Retinal oximetry

Sveinn Hákon Harðarson

Thesis for the degree of Philosophiae Doctor
University of Iceland
Faculty of Medicine
School of Health Sciences
June 2012

Retinal oximetry

Sveinn Hakon Hardarson

Thesis for the degree of Philosophiae Doctor

Supervisor:

Professor Einar Stefánsson, M.D., Ph.D.

Doctoral committee:

Professor Einar Stefánsson, M.D., Ph.D.
Professor Þór Eysteinsson, Ph.D.
Professor Jón Atli Benediktsson, Ph.D.
Aðalbjörn Þorsteinsson, M.D., Ph.D.
Professor Alon Harris, Ph.D.

University of Iceland
School of Health Sciences
Faculty of Medicine
June 2012

Súrefnismælingar í augnbotnum

Sveinn Hákon Harðarson

Ritgerð til doktorsgráðu

Umsjónarkennari:

Einar Stefánsson, prófessor, M.D., Ph.D.

Doktorsnefnd:

Einar Stefánsson, prófessor, M.D., Ph.D.

Þór Eysteinsson, prófessor, Ph.D.

Jón Atli Benediktsson, prófessor, Ph.D.

Aðalbjörn Þorsteinsson, M.D., Ph.D.

Alon Harris, prófessor, Ph.D.

Háskóli Íslands

Heilbrigðisvísindasvið

Læknadeild

Júní 2012

sveinnha@hi.is

ISBN 978-9935-9062-4-3

Printing by Háskólaprent

Reykjavik, Iceland, 2012

Ágrip

Tilgangur

Talið er að truflun á blóðflæði í sjónhimnuæðum og / eða truflun á súrefnisbúskap sjónhimnu tengist ýmsum sjúkdómum, þar á meðal æðalokunum í sjónhimnu, sjónhimnusjúkdómi í sykursýki og gláku. Áreiðanleg tækni til mælinga á súrefnisbúskap sjónhimnu án inngrips hefur verið af skornum skammti og því byggir þekkingin að stórum hluta á dýratilraunum. Í þessari ritgerð er lýst rannsóknum á súrefnismettun í sjónhimnuæðum (1) í ljósi og myrkri í heilbrigðum sjálfboðaliðum, (2) í miðbláæðarlokun í sjónhimnu, (3) í bláæðagreinarlokun í sjónhimnu, (4) í miðslagæðarlokun í sjónhimnu, (5) í sjónhimnusjúkdómi í sykursýki, (6) í sjúklingum, sem undirgengust skurðaðgerð vegna gláku og (7) í sjúklingum sem tóku glákulyf.

Aðferðir

Súrefnismælirinn (Oxymap ehf., Reykjavík) er byggður á augnbotnamyndavél. Við augnbotnamyndavélina er festur mynddeilir, sem gerir kleift að ná fjórum myndum af sama svæði augnbotns samtímis. Tvær myndanna eru notaðar til frekari vinnslu, ein er tekin með 586 nm ljósi en hin með 605 nm ljósi. Ljósgleypni sjónhimnuæða er næm fyrir súrefnismettun við 605 nm en ekki við 586 nm. Meta má súrefnismettun í aðal sjónhimnuæðunum með því að mæla ljósendurkast á þessum tveimur bylgjulengdum. Þetta er gert með aðstoð sérskrifaðs hugbúnaðar.

Niðurstöður

Ljós og myrkur:

Eftir 30 mínútur í myrkri var súrefnismettun í slagæðlingum í sjónhimnu heilbrigðra sjálfboðaliða 92±4% (meðaltal±staðalfrávik, n=15). Eftir 5 mínútur í 80 cd/m^2 ljósi, var mettunin í slagæðlingunum marktækt minni eða 89±5% (p=0,008). Samsvarandi gildi fyrir bláæðlinga í sjónhimnu voru 60±5% í myrkri og 55±10% í ljósi (p=0,020). Sambærilegar niðurstöður fengust þegar mælt var eftir 5 mínútna ljós eða myrkur tímabil til skiptis. Í annarri tilraun (n=19) mældist marktækt minni súrefnismettun í sjónhimnuæðlingum í 100 cd/m^2 ljósi en í myrkri. Ljós af styrknum 1 eða 10 cd/m^2 hafði engin marktæk áhrif.

Miðbláæðarlokun í sjónhimnu:

Súrefnismettun í bláæðlingum framan við miðbláæðarlokunina var 49±12% (meðaltal±staðalfrávik, n=8). Meðalgildið í bláæðlingunum í hinu auganu var 65±6% (p=0,003). Súrefnismettun í slagæðlingum var 99±3% í sjúka auganu en 99±6% í hinu auganu. Mettun í bláæðlingum var mjög breytileg milli sjúkra augna.

Bláæðagreinarlokun í sjónhimnu:

Miðgildi súrefnismettunar í bláæðlingum, sem urðu fyrir áhrifum af bláæðagreinarlokun, var 59% (bil 12-93%, n=22). Miðgildið var 63% (23-80%) í þeim bláæðlingum í sjúka auganu, sem ekki urðu fyrir áhrifum af lokuninni, og 55% (39-80%) í bláæðlingum hins augans. Munurinn var ómarktækur (p>0,05). Marktækur munur var milli slagæðlinga, sem urðu fyrir áhrifum af lokun (miðgildi 101%, bil 89-115%) og slagæðlinga í sama auga, sem ekki urðu fyrir áhrifum af lokun (95%, 85-104%, p<0,05, n=18).

Miðslagæðarlokun í sjónhimnu:

Meðaltal súrefnismettunar í slagæðlingum sjúklings með eins dags sögu um miðslagæðarlokun var 71±9% en meðaltal í bláæðlingum var 63±9%. Einum mánuði síðar og eftir meðferð með prednisólon var meðaltal súrefnismettunar 100±4% í slagæðlingum og 54±5% í bláæðlingum.

Sjónhimnusjúkdómur í sykursýki:

Sjúklingar með sjónhimnusjúkdóm í sykursýki (allir flokkar) mældust með að meðaltali 7-10 prósentustigum hærri súrefnismettun í slagæðlingum en heilbrigðir (p<0,05 fyrir alla flokka, n=6-8 í hverjum flokki). Í bláæðlingum var mettunin 8-12 prósentustigum hærri (p<0,05 fyrir alla flokka).

Skurðaðgerð við gláku:

Súrefnismettun í slagæðlingum sjónhimnu jókst um 2 prósentustig að meðaltali (p=0,046, n=19) eftir aðgerð, sem lækkaði augnþrýsting úr 23±7 mmHg (meðaltal±SD) í 10±4 mmHg (p<0,0001). Engar aðrar marktækar breytingar fundust (p≥0,35).

Dorsólamíð:

Súrefnismettun í slag- og bláæðlingum lækkaði marktækt um 3 prósentustig í slagæðlingum (p<0,01) og bláæðlingum (p<0,05) þegar sjúklingar með gláku eða háan augnþrýsting skiptu úr blöndu af dorsólamíði og tímólóli yfir í tímólól eitt sér (n=6). Engar breytingar fundust hjá sjúklingum, sem skiptu úr tímólóli yfir í blandaða meðferð (p>0,05, n=7).

Ályktanir

Nota má tveggja bylgjulengda súrefnismælingu til að mæla súrefnismettun í sjónhimnuæðum án inngrips í heilbrigðum augum og sjúkum. Niðurstöðurnar benda til þess að súrefnismettun (1) hækki í myrkri, (2) sé lægri í bláæðlingum framan við miðbláæðarlokun, (3) sé breytileg í bláæðagreinarlokun, (4) sé lægri í slagæðlingum sjónhimnu við lokun miðslagæðar, (5) hækki í sjónhimnusjúkdómi í sykursýki, (6-7) breytist lítið við glákuaðgerð eða töku dorsólamíðs.

Lykilorð

Súrefni, sjónhimna, bláæðalokun í sjónhimnu, sjónhimnusjúkdómur í sykursýki, gláka.

Abstract

Purpose

Malfunction of retinal blood flow or oxygenation is believed to be involved in various diseases. Among them are retinal vessel occlusions, diabetic retinopathy and glaucoma. Reliable, non-invasive technology for retinal oxygen measurements has been scarce and most of the knowledge on retinal oxygenation comes from animal studies. This thesis describes human retinal oximetry, performed with novel retinal oximetry technology. The thesis describes studies on retinal vessel oxygen saturation in (1) light and dark in healthy volunteers, (2) central retinal vein occlusion, (3) branch retinal vein occlusion, (4) central retinal artery occlusion, (5) diabetic retinopathy, (6) patients undergoing glaucoma surgery and (7) patients taking glaucoma medication.

Methods

The retinal oximeter (Oxymap ehf., Reykjavik, Iceland) is based on a fundus camera. An attached image splitter allows the simultaneous capture of four images of the same area of the fundus. Two images are used for further analysis, one acquired with 586 nm light and one with 605 nm light. Light absorbance of retinal vessels is sensitive to oxygen saturation at 605 nm but not at 586 nm. Measurement of reflected light at these wavelengths allows estimation of oxygen saturation in the main retinal vessels. This is performed with custom-made analysis software.

Results

Light and dark:

After 30 minutes in the dark, oxygen saturation in retinal arterioles of healthy volunteers was 92±4% (mean±SD, n=15). After 5 minutes in 80 cd/m^2 light, the arteriolar saturation was 89±5%. The decrease was statistically significant (p=0.008). The corresponding values for retinal venules were 60±5% in the dark and 55±10% in the light (p=0.020). Similar results were found after alternating 5 minute periods of darkness and light. In a second experiment (n=19), a significant decrease in retinal vessel oxygen saturation was found in 100 cd/m^2 light compared to darkness but 1 and 10 cd/m^2 light had no significant effect.

Central retinal vein occlusion:

In patients with central retinal vein occlusion, the mean saturation in affected retinal venules was 49±12%, while the mean value for venules in the fellow eye was 65±6% (mean±SD, $p=0.003$, $n=8$). The retinal arteriolar saturation was the same in affected (99±3%) and the unaffected (99±6%) eyes. The venous oxygen saturation showed much variation between affected eyes.

Branch retinal vein occlusion:

Median oxygen saturation in venules affected by branch retinal vein occlusion was 59% (range 12-93%, $n=22$), while it was 63% (23-80%) in unaffected venules in the affected eye and 55% (39-80%) in venules in the fellow eye. The difference was not statistically significant ($p>0.05$). There was a significant difference between affected arterioles (median 101%, range 89-115%) and unaffected arterioles (95%, 85-104%) in the affected eye ($p<0.05$, $n=18$).

Central retinal artery occlusion:

In a patient with a day's history of central retinal artery occlusion due to temporal arteritis, the mean arteriolar saturation was 71±9% and 63±9% in the venules. One month later, after treatment with prednisolone, the mean arteriolar saturation was 100±4% and the venous saturation 54±5%.

Diabetic retinopathy:

When compared with healthy volunteers ($n=31$), patients with all categories of diabetic retinopathy had on average 7-10 percentage points higher saturation in retinal arterioles ($p<0.05$ for all categories, $n=6$-8 in each category). In venules, the saturation was 8-12 percentage points higher ($p<0.05$ for all categories).

Glaucoma surgery:

Oxygen saturation in retinal arterioles increased by 2 percentage points on average ($p=0.046$, $n=19$) with surgery, which lowered intraocular pressure from 23±7 mmHg (mean±SD) to 10±4 mmHg ($p<0.0001$). No other significant changes were found ($p\geq0.35$).

Dorzolamide:

A significant reduction of 3 percentage points was found in arterioles ($p<0.01$) and venules ($p<0.05$) when patients with glaucoma or ocular hypertension changed from dorzolamide-timolol combination eye drops to timolol alone ($n=6$). No change was found in patients, who started on timolol and switched to the combination therapy ($p>0.05$, $n=7$).

Conclusions

Dual wavelength oximetry can be used to non-invasively measure retinal vessel oxygen saturation in health and disease. The results indicate that retinal vessel oxygen saturation is (1) increased in the dark, (2) lower in venules affected by central retinal vein occlusions, (3) variable in branch retinal vein occlusion, (4) lower in retinal arterioles in central retinal artery occlusion, (5) increased in diabetic retinopathy, (6-7) mildly affected by glaucoma surgery or dorzolamide.

Keywords

Oxygen, retina, retinal vein occlusion, diabetic retinopathy, glaucoma.

Acknowledgements

First and foremost I express my gratitude to my supervisor, Einar Stefánsson, for endless inspiration and support. Einar has been a pioneer in the study of retinal oxygenation for over thirty years. He was one of the key persons, who started the non-invasive oximetry project in Iceland, several years before I began working on oximetry. Einar has enthusiastically led the group, and has been an exceptional supervisor.

Einar has played a major role in conceptualising and planning all of the studies described in this thesis. He has supervised all data analysis and has taken part in revising all of the papers, as well as writing the first draft of paper VII (Retinal oximetry in central retinal artery occlusion).

My sincere thanks to my fellow oximetrists, Sindri Traustason and Ólöf Birna Ólafsdóttir. Sindri analysed the data from the study on dorzolamide and wrote the first draft of the paper in collaboration with Thor Eysteinsson and myself. Sindri also participated in all subsequent revisions. Ólöf has collaborated on other oximetry projects (not in this thesis). Thanks to both of them for all the collaboration and discussions on oximetry through the years.

My sincere thanks to María Soffía Gottfreðsdóttir for sharing her expertise in glaucoma. María was instrumental in recruiting participants for both studies on glaucoma and did most of the clinical examinations as well as all of the glaucoma surgery. She participated in writing both papers on glaucoma.

My sincere thanks to Þóra Elísabet Jónsdóttir and Samy Basit for collaboration on the light and dark paper. Þóra collaborated in gathering the data for the second part of the study and did the first analysis of the data. Samy helped designing both parts of the study and participated in writing the paper.

My sincere thanks to Andri Elfarsson and Bjarni Agnarsson for their contribution to paper VII. Andri gathered all clinical information on the patient and Bjarni provided the histology. Both contributed to the revision of the paper.

My sincere thanks to members of my PhD committee; Þór Eysteinsson, Jón Atli Benediktsson, Alon Harris and Aðalbjörn Þorsteinsson. Thanks to Þór for sharing his extensive knowledge on retinal physiology and for helping with the dorzolamide paper. Thanks to Jón Atli for leading the whole Icelandic oximetry project with Einar and supervising the technical work that laid the foundation for the project. Thanks to Alon Harris for collaboration on the dorzolamide project. The initial design of the project was largely done in his lab in

Indianapolis. Thanks to Aðalbjörn Þorsteinsson for sharing his expertise in physiology.

My sincere thanks to all the staff at the Ophthalmology department of Landspítali for all the help, support and companionship through the years.

Special thanks to the engineers, who made the measurements possible. The retinal oximeter, used here, is based on the work of James Beach and collaborators. James has continued to work with the group through the years. The software used for all analysis of oximetry images for this thesis is the work of Gísli Hreinn Halldórsson and Róbert Arnar Karlsson, who have been exceptional collaborators through the years. The technical side of the project was originally run within the Department of Engineering but later in the company Oxymap. My thanks to the staff at Oxymap for collaboration on oximetry in general through the years.

My thanks also to those, who have collaborated on short term oximetry projects, which are not in this thesis: Guðleif Harðardóttir, Renata Blöndal, Ólafur Pálsson, Halldór Reynir Bergvinsson, Margrét Kara Sturludóttir and Stefán Þórarinsson. I used Guðleif's report on haemoglobin as an aid in my literature search for the characteristics of haemoglobin.

My sincere thanks to the "new" people in oximetry, who have not already been mentioned, for joining the project: Jóna Valgerður Kristjánsdóttir, Þórunn Scheving, Ásbjörg Geirsdóttir and Davíð Þór Bragason. Exciting times are ahead!

I am very grateful to those who have financially supported either my studies directly or the oximetry project in general: Eimskip – University Fund, The Icelandic Center for Research (Rannís), Oxymap ehf., The University of Iceland research fund, The Landspítali-University hospital research fund, Helga Jónsdóttir and Sigurliði Kristjánsson memorial fund, Merck Inc.

Last but not least, thanks to everyone, who have helped in any way and I somehow forgot to mention!

Financial disclosure: I and my supervisor both have financial interest in the technology used for the studies. The same is true for some of the other members of the research group. We both have stock in Oxymap ehf. and hold a patent along with other members of the group. Oxymap ehf. financed part of my salary during the Ph.D. studies. Merck Inc. provided medication for the dorzolamide study.

Contents

List of abbreviations

All abbreviations are explained where they are used.

BDR	Background diabetic retinopathy (non-proliferative diabetic retinopathy)
CCD	Charge-coupled device (type of camera sensor)
cd/m^2	Candelas per square metre
cm	Centimetres
d	Diameter
DMO	Diabetic macular oedema
DR	Diabetic retinopathy
F	Flow (or Female in Table 7)
g	Gram
Hb	Deoxygenated haemoglobin
HbO_2	Oxygenated haemoglobin
I	Light intensity on a vessel (brightness value of pixel on a vessel)
I_0	Light intensity of background (brightness value of pixel to the side of a vessel)
k	Constant in Hagen-Poiseuille's law
L	Litre
M	Male
min.	Minutes
mmHg	Millimetres of mercury (unit of pressure)
mmol	Milli moles
n	Number (of subjects studied)
nm	Nanometres
OD	Optical density (measure of light absorbance)
ODR	Optical density ratio (ratio of two optical densities)
p	Probability (of a null hypothesis being true)
Δp	Pressure gradient
PDR	Proliferative diabetic retinopathy
SD	Standard Deviation

List of figures

List of tables

List of papers

This thesis is based on the following original publications:

I. Hardarson, S. H., Basit, S., Jonsdottir, T. E., Eysteinsson, T., Halldorsson, G. H., Karlsson, R. A., Beach, J. M., Benediktsson, J. A., and Stefansson, E. (2009). Oxygen saturation in human retinal vessels is higher in dark than in light. *Invest Ophthalmol Vis Sci, 50*(5), 2308-2311.

II. Traustason, S., Hardarson, S. H., Gottfredsdottir, M. S., Eysteinsson, T., Karlsson, R. A., Stefansson, E. and Harris, A. (2009). Dorzolamide-timolol combination and retinal vessel oxygen saturation in patients with glaucoma or ocular hypertension. *Br J Ophthalmol, 93*(8), 1064-1067.

III. Hardarson, S. H., Gottfredsdottir, M. S., Halldorsson, G. H., Karlsson, R. A., Benediktsson, J. A., Eysteinsson, T., Beach, J. M., Harris, A. and Stefansson, E. Glaucoma filtration surgery and retinal oxygen saturation. (2009). *Invest Ophthalmol Vis Sci, 50*(11), 5247-5250.

IV. Hardarson, S. H. and Stefánsson, E. (2010). Oxygen saturation in central retinal vein occlusion. *Am J Ophthalmol, 150*(6), 871-875.

V. Hardarson, S. H. and Stefansson, E. (2011). Oxygen saturation in branch retinal vein occlusion. *Acta Ophthalmol, Apr 21*, epub ahead of print.

VI. Hardarson, S. H. and Stefansson, E. (2011). Retinal oxygen saturation is altered in diabetic retinopathy. *Br J Ophthalmol, Nov 11*, epub ahead of print.

VII. Hardarson, S. H., Elfarsson, A., Agnarsson, B. A. and Stefansson, E. Retinal oximetry in central retinal artery occlusion. *Acta Ophthalmol*, accepted for publication (diagnosis and therapy section).

The thesis also contains additional unpublished data from studies on light and dark, retinal artery occlusions and diabetic retinopathy. Some of the oximetry images in the thesis have not been published previously.

Declaration of contribution

Below is a declaration of my contribution to each paper, on which the thesis is based. The contribution of co-authors is acknowledged in a special chapter above.

Paper I: Oxygen saturation in human retinal vessels is higher in dark than in light.

This paper is based on two experiments. I planned both experiments under the supervision of Einar Stefánsson and in collaboration with the co-authors. I acquired (almost all) oximetry images for the first experiment. I supervised and took part in acquiring the data for the second experiment. I analysed all oximetry images for the first experiment and repeated the analysis for the second experiment. I performed all statistical analyses, wrote the first draft of the paper and participated in all subsequent revisions.

Paper II: Dorzolamide-timolol combination and retinal vessel oxygen saturation in patients with glaucoma or ocular hypertension.

I acquired all oximetry images, measured blood pressure and visual acuity. I measured visual fields at some of the visits. The statistical analyses were made by the first author and me. I took part in all revisions of the first draft of the manuscript.

Paper III: Glaucoma filtration surgery and retinal oxygen saturation.

Paper IV: Oxygen saturation in central retinal vein occlusion.

Paper V: Oxygen saturation in branch retinal vein occlusion.

Paper VI: Retinal oxygen saturation is altered in diabetic retinopathy

I took part in planning these studies. I acquired and analysed all oximetry images and performed the statistical analyses. I wrote the first draft of the papers and participated in all subsequent revisions.

Paper VII: Retinal oximetry in central retinal artery occlusion:

The paper describes one patient, while the thesis describes three additional patients. I acquired all oximetry images and made all analyses of the images. I took part in all revisions of the manuscript.

1 Introduction

1.1 Why measure retinal oxygenation?

Normal retinal function requires a very high level of energy production and a great supply of oxygen (see for example Alder et al., 1990; Alm and Bill, 1970, , 1972a, , 1972b; Ames et al., 1992; Blair, 2000; Braun et al., 1995; Cringle et al., 2002; Linsenmeier, 1986; L. Wang, Tornquist et al., 1997a, , 1997b). Blood vessels must, however, not obscure the retina and disturb vision. These extreme requirements are met by an unusual arrangement of blood vessels, which allows delivery of great amounts of oxygen and nutrients without greatly affecting the transparency of the retina. Malfunction of the vasculature can result in serious disease. There is considerable evidence for altered retinal blood flow or oxygenation in diseases such as retinal vessel occlusions, diabetic retinopathy and glaucoma.

Measurements of retinal oxygenation are needed for better understanding of normal physiology as well as for studying common and potentially blinding diseases. Human studies require non-invasive and safe measurement techniques. This thesis describes the use of a novel retinal oximeter for the study of retinal oxygenation and the effect of (1) light and dark, (2) central retinal vein occlusion, (3) branch retinal vein occlusion, (4) central retinal artery occlusion, (5) diabetic retinopathy, (6) glaucoma surgery and (7) dorzolamide.

1.1.1 Retinal oxygenation in health and the effect of light and dark

The retina (Figure 1) is oxygenated from two sources; the choroid and the retinal circulation.

The choroid mostly supplies the outer retina with oxygen while the retinal circulation mostly supplies the inner retina. The retina uses more oxygen in the dark than in light (Stefansson et al., 1983) and the division between the oxygen supply of the choroid and retinal circulation changes slightly with light and dark (Ahmed et al., 1993; Birol et al., 2007; Linsenmeier, 1986; Linsenmeier and Braun, 1992). In the dark, the photoreceptors in the outer retina use more oxygen (Ahmed et al., 1993; Ames et al., 1992; Birol et al., 2007; Braun and Linsenmeier, 1995; Braun et al., 1995; Cringle et al., 1999; Haugh-Scheidt, Griff et al., 1995; Haugh-Scheidt, Linsenmeier et al., 1995; Haugh et al., 1990; Linsenmeier, 1986; Linsenmeier and Braun, 1992; Linsenmeier and Yancey, 1989; Medrano and Fox, 1995; L. Wang, Kondo et al., 1997; L. Wang, Tornquist et al., 1997a; Zuckerman and Weiter, 1980). In the dark, oxygen is supplied to the photoreceptors from both sides, i.e. from the choroid and, to a

lesser degree, from the retinal capillaries. In the light, the choroid appears to fully supply the photoreceptors and some of the choroidal oxygen may even reach the inner retina.

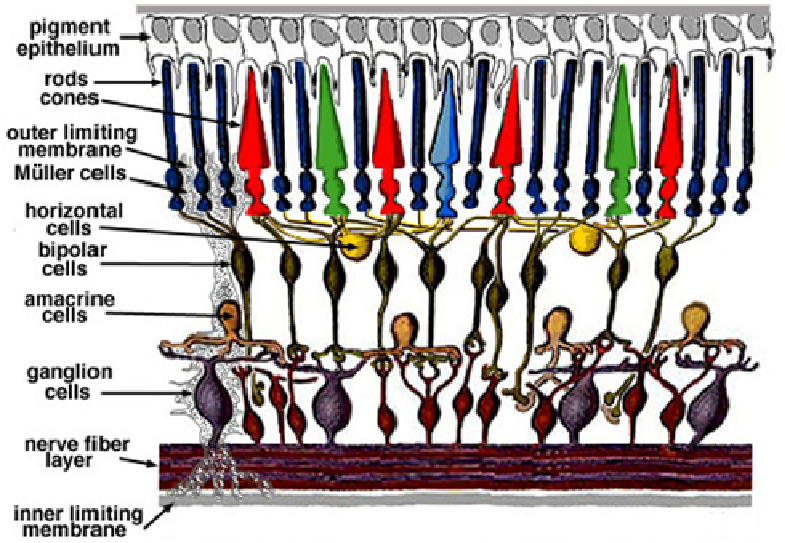

Figure 1. Cross-section of the retina. The retina can be divided into the outer retina, which contains the photoreceptors (rods and cones), and the inner retina, which contains various types of neurons, as displayed in the figure. The retinal capillaries lie in the inner retina, down to the level of the photoreceptors, while the choroid lies outside the retinal pigment epithelium (top of figure). ©Webvision, http://webvision.med.utah.edu/ Non-commercial, no derivative works creative commons license.

The entire blood supply of the eye comes through the ophthalmic artery, which is a branch of the internal carotid artery (Figure 2). The ophthalmic artery gives rise to the ciliary arteries and the central retinal artery. The choroidal circulation originates from the posterior ciliary arteries. The choroidal vessels can be divided, from the outside to the inside, into a layer of large arteries, a layer of medium size arteries and arterioles and the innermost layer of the choriocapillaris (Nickla and Wallman, 2010). The choriocapillaris is a network of wide capillaries adjacent to the outer retina (Hayreh, 1975; Nickla and Wallman, 2010). In healthy eyes, the choroid does not penetrate the retina and does therefore not obscure vision. Oxygen diffuses from the choriocapillaris, through Bruch's membrane and the retinal pigment epithelium to reach the energy intensive photoreceptors. The photoreceptor layer (outer retina) does not contain any capillaries, even if it is more than 100 micrometres

thick according to measurements in the human macula (Loduca et al., 2010). The full thickness of the retina at the fovea does not contain any capillaries.

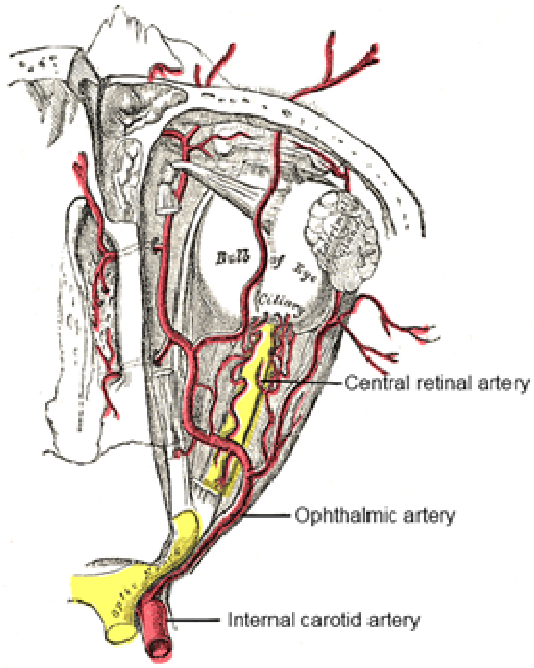

Figure 2. The blood supply of the eye. The ophthalmic artery is a branch of the internal carotid artery and supplies they entire eye. Among the branches of the ophthalmic artery are the ciliary arteries and central retinal artery. The posterior ciliary arteries supply the choroid. The central retinal artery pierces the optic nerve and travels with it to the retina to supply the retinal circulation. Public domain figure, originally reproduced from Gray's anatomy. http://en.wikipedia.org/wiki/Ophthalmic_artery (labels simplified).

The diffusion of oxygen from the choroid to the photoreceptors is driven by a very high concentration gradient for oxygen (Linsenmeier, 1986). The choroid has a very high level of blood flow, which creates high partial pressure of oxygen in the choriocapillaris. In monkeys, blood flow in the choroid was found to be about twenty times the flow in the retinal circulation (Alm et al., 1973). The difference in oxygen saturation in blood entering and leaving the choroid has been found to be only 3% in cats (Alm and Bill, 1970). Despite this, the partial pressure of oxygen reaches almost zero in parts of the outer retina in the dark (Linsenmeier, 1986). This is due to the diffusion distance and the great oxygen consumption of the photoreceptors.

The choroid receives both sympathetic (vasoconstricting) and parasympathetic (vasodilating) innervation (Neuhuber and Schrodl, 2011; Nickla and Wallman, 2010). Earlier animal experiments indicated, however, that choroidal blood flow was not well regulated and that it for example fell almost linearly with decreased perfusion pressure. Later studies on animals and humans, have found that the choroidal blood flow is indeed regulated according to perfusion pressure and even concentration of carbon dioxide (Kiel and Shepherd, 1992; Polska et al., 2007; Schmidl, Garhofer et al., 2011; Schmidl, Weigert et al., 2011). The concentration of oxygen seems to have little effect on choroidal blood flow (for review see Schmidl, Garhofer et al., 2011) although some indirect evidence for a limited regulation during hyperoxia may have been found in cats (Linsenmeier and Yancey, 1989). Recent studies have even shown less choroidal blood flow in dark than in light (Fuchsjager-Mayrl et al., 2003; Fuchsjager-Mayrl et al., 2001; Huemer et al., 2007; Longo et al., 2000). Less choroidal blood flow in the dark may seem counter-intuitive, since the photoreceptors require more oxygen in the dark. It has been postulated that choroidal blood flow is increased in light to dissipate more heat in light than in dark (Nagaoka and Yoshida, 2004; Parver et al., 1980).

The retinal circulation arises from the central retinal artery, which (in most cases) is a branch of the ophthalmic artery (Hayreh, 2011). The central retinal artery travels within the optic nerve until it reaches the retina, where it branches into retinal arterioles, which branch repeatedly and ultimately give rise to the retinal capillaries. The retinal capillaries are generally organised in two layers, one in the nerve fibre layer and the ganglion cell layer and another in the inner nuclear layer (Kong et al., 2010; C. J. Pournaras et al., 2008). The exceptions are that there is an additional superficial layer of capillaries around the optic disc, there is only one layer of capillaries in the peripheral retina and close to the fovea and there are few or no capillaries around retinal arterioles and in the fovea (Hayreh, 2011; Henkind, 1967; Kong et al., 2010; McLeod, 2010; C. J. Pournaras et al., 2008). The retinal capillaries merge into ever larger retinal venules, which finally form the central retinal vein, which leaves the eye with the optic nerve, adjacent to the central retinal artery within the nerve. In a portion of the population, one or more cilioretinal arteries supply a part of the retinal capillaries, most often in the temporal retina. Justice and Lehman (1976) found one or more cilioretinal arteries in about a third of eyes and about half of their patients. The cilioretinal arteries varied greatly in size.

The retinal vasculature is unlike the choroidal circulation in several aspects. The retinal circulation penetrates the retinal tissue and this decreases diffusion distances from capillaries to retinal cells but also means that the retinal vasculature could obscure vision, if it were too dense. Kong et al. (2010)

stained flat-mounted human and monkey retinas for blood vessels and found that, in the four areas tested, the retinal vasculature covered from about 7% to 30% of the total area. The least density was found peripherally but the greatest in the macula. As mentioned above, blood flow in the retinal vasculature is much less than in the choroid. Less blood flow in the retinal circulation is reflected in much greater arteriovenous difference in oxygen saturation. Schweitzer et al. (1999) used a calibrated non-invasive method to measure healthy volunteers and found the saturation to be 92% in retinal arterioles and 58% in retinal venules. In pigs, the retinal venous saturation has been found to be about 55% (Tornquist and Alm, 1979). This means that the arteriovenous difference is a little over 30% in the retinal circulation while it is closer to 3% in the (cat) choroid (Alm and Bill, 1970). Oxygen saturation in the pulmonary artery, representing the mean saturation in venous blood in the body, has been found to be above 70% in healthy individuals at rest (Harms et al., 2003; Sun et al., 2001). The arteriovenous difference in saturation is therefore much less than average in the choroid but greater than average in the retina. The oxygen saturation in the pulmonary artery can decrease dramatically during exercise, even below 30%, which is much lower than in the normal retina (Sun et al., 2001).

Regulation of retinal blood flow has traditionally been considered more effective than the regulation of the choroid, although more evidence is being found of choroidal regulation. The retinal blood flow is adjusted according to demand and is for example increased by flickering light, which is believed to increase inner retinal metabolism (Riva et al., 2005). As opposed to choroidal blood flow, retinal blood flow may be increased in the dark to sustain the increased consumption although this has not been consistently seen (Barcsay et al., 2003; Feke et al., 1983; Havelius et al., 1999; Riva, Grunwald & Petrig, 1983; Riva et al., 1987). Hyperoxia, induced by inhaling oxygen enriched air, leads to constriction of retinal arterioles (C. J. Pournaras et al., 2008; Riva, Grunwald & Sinclair, 1983) and decreased retinal blood flow (Stefansson et al., 1988). The retinal vasoconstriction does not prevent that the retina receives more oxygen during inhalation of pure oxygen since choroidal blood flow is minimally affected (Linsenmeier and Yancey, 1989; C. J. Pournaras et al., 1989; Stefansson, 1981; Stefansson et al., 1983; Wolbarsht et al., 1987). The retinal vessels dilate in response to increased concentration of inspired carbon dioxide (Venkataraman et al., 2008).

The retinal vasculature must also cope with changes in perfusion pressure (see more details in chapter 1.1.5.1). Perfusion pressure in the retinal vasculature is lowered by a decrease in blood pressure or an increase in the intraocular pressure. Lower perfusion pressure leads to an increase in retinal arteriolar diameter while higher perfusion pressure leads to constriction of

arterioles (C. J. Pournaras et al., 2008). This response tends to keep the blood flow adequate and failure of regulation has been implicated in glaucoma, see chapter 1.1.5.1.

The biochemical mechanisms of regulation of retinal blood blow are complicated and the interplay of various factors is not fully understood. This applies to both regulation due to pressure changes and metabolic need (for review see C. J. Pournaras et al., 2008). Most mechanisms are believed to be local as opposed to neural (Delaey and Van De Voorde, 2000) although the extraocular part of the central retinal artery is innervated (Ehinger, 1966; Laties, 1967; Neuhuber and Schrodl, 2011; Ye et al., 1990).

1.1.2 Retinal vascular occlusions (and treatment)

Occlusion of a retinal vessel will obviously disturb retinal blood flow and oxygenation. Occlusions can be broadly divided into vein (venule) occlusions and artery (arteriole) occlusions, the former being more common. Occlusions can be further divided into central occlusions, affecting the central retinal vein or artery, and branch occlusions, affecting branches of the central vessels. Still further classifications are possible; central retinal vein occlusions are for example often divided into ischaemic and the less severe non-ischaemic occlusions, although such classification is not straightforward (Hayreh, 2005). The vascular occlusions considered in this thesis are central retinal vein occlusion and branch retinal vein occlusion. Preliminary data on central retinal artery occlusion is also included.

1.1.3 Central retinal vein occlusion

Central retinal vein occlusion is caused by a thrombus in the central retinal vein. The central retinal vein usually drains all blood from the retinal vasculature and empties into the superior ophthalmic vein or directly into the cavernous sinus (Hayreh, 2011). Occlusion of the central retinal vein can therefore reduce the blood flow in the entire retinal circulation. The occlusion rarely stops the retinal blood flow completely, even if the central retinal vein is usually completely blocked (Hayreh, 2005). The reason is that blood can escape through tributaries of the central retinal vein in the optic nerve, anterior to the occlusion. The amount of remaining blood flow most likely varies with the site of the occlusion and the number and size of tributaries anterior to the occlusion, which can support collateral circulation. Indocyanine green angiography has been used to study the route of the collateral flow and has shown flow from the retina through loops to choroidal veins (Suzuki et al., 2000; Takahashi et al., 1998). The loops are located close to the optic disc.

Opening of the thrombus (re-canalisation) with time after the initial occlusion can also contribute to blood flow. Green et al. (1981) studied enucleated eyes with central retinal vein occlusion and found evidence of re-canalisation in almost all of them. The re-canalisation appears to start early, within days or weeks, and then continue with time. The study is most representative of ischaemic central retinal vein occlusions since most of the eyes were enucleated due to neovascular glaucoma, which can be associated with ischaemic occlusion (see chapter 1.1.4 on the consequences of hypoxia).

Reduction in blood flow in the retina, due to central retinal vein occlusion, is likely to affect the oxygenation of the inner retina. Williamson et al. (2009) used oxygen sensitive electrodes during vitrectomy to measure the partial pressure of oxygen in the vitreous. They found that patients with ischaemic central retinal vein occlusion had lower partial pressure of oxygen above the retina, when compared to patients undergoing vitrectomy for either macular hole or epiretinal membrane removal.

Oxygenation of the retina in patients with central retinal vein occlusion has also been measured non-invasively. Yoneya et al. (2002) used interferometry to measure a spectrum in each pixel in a fundus image. This information was used to calculate oxygen saturation in the fundus and the results show low saturation in areas where fluorescein angiography demonstrates capillary non-perfusion. However, it is very difficult to reliably measure oxygen saturation in the fundus outside the major retinal vessels, for example due to the small amount of blood present in the retinal capillaries and the possible effects of the choroidal background (see chapter 1.2 for more information on methodology). The reliability is especially questionable when there is little perfusion of capillaries. The published colour-coded maps of saturation show great variability and some of them show very high saturation in the tissue / capillaries.

Nevertheless, some degree of retinal hypoxia is most likely present, at least at some stage, in many cases of central retinal vein occlusion, even if direct measurements are scarce and difficult. Further evidence for hypoxia in central retinal vein occlusion can be found by studying the consequences of the occlusion, which are in some cases neovascularisation and oedema. Neovascularisation and oedema can also result from branch retinal vein occlusion or diabetic retinopathy. The link between hypoxia, neovascularisation and oedema is discussed in chapter 1.1.4.

1.1.3.1 Branch retinal vein occlusion

Branch retinal vein occlusion has different aetiology from central retinal vein occlusion. The main risk factor for development of branch retinal vein occlusions is hypertension (Appiah and Trempe, 1989; Klein et al., 2000; The

Eye Disease Case-control Study Group, 1993). The occlusion occurs where a branch retinal artery and vein cross (Christoffersen and Larsen, 1999; Clemett, 1974). At the crossing, both vessels share a common sheath and it is believed that the conditions for thrombus formation and occlusion are created when the arteriole compresses the venule. At the crossing, it is in general more common that the arteriole is above the venule and this is especially common where occlusions occur (Christoffersen and Larsen, 1999; Duker and Brown, 1989; Sekimoto et al., 1992; Weinberg et al., 1990; Zhao et al., 1993).

Several studies have been performed on blood flow in branch retinal vein occlusion and, not surprisingly, the results indicate decreased blood flow. Fujio et al. (1994) used a laser Doppler technique to non-invasively measure decreased blood flow in the arterioles supplying the region affected by branch retinal vein occlusion in two patients and Yoshida et al. (2003) found similar results with similar technology in one patient. Horio and Horiguchi (2004) found decreased blood flow in the retinal segment affected by the occlusion. They measured during vitrectomy in patients with macular oedema due to branch retinal vein occlusion. Avila et al. (1998) found decreased capillary blood flow in affected areas. Kang and Lee (1995) found increased arteriovenous passage time in branch retinal vein occlusion and Noma et al. (Noma, Funatsu, Sakata, Harino, Mimura et al., 2009; Noma, Funatsu, Sakata, Harino, Nagaoka et al., 2009) found decreased blood velocity in perifoveal capillaries. Genevois et al. (2004) studied the capillary network in detail in rats and found that some capillaries closed while at similar time, blood is re-routed through others. According to studies on monkeys (Minamikawa et al., 1993 - English abstract) and miniature pigs (C. J. Pournaras, Tsacopoulos, Strommer et al., 1990a), re-opening of the thrombus does not necessarily re-open closed capillaries.

