Retinal oximetry and systemic arterial oxygen levels

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Thesis for the degree of Philosophiae Doctor

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Ágrip

Tilgangur

Innleiðing púlsoximælinga til samfelldrar mælingar á súrefnismettun blóðs er ein mikilvægasta tækniþróun undanfarinna áratuga fyrir öryggi og vöktun sjúklinga. Þær hafa þó þann veikleika að mæla útæðar (peripheral circulation) og eina leiðin til að mæla súrefnisstyrk í miðlægum æðum er með ífarandi slagæðamælingum. Mæling á súrefnismettun í sjónhimnuæðum augans með sjónhimnu-súrefnismæli er hins vegar mæling á miðlægum æðum án ífarandi tækni. Sjónhimnan er hluti miðtaugakerfisins og eru sjónhimnuæðar því miðlægar æðar, sem samsvara súrefnisástandi miðtaugakerfisins að nokkru leyti.

Meginmarkmið verkefnisins er að meta hvort hægt sé að nota sjónhimnu-súrefnismælingar til að mæla súrefnismettun í miðlægri blóðrás sem hingað til hefur ekki verið mögulegt nema með ífarandi inngripum. Sannreynd er geta tækisins til að meta súrefnismettun í miðlægum æðum með því að skoða þrjá hópa fullorðins fólks; Fólks með miðbláæðarlokun (central retinal vein occlusion, CRVO) sem veldur staðbundnum súrefnisskorti í innri sjónhimnunni, sjúklinga með alvarlega langvinna lungnateppu (chronic obstructive pulmonary disease, COPD) sem einkennist af kerfisbundnum súrefnisskorti og heilbrigða einstaklinga til að meta kerfisbundin áhrif innandaðs súrefnis. Fjórða rannsóknin sem komið er inná var framkvæmd til að meta hvort sjónhimnu-súrefnismælingar eru álitlegur kostur fyrir nýbura.

Aðferðir

Sjónhimnu-súrefnismælingar í miðbláæðarlokun:

Sextán einstaklingar með miðbláæðarlokun tóku þátt í rannsókninni og var súrefnismettun augans með bláæðastífluna borin saman við súrefnismettun í gagnstæða auganu.

Sjónhimnu-súrefnismælingar hjá heilbrigðum við innöndun 100% súrefnis:

Þrjátíu heilbrigðir einstaklingar tóku þátt í rannsókninni og var súrefnismettun sjónhimnuæða við innöndun á andrúmslofti borin saman við innöndun 100% súrefnis.

Sjónhimnu-súrefnismælingar í alvarlegri langvinnri lungnateppu:

Ellefu einstaklingar með alvarlega langvinna lungnateppu með varanlega þörf fyrir súrefni tóku þátt í rannsókninni. Súrefnismettun sjónhimnuæða hægra augans var mæld bæði með og án súrefnismeðferðar**.** Niðurstöðurnar voru bornar saman og jafnframt gerður samanburður án súrefnismeðferðar við blóðsýni frá sveifarslagæð, við fingurmælingu (pulse oximeter) og heilbrigðan samanburðarhóp sem fengin var úr gagnagrunni sem rannsóknarhópurinn hafði áður safnað.

Súrefnismælirinn samanstendur af hefðbundinni augnbotnamyndvél og sérstökum hugbúnaði sem les úr myndunum. Ljósdeilir sér til þess að tvær stafrænarmyndavélar taka samtímis myndir af sama svæðinu með sitthvorri bylgjulengdinni fyrir útreikninga á súrefnismettun sjónhimnuæða.

Sjónhimnu-súrefnismælingar í nýburum:

Að auki voru teknar myndir af 28 fullbura nýburum með laser skanna augnbotnamyndavél og fyrrgreindum hugbúnaði sem búið var að aðlaga laser skanna tækninni, til útreikninga á æðavídd og ljósþéttnihlutfalli í slag- og bláæðlingum.

Niðurstöður

Sjónhimnu-súrefnismælingar í miðbláæðarlokun:

Meðaltal súrefnismettunar í bláæðlingum augna með miðbláæðarlokun mældist 31±12% og 52±11% í gagnstæðum augum (meðaltal±staðalfrávik, n=14, p<0.0001). Mismunur súrefnismettunar í slag- og bláæðlingum mældist 63±11% í augum með miðbláæðarlokun og 43±7% í gagnstæðum augum (p<0.0001). Breytileiki bláæðamettunar reyndist umtalsverður bæði innan augna og milli augna með miðbláæðarlokun. Ekki reyndist munur á súrefnismettun í slagæðlingum augna með miðbláæðarlokun og í gagnstæðum augum (p=0.49).

Sjónhimnu-súrefnismælingar hjá heilbrigðum við innöndun 100% súrefnis:

Innöndun 100% súrefnis jók súrefnismettun slagæðlinga í 94.5±3.8% til samanburðar við 92.0±3.7% áður en hún hófst (n=30, p<0.0001). Í bláæðlingum jókst súrefnismettunin í 76.2±8.0% frá 51.3±5.6% (p<0.0001) áður en innöndunin hófst. Mismunur súrefnismettunar í slag- og bláæðlingum lækkaði marktækt á meðan á innöndun súrefnisins stóð (18.3±9.0% vs. 40.7±5.7% áður, p<0.0001).

Sjónhimnu-súrefnismælingar í alvarlegri langvinnri lungnateppu:

Án súrefnismeðferðar mældist sjónhimnu-súrefnismettunin marktækt lægri hjá fólki með alvarlega langvinna lungnateppu en hjá heilbrigða samanburðarhópnum bæði í slag- (87.2±4.9% vs. 93.4±4.3%, p=0.02,n=11) og í bláæðlingum (45.0±10.3% vs. 55.2±5.5%, p=0.01). Ekki reyndist mærktækur munur á mismuni súrefnismettunar í slag- og bláæðlingum milli þessara hópa (p=0.17). Innöndun súrefnismeðferðar jók marktækt súrefnismettunina í slagæðlingum (87.2±4.9% vs. 89.5±6.0%, p=0.02) en ekki í bláæðlingum (45.0±10.3% vs. 46.7±12.8%, p=0.3). Sjónhimnu-súrefnismælingarnar sýndu lítið eitt lægri gildi en fingurmælingar (mean percentage points difference = -3.1±5.5) og ífarandi slagæðamælingar (-5.0±5.4).

Sjónhimnu-súrefnismælingar í nýburum:

Ljósþéttnihlutfallið í slagæðlingum sjónhimnunnar mældist marktækt lægra en í bláæðlingum (0.256±0.041 vs. 421±0.089, n= 28, p<0.001, parað t-próf). Æðavídd slagæðlinga reyndist marktækt minni en í blæðlingum (14.1±2.7 vs. 19.7±3.7 pixlar, p < 0.001).

Ályktanir

Niðurstöður rannsóknanna sýna að sjónhimnu-súrefnismælirinn er næmur fyrir staðbundnum og kerfisbundnum breytingum á súrefnismettun í miðlægum æðum. Sjónhimnu-súrefnismælingar sýna eilítið lægri gildi en slagæða- og fingurmælingar. Mismuninn má að öllum líkindum rekja til nálægrar legu miðslagæðarinnar við miðbláæðina innan sjóntaugarinnar (countercurrent exchange) og kvörðunar á sjónhimnu-súrefnismælinum. Þrátt fyrir þennan mun, gefa rannsóknirnar vísbendingar um að víkka megi notagildi tækisins yfir í mælingar á súrefnisbúskap í miðlægum æðum blóðrásarinnar. Endurskoða þarf kvörðunina á mælitækinu og með tilkomu tækniframfara er mögulega unnt að sannreyna gildi mæliaðferðarinnar á svæfingadeildum og hjá alvarlega veikum sjúklingum á gjörgæslu.

Rannsóknin á ungabörnunum gefur vísbendingar um að sjónhimnu-súrefnismælingar séu álitlegur kostur til mats á súrefnismettun hjá nýburum.

Lykilorð:

Sjónhimnuæðar, súrefnismælingar, miðlæg blóðrás, miðbláæðalokun í sjónhimnu, langvinn lungnateppa.

Abstract

Purpose

Continuous peripheral pulse oximetry for monitoring adequacy of oxygenation is probably the most important technological advance for patients' monitoring and safety in the last decades. Pulse oximetry has the disadvantage of measuring the peripheral circulation and the only mean to measure oxygen content of the central circulation is by invasive technology. Determination of blood oxyhemoglobin saturation in the retinal vessels of the eye can be achieved non-invasively through spectrophotometric retinal oximetry which provides access to the central nervous system circulation.

The aim of the thesis is to determine whether retinal oximetry technique can be applied for estimation of the central nervous system circulation which until now has only been possible invasively. This was achieved by measuring oxyhemoglobin saturation in three adult subject study groups; in people with central retinal vein occlusion (CRVO) to observe local tissue hypoxia, in patients with severe chronic obstructive pulmonary disease (COPD) on long term oxygen therapy to observe systemic hypoxemia, and in healthy subjects during hyperoxic breathing to observe systemic hyperoxemia. In addition, the fourth study that is mentioned was performed to test whether retinal oximetry is feasible for neonates.

Methods

Retinal oximetry in central retinal vein occlusion:

Sixteen subjects with central retinal vein occlusion participated in the study. The oxyhemoglobin saturation of the central retinal vein occlusion affected eye was compared with the fellow unaffected eye.

Retinal oximetry in healthy people under hyperoxia:

Thirty healthy subjects participated in the study and the oxyhemoglobin saturation of retinal arterioles and venules was compared between normoxic and hyperoxic breathing.

Retinal oximetry in severe chronic obstructive pulmonary disease:

Eleven patients with severe chronic obstructive pulmonary disease participated in the study. Retinal oximetry measurements were made with and without their daily supplemental oxygen therapy. Retinal arteriolar oxyhemoglobin saturation when inspiring ambient air was compared with blood samples from the radial artery and finger pulse oximetry and healthy controls. The healthy control group was assembled from our database for comparison of oxyhemoglobin saturation of retinal arterioles and venules during the ambient air breathing.

The retinal oximeter is based on a conventional fundus camera and a specialized software. A beam splitter coupled with two high resolution digital cameras allows for simultaneous acquisition of retinal images at separative wavelengths for calculation of oxyhemoglobin saturation.

In additon, retinal images of 28 full-term healthy neonates were obtained with scanning laser ophthalmoscope combined with modified Oxymap analysis software for calculation of the optical density ratio and vessel diameter

Results

Retinal oximetry in central retinal vein occlusion:

Mean retinal venous oxyhemoglobin saturation was 31±12% in CRVO eyes and 52±11% in unaffected fellow eyes (mean±SD, n=14, $p<0.0001$). The arteriovenous oxygen difference (AV-difference) was 63±11% in CRVO eyes and 43±7% in fellow eyes ($p<0.0001$). The variability of retinal venous oxyhemoglobin saturation was considerable within and between eyes affected by CRVO. There was no difference in oxyhemoglobin saturation of retinal arterioles between the CRVO eyes and the unaffected eyes ($p=0.49$)

Retinal oximetry in healthy people under hyperoxia:

During hyperoxic breathing the oxyhemoglobin saturation in retinal arterioles increased to 94.5±3.8% as compared with 92.0±3.7% at baseline (n=30, $p<0.0001$). In venules the mean oxyhemoglobin saturation increased to 76.2±8.0% from 51.3±5.6% ($p<0.0001$) at baseline. The AV-difference was markedly lower during hyperoxic breathing as compared with the normoxic breathing (18.3±9.0% vs. 40.7±5.7%, $p<0.0001$).

Retinal oximetry in severe chronic obstructive pulmonary disease:

During ambient air breathing, chronic obstructive pulmonary disease subjects had significantly lower oxyhemoglobin saturation than healthy controls in both

retinal arterioles (87.2±4.9% vs. 93.4±4.3%, p=0.02, n=11) and venules (45.0±10.3% vs. 55.2±5.5%, p=0.01) but the AV-difference was not markedly different (p=0.17). Administration of their prescribed oxygen therapy significantly increased the oxyhemoglobin saturation in retinal arterioles (87.2±4.9% to 89.5±6.0%, p=0.02) but not in venules (45.0±10.3% to 46.7±12.8%, p=0.3). Retinal oximetry values were slightly lower than finger pulse oximetry (mean percentage points difference = -3.1±5.5) and radial artery blood values (-5.0±5.4).

Retinal oximetry study in neonates:

The modified version of the retinal oximetry instrument estimated the optical density ratio in retinal arterioles to be 0.256±0.041 that was significantly different from the 0.421±0.089 in venules (n= 28, p<0.001, paired t-test). The vascular diameter of retinal arterioles was markedly narrower than of venules (14.1±2.7 and 19.7±3.7 pixels, p < 0.001).

Conclusion

The results of this thesis indicate that spectrophotometric retinal oximetry is sensitive to both local and systemic changes in oxyhemoglobin saturation. Retinal oxyhemoglobin saturation values are slightly lower than radial artery blood sample and finger pulse oximetry values. The discrepancies between the different modalities are expected to derive from countercurrent exchange between central retinal artery and vein within the optic nerve but calibration issues cannot be excluded as contributing to this difference. Despite these differences, the findings indicate the potential of retinal oximetry for non-invasive real-time measurements of oxyhemoglobin saturation in central nervous system vessels. Following calibration upgrade and technological improvement, verification retinal oximetry may potentially be applied to critically ill and anesthesia care patients.

The study on combined scanning laser ophthalmoscope and retinal oximetry supports the feasibility of the technique for oximetry analysis in newly born babies.

Keywords:

Retinal vessels, oximetry, systemic circulation, central retinal vein occlusion, chronic obstructive pulmonary disease.

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Conflict of interest:

None

Contents

List of abbreviations

2,3 DPG	2,3 diphosoglycerate
AMD	Age related macular degeneration
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
AV	Arteriovenous
BF	Blood flow
bpm	Beats per minute heart rate
BRVO	Branch retinal vein occlusion
CaO_2	Oxygen content of the arterial blood
CI	Confidence interval
CO	Cardiac output
COHB	Carboxyhemoglobin
CO_2	Carbon dioxide
COPD	Chronic obstructive pulmonary disease
CRA	Central retinal artery
CRV	Central retinal vein
CRVO	Central Retinal Vein Occlusion
CvO_2	Oxygen content of venous blood
DBP	Diastolic blood pressure
dl	Deciliter
DO_2	Global oxygen delivery
EDRF	Endothelium-derived relaxing factor
EtO_2	End-tidal partial pressure of oxygen
$EtCO_2$	End-tidal carbon dioxide (Fraction of expired CO_2)
Fe^{2+}	Normal ferrous iron of hemoglobin for oxygen transport
Fe^{3+}	Oxidized hemoglobin to ferric state incapable of oxygen transport (methemoglobin)
FEV_1	Forced expiratory volume in one second
$FiCO_2$	Fraction of inspired carbon dioxide
FiO_2	Fraction of inspired oxygen
FVC	Forced vital capacity
g	Gram
H^+	Hydrogen ion
Hb	Deoxygenated hemoglobin
HbO_2	Oxygen combined with hemoglobin

HgbA	Normal adult hemoglobin
HgbF	Fetal hemoglobin
HIF	Hypoxia-inducible factor
HR	Heart rate
I	Light intensity on a vessel
I_0	Light intensity of background (to the side of the vessel)
ICU	Intensive care unit
IOP	Intra ocular pressure
L	Length of a vessel
L/min	Liter per minute
MAP	Mean arterial blood pressure
mEq/L	Milliequivalents per liter
MetHb	Methemoglobin
ml	Milliliter
mmHg	Millimetres in mercury (unit of pressure)
MOAP	Mean ophthalmic arterial pressure
MOVP	Mean ophthalmic venous pressure
n	Number of subjects of a study
nm	Nanometres
NO	Nitric oxide
O_2	Oxygen
OD	Optical density (measure of light absorbance)
ODR	Optical density ratio
OEF	Fraction of oxygen extraction
OPP	Ocular perfusion pressure
P50	Oxygen tension at which hemoglobin is 50% satured
PaO_2	Partial pressure of oxygen in arterial blood
PCO_2	Partial pressure of carbon dioxide in mmHg
pH	Negative logarithm of hydorgen ion $[H^+]$ concentration
PO_2	Partial pressure of oxygen
PP	Perfusion pressure in mmHg
RNFL	Retinal nerve fiber layer
ROP	Retinopathy of prematurity
RR	Respiratory rate
RRF	Retinal relaxing factor
SaO_2	Oxyhemoglobin saturation of arterial blood
SO_2	Oxyhemoglobin saturation
SBP	Systolic blood pressure
SD	Standard deviation
SHb	Sulfhemoglobin

SLO	Scanning laser ophthalmoscope
SpO_2	Peripheral pulse oximetry
SV	Stroke volume
SvO_2	Mixed venous blood
TRBF	Total retinal blood flow
VA	Visual acuity
VEGF	Vascular endothelial growth factor
VO_2	Tissue oxygen consumption
vs.	Versus

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I. Eliasdottir, T.S., Bragason, D., Hardarson, S.H., Kristjansdottir, G., Stefánsson, E. (2014).Venous oxygen saturation is reduced and variable in central retinal vein occlusion. *Graefe's Archive for Clinical and Experimental Ophthalmology 253*(10):1653-1661.

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Declaration of contribution

Paper I: **Venous oxygen saturation is reduced and variable in central retinal vein occlusion.**

Þórunn Sch. Elíasdóttir analysed all the oximetry images and performed the statistical analysis. She wrote the manuscript draft and participated in all subsequent revisions.

Paper II: **Retinal vessel oxygen saturation during 100% oxygen breathing in healthy individuals.**

Þórunn Sch. Elíasdóttir took part in planning the study and in the conduct of the study. She monitored the subjects under the study, took part in describing the experimental maneuver and revision of the first draft of the manuscript.

Paper III: **Retinal oximetry measures systemic hypoxia in central nervous system vessels in chronic obstructive pulmonary disease.**

Þórunn Sch. Elíasdóttir took part in planning the study and in the conduct of the study. She monitored the subjects under the study, analysed all the oximetry images and performed the statistical analysis. She wrote the manuscript draft and participated in all subsequent revisions.

Paper IV: **Retinal oximetry with scanning laser ophthalmoscope in infants.**

Þórunn Sch. Elíasdóttir took part in planning the study, in the conduct of the study and participated in revisions of the first draft.

1 Introduction

In situations of caring for patients in the intensive care units (ICU), in acute care settings and under sedation and general anesthesia continuous monitoring of oxyhemoglobin saturation using non-invasive peripheral pulse oximetry has become a standard of care. The peripheral pulse oximetry however, depends on pulsatile arterial blood volume and its measurements are therefore limited by inadequate tissue perfusion accompanying peripheral vasoconstriction. Clinical experience yields it difficult to obtain measurements under such conditions and may leave no other options but invasive measures. Unlike the central nervous system which is protected and preferred in shock and severe illnesse, peripheral pulse oximeter measurements do not represent the central vasculature. The development of a non-invasive retinal oximeter (Hardarson et al., 2006) to measure oxyhemoglobin saturation in retinal vessels provides a prospect for central vascular oximetry. The retinal arterioles are derive from the opthalmic artery which is the first branch from the internal carotid artery, and represents the central vasculature in the central nervous system. Presuming the retinal arterial oxygen content is identical to the systemic circulation, retinal oximetry may provide relevant information on oxygen delivery to the central nervous system. Such a method may enhance the monitoring and treatment of critically ill patients in the ICU, in the field of emergency and anesthesia care. Thus, the aim of the thesis is to determine whether retinal oximetry technique can be applied for estimation of the central nervous system circulation which until now has only been possible through invasive measures.

1.1 Oxyhemoglobin saturation monitoring

For survival of human beings, oxygen delivery to tissues must be sufficient to meet minimal oxygen consumption for cellular metabolism. Insufficient capillary oxygen supply leads to impaired cellular respiration (oxidative phosphorylation) and energy production that may rapidly progress to hypoxic injury and eventually death (Scheufler, 2004). Early recognition of inadequate oxygen delivery and prompt intervention is therefore crucial for survival and health outcome of patients who are critically ill and in unstable hemodynamic conditions (Perel, 2015).

Numerous techniques have been applied to monitoring oxygen delivery and tissue oxygenation but few have proceeded into routine clinical practice. Direct measurements of oxygen partial pressure (PO_2) with polarographic microelectrodes requires a needle to be inserted into the preferred tissue, for example to assess oxygenation of the brain. Reflectance spectrometry is a non-invasive alternative technique for continuous monitoring of microvascular oxyhemoglobin saturation and intracellular oxygen availability (Carreau, El Hafny-Rahbi, Matejuk, Grillon, & Kieda, 2011; Scheufler, 2004). In clinical practice the oxygen delivery by the systemic circulation can either be accessed directly by arterial blood gas analysis of oxygen partial pressure (PaO_2) and oxyhemoglobin saturation or determined indirectly by transcutaneous pulse oximetry.

1.1.1 Arterial blood gas monitoring

Invasive arterial blood gas monitoring necessitates intermittent direct arterial blood sampling for estimation of oxyhemoglobin saturation, most commonly from a peripheral radial artery or femoral artery in the groin. The arterial blood sample is processed on arterial blood gas analyzer which calculates the estimated oxyhemoglobin saturation based on empirical equations, operating PO_2 and pH values. Invasive arterial blood gas analysis is considered the gold standard technique for estimation on oxyhemoglobin saturation (Collins, Rudenski, Gibson, Howard, & O'Driscoll, 2015; Haymond, 2006), especially in critically ill patients where precision and accurate values are necessary for treatment and health outcome.

1.1.2 Transcutaneous pulse oximetry

The global marketing of transcutaneous pulse oximetry in the mid eighties and coinciding reduction (90%) in anesthesia related fatalities (Severinghaus, 2007) marked a milestone in patient monitoring care. Since then, complimentary continuous pulse oximetry has become a routine standard of care whenever tissue oxygenation is jeopardized such as in acute care settings and anesthesia practice. Concurrently, its establishment is considered the most important technological monitoring advances for patients' safety (Severinghaus, 2011) and widely viewed as the fifth vital sign in aformentioned hospital and out-of hospital settings.

Pulse oximetry is based on photoplethysmography which calculate light absorption amplification as transmitted light intensity lessens when peripheral arterial blood volume increase during systolic left ventricular ejection. The stroke volume permits arterial blood saturation to be distinguished from venous

blood saturation and is responsible for the pulsatile nature of this technique. Pulse oximetry incorporates the optical technique of difference in dual light absorption spectra to distinguish the oxyhemoglobin from deoxyhemoglobin in arterial blood. Deoxyhemoglobin absorbs greater amount of red light (660 nm) whereas oxyhemoglobin absorbs greater amount of near-infrared light (940nm). The greater absorbability of near-infrared light and the scattering of red light is the reason for the oxygen rich arterial blood to be distingushed from the oxygen poor venous blood.

The oximeter probe is most commonly put on a finger (also earlobe, toe and nose) where two light emitting diodes emit the two different wavelengths of light through the peripheral vascular bed. A photodiode on the opposite site of the tissue receives the transmitted red and near-infrared light for calculation of their relative amount of oxygenated hemoglobin. Eventually, the arterial oxyhemoglobin saturation is illustrated as photoplethysmographic waveform and digital number display (Alian, Galante, Stachenfeld, Silverman, & Shelley, 2011; Chan & Chan, 2013; Nitzan, Romem, & Koppel, 2014; Sinex, 1999).

Several studies (Golparvar, Naddafnia, & Saghaei, 2002; Shamir, Eidelman, Floman, Kaplan, & Pizov, 1999; Westphal, Silva, Gonçalves, Caldeira Filho, & Poli-de-Figueiredo, 2009) have suggested the possible role of pulse oximetry other than monitoring arterial oxyhemoglobin saturation and pulse rate. In this respect, a pulse oximetry analysis of the plethysmographic wavefore has been proposed to give useful information on hemodynamic changes, including fluid volume status (Cannesson et al., 2007; McGrath, Ryan, Wendelken, Rickards, & Convertino, 2011) and cardiac arrythmias (Cripps, Rocker, & Stradling, 1992; Marinskis et al., 2006) in critically ill patients.

1.1.3 Limitations of peripheral pulse oximetry

For non-invasive pulse oximeter, the empirical calibration route is carried out on healthy volunteers with simultaneous assessment of the standard deviation of difference between oxyhemoglobin saturation values obtained by pulse oximetry and invasive arterial blood sample. In general, the proclaimed accuracy of a pulse oximeter measurement is 2%. Derived from clinical studies on critically ill patients and preterm neonates, this number translates into a probability of an intrinsic error of 3%–4% (Nitzan et al., 2014). Such discrepancy may have enormous impact on patients, when accurate values are warrant for precision of their titrated supplemental oxygen therapy.

Studies have in general, reported good correlations between oxyhemoglobin saturation values obtained by pulse oximetry and invasive arterial blood gas measures over the range of 70% to 100% in healthy people and patients with adequate perfusion. This correlation is however, lost and pulse oximetry readings become inaccurate in patients with inadequate tissue perfusion and under hypoxic condition (Perkins, McAuley, Giles, Routledge, & Gao, 2003; Trivedi, Ghouri, Lai, Shah, & Barker, 1997; Van de Louw et al., 2001; Wilson, Cowan, Lord, Zuege, & Zygun, 2010).

Peripheral vasoconstriction is one of the earliest response to compromised central blood volume (Dutton, 2007; Scheeren, Schober, & Schwarte, 2012), severe hypoxia (Heistad & Abboud, 1980) and acute pain (Hoiseth et al., 2015), signaling the acute sympathetic nervous system response for redistribution of blood flow from lower priority organs to vital organs, including the central nervous system (Dutton, 2007). Because peripheral pulse oximetry depends on pulsatile arterial blood volume their usage can be limited by inadequate tissue perfusion (Chan & Chan, 2013; Nitzan et al., 2014). Under such circumstances it may become difficult or even impossible to obtain sufficient signal for a pulse oximetry reading from peripheral vascular bed. In case of hypoxemia the pulse oximeter measurement may also lag behind the real time oxygen deterioration of arterial blood (Fouzas, Priftis, & Anthracopoulos, 2011).

