	3 men, 3 women
No. with type of diabetes	2 type 1, 4 type 2
Duration of diabetes (years)	17±11
Diabetic macular oedema, no treatment (n=7)	
Age (years)	60±15
Sex	5 men, 2 women
No. with type of diabetes	5 type 1, 2 type 2
Duration of diabetes (years)	19±9
Pre-proliferative/proliferative DR, no treatment (n=7)	
Age (years)	42±14
Sex	6 men, 1 woman
No. with type of diabetes	6 type 1, 1 type 2
Duration of diabetes (years)	20±5

Values are mean±SD.
DR, diabetic retinopathy.

Table 2 Oxygen saturation (%) in retinal arterioles and venules

	Arterioles	Venules	Arteriovenous difference
Healthy volunteers (n=31)	93±4 (91.8 to 94.8)	58±6 (55.8 to 60.2)	35±7 (32.8 to 37.5)
Background DR, no macular oedema (n=6)	103±6 (95.7 to 114.1)	70±4 (65.9 to 74.0)	33±5 (27.9 to 38.5)
Diabetic macular oedema, no treatment (n=7)	102±6 (94.9 to 107.3)	66±9 (57.4 to 74.5)	36±10 (26.2 to 45.0)
Pre-proliferative/proliferative DR, no treatment (n=7)	100±5 (91.6 to 105.8)	67±8 (59.4 to 74.5)	33±5 (27.7 to 37.5)

Values are mean±SD and 95% CIs.
All subgroups with DR had higher arteriolar and venular saturation than the healthy group ($p<0.05$, arterioles and venules, ANOVA and Dunnett's test). The arteriovenous difference was not statistically different between groups.
DR, diabetic retinopathy.

tissue and thereby decreases diffusion, which means that more oxygen is retained in the blood. However, the relative increase in distance may be too small to have a notable effect. Third, diabetic patients have slightly higher levels of glycosylated haemoglobin and this will retain the oxygen in the vasculature somewhat better than in healthy individuals, although changes in 2,3-disphosphoglycerate concentration could either increase or decrease such an effect.[26] This may therefore help to explain the increased saturation in retinal arterioles as well as venules.

While the maldistribution of blood flow and impaired delivery of oxygen from blood to tissue is the most likely reason for the disturbance seen in retinal oxygen metabolism, other possibilities must be considered. These include changes in total supply or demand for blood flow and oxygen in the diabetic retina.

Data on total retinal blood flow or blood velocity in diabetes are conflicting. Several studies have shown increased total retinal blood flow or blood velocity in diabetic retinopathy, while others show either no difference or even decreased blood flow or velocity (for review see Pournaras *et al*[27]). Increased total retinal blood flow might explain the increased saturation seen in venules simply due to the increased supply/demand ratio for oxygen in the retina. Increased total retinal blood flow may also explain the increased saturation seen in arterioles due to relatively reduced loss of oxygen by diffusion through arterial and arteriolar walls to the adjacent tissue or venules.

Decreased demand for oxygen may also play a role but data are lacking. Retinal tissue may have degenerated.[28 29] If the tissue degenerates, oxygen consumption decreases and that may help to explain the increased oxygen saturation in retinal venules.

Oxygen saturation in arterioles was considerably higher in patients with diabetic retinopathy than in healthy individuals. Hammer *et al*[22] found a trend towards higher arteriolar saturation with increased severity of disease, although this was not statistically significant. There are several possible explanations for higher arteriolar saturation in diabetic retinopathy as measured by the two groups.

Both groups measured saturation in large retinal arterioles and averaged measurements along a segment. Oxygen can escape by diffusion through arterial walls as blood flows towards the retina and through the retinal arteriolar walls. There is evidence for such diffusion in various tissues (for a review see Pittman[30]). In the retina, a capillary-free zone surrounds the retinal arterioles, indicating that the tissue adjacent to the arterioles receives enough oxygen directly through the arteriolar walls.[31] In miniature pigs, an oxygen concentration gradient was found away from the retinal arterioles,[32 33] and Schweitzer *et al*[21] found higher oxygen saturation in the centre of arterioles than close to the walls. There appears to be an inward gradient of oxygen concentration towards retinal venules[21 32] and a counter-current mechanism has been proposed, whereby oxygen diffuses from arteries/arterioles into adjacent veins/venules.[33] It should be noted that the central retinal artery lies very close to the central retinal vein within the optic nerve, and this is optimal for counter-current exchange of oxygen.