Decreased blood flow due to branch retinal vein occlusion can be expected to affect retinal oxygenation. Most studies on oxygenation in vein occlusions have been made on artificially induced branch retinal vein occlusions in animals. Such occlusions have been shown to decrease the partial pressure of oxygen, measured in the preretinal vitreous above the affected area (Noergaard et al., 2008; C. J. Pournaras et al., 1997; C. J. Pournaras, Tsacopoulos, Strommer et al. et al., 1990a; J. A. Pournaras et al., 2004; Stefansson et al., 1990). Pournaras et al. have also measured decreased partial pressure of oxygen directly within the inner retina with intraretinal electrodes (C. J. Pournaras, Tsacopoulos, Riva et al., 1990).

Occlusions of branch retinal venules in humans may have different outcome from the experimental occlusions in animals. The experimental occlusions are produced by suddenly blocking a previously healthy venule in

the animal while the constriction may progress more gradually in the human disease. Constriction of venules at the arteriovenous crossing is for example often seen in hypertensive patients, who have no history of occlusion. This is called arteriovenous nicking and is visible on fundus photographs. Development of collateral circulation and re-canalisation of the thrombus as well as atrophy of the tissue may also complicate outcomes of both animal studies and human branch vein occlusions, although not necessarily in the same way. Direct measurements of oxygenation in human patients with branch retinal vein occlusions are necessary to determine how the human condition compares to the animal experiments.

1.1.3.2 Central retinal artery occlusion

Central retinal artery occlusions are generally complete occlusions although there is, in many cases, some blood flow to the retinal vasculature through collateral circulation and through a cilioretinal artery, if such an artery is present. This remaining blood flow is, however, not protective, with the exception of larger cilioretinal arteries (Hayreh, 2011). Central retinal artery occlusion is often due to an embolus although other causes, such as giant cell arteritis are also known (Foroozan et al., 2003; Hayreh, 2011; Hayreh et al., 2009). The symptoms are generally sudden and dramatic loss of vision, although some improvement is possible in the first days after the initial insult (Hayreh and Zimmerman, 2005; Yuzurihara and Iijima, 2004).

As described above (chapter 1.1.1), the retinal vessels nourish the inner retina and occlusion of the retinal vasculature can therefore be expected to affect the inner retina in particular. Preretinal measurements in cats (Stefansson, 1981) and intraretinal measurements in cats (Alder et al., 1990) and rats (Braun and Linsenmeier, 1995; Yu et al., 2007) have indeed shown that the partial pressure of oxygen in the inner retina falls to zero during experimental central (Alder et al., 1990; Stefansson, 1981) or branch (Braun and Linsenmeier, 1995; Yu et al., 2007) retinal artery occlusion while the partial pressure in the outer retina is close to normal or slightly decreased. The choroid is not able to supply the whole retina, particularly not in the dark, when the oxygen consumption in the outer retina is greater.

As mentioned previously, the concentration of oxygen does not seem to have much effect on choroidal blood flow. Inhalation of pure oxygen will therefore raise the partial pressure in the choroid and allow more oxygen to reach the inner retina. Studies on cats and monkeys have shown that during inhalation of 100% oxygen after central artery occlusion, the partial pressure of oxygen in the vitreous, reflecting the partial pressure of oxygen in the inner retina, is raised to normal or higher than normal levels (Landers, 1978;

Stefansson, 1981; Wolbarsht et al., 1987). Landers even saw recovery of the electroretinogram and visual evoked response in the cat with inhalation of pure oxygen after central retinal artery occlusion. Similar results have been found with intraretinal measurements of oxygen tension in cats (Alder et al., 1990; Braun and Linsenmeier, 1995), and rats (Yu et al., 2007) although the inner retinal hypoxia or electrical signs of function were not completely normalised in all cases (Braun and Linsenmeier, 1995; Yu et al., 2007). It should be noted that, during the occlusion, the supply of glucose and other nutrients is of course diminished as well. Nevertheless, only adding supplemental oxygen has beneficial effects on function in experimental retinal artery occlusions, as measured by electrophysiological methods.

Despite the beneficial effects of supplemental oxygen on experimental artery occlusions, there is currently no generally accepted treatment for central retinal artery occlusion, except for steroid treatment in the case of giant cell arteritis. Experiments on monkeys have shown that retinal damage occurs after about one and a half hour and increases after that (Hayreh and Jonas, 2000; Hayreh et al., 2004). Possible treatment will therefore have to be initiated very soon after the occlusion although there are reports of successful treatment in individual cases after longstanding occlusion (Mansour and Younis, 2011; J. A. Pournaras et al., 2010).

1.1.4 Diabetic retinopathy

Diabetic retinopathy eventually affects the great majority of diabetic patients, although the severity varies widely (Williams et al., 2004). The disease is characterised by damage to the retinal capillaries (Cogan and Kuwabara, 1963; Cogan et al., 1961; Kohner, 1993). Capillary basement membranes thicken (Ashton, 1974; Roy et al., 2010), microaneurysms form and some capillaries are obliterated while others become wider (Cogan and Kuwabara, 1963; Kuwabara and Cogan, 1963). All of this is eventually caused by elevated blood glucose. The exact biochemical processes are complex, not fully known and outside the scope of this thesis. The consequences of the capillary damage are poor distribution of blood, which can lead to hypoxia in the retinal tissue.

Hypoxia has been confirmed in long-term diabetic cats. Linsenmeier et al. (1998) used oxygen sensitive intraretinal electrodes and found that the partial pressure of oxygen in the inner retina was decreased even before signs of retinopathy were present. Other studies, on cats (Stefansson et al., 1986), dogs (Ernest et al., 1983; Stefansson et al., 1989) and rats (Alder et al., 1991), have not found evidence of hypoxia in models of diabetes. It, however, remains questionable whether the short-term models, used in these studies, are

comparable enough to the human condition, which develops over years. The animal models do generally not develop visible retinopathy.

Recently, Holekamp et al. (2006) measured partial pressure of oxygen in the vitreous cavity in patients undergoing vitrectomy for either proliferative diabetic retinopathy or non-diabetic disease, i.e. preretinal fibrosis, macular hole and retinal detachment. The partial pressure of oxygen in the diabetic patients was found to be lower than in the non-diabetic patients. This was true for measurements adjacent to the lens as well as in the centre of the vitreous cavity. In a similar experiment, Lange et al. (2011) found higher partial pressure of oxygen at the posterior pole, just above the retina of patients with proliferative diabetic retinopathy, when compared to non-diabetic vitrectomy patients. This was attributed to neovascular complexes, which have previously been shown to be associated with high partial pressure of oxygen (Maeda and Tano, 1996) and the partial pressure of oxygen was similar in the mid-periphery in diabetic patients and controls. Similar to the results of Holekamp et al., (2006) Lange et al. (2011) found lower partial pressure of oxygen in the mid-vitreous in the diabetic patients.

The vitreous gel is avascular and its oxygen is derived from the adjacent vascularised tissues, including the retina. Lower partial pressure of oxygen in the mid-vitreous may therefore indicate that the partial pressure of oxygen in the retina in proliferative diabetic retinopathy is on average decreased although other factors, such as diffusion from the anterior part of the eye, will influence the measurements. It should be noted that all patients, studied by Lange et al. (2011) and some, studied by Holekamp et al. (2006) had undergone panretinal photocoagulation, which may have influenced the results (Stefansson, 2006; Stefansson et al., 1986), even if Holekamp et al. (2006) state that they saw no difference between treated and untreated patients.

Non-invasive measurements of retinal oxygenation have also been performed in diabetic patients. During the first attempts of non-invasive oximetry, Hickam et al. (1959) measured a small group of diabetic patients, most of whom had some retinopathy. They found normal venous oxygen saturation. Tiedeman et al. (1998) measured oxygen saturation in retinal vessels in diabetic patients, who had no retinopathy. They used dual wavelength oximetry and found increased arteriovenous difference in saturation during hyperglycaemia. They interpreted this as increased oxygen consumption of the retina in hyperglycaemia, assuming that blood flow is increased. Greater blood flow with higher blood glucose has been shown in some studies (Bursell et al., 1996; Grunwald et al., 1987; Pemp et al., 2010) but not all (Gilmore, Hudson, Nrusimhadevara, Ridout et al., 2007; Sullivan et al., 1991) and the possible,

direct effects of insulin on ocular blood flow must also be kept in mind (Schmetterer, Muller et al., 1997). Schweitzer et al. (2007) measured retinal vessel oxygen saturation with a multispectral oximeter in patients with mild or moderate non-proliferative retinopathy. At baseline, during inhalation of normal atmosphere, they found slightly higher mean venous saturation in diabetic patients, when compared to healthy volunteers although this did not reach statistical significance. The same group (Hammer, Vilser et al., 2009) used a dual wavelength oximeter in a later study and found that venous saturation was increased in patients with diabetic retinopathy. The saturation increased with severity of retinopathy.

The elevated (or normal) saturation in retinal venules in patients with diabetic retinopathy may seem to contradict the theory of hypoxia in diabetic retinopathy. This is not necessarily the case. Oxygen saturation in retinal vessels can be high at the same time as there is tissue hypoxia due to poor distribution of oxygen by the capillary network and possibly other factors (see further discussion in chapter 5.3.)

Further evidence for hypoxia in diabetic retinopathy is provided in studies where supplemental oxygen is given and the effect on structure or function studied. Oscillatory potentials of the electroretinogram are believed to reflect inner retinal activity (Wachtmeister, 1998). Drasdo et al. (2002) found decreased oscillatory potentials in diabetic patients. The oscillatory potentials were normalised with supplemental oxygen. Similarly, supplemental oxygen improved contrast sensitivity in patients with minimal retinopathy (Harris et al., 1996) and improved colour vision defects in patients with no or minimal retinopathy (Dean et al., 1997). Kurtenbach et al. (2006) found that rod sensitivity was decreased in diabetic patients compared to healthy individuals and that rod sensitivity improved with supplemental oxygen. A small study on patients with diabetic macular oedema indicated that macular thickness was decreased by supplemental oxygen (Nguyen et al., 2004). Supplemental oxygen also leads to less decrease in blood flow in diabetic patients (no retinopathy or various stages of retinopathy) compared to healthy individuals (Gilmore, Hudson, Nrusimhadevara, Harvey et al., 2007; Gilmore, Hudson, Nrusimhadevara, Ridout et al., 2007; Grunwald, Riva, Brucker et al., 1984; Grunwald, Riva, Petrig et al., 1984; Justesen et al., 2010; Patel et al., 1994). The decrease in blood flow may be blunted because the tissue is hypoxic during breathing of normal air and the signalling of vasoconstriction due to added oxygen when breathing oxygen enriched air is not beneficial. However, the cause may also be poor regulation of blood flow.

1.1.5 Consequences and treatment of retinal hypoxia following vein occlusions or diabetic retinopathy

As outlined in chapters 1.1.2 and 1.1.3 above, there is considerable evidence for hypoxia in both retinal vascular occlusions and in diabetic retinopathy. The arguments are summarised below.

1. The pathogenesis of the diseases strongly suggests that hypoxia is involved; occlusions of vessels are obviously likely to interfere with oxygenation and so is dropout of retinal capillaries in diabetic retinopathy.

2. Animal studies have provided evidence for retinal hypoxia in central retinal artery occlusion, branch retinal vein occlusion and long term diabetic retinopathy although there are also studies on short term diabetic animals, which do not show hypoxia.

3. Low partial pressure of oxygen in the mid-vitreous in patients with diabetic retinopathy may indicate less diffusion of oxygen from the retina.

4. High oxygen saturation in retinal vessels may indicate poor distribution of oxygen by retinal capillaries in diabetic retinopathy (see chapter 5.3).

5. Beneficial effects of supplemental oxygen on patients with diabetes are consistent with the role of hypoxia in diabetic retinopathy.

Although retinal vessel occlusions and diabetic retinopathy are different diseases, there are certain similarities between their consequences and symptoms, particularly between retinal vein occlusions and diabetic retinopathy. This is perhaps not surprising since both retinal vein occlusions and diabetic retinopathy seem to cause more or less chronic retinal hypoxia (while the arterial occlusions are more acute). Various mechanisms are activated in retinal hypoxia, many of them through transcription factors called hypoxia inducible factors (HIFs), of which isoform 1 is the best known. Hypoxia inducible factor 1 is stabilised in hypoxia and induces the transcription of many genes, whose products are involved in the response to hypoxia (Arjamaa and Nikinmaa, 2006; Benita et al., 2009; Lange and Bainbridge, 2011). Among the best studied of these is vascular endothelial growth factor (VEGF). There is evidence that concentration of both HIF-1 and VEGF are increased in diabetic retinopathy and retinal vein occlusions (Aiello et al., 1994; Ehlken et al., 2011; Funk et al., 2009; Noma et al., 2011; Noma et al., 2012).

Hypoxia in the retinal tissue poses two major threats to vision, partially through HIF and VEGF dependent mechanisms. These two major threats are oedema and neovascularisation. Treatment, such as laser treatment and vitrectomy, may work by alleviating retinal hypoxia (for review and hypotheses see Stefansson, 2006).

Hypoxia stimulates oedema mainly through two mechanisms; increased permeability of vessels and increased hydrostatic pressure. Normal retinal capillaries form a barrier with tight junctions between endothelial cells and regulate the transport from blood to tissue (Kaur et al., 2008). This barrier breaks down in hypoxia and the permeability of retinal capillaries is increased, partly due to the upregulation of vascular endothelial growth factor during hypoxia. Increased permeability allows molecules that are retained in the vasculature under normal circumstances to escape. This increases the driving force for osmosis and water accumulates in the tissue.

Hypoxia may also lead to increased hydrostatic pressure in the capillaries, which further helps driving water from the vasculature to the tissue. This is because the retinal arterioles dilate in response to hypoxia (see chapter 1.1.1) and the downstream hydrostatic pressure increases. Although vasodilation is a general response to hypoxia, vessel diameter changes in diabetic retinopathy are complex and, in many cases, small (Klein et al., 2006; A. S. Tsai et al., 2011). Hydrostatic pressure may also be increased upstream of a vein occlusion (Attariwala et al., 1997; Hitchings and Spaeth, 1976; Jonas and Harder, 2007; Luckie et al., 1996), simply because of the increased resistance in the vasculature due to the occlusion.

The other major threat posed by hypoxia is neovascularisation. New retinal vessels are formed in an attempt to correct the hypoxia. The new vessels are fragile and create a risk of haemorrhage, which blocks passage of light to the photoreceptors and creates shadows in the visual field. New vessels can also form in the anterior part of the eye, in the iris, and this may be a consequence of retinal hypoxia and diffusion of growth factors from the retina to the iris (Laatikainen, 1977; Stefansson, 2006).

Laser treatment and vitrectomy, used in some cases to combat retinal neovascularisation and oedema, may function by alleviating hypoxia (for review and hypotheses see Stefansson, 2006). The laser destroys part of the retina, particularly the photoreceptors. This decreases oxygen consumption of the retina and increases the available oxygen for the remaining tissue. Beneficial effects on the partial pressure of oxygen above the laser treated retina have been found in animal experiments (Funatsu et al., 1997, Landers et al., 1982; Molnar et al., 1985; Novack et al., 1990; C. J. Pournaras, 1995; C. J. Pournaras, Tsacopoulos, Strommer et al., 1990b; Stefansson et al., 1986;

Stefansson et al., 1981; Yu et al., 2005) and in humans (Stefansson et al., 1992). Recently, Budzynski et al. (2008) found increased partial pressure of oxygen in the inner retina of laser treated cats.

Vitrectomy often results in replacement of the vitreous with less viscous material (silicon oil is an exception). Decreased viscosity may facilitate diffusion and flow of oxygen from well perfused areas of the retina or even from the anterior part of the eye to poorly perfused areas of the retina (Holekamp et al., 2006; Holekamp et al., 2005; Stefansson et al., 1981, , 1982; Stefansson et al., 1990).

The detailed mechanisms, by which hypoxia causes oedema and neovascularisation, and the details of treatment effects are beyond the scope of this thesis. Drug treatment for neovascularisation is also omitted here. The importance of hypoxia in vein occlusions and diabetic retinopathy, however, points to the necessity of practical methods for measuring oxygenation of the retina in patients.

1.1.6 Glaucoma and treatment

1.1.6.1 Glaucoma, intraocular pressure and blood flow

Glaucoma is a complex disease or even a group of diseases. It is characterised by degeneration of the optic nerve and a concomitant loss of visual field (Quigley, 2011). The diagnosis of glaucoma is challenging and there are many types of the disease. Glaucoma can for example be broadly divided into open-angle glaucoma and the less common angle-closure glaucoma, depending on the size of the irido-corneal angle. The causes of glaucoma remain to be fully elucidated and the causes are most likely not the same for all patients. Recent studies have for example shown that patients with open angle exfoliation glaucoma (almost) all have a common sequence variant in a single gene (Thorleifsson et al., 2007) while genetic associations for open angle glaucoma in general appear to be more complex (Ramdas et al., 2011).

Traditionally, glaucoma has been linked with high intraocular pressure although it is now clear that glaucoma does not necessarily develop in an eye with high pressure and many glaucoma patients have normal or low intraocular pressure (Quigley, 2011). Nevertheless, high intraocular pressure remains a risk factor for (some forms of) glaucoma and lowering of the intraocular pressure, pharmacologically or surgically, is the only accepted treatment for glaucoma. Lowering of the intraocular pressure generally slows the progression of glaucoma, even in patients with low pressure.

The effects of intraocular pressure on glaucoma may be more or less direct mechanical effects (Sigal and Ethier, 2009) but they may also be through the

effects of intraocular pressure on ocular blood flow. Decreased ocular blood flow and poor regulation of blood flow have been proposed to play a role in at least some cases of glaucoma (for reviews see Flammer et al., 2002; Grieshaber et al., 2007; Resch et al., 2009; Schmidl, Garhofer et al., 2011) and there is some evidence of hypoxia in the optic nerve head and the retina in glaucoma (Tezel and Wax, 2004). Theoretically, decreased blood flow into the eye may be linked to increased intraocular pressure because the intraocular pressure opposes the blood entering the eye globe. In the retina, for example, the pressure difference between the retinal arterioles and venules drives the blood through the retinal circulation and the pressure in the retinal venules has been found to be close to (Bill, 1963; Morgan et al., 1997; Westlake et al., 2001) or at least positively correlated with (Attariwala et al., 1994; Glucksberg and Dunn, 1993) the intraocular pressure. Other factors being equal, higher intraocular pressure would therefore reduce retinal blood flow. An extreme case of the effect of high intraocular pressure is seen in ophthalmodynamometry, where the intraocular pressure is raised above the retinal arterial blood pressure until the retinal blood flow stops.

Retinal blood flow does, however, not follow changes in intraocular pressure in a linear manner. As mentioned in chapter 1.1.1, retinal blood flow is regulated to compensate for the effect of pressure. The regulation functions by changing the vessel diameter. This can be described (qualitatively) by comparison with Hagen-Poiseuille's law for laminar flow in cylindrical tubes.

The law states that,

$$F = \frac{\Delta P}{k * d^{-4}} \qquad \text{(Equation 1)}$$

where F is flow, ΔP is the pressure gradient along the tube, d is the diameter of the tube and k is a constant, which is related to the viscosity of the fluid and the length of the tube. Feke et al. (1989) found that blood flow in retinal vessels in healthy volunteers increased with diameter of the vessel in approximately the fourth power, consistent with Hagen-Poiseuille's law. If, in the retina, ΔP is decreased, for example due to increased intraocular pressure, well regulated vasculature would increase vessel diameters (d) and compensate so that the flow is not changed. If the regulation is overwhelmed the blood flow will decrease. It has for example been shown that both retinal arteriolar and venular oxygen saturation in the monkey decrease when the intraocular pressure is raised dramatically (Beach et al., 2007; Khoobehi et al., 2004).

The retinal circulation is used above as an example of the possible effects of intraocular pressure on ocular blood flow. Other parts of the vasculature

within the eyeball can also be affected by the intraocular pressure. This includes the choroid (Schmidl, Garhofer et al., 2011) and the optic nerve head circulation. Measurements in the optic nerve head capillaries of monkeys have for example shown that oxygen saturation decreases with dramatic increases in intraocular pressure (Beach et al., 2007; Khoobehi et al., 2004). Experiments on pigs have shown have that the partial pressure of oxygen over the optic nerve head decreases with increased intraocular pressure, although there was evidence of regulation of blood flow at lower pressures (la Cour et al., 2000; Stefansson et al., 2005).

1.1.6.2 Glaucoma treatment and its effect on blood flow and oxygenation

Measurements of blood flow before and after lowering of intraocular pressure are of particular interest if ocular blood flow is affected by intraocular pressure and if ocular blood flow is involved in the pathogenesis of glaucoma. Several studies have been performed on ocular blood flow or blood velocity before and after lowering of intraocular pressure with surgery. The results vary and there is no consensus on whether glaucoma surgery influences ocular blood flow. An increase in blood velocity in the central retinal artery was found in one study (Trible et al., 1994) while another study found no change (Cantor, 2001). Two studies found that surgery increased superficial optic nerve head blood flow (Berisha et al., 2005; Hafez et al., 2003) while two other studies found no change in optic nerve head blood flow (Cantor, 2001) or flow velocity (Tamaki et al., 2001). Furthermore, two studies found no change in capillary blood flow in the peripapillary area (Cantor, 2001; Hafez et al., 2003). Pulsatile ocular blood flow is believed to mostly reflect choroidal blood flow and three studies have shown this flow to be increased after glaucoma surgery (Berisha et al., 2005; Boles Carenini et al., 1997; James, 1994). However, blood velocity in the short posterior ciliary arteries, which partly supply the choroid, has been found to be increased (Trible et al., 1994) or unchanged (Cantor, 2001).

The glaucoma drug dorzolamide lowers intraocular pressure but may also have an effect on retinal blood flow through a different mechanism. Dorzolamide inhibits the enzyme carbonic anhydrase II. Carbonic anhydrases catalyse the formation of bicarbonate and protons from carbon dioxide and water:

$$CO_2 + H_2O \xrightarrow{Carbonic-anhydrase} HCO_3^- + H^+ \qquad \text{(Equation 2)}$$

Inhibition of this reaction reduces the production of aqueous humour in the ciliary body and thereby decreases the intraocular pressure. If dorzolamide

reaches the retina in sufficient quantities and raises the concentration of carbon dioxide, this may lead to an increase in blood flow in retinal vessels by a mechanism independent of intraocular pressure.

Many studies have been performed on the effect of dorzolamide and other carbonic anhydrase inhibitors on ocular blood flow and they have shown varied results. This may partly be due to different methodologies used and different vessels measured. Some instruments, for example, only measure blood velocity and not flow. Some measure at the optic nerve head while others measure in the retina. In a literature review and meta-analysis, Siesky et al. (2009) found that most published studies indicate that ocular blood flow or blood velocities are increased with topical carbonic anhydrase inhibitors.

The effect of dorzolamide on retinal and optic nerve head oxygenation has also been studied. Intravenous dorzolamide (or the related acetazolamide) increased the partial pressure of oxygen over the optic nerve (Kiilgaard et al., 2004; la Cour et al., 2000; Pedersen et al., 2004; Stefansson et al., 1999; Stefansson et al., 2005) and retina (Pedersen et al., 2005) in pigs. Siesky et al. (2008) found indications of higher venous saturation when patients were taking dorzolamide eye drops three times a day compared to the timolol eye drops two times a day. Timolol is a beta blocker, which also reduces intraocular pressure by reducing aqueous humour formation. However, the effects of topical timolol on ocular blood flow seem to be minimal. Timolol has been found to increase (Arend et al., 1998; Bergstrand et al., 2001; Grunwald, 1986, , 1990; Steigerwalt et al., 2001) or decrease (Altan-Yaycioglu et al., 2001; Carenini et al., 1994; Kitaya et al., 1997; Sato et al., 2001; Schmetterer, Strenn et al., 1997; Yoshida et al., 1991; Yoshida et al., 1998) various parameters, related to ocular blood flow, and several studies show no effect (Arend et al., 2003; Evans et al., 1999; Fuchsjager-Mayrl et al., 2005; Galassi et al., 2002; Lubeck et al., 2001; Nicolela et al., 1996; Tamaki et al., 1997; T. H. Wang et al., 1997). In studies on pigs, intravenous timolol has been found to have no effect on optic nerve oxygenation whereas large intravenous doses of dorzolamide clearly elevated partial pressure of oxygen above the porcine optic nerve head (Kiilgaard et al., 2004).

Increasing ocular blood flow with glaucoma treatment is based on the premise that ocular blood flow is insufficient before treatment. Evidence of decreased ocular blood flow in glaucoma does, however, not necessarily mean that blood flow is involved in the pathogenesis of glaucoma. The decreased blood flow may simply be secondary to tissue atrophy (ganglion cell death). Measurements of retinal oxygenation before and after treatment may provide clues to the role of blood flow and oxygenation in glaucoma. If, for example, the retina of a glaucoma patient receives enough blood flow before any

treatment, the lowering of intraocular pressure may simply be matched by regulation of vessel diameter to keep the blood flow similar to what it was before lowering of pressure. If on, the other hand, the retina is hypoxic in glaucoma, the lowering of intraocular pressure may lead to less change in diameter, allowing blood flow and oxygen supply to increase.

1.2 How to measure retinal oxygenation?

Retinal oxygenation can be measured with either invasive or non-invasive methods. Invasive oxygen measurements in the eye are here defined as any measurements that require penetration of the surface of the body. Some of the invasive methods require that an oxygen sensitive probe is inserted into the eye while others are less invasive and require only that oxygen sensitive dyes are injected into the blood stream.

1.2.1 Invasive measurements

Much of our knowledge on retinal oxygenation comes from studies using oxygen sensitive probes that are placed above the retina or even penetrate the retina. In general, there are two types of probes; oxygen sensitive polarographic electrodes and probes that contain an oxygen sensitive dye. The electrode technique is based on an electrochemical reaction, which requires oxygen as a substrate. A polarising voltage is applied across two electrodes. The rate of the reaction is dependent on the concentration of dissolved oxygen in the vicinity of the electrodes and a current, which is proportional to the oxygen concentration, can be measured (see for example Alm and Bill, 1972a; Linsenmeier, 1986; Stefansson, 1981; Stefansson et al., 2005; Tsacopoulos and Lehmenkuhler, 1977; Yu et al., 1990).

The other general type of oxygen probes uses an oxygen sensitive dye, palladium-mesotetra-(4-carboxyphenyl)-porphyrin. The dye is excited by light of a certain wavelength and then re-emits light of a different wavelength, which can be detected. The re-emitted light is affected by the concentration of oxygen (Rumsey et al., 1988; A. G. Tsai et al., 2003; D. F. Wilson et al., 1987). If an oxygen sensitive dye is fixed to the end of an optical fibre, the concentration of oxygen can be measured by sending light to the end of the fibre probe and measuring the light that is returned from the dye. The returning signal can be used to calculate the partial pressure of oxygen at the end of the probe (Stefansson et al., 1989).

The invasive probes, containing either electrodes or an oxygen sensitive dye, can be placed in the vitreous cavity to measure partial pressure of oxygen, for example above the retina or the optic disc. Preretinal measurements of the

partial pressure of oxygen are believed to represent mostly the inner retinal oxygenation (Alder and Cringle, 1990; Stefansson et al., 2005). The location of the probe will greatly affect the results since there is an outward gradient of oxygen concentration from the retinal arterioles and even an inward gradient of oxygen concentration towards the retinal venules. This has been measured in miniature pigs (Molnar et al., 1985; Riva et al., 1986) and in cats (Alder and Cringle, 1990). To obtain a measurement that represents the retinal tissue it may be best to place the probe as far away from large retinal vessels as possible. Intraretinal measurements have also been made with oxygen sensitive electrodes that are small enough to penetrate the retina without too much damage (see for example Alder et al., 1983; Linsenmeier, 1986; Tsacopoulos et al., 1976).

Less invasive techniques for oxygen measurements exist, which are based on injecting an oxygen sensitive dye into the blood stream (Shonat et al., 1992; A. G. Tsai et al., 2003), instead of fixing the dye to the aforementioned fibre optic probes. The injected dye is carried to the retinal and choroidal vasculature, as well as to the rest of the vasculature in the body. The retina and choroid is then illuminated with light of a certain wavelength and the light coming back from the eye will contain information on the oxygen concentration in the vicinity of the dye. The dye is bound to albumin and is therefore mostly confined to the vasculature. Some albumin will escape from the vasculature and the oxygen measurement is not depenent on the concentration of the dye, given some conditions (A. G. Tsai et al., 2003). However, the technique has mostly been used for study of the vasculature. Emission from various depths, for example emission from the choroid and the retina, can be distinguished with optical methods and semi-continuous profiles through the retina of partial pressure of oxygen have been published (Shahidi et al., 2010). However, the depth resolution may be less than for intraretinal microelectrode measurements.

Unfortunately, the injected dye is not safe for human use. Oxygen sensitive probes have been used in several studies on humans (Holekamp et al., 2006; Holekamp et al., 2005; Siegfried et al., 2010; Stefansson et al., 1992) but their use is obviously limited to patients, who are undergoing surgery (vitrectomy). Invasive studies have mostly been performed on animals and have provided wealth of information on oxygenation of the retina and choroid in health, retinal vascular occlusions and diabetic retinopathy (see chapters 1.1.1 -1.1.4 above).

1.2.2 Haemoglobin and oxygen saturation

Non-invasive measurements of retinal oxygenation are based on measurements of the colour of haemoglobin. It is therefore helpful to review the main characteristics of haemoglobin and oxygen transport in blood.

Oxygen is carried in blood either bound to haemoglobin or dissolved in the plasma. During inhalation of normal atmosphere, about 9mmol of oxygen is carried bound to haemoglobin in each litre of arterial blood and a little more than 0.1mmol/L of oxygen is dissolved in the plasma (assuming haemoglobin concentration of 150g/L, calculated assuming standard pH, carbon dioxide concentration and temperature with information from Zijlstra et al. 2000). The fraction of dissolved oxygen is also very small in venous blood. Measuring the amount of oxygen bound to haemoglobin therefore gives a good estimate of the oxygen content in blood. Oxygen saturation is often defined as the proportion (percentage) of haemoglobin that is bound to oxygen,

$$Oxygen\,saturation = \frac{[HbO_2]}{[Hb]+[HbO_2]} \times 100\% \qquad \text{(Equation 3)}$$

, where $[HbO_2]$ is the concentration of oxygenated haemoglobin and [Hb] is the concentration of deoxyhaemoglobin. This definition is a simplification since there are more than two forms of haemoglobin in blood.

The most common type of haemoglobin in adult human blood is haemoglobin A, which is composed of two alpha and two beta globin protein subunits. Each of these four subunits contains a haeme group with one iron ion (Fe^{2+}), which can bind one oxygen molecule. Anywhere from zero to four oxygen molecules can be bound to each molecule of haemoglobin at a particular point in time and these oxygen molecules can be bound to either alpha or beta subunits of haemoglobin A in various arrangements and the ratios between the forms depend non-linearly on the partial pressure of oxygen (Bellelli, 2010; Bellelli and Brunori, 2011, Winslow and Vandegriff, 1997). Oxygen saturation can therefore more accurately be defined as the ratio (or percentage) of occupied binding sites for oxygen. Note that this is a ratio of the total number of functional binding sites. As described below, some forms of haemoglobin do not bind oxygen and these forms should not be included in the calculation of oxygen saturation (Toffaletti and Zijlstra, 2007).

Haemoglobin A usually comprises over 90% of haemoglobin in the blood of a healthy adult and haemoglobin A2, which has delta chains instead of the beta chains, accounts for about 2% (Schechter, 2008), usually less than 3% (Steinberg and Adams, 1991). Foetal haemoglobin (haemoglobin F) accounts for less than 1% on average although this can vary considerably in healthy adults (Rochette et al., 1994; Schechter, 2008). Over 1000 variants of haemoglobin are known. The vast majority of the variants contain single amino acid changes. Some are associated with disease while others cause no

symptoms (Wada, 2002; "Database of Human Hemoglobin Variants and Thalassemias", 2011).

Normal haemoglobin A2 and F transport oxygen but this is not the case for the so called dyshaemoglobins. The most significant dyshaemoglobins are carboxyhaemoglobin and methaemoglobin. Carboxyhaemoglobin has carbon monoxide instead of oxygen bound to the iron ion in the haeme moiety. Carbon monoxide is produced within the body and inhaled in small amounts. Haemoglobin has much greater affinity for carbon monoxide than for oxygen and normal levels of carboxyhaemoglobin are therefore up to 2% of all haemoglobin but can rise to 4-8% in smokers (Fischbach and Dunning, 2009). Methaemoglobin is present at low levels in healthy individuals. The normal level is about 1% (Fischbach and Dunning, 2009) but the levels can rise when certain drugs are taken (Skold et al., 2011). The iron in methaemoglobin is in the form Fe^{3+}, not the Fe^{2+}, which is the form needed for carrying oxygen.

In addition to the above mentioned forms of haemoglobin, it can also be affected by several molecules, which can bind to it outside the haeme moiety and affect its affinity for oxygen. Carbon dioxide and protons for example decrease the affinity of haemoglobin for oxygen (Staub Sr, 1998) while glycated haemoglobin has increased affinity for oxygen (Ditzel, 1976). The concentration of glycated haemoglobin (haemoglobin A1c) is correlated with long term blood glucose levels. Normal values are about 5-7% of total haemoglobin in healthy individuals (Fischbach and Dunning, 2009) but the values can rise in poorly controlled diabetes (Halldorsdottir et al., 2009).

1.2.3 Haemoglobin light absorbance

Non-invasive oximetry utilises the colour of haemoglobin to measure oxygen saturation. Oxygenated and deoxygenated haemoglobin have different colours. This is part of the reason why blood with different oxygen saturation has different colours. A familiar example is that arterial blood has a light red colour while venous blood is darker (see Figure 3).

Figure 4 below shows the absorptivities of several types of haemoglobin as a function of wavelength (absorptivity is a measure of how much light a solution absorbs per unit concentration of the solution and per unit path length through the solution).

As can be seen in Figure 4, light absorptivity of oxygenated and deoxygenated haemoglobin A is different at most wavelengths of light. At some wavelengths, the absorptivity is the same and these wavelengths are called isosbestic wavelengths.

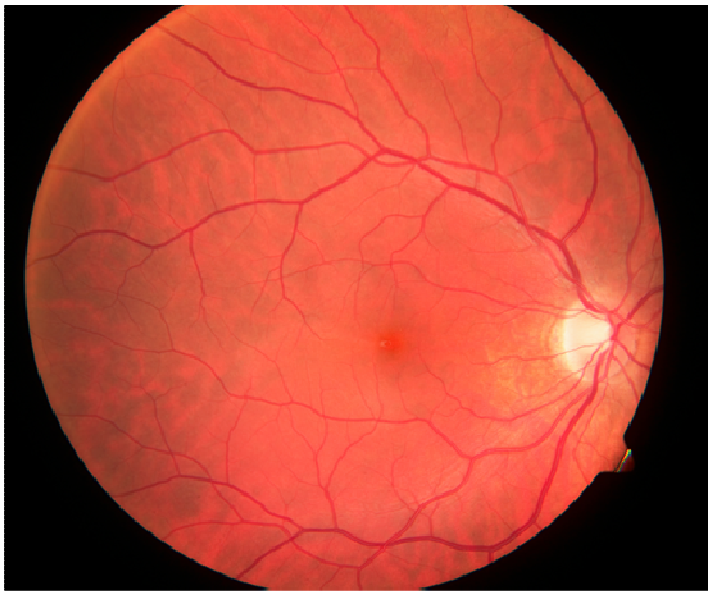

Figure 3. A healthy fundus. The retinal vessels enter and leave with the optic nerve. The bright circle is the end of the optic nerve. Close inspection reveals a colour difference between retinal arterioles and venules.

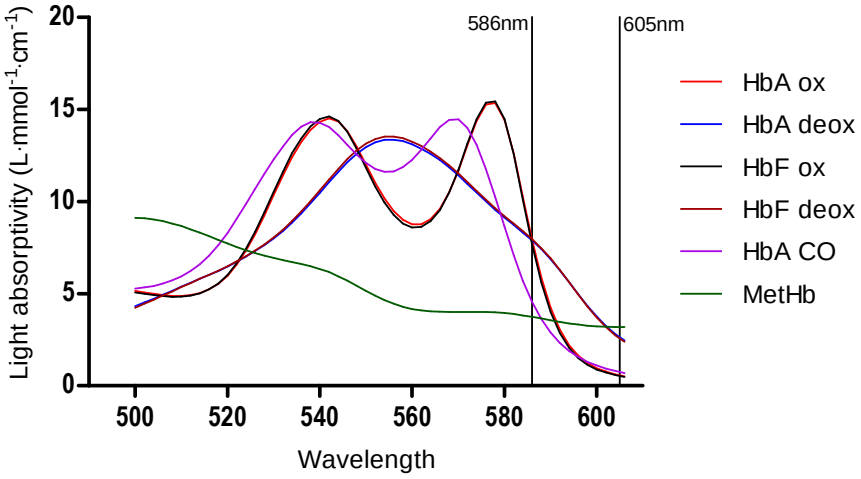

Figure 4. Light absorptivity of several forms of haemoglobin. Light absorptivity is a measure of how much light a solution absorbs per unit concentration of the solution and per unit path length through the solution. The image was prepared from data in Zijlstra et al. (2000).

As described in chapter 1.2.2, blood contains a mixture of haemoglobin derivatives and functional haemoglobin can be bound to various ligands, for example zero to four oxygen molecules as well as carbon dioxide and glucose. The question therefore arises if absorptivities of fully oxygenated and fully deoxygenated haemoglobin A (Figure 4) can be used for analysis of blood.

Firstly, the absorbance changes with oxygenation of haemoglobin have been assumed to be linearly dependent on the overall fraction of occupied haeme moieties. This implies for example that a (hypothetical) solution of haemoglobin molecules would have the same colour if all of the haemoglobin molecules were bound to two oxygen molecules as if half of the haemoglobin molecules were bound to four oxygen molecules and half was devoid of oxygen. This approximation appears to be valid although some non-linearity can be found, at least at wavelengths close to 400 nm (Doyle et al., 1988).

The next question is how haemoglobin types, other than haemoglobin A, are likely to influence the light absorbance of blood. As can be seen from Figure 4 above, haemoglobin A and F have almost the same absorptivities at all wavelengths shown and this is true for both oxygenated and deoxygenated forms. According to Kunkel et al. (1957) haemoglobin A2 has the same light absorbance as haemoglobin A, at least between 520 nm and 590 nm. It is therefore unlikely that haemoglobin F and A2 confound oxygen saturation measurements.

Dyshaemoglobins, such as carboxyhaemoglobin and methaemoglobin, do not affect oxygen saturation, since oxygen saturation is defined as the ratio of occupied binding sites to the number of functional (available) binding sites for oxygen and this depends mostly on the partial pressure of oxygen (Toffaletti and Zijlstra, 2007). However, binding of haemoglobin to carbon monoxide will of course affect the oxygen carrying capacity of blood and the same is true of the concentration of methaemoglobin. Furthermore, if carboxyhaemoglobin and methaemoglobin absorb light of the wavelengths used for oximetry, they may affect the results of oxygen saturation measurements. As can be seen in Figure 4, carboxyhaemoglobin has somewhat similar absorptivity as a function of wavelength as oxygenated haemoglobin A (or F). Methaemoglobin also absorbs considerable light in the wavelength range (Figure 4), although the profile and therefore colour is different (Brunelle et al., 1996).

