

# Retinal Arterial and Venous Oxygen Saturation Is Altered in Diabetic Patients

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**PURPOSE.** To determine the retinal oxygen saturation trend with onset of diabetes and increasing severity of diabetic retinopathy by comparing diabetic groups with and without retinopathy to controls.

**METHODS.** A fundus camera-based dual-wavelength snapshot oximeter imaged retinas of healthy subjects and patients with and without diabetic retinopathy. The images were analyzed to determine oxygen saturation in major retinal arteries and veins, which is inversely proportional to optical density ratio.

**RESULTS.** Control retinal oxygen saturation ( $n = 14$ ) in arteries was  $92.3 \pm 4.2\%$  and in veins,  $57.2 \pm 6.0\%$ . Retinal oxygen saturation for diabetic patients with no signs of diabetic retinopathy (NDR,  $n = 45$ ) in arteries was  $96.3 \pm 8.6\%$  ( $P = 0.662$ ) and in veins,  $58.7 \pm 7.5\%$  ( $P = 0.998$ ). Retinal oxygen saturation for diabetics with mild to moderate nonproliferative diabetic retinopathy (NPDR,  $n = 23$ ) in arteries was  $97.7 \pm 5.8\%$  ( $P = 0.590$ ) and in veins,  $61.1 \pm 7.6\%$  ( $P = 0.658$ ). Retinal oxygen saturation for diabetics with severe NPDR ( $n = 12$ ) in arteries was  $102 \pm 10.2\%$  ( $P = 0.023$ ) and in veins,  $66.8 \pm 8.4\%$  ( $P < 0.001$ ). Retinal oxygen saturation for patients with proliferative diabetic retinopathy (PDR,  $n = 13$ ) in arteries was  $103.6 \pm 8.7\%$  ( $P = 0.003$ ) and in veins,  $66.6 \pm 10.2\%$  ( $P = 0.026$ ). Retinal oxygen saturation for all diabetics with retinopathy combined (all DR,  $n = 48$ ) in arteries was  $100.4 \pm 7.6\%$  ( $P = 0.004$ ) and in veins,  $64.2 \pm 8.4\%$  ( $P = 0.007$ ).

**CONCLUSIONS.** A trend of increasing retinal oxygen saturation was found from controls to NDR group to increasing levels of diabetic retinopathy, though significance was only reached for the comparison of controls to severe-NPDR, PDR, and all-DR groups.

**Keywords:** diabetic retinopathy, dual-wavelength imaging, oximetry, retinal oxygen saturation, functional imaging

Diabetes can lead to retinal tissue death and hypoxia by damaging retinal capillaries.<sup>1,2</sup> Since diabetic retinopathy disturbs the distribution of oxygen to retinal cells, retinal oxygenation has been a focus of research. Linsenmeier et al.<sup>3</sup> have recorded oxygen tension by using intraretinal probes in long-term diabetic cats exhibiting inner retinal hypoxia. Probe placement varies and other studies have measured free oxygen in the vitreous cavity as opposed to the retina.<sup>4-7</sup> The vitreous cavities of vitrectomy patients with diabetic retinopathy have lower oxygen tension, as compared with nondiabetics, according to a study by Holekamp et al.<sup>8</sup>

Retinal oxygen saturation could offer valuable diagnostic information. Retinal venular saturation in diabetics without diabetic retinopathy has been shown to decrease during hyperglycemia challenge in studies using a spectrophotometric technique.<sup>9</sup> Oxygen breathing has also been used in oxygenation studies. Drasdo et al.<sup>10</sup> report a decrease in oscillatory potentials in electroretinograms of non-diabetic retinopathy (NDR) diabetic patients and these potentials are normalized by supplemental oxygen. Oxygen breathing results in elevated contrast sensitivity in diabetic patients with mild retinopathy in a study by Harris et al.<sup>11</sup> Also, 100% oxygen breathing improves diabetic macular edema in a study by Nguyen et al.<sup>12</sup>

Pathogenesis in diabetic retinopathy has been linked to retinal hypoxia, which is thought to provoke neovasculariza-

tion and retinal edema.<sup>13,14</sup> Retinal hypoxia may be eased with laser treatment or by vitrectomy.<sup>15</sup>