1.1.4 Ongoing efforts to improve non-invasive oximetry

Despite recent advances and ongoing efforts to improve the existing technology, non-invasive oximetry modalities are still inferior to invasive monitoring. The drawback of current technology underpins the need for ongoing endeavor to improve and develop non-invasive method for estimation of oxyhemoglobin saturation. Concurrently, Near-infrared spectroscopy has gained increased attention in the acute patient care. Unlike pulse oximetry, cerebral and tissue near-infrared spectroscopy oximetry is independent of the pulsatile blood flow. It uses fixed and relative 70% venous and 30% arterial blood to estimate capillary saturation and therefore tissue oxygen hemoglobin saturation. For that reason transcuteanous cerebral near-infrared spectroscopy offers information on intracerebral tissue oxygen supply and metabolic demand rather than cerebral oxygen delivery itself (Ikeda et al., 2014; Steppan & Hogue, 2014).

1.1.5 Retinal oximetry

Measurements of oxyhemoglobin saturation in retinal vessels may be a more

direct indicator of oxygen delivery to the brain than the peripheral pulse oximetry or a cerebral near-infrared spectroscopy. In numerous clinical situations counting critical care, operating rooms, emergency departments and out-of-hospital traumatic injury, patients may suffer circulatory shock where peripheral vasoconstriction limit the pulse oximetry readings but ocular and cerebral perfusion are preserved (Denninghoff, Smith, Lompado, & Hillman, 2003; Riva, Alm, & Pournaras, 2011). For that reason, measurement of central nervous system vessels with retinal oximetry may be more reliable mean for estimation of oxygen delivery to the brain and to guide resuscitation.

Spectrophotometric retinal oximetry captures images of the retinal circulation for the calculation of oxyhemoglobin saturation and thus, an estimation of oxygen delivery to the central nervous system by the central circulation. Such method might be highly valuable and mark a milestone in patient care. Especially for those patients at risk for central nervous system hypoxia. In addition to arterial oxygen delivery the retinal oximetry allows direct non-invasive assessment of venous oxyhemoglobin saturation and hence has the potential for microcircular hemodynamic assessment as well. Therefore, the method could be an important step in the development of improved approach for measurement oxyhemoglobin saturation of the human body, imaging the only place where arterial and venous blood can be directly imaged with visible light.

1.2 Retina

The transparent structure of the eye allows images as light waves to pass through the cornea, aqueous humor, lens and vitreous humor before hitting the retina that lines the back of the eye **(Figure 1)**. The retina is a part of the central nervous system and formed embryonically as an outgrowth of the forebrain. It is approximately 0.3 mm thick and composed of numerous cell and nerve fiber layers the light must enter before reaching the photoreceptors in the outer retina. Once the light gets to the photoreceptors it is transformed into nerve signals and transmitted by the optic nerve to the cerebral cortex for recreation into a visual image (Kolb; Levin et al., 2011).

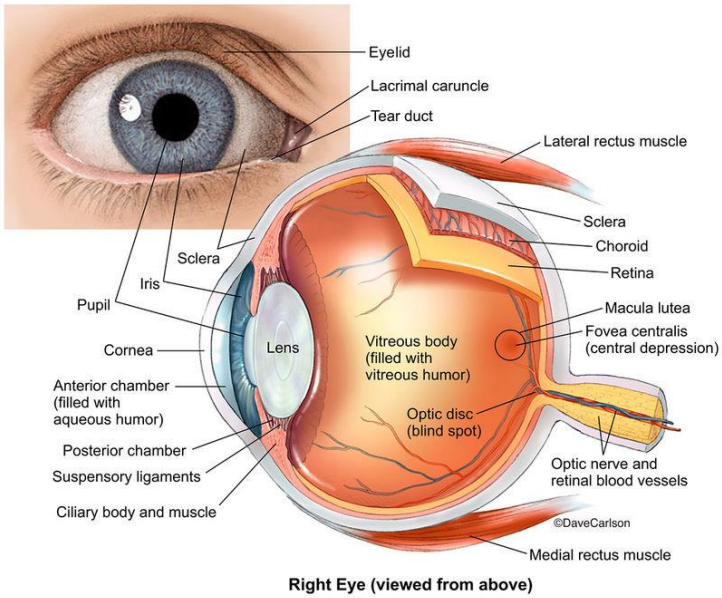


Figure 1. A schematic view of the tissues of the eye. Illustration ©Dave Carlson/CarlsonStockArt.com.

The optic disc (optic nerve head) is a white circular spot in the central retina (**Figure 2**). On the temporal side in the center of the retina is the macula, an avascular oval shaped area, approximately 5 mm in diameter. The macula constitutes the fovea, which is densely packed with cones, the photoreceptors that are essential for sharp and detailed color vision. The rods are the photoreceptors for black and white vision of dim light and are predominantly found outside the macula, in the periphery of the retina.

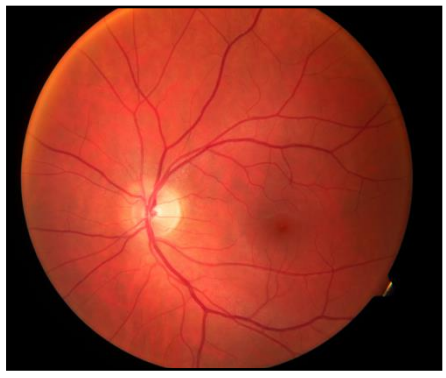


Figure 2. Normal fundus photograph of the left eye with retinal arterioles and venules piercing the optic disc. The dark spot in the centre is the macula consisting the fovea.

1.3 Retinal oxygenation

1.3.1 Ocular blood flow

The human retina is supported by two vascular systems which differ both anatomically and physiologically: the retinal circulation and the choroidal circulation. The retinal circulation supports the inner two-thirds of the retina whereas the choroidal vasculature supplies the outer third of the retina with at least 85% of the total ocular blood flow (Alm & Bill, 1973; Nickla & Wallman, 2010). Both these circulations arise from the ophthalmic artery, which supplies the entire eye. The ophthalmic artery is the first branch emanating the internal carotid artery on its way carrying metabolic substrates and oxygenated blood (Riva et al., 2011) from the aorta to the brain. The ophthalmic artery divides into the central retinal artery and ciliary arteries to supply the inner and outer retina at the back of the eye respectively (Riva et al., 2011).

1.3.1.1 Retinal circulation

The central retinal artery (CRA) penetrates the optic nerve sheath about 10 mm behind the globe (**Figure 3**). It runs centrally within the optic nerve to the optic disc where it bifurcates into superior and inferior retinal branches. These branches in turn divide into temporal and nasal arterioles where each supplies one quadrant of the inner retina (Hayreh, 2011; Pournaras, Rungger-Brandle, Riva, Hardarson, & Stefánsson, 2008). The retinal circulation is an end-arterial circuit without anatomises. Retinal arterioles, arcade by dicthomous and side arm branching toward the periphery until terminating in two layered capillary plexuses connecting precapillary arterioles to postcapillary venules. Larger arterioles and venules are found in the innermost layers of the retina at the inner limiting membrane and the outer plexiform layer. Capillary density is greatest in the centre of the retina with the more superficial capillary plexus situated in the ganglion cell and nerve fiber layer. The second capillary plexus lies deeper, at the boarder of the inner nuclear and outer plexiform layer with a single layered capillary network proceeding until it finally vanish, leaving an avascular zone in the farthest retinal periphery. In addition, a single layered capillary network surrounds the area of the avascular fovea as well as the superficial peripapillary capillaries that enclose the optic disc, to chase the superior and inferior temporal retinal vessels.

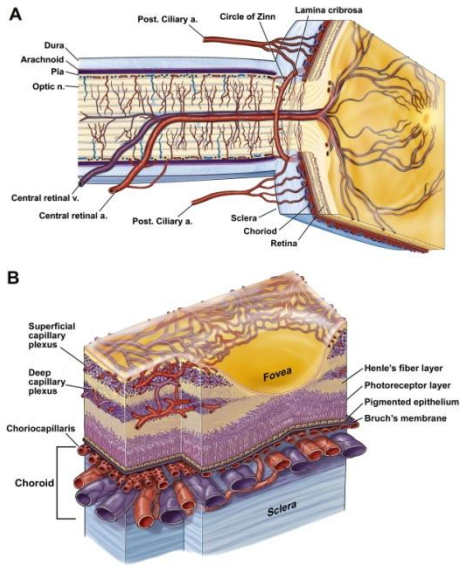


Figure 3. Anatomy of ocular circulation (a-artery, b-vein, n-nerve). A, Cut away drawing along the superior–inferior axis of the human eye through the optic nerve, showing the vascular supply to the retina and choroid. B, Drawing showing vasculature of the retina and choroid. Drawings by Dave Schumick from Anand-Apte and Hollyfield (2009). Reprinted from Prog Retin Eye Res, 31(5), Kur et al., Cellular and physiological mechanisms underlying blood flow regulation in the retina and choroid in health and disease, 377-406, © 2012, with permission of Elsevier Ltd.

The retinal venous system follows a similar pattern to the arteriolar structure. It runs independently in the periphery of the retina but in close proximity to the arterioles with occasionally crossing within the centre of the retina. The postcapillary venules drain into major branch venules that merge at the optic nerve head to form a central retinal vein (CRV). The CRV leaves the eye adjacent (temporal) to the CRA within the optic nerve before emptying either into the ophthalmic vein or directly into the cavernous sinus (Hayreh, 2011; Pournaras et al., 2008; Riva et al., 2011).

1.3.1.2 Choroidal circulation

The choroidal circulation is located between the outer retina and the sclera that constitute the outermost membrane of the eye. It originates in 2-3 main ciliary arteries coming off the ophthalmic artery to supply the temporal and nasal portions of the choroidal sphere. Main ciliary arteries branches into 10-20 short posterior ciliary arteries and two long posterior ciliary arteries to form major choroidal arteries that support the posterior and anterior portion of the choroid respectively. The shortest posterior ciliary arteries aim for a vascular structure

near the macula to nourish the vicinity of fovea. Occasionally a cilioretinal artery arises directly from a short posterior ciliary artery or the peripapillary choroid. It normally pierces the retina temporal to the optic disc to nourish the macular area but the location and contribution to the oxygen supply varies.

The choroid is made of three layers: the outermost Haller´s layer of large vessels, the inner Sattler´s layer of medium and small arteries and arterioles, and the dense innermost network of single choriocapillary layer. Choriocapillary blood drains into the vortex veins before entering orbital veins (Hayreh, 1975, 2011; Nickla & Wallman, 2010; Riva et al., 2011) and eventually emptying into the cavernous sinus.

1.4 Retinal metabolism

The retina is metabolically one of the most active tissue in the body, consuming oxygen faster than the brain whilst lacking the capacity of oxygen reserve (Wangsa-Wirawan & Linsenmeier, 2003). Oxygen delivery by the two separate vascular systems must therefore be highly efficient to meet the metabolic demand of the retinal tissue as reflected by their intrinsic properties in virtue. The retinal circulation is characterized by slow rate of blood flow (Alm & Bill, 1973; Wang et al., 2009; Werkmeister et al., 2015), high oxygen extraction (Werkmeister et al., 2015) and about 35% arteriovenous oxygen difference (Schweitzer et al., 1999). In contrast the choroidal circulation has exceedingly high blood flow (Riva et al., 2011) and very low oxygen extraction fraction or only 3% (Nickla & Wallman, 2010). Choriocapillaries have large diameter (≥ 10 µm) which generate low resistance (compared with 5 µm of retinal capillaries, offering higher resistance) that explains the high blood flow and low rate of oxygen extraction of the choroidal circulation. This create oxygen abundance gradient across the Bruch´s membrane to the avascular zone of outer retinal layers for the energy consuming activity of the photoreceptors (Linsenmeier & Padnick-Silver, 2000).

Because the choroidal circulation is located immediately behind the retina, a sufficient oxygen flux from the choriocapillaries through the Bruch´s membrane and retinal pigment epithelium (**Figure 4**) is of vital importance for normal function of the photoreceptors (Delaey & Van De Voorde, 2000; Jackobiec´s, Miller, Azar, & Blodi, 2008; Linsenmeier & Padnick-Silver, 2000). From choriocapillaries the PO_2 declines sharply across the outer retina until it reaches a very low minimum in the inner segments, at the location of mitochondria in photoreceptors (Linsenmeier & Padnick-Silver, 2000). Photoreceptors carry out the most energy demanding function of the retina

(Buttery, Hinrichsen, Weller, & Haight, 1991) that is influenced by illumination. The metabolic activity is higher in dark than in light when the oxygen consumption exceeds 90% of the blood supply. In darkness thus, approximately 90% of the oxygen supply comes from the choroidal circulation and the remainder 10% diffuses from the retinal circulation. In light however, the oxygen flux from the choroidal circulation fulfills the photoreceptors metabolic need and some oxygen may even reach the inner retina as well (Hardarson et al., 2009; Linsenmeier & Braun, 1992; Linsenmeier & Padnick-Silver, 2000; Nickla & Wallman, 2010; Stefánsson, Wolbarsht, & Landers, 1983).

Unlike choroidal blood flow that appears to be unaffected by increased metabolic activity of the retinal tissue, the blood flow amplifies in the retinal circulation (Garhofer, Huemer, Zawinka, Schmetterer, & Dorner, 2002) to supply photoreceptor oxygen consumption under increased metabolic demand (in a dim light). This is manifested by sharp decline in oxygen diffusion across the outer nuclear layer to the inner segments (Linsenmeier & Padnick-Silver, 2000) at the location of the photoreceptors at the outer retina.

The spatial differences of oxygen diffusion between the retinal and choroidal circulations depend on the location and local metabolic activity within the retinal tissue (Braun, Linsenmeier, & Yancey, 1992; Linsenmeier & Braun, 1992). Studies have shown that the superior and inferior temporal quadrants of the retina receive higher blood flow (Feke et al., 1989) and consume more oxygen (Schweitzer et al., 1999) than the nasal hemisphere that most likely reflects the metabolic activity as previously discussed.

The photoreceptors include rodes and cones and are responsible for light absorption **(Figure 4).** Horizontal and bipolar cells transmit signals from the photoreceptors to the ganglion cells which carry them via optic nerve to the brain. The photorecptors are situated in the outer retina and receive oxygen from the choriocapillaries (ChC) located outside the retinal pigment epithelium, adjacent to the Bruch´s membrane (BrM). The retinal circulation supplies the inner part of the retina with capillaries reaching down to the plane of photreceptors.

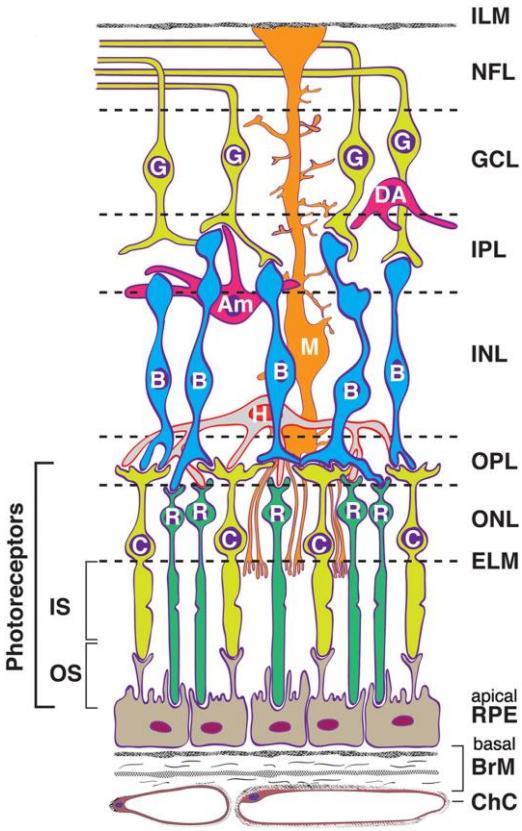


Figure 4. Chorioretinal layers and major cell types. Modified from Zheng et al. (2012). The neurosensory retina has distinct layers (from top to bottom): inner limiting membrane (ILM), nerve fiber layer (NFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), external limiting membrane (ELM), photoreceptor inner segments (IS), and photoceptor outer segments (OS). The retinal pigment epithelium (RPE), lies outside the neurosensory retina but is considered a part of the retina embryologically. The major retinal cell types are ganglion cells (G), diffuse amacrine cells (DA), amacrine cells (Am), Müller cells (M), bipolar cells (B), horizontal cells (H), rods (R), and cones (C). Reprinted from Prog Retin Eye Res, 41(2014),Pikuleva & Curcio.,Cholesterol in the retina: The best is yet to come,64-89,© 2014, with permission of Elsevier Ltd.

1.5 Autoregulation

Vascular beds in organs have an intrinsic capacity to some extent to regulate the perfusion pressure locally. This property is referred to as autoregulation and is produced by an intrinsic capacity for a stretch response of the arteriolar vascular smooth muscle. This ability for a local regulation maintains the blood flow relatively constant despite of variations in the perfusion pressure (Arjamaa & Nikinmaa, 2006) and keeps the PO_2 of the inner retina relatively unaffected by either systemic hypoxemia or hyperoxia. This is an unique characteristic trait of the retinal tissue. Dissimilar to most other tissues of the body, the retinal vessels and the optic nerve head lack autonomic innervations and subsequently relay on the autoregulation for regulation of blood flow. The mechanism behind the retinal autoregulation is not fully understood but is balanced by the effects of myogenic and metabolic factors on the vascular resistance in adjustment of local blood flow to changes in perfusion pressure and the metabolic need of the tissue.

Retinal arteries have remarkably well-developed smooth muscle layer that differ them from arteries of the same size in other organs. Changes in arteriolar diameter is considered the main regulatory component of the retinal vasculature and is triggered either by systemic or local factors (Delaey & Van De Voorde, 2000; Hayreh, 2011; Pournaras et al., 2008). Vascular resistance is generated along the entirely length of the arteriolar wall but terminal arterioles between 20 µm and 50 µm in diameter offer the main resistance, thereby playing a central role in the autoregulatory response either by vessel dilation or vasoconstriction (Hayreh, 2011; Jeppesen, Sanye-Hajari, & Bek, 2007; Schmidl, Garhofer, & Schmetterer, 2011). Contractile properties of intramural perocytes in retinal capillaries also play a role in the autoregulatory response (Anderson, 1996; Anderson & Davis, 1996). Perocytes possess similar properties as smooth muscle cells in that they can dilate and constrict the capillary lumen to some minor extent in response to vasoactive substances or to provocation by local blood gases (Kur & Newman, 2014; Pournaras et al., 2008).

1.5.1 Systemic and local factors

Autoregulation of the retinal blood flow pertains to metabolic and myogenic mechanisms in reaction to activation of systemic and local factors as mentioned above. The autoregulatory mechanism is evoked by vasoactive substances that are released from the endothelium in arterioles and the adherent retinal tissue. Metabolic autoregulation strives for retinal tissue local blood flow regulation in unity with its metabolic requirements. For instance, in the case of accumulation of metabolic wastes in the tissue the rate of blood flow increases. The myogenic autoregulation, however, is activated by

alterations in the transmural pressure, secondary to constriction or stretching on the endothelium in the vessel wall. In return, vasoactive factors are released causing either a dilatation or constriction of the vessel (Delaey & Van De Voorde, 2000; Pournaras et al., 2008).

1.5.1.1 Systemic factors

Systemic blood pressure, circulating hormones, arterial blood gases and pH are among the systemic factors that activate the autonomic local vascular reaction. Local factors incorporate the PO_2 and partial pressure of carbon dioxide (PCO_2), pH, endothelial factors (endothelium derived relaxing factors and constricting factors) and retinal factors (Delaey & Van De Voorde, 2000). Increase in arteriolar mural pressure and mechanical stretch trigger release of the endothelium derived constricting factor, endothelin-1, resulting in vasoconstriction (Polak et al., 2003). Nitric oxide (NO) is one of the major endothelium-derived relaxing factor (EDRF), maintaining the basal vascular tone and mediating vasodilatation by several agonists (Schmetterer & Polak, 2001). Acetylcholine, histamine and bradykinin are all examples of neurotransmitters that modulate the vascular tone by activation of EDRF (Yu, Su, Cringle, & Yu, 2003). The role of retinal relaxing factor (RRF) is yet relatively unknown. It seems to play considerable role as an indirect mediator of the hypoxic response by the retinal tissue itself, independent of other vasoactive mediators (Maenhaut, Boussery, Delaey, & Van de Voorde, 2007). Other local compounds that contribute to retinal blood flow regulation include adenosine and prostacycline by increasing the arteriolar diameter. Angiotensin II, prostaglandin and cyclooxygenase narrow the arteriolar diameter whereas lactate modulates the vascular tone parallel to the local metabolic need either by constricting or widening the vessel (Gidday & Park, 1993; Pournaras et al., 2008; Yamanishi, Katsumura, Kobayashi, & Puro, 2006).

Circulating hormones like endothelin-1 and angiontensin II have negligible effects on the retinal circulation (Flammer & Mozaffarieh, 2008) because the non-fenestrated endothelium of retinal capillaries and the complex network of tight junctions, that resemble the blood-brain barrier (Patton et al., 2005), prevent large molecules penetrating the inner blood-retinal barrier. Conversely, the fenestrated endothelium of choriocapillaries is highly permeable to molecules their size, allowing for their direct effects on smooth muscle cells (Flammer & Mozaffarieh, 2008; Riva et al., 2011).

1.5.1.2 Hypoxemia and hyperoxia

Hypoxemia provokes reactivity in retinal vessels mainly through release of tissue metabolites in response to the abnormally low PO_2 in the arterial blood. Hypoxemia frequently results in hypoxia, the inadequate level of oxygenation

for retinal tissue metabolism. Reports on the impact of adenosine on retinal vascular relaxation are conflicting (Delaey, Boussery, & Van de Voorde, 2000; Gidday & Park, 1993) but prostacycline, lactate and the RRF are all released under hypoxic condition and are found to increase the retinal blood flow secondary to vasodilatatory response (Delaey et al., 2000; Hata et al., 2000; Maenhaut et al., 2007; Pournaras et al., 2008; Yamanishi et al., 2006). Supposedly, RRF is one of the main modulators for hypoxic vascular response. It is independent of endothelial involvement in the arteriolar wall which may explain the slower onset of retinal vascular reaction as compared with that of the cerebral circulation.

In hyperoxia, retinal vasoconstriction has faster onset than the vasodilatation during hypoxemia probably due to faster release of vasoconstrictive substances from the endothelial cells in retinal arterioles (Cheng, 2014; Delaey et al., 2000; Maenhaut et al., 2007).

1.5.1.3 Gas challenges on retinal circulation

Similar to cerebral circulation (Pournaras et al., 2008) the retinal circulation adjust the local retinal blood to changes in arteriolar PO_2 and PCO_2 by widening or narrowing the vascular lumen. Hyperoxia-induced vasoconstriction is greater in retinal vessels than in cerebral vessels, in contrast with the hypercapnic-induced vasodilatation that is greater in the brain (Cheng et al., 2016; Kisilevsky, Hudson, Mardimae, Wong, & Fisher, 2008).

Hyperoxic and hypocapnic gas challenges provoke vasoconstrictions in both retinal arterioles (Gilmore, Hudson, Venkataraman, Preiss, & Fisher, 2004) and venules (Cheng et al., 2016; Jean-Louis, Lovasik, & Kergoat, 2005; Palkovits, Lasta, et al., 2014; Palkovits, Told, Boltz, et al., 2014; Rose, 2016; Werkmeister et al., 2015) whereas hypercapnic (Venkataraman et al., 2008) and hypoxic gas mixtures induce vasodilatation of those vessels (Brinchmann-Hansen, Myhre, & Sandvik, 1989; Cheng et al., 2016; Choudhary, Ball, Fernandez Ramos, McNaught, & Harvey, 2013; Palkovits, Told, Schmidl, et al., 2014; Rose, 2016). The blood flow regulation is found to be relatively stable when PaO_2 is above 32-37 mmHg but beneath these limits the autoregulatory response is found to be lost (Cheng et al., 2016). According to electroretinography studies, the inner retina show more sensitivity to transient hypoxic stress (at the level of the retinal ganglion cells) than the outer retina which is more resistant to the hypoxic challenges (Caprara & Grimm, 2012; Janaky, Grosz, Toth, Benedek, & Benedek, 2007; Tinjust, Kergoat, & Lovasik, 2002).

1.5.1.4 Perfusion pressure

The ability for local autoregulation in vascular beds maintains the retinal blood flow relatively constant and independent of fluctuations in the perfusion pressure, as long as the ocular perfusion pressure (OPP) is within a range of upper and lower limits of the autoregulatory plateau. Beyond these limits the vascular reserve is lost and ocular blood flow becomes directly dependent on the pressure. (Arjamaa & Nikinmaa, 2006). By most studies the upper limits of retinal blood flow regulation is attained when the mean arterial blood pressure (MAP) reaches approximately 40% above baseline (Schmidl, Garhofer, et al., 2011). Other studies have found the autoregulatory limits of the mean OPP in the range of 34 – 60% over baseline (Pournaras et al., 2008), more than 36% below baseline (Riva, Sinclair, & Grunwald, 1981) or when the intraocular pressure (IOP) either reaches about 30 mm Hg (Schulte et al., 1996) or drops below 10 mm Hg (Williamson & Harris, 1994)

1.5.1.5 Calculation of retinal blood flow

Retinal blood flow (BF) through the optic nerve head can be calculated according to the Pouseille's law, which states that blood flow is directly proportional to perfusion pressure and inversely proportional to the vascular resistance:

$$BF = \frac{PP}{R} \qquad \text{Equation 1.}$$

Where perfusion pressure (PP) is the force that drives blood through the vessel, determined by the difference between the arterial and venous pressure (ΔP). The resistance (R) of a vessel wall against the PP is a function of the vascular caliber and the vessel tone (Caprioli & Coleman, 2010). Blood flow resistance can be calculated according to the following equation:

$$R = \frac{8\eta L}{r^4} \qquad \text{Equation 2.}$$

Where, vascular resistance (R) is directly related to the fluid viscosity (η) and the length of a vessel (L), and inversely related to the radius of the vessel in the fourth power (r). For that reason, even small changes in the vascular lumen have considerable effects on blood flow resistance. For instance, lessen the retinal arteriolar lumen by half will 16-fold the increase in OPP.