In addition to the above mentioned forms of haemoglobin, it is conceivable that ligands, which bind outside the haeme moieties, may change the light absorbance of haemoglobin. Large changes in pH appear to have only slight effects on the light absorbance of oxygenated haemoglobin and even less (if any) effect on the light absorbance of deoxyhaemoglobin (Wimberley et al., 1988). The same study found no effect of carbon dioxide concentration on

oximetry readings (the oximeter used 535, 560, 577, 622, 636 and 670 nm wavelengths). This does not preclude the possibility of interference due to carbon dioxide if other wavelengths are used. Incubation of haemoglobin solutions with glucose does affect the light absorbance although the shape of the absorbance curve is not dramatically changed at physiological glucose concentrations (Lazareva and Tuchin, 2007).

1.2.4 Measurement of light absorbance

Light absorbance of a solution, for example a solution of haemoglobin, can be described with optical density. Optical density is defined as

$$OD = log \frac{I_0}{I} \qquad \text{(Equation 4)}$$

, where OD is optical density I_0 is the original light intensity before attenuation of light due to absorbance and I is the light intensity after absorbance in the sample / solution has diminished the light intensity. As can be seen from equation 4, higher optical density means greater absorbance.

Optical density of a blood vessel, measured at an isosbestic (oxygen insensitive, see Figure 4) wavelength will depend on vessel diameter and other factors but not on oxygen saturation. Optical density at a non-isosbestic wavelength (oxygen sensitive), will depend on similar factors as the optical density at an isosbestic wavelength but also on oxygen saturation. The ratio of optical densities at a non-isosbestic and an isosbestic wavelength will therefore be sensitive to oxygen saturation while the effects of other factors, such as vessel width, will tend to cancel out. It can be shown (Beach et al., 1999; Harris et al., 2003; Hickam et al., 1959) that for a solution of haemoglobin in water there is an approximately linear relationship between such a ratio and oxygen saturation;

$$Oxygen\ saturation = a + b \times \frac{OD_{non-isosbestic}}{OD_{isosbestic}} \qquad \text{(Equation 5)}$$

, where a and b are constants. Optical density ratio, ODR, is the ratio of the two optical densities:

$$ODR = \frac{OD_{non-isosbestic}}{OD_{isosbestic}} \qquad \text{(Equation 6)}$$

Substitution into equation 5 gives

$$Oxygen\ \ saturation = a + b \times ODR \qquad \text{(Equation 7)}$$

Equation 7 holds for a solution of haemoglobin and such a two wavelength model assumes that there is only one form of haemoglobin in the solution, which can either be oxygenated or deoxygenated (Zijlstra et al., 2000). Calculation of the optical density ratio requires measurements of light entering and exiting the solution, that is I and I_0 from equation 4, for two wavelengths.

It is considerably more complex to measure blood in retinal vessels than a solution of haemoglobin in a test tube. Light scattering by blood cells and vessel walls makes equation 7 inaccurate (Delori, 1988; Hammer et al., 2001; Hammer et al., 1998; Hammer et al., 2002; Pittman and Duling, 1975; Schweitzer et al., 1995). Furthermore, the light intensities, I and I_0 have to be estimated from reflected light rather than transmitted light and the differences in pigmentation in the retina (within and between subjects) can influence the measurement (Beach et al., 1999; Hammer et al., 2002; Schweitzer et al., 1995; Smith et al., 2001).

The retinal measurements can, for example, be performed with fundus camera based oximeters. The light intensities, I_0 and I, can be estimated from brightness values at chosen locations in the fundus images, see Figure 5.

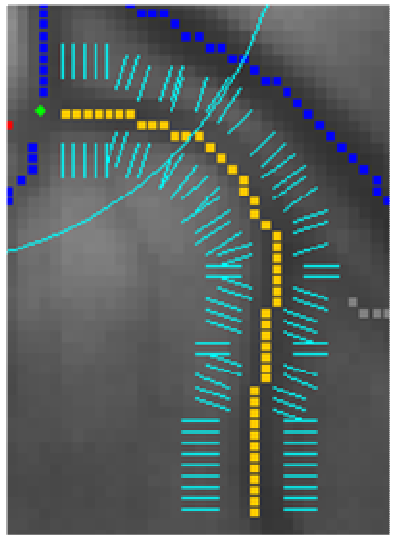

Figure 5. Measurements of light intensities for oximetry. Light intensity is measured to the side of blood vessels to represent light that has not interacted with blood in the vessel (light blue, I_0). Light intensity on the vessel is influenced by light absorbance in the vessel (yellow, I). The ratio of brightness inside and outside the vessel is used for calculation of light absorbance by the vessel, which is then used for calculation of oxygen saturation.

The light intensity after interaction of light with blood is estimated by the brightness value on the vessel. This brightness value is obviously affected by light absorbance by blood in the vessel. The reference light intensity, I_0 is estimated by the brightness value of the image next to the vessel of interest. This brightness value is not affected by light absorbance by the blood vessel. By choosing a brightness value close to the vessel for the reference (I_0), the light intensities, I and I_0, are affected by similar factors except for the light absorbance by the vessel.

Despite the approximations made, it has been shown that relative haemoglobin oxygen saturation in retinal vessels can be estimated by measuring light reflectance at two wavelengths (Beach et al., 1999; Hickam et al., 1959). The method has been shown to be sensitive to changes in saturation as well as giving repeatable results. Increased oxygen saturation in both retinal arterioles and venules was detected when healthy volunteers inhaled 100% oxygen (Beach et al., 1999; Hardarson et al., 2006). The standard deviation between repeated measurements of the same retinal vessels (averaged for one eye) was 3.7% for arterioles and 5.3% for venules (Hardarson et al., 2006).

The method described above is the foundation of the method used in the studies described in this thesis (for further details see methods section, chapters 3.1 and 3.2). Other methods for non-invasive oximetry all use colour changes in haemoglobin to estimate haemoglobin oxygen saturation, even if technical aspects differ.

1.2.5 Development of technology for non-invasive retinal oximetry

1.2.5.1 Retinal vessel oximetry with two wavelengths

Non-invasive blood oximetry was first performed in the 1930s in Germany and the first ear oximeter was made in 1936 (for review see Severinghaus, 2011). Hickam et al. (1959) were the first to attempt to use similar principles in the eye. They applied special filters to obtain fundus images with two wavelengths of light and used densitometric measurements of photographic films to estimate the light intensities, which were then used to calculate retinal vessel oxygen saturation. They demonstrated the sensitivity of their instruments for changes in oxygen saturation by measuring the effect of altered proportion of oxygen in inhaled air. Broadfoot et al. (1961) measured fundus reflectance at three wavelengths in an attempt to develop a choroidal oximeter and found that reflectance changed in the expected direction during various stimuli or experimental conditions *in vitro* and *in vivo* in rabbits and humans. Choroidal oximetry with two wavelengths was later developed by Laing et al. (1969). Preliminary evaluation of the choroidal oximeter indicated sensitivity to

changes in oxygen saturation but the calibration appears to have been crude. Laing et al. (1975) also developed a two wavelength system for retinal oximetry and demonstrated sensitivity to changes in retinal arteriolar saturation in rabbits.

Beach et al. (Beach et al., 1999; Tiedeman et al., 1998) used two wavelengths and similar principles for oximetry calculations as Hickam et al. (1959) did in their early experiments. The main improvements came from the use of digital camera technology and optical solutions that had not been used in this context before. Beach et al. (1999) also attempted to correct for the effect of vessel diameter and fundus pigmentation. The image from a fundus camera was split so that two images of the same area of the fundus were taken at the same time, one with a wavelength sensitive to oxygen saturation and one insensitive (isosbestic). Both images were captured at the same time, with a single camera flash, on the same camera sensor, which eliminated the problem of eye movement.

The oximetry technology used in this thesis is based on the technology of Beach et al. (1999, see more details in chapters 3.1 and 3.2). Computer programs have been developed to analyse the images and return relative haemoglobin oxygen saturation. The automation of the image processing dramatically improved the repeatability of the measurements and the method has been shown to be sensitive to changes in retinal vessel oxygen saturation (Hardarson et al., 2006). A similar two-wavelength approach was used by Crittin et al. (2002), who tested their instrument on two healthy individuals and found changes in the optical density ratio (chapter 1.2.4) in retinal venules when the volunteers inhaled pure oxygen. Recently, Hammer et al. developed an instrument, which also captures two fundus images with two wavelengths of light simultaneously although the optical approach is different. The instrument has been used for measurements of healthy volunteers, diabetic patients and patients with retinal arterial occlusion (Hammer, Riemer et al., 2009; Hammer, Vilser et al., 2009; Hammer et al., 2008).

1.2.5.2 Multiwavelength retinal and optic nerve head oximetry

The approximations made in two-wavelength oximetry make it difficult to achieve absolute oxygen saturation. The results of two-wavelength oximeters can for example be sensitive to differences in vessel diameters and differences in fundus pigmentation. Several groups have attempted to measure absolute retinal oxygen saturation by using more wavelengths and different modelling to estimate the saturation from light intensities. Some of these methods have required long exposure time (image acquisition time) and are mainly suitable for immobilised eyes in animal studies while others have reduced the

measurement area so that accurate data can be recorded in a short time with tolerable light intensities.

Delori (1988) built an instrument, which scans with three wavelengths and detects reflected light electronically. The use of three wavelengths allowed compensation for the effect of light scatter. Vessel tracking was use to minimise the effects of eye movements during scanning. The instrument was used for measurements of patients with neurogenic optic atrophy (Sebag et al., 1989) and it showed, somewhat paradoxically, that arteriovenous difference in oxygen saturation was increased in the affected eye. However, when the decrease in blood flow was taken into account the results showed decreased oxygen delivery in the affected eye. One disadvantage of this method is that it only measured a small area at a time.

Denninghoff et al. later developed at method, which is also based on scanning. Their first instrument performed a linear scan with four wavelengths but a later version of the instrument scanned a small area of the fundus with a different set of four wavelengths. The goal has been to achieve absolute calibration (Smith et al., 2000b). Studies have shown that the retinal saturation measurements are correlated with systemic saturation during blood loss in pigs (Denninghoff et al., 1997; Denninghoff et al., 1998; Denninghoff et al., 2003) and this group has made efforts to control for the effects of fundus pigmentation (Smith et al., 2001) and different light paths (Smith et al., 2000a) on oximetry results.

Schweitzer et al. (1999, 1995, 2001) coupled an imaging spectrograph with a fundus camera. This allows the simultaneous capture of light at multiple wavelengths, down to less than 2 nm apart, over a range of several hundred nanometres. A model was developed for calculation of absolute oxygen saturation. Samples of fully oxygenated blood in glass cuvettes have been accurately measured (Schweitzer et al., 2001). The method may yield absolute measurements of retinal oxygen saturation, although this is of course difficult to confirm experimentally. The main drawback of the method is that only a slit of 1.5 mm x 40 µm is measured at a time. This allows simultaneous measurement of a retinal arteriole and venule if they lie close together.

Several other groups have also developed oximeters, which use multiple wavelengths for measurements. Yoneya et al. (2002) illuminated the retina in patients with central retinal vein occlusion with bright light for six seconds and separated multiple wavelengths with interferometry. The method is claimed to yield oxygen saturation in every pixel of a 35° fundus image. Khoobehi et al. (Beach et al., 2007; Khoobehi et al., 2004; Khoobehi et al., 2011) used a hyperspectral system, which has a spectral resolution of 2.5 nm and a range from

410 nm to 950 nm. Measurements were made in and between retinal arterioles and venules at the optic nerve head of monkeys. The scan takes eight seconds.

One of the main challenges of multispectral (hyperspectral) imaging is gathering information on multiple wavelengths in a short time without exceeding safe light levels. This can be achieved by reducing the area of measurement as Schweitzer et al. (1999) did by measuring only a slit at the fundus. This can also be achieved by increasing the exposure time or light intensity as Yoneya et al. (2002) and Khoobehi et al. (2004) have done but this may limit the methods to animal studies where the eyes can be immobilised and light levels are less crucial. Nevertheless, Yoneya et al. (2002) have performed measurements in humans.

At least three novel approaches have been tried to capture multiple wavelengths in a snapshot. Ramella-Roman et al. (2007, 2008) used a lenslet array and different filters behind each lenslet. This allows capture of an image at several different wavelengths simultaneously. Testing on samples of haemoglobin and on healthy volunteers was carried out with six wavelengths of light. Harvey et al. (2005) have developed an instrument that captures information on eight wavelengths simultaneously by using spectral demultiplexing. The same group has used another system, which records information on different wavelengths sequentially, to develop and test their oximetry calculations (Mordant, Al-Abboud, Muyo, Gorman, Sallam, Ritchie et al., 2011; Mordant, Al-Abboud, Muyo, Gorman, Sallam, Rodmell et al., 2011). Another approach to multi- / hyperspectral retinal oximetry was taken by Johnson et al. (2007), who used a method based on hologram technology to separate up to 50 wavelengths in snapshot images. There has been limited use of the system in humans but validation results from rabbits, using 28 wavelengths, were recently published (Kashani et al., 2011).

Non-invasive oximetry has mainly been used to study blood in the larger retinal vessels. Attempts have been made to measure outside the larger vessels with multi-wavelength oximeters. Such measurements have been made in the optic nerve head (Beach et al., 2007; Khoobehi et al., 2004) and in the retina (Yoneya et al., 2002). The main challenge for these measurements is to isolate a small signal, which comes from the capillaries of interest. As described in chapter 1.1.1, the retinal capillaries are divided into a maximum of three layers. Each capillary is several micrometres wide and the mean distance between them is many times the capillary width. The attenuation of light by the retinal capillaries is therefore minimal (otherwise vision would be disturbed). This attenuation is the signal that must be used to estimate saturation in the retinal capillaries and it should be borne in mind that the signal can be contaminated

by the choroid in the background. At any particular time point the choroid holds much more blood than the retinal circulation and the contribution of this blood to the total signal differs depending on the pigmentation of the fundus. Furthermore, some of the more interesting retinal locations for oxygen measurements contain decreased density of capillaries or even no capillaries, such as parts of retinas in diabetic retinopathy.

Similar issues arise when measuring the optic nerve head capillaries. The superficial layer of the optic nerve head is mostly supplied by the retinal circulation but the posterior ciliary arteries may contribute (Hayreh, 1999). Behind the most superficial layer the origin of blood supply does not include the retinal circulation until, in some cases, behind the lamina cribrosa. Light, which hits the optic nerve head, may therefore be absorbed by vessels in different vascular beds and this may confound the interpretation of the results somewhat.

2 Aims of the studies

The overall aims are to use a newly developed retinal oximeter to test if changes in retinal vessel oxygen saturation can be found when physiological stimuli are applied and if retinal vessel oxygen saturation is changed by certain diseases or treatment. More specifically, the research questions are:

1. Does retinal vessel oxygen saturation change between light and darkness in healthy individuals (paper I)?

2. Is retinal vessel oxygen saturation affected by

   a) central retinal vein occlusion (paper IV)?
   b) branch retinal vein occlusion (paper V)?
   c) central retinal artery occlusion (paper VII)?

3. Is retinal vessel oxygen saturation affected by diabetic retinopathy (of various stages, paper VI)?

4. Is retinal vessel oxygen saturation affected by

   a) glaucoma surgery (paper III)?
   b) dorzolamide (medical treatment of glaucoma, paper II)?

3 Materials and methods

3.1 The retinal oximeter

The retinal oximeter (prototype no. 2, Oxymap ehf., Reykjavik, Iceland) is based on a non-mydriatic fundus camera (Canon CR6-45NM; Canon Inc., Tokyo, Japan). An image splitter (MultiSpec Patho-Imager; Optical Insights, Tucson, Arizona, USA) is attached to the fundus camera with a standard adapter (Canon CR-TA). The image splitter contains two dichroic mirrors and four narrow band-pass light filters. The dichroic mirrors split the beam from the fundus camera into four beams according to the wavelengths of light. The four beams are then further filtered before they all hit the same camera sensor. Therefore, the oximeter simultaneously delivers four images of the same area of the retina with different wavelengths of light (see Figure 6).

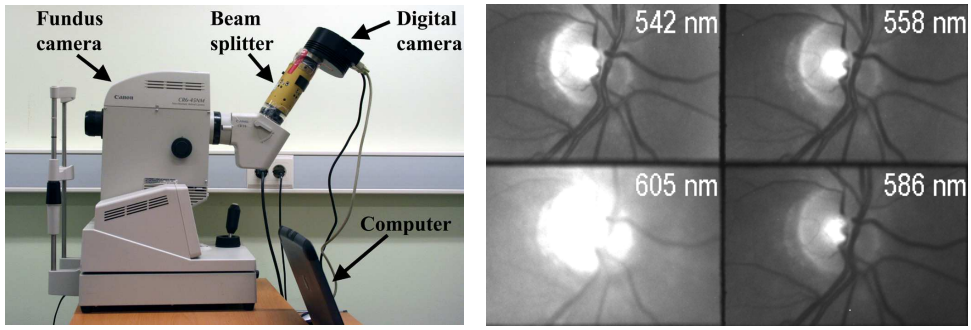

Figure 6. The retinal oximeter (left) and an unprocessed image (right), showing the same area of the fundus with four wavelengths of light.

The filters have centre wavelengths of 542 nm, 558 nm, 586 nm and 605 nm. The full bandwidth at half maximum is 5nm for the latter three filters but 9 nm for the 542 nm filter. This means that the range of transmitted wavelengths, measured between the two wavelengths where the transmittance is half of the maximum transmittance, is 5 nm or 9 nm. The digital camera (SBIG ST-7E, Santa Barbara Instrument Group, Santa Barbara, California), which records the image from the image splitter, has a cooled CCD sensor to reduce noise in the image. The sensor contains 0.4 megapixels. It was operated at 2x2 binning to increase sensitivity. This reduces the resolution down to 0.1 megapixel. The sensor was operated at minus 2°C to reduce the effect of noise. A dark frame, i.e. an image which was taken with the camera shutter closed, was subtracted from each image. This further reduces the systematic part of the sensor noise.

All analysis was performed with the 586 nm (isosbestic) and the 605 nm (non-isosbestic) images. The 542 nm and 558 nm images were not used. Discarding of 542 nm and 558 nm was based on earlier testing, which revealed that optical density ratios, using these wavelengths gave less reliable results.

All images were taken with the 45° setting on the fundus camera adapter. However, the image splitter only allows passage of a much smaller field of view, see Figure 6. Within each experiment, care was taken to align the camera such that the same retinal area was imaged, i.e. the main retinal vessels, close to the optic disc. However, the images of healthy volunteers were taken under a slightly different angle than images of diabetic patients (discussed in chapter 5.3.5). Some adjustment was made in the imaging angle in some cases of branch retinal vein occlusion, so that the occlusion itself could be included.

For all experiments, except for the experiments on the effect of light and dark (see chapter 3.5.1), the light conditions were as follows. Oximetry images were acquired in minimal light. Room lights were off and the infrared filter of the fundus camera was used, allowing only a dim red light to pass to the eye of the subject. Light impermeable curtain blocked the window although some light escaped to the side of the curtain (for ventilation). Dim light came from a laptop computer screen and the screen of the fundus camera, both of which faced away from the subject being measured. Images were acquired with the unmodified xenon flash of the camera. Images were taken at an interval of about one to two minutes (most often closer to one minute). Care was taken to image both eyes of the subject and all subjects in the same experiment under similar conditions.

3.2 Image processing and calibration

The images were processed with a custom made software (Oxymap ehf., Reykjavik, Iceland). The first step is automatic alignment of the four sub-images into the same coordinate system. The software then finds retinal vessels in the image, i.e. uses supervised classifying to define each pixel as belonging to a vessel or not. The software chooses measurement points on the vessel for measurement of light intensity (I) and outside the vessel for the reference light intensity I_0 (see chapter 1.2.4 and Figure 5). The points on the vessel itself are chosen to avoid the central light reflex on the vessels. For each chosen point on the vessel (for intensity I) the program finds a three by three pixels box around the point. The program excludes non-vessel pixels in this box and uses the median value from the remaining pixels. For each such value of I a corresponding value for I_0 was found. A point was found beside the vessel on a

line, which is perpendicular to the vessel direction. Each value for I_0 was a median value from a five by five pixels box around this point.

The program used each pair of I and I_0 values to calculate a single optical density ratio (ODR, see chapter 1.2.4). A vessel segment was chosen by the user (S.H.H. / S.T.) with a mouse click and all optical density ratios along such a segment were then averaged. This average optical density ratio was used as the value for the vessel.

Optical density ratios were transformed into relative haemoglobin oxygen saturation with equation 7 from chapter 1.2.4. Calibration, i.e. the determination of a and b in equation 7, was performed by setting the mean oxygen saturation in main retinal arterioles and venules to values from a calibrated oximeter. The mean value in healthy individuals was set to 92.2% in the main retinal arterioles and 57.9% in the main retinal venules. These values were obtained by Schweitzer et al. (1999) with a calibrated multispectral oximeter. This gives two equations (one for arterioles and one for venules) which can be solved for a and b. The calibration of the current oximeter was performed repeatedly as slightly different versions of the software were used. The relative oxygen saturation values are not completely comparable across studies (papers I-VII) since minor differences may have resulted from changing the version of the analysis program. The same version of the analysis program as well as the same calibration coefficients, a and b from equation 7, were used within each study.

Since the optical density ratios are linearly related to the calculated saturation, the level of statistical significance does not depend on calibration and the p-values are the same for optical density ratios and for calculated saturation.

The program can also be used for vessel diameter measurements. As mentioned above, the program defines points as either belonging to a vessel or not. The program finds the centre pixel on the vessel and a vector, which is perpendicular to the direction of the vessel. The vessel diameter measurement is made by counting the pixels that are defined as belonging to the vessel and lie on the perpendicular cross-section. The effective resolution of the digital camera in the oximeter used in the studies, described in this thesis, is 0.1 megapixels, which means that the larger retinal vessels are about 5 pixels wide. The vessel diameter option has not been systematically tested with the oximeter used in the studies described in this thesis. Testing has been made on a similar algorithm on images from a later, higher resolution version of the oximeter (Blondal et al., 2011)

3.3 Ethical considerations

The studies were approved by the National Bioethics Committee of Iceland and the Icelandic Data Protection Authority and adhered to the tenets of the Declaration of Helsinki. Participants signed an informed consent form after the nature of the study had been explained to them.

3.4 Statistical analyses

Statistical analyses were performed with Prism 5 (Graphpad Software, La Jolla, California) in all cases except for data on glaucoma surgery (paper III), where R, version 2.6.1 was used (provided in the public domain by the R Foundation for Statistical Computing, Vienna, Austria, available at http://www.r-project.org/). The type of statistical test varied according to variable setup of the studies and comparisons and the variable nature of the data in each paper. The test used is mentioned where the results are reported (chapter 4). The choice of statistical tests and the interpretation of the results is discussed in chapter 5.5.

3.5 Light and dark

Two experiments were performed, both on healthy volunteers. The first experiment compared retinal vessel oxygen saturation between a steady light level and darkness. The second experiment compared saturation between different levels of steady light and darkness.

Images were taken after dilation of the subjects' pupil with tropicamide HCl (Mydriacyl; Alcon Inc., Fort Worth, Texas, USA).

Retinal vessel oxygen saturation was measured in one major temporal arteriole and venule. An average saturation was measured in a segment that reached from just outside the optic disc margin and up to or nearly up to the first branching of the vessel. Retinal vessel diameter was measured in the same images and the same vessel segments although this was done at a later time with a later version of the analysis software. In this re-analysis of the data, various ratios of brightness values (I and I_0, see chapter 1.2.4) were calculated to search for possible artefacts (see chapters 4.1.1 and 5.1).

3.5.1 Light levels

Light levels were measured with a digital photometer (Mavolux; Gossen GmbH, Erlangen, Germany). Measurements were made with the luminance attachment of the photometer. The end of the attachment was placed 4-5cm from the lens of the fundus camera, in the same position as the cornea of the eye when an image is taken. The photometer was slowly shifted from left/right

and up/down until a maximum reading was obtained at this distance and this reading was recorded. The aperture angle of the luminance attachment on the photometer is 20°.

Healthy volunteers sat in darkness for 30 minutes to achieve dark adaptation. To avoid interfering with the dark adaptation, infrared light was used when the camera was adjusted. This can be achieved by using the standard infrared filter in the fundus camera, on which the oximeter is based. The fundus camera allows the user to view the fundus on a screen, facing towards the user and away from the subject being imaged. During imaging in the dark, the only sources of light were a slight red glow, which escapes through the infrared filter and dim light from the viewing screen and the screen of a laptop computer. Both screens faced away from the subject being measured. During these conditions, the photometer reading in front of the lens was 0 cd/m^2 (same setup as during light, the photometer measures in whole cd/ m^2).

The aiming light of the fundus camera was used for light adaptation in both experiments. Visible light, from a tungsten bulb was passed through the lens of the fundus camera by removing the infrared filter from the light path. In the first experiment, where only one light level was used, the overhead white fluorescent room lights were also used. The bulbs were Philips 58W/830 New Generation (Royal Philips Electronics NV, Amsterdam, The Netherlands). When the overhead lights were on, the light levels directly to the side of the fundus camera lens were approximately equal to the light levels in front of the lens, measured as described above. In the second experiment, where different light intensities were used, the only source of light was the aiming light of the camera at three different settings (and the dim lights of the screens, mentioned above).

The light used was not evenly distributed over the retina, since the fundus camera does not illuminate the whole retina equally, and neither does ordinary office lighting. The light levels given are therefore approximate and do not represent evenly distributed light (not Ganzfeld levels). Relative intensity of light can, however, be compared since all measurements of light were carefully performed in the same manner.

The oximetry measurement itself requires the use of the xenon flash of the fundus camera, which lasts several milliseconds. A single flash is sufficient for each measurement. The minimum time between measurements was 5 minutes.

3.5.2 Darkness vs. 80 cd/m^2 light

The first light and dark experiment involved comparison of darkness to one light level of approximately 80 cd/m^2. Eighteen healthy volunteers participated but data from three of them were excluded due to poor image quality. Images

during dark adaptation and light adaptation had to be of similar and sufficient quality and one poor quality image required that the whole set from that particular individual was omitted. After exclusion, the mean age was 30 years and the range was 21 to 57 years (13 men and 2 women).

The volunteers were placed in the dark for 30 minutes before the first measurement was made. The volunteers were then placed alternately in light (80 cd/m^2) and dark. Each period of light or dark lasted 5 minutes, and a measurement was performed with the oximeter at the end of each period.

3.5.3 Darkness vs. 1-100 cd/m2 light

The second light and dark experiment compared saturation during darkness and 1, 10 or 100 cd/m^2. Twenty-three volunteers participated in the second experiment. Four individuals were excluded from analysis due to poor image quality. After exclusion, the mean age was 25 years, and the range was 22 to 30 years (11 men and 8 women).

As in the first experiment, the volunteers were placed in the dark for 30 minutes before the first measurement. The volunteers were then placed in light successively at approximately 1, 10, and 100cd/m^2, each period lasting 5 minutes. Finally, the volunteers were adapted to dark for 5 minutes. A measurement was performed with the oximeter at the end of each period.

3.6 Retinal vascular occlusions

Measurements were made of retinal vessel oxygen saturation in patients with three different major types of retinal vascular occlusions; central retinal vein occlusion, branch retinal vein occlusion and central retinal artery occlusion.

3.6.1 Central retinal vein occlusions

Oxygen saturation measurements were made in 10 consecutive patients with unilateral central retinal vein occlusion after referral from their ophthalmologist (convenience sample). Measurements were made before any treatment, except for successful treatment of acute glaucoma in one patient. Data from two patients were excluded because of poor quality of oximetry images. Clinical characteristics of the patients are described with the results (chapter 4.2.1).

Pupils were dilated with 1% tropicamide HCl (Mydriacyl; Alcon Inc., Fort Worth, Texas, USA), which was sometimes supplemented with 10% phenylephrine hydrochloride (AK-Dilate; Akorn Inc, Lake Forest, Illinois, USA), as deemed necessary by the nurses or ophthalmologists in the clinic. The fellow eye (no vein occlusion) did not receive dilating eye drops in three cases.

In these cases, there was adequate dilation of the pupil in darkness and the patients requested that the dilating drops would not be used for this eye.

Oxygen saturation was measured in all main retinal arterioles and venules, for which reliable measurements were possible. As described in chapter 1.2.4, the oximeter uses brightness values inside and outside the retinal vessels to calculate relative oxygen saturation. Care was therefore taken to avoid measuring vessel segments with adjacent haemorrhages, which could have caused artefacts. When possible, measurements of each vessel segment were averaged from close to the optic disc up to or nearly up to the first branching of the vessel. A mean was calculated for arterioles and for venules in each eye. Each mean saturation value was constructed from the same ratio of temporal/nasal vessels as the mean in the other eye in the same patient. In order to achieve this matching, in some cases, two vessel segments (temporal or nasal) were averaged and entered as one for calculation of the mean for the eye.

3.6.2 Branch retinal vein occlusions

Oxygen saturation measurements were performed on 24 consecutive patients with branch retinal vein occlusion after referral from their ophthalmologist (convenience sample). Images were taken after dilation of the subjects' pupil with tropicamide HCl (Mydriacyl; Alcon Inc., Fort Worth, Texas, USA), which was in some cases supplemented with 10% phenylephrine hydrochloride (AK-Dilate; Akorn Inc, Lake Forest, Illinois, USA) as deemed necessary by the nurses or ophthalmologists in the clinic. The pupil of the fellow eye was not dilated in two cases (requested by the patients). The pupils were adequately dilated in the dark for imaging with the infrared aiming light of the fundus camera.

Retinal vessels were divided into three categories: (1) Vessels affected by the occlusion, (2) vessels in the diseased eye, which are not affected by the occlusion and (3) vessels in the fellow eye. Venules affected by the occlusion were either the occluded venules or downstream venules, which collected blood from the occluded venule and another non-occluded venule (two separate analyses performed, see results). Affected arterioles were chosen as the arterioles that supplied the affected area to the greatest degree. The measured vessels, not affected by the occlusion (categories 2 and 3), were chosen so that they were comparable in location to the affected vessels in the same patient. For example, if a major superotemporal venule was occluded, a major inferotemporal venule was chosen for comparison in the same eye and a major temporal venule (preferably superotemporal) in the fellow eye. As in measurements of patients with central retinal vein occlusion (see chapter 3.6.1), care was taken to avoid measurements close to haemorrhages.

All measurements were made before treatment of the affected eye. Measurements could be made in all categories of arterioles in 18 patients and in all categories of venules in 22 patients. This includes venules, which were measured downstream of the occlusion and received contribution of from non-occluded venules. Furthermore, some of the oximetry images were of poor quality. A subgroup analysis was made for patients where the occluded venule could be measured and the image quality was better (n=7).

The mean age of the patients at the time of measurement was 70 years for arteriolar measurements (n=18, 9 males and 9 females) and 67 years for venular measurements (n=22, 12 males, 10 females). The age range was 44–86 years for both arteriolar and venular measurements. The mean duration of the occlusion at the time of measurement was 3 months, range 0–9 months (arteriolar and venular measurements, n=18 for arterioles, n=22 for venules).

3.6.3 Central retinal artery occlusions

Four patients with a history of unilateral central retinal artery occlusion were measured with the retinal oximeter. A description of the patients can be found in Table 10 in chapter 4.2.3 (results section). The patients were invited to participate when visiting their ophthalmologist (convenience sample). Images were taken after dilation of the subjects' pupil with tropicamide HCl (Mydriacyl; Alcon Inc., Fort Worth, Texas, USA). Tropicamide was in some cases supplemented with 10% phenylephrine hydrochloride (AK-Dilate; Akorn Inc, Lake Forest, Illinois, USA) as deemed necessary by the nurses or ophthalmologists in the clinic.

Oxygen saturation was measured in all main retinal arterioles and venules in an attempt to measure (almost) all of the blood entering and leaving the retina. An average was taken for all main arterioles and, separately, for all main venules in each eye. The vessels were measured from just outside the optic disc and up to or nearly up to their branching.

3.7 Diabetic retinopathy

Thirty one healthy volunteers and 28 patients with diabetic retinopathy of various stages participated in the study. Table 1 describes the groups compared in the study.

Images were taken after dilation of the subjects' pupil with tropicamide HCl (Mydriacyl; Alcon Inc., Fort Worth, Texas, USA), which was in some cases supplemented with 10% phenylephrine hydrochloride (AK-Dilate; Akorn Inc, Lake Forest, Illinois, USA) as deemed necessary by the nurses or ophthalmologists in the clinic.

Oxygen saturation was measured in one major temporal (first or second degree) retinal arteriole and venule in one eye in each subject. The measured vessel segment ranged from just outside the optic disc and up to or nearly up to the first major branching of the vessel.

Table 1. Clinical and demographic data for the groups studied. DR is diabetic retinopathy.

Healthy volunteers, n=31	
Age	32±15 years (mean±SD)
Gender	19 males, 12 females
Background DR, no macular oedema, n=6	
Age	57±16 years (mean±SD)
Gender	3 males, 3 females
Number with type of diabetes	2 type I, 4 type II
Duration with diabetes	17±11 years (mean±SD)
Diabetic macular oedema, no treatment, n=7	
Age	60±15 years (mean±SD)
Gender	5 males, 2 females
Number with type of diabetes	5 type I, 2 type II
Duration with diabetes	19±9 years (mean±SD)
Pre-proliferative /proliferative DR, no treatment, n=7	
Age	42±14 years (mean±SD)
Gender	6 males, 1 female
Number with type of diabetes	6 type I, 1 type II
Duration with diabetes	20±5 years (mean±SD)
Proliferative DR, stable after treatment, n=8	
Age	44±17 years (mean±SD)
Gender	6 males, 2 females
Number with type of diabetes	5 type I, 3 type II
Duration with diabetes	21±5 years (mean±SD)

3.8 Glaucoma

Two studies were performed on the effect of glaucoma treatment; one compared oxygen saturation before and after glaucoma surgery and the other was on the effect of the glaucoma drug dorzolamide.

3.8.1 Glaucoma surgery

All consecutive patients with open-angle glaucoma, with and without pseudo-exfoliation syndrome, undergoing glaucoma surgery in Iceland in a six month period were invited to participate in the study. All patients were using topical glaucoma drugs before their surgery, and one was also taking oral acetazolamide (Table 2).

None of the patients used glaucoma drugs at the time of postoperative oximetry. Twenty-five patients were measured before and after surgery. Six patients were excluded from analysis because of poor optical quality of the eye. Of the remaining 19 patients, 12 had primary open-angle glaucoma and seven had exfoliative glaucoma. Fourteen patients underwent trabeculectomy with mitomycin C and five patients underwent glaucoma drainage device surgery with the Ahmed tube. All surgeries were performed by the same surgeon.

Images were taken after dilation of the subjects' pupil with tropicamide HCl (Mydriacyl; Alcon Inc., Fort Worth, Texas, USA), which was in some cases supplemented with 10% phenylephrine hydrochloride (AK-Dilate; Akorn Inc, Lake Forest, Illinois, USA) as deemed necessary by the nurses or ophthalmologists in the clinic.

Oximetry was performed in first and second degree retinal arterioles and venules. Oximetry was performed before glaucoma surgery and again approximately one month after surgery. An average was taken of measurable arterioles and venules in each eye and the same vessels segments were averaged before and after surgery.

3.8.2 Dorzolamide

The study compared oxygen saturation in retinal vessels in patients with chronic glaucoma or ocular hypertension while they were taking either timolol eye drops or drops containing a mixture of timolol and dorzolamide. The difference between the study periods was therefore the addition of dorzolamide to timolol. Dorzolamide could not have been administered alone and compared to placebo drops since at least some of the participants (the glaucoma patients) required treatment.

Table 2. Clinical data for the 19 patients (eyes), who are included in the results of study on glaucoma surgery.

Age	73±7 years (mean±SD)
Gender	12 males, 7 females
Number with pseudo-exfoliation	7
Trabeculectomies, number of eyes	14
Shunt surgery (Ahmed tube), number of eyes	5
Topical medication before glaucoma filtering surgery, no. eyes	
Timolol+Dorzolamide+Latanoprost	7
Latanoprost	3
Betaxolol+Latanoprost	2
Timolol gel+Latanoprost	2
Timolol+Pilocarpine+Latanoprost	1
Timolol+Brimonidine+Latanoprost	1
Pilocarpine+Propine+Travoprost	1
Brimonidine+Latanoprost+Acetazolamide	1
Timolol gel	1

In total, 20 subjects were recruited for the study. The subjects were classified by an ophthalmologist as having open angle glaucoma (11 subjects), or ocular hypertension without a confirmed diagnosis of glaucoma (nine subjects) based on optic nerve head appearance, visual fields and intraocular pressure. The exclusion criteria were allergies or suspicion of other vulnerabilities to any aspect of the study, best corrected vision less than 20/40, history of ocular or orbital trauma and a history of any respiratory disease (such as asthma or emphysema). One subject with ocular hypertension discontinued participation before being randomised because of a planned pregnancy.

The study timeline or setup is shown in Figure 7.

The study was a prospective, randomised, double-blind, 2x2 cross over study. For the first four weeks, all subjects received 0.5% timolol maleate eye drops (Merck, Whitehouse Station, New Jersey). After baseline measurements, the subjects were randomised into two groups. One group (nine subjects)

continued with the same timolol drops and the other group (10 subjects) received a combination of 0.5% timolol maleate and 2% dorzolamide (Merck, Whitehouse Station, New Jersey). Neither the patients, nor the investigators knew who received which drops. Complete blinding was, however not possible, since dorzolamide can cause some ocular discomfort immediately after instillation.

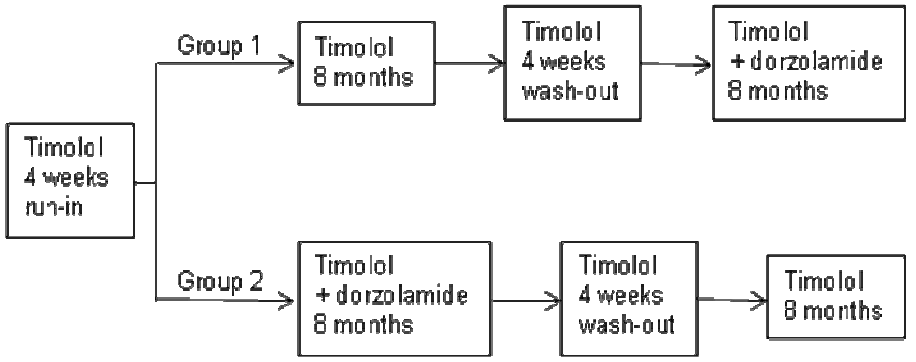

Figure 7. The timeline of the study of the effect of dorzolamide on retinal vessel oxygen saturation. Both groups received timolol or timolol and dorzolamide in combination. Oximetry measurements were made at the end of run-in and washout periods as well as at every two months of the 8 months study periods.

After eight months from the baseline measurements, all subjects received timolol only for four weeks as a wash-out for the second eight months study period, in which the patients received the alternative drops with respect to the first study period. Measurements were made at the baseline, at the end of the washout period and every two months during the study periods.

Six subjects out of nineteen (two with glaucoma, four with ocular hypertension) discontinued participation before having at least one measurement from both test periods. The most common causes for discontinuation were inadequate control of intraocular pressure or irritation from eye-drops. Thirteen subjects (nine with glaucoma, four with ocular hypertension) were able to follow the study protocol until at least two measurements had been performed during the second test period. The age of these subjects was 63±11 years (mean±SD, range 49-81 years, 10 males, 3 females). Ten subjects (seven with glaucoma, three with ocular hypertension) completed all four measurements in both test periods.

Images were taken after dilation of the subjects' pupil with tropicamide HCl (Mydriacyl; Alcon Inc., Fort Worth, Texas, USA).