Hammer et al.<sup>16</sup> have studied hemoglobin oxygen saturation changes that result from longstanding hyperglycemia with an imaging oximeter. The altered microcirculation in diabetes mellitus is hypothesized to have an effect on retinal vessel oxygen saturation. Hammer et al.<sup>16</sup> have found a trend of increasing arterial and venous oxygen saturation correlating to the severity of retinopathy in diabetic patients, though the arterial increase is insignificant. Hammer et al.<sup>17</sup> have also found that flicker light stimulation causes an increase in both central retinal arterial and venous diameter while only causing an increase in venous, and not in arterial, oxygen saturation. Hammer et al.<sup>17</sup> conclude that the flicker response is smaller for NPDR patients than for controls.

Hardarson and Stefánsson<sup>18</sup> have found a significant increase in both arteriolar and venular oxygen saturation in diabetic patients compared to controls by a retinal oximetry method. This retinal oxygen saturation increase in diabetic patients compared to controls does not exhibit a progressively increasing trend correlating to severity of disease, as the study of Hammer et al.<sup>16</sup> has found.

In this study, a noninvasive snapshot retinal oximeter was used to measure retinal vessel oxygen saturation. Healthy controls were compared to diabetic patients without retinop-

**TABLE.** Saturation Values (Mean  $\pm$  Standard Deviation) for All Groups and *P* Values for the Comparison to Healthy Controls

	Arteries	Veins	A-V Difference
Healthy controls ( <i>n</i> = 14)	92.3 $\pm$ 4.2	57.2 $\pm$ 6.0	35.2 $\pm$ 4.6
NDR ( <i>n</i> = 45)	96.3 $\pm$ 8.6 ( <i>P</i> = 0.662)	58.7 $\pm$ 7.5 ( <i>P</i> = 0.998)	37.7 $\pm$ 8.1 ( <i>P</i> = 0.547)
Mild to moderate NPDR ( <i>n</i> = 23)	97.7 $\pm$ 5.8 ( <i>P</i> = 0.590)	61.1 $\pm$ 7.6 ( <i>P</i> = 0.658)	36.6 $\pm$ 7.5 ( <i>P</i> = 1.00)
Severe NPDR ( <i>n</i> = 12)	102 $\pm$ 10.2 ( <i>P</i> = 0.023)	66.8 $\pm$ 8.4 ( <i>P</i> < 0.001)	35.2 $\pm$ 9.4 ( <i>P</i> = 1.00)
PDR ( <i>n</i> = 13)	103.6 $\pm$ 8.7 ( <i>P</i> = 0.003)	66.6 $\pm$ 10.2 ( <i>P</i> = 0.026)	37.3 $\pm$ 8.6 ( <i>P</i> = 0.891)
All DR ( <i>n</i> = 48)	100.4 $\pm$ 7.6 ( <i>P</i> = 0.004)	64.2 $\pm$ 8.4 ( <i>P</i> = 0.007)	36.4 $\pm$ 8.2 ( <i>P</i> = 0.920)

An increasing trend of oxygen saturation can be seen, which is significant for both arteries and veins of the severe-NPDR, PDR, and all-DR groups.

athy (NDR) as well as those with mild, moderate, and severe nonproliferative diabetic retinopathy (NPDR), proliferative diabetic retinopathy (PDR), and a combined retinopathy group (all DR). This is the first study to incorporate such gradual subgroups to investigate disease progression. No previous study of this type has included a diabetic group without retinopathy.

Hardarson and Stefánsson<sup>18</sup> and Hammer et al.<sup>16</sup> have reported conflicting results with respect to 3 aspects. This study will therefore test whether oxygen saturation increases with increasing disease severity, whether the increase in retinal arterial oxygen saturation is significant, and thus whether the change in the difference between arterial and venous saturation (A-V difference) across groups is significant. The groups used by Hardarson and Stefánsson<sup>18</sup> are small, and the statistical power to detect differences between them is low. The use of different subgroups in the studies of Hammer et al.<sup>16</sup> and Hardarson and Stefánsson<sup>18</sup> and of different imaging systems may also affect their differing results.