1.5.1.6 Ocular perfusion pressure

As already brought up, the mean OPP is the pressure driving the blood through

the optic nerve. It is determined by the mean ophthalmic arterial pressure (MOAP) entering the eye, minus the mean ophthalmic venous pressure (MOVP), leaving the eye:

$$OPP = MOAP - MOVP \quad \text{Equation 3.}$$

The MOVP is close to the intraocular pressure (IOP) and therefore, the OPP can be estimated as:

$$OPP = MOAP - IOP = 2/3\ MAP - IOP \quad \text{Equation 4.}$$

The IOP is determined by the rate of aqueous humor production and the drainage of aqueous humor through the trabecular meshwork. The normal lower and upper limits of IOP are 10 and 22 mm Hg respectively and the mean around 16 mm Hg (Thariq Bhatti, 2008; Williamson & Harris, 1994).

1.5.1.7 Mean arterial pressure

Mean OPP is estimated to be 2/3 of the mean brachial blood pressure. The mean arterial pressure is the time weighted average calculation on arterial pressures during one pulse cycle (Butterworth, Mackey, & Wasnick, 2013), determined by the following equation:

$$MAP = \frac{SBP + 2(DBP)}{3} \quad \text{Equation 5.}$$

The MAP calculation is based on the fact that during a pulse cycle, 1/3 of the time is spent near the systolic blood pressure (SBP) and 2/3 near diastolic blood pressure (DBP) (Butterworth et al., 2013)

The OPP may be affected by one or more of the variables that are used to calculate its value. For example, a low systemic arterial blood pressure or a high IOP (≥ 30 - 34 mmHg) will reduce the OPP whereas a high systemic blood pressure or a low IOP (<10 mmHg) will elevate the OPP (Williamson & Harris, 1994). Consequently, the vessel lumen will distend or constrict to the magnitude of fall or rise in IOP respectively until reaching the point the compensatory ability is lost and retinal blood flow becomes a direct function of OPP.

1.6 Choroidal blood flow regulation

Regulation of retinal blood flow is believed to be more efficient than of the choroidal flow by the autoregulatory mechanism. Unlike the retinal circulation, the choroidal vascular bed and the central retinal artery up to the lamina cibrosa are innervated by the autonomic nervous system (Nickla & Wallman, 2010). The choroidal circulation demonstrates limited autoregulatory capacity to changes in IOP (Schmidl et al., 2012; Schmidl, Weigert, et al., 2011) but emerging evidence have verified some autoregulatory efforts to changes in MAP and OPP (Fuchsjager-Mayrl et al., 2003; Luksch et al., 2003; Schmidl et al., 2012).

It is also likely that the choroidal circulation is only autoregulated by changes in PCO_2 but not PO_2 (Thylefors, Piitulainen, & Havelius, 2009). Under system hypoxemic conditions the level of PO_2 drops linearly from the choriocapillaries across the outer retina (Linsenmeier & Braun, 1992) so the oxygen flux becomes insufficient for the outer retina oxygen requirements. In case the systemic PaO_2 become less than 60 mmHg the retinal circulation contributes (10%) by oxygen diffusion to the outer retinal segments (Linsenmeier & Braun, 1992).

Hypercapnia on the other hand, exert strong vasodilatory NO mediated effects on the choroidal vasculature, just like on the retinal circulation (Schmetterer & Polak, 2001). Carbon monoxide seems to affect the rate of choroidal blood flow to some degree whilst hyperoxia has negligible effects on the blood flow (Schmidl, Garhofer, et al., 2011). Due to the lack of choroidal autoregulatory response to system hyperoxia, the abundant oxygen flux for the duration of supplemental oxygen breathing seems beneficiary for the inner retinal tissue by attenuating the effects of hyperoxic vasoconstriction on the retinal circulation (Palkovits, Lasta, et al., 2014).

1.7 Oxygen transportation to tissues

1.7.1 Physiology of oxygenation

Oxygen is transported by the arterial blood to tissues in two forms: dissolved and combined with hemoglobin. Under normal condition, about 97% of the oxygen is bound with hemoglobin and the residual oxygen (3%) is dissolved in plasma. (Guyton, 2000). Oxygen combines reversibly with the heme-portion of the hemoglobin molecule to form oxyhemoglobin. This chemical reaction is reliant on the concentration of PO_2 dissolved in plasma. Each hemoglobin molecule has the capacity to combine with four oxygen molecules.

Accordingly, the percentage of hemoglobin molecules occupied by oxygen molecules to the total oxygen binding capacity is express as oxyhemoglobin saturation (West, 2005).

1.7.1.1 Oxyhemoglobin dissociation curve

The relationship between oxyhemoglobin saturation and the PO_2 can be described by the oxyhemoglobin dissociation curve.

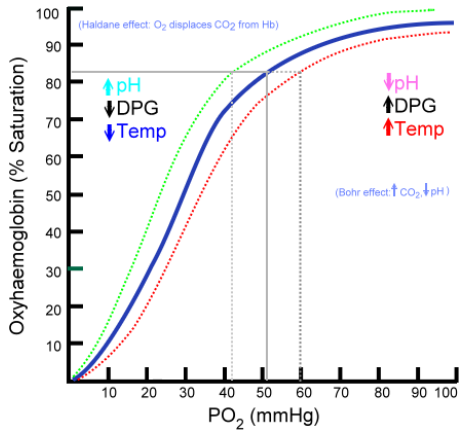


Figure 5. The oxyhemoglobin dissociation curve (blue) illustrates how oxyhemoglobin saturation arises quickly up till approximately PO_2 of 50 mmHg, under normal physiological conditions. From PO_2 of 60 mmHg which represents about 90% oxyhemoglobin saturation the curve becomes flatter because of fewer available oxygen hemoglobin binding sites, until complete saturation is reached at 100%. The red dotted line represents a condition of rightward shift of the curve and the green line leftward shift of the oxyhemoglobin dissociation curve. Public domain figure. https://upload.wikimedia.org/wikipedia/commons/8/8a/Oxyhaemoglobin_dissociation_curve.png

The oxyhemoglobin dissociation curve has a sigmoidal shape representing the dynamic interaction between the oxyhemoglobin saturation and the PO_2 in plasma. The binding of the first oxygen molecule is more difficult than subsequent oxygen-hemoglobin attachments. After the first oxygen join one of the four heme portions, configural changes of the hemoglobin molecule facilitate additional bounding to other groups so the affinity for the fourth oxygen molecule is nearly 300-fold greater than for the first one. The opposite is just true for oxygen release in tissues in that the dissociation of the first oxygen molecule facilitates subsequent oxygen release from the hemoglobin (Butterworth et al., 2013; Guyton, 2000).

The chemical reaction of oxygen-binding is illustrated by the steep sigmoidal upward shift in the region of 20-30 mmHg (Harvey, 2011). Normally, 50% oxyhemoglobin saturation (P50) is reached at approximately 26.6 mmHg. When the hemoglobin affinity for oxygen is increased the 50% saturation is reached at a lower PO_2 with subsequent shifting of the oxyhemoglobin dissociation curve to the left and less availability of oxygen for tissues. The exact opposite is true when the the hemoglobin affinity for oxygen is decreased. Then the oxyhemoglobin dissociation curve is shifted to the right and a higher PO_2 is needed to reach the 50% oxyhemoglobin saturation, and more oxygen become available for tissues. This condition is typical for a normal oxygen delivery in tissues where hydrogen ion (H^+) concentration accompanying elevated CO_2 production secondary to cellular metabolism shifts the oxyhemoglobin dissociation curve to the right. Hence, the hemoglobin affinity is decreased and more oxygen is released at lower PO_2. This condition is represented at the steepest part on the curve where only small changes in PO_2 dislodge oxygen molecules from the hemoglobin. Other known factors that shift the curve to the right include increased 2,3 diphosoglycerate (DPG) and elevated blood temperature (Butterworth et al., 2013; Guyton, 2000).

1.7.1.2 Oxygen content of the blood

As previously stated, the oxyhemoglobin saturation (SO_2) is the percentage of available hemoglobin binding sites that are combined with oxygen given by the following equation:

$$SO_2\ (\%) = \frac{[HbO_2]}{[Hb] + [HbO_2]} \times 100 \qquad \text{Equation 6.}$$

Where HbO_2 is the oxygen combined with hemoglobin and Hb is the deoxygenated hemoglobin. The SO_2 is multiplied by 100 to obtain the percentage. The SO_2 of arterial blood (SaO_2) at 100 mmHg is approximately 97,5 % and 75% for the mixed venous blood (SvO_2) at PaO_2 of 40 mmHg (Guyton, 2000; West, 2005).

According to the Henry´s law, the dissolved concentration of oxygen in the blood is proportional to the partial pressure. For each mmHg of PO_2 there is 0.003 ml oxygen (O_2) dissolved in 100 ml of blood. Therefore, a normal arterial blood (at sea level) with PO_2 of 100 mmHg contains 0.3ml O_2 per 100 ml blood. The oxygen capacity is about 20.8 ml O2 • 100 ml $^{-1}$ of blood because one gram of pure Hb can combine with 1.39 ml O_2 and normal blood has about 15 gram of Hb • 100 ml $^{-1}$. However, under normal physiological conditions of the

body, other species of hemoglobin, namely dyshemoglobins, may exist in the blood as well (West, 2005).

Because in reality the the oxyhemoglobin saturation never reaches the theoretical maximum of 1.39 ml O_2 and some measurements give 1.34 ml, the total oxygen content of blood is given by the following equation:

$$\text{Oxygen concentration} = (1.34 \times Hb \times SaO_2) + 0.003 \times PO_2 \qquad \text{Equation 7.}$$

Where 1.34 is the oxygen carrying capacity (ml/g) of hemoglobin (at sea level), Hb is the amount of hemoglobin in the blood (g/dl), SaO_2 is the oxyhemoglobin saturation of arterial blood at given PO_2 and 0.003 ml O_2 dissolved in 100 ml of blood per mmHg PO_2.

1.7.1.3 Dyshemoglobin

Hemoglobin can be classified as a normal hemoglobin that is capable of carrying oxygen (Hb and HbO_2) and dyshemoglobin that are hemoglobin derivatives and incapable of an oxygen holding. Dyshemoglobin are further classified as methemoglobin (MetHb), carboxyhemoglobin (COHb), and sulfhemoglobin (SHb). Sulfhemoglobin is an uncommon form that is caused by reaction of sulfa-containing compounds, usually from excessive use of sulfa-based drugs (Haymond, 2006). Carboxyhemoglobin carry carbon monoxide that is formed during the metabolic pathway of heme into bilirubin and constitutes normally less than 1 - 3% of the total hemoglobin in the body (McClatchey, 2002). It can however, be as much as five fold in a heavy smoker (Whincup, Papacosta, Lennon, & Haines, 2006). The greater affinity (200 to 300 fold) of carbon monoxide for hemoglobin than oxygen allows COHb to easily displace the HbO_2, shifting the oxyhemoglobin dissociation curve to the left. Methemoglobin is the oxidative deoxy form of normal hemoglobin where the iron of the heme group is in a ferric (Fe^{3+}) form instead of the ferrous (Fe^{2+}) state. The MetHb has no oxygen carrying capacity and similar to the COHb can shift the oxyhemoglobin dissociation curve to the left (Butterworth et al., 2013; McClatchey, 2002).

Some contemporary peripheral co-oximeters differentiate aforementioned abnormal dyshemoglobin structures from normal hemoglobin. However, at 660 nm, the COHb has an absorbance parallel with that of HbO_2 (Miller, 2000) thereby necessitating information's on whether the person is an active smoker before spectroscopic retinal oximetry is performed. The half-life of COHb is 4–6 hours on a room air so smoking should preferentially be abstained for 12-24 hours prior to retinal oximetry. Breathing 100% oxygen shortens COHb half life

to 40-80 minutes and hyperbaric oxygen breathing shorten it still further, or to 15-30 minutes (Nagelhout & Zaglaniczny, 2001).

1.7.1.4 Fetal hemoglobin

Fetal hemoglobin (HgbF) constitutes about 75-84% of the total hemoglobin in new born babies (Chestnut, 2004) and 1% of the normal adult hemoglobin (Schechter, 2008), or hemoglobin A (HgbA). Functionally, HgbF diverge from HgbA in that it has somewhat higher affinity for oxygen because of decreased interaction with 2,3–DPG (Mosca, 2009) with P50 around 19-21 mmHg (Chestnut, 2004) instead of 26,6 mmHg in adults. During the first month of life the HgbF amount is progressively substituted by HgbA until dwindling off by 6 months of life (Edoh, Antwi- Bosaiko, & Amuzu., 2006). As HgbA starts to substitute the HgbF, the level of 2,3DPG raise and the affinity becomes analogous to that of adults within the first few month, although the concentration of HgbF still remains 25% (Chestnut, 2004). HgbF has nearly the same absorption spectrum for oxyhemoglobin and deoxyhemoglobin as HgbA and hence, should not affect the oximetry outcome measures from of adults (Chestnut, 2004; Miller, 2000).

1.7.2 Matching oxygen supply to demand

Oxygen transport to tissues depends on adequate respiratory and circulatory function. Dry air at sea level (barometric pressure of 760 mmHg) has a PO_2 of 20.93% (~21%). The fully satured wapor pressure at a sea level is 47 mmHg at normal body temperature of 37°C. Thus, the PO_2 of an inhaled air at sea level is expressed as follows:

$$\frac{20.93}{100} \times (760 - 47) = 149 \text{ mmHg} \qquad \text{Equation 8.}$$

When the inspired air reaches the alveoli of the lungs the PO_2 has dropped to about 100 mmHg. The reason is that the alveolar PO_2 is a product of the balance between alveolar ventilation and the alveolar gas diffusion across the capillary blood-gas interface (West, 2005).

1.7.2.1 Global oxygen delivery

Global oxygen delivery (DO_2) to tissues is a product of cardiac output (CO), or the blood flow, and the total oxygen content of the arterial blood (CaO_2) (McLellan, 2004). The DO_2 is calculated by the following formula:

$$DO_2 = CO \times CaO_2 \qquad \text{Equation 9.}$$

Where cardiac output is a product of the heart rate (HR) and stroke volume (SV) and the stroke volume is the amount of blood ejected by the left ventricle during one heart beat. The oxygen concentration depends on both oxyhemoglobin saturation and PO_2 dissolved in plasma, as previously discussed. Therefore, an inadequate oxygen delivery may either originate from impaired cardiac output, insufficient PaO_2 or a low hemoglobin count (Butterworth et al., 2013).

Global oxygen delivery to tissues depends on two processes: convection of the blood down the arterial tree and diffusion from capillaries to adjacent tissues (Leach & Treacher, 2002). Elastic properties and caliber of the vessel wall determine the vascular resistance against the cardiac output. Subseqently, the compliance in the arterial system serves the function of damping the pulsatile output from the left ventricle. Hence, the pulsatile pressure is minimized and a continous blood flow facilitated down to the microvascular level (Guyton, 2000).

The rate of oxygen uptake by pulmonary capillaries is governed by oxygen consumption in tissues that is fairly constant under normal resting conditions (West, 2005). The cardiac output is constantly adapted to the overall metabolic need of the body. It is controlled by the sum of various factors (as previosly discussed) that regulate local blood flow according to metabolic need of tissues. These factors add together to make the venous return which in turn is delivered by the pumping activity of the heart and convective flow down the arterial tree to capillaries (Guyton, 2000). From the capillary blood the oxygen diffuses down its concentration gradient to the much lower PO_2 of the mitochondria of individal cells in tissues (Leach & Treacher, 2002).

Presuming the retinal arterial oxygen content is identical to the systemic circulation the oxygen delivery to a retinal tissue can be derived from equation 9, by substituting total retinal blood flow (TRBF) for the cardiac output:

$$DO_2 = TRBF \times CaO_2 \qquad \text{Equation 10.}$$

1.7.2.2 Oxygen consumption

Tissue oxygen consumption (VO_2) is equivalent to the tissue metabolic rate under aerobic conditions per minute. The Fick´s equation describes the relationship between oxygen consumption, cardiac output or retinal blood flow and the oxygen content in both retinal arterioles (CaO_2) and venules (CvO_2):

$$VO_2 = CO \times (CaO_2 - CvO_2) \qquad \text{Equation 11.}$$

or

$$VO_2 = TRBF \times (CaO_2 - CvO_2)$$

Where CaO2 – CvO2 is the difference between the arterial and venous oxygen content (arteriovenous oxygen difference, AV - difference). The global arterial oxygen content is about 20 ml/ 100 ml blood and the venous oxygen content about 15 ml/ 100 ml blood and thus, producting arteriovenous oxygen difference of approximately 5 ml/100 ml blood (Butterworth et al., 2013).

1.7.2.3 Arteriovenous oxygen difference

By rearranging equation 11 it is apparent the oxygen consumption and the local blood flow determine the arteriovenous oxygen difference in the retinal tissue:

$$\text{AV- difference} = \frac{VO_2}{BF} \qquad \text{Equation 12.}$$

The AV-difference is directly related to the local tissue oxygen consumption and inversely to the local blood flow. The relationship is clearly manifested in both retinal and choroidal circulations. In this context, the retinal circulation is characterized by low VO_2/BF ratio and a large AV-difference, resulting in relatively low oxygen content on the venous side of the circulation. In contrast, the choroidal circulation is characterized by high VO_2/BF ratio and low AV-difference with high oxygen content on the venous side of the circulation.

1.7.2.4 Oxygen extraction fraction

The amount of oxygen consumption is reflected by the fraction of oxygen extraction (OEF) across the perfused capillary network. The oxygen extraction fraction can be calculated as follows:

$$OEF = \frac{CaO_2 - CvO_2}{CaO_2} \qquad \text{Equation 13.}$$

Normal oxygen extraction fraction for the majority of tissues is 25% (5ml/20ml). In other words, under normal conditions, most tissues consume only ¼ of the oxygen delivered to the capillary bed. When oxygen supply exceeds the oxygen demand for the metabolic activity the extraction fraction become less than 25%. However, when the oxygen supply is less than the metabolic demand the extraction fraction becomes greater than 25% (Butterworth et al., 2013).

Blood flow distribution varies enormously among tissues, depending on their momentary functional requirements. Some tissues, including the retina

(Wangsa-Wirawan & Linsenmeier, 2003) and the brain have steady energy requirements whilst perfusion and energy utilization of others (for example the liver) is predominated by their activity level (Scheufler, 2004). Consequently, tissues with greater energy expendure, like the inner retina, myocardium and the brain, extract higher oxygen fraction than less active tissues. The oxygen extraction fraction of the cerebral tissue is close to 40% and the arteriovenous oxygen difference is about 34% (Hatazawa et al., 1995; Qin, Grgac, & van Zijl, 2011). Likewise, the inner retina oxygen extraction fraction is 37% and arteriovenous oxygen difference approximately 35% (Felder, Wanek, Blair, & Shahidi, 2015; Schweitzer et al., 1999). Consequently, a duration of reduced oxygen supply (hypoxemia) will directly affect metabolic processes in retinal and cerebral tissue cells (Kergoat, Hérard, & Lemay, 2006; Ozcimen et al., 2016).

The application of non-invasive fundus reflectometry in human studies has made calculation of the inner retinal oxygen extraction achievable. Studies in young healthy individuals indicate no changes in oxygen extraction under acute mild systemic hypoxia (Palkovits, Told, Schmidl, et al., 2014). During 100% oxygen breathing however, the oxygen extraction is lessened by more than 60% from the normoxic breathing (Palkovits, Lasta, et al., 2014). For those reasons the retina autoregulatory response seems to be efficient under both acute system hypoxemic and hyperoxic conditions.

1.7.3 Inadequate tissue oxygenation

Oxygen diffusion from a capillary lumen to tissues is directly related to the capillary area and the PO_2 difference across the vessel wall and is inversely related to the distance between the two sites (Leach & Treacher, 1998, 2002; West, 2005). Normally above 1 to 3 mmHg of oxygen pressure is required to maintain cellular oxidative metabolism which allows for a large safety margin in animals (Guyton, 2000; Leach & Treacher, 1998; Scheufler, 2004). This is true for the inner retina as well which has remarkably higher PO_2 that measures around 20 mmHg in animals with circulatory structure that is analogous to humans (Wangsa-Wirawan & Linsenmeier, 2003).

During the final step of a cellular respiration, oxygen dependent adenosine triphosphate (ATP) is made as an energy basis for the tissue metabolism. Oxygen deficiency depletes the ATP stores and the speed of cellular injury will be determined by the tissue oxygen consumption and the ability to attain ATP under anaerobic conditions. Anaerobic metabolism can only serve as a temporarily reserve because of the inability to match tissue oxygen

consumption. Consequently, the rapidity of cellular damage differs remarkably among tissues where neurologic cells can only survive for few minutes (Guyton, 2000; Leach & Treacher, 1998). This holds true for the photoreceptors and other retinal neurologic cells that require high oxygen consumption for the energy demanding process of transmitting light waves into neurological signs for interpretation by the brain (Caprara & Grimm, 2012). The physiological response of prioritization blood flow to the retina and brain is thus of uttermost importance during hemodynamic stress and make the retina indeed the most advantageous source for non-invasive measures of oxygen delivery and viability of the central nervous system.

1.7.3.1 Similarities between retina and the brain

Embryologically, the retina is a direct outgrowth of the diencephalon, sharing similarities in microvascular structure with the cerebral circulation, blood flow and regulatory mechanisms (Delaey & Van De Voorde, 2000; Patton et al., 2005). Based on the homogeneousity, emerging evident suggests that the retinal vasculature may be a surrogate for pathological changes on the cerobrovasculature and even the cardiovascular system as well (Flammer et al., 2013; McClintic, McClintic, Bisognano, & Block, 2010; Patton et al., 2005). Like in retina, the rate of oxygen consumption by the cerebral tissue is quite constant (3.5 ml/100 gm tissue, or nearly 49 ml O_2/min the entire brain) except under intense brain activity (Clarke & Sokoloff, 1999). Consequently, the high oxygen consumption and the inability to store oxygen and any metabolic products such as glucose, render the cerebral tissue extremely vulnerable to an ischemic insult (Pittman, 2011b). When arterial blood PO_2 level goes below about 60 mmHg (Ainslie & Poulin, 2004), the oxyhemoglobin saturation becomes less than 90% (Gupta, Menon, Czosnyka, Smielewski, & Jones, 1997) and the cerebral tissue PO_2 becomes less than 30 mmHg (normal 35 to 40 mmHg), a prompt provocation of compensatory response with increased cerebral blood flow (Carreau et al., 2011; Guyton, 2000) is initiated secondary to amplification of cardiac output (Zhou, Saidel, & Cabrera, 2007).

As previously discussed the retinal blood is dependent on the blood flow delivered by the carotid arteries to the brain. Impaired global oxygen delivery in case of severe hypoxemia (Heistad & Abboud, 1980) or hemorrhagic shock initiate compensatory response of intense peripheral vasoconstriction to redirect blood flow from lower priority organs to the vital ones (Dutton, 2007) to preserve cerebral and ocular perfusion (Denninghoff et al., 2003; Riva et al., 2011). The initial compensatory response is aimed at increasing oxygen extraction to match the oxygen demand for cellular metabolism as evident by

decreased oxyhemoglobin saturation on the venous site of the circulation. In contrast, a reduced oxygen consumption for example during hypothermia or with tissue necrosis lessen the fraction of oxygen extraction as shown by increased venous oxyhemoglobin saturation (Schober & Schwarte, 2012). Eventually, whenever inadequate oxygenation jeopardizes the normal cellular function, vigilant oxygen monitoring with timely restorative and preventive measures are crucial for viability and functionality of individual tissues and body organs.

1.8 Retinal oximetry

The retina possess highly sophisticated architecture of neurological layers and vascular structure that is analogous to the cerebral circulation. The transparent structure at the front of the eye offers an unique opportunity for a direct non-invasive observation of the retinal circulation. Dual wavelength spectrophotometric retinal oximetry allows for a direct non-invasive assessment of oxyhemoglobin saturation in retinal vessels which serve as a window to the central nervous system. The technique is based on different light absorption of oxyhemoglobin and deoxyhemoglobin and give information on the oxyhemoglobin saturation.

1.8.1 Principles of retinal oximetry

Spectrophotometric oximetry measures the attenuation of light traveling through a blood column as a function of wavelength, based on the fact that oxy- and deoxyhemoglobin have different light absorption spectra (**Figure 6**). Oxyhemoglobin is light red in color whilst deoxyhemoglobin is darker red in color. These differences of shades permit calculation for oxyhemoglobin saturation in retinal arterioles and venules.

Like conventional oxyhemoglobin saturation measurements in a whole blood sample, the retinal oximetry exploits the Beer Lambert law to its system. Supposedly, the light absorption is dependent on the extinction coefficient (molar absorptivity) of the blood (ε); the distance of light has to travel through the sample (d) and the concentration (c):

$$I=I_0 \times 10^{-\varepsilon cd} \qquad \text{Equation: 14}$$

Where I_0, is the original light intensity not interacting with the blood and I, is the intensity of light transmitted through the blood.

Dual wavelength spectrophotometric oximetry simultaneously acquires two automatic monochromatic retinal images at two different wavelengths. One is at the isosbestic point and one at a non-isosbestic wavelength. Vessel points are automatically selected (**Figure 7**) for measurement of light intensity inside (*I*) and outside the vessel (I_O) for calculation of optical density *(OD).* Because of light absorbance by the erythrocytes, the intensity of reflected light from the vessel is less than from the immediate surrounding retina.