The difference in arteriolar saturation between healthy individuals and patients with diabetic retinopathy may be due to increased total retinal blood flow in diabetic retinopathy, which would decrease loss of oxygen from per unit volume of blood. As mentioned above, changes in retinal blood flow in diabetic retinopathy are controversial. Glycosylated haemoglobin has increased affinity for oxygen, which could theoretically increase oxygen saturation in diabetes. However, finger pulse oximetry values, which were available for some subjects, indicated no difference between healthy individuals and diabetic patients.

Both arteriolar saturation and venular saturation were higher in the patients with diabetic retinopathy and the arteriovenous difference was similar. Total retinal oxygen delivery can be approximated by the product of the arteriovenous difference in saturation and retinal blood flow. Unfortunately, data on retinal blood flow in diabetic retinopathy are conflicting.

Unlike Hammer *et al*,[22] we did not find an increase in saturation with the severity of disease. However, the subgroups were small and the statistical power to detect differences between them is low.

Our control group was on average considerably younger than the diabetic groups. Unpublished data from our group shows that retinal oximetry is relatively stable with age in healthy individuals and changes less than 0.1% per year in venules, declining slightly with age. If the current data had been corrected for the age difference, this would make the difference between the healthy controls and diabetic groups slightly larger.

The oxygen saturation values depend on calibration, are not absolute and sometimes exceed 100%. However, the oximeter has been shown to give repeatable results and to be sensitive to changes in oxygen saturation,[17] and relative oxygen saturation can be compared between groups. Traustason *et al*[34] demonstrated that retinal oxygen saturation values correlated well with arterial blood samples.

Differences in vessel diameter may influence measurements of relative oxygen saturation.[20 35] However, earlier studies have indicated that differences in vessel diameter between healthy individuals and patients with various categories of diabetic retinopathy are complex and in most cases small[36 37] in comparison with the changes needed to markedly influence measured saturation.[20]

Oxygen tension and gradients in the retinal tissue are more directly relevant for retinal cells than the oxygen saturation in the larger vessels. However, it is technically not possible to reliably and non-invasively measure oxygen tension in the human retina. When combined with other data retinal vessel oxygen saturation can give valuable insights into disease mechanisms. Our studies do not show which of mechanisms described above are responsible for the differences seen, and it is

possible that more than one mechanism contributes. Blood shunting and bypassing non-perfused capillaries correspond well with findings seen in histology and fluorescein angiography. Maldistribution of oxygen leads to tissue hypoxia and the tissue may then call for more oxygen, that is, total blood flow may increase as a consequence. Degeneration of tissue, regardless of cause, will lead to less oxygen demand and reduce oxygen extraction from blood vessels. All of these phenomena would lead to higher venular oxygen saturation, as confirmed in our data. Whatever the cause is, our data demonstrate that oxygen metabolism is affected in diabetic retinopathy. Non-invasive oximetry allows this metabolic change to be studied in more detail, in larger groups of diabetic patients, and to be correlated with morphological and functional changes in diabetic retinopathy.

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Competing interests Both authors are involved in the development of the instrument (oximeter) used and have a financial interest in the company Oxymap ehf., which makes this instrument. The first author is an employee of the University of Iceland but Oxymap ehf. partly supports the position.

Patient consent Obtained.

Ethics approval Ethics approval was provided by National Bioethics Committee of Iceland and the Icelandic Data Protection Authority.

Contributors Both authors contributed substantially to the conception and design of the study as well as interpretation of the data (analysis made by the first author). Article was drafted by the first author but critically revised by both authors. Both authors approved the final version.

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