Oximetry measurements were performed in first-and second degree retinal vessels in both eyes, and an average was taken for each patient. Blood pressure and heart rate were measured using an automated sphygmomanometer (Omron HEM705CP, Omron, Kyoto, Japan). The mean arterial pressure was calculated from systolic and diastolic pressure values, with

$$Mean\ arterial\ \ pressure = \frac{2}{3}\ Diastolic\ \ pressure + \frac{1}{3}\ Systolic\ \ pressure$$

(Equation 8)

Intraocular pressure measurements were performed as part of a routine ophthalmic examination at two eye clinics in Reykjavik, with Nidek NT-2000 (Nidek, Gamagori, Japan) and Reichert AT555 (Reichert, Depew, New York) tonometers. As described in chapter 1.1.5.1, the pressure in the retinal venules is close to the intraocular pressure. Ocular perfusion pressure values were therefore calculated from mean arterial pressure and intraocular pressure as

$$Ocular\ \ perfusion\ \ pressure = \frac{2}{3}\ Mean\ brachial\ \ artery\ \ pressure - Intraocular\ \ pressure$$

(Equation 9)

Two thirds of the mean brachial artery pressure is used as the standard approximation of ophthalmic artery pressure.

4 Results

4.1 Light and dark

Two experiments were performed to study the effects of light and dark on retinal vessel oxygen saturation. The first experiment involved comparison of retinal vessel oxygen saturation in healthy volunteers in darkness and about 80 cd/m^2 constant light. The second experiment compared saturation in darkness and 1, 10 or 100 cd/m^2 light.

4.1.1 Darkness vs. 80 cd/m2

Table 3 and Figure 8 show retinal vessel oxygen saturation in darkness and about 80 cd/m^2 light. Measurements were made after 30 minutes in dark and then after successive 5 minute periods in light or dark. Statistical comparison is given in Figure 8 (paired t-tests). The arteriovenous difference did not change between light or dark ($p>0.17$, consecutive comparisons of measurements in the same manner as in Figure 8).

Table 3. Retinal vessel oxygen saturation in darkness and light (80 cd/m^2) in 15 healthy volunteers. Mean±SD and 95% confidence intervals.[a]

	30 min. Dark	**35 min. Light**	**40 min. Dark**	**45 min. Light**	**50 min. Dark**	**55 min. Light**
Arterioles	92±4 90-94	89±5 86-92	92±4 90-94	89±4 87-91	91±5 88-93	88±5 85-91
Venules	60±5 58-63	55±10 49-60	59±7 56-63	55±5 52-58	57±7 54-61	54±9 49-59
Arteriovenous difference	32±6 29-35	34±9 29-39	33±6 29-36	34±6 30-37	33±7 30-37	34±6 31-38

[a]The time given is from the start of the first dark adaptation. After the initial 30 minutes in the dark, each light or dark period lasted 5 minutes.

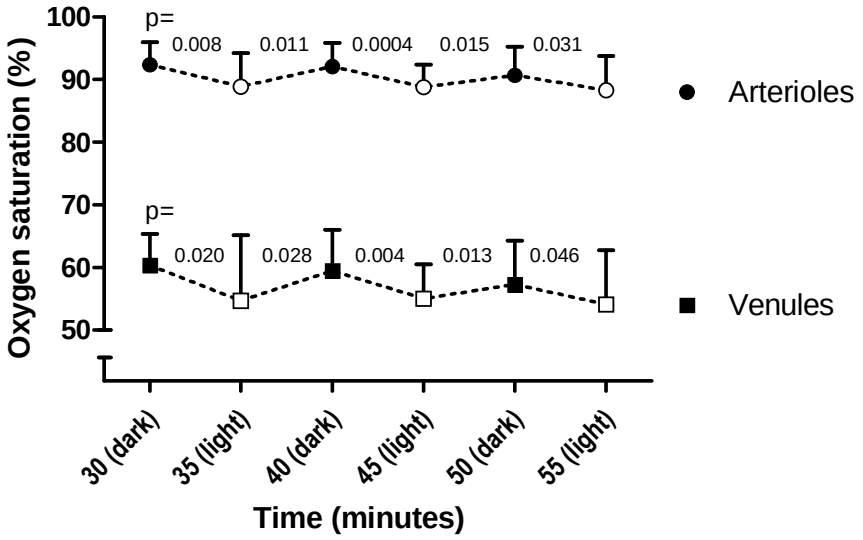

Figure 8. Retinal vessel oxygen saturation in darkness and 80 cd/m^2 light in 15 healthy volunteers. The time given is from the start of the first dark adaptation. The points denote the mean and the bars denote the standard deviation. The p-values are from paired t-tests, where each point in time is compared to the next. Reprinted from paper I (Invest Ophthalmol Vis Sci, 50(5), 2308-2311, ©Association for Research in Vision and Ophthalmology).

Additional measurements of diameter and brightness values were performed with a later version of the analysis software. Table 4 shows the diameter of the same vessel segments as in Table 3 and Figure 8. Figures 9 A-D show the ratios of brightness values (see chapter 1.2.4 for description of I and I_0). The brightness values were extracted to allow examination of possible artefacts in the saturation results (see chapter 5.6.2 for discussion).

Table 4. Retinal vessel diameter in darkness and light (80 cd/m^2) in 15 healthy volunteers

	Time[a] (light/dark)	Vessel diameter (pixels, mean±SD)	Compared to value in the row above[b]
Arterioles	30 min. (dark)	5.3±0.4	
	35 min. (light)	5.2±0.8	p=0.22
	40 min. (dark)	5.4±0.5	p=0.17
	45 min. (light)	5.2±0.6	p=0.07
	50 min. (dark)	5.3±0.6	p=0.35
	55 min. (light)	5.3±0.6	p=0.88
Venules	30 min. (dark)	6.8±0.7	
	35 min. (light)	6.7±0.7	0.26
	40 min. (dark)	6.8±0.7	0.44
	45 min. (light)	6.5±0.6	0.02
	50 min. (dark)	6.8±0.5	0.003
	55 min. (light)	6.8±0.8	0.84

[a] The time given is from the start of the first dark adaptation. After the initial 30 minutes in the dark, each light or dark period lasted 5 minutes

[b] Paired t-test for comparison to the previous value.

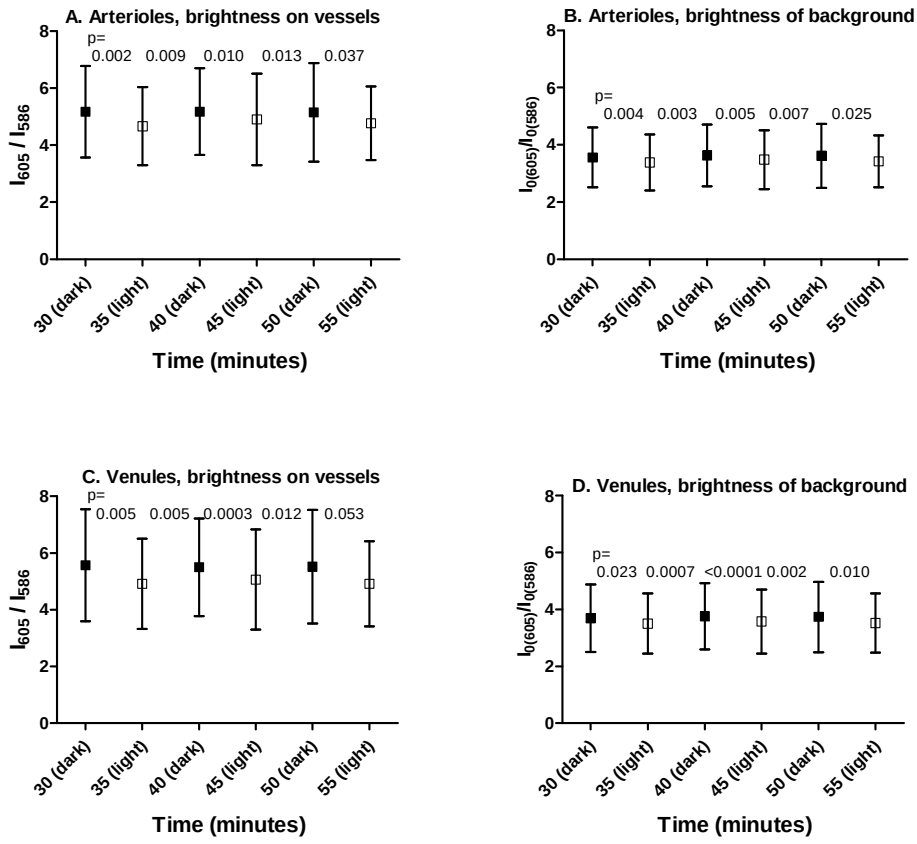

Figure 9. Ratios of brightness values. I is the brightness value, measured on the vessel, and I_0 is the brightness value to the side of the vessel. The subscripts denote whether the brightness was measured at 586 nm or 605 nm. The ratios were calculated to allow analysis of possible artefacts due to bleaching of photopigment during light adaptation (see chapter 5.6.2). The p-values are from paired t-tests, where each point in time is compared to the next.

4.1.2 Darkness vs. 1-100 cd/m2

Table 5 and Figure 10 show retinal vessel oxygen saturation in darkness and successively stronger light. Statistical comparison is given in Figure 10 (paired t-tests). The arteriovenous difference did not change between light or dark, $p>0.63$ for comparisons as made in the same manner as in Figure 10.

Table 5. Retinal vessel oxygen saturation in darkness and three levels of light in 19 healthy volunteers[a]. The table shows mean±standard deviation and 95% confidence intervals. Statistical comparison is given in Figure 10.

Light level	0 cd/m^2	1 cd/m^2	10 cd/m^2	100 cd/m^2	0 cd/m^2
Time (min.)	**30**	**35**	**40**	**45**	**50**
Arterioles	92±4 89-94	91±6 89-94	91±5 89-93	88±7 85-92	91±6 88-94
Venules	59±9 55-64	59±10 55-64	58±7 55-62	55±10 51-60	58±10 53-63
Arteriovenous difference	32±9 28-36	32±8 28-36	33±6 30-36	33±8 29-37	33±7 29-36

[a]The first period of darkness lasted 30 minutes but all successive periods of light and darkness lasted 5 minutes.

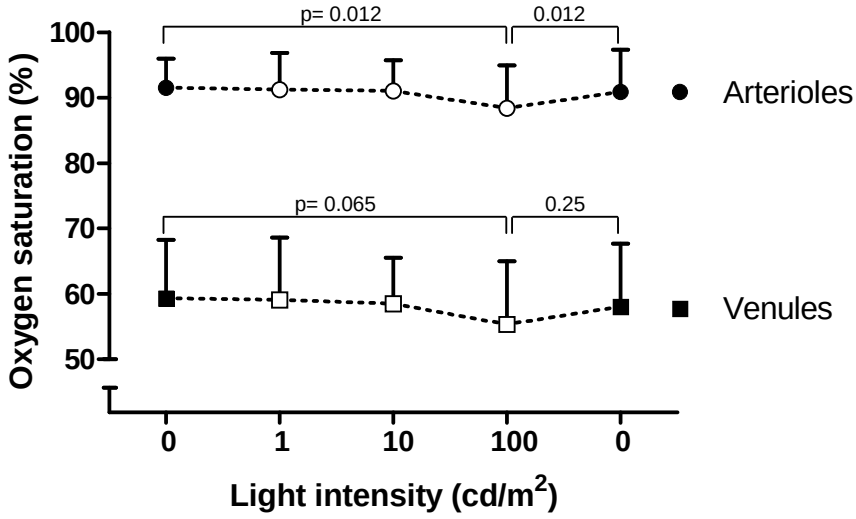

Figure 10. Retinal vessel oxygen saturation in darkness and three light levels in 19 healthy volunteers. The points denote the mean and the bars denote the standard deviation. The p-values are from paired t-tests. Reprinted from paper I (Invest Ophthalmol Vis Sci, 50(5), 2308-2311, ©Association for Research in Vision and Ophthalmology).

Table 6 shows the diameter of the same vessel segments as in Table 5 and Figure 10.

Table 6. Retinal vessel diameter (pixels) in darkness and 1, 10 and 100 cd/m^2 light in 19 healthy volunteers. The table shows mean±SD[a].

	0 cd/m^2 (30 min.)[b]	**1 cd/m^2 (35 min.)**	**10 cd/m^2 (40 min.)**	**100 cd/m^2 (45 min.)**	**0 cd/m^2 (50 min.)**
Arterioles	4.6±0.8	4.6±0.8	4.6±0.9	4.5±0.7	4.5±0.8
Venules	6.0±0.6	6.0±0.5	6.1±0.6	6.2±0.5	5.6±0.6

[a]None of the differences in vessel diameters were statistically significant. The p-values for the difference between 0 cd/m^2 (30 min.) vs. 100 cd/m^2 (45 min.) were 0.65 for arterioles and 0.057 for venules (paired t-test). The p-values for 100 cd/m^2 (45min.) vs. 0 cd/m^2 (50min.) were 0.95 for arterioles and 0.12 for venules.
[b]The first period of darkness lasted 30 minutes but all successive periods of light and darkness lasted 5 minutes.

4.2 Retinal vascular occlusions

Three studies were performed on retinal vascular occlusions; on occlusions of the central retinal vein, branch retinal veins and the central retinal artery.

4.2.1 Central retinal vein occlusions

Table 7 gives age, gender, duration of occlusion and additional information is for each patient with central retinal vein occlusion.

Table 7. Clinical characteristics of patients with central retinal vein occlusion.

Patient no.	**Age (gender)**	**Duration of vein occlusion**	**Comment**
1	72 (M)	1 day	Fellow eye amblyopic
2	81 (M)	1-2 days	
3	62 (F)	2 days	
4	60 (F)	About 1 month	
5	45 (M)	About 3 months	
6	49 (M)	About 3 months	
7	61 (M)	About 3 months	Acute glaucoma 2 months earlier.
8	57 (M)	About 6 months	Affected eye amblyopic.
Mean±SD	61±12		

[a]F is for female and M is for male.

Table 8. Shows that the mean saturation in retinal venules is 49±12% in the eye affected by central retinal vein occlusion and 65±6% in the fellow eye (p=0.003, n=8, paired t-test). The mean saturation in the retinal arterioles was 99±3% in the affected eye and 99±6% in the fellow eye.

Table 8. Retinal vessel oxygen saturation (%) in eight patients with central retinal vein occlusion. The table shows mean±SD and number of measured vessels in each eye (in parenthesis).

Patient no.	Duration of occlusion	Affected eye		Fellow eye	
		Arterioles	Venules	Arterioles	Venules
1	1 day	95±4 (2)	53±2 (3)	94±3 (2)	59±1 (3)
2	1-2 days	99±4 (4)	50±12 (4)	96±3 (3)	62±7 (3)
3	2 days	102±1 (2)	30±25 (5)	103±10 (2)	67±11 (4)
4	About 1 month	101% (1)	54±17 (3)	92 (1)	68±3 (3)
5	About 3 months	96±0.5 (3)	39±10 (5)	96±0.6 (3)	60±9 (5)
6	About 3 months	100±5 (2)	72±7 (5)	98±3 (2)	76±5 (5)
7	About 3 months	102±2 (2)	50±10 (6)	108±2 (2)	64±7 (4)
8	About 6 months	103 (1)	47±13 (4)	105 (1)	66±4 (4)
Mean±SD (n=8)		99±3	49±12[a]	99±6	65±6a

[a] Affected venules have significantly lower saturation than venules in the fellow eye (paired t-test, p=0.003).

Figure 11 shows a plot of the oxygen saturation in retinal vessels of patients with central retinal vein occlusion (data also in Table 8). Figures 12 and 13 show examples of oximetry images from patients with central retinal vein occlusion.

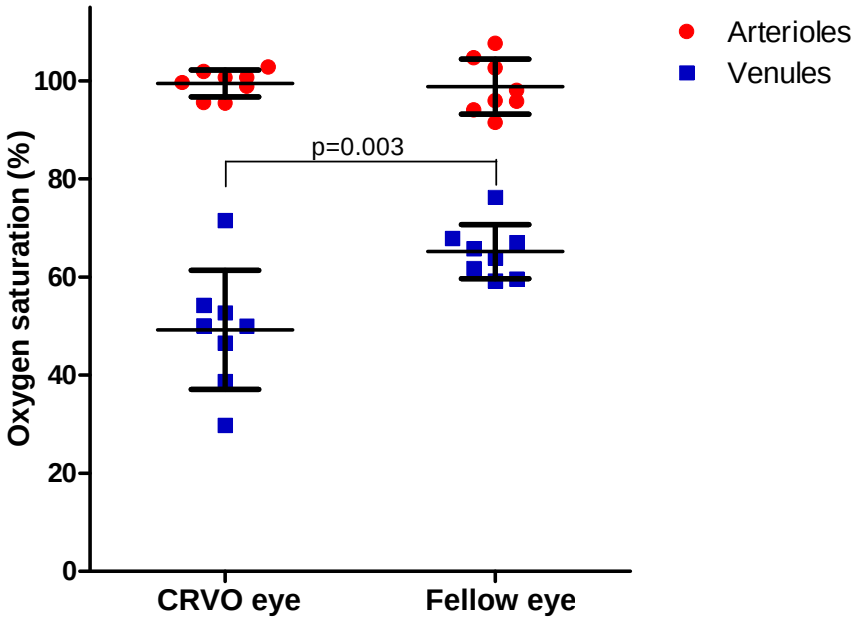

Figure 11. Retinal vessel oxygen saturation in patients with central retinal vein occlusion (CRVO). Each point denotes the mean saturation within an eye. The bars denote mean±SD for all 8 patients. The p-value is from a paired t-test. Reproduced from paper IV (Am J Ophthalmol, 150(6), 871-875, ©Elsevier Inc.)

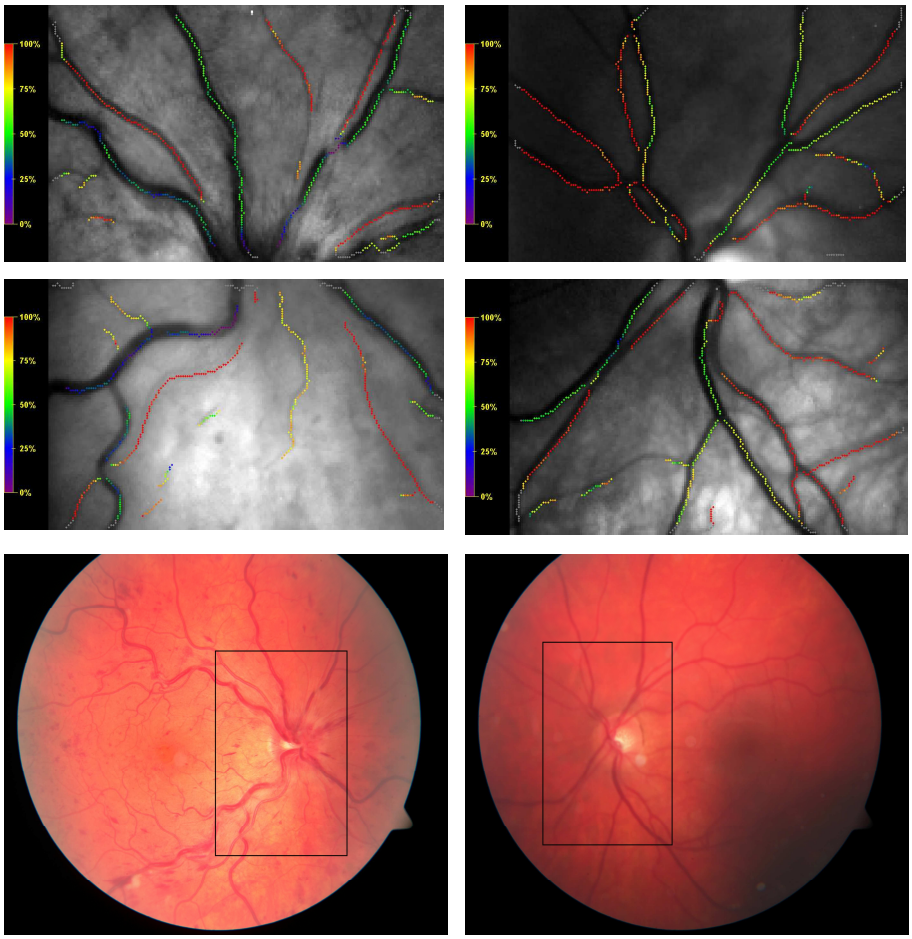

Figure 12. Oxygen saturation map of patient no. 5 in Table 8. The eye affected by central retinal vein occlusion is displayed on the left and the fellow (healthy) eye is on the right. The frames on the colour fundus photographs indicate the retinal area on the oxygen saturation maps above. Reprinted from paper IV (Am J Ophthalmol, 150(6), 871-875, ©Elsevier Inc.)

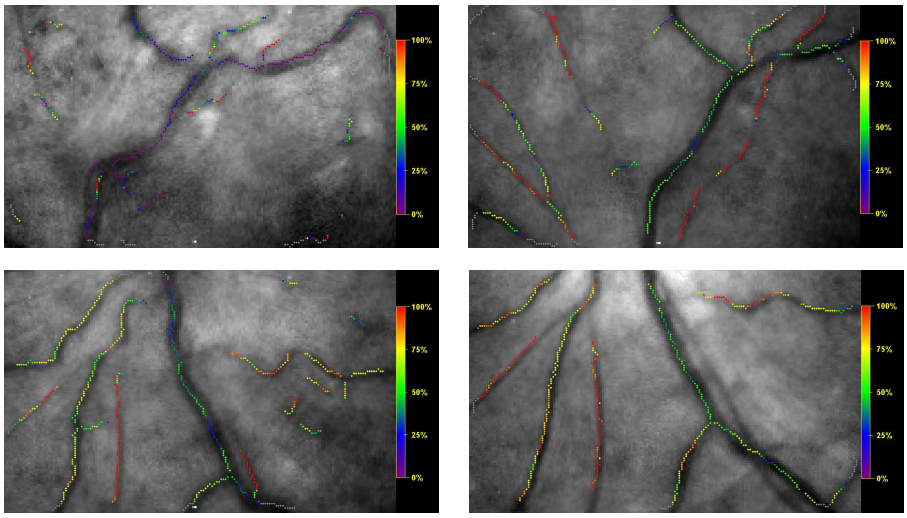

Figure 13. Oxygen saturation map of patient no. 8 in Table 8. The images on the left are of the affected eye before treatment. The images on the right are taken of the same eye three months later after treatment with laser, bevacizumab and triamcinolone

4.2.2 Branch retinal vein occlusions

Table 9 shows the results of saturation measurements for all measureable vessels. Some of the measured affected venular segments received blood also from non-occluded branches, i.e. blood was measured downstream of joining of the occluded venule and a non-occluded venule. The image quality was poor in some cases. A separate analysis was performed of a subgroup where occluded venules could be reliably measured in adequate quality images. The results for both analyses are displayed in Table 9. Figure 14 shows individual values from the subgroup with more reliable measurements of occluded venules (the subgroup with n=7 in Table 9).

Table 9. Retinal vessel oxygen saturation (%) in patients with branch retinal vein occlusion (median and range)

	Affected eye		Fellow eye	Friedman's test for differences in saturation
	Affected vessel	**Unaffected vessel**		
Arterioles (n=18)	101 89-115	95 85-104	98 84-109	p=0.024[a]
Venules (n=22)[b]	59 12-93	63 23-80	55 39-80	p=0.66
Occluded venules only, good image quality (n=7)	49 12-93	63 40-75	53 48-72	p=0.96

[a] The difference between affected arterioles and unaffected arterioles in affected eye was significant according to Dunn's post test ($p<0.05$). No other Dunn's tests showed statistical significance at $p<0.05$.

[b] Some of the affected venules received blood also from non-occluded branches and image quality was poor in some cases. A subgroup analysis is shown in the next row in the table.

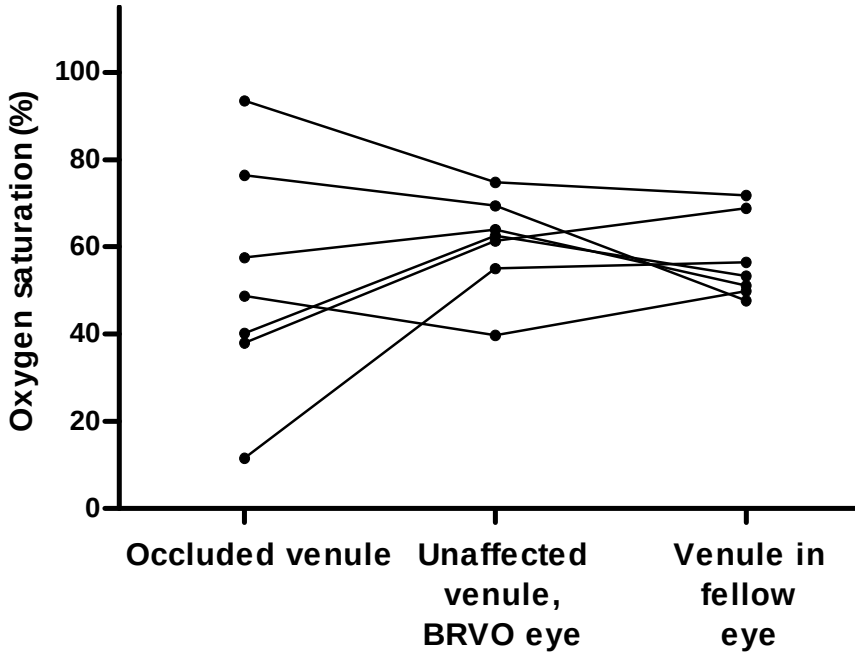

Figure 14. Oxygen saturation in retinal venules in patients with branch retinal vein occlusion. Each point denotes one venule and the lines link points from the same patient. The difference between the three categories is not statistically significant ($p>0.05$, Friedman's and Dunn's tests). Reproduced from paper V (Acta Ophthalmol, 2011, Apr 21, epub ahead of print).

Figures 15 and 16 show examples of oxygen saturation measurements in eyes affected by BRVO.

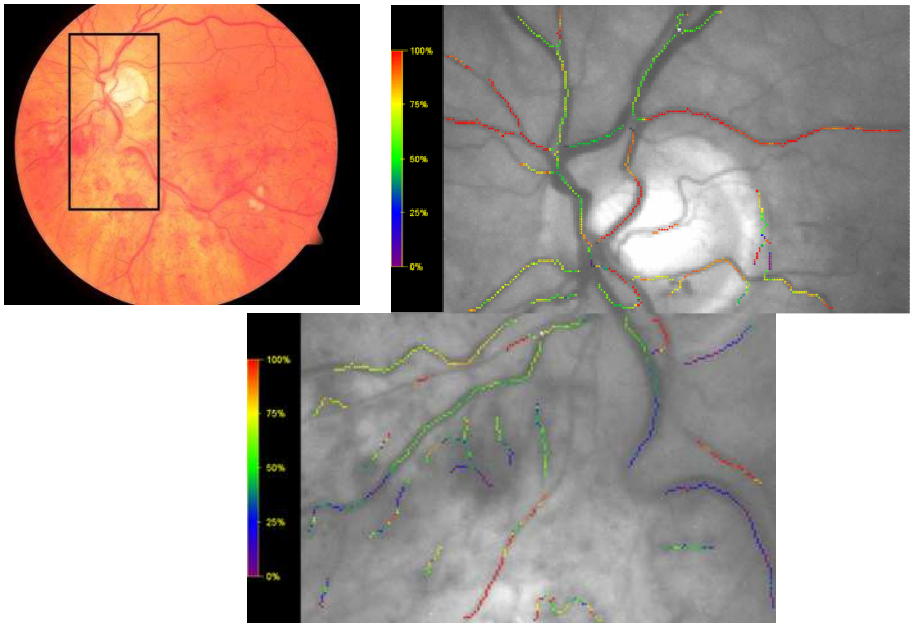

Figure 15. A patient with branch retinal vein occlusion. The frame in the colour fundus image shows the approximate location of the oximetry images. The colours in the oximetry images denote the relative oxygen saturation in the retinal vessels. Reproduced from paper V (Acta Ophthalmol, Apr 21, epub ahead of print).

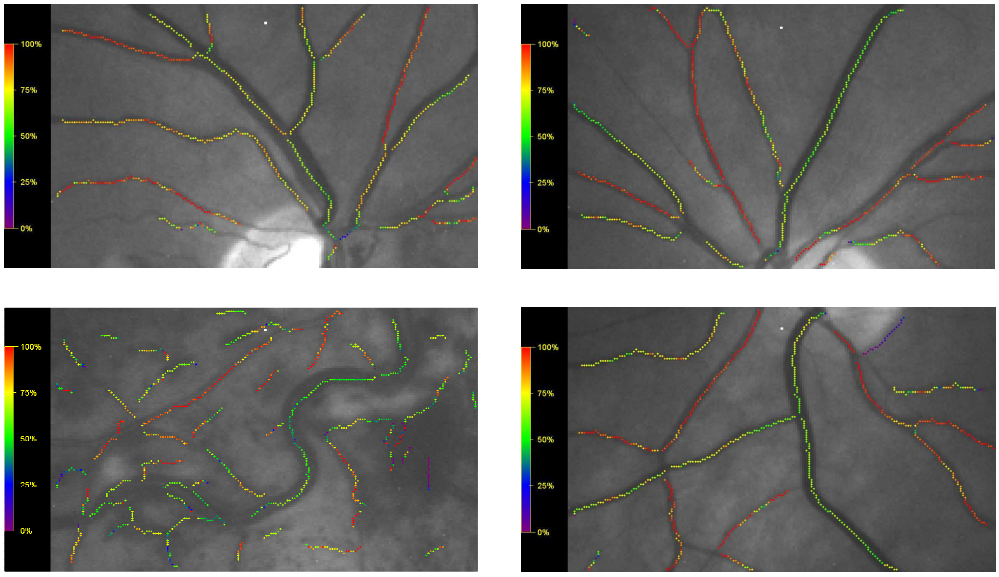

Figure 16. A patient with branch retinal vein occlusion. The images to the left show the affected eye. The occluded and tortuous venule is shown in the lower left image. The images to the right are of the healthy fellow eye.

4.2.3 Central retinal artery occlusions

Four patients with clinically diagnosed central retinal artery occlusion were measured. The patients varied in their clinical characteristics as can be seen in Table 10. Table 11 shows oxygen saturation in retinal vessels in the patients described in Table 10.

Table 10. Clinical characteristics of patients with central artery occlusion

Patient no.	Age (gender)	Duration of occlusion	Visual acuity (affected eye)	Comment
1 (before treatment)	69 (F)	1 day	Light perception	Giant cell arteritis. Very little blood flow (segmented blood column)
1 (after treatment)	69 (F)	1 month	Hand movement	Treated with prednisolone, blood column not segmented anymore
2	79 (M)	1 day	Hand movement	Initially diagnosed as inferotemporal branch retinal artery occlusion. Diagnosis later changed to central retinal artery occlusion.
3	69 (M)	1 month	Finger counting	Re-established blood flow?
4	75 (M)	2 weeks	Finger counting	Two to three months later the visual acuity was 1.0 for the affected eye.

Table 11. Retinal vessel oxygen saturation (%) in four patients with a history of central retinal artery occlusion. The table shows mean±SD for the major retinal vessels in each eye.

Patient no.	Duration of occlusion	Affected eye		Unaffected eye	
		Arterioles	Venules	Arterioles	Venules
1	1 day	71±9	63±9	95±5	66±8
1[a]	1 month	100±4	54±5	100±4	60±6
2	1 day	82±7	34±12	85±3	49±17
3	1 month	101±4	64±8	99±6	60±11
4[b]	2 weeks	93	49±6	97±7	51±5

[a] One patient was measured before and after treatment. More details on the patients can be found in Table 10.

[b] For patient four, only one affected arteriole was measureable

Figure 17 shows the fundus of the patient with giant cell arteritis, the day after vision loss occurred. Interruption of the blood flow can clearly be seen (segmented blood column) as well as low measured saturation in retinal arterioles.

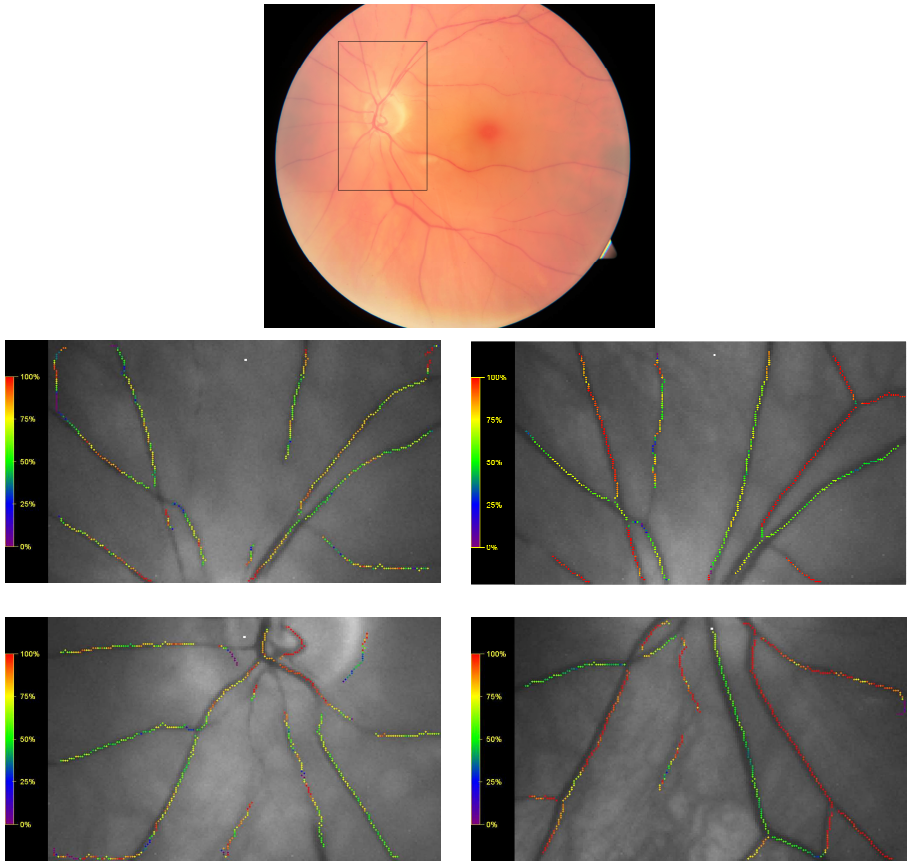

Figure 17. A patient with central retinal artery occlusion due to giant cell arteritis. Above: The fundus image taken one day after occlusion shows "box-caring", i.e. segmentation of the blood column in several vessels, indicating little or no blood flow. The black box in the image indicates the approximate area from which the oximetry measurements were taken. Below to the left: A pseudo-colour map of relative oxygen saturation one day after occlusion. Below to the right: The pseudo-colour map of oxygen saturation one month after occlusion and treatment with prednisolone. Blood flow had improved (no "box-caring"). Reproduced from paper VII (Acta Ophthalmol, accepted for publication).

4.3 Diabetic retinopathy

Retinal vessel oxygen saturation was measured in healthy volunteers and patients with various categories of diabetic retinopathy. Table 1 (chapter 3.7) gives a description of the groups studied. Oxygen saturation was measured in one major temporal arteriole and venule in one eye in each subject.

The saturation in healthy volunteers was 93±4% in arterioles and 58±6% in venules (mean±SD, n=31). In the diabetic patients (whole group, n=20) the oxygen saturation was significantly higher; 101±5% in arterioles and 68±7% in venules. The difference between healthy volunteers and diabetic patients was statistically significant ($p<0.001$ for arterioles and venules, unpaired t-test). The arteriovenous difference was not different between healthy volunteers and diabetic patients ($p=0.53$).

The results for subgroups are shown in Figure 18 and Table 12.

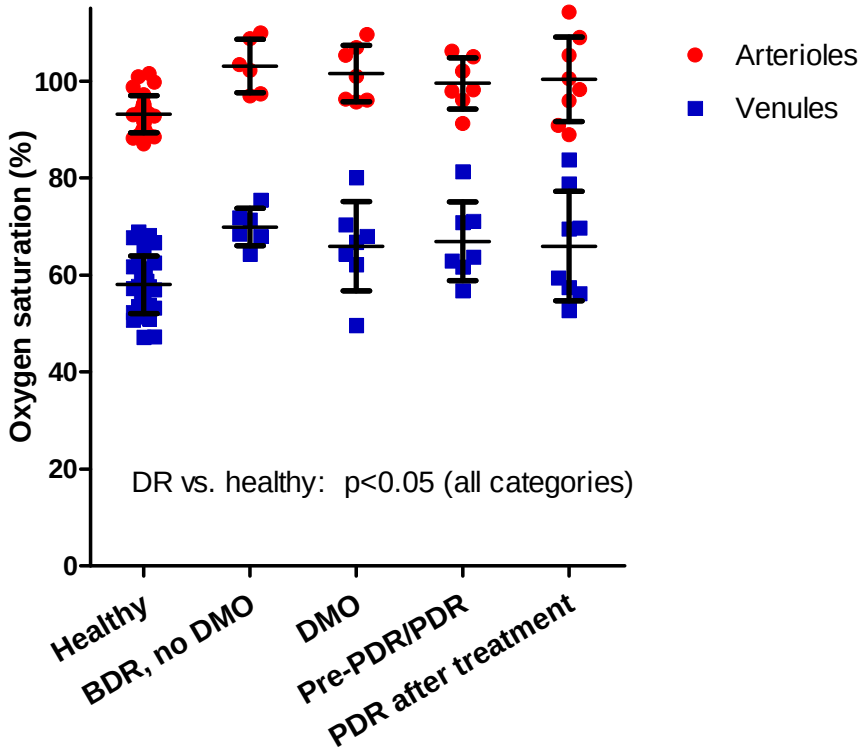

Figure 18. Oxygen saturation in retinal vessels in healthy subjects and in patients with various stages of diabetic retinopathy. Each point denotes one major temporal arteriole or venule. Only one arteriole and one venule were measured for each individual (number of individuals can be found in Table 12). The bars denote means and standard deviations. BDR: Background diabetic retinopathy. DMO: Diabetic macular oedema. PDR: Proliferative diabetic retinopathy. One way ANOVA and Dunnett's tests were used for statistical analysis.

Table 12. Retinal vessel oxygen saturation (%) in retinal arterioles and venules. The table shows mean±SD[a] and 95% confidence intervals. DR: Diabetic retinopathy.

	Arterioles	**Venules**
Healthy volunteers, n=31	93±4 92-95	58±6 56-60
Background DR, no macular edema, n=6	103±6 96-114	70±4 66-74
Diabetic macular oedema, no treatment, n=7	102±6 95-107	66±9 57-75
Pre-proliferative / proliferative DR, no treatment, n=7	100±5 92-106	67±8 59-75
Proliferative DR, stable after treatment, n=8	100±9 93-108	66±11 57-75

[a] All subgroups with DR have higher saturation than the healthy group ($p<0.05$, arterioles and venules, one way ANOVA and Dunnett's test).

Figure 19 shows examples of oximetry images from a patient with diabetic retinopathy and from a healthy volunteer.