## METHODS

The retinal oximeter (Oxymap ehf., Reykjavik, Iceland), based on a fundus camera with a beam splitter and 2 digital cameras, was used to measure oxygen saturation in all major retinal arteries and veins in healthy subjects and in patients with and without diabetic retinopathy. Oxygen-sensitive (600 nm) and oxygen-insensitive (570 nm) wavelengths were used by the retinal oximeter. The oxygen saturation values in all major arteries and veins in each subject were averaged and then all subject values in each category were averaged. An inverse linear relationship between the measured vessel optical density ratio and oxygen saturation was assumed from previous studies.<sup>19</sup> The optical density ratio was calibrated to yield a mean arterial saturation of 92% in normal subjects.<sup>20</sup>

The 6 groups used in this study, as outlined in the introduction, were determined by normal Early Treatment Diabetic Retinopathy Study criteria. The age range for controls was 21 to 60 years and for diabetics, 19 to 91 years. Controls had an average age of 32 years with a standard deviation of 13 years. The NDR group had an average age of 59 years with a standard deviation of 17 years. The mild to moderate NPDR group had an average age of 64 years with a standard deviation of 9 years. The severe-NPDR group had an average age of 61 years with a standard deviation of 5 years. The PDR group had an average age of 59 years with a standard deviation of 8 years.

The all-DR group had an average age of 62 years with a standard deviation of 9 years. The individual groups were not further separated by age or sex owing to the relatively limited sample size.

All analyses that provided *P* values stated in the “Results” and the Table were conducted as completely randomized treatment arrangements in 2-way factorial analyses of variance.<sup>21</sup> In these analyses, oxygen saturation was the dependent variable, and diagnosis of the presence and level of diabetic retinopathy and anatomic source of the oxygen saturation measurement (arterial or venous) were the 2 predictor variables. ANOVA models all included the interaction of the 2 predictor variables. Post hoc testing to separate interaction means was conducted with *t*-tests, and  $\alpha$  level adjustment of these tests was accomplished by using a method of simulation.<sup>22</sup> Analysis of the difference between arterial and venous oxygen saturation was an exception, and these differences were analyzed in a 1-way ANOVA where arterial venous difference was the outcome and diagnosis of diabetic retinopathy presence and severity was the predictor variable. The  $\alpha$  level adjustment was conducted as described above for these 1-way ANOVAs.

## RESULTS

The percentage saturation values as well as *P* values for the comparison to controls are listed in the Table. In healthy subjects, retinal oxygen saturation values for arteries and veins were 92.3  $\pm$  4.2% and 57.2  $\pm$  6%, respectively (*n* = 14). The analogous values in NDR patients were 96.3  $\pm$  8.6% (*P* = 0.662) for arteries and 58.7  $\pm$  7.5% (*P* = 0.998) for veins (*n* = 45). Mild to moderate NPDR patients exhibited 97.7  $\pm$  5.8% (*P* = 0.590) retinal arterial oxygen saturation and 61.1  $\pm$  7.6% (*P* = 0.658) retinal venous oxygen saturation (*n* = 23). Severe-NPDR patients had 102  $\pm$  10.2% (*P* = 0.023) saturation in arteries and 66.8  $\pm$  8.4% (*P* < 0.001) in veins (*n* = 12). Patients with PDR had 103.6  $\pm$  8.7% (*P* = 0.003) saturation in arteries and 66.6  $\pm$  10.2% (*P* = 0.026) in veins (*n* = 13). The all-DR group had 100.4  $\pm$  7.6% (*P* = 0.004) saturation in arteries and 64.2  $\pm$  8.4% (*P* = 0.007) in veins (*n* = 48).

A difference in retinal oxygen saturation was considered to be significant if the *P* value was less than or equal to 0.05. Neither the arterial nor venous saturation values of the NDR group were significant. Neither the arterial nor venous values of the mild to moderate NPDR group were significant. The severe-NPDR group's arterial and venous values were both significant. The PDR group's arterial and venous values were both significant. The all-DR group's arterial and venous values were both significant.