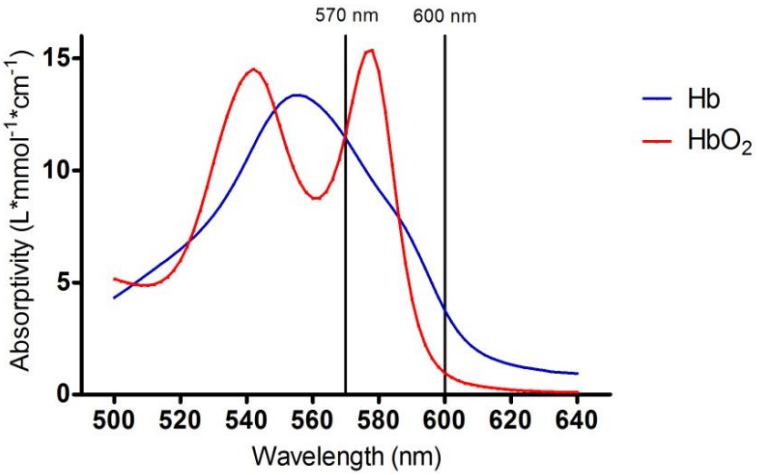


Figure 6. Light absorptivity at isosbestic (570 nm) and non-isosbestic (600 nm) wavelengths. Isosbestic wavelengths are insensitive to oxyhemoglobin saturation whereas non-isosbestic wavelengths are sensitive to distinctive oxyhemoglobin saturation. For the Oxymap T1 retinal oximeter, wavelength of 570 nm is at the reference isosbestic point while 600 nm is sensitive to the oxyhemoglobin. The figure was created by Jona Valgerdur Kristjansdottir based on data from Zijlstra et al. (Zijlstra, Buursam et al.2000).

The OD describes the light absorbance and can be calculated as:

$$OD = \log (I_0/I) \qquad \text{Equation 15.}$$

The higher the OD, the higher is the absorbance. The OD inside a vessel at the isosbestic point depends on the vascular diameter in addition to the distance that the light travels (d) and the erythrocyte concentration (c) but is independent of oxyhemoglobin saturation. In addition to the vascular diameter and the c·d the OD at the non-isosbestic wavelength depends on the oxyhemoglobin saturation. Consequently, the OD ratio at these two wavelengths is sensitive to oxyhemoglobin saturation while the vascular width and c·d tend to cancel out.

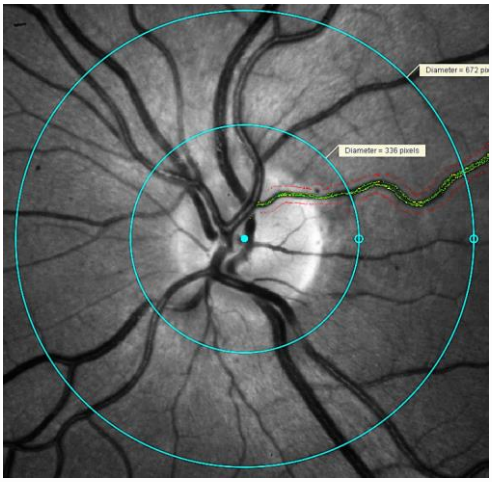


Figure 7. Monochrome retinal oximetry image with a pseudocolor overlay of selected measurepoints along the vessel. Green overlay (600nm) shows the light intensity of the measure inside the vessel *(I).* Red measurepoints illustrate the light intensity of the background, to the sides of the vessel (I_o).

Optical density at the two different wavelengths allows for calculation of an optical density ratio (ODR):

$$ODR = \frac{OD\ \textit{non-isosbestic}}{OD\ \textit{isosbestic}} \qquad \text{Equation 16.}$$

The ODR has nearly linear relationship to the oxyhemoglobin saturation (Beach, Schwenzer, Srinivas, Kim, & Tiedeman, 1999; Hardarson et al., 2006; Harris, Dinn, Kagemann, & Rechtman, 2003) that permits calculation of oxyhemoglobin saturation (SO_2):

$$SO_2 = a + b \times ODR \qquad \text{Equation 17.}$$

In the equation, a and b are calibration constants based. The calibration constant of the retinal oximeter used for the purpose of this thesis will be discussed in chapter 3.2.3.2.

1.8.2 Development of retinal oximetry

Non-invasive spectrophotometric analysis date back to 1959, when Hickam and associates used broadband light filters, two wavelengths of light and standard photographic films to measure oxyhemoglobin saturation in retinal vessels (Hickam, Sieker, & Frayser, 1960). Subsequently photoelectric technique (Delori, 1988), imaging spectroscopy (Schweitzer et al., 1999), multispectral confocal imaging (Smith, Denninghoff, Lompado, & Hillman, 2000) and digital camera systems (Beach et al., 1999) evolved from other research groups. For review see Harris et al. (2003), Geirsdottir et al. (2012) and Beach (2014).

Currently two different systems are commercially available for retinal oximetry that allots non-invasive direct measurement of arterioles and venules: The Imedos system (RVA; Imedos, Jena, Germany) and the Oxymap retinal oximeter (Oxymap; Oxymap ehf. Reykjavik, Iceland).

1.8.2.1 Retinal oximetry with scanning laser ophthalmoscope (SLO)

Recently, a scanning laser ophthalmoscope (SLO) was combined with modified Oxymap analysis software for development of SLO retinal oximetry with a dual-wavelength algorithm. SLO uses low power laser wavelengths (instead of white light) to scan through individual separate retinal layers at once from the inner sensory layers down to the choroid. A green laser (532 nm) scans from the inner retina to the pigment epithelium whereas red laser (366 nm) gives a choroidal view by scanning from the retinal pigment epithelium down to the choroid.

The SLO brings some theoretical advances to retinal oximetry. First, it minimizes any unnecessary light exposure to the fundus by using laser (instead of white light imaging in regular fundus cameras) to obtain the monochromatic images at the dual different wavelengths. Second, it acquires wide-field scanning of nearly the entire fundus but conventional fundus cameras are limited to quite narrow images of the posterior pole. Third, pupil dilation using mydriatic eye drops is unnecessary. Finally, it pierces other optical opacities in the eye such as cataracts better than conventional spectrophotometric fundus camera (Kristjansdottir et al., 2014). In addition,

SLO has a potential for application of retinal oximetry to neonates and infants. This could mark a milestone in management of preterm neonates (and other pediatric groups) who are treated in incubators at neonatal intensive care units.

The risk of retinopathy of prematurity (ROP) is inversely related to gestational age and weight at birth. Extremely premature infants and those with low birthweight are therefore at highest risk for severe ROP and consequently a poor visual outcome due to the immaturity of retina at birth (Cavallaro et al., 2014). The interruption of normal retinal vascular development following transition from the relatively hypoxic intrauterine environment to the oxygen rich ambient air after birth which is further aggravated by administration of supplemental oxygen therapy, is twofold; the first phase is characterized by suppression of insulin-like growth factor-I and normal vascular endothelial growth factor (VEGF). The second phase is characterized by hypoxic induced neovascularization that is analogous to other proliferative retinopathies, as the retina becomes more metabolically demanding (Smith, 2004). Careful titration of supplemental oxygen to preterm neonates to reduce the risk of visual impact (i.e blindness) from hyperoxia is therefore of vital importance for retinal vascular development. Currently, oxygen supplementation to those babies is monitored by continuous peripheral pulse oximetry and intermittent invasive arterial blood gas measurements.

The non-invasive dual wavelength confocal SLO combined with oximetry analysis for retinal oximetry may improve monitoring and management of these babies. Prior to allowing oxyhemoglobin saturation measurements of retinal arterioles and venules it is necessary to determine the ODR in fullterm healthy neonates, shortly after birth.

1.8.3 Retinal oximetry in healthy individuals

Retinal oximetry has shown sensitivity and reliability with high reproducibility and repeatability of retinal oxyhemoglobin saturation measurements during normoxia in healthy humans (Blondal, Sturludottir, Hardarson, Halldorsson, & Stefánsson, 2011; Geirsdottir et al., 2012; Hardarson et al., 2009; Hardarson et al., 2006; Heitmar & Cubbidge, 2013; Man et al., 2013; Man et al., 2014; Palsson et al., 2012; Werkmeister et al., 2015; Yip et al., 2014), rhesus monkeys (Li et al., 2016) and pigs (Traustason et al., 2013) and during induced hypoxemia (Choudhary et al., 2013; Palkovits, Told, Schmidl, et al., 2014; Rose, 2016) and hyperoxia (Palkovits, Lasta, et al., 2014; Rose, 2016; Werkmeister et al., 2015) in humans. Most of these studies have been carried out to test the reproducibility and sensitivity of the technique but also to study

normal physiological reactions to oxygen challanges and retinal metabolism.

Report on the groundwork of SLO with dual-wavelength retinal oximetry revealed sensitivity and repeatability in healthy humans and that the emplacement is technically feasible and promising for advancement of retinal oximetry in the future (Kristjansdottir et al., 2014).

1.8.3.1 Normoxia

Recently, some research reports were published on normal retinal vessel oxyhemoglobin saturation in human subjects of multiethnicities **(Table 1)**. These studies show similar results although some small deviations exist between different versions of retinal oximeters and between different research groups. The differences are attributable to the retinal oximeter hardware, the calibration of the oximeter, the analysis software and the analytical approaches. When comparing oxyhemoglobin saturation measurements between different studies the importance of identical calibration between retinal oximeters devices (Geirsdottir et al., 2012) must be kept in mind and whether methodological procedures for oximetry analysis where according to standardized protocol. None of the studies have found difference in oxyhemoglobin saturation between the left and right eyes but the effects of age on oxyhemoglobin saturation are conflicting.

Geirsdottir et al. (2012) found the oxyhemoglobin saturation in retinal venules to decrease significantly with advanced age, or 1.9% for every 10 years in males and 0.7% for females. The oxyhemoglobin saturation in retinal arterioles was not affected by age. Consequently, the AV-difference increased by 1.5±0.5% for every 10 years in males and 1.0±0.4% in females. The vessel diameter was inversely affected by the age whereas the OPP rose with age which the authors suggested might partly compensate for the increase in vascular resistance caused by the decreased vessel lumen. The oxyhemoglobin saturation measurements of both retinal arterioles and venules were significantly lower in the inferiotemporal quadrants than other retinal quadrants (Geirsdottir et al., 2012). Jani et al. (2014) found no difference across ethnicity, genders nor iris/fundus pigmentations. They found both the arteriolar and venous oxyhemoglobin saturation to decrease with age and an increase in AV-difference. The oxyhemoglobin saturation values were lowest in the inferotemporal quadrant and highest in the superonasal quadrant. Conversely, the AV-difference was greatest in the inferotemporal quadrant and lowest in the superotemporal quadrant (Jani et al., 2014). Yip et al. (2014) reported the age to act as a negative factor for oxyhemoglobin saturation in venules, similar to the finding of Geirsdottir et al. and Jani et al. (Yip et al., 2014). In contrast to the above studies, Mohan et al. (2015) found the arteriolar

and venous oxyhemoglobin saturation to increase with age but the AV-difference to remain unaffected. There was no statistical correlation between the retinal oximeter measurements and finger pulse oximetry readings. They speculated that heavily pigmented fundus like in Indians have more melanin exposed for light absorption which could hypothetically alter the ODR and hence the oximetry measures (Mohan et al., 2015). Yang et al. (2016) obtained difference in oxyhemoglobin saturation measurements between retinal quadrants. The arteriolar measures from inferiotemporal quadrant gave the lowest value followed by the superiotemporal, inferionasal and superionasal qudrants respectively. The retinal venules followed a similar pattern (Yang et al., 2016). Nakano et al. (2016) measured increased oxyhemoglobin saturation in major retinal arteries (0.67% per 10 years) with advanced age but not in venules. They found the mean oxyhemoglobin saturation to be markedly lower in both retinal arterioles and venules in the temporal hemispheres, particularly in the inferiotemporal vessels that are similar to the findings of Geirsdottir et al., Palsson et al., Mohan et al. and Yang et al. (Nakano et al., 2016). Recently, Liu et al.(2017) published the first study on retinal oximetry in children. According to their results the oxyhemoglobin saturation of retinal arterioles and venules is lower in children than in adults. In arterioles, the oxyhemoglobin saturation increased considerable with age and showed a similar trend in venules. They observed a statistical difference between measurements in all retinal quadrants. Being lowest in the inferotemporal region, followed by superotemporal, inferonasal and superonasal qudrants respectively. The AV-difference however, was similar between retinal hemispheres. The authors hypothesised the reason for lower oxyhemoglobin saturation in children is due to their yet not fully developed retina, resulting in a higher oxygen consumption and is inversively related to the retinal nerve fiber layer thickness (Liu et al., 2017) .

Man et al. (2014) reported similar findings, using the Imedos system, to Mohan et al. (2015) on increased oxyhemoglobin saturation in both retinal arterioles and venules by age and unchanged AV-difference. They did not observe any correlation of oxyhemoglobin saturation in retinal vessels with OPP. They reported however, a strong correlation of the finger pulse oximetry readings with oxyhemoglobin saturation in retinal arterioles and the AV-difference which is in contrast with the findings of Geirsdottir et al. (2012) and Mohan et al. (2015).

The results on measured oxyhemoglobin saturation by Kristjansdottir et al. (2014) on the employed SLO retinal oximetry with a dual wavelength algorithm of modified Oxymap analysis software (Kristjansdottir et al., 2014) is in agreement with other publications of retinal oximetry normative data.

Table 1 Published data on retinal oxyhemoglobin saturation (%) in healthy subjects. All values are mean ± standard deviation, except*. *Median of the right eye; SLO: Scanning laser ophthalmoscope.

Author (year)	**Ethnicity** (number of subjects)	**Age**	**Device** (soft-ware)	**Arterioles**	**Venules**	**AV-difference**
Geirsdottir et al. (2012)	Caucasians (120)	18 to 80 median 47	Oxymap T1 (2.2.1)	92.2±3.7	55.6±6.3	36.7±5.4
Jani et al. (2014)	Caucasian (18) Hispanic (13) African-American (17) Asian (13)	19 to 74 mean 44.1±14.7	Oxymap T1 (2.3.1)	90.4±4.3	55.3±7.1	35.4±4.0
Yip et al. (2014)	Asian (118)	≥ 40	Oxymap T1 (2.3.1)	93.64±6.9	54.22±6.9	39.43±8.9
Mohan et al. (2015)	Asian Indian (98)	18 to 63 Mean 33	Oxymap T1 (2.4.2)	90.3±6.6	56.9±6.3	33.2±5.2
Yang et al. (2016)	Chinese	19 to 30	Oxymap T1(2)	93.2±6.3	60.4±5.3	32.9±6.4
Nakano et al. (2016)	Japanese (252)	20 to 93 Mean 61.1±18.8	Oxymap T1 (2.4.2)	97.0 ± 6.9	52.8±8.3	44.2±9.2
Liu et al. (2017)	Chinese (122)	5 to 13 Mean 13.0±2.9	Oxymap T1 (2.4.2 and 2.5.0)	85.5 **±** 7.1	48.2 **±** 5.5	37.3 **±** 6.5
Man et al. (2013)	Caucasian (20)	19 to 45 Mean 30.1±7.56 years	Imedos	94.0±4.9	61.8±4.99	
Man et al. (2014)	Caucasian (50)	18 to 58 Median 26	Imedos	*95.94 range: 91.53-98.49	*62.35 range 57.65-64.17	33.79±3.37
Kristjansdottir et al. (2014)	Caucasian (11)	Mean: 34±10	SLO (modi-fied Oxymap analyzer Soft-ware)	92±13	57±12	

1.8.3.2 Induced hypoxemia

Retinal oximetry studies on induced acute systemic hypoxemia in healthy young adults show sensitivity to changes of oxygen concentration in both retinal arterioles and venules. In other words, these studies show the subnormal oxygen level delivered by the systemic circulation is being manifested in the central circulation by retinal oximetry.

Choudhary et al. (2013) used multispectral image-replicating imaging spectrometer to measure the effects of acute mild hypoxemia on retinal vessel oxyhemoglobin saturation in 10 healthy subjects with the mean age of 25±5 years. During normoxic breathing the mean oxyhemoglobin saturation of retinal arterioles was 98.5±1.6%, 70.7±2.7% in venules and the AV- difference was 27.8±2.9%. During hypoxic breathing of 15% oxygen the oxyhemoglobin saturation in retinal arterioles decreased to 90.3±2.0% and 62.4±2.2% in venules. The AV-difference however, remained unchanged whilst the vessel diameter increased by 3% and 4% respectively. The oxyhemoglobin saturation in retinal arterioles was similar to their finger pulse oximetry readings, or 98.5±1.6% under normoxic breathing and 89.6±0.5% with hypoxic breathing (Choudhary et al., 2013)

Palkovits et al. (2014) included 27 healthy Caucasians in a statically analysis of retinal oximeter measurements and blood flow velocity during normoxia and different breathing regimens of hypoxic mixture. The participants age ranged from 18 to 35 years (mean 25.2±3.9 years). The Imedos retinal oximetry system was used to measure oxyhemoglobin saturation and vessel diameter in one major temporal retinal artery and venule. Blood velocity was measured by bidirectional laser Doppler velocimetry at the same location as oxyhemoglobin saturation and diameter were quantified. The study was performed in randomized two-way crossover design and parameters were obtained under normoxia and under isocapnic hypoxia when participants inhaled oxygen 12% + Nitrogen 88% or oxygen 15% + Nitrogen 85%. Oxyhemoglobin saturation in both retinal arterioles and venules, the AV-difference and finger pulse oximetry readings all decreased during the hypoxic gas mixtures breathing. The PO_2 of a capillary earlobe blood measured 45.5±7.0 mmHg and 56.9±4.3 mmHg during 12% and 15% oxygen breathing respectively. The hypoxic breathing regimen induced vasodilatation in both retinal arteries and venules as compared with normoxia that was more prominent during 12% oxygen breathing. The blood flow increased during both breathing regimens but was more augmented during 12% oxygen breathing (Palkovits, Told, Schmidl, et al., 2014). It appears that the retinal circulation

was well autoregulated during the hypoxic challenges which is in agreement with earlier statements in that above a PaO_2 of 32-37 mmHg the autoregulatory response of the retinal circulation is well preserved. The authors concluded that the retinal oxygen extraction fraction was unaffected by the graded systemic hypoxia. Rose et al. (2016) used Metabolic Hyperspectral Retinal Camera to measure oxyhemoglobin saturation of retinal arterioles and venules of one eye in 11 healthy people, under conditions of normoxia, isocapnic hyperoxia, and isocapnic hypoxia. Total retinal blood flow was quantified with Doppler Spectral-Domain Optical Coherence Tomography (SD-OCT). Participants mean age was 33.36±6.03 years. When the end tidal partial pressure of oxygen (E_tO_2), which is an indicator of arterial PO_2, was reduced from baseline (100 mm Hg) to 80, 60, and 50 mm Hg, the retinal arterial and venous oxyhemoglobin saturation decreased from 99.3±5.8% and 56.3±4.2% to 95.6±5.1% and 52.5±4.1%, 89.6±2.8% and 49.5±2.9%, 83.3±3.9% and 45.0±6.1% respectively. The retinal blood flow increased markedly during the hypoxic gas mixture breathing as compared with the normoxic baseline breathing (Rose, 2016).

1.8.3.3 Induced hyperoxia

Like studies on induced hypoxemia, retinal oximetry demonstrates sensitivity to changes of oxyhemoglobin saturation for the period of acute systemic hyperoxia.

Palkovits et al. (2014) used the Imedos system to measure oxyhemo–globin saturation of one major temporal retinal artery and vein in 41 healthy humans during isocapnic 100% oxygen breathing. The age of the participants ranged from 18 to 35 years. Retinal venous blood velocity was measured using bidirectional laser Doppler velocimetry and the blood flow was calculated. At baseline during normoxic breathing the oxyhemoglobin saturation in retinal arterioles measured 92.3±3.9%, 61.8±4.4% in venules and the AV-difference 30.5±7.9%. During 100% oxygen breathing these values increased to 96.4±3.1%, 73.9±10.0% and 22.4±10.4% respectively. The increase in oxyhemoglobin saturation was greater in retinal venules than arterioles and the calculated blood flow was markedly reduced during the hyperoxic breathing (Palkovits, Lasta, et al., 2014). Werkmeister et al. (2015) used the Imedos system to measure the oxyhemoglobin saturation and vessel diameter in retinal arterioles and venules and dual-beam Doppler FD-OCT system for retinal blood velocities. Measurements were performed in one eye during both normoxic and 100% oxygen breathing. Participants included eight healthy persons between the age of 18 and 35 years. During normoxic breathing, the

mean oxygen saturation of retinal arterioles was 95.3 ±1.9%, and 68.0±3.9% of venules. During hyperoxic breathing these values raise to 99.4 ± 0.3% and 76.6±5.2% respectively with a drop in the AV-difference (Werkmeister et al., 2015)

Rose et al (2016) reported marked increase in oxyhemoglobin saturation of retinal venules during baseline level versus 200 mm Hg and baseline level versus 300 mm Hg (above mentioned method in 1.8.3.2). The oxyhemoglobin saturation in retinal arterioles at 200 and 300 mmHg remained unchanged as compared with 100 mmHg at baseline. Yet, the total retinal blood flow did significantly decrease under the hyperoxic breathing (Rose, 2016). These results of increased oxyhemoglobin saturation on the venous site of the circulation and reduced blood flow under system hyperoxia indicate the effective autoregulatory response and reduced oxygen extraction of the inner retina with enhanced global oxygen delivery as already stated in former chapters.

1.8.4 Retinal oximetry in eye diseases

Retinal oximetry has been widely used for retinal imaging and appraisal of retinal vessel oxyhemoglobin saturation in patients with variety of eye diseases. Those include glaucoma (Michelson & Scibor, 2006; Olafsdottir, Hardarson, Gottfredsdottir, Harris, & Stefánsson, 2011; Vandewalle et al., 2013; Vandewalle, Abegao Pinto, Olafsdottir, & Stalmans, 2012), diabetic retinopathy (Dong et al., 2016; Hardarson & Stefánsson, 2012b; Jørgensen & Bek, 2014; Jørgensen, Hardarson, & Bek, 2014; Kashani et al., 2014; Khoobehi, Firn, Thompson, Reinoso, & Beach, 2013; Klefter et al., 2016; Klefter, Vilsboll, Knop, & Larsen, 2015; Man et al., 2015; Šín et al., 2016), retinitis pigmentosa (Battu, Mohan, Khanna, Kumar, & Shetty, 2015; Eysteinsson, Hardarson, Bragason, & Stefánsson, 2014; Ueda-Consolvo, Fuchizawa, Otsuka, Nakagawa, & Hayashi, 2015; Zong et al., 2016) age related macular degeneration (AMD) (Geirsdottir, Hardarson, Olafsdottir, & Stefánsson, 2014), retinal vein occlusions (Hardarson & Stefánsson, 2010, 2012a; Lin et al., 2016) and retinal arterial occlusions (Hardarson, Elfarsson, Agnarsson, & Stefánsson, 2013). All these studies reveal notable changes in retinal vessel oxyhemoglobin saturation and retinal tissue metabolism. For instance, in severe glaucomatous eyes, increased venous oxyhemoglobin saturation and reduced AV-difference indicate reduced oxygen extraction secondary to retinal tissue atrophy (Olafsdottir et al., 2011; Vandewalle et al., 2013). In patients with diabetic retinopathies increased oxyhemoglobin saturation in both retinal arterioles and venules in addition to reduced oxygen

extraction and thus, unaltered AV-difference (Hardarson & Stefánsson, 2012b; Jørgensen et al., 2014; Man et al., 2015) are indicative of disease progression to more advanced stages. In retinitis pigmentosa, changes in retinal oximetry values and reduced vascular caliber most likely mirror retinal tissue atrophy and decrease in oxygen consumption (Battu et al., 2015; Eysteinsson et al., 2014). In patients with exudative AMD an increase in both retinal venous oxyhemoglobin saturation and AV-difference indicate abnormally low oxygen consumption of the inner retina (Geirsdottir et al., 2014). In both retinal artery occlusions and retinal vein occlusions, retinal oximeter measurements give valuable information on the oxygen changes and the effect on retinal tissue metabolic function by comparing the affected eye with the fellow unaffected eye. In central retinal vein occlusion (CRVO) the mean oxyhemoglobin saturation has shown to be markedly lower and the inter eye variability greater than of the fellow unaffected eye (Hardarson & Stefánsson, 2010; Traustason, la Cour, & Larsen, 2014). In branch retinal vein occlusion (BRVO) the oxyhemoglobin saturation of retinal venules is decreased to a variable degree and in some cases the arteriolar saturation is higher in the affected eye than in the opposite unaffected eye (Hardarson & Stefánsson, 2012a; Lin et al., 2016).

Up to date only a few studies have been published on retinal oximetry in people with branch and central retinal artery occlusion. These reports give novel insight of the consequences of obstructed arterial blood flow on the retinal tissue oxygen supply. Initially, after the clinical onset of symptoms the oxyhemoglobin saturation of retinal arterioles is subnormal but gets better with time and treatment (Hammer, Riemer, Vilser, Gehlert, & Schweitzer, 2009; Hardarson et al., 2013) signaling facilitation of the local blood flow and hence improved oxygen delivery to the inner retina.

1.8.5 Central retinal vein occlusion (CRVO)

Central retinal vein occlusion is a sight threatening disease that is a frequent cause for visual loss in humans. It usually affects only one eye (McAllister, 2012) although the incidence of CRVO in the contralateral eye may reach 7% within 5 years from the development in the first eye (Hahn, Mruthyunajaya, & Fekrat, 2013). The clinical existence of the disease has been known since 1878 (Hayreh, 2014) but its pathophysiology is yet to be fully elucidated (Glueck, Wang, Bell, Rangaraj, & Goldenberg, 2005; Hayreh, Zimmerman, & Podhajsky, 2012; Kang et al., 2011). The risk factors have been associated with variety of systemic factors such as hypertension and diabetic mellitus; hematologic and local factors, including ocular hypertension and glaucoma

(Elman, Bhatt, Quinlan, & Enger, 1990; Hayreh, Zimmerman, McCarthy, & Podhajsky, 2001; Hayreh, Zimmerman, Beri, & Podhajsky, 2004). In 2008 CRVO was estimated to affect 2.5 million people worldwide, based on a prevalence ratio of 0,8 per 1000 individuals from population derived studies around the world (Rogers et al., 2010). As an advanced age has been identified as an important risk factor (McAllister, 2012) for CRVO the prevalence and disease burden may be expected to rise with the growth rate of the aging population during the 21st century.