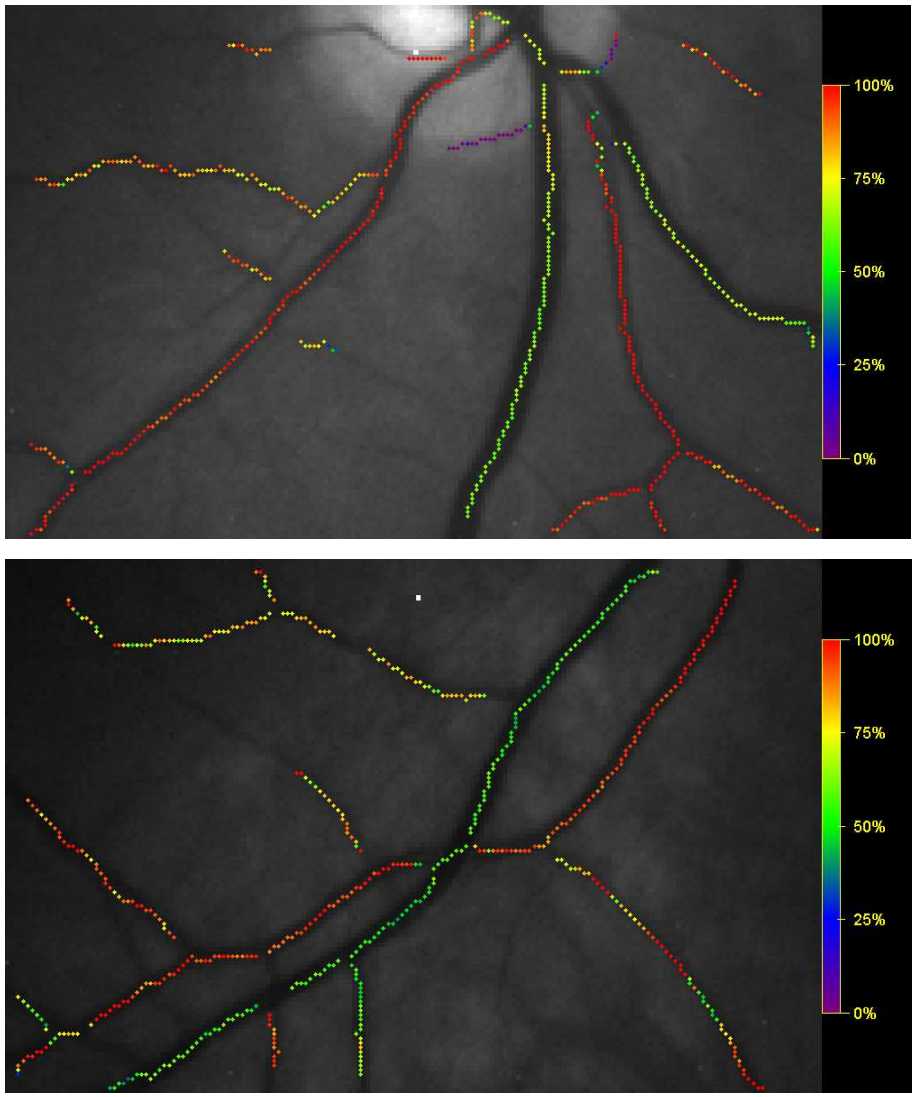

Figure 19. Retinal vessel oxygen saturation in diabetic retinopathy compared to a healthy retina. The colour scale denotes relative oxygen saturation. Above: A patient with proliferative diabetic retinopathy (no treatment) Below: A healthy volunteer. Both images show the vessels just inferior to the optic disc in the right eye.

4.4 Glaucoma treatment

Two studies were performed on the effect of glaucoma treatment on retinal vessel oxygen saturation; one on the effect of glaucoma surgery and the other on the effect of dorzolamide eye drops.

4.4.1 Glaucoma surgery

Oxygen saturation in retinal arterioles in the operated eye increased by 2 percentage points ($p=0.046$, $n=19$, paired t-test) after surgery, which lowered intraocular pressure from 23±7 mmHg (mean±SD) to 10±4 mmHg ($p<0.0001$). No other significant changes in oxygen saturation were found ($p\geq0.35$). Table 13 shows means and standard deviations while Figure 20 shows the individual data points.

Table 13. Retinal vessel oxygen saturation (in %, mean ± SD) in operated and fellow eyes in 19 patients before and after glaucoma filtering surgery.

	Operated eye		**Fellow eye**	
	Before surgery	**After surgery**	**Before surgery**	**After surgery**
Arterioles	97±4[a]	99±6[a]	96±5	96±5
Venules	63±5	64±6	64±6	63±8
Arteriovenous difference	34±6	36±8	32±6	32±8

[a] The increase in arterioles in operated eye was statistically significant, $p=0.046$ (paired t-test).

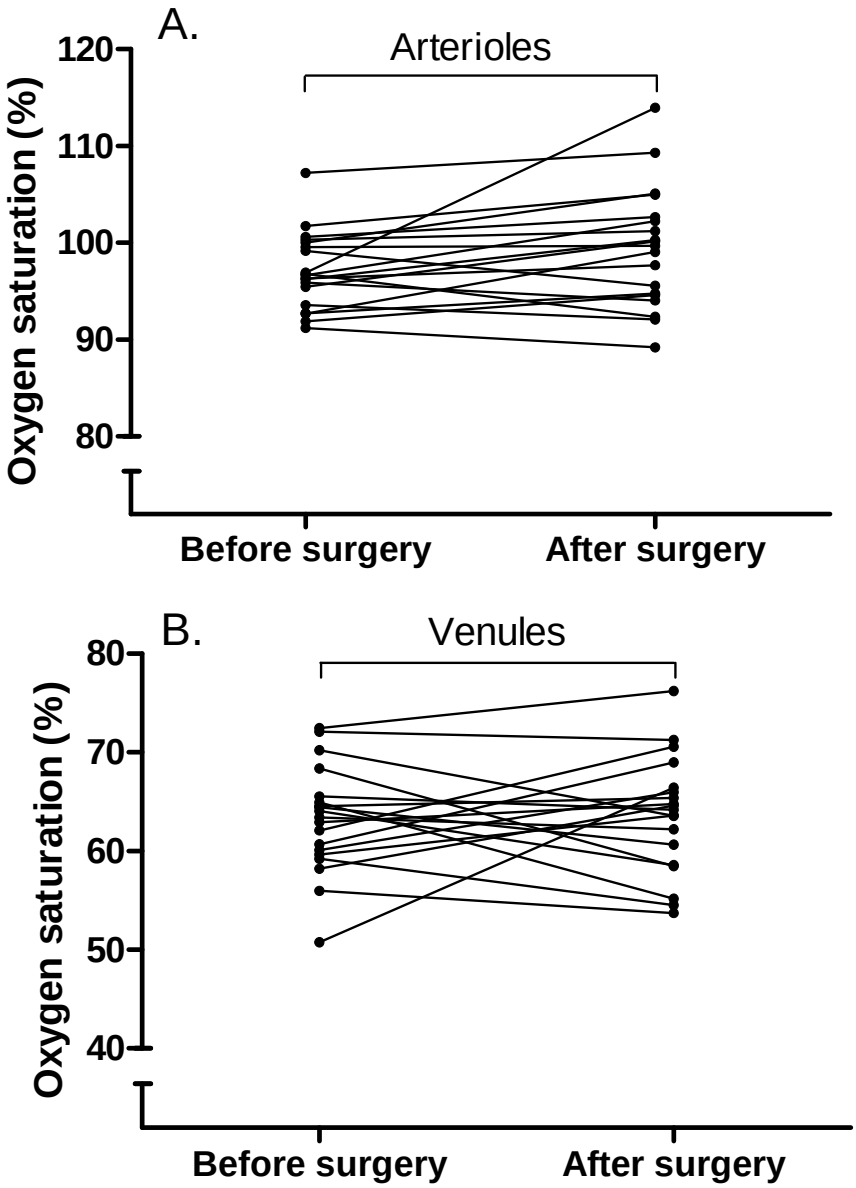

Figure 20. Oxygen saturation in (A) retinal arterioles and (B) venules before and after glaucoma filtering surgery. The pre- and post-operative data points for each eye are connected with a line. Same measurements as in Table 13.

A post-hoc power analysis was performed to estimate the possibility that a real difference in saturation was undetected due to low statistical power. The result of the analysis is that the probability of detecting a difference of 5 percentage points with surgery was 99% for arterioles, 87% for venules, and 70% for arteriovenous difference.

Figure 21 shows an example of oximetry images from the study on glaucoma surgery:

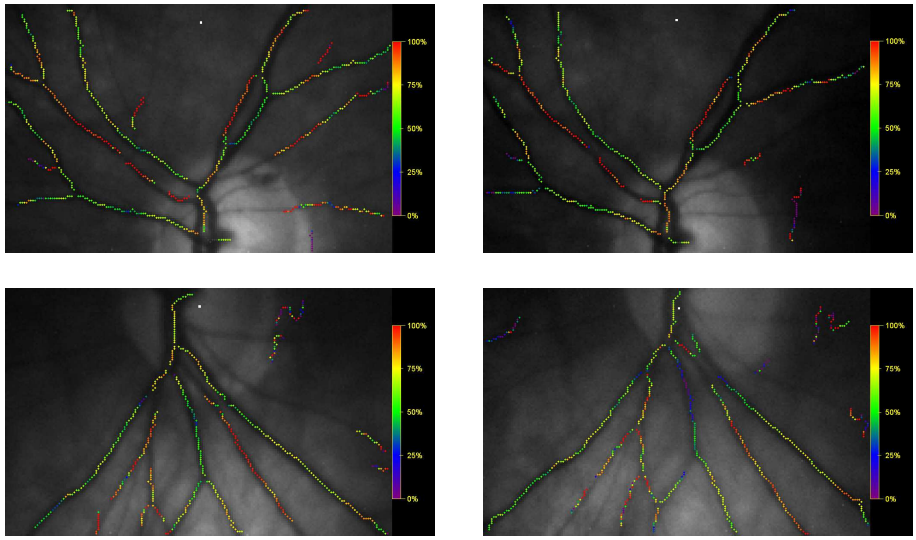

Figure 21. Retinal vessel oxygen saturation before (left) and after (right) glaucoma surgery. The colour scale on the right shows the relative oxygen saturation.

4.4.2 Dorzolamide

Table 14 shows the mean saturation values for each study period, where the subjects took either timolol alone or a combination of dorzolamide and timolol. Out of the 13 subjects in Table 14, three did not complete the whole study protocol. For each subject, an average was taken of all available oximetry measurements within each study period.

Table 14. Retinal vessel oxygen saturation in retinal vessels and other physiological parameters during the two different drug treatments (n=13, mean±SD)

	Dorzolamide-timolol combination	Timolol monotherapy
Retinal arteriolar saturation	97±2%	96%±2%
Retinal venular saturation	66±5%	65%±6%
Arteriovenous difference	31±4%	31±5%
Intraocular pressure[a]	14±2 mmHg	17±3 mmHg
Mean arterial blood pressure	96±11 mmHg	98±9 mmHg
Ocular perfusion pressure	49±6 mmHg	49±6 mmHg
Finger pulse oximetry value	96±1%	96±1%

[a]The intraocular pressure was significantly lower during the combination treatment (p=0.001, paired t-test).

Table 15 shows the oxygen saturation values, categorised by order of drug treatments. Subjects, who started on dorzolamide-timolol combination, showed a significant reduction in arteriolar and venular saturation when changing to timolol monotherapy. No significant changes were noted in subjects, who started on timolol and changed to dorzolamide-timolol combination.

Table 15. Retinal vessel oxygen saturation (%), categorised by order of drug treatments (mean±SD)

	Timolol in period 1 (n = 7)		Dorzolamide-timolol in period 1 (n=6)	
	Timolol monotherapy	**Dorzolamide-timolol combination**	**Dorzolamide-timolol combination**	**Timolol monotherapy**
Arterioles	97±2	96±2	98±2[a]	95±2[a]
Venules	64±7	64±5	69±5[b]	66±6[b]
AV difference	32±5	32±4	29±4	30±5

[a]Significant difference between periods, p< 0.01 (Bonferroni post-test after two-way repeated measures ANOVA).

[b]Significant difference between periods, p< 0.05 (Bonferroni post-test after two-way repeated measures ANOVA).

Ten subjects completed the entire study protocol, i.e. were measured four times during the dorzolamide-timolol period and four times during the timolol period . Figures 22 and 23 show results of their measurements plotted over time.

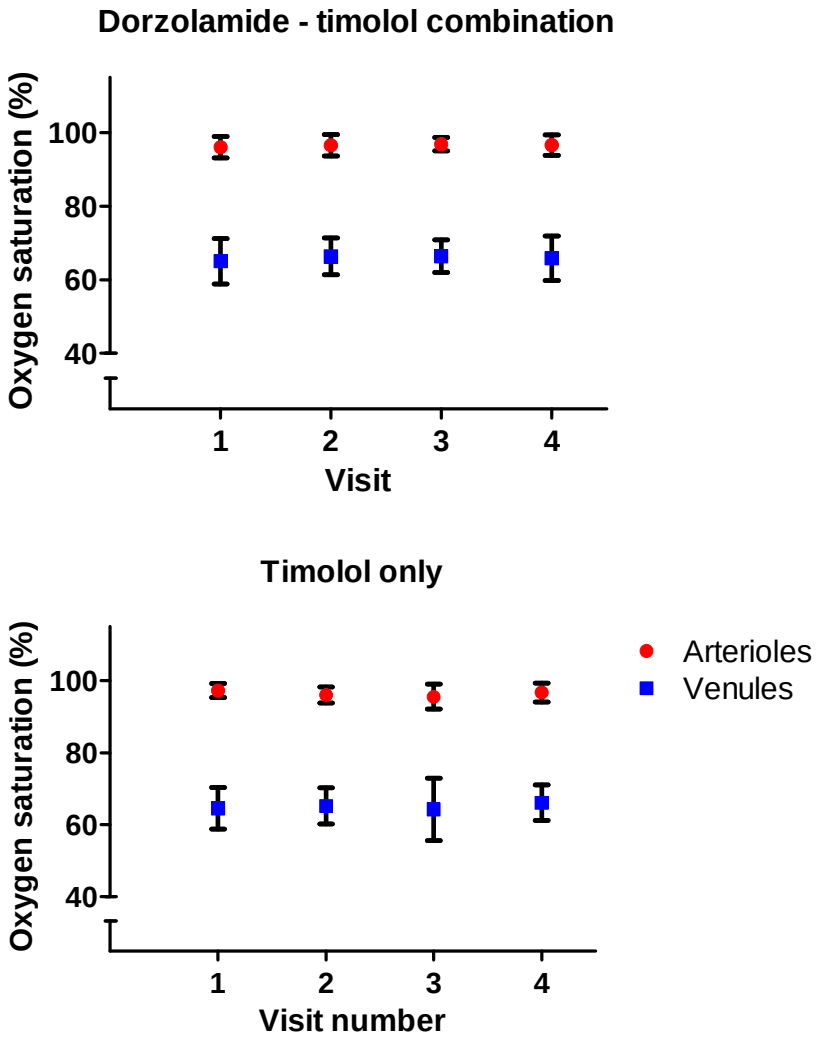

Figure 22. Retinal vessel oxygen saturation at the four study visits in each period of the dorzolamide study. Top: Dorzolamide-timolol period. Bottom: Timolol period. Only the ten subjects, who completed the whole study protocol are included. The graph shows means and standard deviations. Reproduced from paper II (Br J Ophthalmol, 93(8), 1064-1067).

The stability of the oxygen saturation measurements over time can be examined by tracing the lines for each subject in Figure 23. Table 16 shows a numerical measure of the stability, i.e. the standard deviation of oximetry measurements over time.

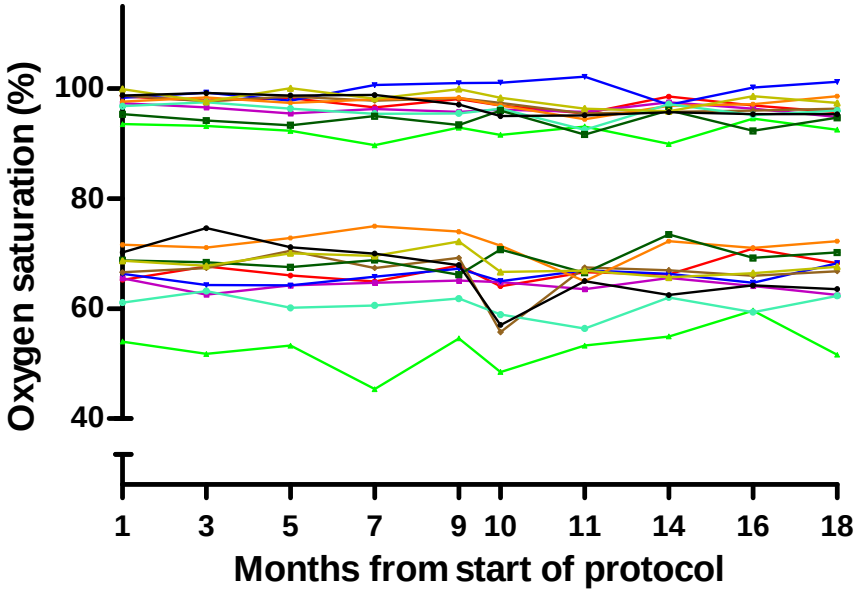

Figure 23. Retinal vessel oxygen saturation at each study visit in the dorzolamide study. Each line shows changes with time in oxygen saturation in arterioles (above) and venules (below) in a single subject. After run-in (one month of timolol) the subjects received either timolol or dorzolamide-timolol combination (see Figure 7). After washout (month 9-10) the subjects changed to the other treatment. Only the ten subjects, who completed the whole study protocol are included. Reproduced from paper II (Br J Ophthalmol, 93(8), 1064-1067).

Table 16. Standard deviation of measurements over time in study of dorzolamide. For each individual, the standard deviation was calculated between 10 measurements, one or two months apart in about 17 months period (see Figure 23 for each individual). The numbers are in percentage of haemoglobin saturation.

	Standard deviation (median, range, n=10 subjects)
Arterioles	1.4 (0.8-1.8)
Venules	2.1 (1.4-5.1)

5 Discussion

The results show that dual wavelength oximetry can be used for studies of normal retinal physiology as well as for studies on retinal vascular occlusions, diabetic retinopathy and glaucoma. The major findings are discussed below.

5.1 Light and dark

The two experiments, performed to study the effect of light and darkness, both support the conclusion that oxygen saturation in the main retinal vessels is higher in dark than in light. Saturation increases in both arterioles and venules and the arteriovenous difference is unchanged. The effect was clearer in the first experiment, where arteriolar and venular saturation in the dark was repeatedly measured higher than in 80 cd/m^2 light. There was no statistically significant difference between dark and the lower light intensities, 1 and 10 cd/m^2, in the second experiment. At 100 cd/m^2 the saturation was lower than in the dark although this was not quite significant in the venules. The saturation was only a few percent higher in dark than in 80 or 100 cd/m^2. This is similar to the standard deviation of repeated measurements with the oximeter (Hardarson et al., 2006). A larger group of volunteers is therefore needed to investigate if 1 and 10 cd/m^2 light causes smaller changes in saturation.

Increased retinal oxygen consumption in the dark was first shown *in vivo* in monkeys (Stefansson, E., 1981). An increase in oxygen saturation in retinal vessels in the dark, seen in the present studies, may at first seem to contradict these results and other studies, which show increased outer (Ahmed et al., 1993; Ames et al., 1992; Birol et al., 2007; Braun and Linsenmeier, 1995; Braun et al., 1995; Cringle et al., 1999; Haugh-Scheidt, Griff et al., 1995; Haugh-Scheidt, Linsenmeier et al., 1995; Haugh et al., 1990; Linsenmeier, 1986; Linsenmeier and Braun, 1992; Linsenmeier and Yancey, 1989; Medrano and Fox, 1995; L. Wang, Kondo et al., 1997; L. Wang, Tornquist et al., 1997a; Zuckerman and Weiter, 1980) and total retinal (Alder and Cringle, 1990; Medrano and Fox, 1995; Murray et al., 1991; Stefansson, 1988; Stefansson et al., 1983; Tillis et al., 1988; Zuckerman and Weiter, 1980) oxygen consumption in the dark. However, the increased saturation may well be a consequence of increased demand for oxygen in the outer retina. It is well established that the photoreceptors in the outer retina have high demand for energy, particularly in the dark when their cGMP-gated cation channels are open and the cell membrane is depolarised (Ames et al., 1992; Zuckerman and Weiter, 1980). The Na/K ATPase pump maintains balance while the cation channels are open and this explains a large portion of the energy requirements

of the photoreceptors in the dark. The mitochondrial rich inner segments of the photoreceptors produce the ATP needed and consume oxygen in the process. This consumption and the distance from the oxygen source (choroid and retinal capillaries) cause the partial pressure of oxygen to fall to almost zero in the dark in a part of the outer retina, most likely at the inner segments of the photoreceptors or on their inner side (Birol et al., 2007; Linsenmeier, 1986). Transretinal profiles for partial pressure of oxygen in the animals show that, in the dark, there is an oxygen concentration (pressure) gradient towards this minimum in the outer retina from both the retinal capillaries and the choroid. From these profiles, it has been estimated that the outer retina receives about 7 to 15% of its oxygen from the retinal vasculature in the dark (Ahmed et al., 1993; Birol et al., 2007; Braun and Linsenmeier, 1995; Linsenmeier and Braun, 1992). In the light, on the other hand, the photoreceptors use much less oxygen so that the gradient from the choroid has been found to extend into the inner retina in most studies and the calculated contribution of choroid to the inner retina in the light is 0-11% (Ahmed et al., 1993; Birol et al., 2007; Braun and Linsenmeier, 1995; Linsenmeier and Braun, 1992). The choroid does not seem to react to the increased need for oxygen. Although this has not been studied in detail, published transretinal profiles seem to indicate that the partial pressure of oxygen in the choroid is similar (and high) in both dark and light (Ahmed et al., 1993; Birol et al., 2007; Braun and Linsenmeier, 1995; Haugh et al., 1990; Linsenmeier, 1986; Linsenmeier and Braun, 1992; Linsenmeier and Yancey, 1989), even if the choroidal blood flow may increase in the light, possibly to dissipate heat (Nagaoka and Yoshida, 2004; Parver et al., 1980).

The retinal vasculature must therefore supply the inner retina and a part of the outer retina with oxygen in darkness while it has to supply less than the all of the inner retina in constant light. The inner retinal oxygen consumption appears to be about the same in constant light and darkness (Braun et al., 1995; Medrano and Fox, 1995) so the net transfer of oxygen from the retinal capillaries to the retinal tissue has to increase in the dark due to the contribution that the retinal capillaries make to the outer retina in the dark. A net increase in diffusion of oxygen from the retinal capillaries to the (outer) retinal tissue can only occur by an increase in the concentration (pressure) gradient for oxygen. Lower partial pressure of oxygen in the outer retina in the dark will tend to increase the diffusion but so will an increase in partial pressure in the retinal capillaries.

An increase in partial pressure of oxygen in the retinal capillaries in the dark would therefore assist the choroid in supplying the outer retina with enough oxygen. The increase in partial pressure of oxygen in retinal capillaries could be accomplished by an increase in retinal blood flow. The original

studies on retinal blood flow in light and dark indicated that blood flow was increased in the dark (Feke et al., 1983; Riva, Grunwald & Petrig, 1983). However, the studies used visible laser light for the measurements and this may have confounded the measurements. A later study with near infrared laser light found only a transient difference (Riva et al., 1987). Another study found increased blood velocity in the central retinal artery in the dark (Havelius et al., 1999).

Barcsay et al. (2003) found small changes in diameter between light and dark and the results were not the same for arterioles and venules. Their conclusion was that diameter changes were too small to be clinically significant. In the present study, a weak trend was found towards dilation in darkness compared to light. This was, however, not consistent and not seen in the second part of the studies, where diameter was compared between darkness and 1, 10 and 100 cd/m^2 light. It should be noted that the resolution of the oximeter is low for diameter measurements (see chapter 3.2). There is therefore some evidence, from earlier and present studies, for increased blood flow in the dark although the measurements are difficult and the results somewhat contradictory.

Even if studies on blood flow give contradictory results, there are other signs of increased blood flow. An increase in partial pressure of oxygen in the inner retina in the dark has been shown in a study in cats (Linsenmeier and Braun, 1992), although this was not confirmed in a study in a monkey (Birol et al., 2007). Inner retinal consumption is most likely similar in light and dark (Braun et al., 1995; Medrano and Fox, 1995). An increase in the partial pressure of oxygen in the inner retina, if real, would therefore most likely be caused by increased blood flow and not decreased consumption in the dark.

Another sign of increased blood flow in the dark is the elevated oxygen saturation in retinal arterioles, found in both of the present studies on light and dark. Oxygen saturation was measured along main temporal retinal vessels, starting from close to the optic disc and up to the first branching of the vessel. The walls of arteries and arterioles are of course not impermeable to oxygen and various signs of diffusion directly from retinal arterioles have been found. In miniature pigs (Molnar et al., 1985; Riva et al., 1986) and in cats (Alder and Cringle, 1990) an oxygen concentration gradient was found away from the retinal arterioles. Schweitzer et al. (1999) measured oxygen saturation on a cross section of retinal vessels and found higher oxygen saturation in the centre of retinal arterioles than close to the walls. Additionally, the density of retinal capillaries is markedly decreased around the arterioles, indicating that diffusion through the retinal arteriolar walls is sufficient for the tissue adjacent to the

arterioles (McLeod, 2010). Oxygen may be delivered to the surrounding tissue from the retinal arterioles. There appears to be an inward gradient towards retinal venules (Alder and Cringle, 1990; Molnar et al., 1985; Schweitzer et al., 1999) and a counter-current mechanism has been proposed, whereby oxygen diffuses from arteries/arterioles into adjacent veins/venules (Buerk et al., 1993; Karlsson et al., 2007; Riva et al., 1986, Teng et al., 2012). It should be noted that loss of oxygen can occur both along the measured arteriolar segment and upstream, for example in the optic nerve where the central retinal artery and central retinal vein lie close together. The loss of oxygen from arteries and arterioles may be dependent on blood flow, i.e. less oxygen being lost from per unit volume of blood as the flow increases and this may help to explain the increased saturation in the retinal arterioles in the dark, provided that the blood flow is increased in the dark.

The hypothesis to explain the increased saturation in the dark is therefore that retinal blood flow is increased in the dark in response to increased outer and total retinal oxygen consumption in the dark. The increased blood flow increases the partial pressure of oxygen in the retinal capillaries, which facilitates diffusion of oxygen from the capillaries to the outer retina. The measured consequence of the increased blood flow is the increase in saturation in both arterioles and venules. An unchanged arteriovenous difference in saturation coupled with a postulated increase in blood flow yields increased delivery of oxygen to the tissue.

Both experiments on the effect of light and dark had several weaknesses although none of the weaknesses is likely to have changed the conclusions. First, the light used for adaptation was not uniform and the intensity could not be very accurately measured, as the result of the measurement depended on how the eye and the photometer were aligned. An effort was made to standardise the alignment of the eye, with respect to the light sources and to mimic this alignment with the photometer. Even if the intensity of the light could not be accurately measured and the light was not uniform it is clear that differences between dark and moderate light intensities (up to office light values) were tested and this served the purpose of the experiment, i.e. to test if light or dark had an effect on saturation. Uniform lighting of known intensity would have been preferred but this requires extensive technical modifications as the light source of the fundus camera has to be a part of the lighting.

Another possible weakness is the possible contamination of the dark adaptation by lights from the screen of the fundus camera and the screen of the laptop computer used as well as from the small amount of light that leaked through the infrared filter of the fundus camera. The light was clearly visible

and this may have affected the results somewhat. However, the light was of very low intensity and in fact not measureable by the photometer (0 cd/m^2 reading). The lower light settings tested (1 and 10 cd/m^2) did not give significant changes in saturation and this may indicate that the response is related to intensity. Therefore, a very low light level during the dark periods is unlikely to have had a large effect.

The completeness of the dark adaptation may also have been influenced by the xenon flashes used and the short time between some of the measurements (five minutes). The first dark adapted image was taken after 30 minutes in darkness. The xenon flash and the background light during the periods of light, adapted the retina to light and a clear afterimage was seen by the subjects after the flash. However, the results were similar after dark adaptation for 30 minutes or five minutes.

Finally, the saturation measurements could have been affected by changes in the colour of the fundus (bleaching of photopigment) or by changes in vessel diameter, even if we did not conclusively demonstrate the latter. These factors may have had a small effect in all of the studies in this thesis and are discussed in chapter 5.6.

5.2 Retinal vascular occlusions

5.2.1 Central retinal vein occlusions

Oxygen saturation in the main retinal venules was found to be significantly lower in eyes affected by central retinal vein occlusion than in the fellow eyes in the same patients. No difference was found in retinal arterioles. There was considerable variability between affected eyes (see Table 8) as well as between venules within the same eye. The variation within eyes can be seen by examining the standard deviations in Table 8 and the examples in Figures 12 and 13.

The most likely explanation for low oxygen saturation in venules in eyes affected by occlusion is that more oxygen is lost from per unit volume of blood to the tissue as less blood flows through the retinal capillaries after the occlusion. A decrease in venous saturation in central retinal vein occlusion is in agreement with invasive measurements with oxygen sensitive electrodes, which showed decreased partial pressure of oxygen in the vitreous above the retina (Williamson et al., 2009). Decreased oxygen saturation in central retinal vein occlusion was also seen by Yoneya et al. (2002), although the interpretation of their saturation measurements is difficult (see chapters 1.2.5.2).

The reasons for the apparently modest decrease in saturation in some affected eyes are unclear. Collateral blood flow may vary between patients due

to different location of the occlusion within the central retinal vein (Hayreh, 2005). Blood flow can also vary with the extent of re-canalisation of the thrombus (Green et al., 1981). Furthermore, the demand for oxygen may decrease after the occlusion and this may affect the saturation. Wolter (1961), for example, reported death of inner retinal neurons in a histological study of two eyes with central retinal vein occlusion. Cell death and decreased function will decrease the demand for oxygen. If the blood flow increases again, through maturation of collateral circulation and / or due to re-canalisation of the thrombus, the venous saturation will rise and reflect a new balance between blood supply and oxygen consumption. It should be noted that ischaemia may also increase with time in some cases (Hayreh et al., 1994; The Central Vein Occlusion Study Group, 1997).

The variability between venules within the same eye was somewhat surprising. The saturation in each retinal venule reflects the balance of oxygen supply and demand for the retinal area drained by the particular venule and different saturation in the venules reflects either or both different blood flow or different oxygen consumption. It is well known that the distribution of retinal cells is not uniform and it is therefore likely that oxygen consumption is heterogeneous as well. However, retinal blood flow is at least to some extent adjusted to match this heterogeneity. The pressure rises in the venules upstream of the occlusion and this increase appears to be greater in ischaemic than non-ischaemic occlusions (Jonas and Harder, 2007). It is unknown whether different haemodynamics produce different relative decrease of blood flow in different vessels.

Unfortunately, neither fluorescein angiography nor a measure of blood flow was available for the patients measured and explanations of the variability in venous saturation between and within eyes will therefore be based on speculation. It should be noted that technical variability may be a part of the explanation, even if care was taken to avoid the effects of haemorrhages on the measurements. Vessel diameter will for example vary and this can have some effect on the measurement (see chapter 5.6.3) and image quality was not always optimal (see for example Figure 13).

The mean arteriolar saturation was exactly the same in the affected and the unaffected eyes. In other studies in this thesis, changes have been seen in arteriolar saturation and the suggested explanation is changes in blood flow. The increased saturation in darkness in healthy volunteers for example, may for example be explained by increased blood flow and, consequently, decreased loss of oxygen from per unit volume of blood by diffusion through arteriolar walls (see chapter 5.1). A decrease in retinal blood flow could, therefore, have

been expected to decrease the arteriolar saturation in eyes affected by central retinal vein occlusion. The reason for the lack of change is unclear. A decrease in oxygen consumption in the retinal tissue around the retinal arteriole may possibly play some role although this is only a hypothesis. Decreased arteriolar diameter would also tend to raise the saturation measurement (artefact, chapter 5.6.3) and there is evidence of decreased arteriolar diameter after experimental branch retinal vein occlusion (chapter 5.2.2 below). Whether changes in arteriolar diameter play a role after central retinal vein occlusion is unclear.

The results show that changes in retinal vessel oxygen saturation are more complex than a uniform decrease. Further studies are needed with more patients and more information on blood flow so that the effects of central retinal vein occlusions on retinal oxygenation can be clarified.

5.2.2 Branch retinal vein occlusions

Oxygen saturation is variable in venules, affected by a branch retinal vein occlusion, while affected arterioles showed slightly higher saturation values than unaffected arterioles in the affected eye. Saturation in occluded venules was measured down to 12% and up to 93%. Although some technical variability may have affected these extremes, it is clear that there is not a uniform decrease in saturation in occluded venules.

The possible explanations for either low or high oxygen saturation in affected venules are similar to those already discussed for central retinal vein occlusion. The reason for low venous saturation in occluded venules is most likely that more oxygen is lost from per unit volume of blood as the blood flows in less quantity than before the occlusion. High (or normal) saturation, on the other hand, may be explained by collateral blood flow / shunting of blood (Christoffersen and Larsen, 1999; Danis and Wallow, 1987; Frangieh et al., 1982; Genevois et al., 2004; Hamilton et al., 1979; Hamilton et al., 1974; Pieris and Hill, 1982), re-canalisation of the thrombus (Frangieh et al., 1982) and/or decreased oxygen consumption due to cell death after the occlusion (Donati et al., 2008; Frangieh et al., 1982; Hamilton et al., 1979; Hockley et al., 1979). These mechanisms may have an effect soon after the occlusion as well as more gradual effects with time. Donati et al. (2008) have for example shown that cell death starts within hours after experimental branch retinal vein occlusion in miniature pigs and progressive atrophy appears to continue for weeks (studied up to three weeks). Re-routing of blood in the retinal capillary network starts immediately after experimental venous occlusion in the rat and re-modelling of the vasculature continued for 30 days even if the occlusion had re-canalised in three days (Genevois et al., 2004). Pieris et al. (1982) reported appearance of collaterals two to eight months after the occlusion and the collateral formation

was increased if the thrombus did not re-canalise. It should be noted that re-routing or shunting of blood away from the occlusion may mean that nearby tissue receives more blood than under normal conditions and the blood coming from this tissue may therefore have higher saturation than under normal conditions. Raised venous saturation, in some cases of branch retinal vein occlusion may therefore partly be explained by similar mechanisms as proposed for diabetic retinopathy in chapter 5.3 below.

The response of the retina to branch retinal vein occlusion is therefore dynamic and multi-faceted and different mechanisms may contribute in different ratios in different patients. This response may explain why some occluded venules have high oxygen saturation while others have low saturation. A sudden decrease in consumption after occlusion followed by a gradual increase in blood flow or shunting may, hypothetically, explain the highest saturation values in the occluded venules. The mechanisms of neovascularisation and oedema after branch retinal vein occlusion are discussed in chapter 1.1.4.

The cases, measured with low saturation here, are comparable to results from animal studies. Decreased partial pressure of oxygen in the vitreous above the affected area has been found in miniature pigs (C. J. Pournaras, Tsacopoulos, Strommer et al., 1990a), pigs (Noergaard et al., 2008), cats (Stefansson et al., 1990) and monkeys (Pournaras et al. 1997). One study on monkeys showed no difference in the partial pressure of oxygen over affected and non-affected areas (Ernest and Archer, 1979). In that study, five of the six occlusions were examined several months after the occlusion and atrophy of the tissue had occurred, which most likely decreases the oxygen consumption and raises the partial pressure on the inner side of the retina. However, one monkey was measured 30 minutes after the occlusion and showed similar results.

The partial pressure of oxygen in the vitreous, just above the retina, is believed to reflect the oxygen pressure within the (inner) retina (Alm and Bill, 1972a). Pournaras et al. (C. J. Pournaras, Tsacopoulos, Riva, 1990) have also measured decreased partial pressure of oxygen directly within the inner retina following experimental branch retinal vein occlusion in miniature pigs.

The arterioles, which supplied the affected area to the greatest degree, had slightly higher saturation than the unaffected arterioles in the affected eye. It is likely that blood flow decreases in the affected arterioles and this could have led to a decrease in saturation due to more diffusion of oxygen from per unit volume of blood from the arteriole to the surrounding tissue. The observed increase in saturation is therefore difficult to explain but it should be noted that the affected arteriole was simply chosen from fundus photographs as the

arteriole, which appeared to supply the affected area to the greatest degree. Fluorescein angiography data may be necessary to choose the affected arteriole more precisely. Furthermore, the arteriole may become narrower (Donati et al., 1997; Donati et al., 1998) and sheathed with time after occlusion (Hamilton et al., 1974). This was not observed in the current study but accurate vessel diameter measurements were not possible. Narrowing of arterioles could potentially elevate the measured saturation artefactually (see chapter 5.6.3).

Measurements of oxygen saturation in occluded branch retinal venules are technically challenging and sometimes impossible with the technology used here. The occluded venule was in some cases small and surrounded by haemorrhage and the image quality was in some cases poor. The oximeter can only measure larger first or second degree vessels reliably and vessel diameter may affect the results of measurements (see chapter 5.6.3). Haemorrhages, which are close to the measured vessel segment, can influence the measured brightness, used as a reference in the saturation calculation (see chapter 1.2.4). These technical limitations meant that, in 15 of 22 eyes, the occluded venule itself could not be reliably measured. In these cases, a downstream venule was measured. The downstream venule will have carried some blood from the affected area, either by collateral circulation bypassing the occlusion or through re-canalised occlusions.

The effect of the occlusion may therefore appear in the downstream venule although this measurement is not ideal and the downstream venule may contain blood from well perfused areas of the retina. A subgroup of patients, where the occluded venule could be more reliably measured, was analysed separately and the results were similar to the results for the entire group, i.e. the saturation in affected venules varied from very low to very high while the affected arterioles showed slightly elevated saturation.

5.2.3 Central retinal artery occlusions

Central artery occlusion is a rather rare occurrence and only four patients with variably long history of occlusion were measured. The results were also rather variable. The patient with giant cell arteritis showed markedly lowered saturation in retinal arterioles in the affected eye but the venules had similar saturation in both eyes. Fundus photographs showed that the blood column was segmented in several vessels in the affected eye, which indicates that blood flow was very slow or completely stagnant. It is therefore not surprising that the arterioles have only slightly higher saturation than the venules. Oxygen will diffuse through the vessel walls and the blood will reach equilibrium with the surrounding tissue. What is perhaps surprising is that the saturation in the affected eye is similar to the venous saturation in the unaffected eye.

Measurements after experimental occlusion of retinal arteries in animals have indicated that the partial pressure in the inner retina is close to zero (Alder et al., 1990; Braun and Linsenmeier, 1995; Yu et al., 2007) and if this was true in the patient with giant cell arteritis the saturation of stagnant blood in (inner) retinal blood vessels should be much lower. One possible explanation for this discrepancy is that more time elapsed between the occlusion and the measurement in the case of the patient (one day) compared to the experimental animals. Oxygen consumption in the inner retina may therefore have decreased more in the patient due to cell death and this would lead to more oxygen reaching the inner retina from the choroidal circulation. It is, however, unlikely that this fully accounts for the high saturation since the diffusion distance from the choroid to the retinal vessels is great and the outer retina is likely to consume oxygen after the occlusion as well. It is also possible that a decrease in vessel diameter in the affected eye contributed somewhat to the high measured saturation. Decreased vessel diameter will tend to elevate the measured saturation – see discussion on this artefact in chapter 5.6.3.

After the patient with giant cell arteritis had been treated with prednisolone, blood flow was re-established, i.e. no segmentation of the blood column was seen and the vessels appeared wider. The saturation in arterioles and venules was then close to normal and even lower than just after the occlusion. This is surprising since cell death in the inner retina is likely to have decreased the inner retinal oxygen consumption and a high retinal venous saturation could have been expected after the return of blood flow. No quantitative measurements were made of blood flow but even a modest increase in blood flow could have been expected to raise the venous saturation if the inner retinal oxygen consumption was unchanged (diminished from before occlusion). An increase in inner retinal consumption after treatment is possible but remains speculative.

One additional patient was measured one day after occlusion. Arterial and venous saturation were low in both eyes of this patient and a part of the reason may be rather poor image quality (see discussion on the effect of image quality in chapter 5.6.4). His venous saturation was very low in the affected eye, considerably much lower than in the unaffected eye. The clinical information available on this patient was rather vague and he was initially diagnosed with branch retinal artery occlusion. Some remaining blood flow through collateral circulation may explain why there was considerable arteriovenous saturation difference. This cannot be confirmed, since neither measurement of blood flow nor fluorescein angiography was available.