## DISCUSSION

These results showed that retinal oxygen saturation in major branch arteries and veins exhibits a progressively increasing saturation trend from the control group to the NDR group to the retinopathy groups. The increase was more pronounced with increasing severity of retinopathy. However, significance was only reached for severe-NPDR, PDR, and all-DR groups. This may be due to outliers in the data. Noninvasive oximetry with large subgroups allows this metabolic change to be studied in more detail. The study in a large group of diabetic patients will be correlated with morphologic and functional changes in diabetic retinopathy.

Hardarson and Stefánsson<sup>18</sup> have also shown an oxygen saturation increase in diabetes, using controls and retinopathy groups, but that increase does not follow an increasing trend with disease severity. Hammer et al.,<sup>16</sup> however, do show an

increasing oxygenation trend for arteries and veins, consistent with these results. This study is the first of its type to include a diabetic group without retinopathy to investigate oxygen saturation in that case.

The control group had a much lower average age than the diabetic groups. Retinal venous oxygen saturation decreases by 1.59% per decade, and retinal arterial oxygen saturation decreases by 1.02% per decade.<sup>23</sup> Therefore, if the ages of the groups were matched, the difference in retinal oxygen saturation between controls and diabetic groups would be even more pronounced. Sex has been shown to have no effect on retinal oxygen saturation.<sup>23</sup>

Since the severe-NPDR and PDR groups exhibited significantly higher arterial and venous retinal oxygen saturation than healthy controls, oxygen metabolism seems to be affected in diabetic patients. The higher arterial saturation seen in these data did not result in a decreased A-V difference, since venous saturation also increased.

This study's arterial and venous trends were analogous to the arteriolar and venular trends that have been reported by Hammer et al.<sup>16</sup> However, since Hammer et al.<sup>16</sup> and colleagues have found the venular but not arteriolar trends to be significant, they find a significant decrease in the A-V difference between controls and diabetic retinopathy patients. We found no significant A-V difference decrease between controls and either NDR patients or diabetic retinopathy patients. Hardarson and Stefánsson<sup>18</sup> also find no change in the A-V difference between controls and diabetic retinopathy patients.

The exact causes of the elevated levels of oxygen saturation in diabetic retinopathy are still uncertain. A major limitation of this study, which may affect the interpretation of results, was the incomplete data on blood pressure, glycemic status, and vessel diameter. The speculations about blood flow and vessel wall changes are therefore included to guide future studies with more complete background data.

Blood flow due to altered supply and demand as well as distribution of oxygen may affect retinal oxygenation. Oxygen distribution may be affected by at least 3 mechanisms, including shunting and capillary nonperfusion, thickening of capillary basement membranes, and greater oxygen affinity of hemoglobin in diabetic patients.

Blood may bypass the capillary network if it is shunted through dilated channels. In this mechanism, some capillaries are closed off and some are enlarged for preferential passage.<sup>1</sup> Fluorescein angiography studies have shown that enlarged capillaries cause blood to be shunted from arterioles to venules in the retina, leading to capillary nonperfusion. This transport via dilated capillaries is too quick to allow oxygen flow to tissue and therefore, the venous blood becomes hyperoxic and the retinal tissue becomes hypoxic in such nonperfused areas. Shunting and capillary nonperfusion disturb the normal blood flow and may lead to altered oxygenation, which is a risk factor for pathology. Nonperfused areas do not extract the oxygen from the hemoglobin, so these venules have higher oxygenation levels and this tissue is hypoxic and ischemic.

Capillary walls have been shown to thicken in diabetic retinopathy, which can lower oxygen delivery levels.<sup>24,25</sup> In this case, oxygen is inhibited from efficiently diffusing and perfusing vessels, possibly contributing slightly to the elevated oxygenation of blood. The typical differences of larger vessels have been shown to be small<sup>26,27</sup> and we speculate that the differences in capillary thickness are not theoretically large enough to affect these measurements.<sup>19</sup> Our oximetry software did not show significant vessel diameter differences between groups, though data were incomplete. Future studies should document vessel diameter more thoroughly.