1.8.5.1 Pathophysiology

CRVO is caused by thrombotic occlusion of the central retinal vein most commonly within the optic nerve at a variable location posterior to the lamina cribrosa. Color Doppler imaging and fluorescein angiography demonstrate central retinal venous outflow obstruction and retinal capillary non perfusion to various degrees (Hayreh, 2005), along with reduced venous blood flow velocity and prolonged arteriovenous transit time (Arsene et al., 2002). Clinical entities of the disease consists as well of visual impairment (Martinet et al., 2012), intraretinal hemorrhage, venous tortuosity, vascular congestion and macular edema (London & Brown, 2011). The impediment to the venous blood flow amplifies the up-stream intraluminal pressure (venous dilatation) which elevates the hydrostatic pressure causing extravasations of fluid into the extracellular space (Kaur, Foulds, & Ling, 2008) resulting in retinal tissue edema. Retinal hypoxia stimulates VEGF production, which increases vascular permeability and leakage of osmotically active plasma proteins into tissue. Increased hydrostatic difference between vasculature and tissue and reduced osmotic pressure difference combine to cause retinal edema according to Starling´s law. Eventually, the extent of the venous stasis retinopathy together with or without tissue hypoxia, depends on the severity (Hayreh, Podhajsky, & Zimmerman, 2011) and location of the obstruction against blood flow within the central retinal vein.

Depending on the implicated retinal tissue hypoxic injury the temporary and long term visual morbidities are primarily caused by vitreous hemorrhage, the magnitude of macular edema, concomitant development of neovascularization and the progression to neovascular glaucoma (McIntosh et al., 2010).

1.8.5.2 Retinal blood flow in CRVO

Fluorescein angiographies always manifest some evidence of blood flow within the vascular bed in CRVO. The obstruction to venous outflow is determined by the number of tributaries anterior to the occlusion in the central retinal vein for

collateral flow. The farther away from lamina cribrosa, the greater the amount of tributaries situated anterior to the occlusion to create anastomosis with nearby veins for re-routing the blood around the thrombus. On the contrary, the closer the thrombus is situated to the lamina cribrosa, fewer tributaries are available for this collateral flow (Hayreh et al., 2011). In addition, histopathological studies on enucleated eyes due to neovascular glaucoma after CRVO have shown evidence of recanilization of the thrombus that most likely have an onset early on and proceeds over time (Green, Chan, Hutchins, & Terry, 1981).

1.8.5.3 Classification of CRVO

CRVO is classified as either non ischemic or ischemic type of CRVO. In the former the thrombi is located more distal in the optic nerve whereas the occlusion is closer to the lamina cribrosa in the ischemic type. Subsequently, the clinical entity and visual outcome of the non ischemic and ischemic CRVO is very divergent based on the number of tributaries anterior to the occlusion for venous blood shunting (Hayreh et al., 2011).

Non ischemic CRVO is relatively benign with better prognosis and visual outcome than the ischemic type which has poor prognosis (Hayreh, 2014). Macular edema is seen in both types but is markedly less and not always present in the non ischemic form. Unlike the ischemic type, neovascularization never develops in non ischemic CRVO eyes. Transient visual deterioration (central scotoma) secondary to macular edema is the major complication in non ischemic CRVO that tends to resolve over time. Ischemic CRVO on the other hand is characterized by severe hypoxia and capillary closure with the risk of neovascular glaucoma and permanent blindness due to an irreversible ischemic damage of macular ganglion cells (Hayreh, 2014; Hayreh, Rojas, Podhajsky, Montague, & Woolson, 1983).

1.8.5.4 Tissue hypoxia

Similar to chronic systemic hypoxic condition in cardio- and pulmonary diseases (Arjamaa & Nikinmaa, 2006), vascular disorders like CRVO may result in local hypoxia of the inner retina tissue (Williamson et al., 2009). The local hypoxia involves only the retinal circulation secondary to the obstruction of the central retinal vein whereas the oxygen delivery by the systemic circulation is normal.

Hypoxia sets off cascade of hypoxia signals, primarily by the hypoxia-inducible factor (HIF) protein. HIF is a key factor and a common denominator

for all hypoxia-dependent events in cells including angiogenesis regulation and transcription of several others genes. HIF comprises two subunits: a stable HIF-β unit which is continuously expressed and a labile HIF-α unit which is regulated and stabilized by normal cellular oxygen tension. Under hypoxic conditions, HIF-1α evades its degrading and starts to build up before moving into the nucleus of the cell where it proceeds as a transcription factor for several gene arrays (Arjamaa & Nikinmaa, 2006). These target genes direct glucose transport to tissue cells, glycolysis, a modulation of the vascular tone and an erythropoiesis for homeostasis and survival of retinal cells (Carreau et al., 2011). In oxygen depleted tissues the HIF-1α is a chief stimulant for up-regulation of VEGF for neovascular sprouting (Pages & Pouyssegur, 2005) and angiogenesis throughout the body. The VEGF expression and inflammatory induction disrupts the blood retinal barrier (Arjamaa & Nikinmaa, 2006; Flammer et al., 2013; Kaur et al., 2008) that is analogous to the breakdown of the blood brain barrier (Schoch, Fischer, & Marti, 2002; Yeh, Lu, Lin, Liou, & Fu, 2007) in cerebral ischemia (Croll et al., 2004). Consequently, the inflammatory mediators (Kaur et al., 2008) and VEGF expression jointly add to the vascular hyperpermability, retinal edema, intraretinal hemorrhage and the retinal capillary non perfusion manifested in CRVO (Boyd et al., 2002; Campochiaro, 2012; Campochiaro et al., 2008; Noma, Funatsu, Mimura, Harino, & Hori, 2009).

In ischemic CRVO the angiogenic factors diffuse across the disrupted blood retinal barrier to establish neovascularization at remote locations throughout the intraocular tissues (Kaur et al., 2008); on the optic nerve head and the iris which carries the risk for neovascular glaucoma. For instance, the concentration of VEGF in the aqueous humor was found to be directly correlated with the onset and progression of neovascularization of the iris (Boyd et al., 2002) and to be inversely related with the patient´s visual outcome (Campochiaro, 2012). Because the VEGF up-regulation is a chief stimulant for blood retinal barrier breakdown a treatment for anti-VEGF formation is considered an effective means for improving visual outcome in CRVO affected eyes (Campochiaro et al., 2011; Epstein, Algvere, von Wendt, Seregard, & Kvanta, 2012) either as a monotheraphy or in conjunction with other forms of treatment such as vitrectomy or a panretinal photocoagulation.

1.8.5.5 Oxygen measurements in CRVO

Four studies have published their findings on oxygen content in humans with CRVO. All of them quantified lower oxygenation of the inner retina in CRVO affected eyes than in the opposite unaffected eyes. In 2002, Yoneya and

associates used spectral imaging to measure oxyhemoglobin saturation of retinal vessel in eyes affected by ischemic CRVO. They reported semi-quantitative correlation between fluorescein angiography and decreased oxyhemoglobin saturation of retinal venules (less than 40%) in patients with ischemic CRVO. They also noted decreased oxyhemoglobin saturation in adjacent capillary areas that seemed to be circulatory intact. In addition, unaffected retinal hemispheres in CRVO eyes appeared to be influenced by the circulatory disruption (Yoneya et al., 2002). In 2009, Williamson and associates used oxygen sensitive electrode probes to measure intravitreal PO_2 during vitrectomy. Patients with ischemic CRVO had lower preretinal PO_2 than patients undergoing vitrectomy for either epiretinal membrane removal or macular hole (Williamson et al., 2009). In 2010, Hardarson and Stefánsson used the the Oxymap, non-invasive spectrophotometric retinal oximeter (2nd version), with a 45 degree view of the fundus to measure retinal vessel oxyhemoglobin saturation in eight patients with unilateral CRVO. The mean oxyhemoglobin saturation in venules was 49±12% in CRVO eyes and 65±6% in the unaffected eyes (p=0.003). There was no difference however, in arteriolar oxyhemoglobin saturation (99%) of CRVO affected and unaffected fellow eyes (Hardarson & Stefánsson, 2010). Most recently, Traustason et al. (2014) used a similar non-invasive spectrophotometric technique (Oxymap Retinal Oximeter P3) for retinal oximetry in 11 patients with unilateral CRVO. At baseline, before intravitreal VEGF inhibitor treatment (Ranibizumab) was initiated, the mean oxyhemoglobin saturation of retinal venules was 32±12% and 59±10% in the unaffected eyes (p=0.001). Concurrently, the oxyhemoglobin saturation of retinal arterioles was significantly higher in CRVO eyes than in fellow eyes, or 95±8% and 91±3% respectively (p=0.04). The oxyhemoglobin saturation in CRVO eyes improved with time and intravitreal anti-VEGF treatment but still remained subnormal or roughly halfway normalized at 3 and 6 months follow-up point in time (Traustason et al., 2014).

Hayreh and associates have studied the effectiveness of different parameters for delineating ischemic CRVO from non ischemic CRVO in the early stages of the disease. They found fluorescein angiography to provide optimal reliable information about retinal capillary non perfusion in only 50 – 60% of patients whereas combining information from electroretinography and relative afferent papillary defect captured 97% of the cases and hence, to be the best tests (Hayreh, 2014). Information on retinal oxyhemoglobin saturation may be helpful in classification of CRVO in the early stages of the disease although the value of this is beyond the scope of this thesis. Retinal oximetry may also be valuable in the management and observation of CRVO patients

over time. Timely intervention on retinal tissue hypoxia is supposedly essential for preventing and interrupting any detrimental effects of the hypoxia signaling cascade in CRVO where retinal oximetry may possibly play a vital role in the future.

Earlier oximetry studies on CRVO have been confined to imaging a small portion of the fundus. In this thesis we use the Oxymap T1 oximeter to aquire wider images of the fundus, than previous studies, to analyse oxyhemoglobin saturation in retinal vessels in patients with CRVO and to observe the disease process over time.

1.8.6 Retinal oximetry in systemic diseases

Retinal oximetry has been shown to be reliable and valid in patients suffering from chronic systemic hypoxia secondary to Eisenmenger syndrome and in patients with severe chronic obstructive pulmonary disease (COPD).

Traustason et al. (2011) utilized the Oxymap device to detect hypoxemia of both retinal arterioles and venules in clinically stable patients with Eisenmenger syndrome, a congenital cyanotic cardiac defect. The oxyhemoglobin saturation in retinal arterioles was 81±9% and 44±12% in venules as compared with 93±3% ($P<0.001$) and 59±5% ($P<0.001$) respectively in healthy controls. The AV-difference was not markedly different between the groups (37%±6% and 34%±5%, respectively). The oxyhemoglobin saturation of retinal arterioles in the Eisenmenger group correlated with both intra-femoral artery oxyhemoglobin saturation (83±5%, $p=0.82$; $P < 0.001$) and finger pulse oximetry (88±5%). Also, the decrease in retinal venous oxyhemoglobin saturation was decreased in proportion to the decrease in femoral artery saturation. Palkovits and associates (2013) reported retinal vessel hypoxia in patients with severe COPD during cessation of their supplemental oxygen therapy, using the Imedos device. The AV-difference remained unchanged during both breathing regimens of the ambient air and oxygen supplementation. According to their findings the retinal oxyhemoglobin saturation correlated with both capillary earlobe blood gas sample and finger pulse oximeter measurements (Palkovits et al., 2013).

In people with Giant cell arteritis, retinal oximetry has revealed reduced oxyhemoglobin saturation in retinal arterioles and elevation in venules despite no ophthalmogical manifestation of the disease (Turksever, Daikeler, Konieczka, & Todorova, 2014). Mild to moderate Alzheimer disease also appears to be expressed by elevated oxyhemoglobin saturation of both retinal

arterioles and venules as compared with healthy control group (Einarsdottir et al., 2015).

Although the literature is sparse on retinal oximetry in systemic diseases, those results suggests the retinal circulation represents the systemic and central nervous system involvement. Retinal oximetry imaging opens the opportunity to examine the central nervous oxyhemoglobin saturation in systemic diseases and thus, gain new insight into oxygen delivery and retinal metabolism in systemic disorders.

1.8.7 Chronic obstructive pulmonary disease (COPD)

Chronic obstructive pulmonary disease is characterized by progressive airflow limitation ("From the Global Strategy for the Diagnosis, Management and Prevention of COPD. Global Initiative for Chronic Obstructive Lung Disease (GOLD)," 2016), chronic inflammation of the airways and systemic inflammatory response (van Eeden & Sin, 2008). The airflow limitation eventually creates ventilation-perfusion mismatching that unavoidable leads to systemic hypoxemia, either with or without CO_2 retention (West, 2003). The severity of airflow limitation is classified by spirometry measurement of a forced expiratory volume in one second (FEV_1) to the forced vital capacity (FVC), after maximum inspiration. Patients with less than 50% and 30% of the predicted forced expiratory volume in one second (FEV_1/FVC) are classified with severe (stage 3) and very severe (stage 4) COPD respectively. Long term oxygen therapy (> 15 hours/day) has shown to increase survival in patients with severe resting hypoxemia and is recommended for patients with a resting PO_2 at or below (≤) 55 mmHg or arterial oxyhemoglobin saturation ≤ 88%, with or without hypercapnia ("From the Global Strategy for the Diagnosis, Management and Prevention of COPD. Global Initiative for Chronic Obstructive Lung Disease (GOLD)," 2016). The aim of the supplemental oxygen therapy is to target the pulmonary oxygen concentration between 60-80 mm Hg and the oxygen flow titrated according to arterial blood gas and peripheral pulse oximeter measurements (Hines & Marschall, 2012).

The magnitude of systemic inflammation in COPD has been affiliated with the severity of the airflow obstruction and is believed to underlie the extrapulmonary pathogenesis of the disease (Clarenbach, Thurnheer, & Kohler, 2012). Many organ systems are negatively affected by the systemic inflammatory response including the cardiovascular and autonomic nervous system (van Gestel & Steier, 2010). Cardiovascular events are considered a frequent reason for mortality in patients classified with mild to moderate COPD

(Sin, Anthonisen, Soriano, & Agusti, 2006; van Eeden & Sin, 2008). Presumably, the systemic inflammatory response in addition to the systemic hypoxia, oxidative stress and sympathetic activation lead to vascular dysfunction and hence, a cardiovascular disease (Clarenbach et al., 2012). Hypoxia seems to provoke vascular dysfunction by disrupting the physiological equilibrium between vascular constriction and vasodilatation by means of upregulaton of vasoconstrictive mediators, such as endothelin-1 and inhibition of nitrous oxide activity (McQuillan, Leung, Marsden, Kostyk, & Kourembanas, 1994).

It has been speculated that hypoxemia is a key modulator for development of polyneuropathy in COPD patients (Oncel et al., 2010; Ozge, Ozge, Yilmaz, Yalçinkaya, & Calikoğlu, 2005), and thus, possibly playing role in the optic nerve and retinal involvement (Demir et al., 2012) in the disease. As already mentioned, Palkovits and associates (2013) reported markedly reduced oxyhemoglobin saturation of both retinal arterioles and venules in patients with severe COPD. According to their findings there was a significant positive correlation of oxyhemoglobin saturation in retinal arterioles with capillary earlobe blood measures and finger pulse oximetry (Palkovits et al., 2013). Based on their findings, retinal oximetry has the ability to identify systemic hypoxemia of the retinal circulation which supports its potential for applicability to acute patients´ care settings in the future.

2 Aims

The overall aims of this thesis are to test whether the retinal oximetry can be applied to measuring systemic oxygen levels in healthy subjects as well as subjects with compromised oxygenation in order to improve non-invasive monitoring of critically ill and patients in anesthesia care in the future.

The specific objective is to test whether oxyhemoglobin saturation of the systemic circulation can be measured through the retinal circulation in health and disease. The research questions and hypothesis are the following:

Research question 1: Is the retinal oximeter sensitive to changes in oxyhemoglobin saturation (paper I-V)?

a. Is the retinal oximeter sensitive to local hypoxia in retina, i.e. CRVO (paper I)?
b. Is the retinal oximeter sensitive to systemic changes in oxygen levels? (paper II and III)?
    i. Is the oxyhemoglobin saturation in retinal vessels affected by the system hypoxemia and supplemental oxygen breathing in people with severe chronic obstructive pulmonary disease (paper III)?
    ii. Is oxyhemoglobin saturation in retinal vessels affected by hyperoxic breathing in healthy individuals (paper II)
c. Is a retinal oximetry applicable to infants (paper IV)?

Hypothesis 1:

a. The retinal oximeter is sensitive to the various extent of retinal tissue hypoxia in people with CRVO (paper I).
b. The retinal oximeter is sensitive to changes in systemic arterial oxygen content.
    i. Oxyhemoglobin saturation in retinal vessels is affected by systemic hypoxemia in people with severe COPD, and the oxyhemoglobin saturation improves with supplemental oxygen breathing (paper III).

ii. Oxyhemoglobin saturation of retinal vessels is increased from normal baseline level during systemic hyperoxia (paper II).

c. Combined scanning laser opththalmoscope and retinal oximetry is sensitive to different oxygen content in retinal arterioles and venules. The modified version of retinal oximetry is feasible for neonates. (paper IV)

Research question 2: Is retinal oximetry comparable to radial artery blood measures and finger pulse oximetry in people with chronic hypoxia secondary to severe COPD (paper III)?

Hypothesis 2: Spectrophotometric retinal oximetry is at least as good indicator of the systemic oxyhemoglobin saturation as invasive radial artery blood sample measurements and peripheral finger phlethysmography in people with severe COPD (paper III).

3 Materials and methods

3.1 Protection of human subjects and ethical standards

The studies were approved by the National Bioethics Committee of Iceland and the Icelandic Data Protection Authority. Protocols were in compliance with the tenets of Declaration of Helsinki. A signed informed consent was obtained from all adult participants (paper I-III) and parents of the neonates (paper IV) prior to the study enrollment.

3.2 Retinal oximetry studies

3.2.1 Study population

3.2.1.1 CRVO patients (Paper I)

Nineteen consecutive Caucasian patients who presented with symptoms of unilateral CRVO at the Department of Ophthalmology at the Landspitali University Hospital in Iceland enrolled in the study. All patients were recruited using convenience sampling of referral by their ophthalmologist for retinal oximetry. The study design is a prospective observational case-series.

Three study subjects were excluded from the analysis due to either insufficient oximetry image quality or lack of images from the first visit. Of the 16 patients included in the analysis eleven were males and five females. The age ranged from 48-81 years with the mean of 64±9 years (mean ± SD). Five patients were referred by their ophthalmologist for repeated oximetry imaging over time.

Four patients were treated for glaucoma, two patients had COPD, two had diabetes mellitus, five had ischemic heart disease, four were on a treatment for arterial hypertension, and single patients had aortic valve replacement, atrial fibrillation, ipsilateral carotid endarterectomy, chronic renal failure, renal cancer and migraine. Oxyhemoglobin saturation measurements of retinal vessels of both eyes were made before initiating any treatment (except for seven days of latanoprost eye drops in one case).

3.2.1.2 Healthy people under hyperoxia (Paper II)

In total 33 healthy individuals with healthy eyes were recruited through advertisement for the study. Thirty subjects were included in the analysis. Eleven were males and 19 females with the mean age of 44±18 years.

Exclusion criteria included any eye disease, stroke, epilepsy and seizure disorders, smoking and systemic diseases. Systemic diseases pertained to disorders that could have pathological effects on the eye such as diabetes mellitus or affecting the systemic oxygen content like cardiopulmonary diseases including coronary artery disease and COPD.

It was required that expiratory end tidal oxygen plateau was reached during the hyperoxic breathing (100% oxygen) period. Three participants were precluded from the study; two of them did not reach end tidal oxygen plateau and one was considered a glaucoma suspect. All study subjects underwent eye examination by ophthalmologist within seven months before participation in the study.

3.2.1.3 COPD patients (Paper III)

Eleven Caucasian (7 female, 4 male) people with severe COPD [GOLD (Global Initiative in Obstructive Lung Disease) stage 3 or 4] as classified by a forced expiratory volume of less than 50% of predicted in one second (FEV_1 < 50 %) were recruited in the study. The mean age was 70.4±5.4 years (ranging 66 to 82 years). All COPD participants were on long term oxygen therapy and had lightweight portable concentrator devices that supplied their prescribed oxygen (Luxfer, Salford M50 3XE, UK). The long term oxygen therapy was based on meeting the international criteria for sustained hypoxia, generally defined as an arterial oxyhemoglobin saturation of <90%. All COPD participants received their ambulatory pulmonary care at the National University Hospital in Iceland and were clinically evaluated to have sufficient respiratory reserve capacity to endure ceasing their supplemental oxygen for 15 minutes. They were all in a stable condition and had a baseline finger pulse oximetry of greater than 89% on their prescribed oxygen.

Study exclusion criteria included: signs or symptoms of a coronary arterial disease; history of atrial fibrillation, congestive heart failure, carotid stenosis, brain tumor, stroke, anticoagulation treatment with blood test coagulation factors outside the normal range; diabetes mellitus, mental illness or any eye disease. Before participating in the study, all the COPD subjects underwent a complete eye examination.

Eleven age and gender matched healthy subjects served as a control group data. The control group was selected from a set of 120 healthy subjects in a database who had undergone retinal oximetry analysis of oxyhemoglobin saturation while breathing ambient air prior to the current investigation.

3.2.1.4 Neonates (Paper IV)

Fifty nine full-term healthy neonates were recruited at the pediatric department

of the University Hospital in Iceland during a routine fifth day postpartum infant examination. The mean gestational age was 40 weeks. Thirty four were female and 25 male gender. The mean age on the day of the study was 16±4.8 days. Inclusion criteria included healthy neonates with gestational age of 37 to 42 weeks and normal weight at birth. Exclusion criteria included any complications of the mother or the fetus during pregnancy or in the perinatal period.

Successively retinal images were obtained from 55 babies. Three babies were excluded from the study because they did not collaborate in opening their eyes for image acquisition. One was precluded due to prematurity at birth.

3.2.2 Study protocol (Paper I-IV)

All studies were conducted according to a standard protocol. Medical histories including smoking were obtained from self reported questioners (paper I-III) and patient records (paper I & III). Prior to the study, participants sat comfortable on a chair for measurement of vital signs at baseline. Pupil dilation (mydriasis) was achieved using 1% tropicamide (Mydriacyl, S.A. Alcon-Couvreur N.V., Puurs, Belgium) followed by retinal oximetry. In neonates, a detail on the maternal and fetal health was obtained prior to the study (paper IV).

3.2.2.1 CRVO (Paper I)

A finger pulse oximetry reading (Ohmeda Biox 3700; Ohmeda, Boulder, CO), blood pressure and pulse rate measurements (Omron M6 Comfort [HEM-7000-E]; Omron Healthcare Europe, Hoofddorp, The Netherlands) were obtained prior to and following retinal oximetry and recorded on the patient chart.

3.2.2.2 Hyperoxia (Paper II)

Oxyhemoglobin saturation of retinal arterioles and venules by retinal oximetry imaging were compared between three inspired gas conditions: 1) ambient air (baseline); 2) hyperoxic breathing for ten minutes and; 3) ambient breathing for ten minutes (recovery). Under each circumstances; finger pulse oximetry, pulse rate, blood pressure, fraction of inspired oxygen (FiO_2), end fraction of expired oxygen (EtO_2), end-tidal carbon dioxide ($EtCO_2$), fraction of inspired carbon dioxide ($FiCO_2$) and respiratory rate (RR) were measured.

First, both pupils were dilated for measurement of IOP (iCare TAO1 Tonometer; Tiolat Oy, Helsinki, Finland). Next, with the study participant sitting comfortable in a chair, a finger pulse oximetry, pulse rate (Datex Ohmeda,

Oxytip+ Healthcare, Finland) and blood pressure readings ([HEM-7221-E]; Omron Healthcare Europe, Hoofddorp, Netherlands) were obtained followed by retinal oximetry imaging for baseline.

Then an appropriately sized inflatable soft cushion face mask (Flexicare, Flexicare Medical Ltd., Mountain Ash., UK) was placed over the mouth and nose of the subject´s face. The face mask was connected to a circle system with carbon dioxide absorber of an anesthesia machine (Dameca: Siesta 10770, Roedovre, Denmark). Airtight seal was created by fasten a head strap to retaining hooks surrounding the facial mask orifice which was further supported by the participant´s hand to prevent leakage. The adequacy of the face mask fitting was tested by checking the tidal volume and capnography waveform with oxygen flowing through the anesthesia machine (Dameca: Siesta 10770, Roedovre, Denmark). Subsequently the oxygen flow was set to 6 L/min and 100% concentration inhaled for 10 minutes that was immediately followed by a second session of retinal oximetry imaging. Because the face mask did not fit in front of the fundus camera it was removed and the study subjects held their breath whilst the retinal oximetry images were captured (about 30 seconds).

The face mask was also attached to a respiratory gas analyzer (Datex-Ohmeda D-LCC15.03, Planar Systems Inc., Beaverton Oregon, USA) with the ability to continuously monitor the subjects' respiratory parameters (FiO_2, EtO_2, $FiCO_2$, $EtCO_2$, RR) throughout the study. Blood pressure cuff was applied over the brachial artery and the blood pressure measured under the three breathing regimens; 1) on ambient air at baseline 2) nine minutes into hyperoxic breathing 3) recovery ambient air breathing for ten minutes. Systolic and diastolic blood pressures were used to calculate mean arterial pressure (MAP) using the formula: (1/3 systolic blood pressure) + (2/3 diastolic blood pressure). The ocular perfusion pressure (OPP) was calculated as 2/3 MAP - IOP.

Two male study subjects, 24 and 33 years old, underwent retinal oximetry imaging of the left eye every 5 seconds for a total of 120 seconds. The procedure started at once when the supplemental oxygen was halted and lasted into the first two minutes of the recovery ambient air breathing.

3.2.2.3 COPD patients (Paper III)

Oxyhemoglobin saturation of retinal arterioles and venules in subjects with severe COPD were compared to healthy controls while breathing ambient air. Additionally, the COPD subjects were exposed to three inspired gas conditions: 1) prescribed supplemental oxygen; 2) ambient air and; 3) prescribed supplemental oxygen. Under each breathing regimen, finger pulse oximetry, pulse rate, blood pressure, fraction of inspired and expired oxygen (FiO_2/EtO_2), inspired and end-tidal carbon dioxide ($FiCO_2/EtCO_2$), respiratory rate and retinal oximetry images of both eyes were obtained (**Figure 8**). Radial arterial blood samples for blood gas analysis were drawn under the ambient air breathing period only.