The remaining two patients had a longer history of occlusion (two weeks to one month) and their saturation was similar between the affected and the

unaffected eye, indicating that the balance between oxygen supply (blood flow) and oxygen consumption is similar in the affected and the unaffected eye. One of these patient had normal visual acuity about 2 months after the occlusion, which indicates that this was not a usual central retinal artery occlusion. The changes in either blood flow or oxygen consumption are unknown. It is therefore difficult to determine why the vessel saturation in these cases, as in the case with giant cell arteritis after treatment, is close to normal.

Hammer et al. (Hammer, Riemer et al., 2009) investigated branch and central retinal artery occlusions with a two wavelength oximeter, based on the same principles as the oximeter used in the studies described here. As in the current study, only a few patients were included and no statistical analysis was performed. However, mean values for arterioles (98%) and venules (65%) in healthy individuals, published in the same paper, can be used as a rough reference. At the time of diagnosis, which was within 48 hours of the occlusion, they found low arterial saturation (78%) in the occluded branch artery and a considerable increase (91%) five days later, after treatment with pentoxifylline for improvement of blood rheology. Saturation after central retinal artery occlusion was, however initially close to normal (93%) although it did increase after five days and treatment (103%). Venous saturation in central retinal artery occlusion was initially slightly below normal (55%) and had increased to 70% five days later. A later paper by the same group confirmed the results for saturation in occluded branch retinal arterioles (Gehlert et al., 2010).

Rather high saturation values, reported by Hammer et al. (Hammer, Riemer et al., 2009), in both retinal arterioles and venules in patients with central retinal artery occlusion compare well with the patients measured here two weeks or one month after the occlusion. Whether these patients have considerable retinal blood flow and / or decreased inner retinal oxygen consumption is difficult to determine. Inner retinal atrophy is most likely partly responsible for the outcome (Hayreh and Jonas, 2000; Ikeda and Kishi, 2010). The remaining two patients, described here, were measured soon after the occlusion and had low saturation values, the arterial values being similar to the values reported by Hammer et al. (Hammer, Riemer et al., 2009) and Gehlert et al. (2010) for occluded branch arterioles before treatment.

Several variables are likely to have an effect on the saturation values, most notably the magnitude of the remaining blood flow, the time between occlusion and measurement and changes in blood flow and oxygen consumption with time. Retinal vessel oximetry may be useful to evaluate the physiological consequences of an occlusion and the above mentioned factors. Central retinal

artery occlusion is, fortunately, a rather rare disease and this is reflected in the low number of patients studied here and in the previous publication by Hammer et al. (Hammer, Riemer et al., 2009). Measurements of larger groups of patients, preferentially with information on blood flow, are needed to clarify the interplay between retinal vessel oxygen saturation and factors associated with central retinal artery occlusions.

5.3 Diabetic retinopathy

The results show that oxygen saturation in major temporal retinal vessels is higher in patients with diabetic retinopathy than in healthy volunteers. This applies for both arterioles and venules and for all categories of diabetic retinopathy tested. The categories tested were background diabetic retinopathy without macular oedema, macular oedema, pre-proliferative / proliferative retinopathy before treatment and proliferative diabetic retinopathy after treatment.

Higher retinal vessel oxygen saturation in diabetic retinopathy may seem to contradict evidence of hypoxia in the retina. This is not necessarily the case. As explained below, high saturation in retinal vessels can be caused by 1) poor distribution of oxygen to the tissue 2) increased (or normal) oxygen supply, and 3) less consumption of oxygen by the tissue.

5.3.1 Distribution of oxygen to the retina in diabetic retinopathy

Poor distribution of oxygen can be caused by a) capillary closure and shunting of blood, b) thickening of capillary walls and c) greater affinity of glycosylated haemoglobin for oxygen.

One of the main characteristics of diabetic retinopathy is damage to retinal capillaries (Cogan and Kuwabara, 1963; Cogan et al., 1961; Kohner, 1993), which is believed to create hypoxia in the affected tissue (Linsenmeier et al., 1998). The hypoxia can then cause macular oedema and retinal neovascularisation through the mechanisms described in chapter 1.1.4. While some capillaries close, there is evidence that others become wider and act as shunt vessels or preferential channels from the arterioles to the venules (Cogan and Kuwabara, 1963). The shunted blood will probably loose less oxygen than blood travelling through a normal retinal capillary network and will therefore tend to raise the saturation in the larger retinal venules, which were measured in the current study. Additionally, capillaries may, in some cases, close and later re-open (Yamana et al., 1988). If cell death occurs while the capillaries are closed, the consumption is decreased and will not reach the same level even if blood flow is restored.

According to this hypothesis, which is summarised in Figure 24, the net result of capillary closure and formation of preferential channels is that parts of the retinal tissue are hypoxic while, at the same time, the oxygen saturation in the larger retinal vessels is normal or even higher than normal. It should be noted that shunt vessels may allow the total retinal blood flow to stay constant or even increase (see below), even if some parts of the tissue do not receive enough blood.

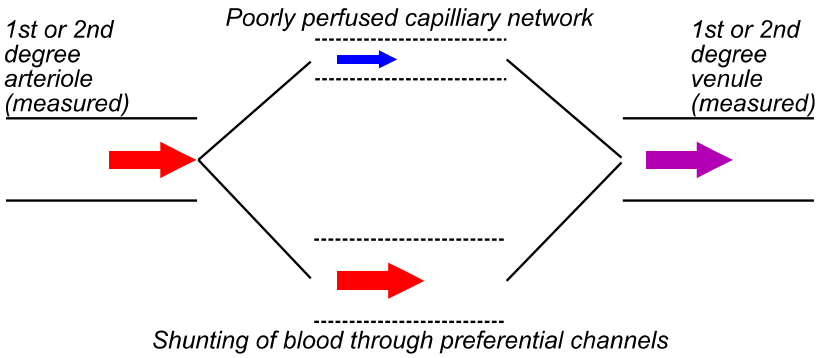

Figure 24. Poor distribution of blood in diabetic retinopathy. The diagram explains how poor distribution of blood and oxygen can lead to normal or high oxygen saturation in the larger retinal vessels, while parts of the retinal tissue are hypoxic. Capillary closure leads to tissue hypoxia. Preferential channels, formed in the capillary network, shunt blood from the arteriolar side to the venular side and the net result is normal or high oxygen saturation in the larger retinal vessels.

Another factor that can adversely affect distribution of oxygen to the retina is thickening of the capillary walls. Thickening of the capillary wall is well established in diabetic retinopathy (for review see Ashton, 1974; Roy et al., 2010). Oxygen diffusion is inversely related to the diffusion distance and thickening of the capillary walls increases the distance between the capillary lumen and the tissue outside the capillary wall. However, the distance between the retinal capillaries (in the plane of the retina) is many times the thickness of the walls, and in fact many times the thickness of an entire capillary. Increases in diffusion distances due to capillary wall thickening will therefore be very small and the effects probably negligible (unless the diffusion coefficient for oxygen in the capillary wall is much less favourable than the diffusion coefficient in the retinal tissue).

Higher concentration of glycosylated haemoglobin in the diabetic patients may also play a minor role since glycosylation increases the affinity of

haemoglobin for oxygen. This may help to explain the higher saturation in retinal arterioles as well as in venules. Unfortunately, the concentration of glycosylated haemoglobin was not measured for the participants. It was previously found (Bjoernsdottir et al., 2004) that Icelandic patients with type 2 diabetes had 7% of their haemoglobin glycosylated. Young Icelandic adults (20-30 years) with type 1 diabetes had on average 9% of glycosylated haemoglobin (Halldorsdottir et al., 2009). Normal values in healthy individuals are about 5-7% (Fischbach and Dunning, 2009).

The increased affinity of glycosylated haemoglobin for oxygen is unlikely to play a major role in the present results, simply because the difference in concentration between diabetic patients and the healthy comparison group is likely to be small. Furthermore, the affinity of haemoglobin for oxygen is decreased by 2,3-disphosphoglycerate and the concentration of 2,3-disphosphoglycerate can fluctuate in diabetic patients (Ditzel, 1976). The end result may be that oxygen affinity of blood in diabetic patients is similar to normal and the full explanation for this is not necessarily given by concentration changes in glycosylated haemoglobin or 2,3-disphosphoglycerate.

The high oxygen saturation in the larger retinal vessels can, according to the above, be explained by poor distribution of oxygen to the tissue and it is likely that shunting of blood is the major factor while changes in capillary wall thickness and affinity of haemoglobin for oxygen may play a minor role

5.3.2 Supply of oxygen to the retina in diabetic retinopathy

The high saturation found may also be explained by increased oxygen supply. The hypoxic tissue signals the need for more oxygen and this may lead to increased total retinal blood flow even if the distribution of blood through the capillary network is still dysfunctional. However, measurements of total retinal blood flow have given contradictory results. The author of this thesis reviewed the literature on blood flow in diabetic retinopathy in 2008 (see in C. J. Pournaras et al., 2008). At that time, published reports on blood flow in diabetic retinopathy were conflicting. Blood flow had been found to be either more, less or the same in patients with diabetes, when compared with healthy individuals. This held true for patients with no retinopathy, with background diabetic retinopathy, macular oedema or proliferative retinopathy before treatment. After laser treatment for proliferative retinopathy, however, all studies agreed that the blood flow is less than in healthy individuals.

Since this review was made, several studies have been published, where comparison is made between retinal blood flow in healthy individuals and in patients with diabetes. These have not resolved the confusion. Burgansky-

Eliash et al. (2012) found increased blood velocity in small retinal vessels in patients with diabetes but no retinopathy. On the other hand, Nagaoka et al. (2010) found decreased velocity and flow in a main temporal retinal arteriole in patients with diabetes and no retinopathy. Nagaoka et al. (2010) also found decreased velocity and flow in patients with mild non-proliferative retinopathy and this agrees with another study by Burgansky-Eliash et al. (2010), where blood velocity was less in small retinal vessels in non-proliferative retinopathy, when compared to normal. However, Pemp et al. (2010) found increased blood flow in a combined group of patients with no retinopathy or non-proliferative retinopathy, even if this difference was less and not statistically significant after blood glucose had been lowered in the diabetic patients.

Possible changes in total retinal blood flow are therefore not completely clear and the same is true for choroidal blood flow in diabetes (for review see Pemp and Schmetterer, 2008). Increased oxygen saturation in retinal vessels, found in the present study is, however, consistent with increased total retinal blood flow. If blood flow is indeed increased, this would help to explain the increased saturation in retinal arterioles as well as venules. This is because saturation measurements were averaged along vessel segments and oxygen can escape with diffusion through the arteriolar walls to the adjacent tissue or to an adjacent venule. Increased blood flow would tend to decrease the loss of oxygen through arteriolar walls from per unit volume of blood.

5.3.3 Oxygen consumption in diabetic retinopathy

The final major factor, which can help explain high oxygen saturation in retinal vessels in diabetic retinopathy, is decreased oxygen consumption. The primary damage in diabetic retinopathy is most often thought to occur in the vasculature and any degeneration of the retinal tissue or loss of function is believed to be secondary to the vascular damage. However, there is some evidence for primary degeneration of the neural retina. Histological studies on diabetic rats and on human retinas have revealed that apoptosis of both vascular and neural cells takes place in the diabetic retina and the neural apoptosis is not necessarily dependent on vascular changes (for review see Barber et al., 2011). Studies on zebrafish even indicate dysfunction of cones, which may be independent of vascular damage (Alvarez et al., 2010).

Measurements on retinal oxygen consumption in diabetic retinopathy are scarce. Sutherland et al. (1990) found that oxygen consumption was decreased in diabetic rabbit retina *in vitro*, compared to non-diabetic rabbits. Tiedeman et al. (1998) found that venous oxygen saturation decreased with hyperglycaemia in diabetic patients with no retinopathy and concluded that oxygen consumption was increased, assuming increased blood flow in hyperglycaemia.

However, no comparison is available on oxygen consumption in healthy individuals and in diabetic patients.

High venous saturation, found in the present study, could be explained by a decrease in oxygen consumption. However, further studies are needed to determine if the oxygen consumption is different between diabetic patients and healthy individuals. The interplay of neural and vascular damage (or lack of interplay) also remains to be better elucidated.

5.3.4 Other studies on oxygenation in diabetic retinopathy

The present results are in general agreement with other studies on retinal vessel oxygen saturation in diabetic patients. Hickam et al. (1959) found no difference in venous saturation between diabetic patients and healthy individuals. However, their small group of diabetic patients was mixed, i.e. included patients with and without retinopathy. Schweitzer et al. (2007) used a multispectral, calibrated instrument to measure saturation in large retinal vessels in patients with mild or moderate non-proliferative diabetic retinopathy. They found that venous oxygen saturation was slightly higher in the diabetic patients, when compared to normal, although the difference was not statistically significant. Hammer et al. (Hammer, Vilser et al., 2009) used a two wavelength instrument, similar to the oximeter used in the present study, to measure oxygen saturation in all major retinal arterioles and venules around the optic disc. The groups measured were healthy individuals, patients with mild non-proliferative retinopathy, moderate non-proliferative retinopathy, severe non-proliferative retinopathy and proliferative retinopathy (some but not all patients in the last group had a history of laser treatment). Venous oxygen saturation increased with severity of retinopathy although not all comparisons with healthy showed a statistically significant difference. Arteriolar saturation was increased with severity of retinopathy but this was not statistically significant, compared to normal.

5.3.5 Limitations of the present study on diabetic retinopathy

The main limitations of the present study were that the healthy individuals were not matched for age or gender and that the oximetry images of the healthy volunteers were taken under a slightly different angle than the oximetry images of the patients with diabetic retinopathy (see Figure 19). Furthermore, it is uncertain if changes in vessel diameter or the central reflex from the retinal vessels in diabetic retinopathy (or other retinal changes) may produce artefacts.

The difference in age is unlikely to have created much bias in the results. The diabetic patients were, on average, older than the healthy individuals. Results from a later oximeter, based on similar principles as the one used in the present study,

indicate that saturation decreases slightly with age in healthy individuals (Geirsdottir et al., paper in preparation). The effect of the age difference between groups in the present study may therefore have been to decrease the observed difference slightly but not change the overall conclusion. It should also be noted that Schweitzer et al. (2007) found a slight increase in venous saturation with age in patients with mild or moderate non-proliferative retinopathy but no change in healthy individuals. Whether this reflects the progress of the disease with time or is linked to age in a different manner is unknown.

The difference in imaging angle between the normal group and the diabetic patients may have caused some bias in the results. However, recent preliminary results from a later oximeter show very similar results as presented here, even if there is no difference in the imaging protocol (Hardarson and Stefansson, 2011)

As is discussed in chapter 5.6.3 below, increased vessel diameter may result in decreased measured saturation (artefact). Differences in diameter were not analysed in the present study. 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 (Klein et al., 2006; A. S. Tsai et al., 2011) in comparison with the changes needed to markedly influence measured saturation (J. M. Beach et al., 1999 and unpublished own data) although there may be exceptions, especially in the case of narrowing after laser treatment (Gottfredsdottir et al., 1993; Kristinsson et al., 1997, C. A. Wilson et al., 1988).

It should also be mentioned that the central reflex, which appears on the retinal vessels has been found to be decreased in diabetic patients (Brinchmann-Hansen et al., 1987). The central reflex was not studied systematically in the present study. However, subjectively evaluated, it may have been brighter in the diabetic patients. The software, used for analysis, avoids the influence of the central reflex on the light intensity measurements. However, differences between groups in this reflex are unfortunate and some effect on the results cannot be ruled out.

In summary, several factors may have confounded the results. However, the increase in saturation in diabetic retinopathy is clear and statistically significant for all categories of diabetic patients and for both arterioles and venules. The limitations of the study are not likely to have changed the major conclusions that the retinal vessel oxygen saturation is increased in diabetic retinopathy.

5.4 Glaucoma treatment

The main result of both studies on glaucoma treatment was that neither glaucoma surgery nor addition of dorzolamide to timolol had a major effect on retinal vessel oxygen saturation.

5.4.1 The implications of small changes in saturation with glaucoma treatment

Small changes were observed in retinal vessel oxygen saturation with either glaucoma surgery or with dorzolamide treatment. Oxygen saturation in retinal arterioles was increased by two percentage points after surgery and both retinal arteriolar and venular saturation decreased by three percentage points in patients switching from dorzolamide-timolol combination to timolol alone. No change was observed in the subgroup, which started taking timolol and switched to the dorzolamide-timolol combination in the second period.

An increase in arteriolar saturation, with surgery or in a subgroup taking dorzolamide in the first study period, is consistent with increased retinal blood flow. Oxygen escapes from retinal arteries and arterioles by diffusion (see chapter 5.1) and the oximetry measurements may be affected by this as an average is taken of saturation along retinal vessel segments. Increased retinal blood flow may lead to decreased loss of oxygen from per unit volume of blood by diffusion through arterial and arteriolar walls. As described in chapter 1.1.5.2, earlier studies on the effect of glaucoma surgery or dorzolamide on ocular blood flow give contradictory results although the result of a recent meta-analysis (Siesky et al., 2009) was that carbonic anhydrase inhibitors, such as dorzolamide, do indeed increase ocular blood flow (various parameters).

The present results are therefore consistent with increased retinal blood flow even if no direct measurement was made of flow. This is similar to the results for arterioles in dark and in diabetic retinopathy (chapters 4.1 and 4.3) although other explanations may apply in diabetic retinopathy, besides increased blood flow. If blood flow is indeed increased with surgery or with dorzolamide, the unchanged arteriovenous difference in saturation, found in both studies here, translates into increased oxygen delivery to the tissue. To confirm this, simultaneous measurements of blood flow and oxygen saturation are needed.

The lack of large and consistent changes in retinal vessel oxygen saturation with glaucoma treatment may provide indirect clues to the role of retinal oxygenation in the pathophysiology of glaucoma. As mentioned in the introduction (chapter 1.1.5.1), there is evidence of decreased ocular blood flow in glaucoma (Flammer et al., 2002). If decreased retinal blood flow is a primary

event, leading to hypoxia in the retinal tissue, retinal venous oxygen saturation might be expected to be low before treatment, the blood flow might be expected to rise with decreased intraocular pressure after treatment and lead to elevated retinal venous oxygen saturation. The fact that retinal venous saturation seems to be normal and changes very little with treatment does not support the hypothesis that glaucomatous retinas suffer from lack of blood flow and consequent hypoxia. A recent study by Olafsdottir et al. (2011) gives more direct evidence against ischaemia / hypoxia in glaucoma. They found that retinal venous oxygen saturation increased with severity of glaucoma, i.e. with worsening visual fields. The most likely explanation is that glaucomatous atrophy decreases the demand for oxygen and therefore increases retinal venous oxygen saturation. Michelson and Scibor (2006) found that retinal venous saturation was normal in patients with normal tension glaucoma, providing further evidence against the role of retinal hypoxia in glaucoma. They found slightly lower arteriolar saturation in normal tension glaucoma, compared to healthy volunteers and this may be related to less blood flow in the glaucoma patients. In short, oximetry results in glaucoma indicate that changes in retinal vessel oxygen saturation are small and most likely secondary to the disease and not a primary cause of glaucoma.

Although the oxygen saturation measurements, viewed in the context of earlier blood flow measurements, provide clues to the lack of role for oxygen in the pathophysiology of glaucoma, this is by no means conclusive evidence against the ischaemia / hypoxia theory on glaucoma. Longitudinal studies are needed to clarify the potential role of hypoxia in glaucoma. Furthermore, oxygen saturation was only measured in major retinal vessels and without stimuli or conditions that may challenge the retinal or optic nerve blood flow. The most likely site for the major glaucomatous damage is at the optic nerve head, since ganglion cells grouped together there are lost at the same time (Quigley, 2011). The metabolic changes in the retina are therefore most likely a consequence of ganglion cell death, initiated at the optic nerve head, rather than a primary cause of glaucomatous damage.

It should also be noted that the choroidal circulation may be affected by the lowering of intraocular pressure. Earlier studies indicated that choroidal blood flow fell linearly with increased intraocular pressure although later studies have found evidence of regulation to cope with the effect of pressure (Kiel and Shepherd, 1992; Polska et al., 2007; Schmidl, Garhofer et al., 2011; Schmidl, Weigert et al., 2011). Although blood flow was not measured in the current studies, the possibility still exists that choroidal blood flow is increased after glaucoma treatment (Berisha et al., 2005; Bernd et al., 2001; Boles Carenini et

al., 1997; Fuchsjager-Mayrl et al., 2005; James, 1994) and if this is the case, oxygen delivery to the retina may also increase.

The conditions, under which the oximetry measurements were made, were not designed to challenge the regulation of blood flow. The measurements were made in a dark room and aiming of the oximeter was achieved with infrared light. The major stimulus to the retina was the camera flash. The time between repeated images of the same eye was about one to two minutes. The patients were of course awake and sitting upright. The possibility should be mentioned, that glaucoma treatment may have improved blood flow and oxygenation under more challenging conditions, for example when blood pressure decreases during the night.

It is unclear why retinal vessel oxygen saturation only changed in the subgroup, which took the combination of dorzolamide and timolol first and then switched to timolol. One theoretical possibility is enzyme induction. By this hypothesis, carbonic anhydrase would be upregulated during the inhibition by dorzolamide. Due to this upregulation of the enzyme, the effect of stopping the dorzolamide administration would be greater than the reverse effect of starting dorzolamide administration. However, no evidence for this hypothesis has been found in the literature. It also has to be kept in mind that the subgroups are very small.

5.4.2 Earlier studies on the effect of glaucoma treatment on ocular oxygenation

No published papers have been found on human retinal oxygenation before and after glaucoma surgery. Spectrophotometric measurements in monkeys have shown decreased oxygen saturation in retinal arterioles and venules as well as in the optic nerve head when intraocular pressure is increased dramatically (Beach et al., 2007). Experiments on pigs have shown that the partial pressure of oxygen over the optic nerve head is decreased when the intraocular pressure is raised (la Cour et al., 2000). This may seem to contradict the present results, which show little change in saturation with change in intraocular pressure. However, the circulation in healthy animals, especially in the optic nerve head, is not directly comparable to the major retinal vessels in a glaucoma patient. Furthermore, the decrease in oxygen pressure in the pig was minimal until the intraocular pressure is raised considerably, most likely due to autoregulation blood flow.

Similar experiments on pigs have shown that dorzolamide (and acetazolamide) can increase the partial pressure of oxygen over the optic nerve head (Kiilgaard et al., 2004; la Cour et al., 2000; Pedersen et al., 2004; Stefansson et al., 1999; Stefansson et al., 2005) and over the retina (Pedersen et

al., 2005). Again, the circulation in the pig optic nerve head may react differently from the human retinal circulation and it has to be kept in mind that large intravenous doses were used in the pig experiments, compared to the drops used twice a day eye in the present study.

A study on the effect of adding dorzolamide to timolol was performed by collaborators in Indianapolis with a similar protocol and at the same time as the study described here (Siesky et al., 2010). They used a similar oximeter and the results showed no changes in optical density ratio with the addition of dorzolamide to timolol. Optical density ratio, as calculated in this study, is related to oxygen saturation (chapter 1.2.4). Additionally, blood flow parameters were measured with Heidelberg retinal flowmetry (peripapillary capillary beds) and colour Doppler imaging (blood velocity in retrobulbar vessels). The number of zero flow pixels on Heidelberg retinal flowmetry was decreased during the dorzolamide-timolol period, but no other parameter changed with addition of dorzolamide to timolol.

The two studies on addition of dorzolamide therefore show small (present study) or no changes (Siesky et al., 2010) in oxygen saturation. However, in an earlier study, Siesky et al. (2008) found evidence of increased oxygen saturation in retinal venules in glaucoma patients taking dorzolamide or brinzolamide, compared to when the same patients were taking timolol alone. It is unclear why this result is so different from the other two studies (present study and Siesky et al., 2010) on dorzolamide and retinal oxygen saturation. The main difference is that in the earlier study, dorzolamide (or brinzolamide) alone, given three times a day, was compared to timolol alone, given twice a day. In the present study and the later study by Siesky et al. (2010), drops were given twice a day and dorzolamide-timolol combination was compared to timolol alone. As described in chapter 1.1.5.2, timolol is not believed to have significant effects on blood flow or oxygenation. In a study on pigs, no effects were found of timolol on optic nerve head oxygen tension, regardless of whether timolol was given alone or in combination with dorzolamide (Kiilgaard et al., 2004).

5.4.3 Stability of oximetry over time

In the study of the effects of dorzolamide on retinal vessel oxygen saturation, ten of the patients were followed for 18 months and measured with one or two months' interval. As the effect of the different drugs on saturation was minimal, the measurements can be viewed as repeated measurements under similar conditions over a long time period. The standard deviation of these repeated measurements was very low (Table 16 in chapter 4.4.2). It can be compared to two earlier repeatability studies, although it has to be kept in mind

that the current standard deviation is for measurements of mean arteriolar and mean venular saturation for an eye while the other two studies measured single vessels repeatedly. The current standard deviation is about two to three percentage points lower than the standard deviation between images of patients with better optical quality of the eye, taken within the the same session in an earlier study with the same instrument (Hardarson et al., 2006). Hammer et al. measured the standard deviation of repeated measurements with their oximeter in healthy volunteers (one session, single vessels) and found it to be about one percentage point higher than the standard deviations found here (Hammer, Riemer et al., 2009).

5.4.4 Limitations of the studies on the effect of glaucoma treatment on retinal vessel oxygen saturation

Glaucoma is a complex disease and it is a limitation of both studies of its treatment that the groups were small and not rigorously defined. In the study of glaucoma surgery, all patients, who were undergoing filtering surgery for open angle glaucoma were invited to participate. All of them had severe glaucoma. However, some had exfoliation glaucoma whereas others had not.

In the study of dorzolamide, patients were classified as having open angle glaucoma or optic nerve hypertension by their ophthalmologist, based on optic nerve head appearance, visual fields and intraocular pressure. The diagnosis was made by two ophthalmologists and the glaucoma patients had mild glaucoma. The reason for this loose inclusion criteria for the dorzolamide study was that recruitment was difficult for the 18 month study. The effect on the results is unclear. However, in the parallel study in Indianapolis (Siesky et al., 2010), neither the group with glaucoma nor the healthy control group showed any effect of dorzolamide on retinal oxygen saturation.

Another disadvantage of both studies is the possible effect of other treatments. The effects of dorzolamide had to be investigated by comparing the combination of dorzolamide and timolol to timolol alone simply because it would have been unethical to leave the glaucoma patients untreated. As mentioned in chapter 1.1.5.2, timolol is believed to have little effects on ocular blood flow or oxygenation and it is therefore likely that the comparison of dorzolamide and timolol combination to timolol alone would have revealed the possible effects of dorzolamide on oxygen saturation.

Stopping the glaucoma medication before the study on the effect of surgery would also have been unethical. All patients undergoing surgery took their regular glaucoma medication before the surgery but not after surgery. Despite this, the intraocular pressure was decreased considerably and it is unlikely that

stopping the glaucoma medication after surgery changed the conclusions of the study. Nevertheless, some confounding effects of for example dorzolamide before surgery cannot be ruled out.

Finally, the double-blinding of the dorzolamide study was not perfect since the patients would frequently notice more irritation due to the dorzolamide-timolol combination than due to timolol alone and complain about this to the researchers. Nevertheless, the analysis of oximetry images was performed without knowledge of the study period in which the images were taken.

5.5 Statistical analysis

The statistical tests used are mentioned where the results are reported (Chapter 4). In most cases, the set up of the studies or the nature of the data meant that more than one approach could have been taken to perform statistical analysis. Below is a brief discussion of the main statistical issues.

In the light and dark experiments, the main interest was the simple question of whether saturation changes between light and dark. A simple statistical approach was taken in the first experiment, i.e. to compare a measurement in the dark to the next measurement in light. This gave the same answer five times in a row and the p-value was always lower than 0.05 (Figure 8). Interpretation of the differences in saturation, found in the first light and dark experiment, is therefore not complicated by the multiple tests. In the second light and dark experiment, the results for the bright light (100 cd/m^2), were similar to the results for the 80 cd/m^2 light in the first experiment, although not quite statistically significant for venules (Figure 10). When viewed together, these results clearly indicate that oxygen saturation is greater in darkness than in 80-100 cd/m^2 light. The probability of repeatedly obtaining these low p-values if there was no effect of light and dark on oxygen saturation is extremely low.

Other aspects of the light and dark experiments were analysed in the same manner for consistency. Interpretation of the low p-values for differences in brightness ratios is straight-forward (Figure 9, all values low). The differences in diameter were not at all consistent and no strong conclusions were therefore drawn. It was known beforehand that the diameter measurements were crude due to low resolution.

Other statistical tests could have been used for analysis of light and dark experiments, particularly the second experiment where different light levels were compared to dark. Analysis of variance for repeated measurements with Dunnett's post tests would have been more appropriate for this experiment but the simpler approach of t-tests was used for consistency with previous experiment. The analysis of variance was tested (not shown) and gave the same

statistical significance, except that difference in venous saturation between darkness and 100 cd/m^2 light was significant, instead of being borderline significant. For experiment one (alternating light and dark), using repeated measures analysis of variance would have been problematic. The time points in darkness were for example not equivalent since the dark adaptation was 30 minutes in one case and five minutes in the other cases. The magnitude of the effect could have depended on duration of dark adaptation and history of dark / light exposure. Treating all time points in the first light and dark experiment equally in a combined test would have been questionable.

The choice of parametric or non-parametric tests for retinal vein occlusion measurements was not straight-forward. All the data passed the Shapiro-Wilk normality test. However, the samples are small, which means that the normality tests are not powerful. Non-parmetric tests were chosen for branch retinal vein occlusions since the whole group included data, which was affected by poor image quality in a fashion that was expected to be non-random. This means that it is questionable whether measured saturation in branch retinal vein occlusion data is normally distributed. The subset of patients with good quality images was analysed with non-parametric tests for consistency. Parametric tests were applied to the branch retinal vein occlusion data (not shown) and gave the same conclusions, except that the difference between affected arterioles and arterioles in the fellow eye became significant.

Parametric test were used for central retinal vein occlusion data since they were generally based on better quality images than the branch retinal vein occlusions and were, in most cases, averages of several vessels from each eye. Non-parametric tests (not shown) gave the same conclusion.

For analysis of glaucoma surgery, two-way analysis of variance with Bonferroni post-tests could have been more appropriate than the simple t-tests used. This approach has been tested (not shown) and gave the same conclusions.

5.6 Technical aspects of oximetry measurements

5.6.1 Calibration and interpretation of retinal oximetry values

As is described in chapter 1.2.4, the oximeter relies on reflected light on two wavelengths to calculate the oxygen saturation. This requires several approximations and the calibration is done by comparison with literature values from a multispectral device (Schweitzer et al., 1999). Calibration of retinal oximeters will always be challenging since accurate measurements *in vivo* are not possible. Schweitzer et al. (1999) used glass capillaries for calibration, which may be more appropriate for their instrument than the one used here, because their measurements do not rely on background reflectance. A single

oximetry measurement should be taken with caution because factors such as vessel diameter and fundus pigmentation may influence the results (Beach et al., 1999). The imperfection of the calibration is for example seen when single oxygen saturation values are above 100%. However, the oximeter has been shown to give repeatable measurements and to be sensitive to changes in oxygen saturation (Hardarson et al., 2006). Provided that known confounding factors are kept in mind, the oximeter can be used for comparison of groups (as is most often the case in this thesis) or to detect large deviations in retinal oxygen saturation in individuals. The calibration parameters used to calculate oxygen saturation from optical density ratios do not influence the results (p-values) of statistical tests because the relationship between optical density ratio and the calculated oxygen saturation is linear.

5.6.2 The effect of variable fundus reflectance on oximetry

The oximetry calculations (chapter 1.2.4) are based on comparison of light intensities at the retinal vessels (I in equation 4) and beside the retinal vessels (I_0). Light intensity to the side of the vessel is obviously dependent on fundus reflectance. Light intensity at the retinal vessel may be dependent on the fundus reflectance to a lesser degree, simply because part of the light is absorbed or reflected without ever interacting with the fundus outside the vessel (Hammer et al., 2001). Differences in fundus pigmentation within or between eyes can therefore create differences in calculated saturation within or between eyes.

Delori and Pflibsen (1989) found different fundus reflectance in people of different race and with different iris colour. Different melanin concentration, especially in the choroid appears to have a significant effect on fundus reflectance. The difference in reflectance was more pronounced for red light than for shorter wavelengths. This wavelength dependency means that calculated optical density in retinal oximetry is differently affected at the two wavelengths and the resulting optical density ratio and calculated saturation is affected. Beach et al. (1999) and Hammer et al. (2008) have both made attempts to correct dual wavelength oximetry for the influence of fundus pigmentation.

Despite the influence of fundus pigmentation on individual retinal oximetry results, it is unlikely that the conclusions of the studies reported herein are affected. The population studied consists predominantly of lightly pigmented Caucasians. There is little reason to believe that pigmentation is on average dramatically different between diabetic patients and the healthy comparison group. In all other studies, each participant was compared with him- /herself.

Even if melanin in the fundus is unlikely to have affected the conclusions of the current studies, the possibility remains that bleaching of rhodopsin in light and dark experiments could have created an error. Ripps et al. (1981) found that fundus reflectance in the cat retina changed with light adaptation. The change was small at the wavelengths used in the retinal oximeter. However, the change in light absorbance was slightly greater at 586 nm than at 605 nm and this may create artefactual difference when calculated saturation in light is compared to saturation in dark.

A more detailed analysis of the light and dark experiment was performed to address the possibility that fundus absorbance and therefore reflectance change and affect the oximetry results. Figures 9A and C show the ratio between light intensities inside retinal vessels at the two wavelengths of the oximeter (I_{605}/I_{586}). Since only light reflectance at 605 nm should change (increase) with oxygen saturation, this ratio should be sensitive for oxygen saturation. As can be seen in figures 9A and C the ratio is higher in the dark than in the light, which is in an agreement with increased oxygen saturation in the dark. The ratio of light intensities outside the vessel ($I_{0\text{-}605}/I_{0\text{-}586}$) is also higher in the dark (figure 9B and D) and this could create some bias. However, it can be seen from equations 4 and 6 in chapter 1.2.4 that this change in I_0 will tend to increase the optical density ratio in the dark and decrease the resulting saturation in the dark. This is opposite to the result. Therefore, this artefact will tend to attenuate the differences seen in saturation and not affect the conclusion of the study. The reason for the higher ratio $I_{0\text{-}605}/I_{0\text{-}586}$ in the dark is unclear but may be either or both, bleaching of photopigment or increased oxygen saturation in retinal capillaries and choroidal vasculature in the dark. Lack of bleaching in the dark will probably decrease the brightness less at 605 nm than on 586 nm. Increased oxygen saturation in capillaries and in the choroid will increase the brightness only at 605 nm.

In summary, global differences in fundus reflectance or fundus absorbance are not likely to have changed the conclusions of any study reported herein. The influence of extravascular haemorrhage is discussed in chapter 5.6.4 below.

5.6.3 The effect of vessel diameter on retinal oximetry

Beach et al. (1999) and Hammer et al. (2008) have found that oxygen saturation, measured with a dual wavelength oximeter, decreased with increased vessel diameter. Beach et al. could demonstrate that this is most likely a technical error since the relationship is present when healthy subjects inhale 100% oxygen and oxygen saturation in all major retinal arterioles is likely to be very close to 100%. The reason for the relationship between vessel diameter and measured saturation may be that light paths are different for

different sizes of vessels (Hammer et al., 2001). In wider vessels, for example, a greater proportion of the light coming from the vessel is backscattered from the blood while, in smaller vessels a greater proportion travels through the whole blood column, even twice. The contribution of each light path to the final signal is also dependent on the wavelength of the light.

The effect of vessel diameter on the current results has not been systematically tested. Nevertheless, it is most likely that the same relationship exists, i.e. decreased measured saturation with increased diameter. This is in agreement with experience, gained during the use of the instrument. With a later version of the oximeter used here, the artefactual decrease in saturation with increased vessel diameter was found to be about 1% for every 10% change in vessel diameter (unpublished own data)

A small decrease in measured saturation with increased vessel diameter is unlikely to have changed the conclusions of the studies, presented here. Theoretically, the small increase in arteriolar saturation after glaucoma surgery may have been exaggerated by a decrease in vessel diameter after surgery. Decreased saturation in some venous occlusions may likewise have been exaggerated if the affected venules were wide. However, the vessel diameter was not measured in either case. In other studies the effect of vessel diameter is more likely to have attenuated the differences in saturation, which were found. Dorzolamide would for example tend to increase diameter rather than reduce it and this would tend to decrease the measured saturation.

5.6.4 The effect of image quality on retinal oximetry

The effects of image quality on oximetry measurements are more difficult to quantify and correct for. Experience indicates that poor contrast, for example due to cataract, tends to lead to lower measured oxygen saturation. Poor focus seems to have less effect on results. This can be concluded by inspecting images of different quality of the same retina under the same conditions.

In most studies, described herein, a systematic difference in image quality between the groups or time-points is unlikely. Poor images were excluded from analysis. The most notable exceptions are the studies on retinal vein occlusions, where the haemorrhages can interfere with measurements. To minimise confounding, care was taken to choose measurement points where there was no haemorrhage and to use the better quality images. The better quality images may, however, be of those with worse disease. Therefore, a separate analysis was made on data from branch retinal vein occlusion, where more spurious images were also included. This did not change the conclusion of the study.

5.6.5 Other physiological confounders

In addition to fundus pigmentation and vessel diameter, several physiological factors may influence the measurements of retinal vessel oxygen saturation. These factors include different haemoglobin types, the effect of light exposure during measurements and the effect of pupil dilation.

The different haemoglobin types are described in chapter 1.2.3. Measurements of their concentration requires multiple wavelengths of light (Zijlstra et al., 2000). Such measurements can best be performed in blood samples although recent advances in pulse oximetry allow at least approximate non-invasive measurements of carboxyhaemoglobin and methaemoglobin (Barker and Badal, 2008). Examination of the equations, used for oximetry in this thesis (chapter 1.2.4), shows that carboxyhaemoglobin will increase measured saturation and methaemoglobin is more likely to decrease it. No measurements were made of different haemoglobin types in the current studies and finger oximetry values were not analysed. Neither finger oximetry values nor the ratio of different haemoglobin types is likely to be different between groups or time-points in the studies with the exception that glycosylated haemoglobin may have had slightly higher concentration in diabetic patients than in the healthy comparison group.

As is reported in chapter 4.1, retinal vessel oxygen saturation changes between light and dark. Therefore, light conditions during measurements must be standardised. The ambient light level in the room was kept low (chapter 3.1). The effect of repeated measurement flashes is more likely to have an effect although no such effect was detected. Values, which were compared, were obtained with the same imaging protocol and differences in flash exposure are therefore minimal.

Dilation of pupils was performed for almost all measurements and is therefore unlikely to confound comparisons. In some of the studies, phenylephrine was added to tropicamide when deemed necessary by nurses or ophthalmologists during a regular clinical visit. Better standardisation of dilation would have been preferred. Furthermore, a potential increase in intraocular pressure with dilation (Shaw and Lewis, 1986) or direct effects of dilating drops on retinal vessels should be kept in mind.

6 Conclusions and future perspectives

This thesis describes non-invasive measurements of retinal vessel oxygen saturation in health and disease. At the initiation of the project, few measurements were available on human retinal oxygenation and most of the knowledge on the potential role of oxygenation in disease was derived from animal studies and indirect evidence. The reason was that technology for non-invasive measurements of retinal oxygenation was scarce.