Diabetic patients exhibit higher levels of glycosylated hemoglobin (HbA1c), which will retain oxygen more efficiently than hemoglobin in healthy vasculature. This may be augmented or decreased by changes in the concentration of 2,3-diphosphoglycerate.<sup>28</sup> This increased oxygen affinity would explain higher saturation in arteries and veins. However, if this were the factor responsible for the elevated oxygenation in the retina, then there should be higher oxygenation throughout the body owing to this ubiquitous glycosylated hemoglobin. Finger pulse oximetry does not support this because no difference has been detected between controls and diabetic patients with respect to systemic oxygenation.<sup>18</sup>

Other possible reasons for the disturbance of oxygen saturation include changes in total blood flow and oxygen supply and demand in the diabetic retina. Studies have shown total retinal blood flow in diabetes to increase, decrease, or show no difference and are thus inconclusive.<sup>29</sup> Increased arterial and venous saturation may be due to increased total retinal blood flow and a decreased demand for oxygen in the retina and relatively reduced oxygen diffusion to adjacent tissue. Some studies suggest that degenerated tissue in diabetic retinopathy extracts less oxygen from the arteries, leading to hyperoxia in retinal veins.<sup>30,31</sup>

In the poorly perfused areas of the retina, the hypoxic conditions and high glucose levels of diabetics would highly favor anaerobic glycolysis, and little oxygen would be consumed. In areas with better perfusion, reduced oxygen consumption may also occur as a result of mitochondrial dysfunction. Mitochondrial dysfunction and impaired mitochondrial metabolism will contribute to reduced oxidative phosphorylation and oxygen consumption in the retinal tissue of diabetics.<sup>32,33</sup>

The oxygen saturation values obtained with the retinal oximeter are relative, as they sometimes exceed 100% depending on calibration.<sup>18</sup> Diabetic patients may exhibit altered optical densities, possibly contributing to supranormal oxygen saturation values. Other groups have also reported oxygen saturation values above complete saturation.<sup>16,18</sup> Measurements of this type have been shown to be repeatable.<sup>34</sup> Further, a study by Traustason et al.<sup>35</sup> correlates arterial blood sample oxygen saturation with the retinal oximeter's oxygen saturation values, providing support for the assurance of reliability and validity of comparison between groups.

The higher values of oxygen saturation in retinal arteries and veins in diabetic patients may be due in part to different spectral reflectance between control and different diabetic subgroups. To rule out this artifact, individual monochromatic images (570 and 600 nm) of controls and diabetics have to be separately analyzed. Another way to alleviate this issue is to control for all other factors besides disease state, such as age and pigmentation, and introduce oxygen challenge. By breathing pure oxygen, a healthy control and a diabetic patient would both be brought to 100% oxygen saturation. Any difference found in the optical density ratio could then be attributed to differing spectral reflectance based on retinal health.

More than one of the mechanisms outlined may contribute to the oxygenation differences detected in this study. Findings in histology and fluorescein angiography support blood shunting as well as bypassing nonperfused capillaries.<sup>18</sup> If oxygen is maldistributed in the disease state, the tissue becomes hypoxic and in turn demands an increase in blood flow to deliver more oxygen. This is one way total blood flow might increase in diabetic retinopathy.<sup>18</sup> Dead tissue cannot consume oxygen. Tissue degeneration therefore results in a lower amount of oxygen extracted from blood vessels and increased venous/venular oxygen saturation as detected by the retinal oximeter.

From this study, we believe the dual-wavelength retinal oximetry will significantly aid in the screening of diabetic retinopathy by identifying those diabetic patients potentially at risk for retinal vascular changes. A supranormal reading of O<sub>2</sub> saturation is not by itself an indicator for diabetic retinopathy. Other conditions, in particular those raising blood flow, such as the use of certain glaucoma medications, can cause similar changes to the retinal oxygen saturation. However, our findings as well as those of others indicate that a higher saturation reading, relative to an earlier reading from the same vessel in a diabetic patient who is known to be at risk for progression of diabetic retinopathy, could provide a warning sign for clinicians. That patient should be carefully watched. We feel that further research focusing on the sensitivity and reliability of saturation changes in relation to latent progression of diabetic retinopathy could lead to significantly improved care management for diabetic retinopathy patients.

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