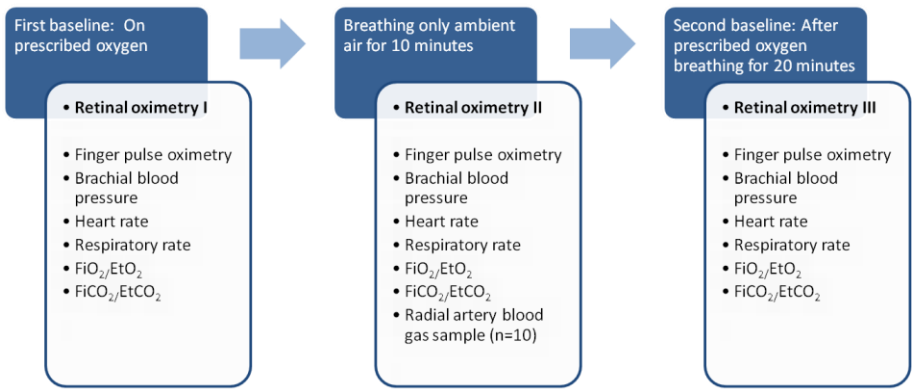


Figure 8. Order of experimental protocol.

Subjects were seated comfortably in a chair while breathing their prescribed supplemental oxygen from a lightweight portable oxygen concentrator. Finger oximetry sensor was applied to a finger for which a stable reading could be obtained and the first peripheral arterial oxyhemoglobin saturation value and pulse rate were measured (Datex- Ohmeda OxyTip+ Healthcare, Finland). The patient's portable oxygen concentrator device and nasal cannula were then substituted with a dual nasal cannula system (Flexicare Medical Limited, UK) connected to an oxygen cylinder with the flow meter set at the subjects prescribed rate of flow. In addition, the cannula was connected to a respiratory gas analyzer (Datex-Ohmeda D-LCC15.03, Planar Systems Inc., Beaverton Oregon, USA) with the ability to continuously monitor the respiratory rate and measure the respiratory parameters. Finger pulse oximetry, pulse rate,

respiratory rate, $EtCO_2$ and FiO_2 were continuously measured during the study. Non-invasive blood pressure cuff was applied over the brachial artery (Omron M6 Comfort [HEM-7000-E]; Omron Healthcare Europe, Hoofddorp, The Netherlands) on the opposite arm to the finger pulse oximeter. Blood pressure measurements were obtained at three different time points in the study. Mean arterial pressure was calculated from systolic and diastolic blood pressure values, using the formula: (1/3 systolic blood pressure) + (2/3 diastolic blood pressure).

After pupil dilation, baseline retinal oximetry images were obtained with subjects inhaling their prescribed supplemental oxygen (first baseline period). Then the supplemental oxygen was discontinued and the subject inhaled ambient air (ambient air period) followed by acquisition of the second session of retinal oximetry images. The prescribed supplemental oxygen was then re-instituted for a period of 20 minutes (second baseline period) followed by acquisition of the final session of retinal oximetry images.

Prior to the second retinal oximetry session, while on ambient air for 10 minutes, a modified Allen´s test (Habib, Baetz, & Satiani, 2012) was made on the non-dominant hand to verify the arterial competency. Instantaneously after the oximetry imaging an radial artery blood sample was drawn (BD Preset with needle, Becton, Dickinson and Company, UK) and sent for immediate blood gas analysis using co-oximetric blood gas analysis (ABL 800,Radiometer A/S, Husum, Denmark).

3.2.2.4 Neonates (Paper IV)

The neonate lay in a prone position on the lower arm of the parent. The parent stabilized the back by supporting the chin and chest with the other hand (modified flying baby position). The researcher assisted the parent by aligning the head of the baby toward the SLO device with the adequacy of alignment confirmed on the Optomap monitor. The researcher spread the infants' eyelid by hand using a rubber glove or a cotton tip. A different researcher obtained images of this one eye.

3.2.3 Oxymap retinal oximeter (Paper I-III)

The non-invasive dual wavelength Oxymap T1 retinal oximeter (Oxymap ehf., Reykjavik, Iceland) is based on a conventional fundus camera (Topcon TRC-50DX; Topcon Corporation, Tokyo, Japan) connected with a costume-made optical adapter (**Figure 9**). Two narrow band-pass filters (5nm) and a beam splitter is coupled with two high resolution digital cameras (Insight IN 1800,

1600 x 1200 square pixels; Diagnostic Instruments Inc., Sterling Heights, MI) that generate 50° view of the fundus.

Figure 9. The retinal Oxymap T1 oximeter.

First, the fundus camera briefly illuminates the ocular fundus with white light for retinal oximetry. Subsequently, the two narrow band filters allows the retinal oximeter to simultaneously acquire two monochromatic retinal images at 570nm and 600nm for spectrophotometric analysis. A broader 80 nm bandpass filter is situated in the light path of the fundus camera (585 nm center wavelength) in order to restrict redundant light exposure to the subjects' eyes by permitting only 545 to 625 nm light to exit the camera lens.

Spectrophotometric oximetry is based on the fact that oxyhemoglobin and deoxyhemoglobin have different light absorption spectra. The monochrome image at 570 nm is at isosbestic wavelength that is insensitive to oxyhemoglobin. The absorbance of arterioles and venules is similar at this point so they come into view equally dark on the image. The monochromatic image at 600 nm wavelength however, is at non-isosbestic point that is sensitive to oxyhemoglobin (**Figure 10**). The absorbance of the arterioles at this wavelength is less than of venules so the arterioles appear brighter than venules on the image**.**

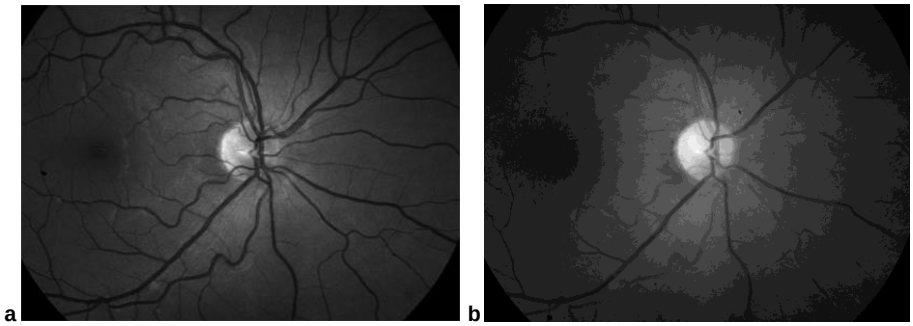


Figure 10. Oxymap T1 acquisition of two monochromatic fundus images at different wavelengths with the optic disc in the centre. (a) Isosbestic wavelength (570 nm) that is not sensitive to oxygen. (b) Oxyhemoglobin sensitive wavelength (600 nm). The arterioles and venules are of similar density on the 570 nm image (left), but on the 600 nm image (right) the arterioles have a much lower optical density and appear brighter, due to lower absorption of oxyhemoglobin at 600 nm.

3.2.3.1 Oximetry imaging

The room was dimmed with window blinds by the only light source coming from the fundus camera and a computer screen that was configured at the dimmest setting. Image acquisition was performed according to a standard protocol with the study subjects sitting at the fundus camera with their chin on a chin rest and forehead against the head bar in front of them. The same experienced researcher took all the images with participants sitting in front of the fundus camera. Five images of the right eye were taken first and then of the left eye in paper II and III. In paper I these five images were first taken of the CRVO affected eye and then the opposite unaffected eye. The first image was centred on the macula. Second image was centred on the optic disc. On the third image the subject gazed up and on the fourth image down. The fifth image was replication of the second image with the optic disc in the centre. The average time for obtaining these five images of each eye was approximately 30 seconds. The first good quality image with the optic disc in centre was selected manually for analysis.

3.2.3.2 Image processing

Specialized software (Oxymap Analyzer software 2.2.1, paper I and II, and 2.4 paper III, version 3847) automatically selects vessel points on the monochromatic images for measurement of light intensity inside (I) and outside the vessel (I_O) for calculation of optical density *(OD)* and optical density ratio (ODR) for estimation of oxyhemoglobin saturation. The calibration of the retinal

oximeter is based on calibration constants where a = -1,28 and b = 1,24. These calibration factors are derived from the work of Schweitzer et al., (1999) who measured the average oxyhemoglobin saturation of whole blood in vivo and vitro for healthy people using calibrated imaging spectroscopy. Their findings of 92.2% for retinal arterioles and 57.9% for venules are used to extrapolate the oxyhemoglobin saturation in subjects' vessel by comparison of the ODR to that of the calibration settings. The calculated oxyhemoglobin saturation is then automatically presented as a pseudocolor overlay (**Figure 11**) on the fundus image.

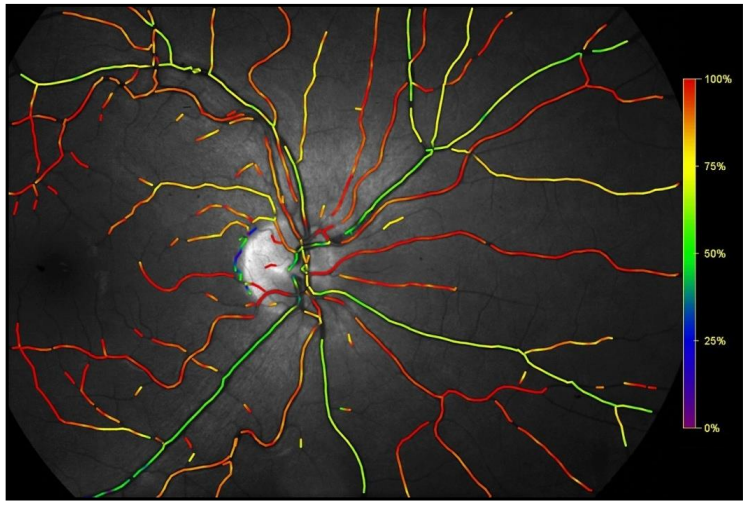


Figure 11. Pseudocolor image of the right fundus in a healthy subject. The colors indicate oxyhemoglobin saturation as seen on the scale on the right. In general, arterioles are orange to red, indicating oxygen saturation about 90-100%. Venules may vary from bluish to yellow but are normally green, indicating normal oxyhemoglobin saturation around 50-60%.

The software also quantifies the vessel width at particular point by counting the pixels on an orthogonal cross-section of the vessel. These numbers of pixels are then averaged for over 100 cross-sections along the measured vessel to obtain the mean.

3.2.3.3 Data analysis

First and second degree arterioles and venules in each quadrant on the fundus images were selected for analysis and calculation of mean oxyhemoglobin saturation values. Vessels were matched, so that measured vessels in the

fellow eye were parallel to the CRVO eye (paper I), within the eye between oximetry sessions (paper II and III) or between the eye of the COPD subject and of a healthy subject from the control group (paper III). To get this matching two vessel segments were sometimes averaged and inserted as one for calculation of the mean for the eye. For example in CRVO, if a selected vessel segment in the inferior temporal venule in the fellow eye was too short (<100 pixels) before branching, both daughter vessels on the other side of the bifurcation were measured. The calculated mean average was then inserted as one and compared with the mean value of the matching parent vessel before branching (if ≥ 100 pixels) in the CRVO affected eye.

The AV- difference was represented as the calculated difference between arterial and venous oxyhemoglobin saturation.

3.2.3.3.1 CRVO (Paper I)

Vessel segments close to haemorrhages were excluded to bypass artefacts. Vessels were matched, so that measured vessels in the fellow eye were parallel to the CRVO eye.

Of the sixteen patients included in the analysis, single measurement was made in 11 cases and repeated measurements in 5 cases over a time period ranging from two weeks to 20 months. Data from two patients were not included in the comparative statistical analysis because images of the unaffected eye were lacking. Hence, the values of their oxyhemoglobin saturation and numbers of measured vessels are given in the result **Table 4** (chapter 4.1) for information only.

3.2.3.3.2 Hyperoxia (Paper II)

Oxyhemoglobin saturation was calculated for all first or second degree arterioles and venules in each quadrant of the right eye. Vessels were matched between retinal images of the three breathing regimens.

3.2.3.3.3 COPD (Paper III)

First and second degree arterioles and venules in each quadrant of the right eye were matched between the retinal images of the three breathing conditions and with a retinal image of a healthy control subject. Identical image acquisition and oximetric analyses were performed for the healthy subjects.

Of the 11 COPD participants, one was not breathing the prescribed oxygen on arrival so there was no measurement at first baseline for this subject and

we were unable to draw arterial blood sample from another. Hence, data from 10 subjects within the COPD group underwent statistical analysis at each study time period for comparison of mean and standard deviation. Furthermore, one COPD patient was “a mouth breather” and therefore it was not possible to get adequate $EtCO_2$ and FiO_2 data from the dual nasal cannula at any of the three study period. All 11 COPD subjects were included in the comparison with healthy controls under ambient air breathing.

Table 2. Selection criteria for retinal vessels segment measurement of oxyhemoglobin saturation with the Oxymap T1 retinal oximeter. One pixel is approximately 9.3 µm.

Begin vessel selection	Paper I and II: a) at least 15 pixels around the optic disc excluded b) at least 15 pixels on a border of any bright area around the optic disc excluded c) start as close to the optic disc as possible Paper III: An area that was demarcated 1.5 disc diameters (344 pixels)
End vessel selection	Paper I and II: Never closer than 30 pixels to the rim of the image Paper III: 3 disc diameters (690 pixels)
Minimum vessel diameter	Paper I and III : 8.0 pixels (~75 µm) Paper II: 6.0 pixels (~56 µm)
Vessel length	Paper I: 100 – 300 pixels (as close to 300 pixels as possible) Paper II: 50 – 200 pixels (as close to 200 pixels as possible) Paper III: 50 pixels at minimum but as close to the total length of the segment between the two circles (1.5 and 3 disc diameters) as possible.
Vessel segment exclusion criteria	a)Vessel branching with 15 pixels b)Vessel crossing c) Any extremes in background brightness such as hemorrhage to avoid artifacts

3.2.4 Neonates (Paper IV)

The Optomap 200Tx scanning laser ophthalmoscope (Optos plc., Dunfermline, Scotland, UK) for the oximetry imaging in neonates, uses 532nm and 633nm laser wavelengths of light to obtain two monochrome spectral images of the fundus. Although the reference wavelength at 532nm nearer an isosbestic point, it is not completely isosbestic. The great distinction between oxy- and deoxyhemoglobin light absorption at 532nm and 633nm (oxygen sensitive wavelength) allows for application of SLO for retinal oximetry. The ODR is calculated according to the following equation:

$$ODR = \frac{OD\ 633nm}{OD\ 532nm} \qquad \text{Equation: 18}$$

The calculated oxyhemoglobin saturation is then inversely and near linearly related to the ODR.

3.2.4.1 Oximetry imaging of neonates

The room was dimmed with window blinds and the only light source coming from the SLO and a computer screen that was configured at the dimmest setting. The same researcher took all the images with the babies aligned in front of the SLO in a modified baby flying position. Both Ultra-Widefield (200°) and ResMax (100°) retinal images were obtained of a unilateral eye. For the purpose of the study 250 ResMax images were acquired in total with the median of 4 images (range 0-8) per neonate. Ultra-Widefield images were obtained for further research purpose only.

3.2.4.2 Image processing

A modified version of the dual wavelength Oxymap Analyzer software 2.3 (version 5206) algorithms was used for the spectral imaging and to process the monochromatic ResMax images for calculation of ODR and vessel diameter. The calibration of the modified version of Oxymap Analyzer software is based on calibration constants where a= -2.4733 and b =1.4388. These calibration factors are derived from the work of Kristjansdottir et al. (2014) which based on the the work of Schweitzer et al. (1999) for mean oxyhemoglobin saturation of 92.2% for retinal arterioles and of 57.9% for venules for healthy people as has previously been discussed (chapter 3.2.3.2). The oxyhemoglobin saturation can then be calculated according to the (a x ODR + b) x 100. The calculated oxyhemoglobin saturation was automatically presented as a pseudocolor map on a 100° oximetry fundus image (**Figure 12**).

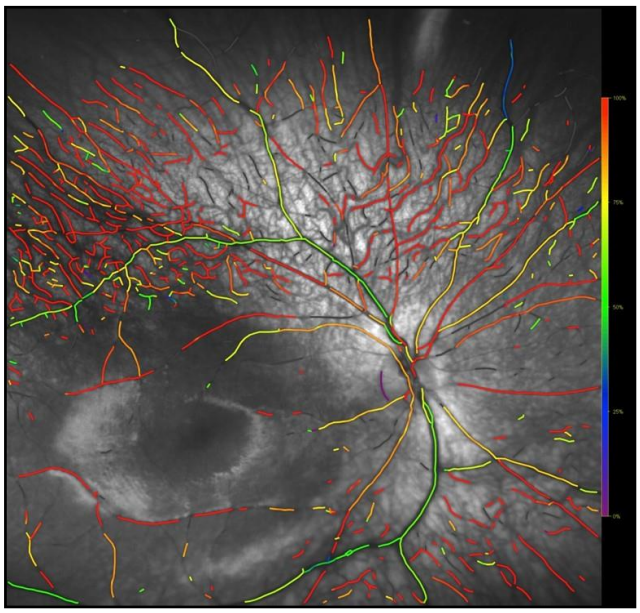


Figure 12. Pseudocolor overlay, using Oxymap algorithm, over monochrome spectral SLO image of the fundus.

3.2.4.3 Image analysis

In total, 28 fundus images of 28 neonates were manually selected for analysis by the modified Oxymap Analyzer software for calculation of ODR and vessel diameter. The image analysis was standardized prior to the study. The image of the best contrast and focus quality of the main superotemporal arteriole and venule with the optic disc in the centre was manually selected for oximetry analysis.

Table 3. Selection criteria for retinal vessels segments for measurement of oxyhemoglobin saturation with the combined SLO and modified Oxymap analysis software.

Begin vessel selection	a) at least 15 pixels around the optic disc excluded b) at least 15 pixels on a border of any bright area around the optic disc excluded c) start as close to the optic disc as possible
End vessel selection	Never closer than 30 pixels to the rim of the image
Minimum vessel diameter	6.0 pixels
Vessel length	60-350 pixels (as close to 360 pixels as possible)
Vessel segment exclusion criteria	a) Vessel branching with 15 pixels b) Vessel crossing c) Any extremes in background brightness

If the superotemporal vessel segment from the optic disc rim to the first branching was shorter than 60 pixels, the segment was excluded. The vessels segment (≥ 60 pixels) posterior to the branching until the second branching was selected instead for oximetry analysis.

3.3 Statistical analysis (Paper I-IV)

Statistical data analysis for paper I-III was carried out with Prism version 5 (GraphPad Software Inc, LaJolla, California, USA) for comparison of means by using 2-tailed paired *t* tests. Resulting data are presented as mean ± SD. P value of <0.05 was considered to be statistically significant.

For paper III the resultant data was in addition to the 2-tailed paired *t* tests, presented as repeated measures one–way ANOVA for comparison of means. Dunnett's and Tukey´s multiple comparison post tests were performed to compare the means of group pairs. A p value of < 0.05 was considered to be statistical significant and the data presented as mean ± SD (95% confidence interval (CI)). Bland-Altman plots were used to determine the level of agreement between different measurement devices, i.e. retinal oximetry, pulse oximetry and radial artery blood samples. The degree of error, defined as the difference between the measured values, was reviewed in terms of bias and

variability, where bias was calculated as the average difference between the measurements and the variability as the mean bias ± 1.96 standard deviations. A power analysis indicated that seven subjects were necessary for paper III to identify a difference of 3 percentage points (%) in oxyhemoglobin saturation between retinal oximetry measurements of subjects inspiring supplemental oxygen versus ambient air with 90% power.

For paper IV, statistical data analysis was carried out using SPSS version 22 (Release 22.0.0.0, IBM). A 2-tailed paired *t* test was used for comparison of the mean oxyhemoglobin saturation in superotemporal arterioles and venules. Resulting data are presented as mean ± SD. P value of <0.05 was determined to be statistically significant.

4 Results

Three studies on retinal oximetry (paper I-III) were carried out to test whether the Oxymap T1 retinal oximeter is sensitive to changes in oxyhemoglobin saturation. The first study was on retinal vessel hypoxia in people affected by CRVO (Paper I). The second study was on changes of oxyhemoglobin saturation in retinal arterioles and venules in healthy people during hyperoxic breathing (Paper II). The third study was on retinal vessel oxyhemoglobin saturation in people with systemic hypoxemia with and without their supplemental oxygen therapy. In that study a comparison was made with a healthy cohort and invasive radial artery blood gas and finger pulse oximetry values during ambient air breathing (Paper III). The fourth study was adjunctive to the former studies in order to learn whether a retinal oximetry is applicable to infants (paper IV).

4.1 Retinal oximetry in CRVO patients (Paper I)

For the 14 eyes affected by CRVO, the oxyhemoglobin saturation of retinal arterioles was 95±5% and 31±12% in venules (mean±SD). In unaffected fellow eyes of the same patients the oxyhemoglobin saturation of retinal arterioles was 94±6% and 52±11% in venules. The venous oxyhemoglobin saturation in the CRVO affected eyes was significantly lower than in the fellow unaffected eyes (31±12% versus 52±11%, $p< 0.0001$, paired t test) (**Figure 13**). There was no statistical difference between oxyhemoglobin saturation of retinal arterioles in eyes affected by CRVO and the fellow unaffected eyes ($p=0.49$).

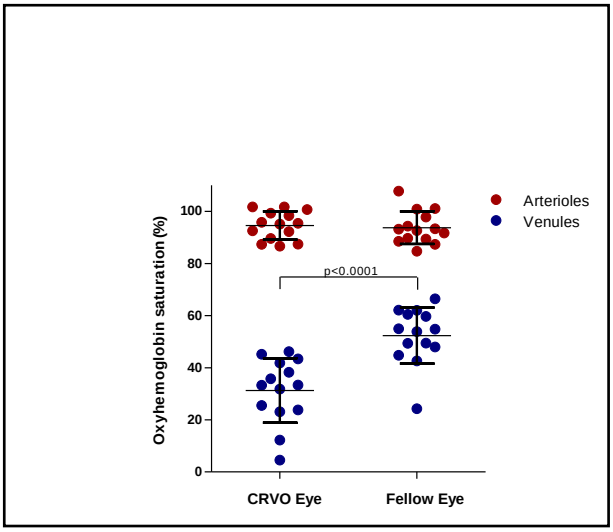


Figure 13. Oxyhemoglobin saturation in retinal arterioles (red dots) and venules (blue dots) in CRVO eyes and unaffected fellow eyes. Each dot stands for mean oxyhemoglobin saturation (%) of individual eye in first and second degree retinal vessels. Bars designate mean ± SD (n=14, p< 0.0001).

Greater variability of retinal venous oxyhemoglobin saturation was observed in CRVO eyes as compared with unaffected fellow eyes (**Table 4**). The AV-difference in eyes with CRVO was 63±11% compared with 43±7% in unaffected fellow eyes (p< 0.0001). The mean arteriolar vessel diameter in pixels (each pixel is about 9,3 µm) in CRVO eyes was 10±1 and 12±1 in fellow eyes (p< 0.0001). The diameter of retinal venules in eyes with CRVO was 17±2 and 16±2 in unaffected fellow eyes (p=0.02).

Table 4 Oxyhemoglobin Saturation (%) in first and second degree retinal arterioles and venules in 16 patients with central retinal vein occlusion. The values show mean ± standard deviation and number of measured retinal vessels (parentheses). SpO_2 is finger pulse oximetry. The diameter of retinal venules is shown in pixels. Patients' number 15 and 16 were not included in statistical analysis. Reprinted from Paper I (Graefes Arch Clin Exp Ophthalmol, 253(10), 1653-61,© 2015, with permission of Graefe's Archive for Clinical and Experimental Ophthalmology)

Patient No.	CRVO Eye		Fellow Unaffected Eye			CRVO Eye	Fellow Eye
	Arterioles	Venules	Arterioles	Venules	SpO_2	Retinal venule diameter	Retinal venule diameter
1	87 ± 5 (2)	23 ± 26 (4)	87 ± 6 (2)	49 ± 6 (4)	94	16	17
2	87 ± 3 (6)	5 ± 32 (3)	85 ± 8 (5)	24 ± 6 (3)	93	14	14
3	101 ± 4 (6)	24 ± 12 (4)	101 ± 8 (6)	62 ± 4 (4)	98	20	16
4	96 ± 16 (4)	33 ± 22 (3)	101 ± 6 (4)	54 ± 5 (3)	95	18	15
5	95 ± 5 (5)	42 ± 13 (5)	93 ± 11 (5)	61 ± 4 (4)	97	16	16
6	98 ± 7 (3)	38 ± 17 (5)	94 ± 1 (3)	55 ± 6 (5)	97	13	13
7	95 ± 3 (7)	45 ± 12 (4)	98 ± 7 (7)	60 ± 7 (4)	95	18	20
8	92 ± 12 (5)	46 ± 24 (2)	90 ± 4 (6)	55 ± 2 (2)	97	19	17
9	102 ± 8 (3)	33 ± 24 (5)	108 ± 2 (3)	62 ± 13 (5)	97	17	17
10	87 ± 10 (6)	12 ± 15 (6)	89 ± 5 (6)	43 ± 6 (6)	95	18	14
11	102 ± 7 (7)	32 ± 22 (6)	92 ± 11(7)	48 ± 9 (6)	94	19	17
12	93 ± 7 (4)	25 ± 14 (3)	89 ± 3 (4)	49 ± 7 (3)	96	14	16
13	99 ± 6 (5)	36 ± 6 (5)	93 ± 7 (5)	45 ± 5 (4)		20	16
14	90 ± 6 (5)	43 ± 6 (6)	93 ± 4 (5)	66 ± 4 (6)		16	15
15	95 ± 5 (7)	34 ± 3 (4)					
16	102 ± 9 (4)	50 ± 8 (4)			96		
Patients 1-14 Mean±SD	95 ± 5	31 ± 12	94 ± 6	52 ± 11		17 ± 2	16 ± 2
Patients 1-16 Mean ± SD	95 ± 5	33 ± 12					

The standard deviation reflects the oxyhemoglobin saturation variation within individual eyes

There were no statistical differences between finger pulse oximetry (96%±2%, n=13) and oxyhemoglobin saturation of retinal arterioles either in the CRVO eye (p = 0.28) or the fellow eye (p = 0.24).