Studies in recent years, among them the studies described here, are beginning to further our understanding of human retinal oxygenation in health and disease. We now have evidence for increased retinal vessel oxygen saturation in the dark. Together with earlier results from animal studies and from (limited) studies on retinal blood flow, the present results clarify how the retina copes with the large change in energy demand between light and dark. The results of studies on retinal vessel occlusions confirm hypoxia in some cases but not all and this may point to a variable degree of recovery of flow or decreased oxygen demand. The results of measurements of diabetic retinopathy are more uniform; patients with all categories of diabetic retinopathy appear to have high retinal vessel oxygen saturation and this may be due to poor distribution of oxygen and other factors. Glaucoma treatment does not seem to have large effect on oxygen saturation and this is in agreement with recent evidence against hypoxia in glaucoma. Longitudinal studies are needed for further clarification and it is still unclear whether intermittent hypoxia may play a role in glaucoma, for example during periods of low blood pressure.

The present studies are therefore steps towards better understanding of retinal oxygenation in various diseases and in health. They have shown that dual wavelength non-invasive retinal oximetry can provide valuable data. To further build on these studies, a new retinal oximeter has recently been developed. It is based on the same principles but has better optics, more resolution and wider field of view. This will allow more detailed studies of the diseases studied here as well as other diseases. Among the challenges ahead are larger studies, which allow more comparison with clinical features and the use of vessel diameter measurements to help interpret the oxygen saturation changes found. Refinement of the measurement technique itself by correcting for known confounders will also be a major task.

Many common and serious retinal and optic nerve diseases are believed to involve disturbance in blood flow and / or oxygenation and the current thesis adds to the available evidence. The studies described herein and similar on-going studies aim at better understanding the pathophysiology. The final aim is,

however, to be able to use the knowledge and technology for better diagnosis, monitoring and treatment of disease.

7 References

Ahmed, J., Braun, R. D., Dunn, R., Jr. and Linsenmeier, R. A. (1993). Oxygen distribution in the macaque retina. *Invest Ophthalmol Vis Sci, 34*(3), 516-521.

Aiello, L. P., Avery, R. L., Arrigg, P. G., Keyt, B. A., Jampel, H. D., Shah, S. T., Pasquale, L. R., Thieme, H., Iwamoto, M. A., Park, J. E. and et al. (1994). Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *N Engl J Med, 331*(22), 1480-1487.

Alder, V. A., Ben-Nun, J. and Cringle, S. J. (1990). PO2 profiles and oxygen consumption in cat retina with an occluded retinal circulation. *Invest Ophthalmol Vis Sci, 31*(6), 1029-1034.

Alder, V. A. and Cringle, S. J. (1990). Vitreal and retinal oxygenation. *Graefes Arch Clin Exp Ophthalmol, 228*(2), 151-157.

Alder, V. A., Cringle, S. J. and Constable, I. J. (1983). The retinal oxygen profile in cats. *Invest Ophthalmol Vis Sci, 24*(1), 30-36.

Alder, V. A., Yu, D. Y., Cringle, S. J. and Su, E. N. (1991). Changes in vitreal oxygen tension distribution in the streptozotocin diabetic rat. *Diabetologia, 34*(7), 469-476.

Alm, A. and Bill, A. (1970). Blood flow and oxygen extraction in the cat uvea at normal and high intraocular pressures. *Acta Physiol Scand, 80*(1), 19-28.

Alm, A. and Bill, A. (1972a). The oxygen supply to the retina. I. Effects of changes in intraocular and arterial blood pressures, and in arterial P O2 and P CO2 on the oxygen tension in the vitreous body of the cat. *Acta Physiol Scand, 84*(2), 261-274.

Alm, A. and Bill, A. (1972b). The oxygen supply to the retina. II. Effects of high intraocular pressure and of increased arterial carbon dioxide tension on uveal and retinal blood flow in cats. A study with radioactively labelled microspheres including flow determinations in brain and some other tissues. *Acta Physiol Scand, 84*(3), 306-319.

Alm, A., Bill, A. and Young, F. A. (1973). The effects of pilocarpine and neostigmine on the blood flow through the anterior uvea in monkeys. A study with radioactively labelled microspheres. *Exp Eye Res, 15*(1), 31-36.

Altan-Yaycioglu, R., Turker, G., Akdol, S., Acunas, G. and Izgi, B. (2001). The effects of beta-blockers on ocular blood flow in patients with primary open angle glaucoma: a color Doppler imaging study. *Eur J Ophthalmol, 11*(1), 37-46.

Alvarez, Y., Chen, K., Reynolds, A. L., Waghorne, N., O'Connor, J. J. and Kennedy, B. N. Predominant cone photoreceptor dysfunction in a hyperglycaemic model of non-proliferative diabetic retinopathy. (2010). *Dis Model Mech, 3*(3-4), 236-245.

Ames, A., 3rd, Li, Y. Y., Heher, E. C. and Kimble, C. R. (1992). Energy metabolism of rabbit retina as related to function: high cost of Na+ transport. *J Neurosci, 12*(3), 840-853.

Appiah, A. P. and Trempe, C. L. (1989). Risk factors associated with branch vs. central retinal vein occlusion. *Ann Ophthalmol, 21*(4), 153-155, 157.

Arend, O., Harris, A., Arend, S., Remky, A. and Martin, B. J. (1998). The acute effect of topical beta-adrenoreceptor blocking agents on retinal and optic nerve head circulation. *Acta Ophthalmol Scand, 76*(1), 43-49.

Arend, O., Harris, A., Wolter, P. and Remky, A. (2003). Evaluation of retinal haemodynamics and retinal function after application of dorzolamide, timolol and latanoprost in newly diagnosed open-angle glaucoma patients. *Acta Ophthalmol Scand, 81*(5), 474-479.

Arjamaa, O. and Nikinmaa, M. (2006). Oxygen-dependent diseases in the retina: role of hypoxia-inducible factors. *Exp Eye Res, 83*(3), 473-483.

Ashton, N. (1974). Vascular basement membrane changes in diabetic retinopathy. Montgomery lecture, 1973. *Br J Ophthalmol, 58*(4), 344-366.

Attariwala, R., Giebs, C. P. and Glucksberg, M. R. (1994). The influence of elevated intraocular pressure on vascular pressures in the cat retina. *Invest Ophthalmol Vis Sci, 35*(3), 1019-1025.

Attariwala, R., Jensen, P. S. and Glucksberg, M. R. (1997). The effect of acute experimental retinal vein occlusion on cat retinal vein pressures. *Invest Ophthalmol Vis Sci, 38*(13), 2742-2749.

Avila, C. P., Jr., Bartsch, D. U., Bitner, D. G., Cheng, L., Mueller, A. J., Karavellas, M. P. and Freeman, W. R. (1998). Retinal blood flow measurements in branch retinal vein occlusion using scanning laser Doppler flowmetry. *Am J Ophthalmol, 126*(5), 683-690.

Barber, A. J., Gardner, T. W. and Abcouwer, S. F. The significance of vascular and neural apoptosis to the pathology of diabetic retinopathy. (2011). *Invest Ophthalmol Vis Sci, 52*(2), 1156-1163.

Barcsay, G., Seres, A. and Nemeth, J. (2003). The diameters of the human retinal branch vessels do not change in darkness. *Invest Ophthalmol Vis Sci, 44*(7), 3115-3118.

Barker, S. J. and Badal, J. J. (2008). The measurement of dyshemoglobins and total hemoglobin by pulse oximetry. *Curr Opin Anaesthesiol, 21*(6), 805-810.

Beach, J., Ning, J. and Khoobehi, B. (2007). Oxygen saturation in optic nerve head structures by hyperspectral image analysis. *Curr Eye Res, 32*(2), 161-170.

Beach, J. M., Schwenzer, K. J., Srinivas, S., Kim, D. and Tiedeman, J. S. (1999). Oximetry of retinal vessels by dual-wavelength imaging: calibration and influence of pigmentation. *J Appl Physiol, 86*(2), 748-758.

Bellelli, A. Hemoglobin and cooperativity: Experiments and theories. (2010). *Curr Protein Pept Sci, 11*(1), 2-36.

Bellelli, A. and Brunori, M. Hemoglobin allostery: variations on the theme. (2011). *Biochim Biophys Acta, 1807*(10), 1262-1272.

Benita, Y., Kikuchi, H., Smith, A. D., Zhang, M. Q., Chung, D. C. and Xavier, R. J. (2009). An integrative genomics approach identifies Hypoxia Inducible Factor-1 (HIF-1)-target genes that form the core response to hypoxia. *Nucleic Acids Res, 37*(14), 4587-4602.

Bergstrand, I. C., Heijl, A., Wollmer, P., Hansen, F. and Harris, A. (2001). Timolol increased retrobulbar flow velocities in untreated glaucoma eyes but not in ocular hypertension. *Acta Ophthalmol Scand, 79*(5), 455-461.

Berisha, F., Schmetterer, K., Vass, C., Dallinger, S., Rainer, G., Findl, O., Kiss, B. and Schmetterer, L. (2005). Effect of trabeculectomy on ocular blood flow. *Br J Ophthalmol, 89*(2), 185-188.

Bernd, A. S., Pillunat, L. E., Bohm, A. G., Schmidt, K. G. and Richard, G. (2001). [Ocular hemodynamics and visual field in glaucoma treated with dorzolamide]. *Ophthalmologe, 98*(5), 451-455. Paper in German.

Bill, A. (1963). The uveal venous pressure. *Arch Ophthalmol, 69*, 780-782.

Birol, G., Wang, S., Budzynski, E., Wangsa-Wirawan, N. D. and Linsenmeier, R. A. (2007). Oxygen Distribution and Consumption in the Macaque Retina. *Am J Physiol Heart Circ Physiol*.

Bjoernsdottir, S., Rossberger, J., Guethbjoernsdottir, H. S. and Hreietharsson, A. B. (2004). [Treatment pattern and results in an outpatient population with type 2 diabetes in Iceland.]. *Laeknabladid, 90*(9), 623-627.

Blair, N. P. (2000). Ocular oxygen consumption during vitreoperfusion in the cat. *Trans Am Ophthalmol Soc, 98*, 305-329.

Blondal, R., Sturludottir, M. K., Hardarson, S. H., Halldorsson, G. H. and Stefansson, E. (2011). Reliability of vessel diameter measurements with a retinal oximeter. *Graefes Arch Clin Exp Ophthalmol*.

Boles Carenini, A., Brogliatti, B., Sibour, G., Bellone, A. and Valli, A. (1997). Evaluation of the choroidal and retinal blood flows by means of the pOBF system and the Eco-Color-Doppler in glaucomatous patients after trabeculectomy surgery. *Acta Ophthalmol Scand Suppl*(224), 41-42.

Braun, R. D. and Linsenmeier, R. A. (1995). Retinal oxygen tension and the electroretinogram during arterial occlusion in the cat. *Invest Ophthalmol Vis Sci, 36*(3), 523-541.

Braun, R. D., Linsenmeier, R. A. and Goldstick, T. K. (1995). Oxygen consumption in the inner and outer retina of the cat. *Invest Ophthalmol Vis Sci, 36*(3), 542-554.

Brinchmann-Hansen, O., Myhre, K., Dahl-Jorgensen, K., Hanssen, K. F. and Sandvik, L. (1987). The central light reflex of retinal arteries and veins in insulin-dependent diabetic subjects. *Acta Ophthalmol (Copenh), 65*(4), 474-480.

Broadfoot, K. D., Gloster, J. and Greaves, D. P. (1961). Photoelectric method of investigating the amount and oxygenation of the blood in the fundus oculi. *Br J Ophthalmol, 45*(3), 161-182.

Brunelle, J. A., Degtiarov, A. M., Moran, R. F. and Race, L. A. (1996). Simultaneous measurement of total hemoglobin and its derivatives in blood using CO-oximeters: analytical principles; their application in selecting analytical wavelengths and reference methods; a comparison of the results of the choices made. *Scand J Clin Lab Invest Suppl, 224*, 47-69.

Budzynski, E., Smith, J. H., Bryar, P., Birol, G. and Linsenmeier, R. A. (2008). Effects of photocoagulation on intraretinal PO2 in cat. *Invest Ophthalmol Vis Sci, 49*(1), 380-389.

Buerk, D. G., Shonat, R. D., Riva, C. E. and Cranstoun, S. D. (1993). O2 gradients and countercurrent exchange in the cat vitreous humor near retinal arterioles and venules. *Microvasc Res, 45*(2), 134-148.

Burgansky-Eliash, Z., Barak, A., Barash, H., Nelson, D. A., Pupko, O., Lowenstein, A., Grinvald, A. and Rubinstein, A. Increased Retinal Blood Flow Velocity in Patients with Early Diabetes Mellitus. (2012). *Retina, 32*(1):112-119.

Burgansky-Eliash, Z., Nelson, D. A., Bar-Tal, O. P., Lowenstein, A., Grinvald, A. and Barak, A. Reduced retinal blood flow velocity in diabetic retinopathy. (2010). *Retina, 30*(5), 765-773.

Bursell, S. E., Clermont, A. C., Kinsley, B. T., Simonson, D. C., Aiello, L. M. and Wolpert, H. A. (1996). Retinal blood flow changes in patients with insulin-dependent diabetes mellitus and no diabetic retinopathy. *Invest Ophthalmol Vis Sci, 37*(5), 886-897.

Cantor, L. B. (2001). The effect of trabeculectomy on ocular hemodynamics. *Trans Am Ophthalmol Soc, 99*, 241-252.

Carenini, A. B., Sibour, G. and Boles Carenini, B. (1994). Differences in the longterm effect of timolol and betaxolol on the pulsatile ocular blood flow. *Surv Ophthalmol, 38 Suppl*, S118-124.

Christoffersen, N. L. and Larsen, M. (1999). Pathophysiology and hemodynamics of branch retinal vein occlusion. *Ophthalmology, 106*(11), 2054-2062.

Clemett, R. S. (1974). Retinal branch vein occlusion. Changes at the site of obstruction. *Br J Ophthalmol, 58*(5), 548-554.

Cogan, D. G. and Kuwabara, T. (1963). Capillary Shunts in the Pathogenesis of Diabetic Retinopathy. *Diabetes, 12*, 293-300.

Cogan, D. G., Toussaint, D. and Kuwabara, T. (1961). Retinal vascular patterns. IV. Diabetic retinopathy. *Arch Ophthalmol, 66*, 366-378.

Cringle, S. J., Yu, D. Y., Alder, V. and Su, E. N. (1999). Light and choroidal PO2 modulation of intraretinal oxygen levels in an avascular retina. *Invest Ophthalmol Vis Sci, 40*(10), 2307-2313.

Cringle, S. J., Yu, D. Y., Yu, P. K. and Su, E. N. (2002). Intraretinal oxygen consumption in the rat in vivo. *Invest Ophthalmol Vis Sci, 43*(6), 1922-1927.

Crittin, M., Schmidt, H. and Riva, C. E. (2002). Hemoglobin oxygen saturation (So2) in the human ocular fundus measured by reflectance oximetry: preliminary data in retinal veins. *Klin Monatsbl Augenheilkd, 219*(4), 289-291.

Danis, R. P. and Wallow, I. H. (1987). Microvascular changes in experimental branch retinal vein occlusion. *Ophthalmology, 94*(10), 1213-1221.

Database of Human Hemoglobin Variants and Thalassemias, http://globin.bx.psu.edu/hbvar/.

Dean, F. M., Arden, G. B. and Dornhorst, A. (1997). Partial reversal of protan and tritan colour defects with inhaled oxygen in insulin dependent diabetic subjects. *Br J Ophthalmol, 81*(1), 27-30.

Delaey, C. and Van De Voorde, J. (2000). Regulatory mechanisms in the retinal and choroidal circulation. *Ophthalmic Res, 32*(6), 249-256.

Delori, F. C. (1988). Noninvasive techniques for oximetry of blood in retinal vessels. *Applied Optics, 27*(6), 1113-1125.

Delori, F. C. and Pflibsen, K. (1989). Spectral reflectance of the human ocular fundus. *Applied Optics, 28*(6), 1061-1077.

Denninghoff, K. R., Smith, M. H., Chipman, R. A., Hillman, L. W., Jester, P. M., Hughes, C. E., Kuhn, F. and Rue, L. W. (1997). Retinal large vessel oxygen saturations correlate with early blood loss and hypoxia in anesthetized swine. *J Trauma, 43*(1), 29-34.

Denninghoff, K. R., Smith, M. H., Hillman, L. W., Redden, D. and Rue, L. W. (1998). Retinal venous oxygen saturation correlates with blood volume. *Acad Emerg Med, 5*(6), 577-582.

Denninghoff, K. R., Smith, M. H., Lompado, A. and Hillman, L. W. (2003). Retinal venous oxygen saturation and cardiac output during controlled hemorrhage and resuscitation. *J Appl Physiol, 94*(3), 891-896.

Ditzel, J. (1976). Oxygen transport impairment in diabetes. *Diabetes, 25*(2 SUPPL), 832-838.

Donati, G., Kapetanios, A., Dubois-Dauphin, M. and Pournaras, C. J. (2008). Caspase-related apoptosis in chronic ischaemic microangiopathy following experimental vein occlusion in mini-pigs. *Acta Ophthalmol, 86*(3), 302-306.

Donati, G., Pournaras, C. J., Pizzolato, G. P. and Tsacopoulos, M. (1997). Decreased nitric oxide production accounts for secondary arteriolar constriction after retinal branch vein occlusion. *Invest Ophthalmol Vis Sci, 38*(7), 1450-1457.

Donati, G., Pournaras, C. J. and Tsacopoulos, M. (1998). Effect of nitroprusside on arteriolar constriction after retinal branch vein occlusion. *Invest Ophthalmol Vis Sci, 39*(10), 1910-1917.

Doyle, M. L., Di Cera, E. and Gill, S. J. (1988). Effect of differences in optical properties of intermediate oxygenated species of hemoglobin A0 on Adair constant determination. *Biochemistry, 27*(2), 820-824.

Drasdo, N., Chiti, Z., Owens, D. R. and North, R. V. (2002). Effect of darkness on inner retinal hypoxia in diabetes. *Lancet, 359*(9325), 2251-2253.

Duker, J. S. and Brown, G. C. (1989). Anterior location of the crossing artery in branch retinal vein obstruction. *Arch Ophthalmol, 107*(7), 998-1000.

Ehinger, B. (1966). Adrenergic nerves to the eye and related structures in man and in the cynomolgus monkey (Macaca Irus). *Invest Ophthalmol Vis Sci, 5*(1), 42-52.

Ehlken, C., Rennel, E. S., Michels, D., Grundel, B., Pielen, A., Junker, B., Stahl, A., Hansen, L. L., Feltgen, N., Agostini, H. T. and Martin, G. Levels of VEGF but not VEGF(165b) are increased in the vitreous of patients with retinal vein occlusion. (2011). *Am J Ophthalmol, 152*(2), 298-303.

Ernest, J. T. and Archer, D. B. (1979). Vitreous body oxygen tension following experimental branch retinal vein obstruction. *Invest Ophthalmol Vis Sci, 18*(10), 1025-1029.

Ernest, J. T., Goldstick, T. K. and Engerman, R. L. (1983). Hyperglycemia impairs retinal oxygen autoregulation in normal and diabetic dogs. *Invest Ophthalmol Vis Sci, 24*(7), 985-989.

Evans, D. W., Harris, A. and Cantor, L. B. (1999). Primary open-angle glaucoma patients characterized by ocular vasospasm demonstrate a different ocular vascular response to timolol versus betaxolol. *J Ocul Pharmacol Ther, 15*(6), 479-487.

Feke, G. T., Tagawa, H., Deupree, D. M., Goger, D. G., Sebag, J. and Weiter, J. J. (1989). Blood flow in the normal human retina. *Invest Ophthalmol Vis Sci, 30*(1), 58-65.

Feke, G. T., Zuckerman, R., Green, G. J. and Weiter, J. J. (1983). Response of human retinal blood flow to light and dark. *Invest Ophthalmol Vis Sci, 24*(1), 136-141.

Fischbach, F. T. and Dunning, M. B. (2009). *A manual of laboratory and diagnostic tests* (8 ed.). Philadelphia: Wolters Kluwer Health / Lippincott Williams & Wilkins.

Flammer, J., Orgul, S., Costa, V. P., Orzalesi, N., Krieglstein, G. K., Serra, L. M., Renard, J. P. and Stefansson, E. (2002). The impact of ocular blood flow in glaucoma. *Prog Retin Eye Res, 21*(4), 359-393.

Foroozan, R., Deramo, V. A., Buono, L. M., Jayamanne, D. G., Sergott, R. C., Danesh-Meyer, H. and Savino, P. J. (2003). Recovery of visual function in patients with biopsy-proven giant cell arteritis. *Ophthalmology, 110*(3), 539-542.

Frangieh, G. T., Green, W. R., Barraquer-Somers, E. and Finkelstein, D. (1982). Histopathologic study of nine branch retinal vein occlusions. *Arch Ophthalmol, 100*(7), 1132-1140.

Fuchsjager-Mayrl, G., Malec, M., Amoako-Mensah, T., Kolodjaschna, J. and Schmetterer, L. (2003). Changes in choroidal blood flow during light/dark transitions are not altered by atropine or propranolol in healthy subjects. *Vision Res, 43*(20), 2185-2190.

Fuchsjager-Mayrl, G., Polska, E., Malec, M. and Schmetterer, L. (2001). Unilateral light-dark transitions affect choroidal blood flow in both eyes. *Vision Res, 41*(22), 2919-2924.

Fuchsjager-Mayrl, G., Wally, B., Rainer, G., Buehl, W., Aggermann, T., Kolodjaschna, J., Weigert, G., Polska, E., Eichler, H. G., Vass, C. and Schmetterer, L. (2005). Effect of dorzolamide and timolol on ocular blood flow in patients with primary open angle glaucoma and ocular hypertension. *Br J Ophthalmol, 89*(10), 1293-1297.

Fujio, N., Feke, G. T., Ogasawara, H., Goger, D. G., Yoshida, A. and McMeel, J. W. (1994). Quantitative circulatory measurements in branch retinal vessel occlusion. *Eye, 8 (Pt 3)*, 324-328.

Funatsu, H., Wilson, C. A., Berkowitz, B. A. and Sonkin, P. L. (1997). A comparative study on the effects of argon and diode laser photocoagulation on retinal oxygenation. *Graefes Arch Clin Exp Ophthalmol, 235*(3), 168-175.

Funk, M., Kriechbaum, K., Prager, F., Benesch, T., Georgopoulos, M., Zlabinger, G. J. and Schmidt-Erfurth, U. (2009). Intraocular concentrations of growth factors and cytokines in retinal vein occlusion and the effect of therapy with bevacizumab. *Invest Ophthalmol Vis Sci, 50*(3), 1025-1032.

Galassi, F., Sodi, A., Renieri, G., Ucci, F., Pieri, B., Harris, A. and Siesky, B. (2002). Effects of timolol and dorzolamide on retrobulbar hemodynamics in patients with newly diagnosed primary open-angle glaucoma. *Ophthalmologica, 216*(2), 123-128.

Gehlert, S., Dawczynski, J., Hammer, M. and Strobel, J. (2010). [Haemoglobin oxygenation of retinal vessels in branch retinal artery occlusions over time and correlation with clinical outcome]. *Klin Monbl Augenheilkd, 227*(12), 976-980.

Genevois, O., Paques, M., Simonutti, M., Sercombe, R., Seylaz, J., Gaudric, A., Brouland, J. P., Sahel, J. and Vicaut, E. (2004). Microvascular remodeling after occlusion-recanalization of a branch retinal vein in rats. *Invest Ophthalmol Vis Sci, 45*(2), 594-600.

Gilmore, E. D., Hudson, C., Nrusimhadevara, R. K., Harvey, P. T., Mandelcorn, M., Lam, W. C. and Devenyi, R. G. (2007). Retinal arteriolar diameter, blood velocity, and blood flow response to an isocapnic hyperoxic provocation in early sight-threatening diabetic retinopathy. *Invest Ophthalmol Vis Sci, 48*(4), 1744-1750.

Gilmore, E. D., Hudson, C., Nrusimhadevara, R. K., Ridout, R., Harvey, P. T., Mandelcorn, M., Lam, W. C. and Devenyi, R. G. (2007). Retinal arteriolar hemodynamic response to an acute hyperglycemic provocation in early and sight-threatening diabetic retinopathy. *Microvasc Res, 73*(3), 191-197.

Glucksberg, M. R. and Dunn, R. (1993). Direct measurement of retinal microvascular pressures in the live, anesthetized cat. *Microvasc Res, 45*(2), 158-165.

Gottfredsdottir, M. S., Stefansson, E., Jonasson, F. and Gislason, I. (1993). Retinal vasoconstriction after laser treatment for diabetic macular edema. *Am J Ophthalmol, 115*(1), 64-67.

Green, W. R., Chan, C. C., Hutchins, G. M. and Terry, J. M. (1981). Central retinal vein occlusion: a prospective histopathologic study of 29 eyes in 28 cases. *Trans Am Ophthalmol Soc, 79*, 371-422.

Grieshaber, M. C., Mozaffarieh, M. and Flammer, J. (2007). What is the link between vascular dysregulation and glaucoma? *Surv Ophthalmol, 52 Suppl 2*, S144-154.

Grunwald, J. E. (1986). Effect of topical timolol on the human retinal circulation. *Invest Ophthalmol Vis Sci, 27*(12), 1713-1719.

Grunwald, J. E. (1990). Effect of timolol maleate on the retinal circulation of human eyes with ocular hypertension. *Invest Ophthalmol Vis Sci, 31*(3), 521-526.

Grunwald, J. E., Riva, C. E., Brucker, A. J., Sinclair, S. H. and Petrig, B. L. (1984). Altered retinal vascular response to 100% oxygen breathing in diabetes mellitus. *Ophthalmology, 91*(12), 1447-1452.

Grunwald, J. E., Riva, C. E., Martin, D. B., Quint, A. R. and Epstein, P. A. (1987). Effect of an insulin-induced decrease in blood glucose on the human diabetic retinal circulation. *Ophthalmology, 94*(12), 1614-1620.

Grunwald, J. E., Riva, C. E., Petrig, B. L., Sinclair, S. H. and Brucker, A. J. (1984). Effect of pure O2-breathing on retinal blood flow in normals and in patients with background diabetic retinopathy. *Curr Eye Res, 3*(1), 239-241.

Hafez, A. S., Bizzarro, R. L., Rivard, M. and Lesk, M. R. (2003). Changes in optic nerve head blood flow after therapeutic intraocular pressure reduction in glaucoma patients and ocular hypertensives. *Ophthalmology, 110*(1), 201-210.

Halldorsdottir, H., Steinsdottir, F. K., Gudmundsdottir, A., Smari, J. and Arnarson, E. O. (2009). [Clinical status and treatment adherence of young adults with type one diabetes mellitus following transition to adult health care]. *Laeknabladid, 95*(11), 755-761. Paper in Icelandic.

Hamilton, A. M., Kohner, E. M., Rosen, D., Bird, A. C. and Dollery, C. T. (1979). Experimental retinal branch vein occlusion in rhesus monkeys. I. Clinical appearances. *Br J Ophthalmol, 63*(6), 377-387.

Hamilton, A. M., Kohner, E. M., Rosen, D. and Bowbyes, J. A. (1974). Experimental venous occlusion. *Proc R Soc Med, 67*(10), 1045-1048.

Hammer, M., Leistritz, S., Leistritz, L. and Schweitzer, D. (2001). Light paths in retinal vessel oxymetry. *IEEE Trans Biomed Eng, 48*(5), 592-598.

Hammer, M., Riemer, T., Vilser, W., Gehlert, S. and Schweitzer, D. (2009, Saturday 24 January 2009). *A new imaging technique for retinal vessel oximetry: principles and first clinical results in patients with retinal arterial occlusion and diabetic retinopathy.* Paper presented at the Ophthalmic Technologies XIX San Jose, CA, USA

Hammer, M., Schweitzer, D., Michel, B., Thamm, E. and Kolb, A. (1998). Single scattering by red blood cells. *Applied Optics, 37*(31), 7410-7418.

Hammer, M., Thamm, E. and Schweitzer, D. (2002). A simple algorithm for in vivo ocular fundus oximetry compensating for non-haemoglobin absorption and scattering. *Phys Med Biol, 47*(17), N233-238.

Hammer, M., Vilser, W., Riemer, T., Mandecka, A., Schweitzer, D., Kuhn, U., Dawczynski, J., Liemt, F. and Strobel, J. (2009). Diabetic patients with retinopathy show increased retinal venous oxygen saturation. *Graefes Arch Clin Exp Ophthalmol*.

Hammer, M., Vilser, W., Riemer, T. and Schweitzer, D. (2008). Retinal vessel oximetry-calibration, compensation for vessel diameter and fundus pigmentation, and reproducibility. *Journal of Biomedical Optics, 13*(5), -.

Hardarson, S. H., Harris, A., Karlsson, R. A., Halldorsson, G. H., Kagemann, L., Rechtman, E., Zoega, G. M., Eysteinsson, T., Benediktsson, J. A., Thorsteinsson, A., Jensen, P. K., Beach, J. and Stefansson, E. (2006). Automatic retinal oximetry. *Invest Ophthalmol Vis Sci, 47*(11), 5011-5016.

Hardarson, S. H. and Stefansson, E. (2011). Retinal oxygenation in diabetic retinopathy [ARVO conference abstract]. *Invest Ophthalmol Vis Sci, 52*. E-abstract 1275

Harms, M. P., van Lieshout, J. J., Jenstrup, M., Pott, F. and Secher, N. H. (2003). Postural effects on cardiac output and mixed venous oxygen saturation in humans. *Exp Physiol, 88*(5), 611-616.

Harris, A., Arend, O., Danis, R. P., Evans, D., Wolf, S. and Martin, B. J. (1996). Hyperoxia improves contrast sensitivity in early diabetic retinopathy. *Br J Ophthalmol, 80*(3), 209-213.

Harris, A., Dinn, R. B., Kagemann, L. and Rechtman, E. (2003). A review of methods for human retinal oximetry. *Ophthalmic Surg Lasers Imaging, 34*(2), 152-164.

Harvey, A. R., Fletcher-Holmes, D. W., Gorman, A., Altenbach, K., Arlt, J. and Read, N. D. (2005). Spectral imaging in a snapshot. *Spectral Imaging: Instrumentation, Applications, and Analysis III. Proceedings of the SPIE*(5694), 110-119.

Haugh-Scheidt, L. M., Griff, E. R. and Linsenmeier, R. A. (1995). Light-evoked oxygen responses in the isolated toad retina. *Exp Eye Res, 61*(1), 73-81.

Haugh-Scheidt, L. M., Linsenmeier, R. A. and Griff, E. R. (1995). Oxygen consumption in the isolated toad retina. *Exp Eye Res, 61*(1), 63-72.

Haugh, L. M., Linsenmeier, R. A. and Goldstick, T. K. (1990). Mathematical models of the spatial distribution of retinal oxygen tension and consumption, including changes upon illumination. *Ann Biomed Eng, 18*(1), 19-36.

Havelius, U., Hansen, F., Hindfelt, B. and Krakau, T. (1999). Human ocular vasodynamic changes in light and darkness. *Invest Ophthalmol Vis Sci, 40*(8), 1850-1855.

Hayreh, S. S. (2011). Acute retinal arterial occlusive disorders. *Prog Retin Eye Res, 30*(5), 359-394.

Hayreh, S. S. (1975). Segmental nature of the choroidal vasculature. *Br J Ophthalmol, 59*(11), 631-648.

Hayreh, S. S. (1999). Blood supply of the optic nerve head. A 'reality check'. In L. E. Pillunat, A. Harris, D. R. Anderson & E. L. Greve (Eds.), *Current concepts on ocular blood flow in glaucoma* (pp. 3-31). The Hague: Kugler Publications.

Hayreh, S. S. (2005). Prevalent misconceptions about acute retinal vascular occlusive disorders. *Prog Retin Eye Res, 24*(4), 493-519.

Hayreh, S. S. and Jonas, J. B. (2000). Optic disk and retinal nerve fiber layer damage after transient central retinal artery occlusion: an experimental study in rhesus monkeys. *Am J Ophthalmol, 129*(6), 786-795.

Hayreh, S. S., Podhajsky, P. A. and Zimmerman, M. B. (2009). Retinal artery occlusion: associated systemic and ophthalmic abnormalities. *Ophthalmology, 116*(10), 1928-1936.

Hayreh, S. S. and Zimmerman, M. B. (2005). Central retinal artery occlusion: visual outcome. *Am J Ophthalmol, 140*(3), 376-391.

Hayreh, S. S., Zimmerman, M. B., Kimura, A. and Sanon, A. (2004). Central retinal artery occlusion. Retinal survival time. *Exp Eye Res, 78*(3), 723-736.

Hayreh, S. S., Zimmerman, M. B. and Podhajsky, P. (1994). Incidence of various types of retinal vein occlusion and their recurrence and demographic characteristics. *Am J Ophthalmol, 117*(4), 429-441.

Henkind, P. (1967). Radial peripapillary capillaries of the retina. I. Anatomy: human and comparative. *Br J Ophthalmol, 51*(2), 115-123.

Hickam, J. B., Sieker, H. O. and Frayser, R. (1959). Studies of retinal circulation and A-V oxygen difference in man. *Trans Am Clin Climatol Assoc, 71*, 34-44.

Hitchings, R. A. and Spaeth, G. L. (1976). Chronic retinal vein occlusion in glaucoma. *Br J Ophthalmol, 60*(10), 694-699.

Hockley, D. J., Tripathi, R. C. and Ashton, N. (1979). Experimental retinal branch vein occlusion in rhesus monkeys. III. Histopathological and electron microscopical studies. *Br J Ophthalmol, 63*(6), 393-411.

Holekamp, N. M., Shui, Y. B. and Beebe, D. (2006). Lower intraocular oxygen tension in diabetic patients: possible contribution to decreased incidence of nuclear sclerotic cataract. *Am J Ophthalmol, 141*(6), 1027-1032.

Holekamp, N. M., Shui, Y. B. and Beebe, D. C. (2005). Vitrectomy surgery increases oxygen exposure to the lens: a possible mechanism for nuclear cataract formation. *Am J Ophthalmol, 139*(2), 302-310.

Horio, N. and Horiguchi, M. (2004). Retinal blood flow analysis using intraoperative video fluorescein angiography combined with optical fiber-free intravitreal surgery system. *Am J Ophthalmol, 138*(6), 1082-1083.

Huemer, K. H., Garhofer, G., Aggermann, T., Kolodjaschna, J., Schmetterer, L. and Fuchsjager-Mayrl, G. (2007). Role of nitric oxide in choroidal blood flow regulation during light/dark transitions. *Invest Ophthalmol Vis Sci, 48*(9), 4215-4219.

Ikeda, F. and Kishi, S. Inner neural retina loss in central retinal artery occlusion. (2010). *Jpn J Ophthalmol, 54*(5), 423-429.

James, C. B. (1994). Effect of trabeculectomy on pulsatile ocular blood flow. *Br J Ophthalmol, 78*(11), 818-822.

Johnson, W. R., Wilson, D. W., Fink, W., Humayun, M. and Bearman, G. (2007). Snapshot hyperspectral imaging in ophthalmology. *J Biomed Opt, 12*(1), 014036.

Jonas, J. B. and Harder, B. (2007). Ophthalmodynamometric differences between ischemic vs nonischemic retinal vein occlusion. *Am J Ophthalmol, 143*(1), 112-116.

Justesen, B. L., Mistry, P., Chaturvedi, N., Thom, S. A., Witt, N., Kohler, D., Hughes, A. D. and Sjolie, A. K. Retinal arterioles have impaired reactivity to hyperoxia in type 1 diabetes. (2010). *Acta Ophthalmol, 88*(4), 453-457.

Justice, J., Jr. and Lehmann, R. P. (1976). Cilioretinal arteries. A study based on review of stereo fundus photographs and fluorescein angiographic findings. *Arch Ophthalmol, 94*(8), 1355-1358.

Kang, G. and Lee, J. (1995). Retinal circulation times in branch retinal vein occlusion. *Korean J Ophthalmol, 9*(2), 107-110.

Karlsson, R. A., Hardarson, S. H., Stefansson, E., Halldorsson, G. H., Basit, S., Eysteinsson, T., Benediktsson, J. A., Harris, A. and Beach J. M. Counter-current oxygen flux from arterioles to venules in the human retinal circulation [ARVO conference abstract]. (2007). *Invest Ophthalmol Vis Sci, 48*. E-Abstract 2290.

Kashani, A. H., Kirkman, E., Martin, G. and Humayun, M. S. (2011). Hyperspectral computed tomographic imaging spectroscopy of vascular oxygen gradients in the rabbit retina in vivo. *PLoS One*, *6*(9), e24482.

Kaur, C., Foulds, W. S. and Ling, E. A. (2008). Blood-retinal barrier in hypoxic ischaemic conditions: basic concepts, clinical features and management. *Prog Retin Eye Res*, *27*(6), 622-647.

Khoobehi, B., Beach, J. M. and Kawano, H. (2004). Hyperspectral imaging for measurement of oxygen saturation in the optic nerve head. *Invest Ophthalmol Vis Sci*, *45*(5), 1464-1472.

Khoobehi, B., Chiroli, V., Ronchetti, D., Miglietta, D., Thompson, H., Ongini, E. and Impagnatiello, F. Enhanced oxygen saturation in optic nerve head of non-human primate eyes following the intravitreal injection of NCX 434, an innovative nitric oxide-donating glucocorticoid. (2011). *J Ocul Pharmacol Ther*, *27*(2), 115-121.

Kiel, J. W. and Shepherd, A. P. (1992). Autoregulation of choroidal blood flow in the rabbit. *Invest Ophthalmol Vis Sci*, *33*(8), 2399-2410.

Kiilgaard, J. F., Pedersen, D. B., Eysteinsson, T., la Cour, M., Bang, K., Jensen, P. K. and Stefansson, E. (2004). Optic nerve oxygen tension: the effects of timolol and dorzolamide. *Br J Ophthalmol*, *88*(2), 276-279.

Kitaya, N., Yoshida, A., Ishiko, S., Mori, F., Abiko, T., Ogasawara, H., Kato, Y. and Nagaoka, T. (1997). Effect of timolol and UF-021 (a prostaglandin-related compound) on pulsatile ocular blood flow in normal volunteers. *Ophthalmic Res*, *29*(3), 139-144.

Klein, R., Klein, B. E., Moss, S. E. and Meuer, S. M. (2000). The epidemiology of retinal vein occlusion: the Beaver Dam Eye Study. *Trans Am Ophthalmol Soc*, *98*, 133-141; discussion 141-133.

Klein, R., Klein, B. E., Moss, S. E., Wong, T. Y. and Sharrett, A. R. (2006). Retinal vascular caliber in persons with type 2 diabetes: the Wisconsin Epidemiological Study of Diabetic Retinopathy: XX. *Ophthalmology*, *113*(9), 1488-1498.

Kohner, E. M. (1993). Diabetic retinopathy. *Bmj*, *307*(6913), 1195-1199.

Kong, X., Wang, K., Sun, X. and Witt, R. E. (2010). Comparative study of the retinal vessel anatomy of rhesus monkeys and humans. *Clin Experiment Ophthalmol*, *38*(6), 629-634.

Kristinsson, J. K., Gottfredsdottir, M. S. and Stefansson, E. (1997). Retinal vessel dilatation and elongation precedes diabetic macular oedema. *Br J Ophthalmol*, *81*(4), 274-278.

Kunkel, H. G., Ceppellini, R., Müller-Eberhard, U. and Wolf, J. (1957). Observations on the minor basic hemoglobin component in the blood of normal individuals and patients with thalassemia. *J Clin Invest*, *36*(11), 1615-1625.

Kurtenbach, A., Mayser, H. M., Jagle, H., Fritsche, A. and Zrenner, E. (2006). Hyperoxia, hyperglycemia, and photoreceptor sensitivity in normal and diabetic subjects. *Vis Neurosci*, *23*(3-4), 651-661.

Kuwabara, T. and Cogan, D. G. (1963). Retinal vascular patterns. VI. Mural cells of the retinal capillaries. *Arch Ophthalmol*, *69*, 492-502.

la Cour, M., Kiilgaard, J. F., Eysteinsson, T., Wiencke, A. K., Bang, K., Dollerup, J., Jensen, P. K. and Stefansson, E. (2000). Optic nerve oxygen tension: effects of intraocular pressure and dorzolamide. *Br J Ophthalmol*, *84*(9), 1045-1049.

Laatikainen, L. (1977). Preliminary report on effect of retinal panphotocoagulation on rubeosis iridis and neovascular glaucoma. *Br J Ophthalmol, 61*(4), 278-284.

Laing, R. A., Cohen, A. J. and Friedman E. (1975). Photographic measurements of retinal blood oxygen saturation: falling saturation rabbit experiments. *Invest Ophthalmol, 14*(8), 606-610.

Laing, R. A., Danisch L. A. and Young R. (1969). Non-invasive, multichromatic eye oximeter. Retrieved from www.archive.org/details/nasa_techdoc_19700011123

Landers, M. B., 3rd. (1978). Retinal oxygenation via the choroidal circulation. *Trans Am Ophthalmol Soc, 76*, 528-556.

Landers, M. B., 3rd, Stefansson, E. and Wolbarsht, M. L. (1982). Panretinal photocoagulation and retinal oxygenation. *Retina, 2*(3), 167-175.

Lange, C. A. and Bainbridge, J. W. (2011). Oxygen Sensing in Retinal Health and Disease. *Ophthalmologica*.

Lange, C. A., Stavrakas, P., Luhmann, U. F., de Silva, D. J., Ali, R. R., Gregor, Z. J. and Bainbridge, J. W. (2011). Intraocular oxygen distribution in advanced proliferative diabetic retinopathy. *Am J Ophthalmol, 152*(3), 406-412.

Laties, A. M. (1967). Central retinal artery innervation. Absence of adrenergic innervation to the intraocular branches. *Arch Ophthalmol, 77*(3), 405-409.

Lazareva, E. N. and Tuchin, V. V. (2007). *Dynamics of visible absorbance spectrum of hemoglobin solution incubated with glucose.* Paper presented at the The 6th international conference on photonics and imaging in biology and medicine, Wuhan, China.

Linsenmeier, R. A. (1986). Effects of light and darkness on oxygen distribution and consumption in the cat retina. *J Gen Physiol, 88*(4), 521-542.

Linsenmeier, R. A. and Braun, R. D. (1992). Oxygen distribution and consumption in the cat retina during normoxia and hypoxemia. *J Gen Physiol, 99*(2), 177-197.

Linsenmeier, R. A., Braun, R. D., McRipley, M. A., Padnick, L. B., Ahmed, J., Hatchell, D. L., McLeod, D. S. and Lutty, G. A. (1998). Retinal hypoxia in long-term diabetic cats. *Invest Ophthalmol Vis Sci, 39*(9), 1647-1657.

Linsenmeier, R. A. and Yancey, C. M. (1989). Effects of hyperoxia on the oxygen distribution in the intact cat retina. *Invest Ophthalmol Vis Sci, 30*(4), 612-618.

Loduca, A. L., Zhang, C., Zelkha, R. and Shahidi, M. (2010). Thickness mapping of retinal layers by spectral-domain optical coherence tomography. *Am J Ophthalmol, 150*(6), 849-855.

Longo, A., Geiser, M. and Riva, C. E. (2000). Subfoveal choroidal blood flow in response to light-dark exposure. *Invest Ophthalmol Vis Sci, 41*(9), 2678-2683.

Lubeck, P., Orgul, S., Gugleta, K., Gherghel, D., Gekkieva, M. and Flammer, J. (2001). Effect of timolol on anterior optic nerve blood flow in patients with primary open-angle glaucoma as assessed by the Heidelberg retina flowmeter. *J Glaucoma, 10*(1), 13-17.

Luckie, A. P., Wroblewski, J. J., Bird, A. C., Hamilton, A. M., Sanders, M. D., Green, W. and Slater, N. G. (1996). The venous closing pressure in central retinal vein obstruction. *Aust N Z J Ophthalmol, 24*(3), 233-238.

Maeda, N. and Tano, Y. (1996). Intraocular oxygen tension in eyes with proliferative diabetic retinopathy with and without vitreous. *Graefes Arch Clin Exp Ophthalmol, 234 Suppl 1*, S66-69.

Mansour, A. M. and Younis, M. H. (2011). Reappraisal of maximal paracentesis in central retinal artery occlusion. *Acta Ophthalmol, 89*(2), e207-208.

McLeod, D. (2010). Krogh cylinders in retinal development, panretinal hypoperfusion and diabetic retinopathy. *Acta Ophthalmol, 88*(8), 817-835.

Medrano, C. J. and Fox, D. A. (1995). Oxygen consumption in the rat outer and inner retina: light- and pharmacologically-induced inhibition. *Exp Eye Res, 61*(3), 273-284.

Michelson, G. and Scibor, M. (2006). Intravascular oxygen saturation in retinal vessels in normal subjects and open-angle glaucoma subjects. *Acta Ophthalmol Scand, 84*(3), 289-295.

Minamikawa, M., Yamamoto, K. and Okuma, H. (1993). [Experimental retinal branch vein occlusion. 4. Pathological changes in the middle and late stage]. *Nippon Ganka Gakkai Zasshi, 97*(8), 920-927. Article in Japanese.

Molnar, I., Poitry, S., Tsacopoulos, M., Gilodi, N. and Leuenberger, P. M. (1985). Effect of laser photocoagulation on oxygenation of the retina in miniature pigs. *Invest Ophthalmol Vis Sci, 26*(10), 1410-1414.

Mordant, D. J., Al-Abboud, I., Muyo, G., Gorman, A., Sallam, A., Ritchie, P., Harvey, A. R. and McNaught, A. I. (2011). Spectral imaging of the retina. *Eye (Lond), 25*(3), 309-320.

Mordant, D. J., Al-Abboud, I., Muyo, G., Gorman, A., Sallam, A., Rodmell, P., Crowe, J., Morgan, S., Ritchie, P., Harvey, A. R. and McNaught, A. I. (2011). Validation of human whole blood oximetry, using a hyperspectral fundus camera with a model eye. *Invest Ophthalmol Vis Sci, 52*(5), 2851-2859.

Morgan, W. H., Yu, D. Y., Cooper, R. L., Alder, V. A., Cringle, S. J. and Constable, I. J. (1997). Retinal artery and vein pressures in the dog and their relationship to aortic, intraocular, and cerebrospinal fluid pressures. *Microvasc Res, 53*(3), 211-221.

Murray, D. L., Feke, G. T. and Weiter, J. J. (1991). Preretinal pH changes in the rabbit under conditions of light and dark. *Exp Eye Res, 53*(6), 717-722.

Nagaoka, T., Sato, E., Takahashi, A., Yokota, H., Sogawa, K. and Yoshida, A. (2010). Impaired retinal circulation in patients with type 2 diabetes mellitus: retinal laser Doppler velocimetry study. *Invest Ophthalmol Vis Sci, 51*(12), 6729-6734.

Nagaoka, T. and Yoshida, A. (2004). The effect of ocular warming on ocular circulation in healthy humans. *Arch Ophthalmol, 122*(10), 1477-1481.

Neuhuber, W. and Schrodl, F. (2011). Autonomic control of the eye and the iris. *Auton Neurosci, 165*(1), 67-69.

Nguyen, Q. D., Shah, S. M., Van Anden, E., Sung, J. U., Vitale, S. and Campochiaro, P. A. (2004). Supplemental oxygen improves diabetic macular edema: a pilot study. *Invest Ophthalmol Vis Sci, 45*(2), 617-624.

Nickla, D. L. and Wallman, J. The multifunctional choroid. (2010). *Prog Retin Eye Res, 29*(2), 144-168.

Nicolela, M. T., Buckley, A. R., Walman, B. E. and Drance, S. M. (1996). A comparative study of the effects of timolol and latanoprost on blood flow velocity of the retrobulbar vessels. *Am J Ophthalmol, 122*(6), 784-789.

Noergaard, M. H., Bach-Holm, D., Scherfig, E., Bang, K., Jensen, P. K., Kiilgaard, J. F., Stefansson, E. and la Cour, M. (2008). Dorzolamide increases retinal oxygen tension after branch retinal vein occlusion. *Invest Ophthalmol Vis Sci, 49*(3), 1136-1141.

Noma, H., Funatsu, H., Mimura, T., Eguchi, S. and Hori, S. (2011). Soluble Vascular Endothelial Growth Factor Receptor-2 and Inflammatory Factors in Macular Edema with Branch Retinal Vein Occlusion. *Am J Ophthalmol, 152*(4): 669-677.

Noma, H., Funatsu, H., Mimura, T., Tatsugawa, M., Shimada, K. and Eguchi, S. (2012). Vitreous Inflammatory Factors and Serous Macular Detachment in Branch Retinal Vein Occlusion. *Retina, 32*(1), 86-91.

Noma, H., Funatsu, H., Sakata, K., Harino, S., Mimura, T. and Hori, S. (2009). Macular microcirculation in hypertensive patients with and without branch retinal vein occlusion. *Acta Ophthalmol, 87*(6), 638-642.

Noma, H., Funatsu, H., Sakata, K., Harino, S., Nagaoka, T., Mimura, T., Sone, T. and Hori, S. (2009). Macular microcirculation and macular oedema in branch retinal vein occlusion. *Br J Ophthalmol, 93*(5), 630-633.

Novack, R. L., Stefansson, E. and Hatchell, D. L. (1990). The effect of photocoagulation on the oxygenation and ultrastructure of avascular retina. *Exp Eye Res, 50*(3), 289-296.

Olafsdottir, O. B., Hardarson, S. H., Gottfredsdottir, M. S., Harris, A. and Stefansson, E. (2011). Retinal oximetry in primary open-angle glaucoma. *Invest Ophthalmol Vis Sci, 52*(9), 6409-6413.

Parver, L. M., Auker, C. and Carpenter, D. O. (1980). Choroidal blood flow as a heat dissipating mechanism in the macula. *Am J Ophthalmol, 89*(5), 641-646.

Patel, V., Rassam, S. M., Chen, H. C. and Kohner, E. M. (1994). Oxygen reactivity in diabetes mellitus: effect of hypertension and hyperglycaemia. *Clin Sci (Lond), 86*(6), 689-695.

Pedersen, D. B., Eysteinsson, T., Stefansson, E., Kiilgaard, J. F., La Cour, M., Bang, K. and Jensen, P. K. (2004). Indomethacin lowers optic nerve oxygen tension and reduces the effect of carbonic anhydrase inhibition and carbon dioxide breathing. *Br J Ophthalmol, 88*(8), 1088-1091.

Pedersen, D. B., Koch Jensen, P., la Cour, M., Kiilgaard, J. F., Eysteinsson, T., Bang, K., Wiencke, A. K. and Stefansson, E. (2005). Carbonic anhydrase inhibition increases retinal oxygen tension and dilates retinal vessels. *Graefes Arch Clin Exp Ophthalmol, 243*(2), 163-168.

Pemp, B., Polska, E., Garhofer, G., Bayerle-Eder, M., Kautzky-Willer, A. and Schmetterer, L. (2010). Retinal blood flow in type 1 diabetic patients with no or mild diabetic retinopathy during euglycemic clamp. *Diabetes Care, 33*(9), 2038-2042.

Pemp, B. and Schmetterer, L. (2008). Ocular blood flow in diabetes and age-related macular degeneration. *Can J Ophthalmol, 43*(3), 295-301.

Pieris, S. J. and Hill, D. W. (1982). Collateral vessels in branch retinal vein occlusion. *Trans Ophthalmol Soc U K, 102 (Pt 1)*, 178-181.

Pittman, R. N. and Duling, B. R. (1975). A new method for the measurement of percent oxyhemoglobin. *J Appl Physiol, 38*(2), 315-320.

Polska, E., Simader, C., Weigert, G., Doelemeyer, A., Kolodjaschna, J., Scharmann, O. and Schmetterer, L. (2007). Regulation of choroidal blood flow during combined changes in intraocular pressure and arterial blood pressure. *Invest Ophthalmol Vis Sci, 48*(8), 3768-3774.

Pournaras, C. J. (1995). Retinal oxygen distribution. Its role in the physiopathology of vasoproliferative microangiopathies. *Retina, 15*(4), 332-347.

Pournaras, C. J., Miller, J. W., Gragoudas, E. S., Husain, D., Munoz, J. L., Tolentino, M. J., Kuroki, M. and Adamis, A. P. (1997). Systemic hyperoxia decreases vascular endothelial growth factor gene expression in ischemic primate retina. *Arch Ophthalmol, 115*(12), 1553-1558.

Pournaras, C. J., Riva, C. E., Tsacopoulos, M. and Strommer, K. (1989). Diffusion of O2 in the retina of anesthetized miniature pigs in normoxia and hyperoxia. *Exp Eye Res, 49*(3), 347-360.

Pournaras, C. J., Rungger-Brandle, E., Riva, C. E., Hardarson, S. H. and Stefansson, E. (2008). Regulation of retinal blood flow in health and disease. *Prog Retin Eye Res, 27*(3), 284-330.

Pournaras, C. J., Roth, A., Munoz, J. L. and Abdesselem, R. (1990). [Experimental venous branch occlusion: change in the preretinal oxygen pressure pO2 by dexamethasone]. *Klin Monbl Augenheilkd, 196*(6), 475-480. Article in German.

Pournaras, C. J., Tsacopoulos, M., Riva, C. E. and Roth, A. (1990). Diffusion of O2 in normal and ischemic retinas of anesthetized miniature pigs in normoxia and hyperoxia. *Graefes Arch Clin Exp Ophthalmol, 228*(2), 138-142.

Pournaras, C. J., Tsacopoulos, M., Strommer, K., Gilodi, N. and Leuenberger, P. M. (1990a). Experimental retinal branch vein occlusion in miniature pigs induces local tissue hypoxia and vasoproliferative microangiopathy. *Ophthalmology, 97*(10), 1321-1328.

Pournaras, C. J., Tsacopoulos, M., Strommer, K., Gilodi, N. and Leuenberger, P. M. (1990b). Scatter photocoagulation restores tissue hypoxia in experimental vasoproliferative microangiopathy in miniature pigs. *Ophthalmology, 97*(10), 1329-1333.

Pournaras, J. A., Nguyen, C., Mameletzi, E., Zografos, L. and Wolfensberger, T. J. (2010). Successful treatment of longstanding vasospastic central retinal artery occlusion. *Acta Ophthalmol, 88*(2), e34-35.

Pournaras, J. A., Petropoulos, I. K., Munoz, J. L. and Pournaras, C. J. (2004). Experimental retinal vein occlusion: effect of acetazolamide and carbogen (95% O2/5% CO2) on preretinal PO2. *Invest Ophthalmol Vis Sci, 45*(10), 3669-3677.

Quigley, H. A. (2011). Glaucoma. *Lancet, 377*(9774), 1367-1377.

Ramdas, W. D., van Koolwijk, L. M., Lemij, H. G., Pasutto, F., Cree, A. J., Thorleifsson, G., Janssen, S. F., Jacoline, T. B., Amin, N., Rivadeneira, F., Wolfs, R. C., Walters, G. B., Jonasson, F., Weisschuh, N., Mardin, C. Y., Gibson, J., Zegers, R. H., Hofman, A., de Jong, P. T., Uitterlinden, A. G., Oostra, B. A., Thorsteinsdottir, U., Gramer, E., Welgen-Lussen, U. C., Kirwan, J. F., Bergen, A. A., Reis, A., Stefansson, K., Lotery, A. J., Vingerling, J. R., Jansonius, N. M., Klaver, C. C. and van Duijn, C. M. (2011). Common genetic variants associated with open-angle glaucoma. *Hum Mol Genet, 20*(12), 2464-2471.

Ramella-Roman, J. C. and Mathews, S. A. (2007). Spectroscopic measurements of oxygen saturation in the retina. *Ieee Journal of Selected Topics in Quantum Electronics, 13*(6), 1697-1703.

Ramella-Roman, J. C., Mathews, S. A., Kandimalla, H., Nabili, A., Duncan, D. D., D'Anna, S. A., Shah, S. M. and Nguyen, Q. D. (2008). Measurement of oxygen saturation in the retina with a spectroscopic sensitive multi aperture camera. *Opt Express, 16*(9), 6170-6182.

Resch, H., Garhofer, G., Fuchsjager-Mayrl, G., Hommer, A. and Schmetterer, L. (2009). Endothelial dysfunction in glaucoma. *Acta Ophthalmol, 87*(1), 4-12.

Ripps, H., Mehaffey, L., 3rd and Siegel, I. M. (1981). Rhodopsin kinetics in the cat retina. *J Gen Physiol, 77*(3), 317-334.

Riva, C. E., Grunwald, J. E. and Petrig, B. L. (1983). Reactivity of the human retinal circulation to darkness: a laser Doppler velocimetry study. *Invest Ophthalmol Vis Sci, 24*(6), 737-740.

Riva, C. E., Grunwald, J. E. and Sinclair, S. H. (1983). Laser Doppler Velocimetry study of the effect of pure oxygen breathing on retinal blood flow. *Invest Ophthalmol Vis Sci, 24*(1), 47-51.

Riva, C. E., Logean, E. and Falsini, B. (2005). Visually evoked hemodynamical response and assessment of neurovascular coupling in the optic nerve and retina. *Prog Retin Eye Res, 24*(2), 183-215.

Riva, C. E., Petrig, B. L. and Grunwald, J. E. (1987). Near infrared retinal laser Doppler velocimetry. *Lasers in Ophthalmology, 1*(4), 211-215.

Riva, C. E., Pournaras, C. J. and Tsacopoulos, M. (1986). Regulation of local oxygen tension and blood flow in the inner retina during hyperoxia. *J Appl Physiol, 61*(2), 592-598.

Rochette, J., Craig, J. E. and Thein, S. L. (1994). Fetal hemoglobin levels in adults. *Blood Rev, 8*(4), 213-224.

Roy, S., Ha, J., Trudeau, K. and Beglova, E. (2010). Vascular Basement Membrane Thickening in Diabetic Retinopathy. *Curr Eye Res*.

Rumsey, W. L., Vanderkooi, J. M. and Wilson, D. F. (1988). Imaging of phosphorescence: a novel method for measuring oxygen distribution in perfused tissue. *Science, 241*(4873), 1649-1651.

Sato, T., Muto, T., Ishibashi, Y. and Roy, S. (2001). Short-term effect of beta-adrenoreceptor blocking agents on ocular blood flow. *Curr Eye Res, 23*(4), 298-306.

Schechter, A. N. (2008). Hemoglobin research and the origins of molecular medicine. *Blood, 112*(10), 3927-3938.

Schmetterer, L., Muller, M., Fasching, P., Diepolder, C., Gallenkamp, A., Zanaschka, G., Findl, O., Strenn, K., Mensik, C., Tschernko, E., Eichler, H. G. and Wolzt, M. (1997). Renal and ocular hemodynamic effects of insulin. *Diabetes, 46*(11), 1868-1874.

Schmetterer, L., Strenn, K., Findl, O., Breiteneder, H., Graselli, U., Agneter, E., Eichler, H. G. and Wolzt, M. (1997). Effects of antiglaucoma drugs on ocular hemodynamics in healthy volunteers. *Clin Pharmacol Ther, 61*(5), 583-595.

Schmidl, D., Garhofer, G. and Schmetterer, L. (2011). The complex interaction between ocular perfusion pressure and ocular blood flow - Relevance for glaucoma. *Exp Eye Res, 93*(2), 141-155.

Schmidl, D., Weigert, G., Dorner, G. T., Resch, H., Kolodjaschna, J., Wolzt, M., Garhofer, G. and Schmetterer, L. (2011). Role of adenosine in the control of choroidal blood flow during changes in ocular perfusion pressure. *Invest Ophthalmol Vis Sci, 52*(8), 6035-6039.

Schweitzer, D., Hammer, M., Kraft, J., Thamm, E., Konigsdorffer, E. and Strobel, J. (1999). In vivo measurement of the oxygen saturation of retinal vessels in healthy volunteers. *IEEE Trans Biomed Eng, 46*(12), 1454-1465.

Schweitzer, D., Lasch, A., van der Vorst, S., Wildner, K., Hammer, M., Voigt, U., Jutte, M. and Muller, U. A. (2007). [Change of Retinal Oxygen Saturation in Healthy Subjects and in Early Stages of Diabetic Retinopathy during Breathing of 100 % Oxygen.]. *Klin Monatsbl Augenheilkd, 224*(5), 402-410.

Schweitzer, D., Leistritz, L., Hammer, M., Scibor, M., Bartsch, U. and Strobel, J. (1995). Calibration-free measurement of the oxygen saturation in human retinal vessels. *Ophthalmic Technologies V. Proc. SPIE, 2393*, 210-218.

Schweitzer, D., Thamm, E., Hammer, M. and Kraft, J. (2001). A new method for the measurement of oxygen saturation at the human ocular fundus. *Int Ophthalmol, 23*(4-6), 347-353.

Sebag, J., Delori, F. C., Feke, G. T. and Weiter, J. J. (1989). Effects of optic atrophy on retinal blood flow and oxygen saturation in humans. *Arch Ophthalmol, 107*(2), 222-226.

Sekimoto, M., Hayasaka, S. and Setogawa, T. (1992). Type of arteriovenous crossing at site of branch retinal vein occlusion. *Jpn J Ophthalmol, 36*(2), 192-196.

Severinghaus, J. W. (2011). Monitoring oxygenation. *J Clin Monit Comput, 25*(3), 155-161.

Shahidi, M., Wanek, J., Blair, N. P., Little, D. M. and Wu, T. (2010). Retinal tissue oxygen tension imaging in the rat. *Invest Ophthalmol Vis Sci, 51*(9), 4766-4770.

Shaw, B. R. and Lewis, R. A. (1986). Intraocular pressure elevation after pupillary dilation in open angle glaucoma. *Arch Ophthalmol, 104*(8), 1185-1188.

Shonat, R. D., Wilson, D. F., Riva, C. E. and Cranstoun, S. D. (1992). Effect of acute increases in intraocular pressure on intravascular optic nerve head oxygen tension in cats. *Invest Ophthalmol Vis Sci, 33*(11), 3174-3180.

Siegfried, C. J., Shui, Y. B., Holekamp, N. M., Bai, F. and Beebe, D. C. (2010). Oxygen distribution in the human eye: relevance to the etiology of open-angle glaucoma after vitrectomy. *Invest Ophthalmol Vis Sci, 51*(11), 5731-5738.

Siesky, B., Harris, A., Brizendine, E., Marques, C., Loh, J., Mackey, J., Overton, J. and Netland, P. (2009). Literature review and meta-analysis of topical carbonic anhydrase inhibitors and ocular blood flow. *Surv Ophthalmol, 54*(1), 33-46.

Siesky, B., Harris, A., Cantor, L. B., Kagemann, L., Weitzman, Y., McCranor, L., Marques, C., Werne, A. and Stefansson, E. (2008). A comparative study of the effects of brinzolamide and dorzolamide on retinal oxygen saturation and ocular microcirculation in patients with primary open-angle glaucoma. *Br J Ophthalmol, 92*(4), 500-504.

Siesky, B., Harris, A., Kagemann, L., Stefansson, E., McCranor, L., Miller, B., Bwatwa, J., Regev, G. and Ehrlich, R. (2010). Ocular blood flow and oxygen delivery to the retina in primary open-angle glaucoma patients: the addition of dorzolamide to timolol monotherapy. *Acta Ophthalmol, 88*(1), 142-149.

Sigal, I. A. and Ethier, C. R. (2009). Biomechanics of the optic nerve head. *Exp Eye Res, 88*(4), 799-807.

Skold, A., Cosco, D. L. and Klein, R. (2011). Methemoglobinemia: pathogenesis, diagnosis, and management. *South Med J, 104*(11), 757-761.

Smith, M. H., Denninghoff, K. R., Lompado, A. and Hillman, L. W. (2000a). Effect of multiple light paths on retinal vessel oximetry. *Applied Optics, 39*(7), 1183-1193.

Smith, M. H., Denninghoff, K. R., Lompado, A. and Hillman, L. W. (2000b). Retinal vessel oximetry: Toward absolute calibration. *Proceedings of SPIE, 3908*.

Smith, M. H., Denninghoff, K. R., Lompado, A., Woodruff, J. B. and Hillman, L. W. (2001). Minimizing the influence of fundus pigmentation on retinal vessel oximetry measurements. *Proc. SPIE, 4245*, 135-145.

Staub Sr, N. C. (1998). Transport of oxygen and carbon dioxide: Tissue oxygenation. In R. M. Berne, M. N. Levy, B. M. Koeppen & B. A. Stanton (Eds.), *Physiology 4th edition* (pp. 561-571). St. Louis, USA: Mosby Inc.

Stefansson, E. (1981). *Ocular oxygenation and neovascularization*. Durham, NC: Duke University.

Stefansson, E. (1988). Retinal oxygen tension is higher in light than dark. *Pediatr Res, 23*(1), 5-8.

Stefansson, E. (2006). Ocular oxygenation and the treatment of diabetic retinopathy. *Surv Ophthalmol, 51*(4), 364-380.

Stefansson, E., Hatchell, D. L., Fisher, B. L., Sutherland, F. S. and Machemer, R. (1986). Panretinal photocoagulation and retinal oxygenation in normal and diabetic cats. *Am J Ophthalmol, 101*(6), 657-664.

Stefansson, E., Jensen, P. K., Eysteinsson, T., Bang, K., Kiilgaard, J. F., Dollerup, J., Scherfig, E. and la Cour, M. (1999). Optic nerve oxygen tension in pigs and the effect of carbonic anhydrase inhibitors. *Invest Ophthalmol Vis Sci, 40*(11), 2756-2761.

Stefansson, E., Landers, M. B., 3rd and Wolbarsht, M. L. (1981). Increased retinal oxygen supply following pan-retinal photocoagulation and vitrectomy and lensectomy. *Trans Am Ophthalmol Soc, 79*, 307-334.

Stefansson, E., Landers, M. B., 3rd and Wolbarsht, M. L. (1982). Vitrectomy, lensectomy, and ocular oxygenation. *Retina, 2*(3), 159-166.

Stefansson, E., Machemer, R., de Juan, E., Jr., McCuen, B. W., 2nd and Peterson, J. (1992). Retinal oxygenation and laser treatment in patients with diabetic retinopathy. *Am J Ophthalmol, 113*(1), 36-38.

Stefansson, E., Novack, R. L. and Hatchell, D. L. (1990). Vitrectomy prevents retinal hypoxia in branch retinal vein occlusion. *Invest Ophthalmol Vis Sci, 31*(2), 284-289.

Stefansson, E., Pedersen, D. B., Jensen, P. K., la Cour, M., Kiilgaard, J. F., Bang, K. and Eysteinsson, T. (2005). Optic nerve oxygenation. *Prog Retin Eye Res, 24*(3), 307-332.

Stefansson, E., Peterson, J. I. and Wang, Y. H. (1989). Intraocular oxygen tension measured with a fiber-optic sensor in normal and diabetic dogs. *Am J Physiol, 256*(4 Pt 2), H1127-1133.

Stefansson, E., Wagner, H. G. and Seida, M. (1988). Retinal blood flow and its autoregulation measured by intraocular hydrogen clearance. *Exp Eye Res, 47*(5), 669-678.

Stefansson, E., Wolbarsht, M. L. and Landers, M. B., 3rd. (1983). In vivo O2 consumption in rhesus monkeys in light and dark. *Exp Eye Res, 37*(3), 251-256.

Steigerwalt, R. D., Jr., Laurora, G., Belcaro, G. V., Cesarone, M. R., De Sanctis, M. T., Incandela, L. and Minicucci, R. (2001). Ocular and retrobulbar blood flow in ocular hypertensives treated with topical timolol, betaxolol and carteolol. *J Ocul Pharmacol Ther, 17*(6), 537-544.

Steinberg, M. H. and Adams, J. G., 3rd. (1991). Hemoglobin A2: origin, evolution, and aftermath. *Blood, 78*(9), 2165-2177.

Sullivan, P. M., Parfitt, V. J., Jagoe, R., Newsom, R. and Kohner, E. M. (1991). Effect of meal on retinal blood flow in IDDM patients. *Diabetes Care, 14*(8), 756-758.

Sun, X. G., Hansen, J. E., Stringer, W. W., Ting, H. and Wasserman, K. (2001). Carbon dioxide pressure-concentration relationship in arterial and mixed venous blood during exercise. *J Appl Physiol, 90*(5), 1798-1810.

Sutherland, F. S., Stefansson, E., Hatchell, D. L. and Reiser, H. (1990). Retinal oxygen consumption in vitro. The effect of diabetes mellitus, oxygen and glucose. *Acta Ophthalmol (Copenh), 68*(6), 715-720.

Suzuki, A., Okamoto, N., Ohnishi, M., Tsubakimori, S. and Fukuda, M. (2000). Investigation of blood flow velocity by color Doppler imaging in nonischemic central retinal vein occlusion with collateral veins. *Jpn J Ophthalmol, 44*(6), 685-687.

Takahashi, K., Muraoka, K., Kishi, S. and Shimizu, K. (1998). Formation of retinochoroidal collaterals in central retinal vein occlusion. *Am J Ophthalmol, 126*(1), 91-99.

Tamaki, Y., Araie, M., Hasegawa, T. and Nagahara, M. (2001). Optic nerve head circulation after intraocular pressure reduction achieved by trabeculectomy. *Ophthalmology, 108*(3), 627-632.

Tamaki, Y., Araie, M., Tomita, K., Nagahara, M. and Tomidokoro, A. (1997). Effect of topical beta-blockers on tissue blood flow in the human optic nerve head. *Curr Eye Res, 16*(11), 1102-1110.

Teng, P. Y., Blair, N. P., Wanek, J. and Shahidi, M. (2012). Oxygen tension and gradient measurements in the retinal microvasculature of rats. *Graefes Arch Clin Exp Ophthalmol, 250*(3), 361-370.

Tezel, G. and Wax, M. B. (2004). Hypoxia-inducible factor 1alpha in the glaucomatous retina and optic nerve head. *Arch Ophthalmol, 122*(9), 1348-1356.

The Central Vein Occlusion Study Group. Natural history and clinical management of central retinal vein occlusion. (1997). *Arch Ophthalmol, 115*(4), 486-491.

The Eye Disease Case-control Study Group. (1993). Risk factors for branch retinal vein occlusion. *Am J Ophthalmol, 116*(3), 286-296.

Thorleifsson, G., Magnusson, K. P., Sulem, P., Walters, G. B., Gudbjartsson, D. F., Stefansson, H., Jonsson, T., Jonasdottir, A., Jonasdottir, A., Stefansdottir, G., Masson, G., Hardarson, G. A., Petursson, H., Arnarsson, A., Motallebipour, M., Wallerman, O., Wadelius, C., Gulcher, J. R., Thorsteinsdottir, U., Kong, A., Jonasson, F. and Stefansson, K. (2007). Common sequence variants in the LOXL1 gene confer susceptibility to exfoliation glaucoma. *Science, 317*(5843), 1397-1400.

Tiedeman, J. S., Kirk, S. E., Srinivas, S. and Beach, J. M. (1998). Retinal oxygen consumption during hyperglycemia in patients with diabetes without retinopathy. *Ophthalmology, 105*(1), 31-36.

Tillis, T. N., Murray, D. L., Schmidt, G. J. and Weiter, J. J. (1988). Preretinal oxygen changes in the rabbit under conditions of light and dark. *Invest Ophthalmol Vis Sci, 29*(6), 988-991.

Toffaletti, J. and Zijlstra, W. G. (2007). Misconceptions in reporting oxygen saturation. *Anesth Analg, 105*(6 Suppl), S5-9.

Tornquist, P. and Alm, A. (1979). Retinal and choroidal contribution to retinal metabolism in vivo. A study in pigs. *Acta Physiol Scand, 106*(3), 351-357.

Trible, J. R., Sergott, R. C., Spaeth, G. L., Wilson, R. P., Katz, L. J., Moster, M. R. and Schmidt, C. M. (1994). Trabeculectomy is associated with retrobulbar hemodynamic changes. A color Doppler analysis. *Ophthalmology, 101*(2), 340-351.

Tsacopoulos, M., Baker, R. and Levy, S. (1976). Studies on retinal oxygenation. *Adv Exp Med Biol, 75*, 413-416.

Tsacopoulos, M. and Lehmenkuhler, A. (1977). A double-barrelled Pt-microelectrode for simultaneous measurement of PO2 and bioelectrical activity in excitable tissues. *Experientia, 33*(10), 1337-1338.

Tsai, A. G., Johnson, P. C. and Intaglietta, M. (2003). Oxygen gradients in the microcirculation. *Physiol Rev, 83*(3), 933-963.

Tsai, A. S., Wong, T. Y., Lavanya, R., Zhang, R., Hamzah, H., Tai, E. S. and Cheung, C. Y. (2011). Differential association of retinal arteriolar and venular caliber with diabetes and retinopathy. *Diabetes Res Clin Pract, 94*(2), 291-298.

Venkataraman, S. T., Hudson, C., Fisher, J. A., Rodrigues, L., Mardimae, A. and Flanagan, J. G. (2008). Retinal arteriolar and capillary vascular reactivity in response to isoxic hypercapnia. *Exp Eye Res, 87*(6), 535-542.

Wachtmeister, L. (1998). Oscillatory potentials in the retina: what do they reveal. *Prog Retin Eye Res, 17*(4), 485-521.

Wada, Y. (2002). Advanced analytical methods for hemoglobin variants. *J Chromatogr B Analyt Technol Biomed Life Sci, 781*(1-2), 291-301.

Wang, L., Kondo, M. and Bill, A. (1997). Glucose metabolism in cat outer retina. Effects of light and hyperoxia. *Invest Ophthalmol Vis Sci, 38*(1), 48-55.

Wang, L., Tornquist, P. and Bill, A. (1997a). Glucose metabolism in pig outer retina in light and darkness. *Acta Physiol Scand, 160*(1), 75-81.

Wang, L., Tornquist, P. and Bill, A. (1997b). Glucose metabolism of the inner retina in pigs in darkness and light. *Acta Physiol Scand, 160*(1), 71-74.

Wang, T. H., Hung, P. T., Huang, J. K. and Shih, Y. F. (1997). The effect of 0.5% timolol maleate on the ocular perfusion of ocular hypertensive patients by scanning laser flowmetry. *J Ocul Pharmacol Ther, 13*(3), 225-233.

Weinberg, D., Dodwell, D. G. and Fern, S. A. (1990). Anatomy of arteriovenous crossings in branch retinal vein occlusion. *Am J Ophthalmol, 109*(3), 298-302.

Westlake, W. H., Morgan, W. H. and Yu, D. Y. (2001). A pilot study of in vivo venous pressures in the pig retinal circulation. *Clin Experiment Ophthalmol, 29*(3), 167-170.

Williams, R., Airey, M., Baxter, H., Forrester, J., Kennedy-Martin, T. and Girach, A. (2004). Epidemiology of diabetic retinopathy and macular oedema: a systematic review. *Eye, 18*(10), 963-983.

Williamson, T. H., Grewal, J., Gupta, B., Mokete, B., Lim, M. and Fry, C. H. (2009). Measurement of PO2 during vitrectomy for central retinal vein occlusion, a pilot study. *Graefes Arch Clin Exp Ophthalmol, 247*(8), 1019-1023.

Wilson, C. A., Stefansson, E., Klombers, L., Hubbard, L. D., Kaufman, S. C. and Ferris F. L., 3rd. (1988). Optic disk neovascularization and retinal vessel diameter in diabetic retinopathy. *Am J Ophthalmol, 106*(2), 131-134.

Wilson, D. F., Vanderkooi, J. M., Green, T. J., Maniara, G., DeFeo, S. P. and Bloomgarden, D. C. (1987). A versatile and sensitive method for measuring oxygen. *Adv Exp Med Biol, 215*, 71-77.

Wimberley, P. D., Fogh-Andersen, N., Siggaard-Andersen, O., Lundsgaard, F. C. and Zijlstra, W. G. (1988). Effect of pH on the absorption spectrum of human oxyhemoglobin: a potential source of error in measuring the oxygen saturation of hemoglobin. *Clin Chem, 34*(4), 750-754.

Winslow, R. M. and Vandegriff, K. D. (1997). Oxygen-haemoglobin dissociation curve. In R. G. Crystal (Ed.), *The Lung: Scientific foundations* (pp 1625-1632). Philadelphia: Lippincott Williams & Wilkins.

Wolbarsht, M. L., Stefansson, E. and Landers, M. B., 3rd. (1987). Retinal oxygenation from the choroid in hyperoxia. *Exp Biol, 47*(1), 49-52.

Wolter, J. R. (1961). Retinal Pathology after Central Retinal Vein Occlusion. *Br J Ophthalmol, 45*(10), 683-694.

Yamana, Y., Oka, Y., Ohnishi, Y., Ishibashi, T. and Inoguchi, T. (1988). Reflow of obstructed capillaries in the maculae of humans with diabetic retinopathy, observed by fluorescein angiography. *Br J Ophthalmol, 72*(9), 660-665.

Ye, X. D., Laties, A. M. and Stone, R. A. (1990). Peptidergic innervation of the retinal vasculature and optic nerve head. *Invest Ophthalmol Vis Sci, 31*(9), 1731-1737.

Yoneya, S., Saito, T., Nishiyama, Y., Deguchi, T., Takasu, M., Gil, T. and Horn, E. (2002). Retinal oxygen saturation levels in patients with central retinal vein occlusion. *Ophthalmology, 109*(8), 1521-1526.

Yoshida, A., Feke, G. T., Mori, F., Nagaoka, T., Fujio, N., Ogasawara, H., Konno, S. and McMeel, J. W. (2003). Reproducibility and clinical application of a newly developed stabilized retinal laser Doppler instrument. *Am J Ophthalmol, 135*(3), 356-361.

Yoshida, A., Feke, G. T., Ogasawara, H., Goger, D. G., Murray, D. L. and McMeel, J. W. (1991). Effect of timolol on human retinal, choroidal and optic nerve head circulation. *Ophthalmic Res, 23*(3), 162-170.

Yoshida, A., Ogasawara, H., Fujio, N., Konno, S., Ishiko, S., Kitaya, N., Kagokawa, H., Nagaoka, T. and Hirokawa, H. (1998). Comparison of short- and long-term effects of betaxolol and timolol on human retinal circulation. *Eye, 12 (Pt 5)*, 848-853.

Yu, D. Y., Cringle, S. J. and Alder, V. A. (1990). The response of rat vitreal oxygen tension to stepwise increases in inspired percentage oxygen. *Invest Ophthalmol Vis Sci, 31*(12), 2493-2499.

Yu, D. Y., Cringle, S. J., Su, E., Yu, P. K., Humayun, M. S. and Dorin, G. (2005). Laser-induced changes in intraretinal oxygen distribution in pigmented rabbits. *Invest Ophthalmol Vis Sci, 46*(3), 988-999.

Yu, D. Y., Cringle, S. J., Yu, P. K. and Su, E. N. (2007). Intraretinal oxygen distribution and consumption during retinal artery occlusion and graded hyperoxic ventilation in the rat. *Invest Ophthalmol Vis Sci, 48*(5), 2290-2296.

Yuzurihara, D. and Iijima, H. (2004). Visual outcome in central retinal and branch retinal artery occlusion. *Jpn J Ophthalmol, 48*(5), 490-492.

Zhao, J., Sastry, S. M., Sperduto, R. D., Chew, E. Y. and Remaley, N. A. (1993). Arteriovenous crossing patterns in branch retinal vein occlusion. The Eye Disease Case-Control Study Group. *Ophthalmology, 100*(3), 423-428.

Zijlstra, W. G., Buursma, A. and Van Assendelft, O. W. (2000). *Visible and near infrared absorption spectra of human and animal haemoglobin - Determination and application*. Utrecht: VSP.

Zuckerman, R. and Weiter, J. J. (1980). Oxygen transport in the bullfrog retina. *Exp Eye Res, 30*(2), 117-127.