4.1.1 Patients follow-up

All five patients that were followed with repeated retinal oximetry images received treatment; dorzolamide eye drops, intravitreal anti-VEGF bevacizumab and/or laser photocoagulation. In all five CRVO cases the venular oxyhemoglobin saturation improved over time (**Figure 14**). In two patients, the clinical situation improved and the venous oxyhemoglobin saturation recovered. In two eyes the clinical signs and symptoms of CRVO completely resolved but the venular oxyhemoglobin saturation did not return to normal. One eye developed iris neovascularisation, glaucoma and poor visual outcome where the venous oxyhemoglobin saturation remained slightly subnormal. Measured values at the first and last visit are listed in **Table 5**.

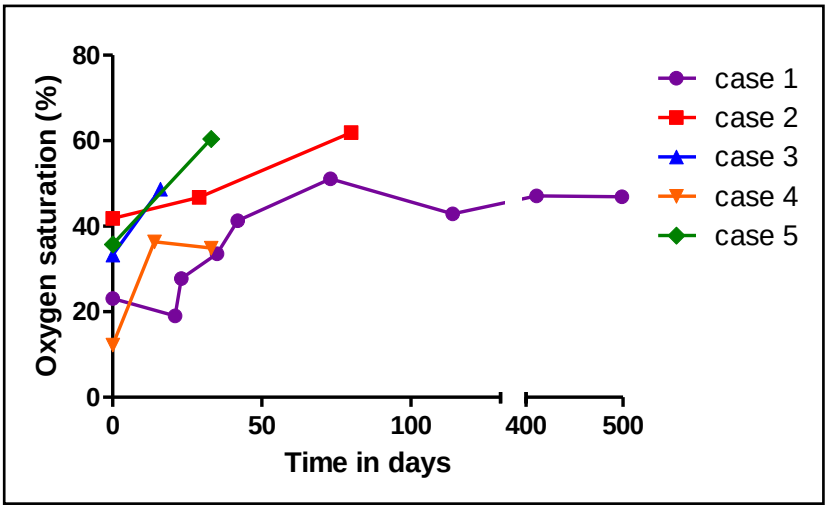


Figure 14. Mean oxyhemoglobin saturation of retinal venules during the follow-up period of five patients with CRVO. Each dot indicates the oxyhemoglobin saturation in first and second degree venules at a point in time. Case1. The patient developed iris neovascularisation, glaucoma (**Figure 15**) and poor visual outcome. Case 2. The patient received dorzolamide eye drops. Clinical signs of CRVO resolved (**Figure 16**). Case 3. The patient presented with ischemic CRVO, macular edema, and elevated intraocular pressure. Treatment consisted of dorzolamide eye drops, intravitreal bevacizumab, and panretinal photocoagulation. At week two the mean retinal venous oxyhemoglobin saturation had improved. Case 4. The patient was treated with dorzolamide eye drops for 4 weeks. During the 8-week follow-up period, clinical signs and symptoms resolved. Case 5. The patient presented with mild CRVO on arrival with no macular edema. The patient was treated with dorzolamide eye drops and established full recovery.

Table 5. Retinal venous oxyhemoglobin saturation values (%) in the five patients that were followed with repeated oximetry imaging.

Case	Follow up period	On arrival	Last image
1	20 months	23±26	40±23
2	80 days	42±13	62±8
3	16 days	33±23	49±12
4	76 days	12±15	35±10
5	33 days	36±6	60±7

The values show mean±standard deviation

Retinal oximetry images on the patient who developed iris neovascularisation, glaucoma and poor visual outcome are presented in **Figure 15**. Images on the patient who resolved from the clinical signs and symptoms are presented in **Figure 16**.

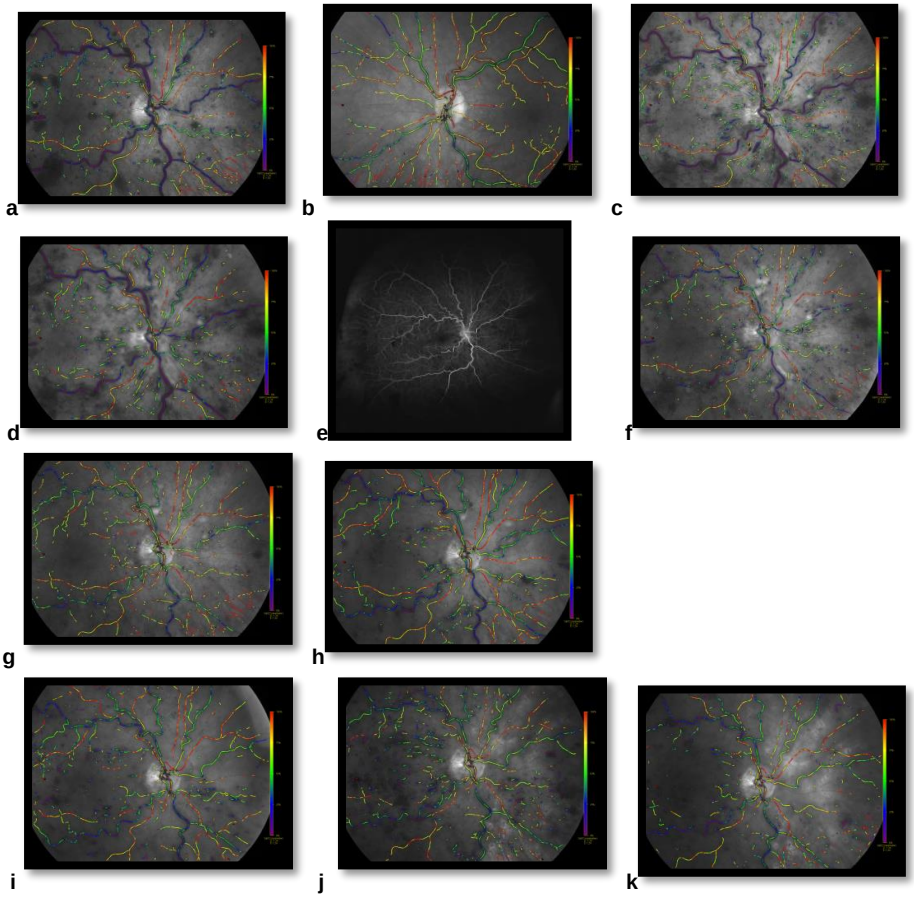


Figure 15. The patient was followed with repeated oximetry images (Figure 14, case 1) for twenty months. In the first oximetry image (a), oxygen saturation of retinal venules ranged from minus 4% to 49% as indicated by blue and green colours respectively on the image. Three weeks later (c) mean oxygen saturation of retinal venules had decreased. Clinical evaluation revealed cystoid macular edema and worsened visual acuity. Three days later (d), after the first dosage of intravitreal bevacizumab, retinal venous oxygen saturation and macular edema improved. At week five (f) and fifteen (g) retinal venous oxygen saturation had improved but regional variability remained high. From week twenty-one (h) clinical symptoms worsened. At twenty months (k), after twelve intravitreal bevacizumab injections and three sessions of panretinal photocoagulation, the CRVO eye had developed neovascular glaucoma.

a First retinal oximetry image of the CRVO eye, two weeks after onset of symptoms. Retinal venous oxyhemoglobin saturation is 23±26% (mean ± SD) and retinal arteriolar oxyhemoglobin saturation 87±5%. Visual acuity (VA) 0.7. No macular edema. Central macular thickness 160 µm. **b** Unaffected left fellow eye. Oxyhemoglobin saturation in retinal venules is 49±6% and 87±6% in retinal arterioles. VA 0.7. **c** Three weeks after the first retinal oximetry image. Retinal venous oxyhemoglobin saturation is 19±22%. VA has deteriorated to 0.1. Macular edema. Central macular thickness 626 µm. **d** and **e** At three and a half weeks, three days after first bevacizumab injection. Retinal venous oxyhemoglobin saturation is 28±21%. Macular edema has resolved. Central macular thickness 190 µm. Fluorescein angiography at 72 seconds after injection shows poorly perfused areas of the retina. **f** At five weeks. Clinical signs improving. Retinal venous oxyhemoglobin saturation is 34±20%. VA 0.3. No macular edema. Central macular thickness 170 µm. **g** At fifteen weeks, after three bevacizumab injections. Retinal venous oxyhemoglobin saturation is 51±5%. VA 0.2. No macular edema. Central macular thickness 132 µm. **h** At week twenty-one. Worsening clinical signs. Retinal venous oxyhemoglobin saturation is 43±21%. VA 0.1. Recurrence of macular edema. **i** At thirteen months, three months after ninth bevacizumab injection. Retinal venous oxyhemoglobin saturation is 47±18%. VA 0.3. Central macular thickness 494 µm. **j** At sixteen months, after ten bevacizumab injections and panretinal photocoagulation. Retinal venous oxyhemoglobin saturation is 47±20%. VA 0.17. Central macular thickness 316 µm. **k** At twenty months. Neovascular glaucoma. Retinal venous oxyhemoglobin saturation is 40±23% and retinal arteriolar oxyhemoglobin saturation 88±5%. VA 0.3.Central macular thickness 140 µm. Reprinted from Paper I (Graefes Arch Clin Exp Ophthalmol, 253(10), 1653-61,© 2015, with permission of Graefe's Archive for Clinical and Experimental Ophthalmology).

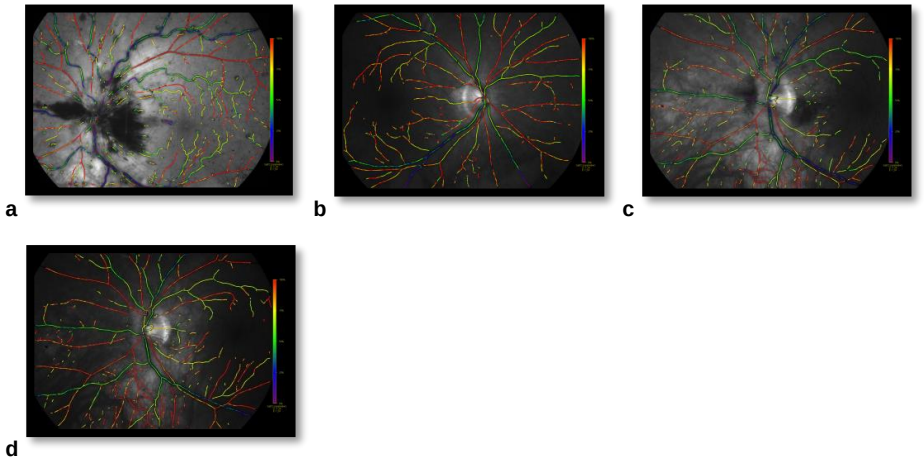


Figure 16. The patient suffered vision loss six weeks before presenting to the University Hospital. The person was followed with repeated oximetry images for a period of 11 weeks (Figure 14, case 2). Clinical evaluation confirmed left eye CRVO (a) and hemorrhagic fundus without much edema. Dorzolamide eye drop treatment was implemented. At week eleven (d) clinical signs and symptoms had resolved.

a CRVO eye at presentation, six weeks after onset of symptoms. Retinal venous oxyhemoglobin saturation is 42±13% (mean ± SD). Retinal arteriolar oxyhemoglobin saturation is 95±5%. VA 0.2 to 0.4. **b** Fellow eye. Retinal venous oxyhemoglobin saturation is 61±4% and retinal arteriolar oxyhemoglobin saturation 93±11%. VA 1.0. **c** At four weeks. Less retinal hemorrhage. Retinal venous oxyhemoglobin saturation is 47±13%. VA 0.6. **d** At eleven weeks. Clinical signs and symptoms resolved. Retinal venous oxyhemoglobin saturation is 62±8% and retinal arteriolar oxyhemoglobin saturation 100±3%. VA 0.9-1.0. Reprinted from Paper I (Graefes Arch Clin Exp Ophthalmol, 253(10), 1653-61,© 2015, with permission of Graefe's Archive for Clinical and Experimental Ophthalmology)

4.2 Retinal oximetry in healthy under hyperoxemia (Paper II)

After ten minutes of hyperoxic facemask breathing the mean FiO_2 was 96±2% and the EtO_2 91±4% (mean ± SD, n=30). The $EtCO_2$ measured 36±2 mmHg (n=29). The physiological parameters are presented in table **Table 6**.

Both the brachial blood pressure and the heart rate were unaffected by hyperoxic breathing as compared with baseline normoxic breathing (p=0.86, n=30 and p=0.17,n=26 respectively).

Table 6 Physiological parameters (mean ± SD) in 30 healthy subjects at baseline, under experimental condition of hyperoxic breathing for 10 minutes and after recovery period on ambient air for 10 minutes.

Physiological Parameters	Baseline	Hyperoxia	Recovery
FiO_2 / EtO_2 (%)	-	96±2 / 91±4	-
$EtCO_2$ (mm Hg)	-	36±2[1]	-
Heart rate (bpm)	72±11	70±11	-
SBP (mm Hg)	132±20	127±22	128±19
DBP (mm Hg)	84±12	86±14	84±14
MAP (mm Hg)	100±14	100±15	99±15
SpO2 (%)	97.5±0.7	99.1±0.3	-
IOP (mm Hg)	15±4	-	-
OPP (mm Hg)	52±10	-	-

FiO_2, concentration of inhaled oxygen; EtO_2 concentration of exhaled oxygen; $EtCO_2$, end-tidal carbon dioxide; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; SpO2, finger pulse oximetry; IOP, intra ocular pressure; OPP; ocular perfusion pressure.

[1] EtCO2 is missing from one of the study participants.

The oxyhemoglobin saturation of retinal arterioles was 92.0±3.7% at baseline ambient air breathing and increased to 94.5±3.8% during the hyperoxic breathing (n=30, p<0.0001). The oxyhemoglobin saturation of retinal venules was 51.3±5.6% at the baseline and increased to 76.2±8.0% during the hyperoxic breathing (p<0.0001). Concurrently, the AV-difference measured 40.7±5.7% at baseline versus 18.3±9.0% during the hyperoxic breathing (p<0.0001). There were no statistical differences between oxyhemoglobin saturation measurements and the AV-difference during ambient air breathing at baseline and ten minutes of recovery breathing (p=0.2 and 0.8).

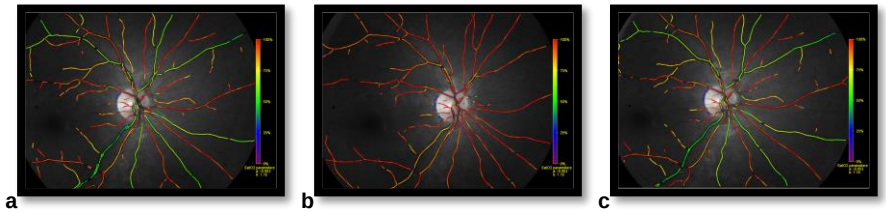


Figure 17. Retinal oxyhemoglobins saturation in retinal arterioles and venules under the different breathing regimens. **a** Baseline ambient air breathing. **b** After 10 minutes of hyperoxic breathing **c** Recovery ambient air breathing for 10 minutes.

Under hyperoxic condition the vessel diameter narrowed in both retinal arterioles and venules. In arterioles, the diameter decreased from 10.3±1.3 pixels at baseline to 9.7±1.4 pixels ($p<0.0001$) during hyperoxic breathing. In venules, the diameter of the vessel wall decreased from 13.3±1.5 pixels at baseline to 11.4±1.2 pixels ($p<0.0001$) with hyperoxic breathing. The retinal venules´ diameter was slightly narrower at recovery as compared with baseline breathing (13.1±1.4 vs. 13.3±1.5, $p = 0.007$). There was no difference in arteriolar diameter between baseline and recovery breathing ($p = 0.3$).

During hyperoxic breathing (**Figure 18**), the mean oxyhemoglobin saturation in retinal arterioles was markedly different from finger pulse oximeter measurements (n=30, 94.5±3.8% and 99.1±0.3 respectively, $p<0.0001$, paired t-test).

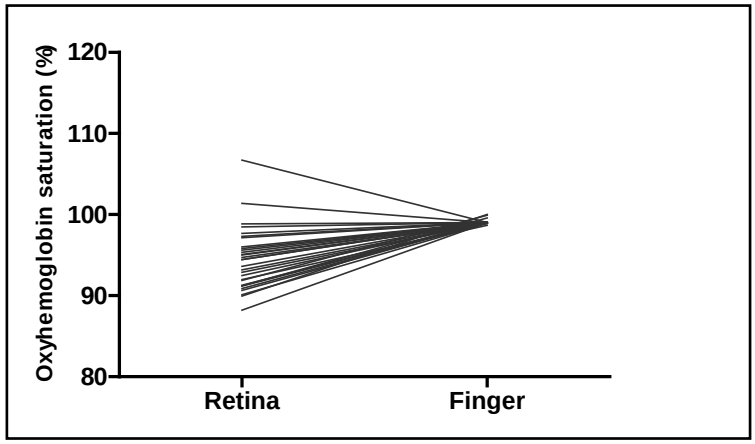


Figure 18. The graph show individual oxyhemoglobin saturation measurements by retinal and peripheral pulse oximetry in hyperoxic breathing.

During ambient air breathing, the mean oxyhemoglobin saturation in retinal arterioles showed also difference from finger pulse oximeter measurements (n=27, 92.0±3.7 versus 97.5±0.7 respectively, $p < 0.0001$).

The finger pulse oximetry increased during hyperoxic breathing as compared with baseline ambient air breathing (n=27, $p< 0.0001$).

Figure 19 show retinal oximetry analysis of the images that were captured every five seconds of two healthy male subjects. The oximetry image session started immediately after cessation of hyperoxic breathing and lasted into the first two minutes of recovery breathing. The oxyhemoglobin saturation of both retinal arterioles and venules returned to baseline values within these two minutes.

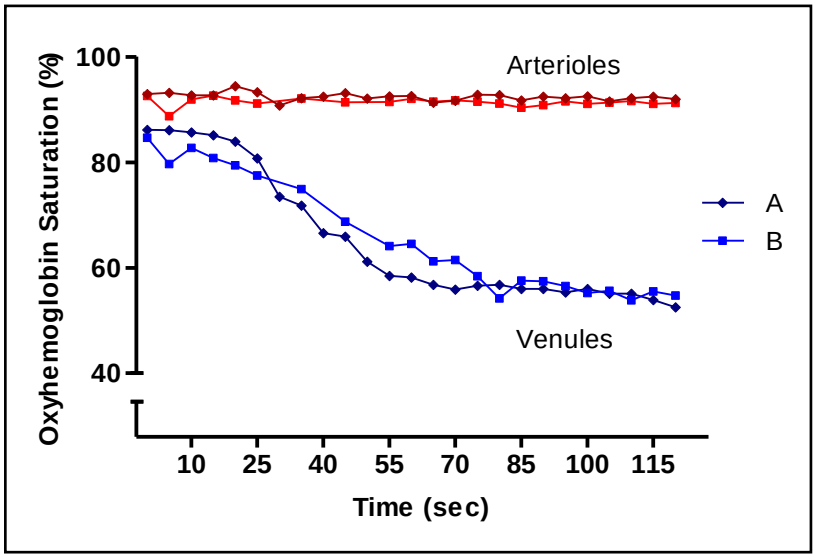


Figure 19. The slope illustrate the return of oxyhemoglobin saturation of retinal vessels down to baseline values in two study subjects after cessation of face mask oxygen breathing.

4.3 Retinal oximetry in COPD patients (Paper III)

All enrolled COPD subjects finished the study and no adverse reactions were observed. Physiological parameters are presented in **Table 7**.

Table 7 Basic physiological parameters (mean ± SD) at first baseline (on prescribed oxygen prior to the first retinal oximetry image), then after 10 minutes of only ambient air breathing and at second baseline after a recovery period of 20 minutes with oxygen breathing.

	Oxygen therapy First Baseline n=10[1]	**Ambient air Breathing n=11**	**Oxygen therapy Second baseline n=11**
SBP (mmHg)	133 ± 21	127 ± 19	129 ± 13
DBP (mmHg)	82 ± 15	78 ± 10	82 ± 11
MAP (mmHg)	99 ± 15	94 ± 11	97 ± 11
SpO_2 (%)	94 ± 4	90 ±3	95 ± 2
Heart rate (bpm)	82± 10	77 ± 13	76 ± 12
RR (min^{-1})	18 ± 4	15 ± 3	15 ± 3
	n=9[2]	**n=10**	**n=10**
FiO_2 (%)	39 ± 20	21 ± 3	43 ± 15
$EtCO_2$ (%)	34 ± 8	33 ± 7	33 ± 8

SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; SpO2, finger pulse oximetry; RR, Respiratory rate; FiO_2, concentration of inhaled oxygen; $EtCO_2$, end-tidal carbon dioxide.

[1] One participant was not breathing the prescribed supplemental oxygen on arrival. Therefore no basic physiologal parameters at first baseline from that subject are presented on the table.

[2] Reliable measures of FiO2 and EtCO2 from one of the participants were not possible to acquire due to the subject being a mouth breather.

4.3.1 COPD subjects compared to healthy control subjects

COPD patients (n=11) breathing ambient air had significantly lower mean oxyhemoglobin saturation as compared with healthy controls in both retinal arterioles (87.2±4.9% vs. control = 93.4 ±4.3%, 95% CI:-11.31 to -1.02, p=0.02, paired t test) and venules (45.0±10.3% vs. control = 55.2±5.5%, 95% CI: -17.95 to -2.37, p= 0.01) (**Figure 20**). The AV-difference was not markedly different between the COPD group and the healthy subject group (42.2±8.0% versus control = 38.2±4.0%, 95% CI: -1.98 to 9.98, p= 0.17).

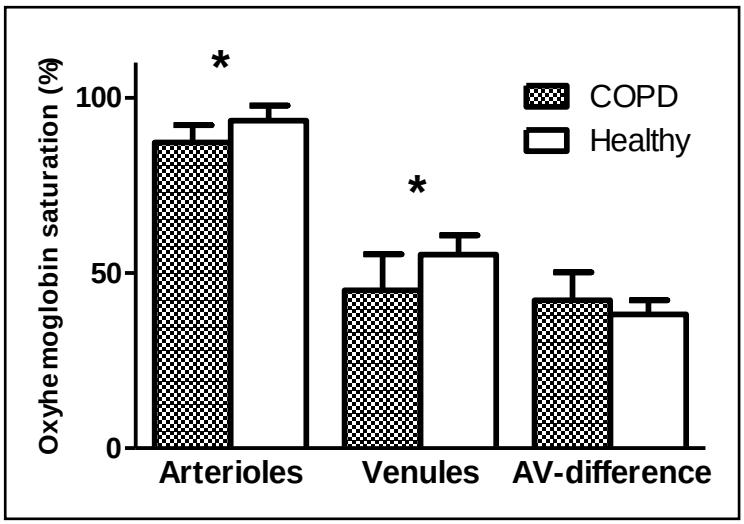


Figure 20. COPD subjects (n=11) breathing ambient air had significantly lower mean oxyhemoglobin saturation in both retinal arterioles and venules as compared with healthy controls. Oxyhemoglobin values are presented as mean ± standard deviation. *Signifiacant difference between COPD patients and healthy control subjets.

Administration of the prescribed supplemental oxygen increased the oxyhemoglobin saturation in retinal arterioles (87.2%±4.9% to 89.5%±6.0 %, 95% CI:-4.13 to -0.31, p=0.02) but not in venules (45.0%±10.3% to 46.7%±12.8%, 95% CI: -5.15 to 1.76, p=0.3). When COPD subjects were on their supplemental oxygen the difference in oxyhemoglobin saturation showed a nonsignificance as compared with the healthy group in retinal arterioles (95% CI: -9.37 to 1.49, p=0.14).

No differences were observed between COPD subjects and healthy controls respectively in vessel diameter of arterioles (106.6±10.6 µm vs.114.2±10.9 µm, 95% CI: -17.06 to 2.00, p=0.11) or venules (147.7±14.1 µm vs. 153.4±15.1 µm, 95% CI: -24.28 to 12.85, p=0.51).

4.3.2 COPD subjects under experimental protocol

After termination of inspiring supplemental oxygen and 10 minutes of ambient air breathing, the oxyhemoglobin saturation in retinal arterioles and finger oximetry reading decreased considerably (**Table 8**). Inhalation of supplemental oxygen for 20 minutes (second baseline period) returned the oxyhemoglobin saturation in retinal arterioles and finger nearly to the values at first baseline. Cessation or reapplication of supplemental oxygen breathing neither significantly affected the retinal venule oxyhemoglobin saturation, AV-difference,

nor retinal vessel diameter. No significant differences were found between oximetric measurements at first and second baseline.

Table 8 Comparison of oxyhemoglobin saturation (%) between retinal vessels, finger pulse oximetry and radial artery blood with and without oxygen therapy in 10 patients with severe COPD. Arteriole and venule oxyhemoglobin saturation difference and diameters are also shown.

n=10	Oxygen therapy First baseline	Ambient Air 10 minutes	Oxygen therapy Recovery Second baseline	95% CI of difference
Retinal arterioles	91.0 ± 4.5	87.5 ± 5.1	90.0 ± 5.9	* 1.03 to 6.05[a] * -5.09 to -0.07[b]
Finger pulse oximetry	93.7 ± 3.6	90.6 ± 2.8	94.7 ± 2.5	* 0.14 to 6.05 [a] * -7.05 to -1.14[b]
Radial artery	—	92.5 ± 3.6[1]	—	
Retinal venules	47.6 ± 12.7	45.6±10.6	46.5 ± 13.5	ns -2.13 to 6.11[a] ns -4.96 to 3.28[b]
AV-difference	43.4 ± 10.6	41.8 ± 8.3	43.5 ± 9.6	ns -2.68 to 5.79[a] ns -5.98 to 2.49[b]
Arteriolar diameter	107.8± 18.4	104.7± 8.7	102.6±10.1	ns -4.26 to 10.63[a] ns -5.42 to 9.47 [b]
Venular diameter	142.7± 19.2	147.3± 14.8	143.5±17.5	ns -13.10 to 3.85[a] ns -4.66 to 12.29[b]

[a] Oxygen therapy at first baseline versus ambient air breathing for 10 minutes; [b] Ambient air breathing versus oxygen therapy at second baseline.
Values are mean ± SD. Repeated measures one-way ANOVA and Tukey´s multiple comparison post test. (CI: 95% Confidence interval of the difference). *p < 0.05; ns, non-significant.

The comparison of retinal arteriolar oximetry with finger pulse oximetry and radial artery co-oximetry in the COPD subjects during the ambient air breathing is shown in **Figure 21**.

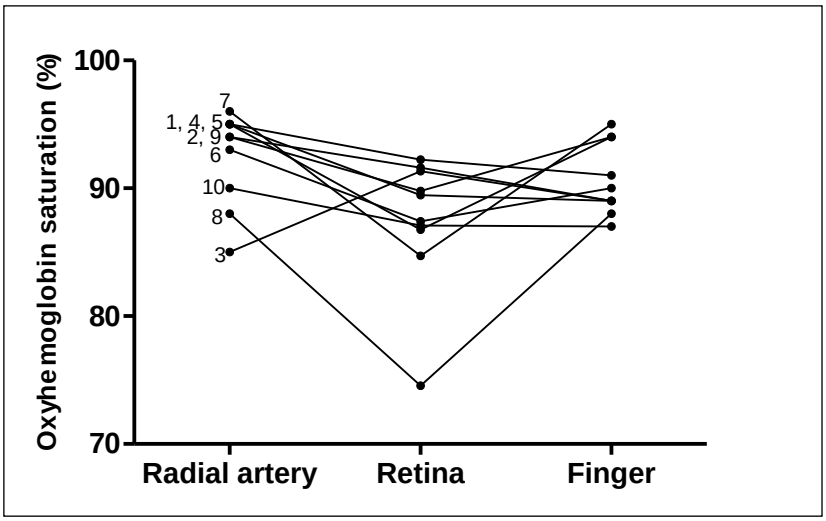


Figure 21. Comparison of 10 individual COPD subjects´ oxyhemoglobin saturation in retinal arterioles, radial artery blood sample and finger after 10 minutes of ambient air breathing. The mean oxyhemoglobin saturation in retinal arterioles was 87.5±5.1% compared to 92.5±3.6% in the radial artery (95% CI: -8.65 to -1.35, $p<0.05$) and 90.6±2.8% in the finger (95% CI: -6.75 to 0.54, $p >0.05$). Each number indicates a COPD subject with reference to **Table 9**. Each data point represents mean oxyhemoglobin saturation (%) in a single COPD subject. Repeated measures one-way ANOVA and Dunnett´s multiple comparison post test.

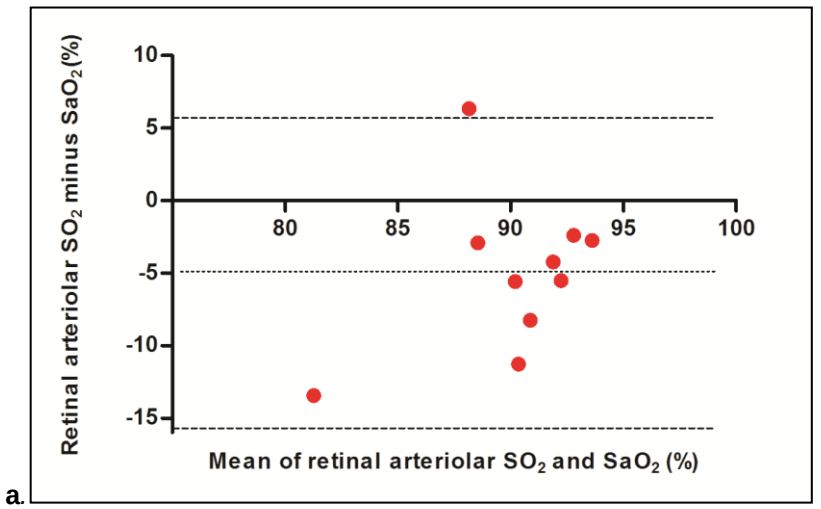
Individual radial artery blood gas measurements are presented in **Table 9**. For the COPD subjects as a group the mean arterial blood gas PaO_2 was 61.3±10.5mm Hg and PCO_2 it was 42.2%±5.9 mm Hg. The mean value for bicarbonate was 26.4 ±3.4 mEq/L, and the pH 7.4±0.0.

Table 9 Selected characteristics of the COPD patients: Arterial blood gas analysis, retinal arteriolar oxyhemoglobin saturation and finger pulse oximetry.

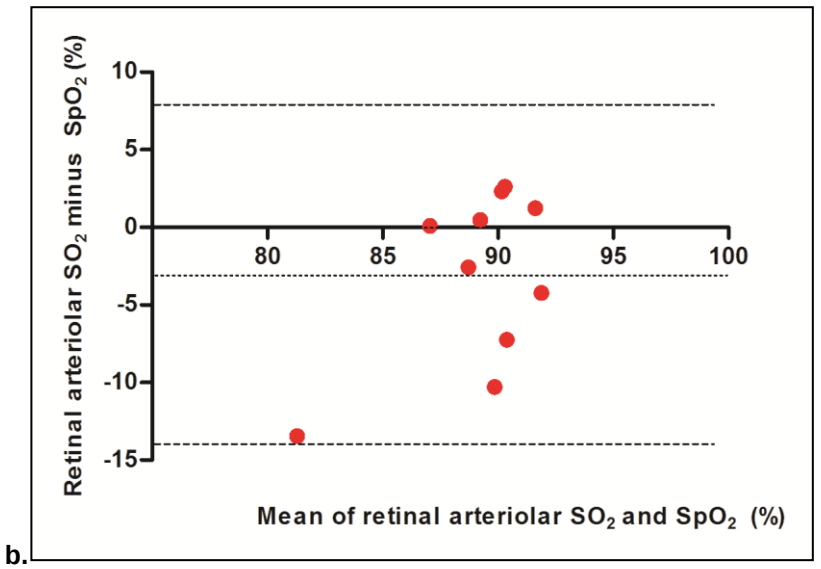
Subject	Age	SaO_2	Retina	SpO_2	PaO_2	PCO_2	pH	bicarb	O_2oxyhgl
1	68	95.0	92.2	91.0	64	43	7.43	28	92
2	64	94.0	89.8	94.0	68	43	7.39	25	92
3	71	85.0	91.3	89.0	42	52	7.44	33	84
4	67	95.0	86.7	94.0	78	38	7.37	22	94
5	77	95.0	89.5	89.0	60	40	7.45	27	94
6	76	93.0	87.4	90.0	69	49	7.4	29	92
7	68	96.0	84.7	95.0	66	42	7.39	25	95
8	66	88.0	74.5	88.0	55	43	7. 43	27	86
9	82	94.0	91.6	89.0	63	42	7.43	27	92
10	68	90.0	87.0	87.0	53	30	7.41	21	90

SaO_2, arterial oxyhemoglobin saturation; Retina, arterial oxyhemoglobin saturation; SpO_2, finger pulse oximetry; PaO_2, mean partial pressure of oxygen; $PaCO_2$, mean partial pressure of carbon dioxide; Bicarb, bicarbonate; O_2oxyhgl, oxyhemoglobin.

Bland Altman plots compared retinal arteriolar oximetric oxyhemoglobin saturation values with radial artery blood gas and finger pulse oximetry under ambient air breathing. Radial artery blood gas measurement and finger pulse oximetry revealed a bias and limit of agreement of -3.1±5.5; 95% CI: -14.05 to 7.84 and -5.0±5.4; 95% CI: -15.68 to 5.67 respectively (**Figure 22** a and b).



a.



b.

Figure 22. Bland Altman plots comparing arteriole oxyhemoglobin saturation values of retinal oximetry with a) peripheral pulse oximetry from finger and b) radial artery blood during ambient air breathing in 10 patients with systemic hypoxemia secondary to severe COPD. Dotted lines indicate mean difference between measurements and dash lines indicate 95% limits of agreement. SO_2, retinal arteriole oxyhemoglobin saturation; SpO_2, finger pulse oximetry; SaO_2, Radial artery oxyhemoglobin saturation.

4.4 Retinal oximetry in neonates with SLO (Paper IV)

The mean ODR of 0.256 ± 0.041 for arterioles and 0.421 ± 0.089 for venules is considerably different (n= 28, p<0.001, paired t test). The median values were 0.255 (range 0.150–0.337) and 0.409 (range 0.268–0.626) respectively. The average vessel diameter measured 14.1 ± 2.7 pixels for arterioles and 19.7 ± 3.7 pixels for venules (n = 28, p<0.001).

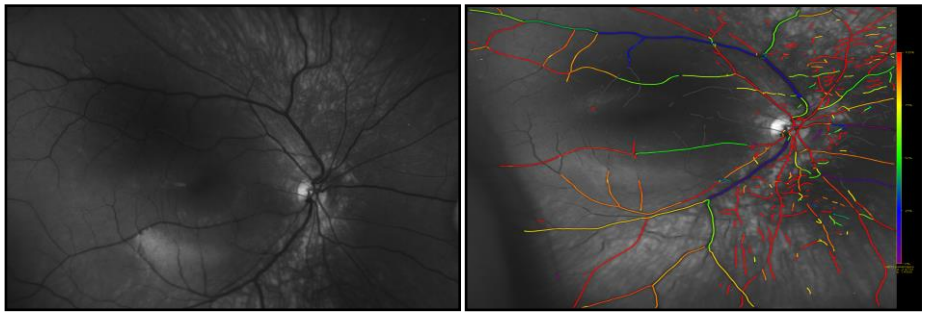


Figure 23 Retinal oximetry image of an infant (left) without and (right) with a pseudocolor overlay.

5 Discussion

The outcome of this thesis suggest that oxyhemoglobin saturation of the systemic circulation can be measured through the retinal circulation. The Oxymap T1 retinal oximeter is shown to be sensitive to the changes in oxyhemoglobin saturation with hypoxemia and systemic hyperoxia. In the following discussion the main focus is on the clinical studies in the adult subjects (paper I-III) followed by a brief deliberation on the results of the neonatal study (paper IV). The neonatal study gives indications that the retinal oximetry may also be applicable to newborns when combined with scanning laser ophthalmoscope.

The major findings of each study will be discussed independently before concluding on the results.

5.1 Retinal oximetry in CRVO patients (Paper I)

The retinal oximetry study on CRVO patients was carried out to test if the retinal oximeter is sensitive to retinal tissue hypoxia. Central retinal vein occlusion creates obstruction to the venous outflow with disturbances against blood flow and retinal tissue hypoxia to a variable degree. Earlier oximetry studies on CRVO were restricted to 20 degree field of view images on the fundus. The Oxymap T1 oximeter that was used in this thesis yield 50-degree field of view and thereby wider fundus images than previous studies for estimation of oxyhemoglobin saturation of retinal vessels in CRVO. Following up on patients with repeated oximetry imaging provides a novel insight on the changes of oxyhemoglobin saturation with the course of the CRVO disease over time.

Earlier studies on CRVO patients with the older versions of the retinal oximeter had revealed markedly lower oxyhemoglobin saturation of retinal venules in CRVO affected eyes than in fellow unaffected eyes (Hardarson & Stefánsson, 2010; Traustason et al., 2014). Our results of lower mean oxyhemoglobin saturation of 31±12% in CRVO eyes as compared with 52±11% in the fellow eyes verify these earlier findings. The results are also in agreement with the semi-quantitative findings reported by Yoneya and associates (2002) of retinal tissue hypoxia in CRVO affected human eyes.

The retinal oximeter captured local hypoxic areas to a variable degree in CRVO affected eyes. The oxyhemoglobin saturation in some of the venules was 6% which is equivalent to the average PO_2 of 8 mmHg previously measured with invasive oxygen probe at the retinal surface in patients with CRVO (Williamson et al., 2009). Animal studies have shown that the preretinal PO_2 is overall a good indicator of the intraretinal PO_2 because the retinal circulation is the main oxygen source for the vitreous (Wangsa-Wirawan & Linsenmeier, 2003). The difference between mean retinal oximetry values and preretinal findings can at least be partially explained by different methods used in calculating their values; at one hand, invasive PO_2 values are determined by closeness of the oxygen probe to blood vessels across the retinal surface. On the other hand, retinal venous oxyhemoglobin saturation is the weighted average of all venular segments that are analysed in the pertaining eye.

Although calculations of PO_2 from the hemoglobin dissociation curve holds for retinal vessels only, the mid-vitreous cavity PO_2 of 19.8 mmHg that had previously been measured in CRVO eyes (Williamson et al., 2009) matches our findings of mean oxyhemoglobin saturation of 31% in CRVO affected eyes. In healthy cats an uniformity has been found between retinal venous PO_2 and mid-vitreous PO_2 (Alder & Cringle, 1985) that becomes disruptured under pathological condition like diabetic retinopathy (Linsenmeier et al., 1998). This is probably the case in human CRVO affected eyes because the direction of oxygen gradient is reversed and the vitreous may indeed provide oxygen support for the hypoxic retina instead (Williamson et al., 2009).

Our hypoxic measures in obstructed venules are also in agreement with invasive measures on preretinal PO_2 of BRVO areas in pigs, miniature pigs (Noergaard et al., 2008; Pournaras, Petropoulos, Munoz, & Pournaras, 2004; Pournaras, Tsacopoulos, Strommer, Gilodi, & Leuenberger, 1990) and cats (Stefánsson, Novack, & Hatchell, 1990). In addition, our mean oxyhemoglobin saturation findings of 52% in the fellow unaffected eyes and 55.6% in healthy subjects (Geirsdottir et al., 2012) do convert into 28 and 29 mmHg PO_2 respectively that fit nicely to the preretinal PO_2 of pigmented Long-Evans rats (Lau & Linsenmeier, 2012).

5.1.1 Effects of the venous occlusion on the oxygen extraction

The retinal tissue has higher oxygen extraction under normal condition (8ml per 100 ml blood) with lower retinal venous and tissue PO_2 than most other tissues in the body (Wangsa-Wirawan & Linsenmeier, 2003). The subnormal venous oxyhemoglobin saturation in CRVO eyes indicate the effects of the

venous obstruction on the retinal circulatory closed circuit, where the venous pressure is increased and the perfusion pressure decreased. Consequently, more oxygen is extracted per volume as the sluggish blood flow moves through the microcirculatory network. The PO_2 differences create a higher gradient for oxygen to diffuse from the retinal capillaries into the hypoxic tissue, to be consumed in the oxidative synthesis of ATP for cellular metabolism. Moreover, the obstruction of the retinal circulation will reduce the clearance rate of H^+ and most likely induce H^+ accumulation from the lactate production in the anaerobic glycolysis as well (Birol, Budzynski, Wangsa-Wirawan, & Linsenmeier, 2005; Nielsen, Madsen, Svendsen, Roach, & Secher, 1998). Consequently, the elevated H^+ concentration and acidification of the inner retina shifts the oxyhemoglobin dissociation curve further to the right. As the oxygen´s affinity for hemoglobin decrease more oxygen is unloaded from retinal capillaries to the hypoxic tissue that adding to the subnormal venous oxyhemoglobin saturation as shown by the retinal oximetry results. The increased AV-difference in CRVO is ultimately a biomarker of acute retinal tissue hypoxia that might become reversible with timely restoration of oxygen supply for the retinal tissue oxygen demand.

5.1.2 Increased AV-difference in CRVO

The enlarged AV-difference in CRVO eyes as compared with opposite unaffected eyes is in line with previous findings of Hardarson & Stefánsson (2010) and Traustason et al., (2014). The mean AV-difference is bigger in fellow unaffected eyes (43±7%) of CRVO patients than reported on healthy people (36.7±5.4%) with healthy eyes (Geirsdottir et al., 2012). The reason is unclear but may partially be explained by lack of control for confounding variables whereas the healthy group is younger (median age 47 years) and systemic risk factors in the CRVO subjects are not controlled for in this study. Some of the risk factors may indeed lower the oxyhemoglobin saturation of retinal venules. A possible explanation could also be if the blood flow is slower in the fellow unaffected eyes of people with CRVO than of healthy subjects with healthy eyes. We did not quantify the retinal blood flow so this speculation remains to be elucidated.

5.1.3 Intra- and inter individual variability

The retinal venous oxyhemoglobin saturation is variable within each eye and between individual eyes as shown by the standard deviation. The inconsistency is markedly greater in eyes with CRVO than in unaffected fellow eyes. The variability in the oxyhemoglobin saturation is shown by the colour

coded map on the oximetry images, changing from normoxic green to bluish and purple, determined by the hypoxic state of the inner retina. This wide range of intra- and inter individual oxyhemoglobin saturation of retinal venules is similar to those previously published by Hardarson & Stefánsson (2012). The variability most certainly signifies the individual distinction of the magnitude in retinal venous obstruction that is determined by the location (Hayreh, 2005) and the various reparatory mechanisms engaged against the occlusion. Collateral pathways for establishment of venous drainage (Hayreh et al., 2011; Paques & Gaudric, 2002; Takahashi, Muraoka, Kishi, & Shimizu, 1998) is undertaken to variable degree and progressive channelizing of the thrombus may start within days or weeks from the initial onset of the occlusion (Green et al., 1981), potentially contributing to the retinal oximetry and clinical improvement over course of the disease.

5.1.4 Vessel diameter

Retinal venules in the CRVO affected eyes are characterized by vessel dilation and venous tortuosity most likely due to elevated intraluminal pressure (Kristinsson, Gottfredsdottir, & Stefánsson, 1997), resulting from back up pressure because of the obstruction to the central venous outflow. The mean difference of venular diameter by only one pixel is probably a matter of accuracy from resolution of measurements. In some CRVO patients the mean vessel diameter is wider in the unaffected fellow eye than the CRVO eye. Some of the patients have comorbidites that are known to influence the retinal vessel calibre and may contribute to increased venous diameter and tortuosity of the fellow unaffected eye.

5.1.5 Retinal arterioles

The oxyhemoglobin saturation in retinal arterioles is unaffected by the CRVO, as compared with the fellow control eye. The finding is in agreement with the previous finding by Hardarson & Stefánsson (2010) but Traustason et al., (2014) found the oxyhemoglobin saturation to be slightly elevated in CRVO affected eyes. They speculated the elevation may in part be explained by technique error since the numbers of very high values were more frequently observed in CRVO eyes than in contralateral eyes. In this thesis, the extremes of intra- and inter individual variability is similar between CRVO eyes and unaffected control eyes.

In arterioles, the mean vessel diameter was narrower in CRVO eyes than in unaffected eyes. The reason for the unchanged arteriolar oxyhemoglobin saturation in CRVO eyes and narrowness of the vessel lumen is unclear.

Whether it is of technological origin or perhaps a natural course of the disease remains to be answered. This latest version of the Oxymap T1 software automatically correct for any artifactual changes in the oxyhemoglobin saturation derived from widening or narrowing of the vascular diameter. As such, the calculation of oxyhemoglobin saturation of the inner retina should be unaffected by the vascular width of both retinal arterioles and venules. Ultimately, reduced retinal arteriolar calibre in CRVO eyes points toward diminished oxygen delivery of the inner retina secondary to reduced blood flow, winding-up with higher oxygen extraction and increased arteriovenous oxygen difference than in the fellow unaffected eyes.

5.1.6 Follow-up on patients over time

The retinal oximeter detects temporal and topographical variance of oxyhemoglobin saturation of the retinal circulation over time. By following up on patients it become evident that venous oxyhemoglobin saturation is lowest at first and improves with time and treatment as had previously been reported by Traustason et al., (2014). It also appears that even though clinical signs and symptoms resolve, the venous oxyhemoglobin saturation remain lower than normal. As was previously discussed, different compensatory mechanisms against the vein occlusion are expected to be underlying the improved oxyhemoglobin saturation. Increased oxyhemoglobin saturation is a sign of better balance between oxygen supply and demand of the retinal tissue despite of the impending hypoxic injury secondary to CRVO.

5.1.7 Limitations of the retinal oximetry study on CRVO

The study has several limitations some of which have already been discussed. Confounding variables were not controlled for and therefore systemic co-existing diseases may have influenced the resultant differences between the CRVO eye and fellow control eye. The subject group is small and control group for the unaffected eye is lacking. Affiliated blood flow measures were not performed and hence its correlation with variability of venous oxyhemoglobin saturations within and between eyes is based on assumptions. However, one patient underwent fluorescein angiography that revealed capillary non-perfusion that was consistent with hypoxic areas on retinal oximetry images. Although a certain measures were taken to avoid technical limitations they could not be entirely avoided and some of the images were of poor quality. Increased age and some systemic (and ocular) diseases are known to alter the media transparency. Cataract is known to alter lens morphology and thus, creating more artificial scattering of light which affects the oxyhemoglobin

saturation (Patel, Hudson, Flanagan, & Heitmar, 2013) and lower measurements of venules (Hardarson et al., 2015). Despite the abovementioned limitations, spectrophotometric retinal oximetry enables analysis of oxyhemoglobin saturation in the inner retinal vasculature and our estimation of oxygen levels in CRVO seems realistically accurate. In the future, study on larger patient group with CRVO is needed in order to investigate the impact of the disease on retinal vessel oxyhemoglobin saturation and to observe the natural course of the disease and the effectiveness of treatment over time.

5.2 Retinal oximetry in healthy under hyperoxemia (Paper II)

The retinal oximetry study on healthy subjects was performed to test if the retinal oximeter is sensitive to hyperoxic changes of the retinal circulation. Inhalation of high oxygen concentration elevates the systemic arterial oxygen content and optimizes the oxyhemoglobin saturation. The increased oxygen delivery is supposedly reflected in increased oxyhemoglobin saturation of retinal vessels by retinal oximeter measurements.

The retinal oximeter calculates stable oxyhemoglobin saturation level at baseline. System hyperoxia elevates the oxyhemoglobin saturation and narrows the vascular lumen of both retinal arterioles and venules. These changes are more pronounced on the venous site than the arteriolar site of the retinal circulation. Systemic hyperoxia markedly lessen the AV-difference. Retinal oximeter measurements demonstrate lower oxyhemoglobin saturation values than finger oximetry during both ambient air and supplemental oxygen breathing.

5.2.1 Choroidal interaction on the inner retina during hyperoxia

Increased oxyhemoglobin saturation of retinal arterioles and venules is in agreement with recent reports (Palkovits, Lasta, et al., 2014; Werkmeister et al., 2015) on the ability of retinal oximetry to recognize those systemic changes of oxyhemoglobin saturation. Continual retinal oximetry imaging reveals prompt retinal circulatory recovery when supplemental oxygen is halted. Utilizing rebreathing circuit made possible to induce isocapnic hyperoxia and thus, the vasoconstriction of both retinal arterioles and venules is a marker of the effective autoregulatory response from the hyperoxic provocation. The pronounced elevation of venous oxyhemoglobin saturation supports the former belief which is based on invasive PO_2 animal studies (Linsenmeier & Yancey, 1989; Pournaras, Riva, Tsacopoulos, & Strommer, 1989), that the high

systemic oxygen concentration causes oxygen to flux from the poorly regulated choroidal circulation to the innermost layers of the retinal tissue. It is likely that the influx contributes to the metabolic need of the inner retinal tissue and some oxygen molecules end up in the retinal veins as well. Because the retinal vessel diameter is reduced and the blood flow is known to diminish under hyperoxic condition (Palkovits, Lasta, et al., 2014; Werkmeister et al., 2015), the concomitant decrease of oxygen extraction from the retinal circulation is the proposed mechanism behind the reduced AV-difference seen in our results.

5.2.2 Retinal oximetry readings during acute hyperoxia

Both retinal oximetry and finger pulse oximetry measured considerable elevated oxyhemoglobin saturation from baseline with supplemental oxygen breathing. Retinal oximeter measurements display relatively lower average oxyhemoglobin saturation values than peripheral pulse oximeter measurements, both at a baseline and during system hyperoxia. It would be expected to see the oxyhemoglobin binding sites of retinal arterioles nearly fully saturated during system hyperoxia so the results are somewhat perplexing and the reason for the disparity between the two methods is unclear. The causation can be twofold, either technical or physiological, and both; The former reason is based on the fact that the calibration factor of the instrument is derived from laboratory values that were calibrated in vivo and are somewhat lower than given values in the systemic circulation (Gyton, 2000). Palkovits et al., (2014) got similar results on induced systemic hyperoxia, using the Imedos system that utilizes the same calibration factor as ours. The latter reason is probable, due to countercurrent exchange between the central retinal artery and the central retinal vein as they run adjacent each other within the optic nerve.

5.2.3 Limitations of oximetry study on healthy subjects

Measurements on arteriolar oxyhemoglobin saturation with the retinal oximeter give lower values than expected during systemic hyperoxia. This is most likely due to the above mentioned biological countercurrent exchange mechanism but some calibration issues of the instrument can not be ruled out.

The Oxymap T1 retinal oximeter quantifies retinal oxyhemoglobin saturation based on calibration factors presuming that average oxyhemoglobin saturation in healthy individuals is 92.2% for arterioles and 57.9% for venules. These reference values were initially attained with a laboratory oximeter, which was calibrated in vitro and are somewhat lower than quoted normal values in

the systemic circulation (Guyton, 2000). Inhalation of 100% oxygen by facemask (FiO_2 of approximately 96%) in healthy subjects in this thesis, increase the retinal arteriolar oxyhemoglobin saturation to 94.5±3.8 and 76.2±8.0% in venules which is closer to normal mixed venous oxyhemoglobin saturation during ambient air breathing at sea level. Hence, the calibration factor may be accountable for the lower retinal arteriolar oxyhemoglobin saturation than expected. The notion of lower estimated values during systemic hyperoxia than expected is further supported by the findings of Palkovits and associates (2014) with their arteriolar oxyhemoglobin saturation results of 96.4±3.1%. Of interest, their retinal oximeter (Imedos) is based on the same calibration factor as the Oxymap T1 instrument. They speculated the reason for not reaching oxyhemoglobin saurian of 100% would most likely be due to the calibration or the countercurrent exchange of oxygen within the optic nerve.

Although it may be speculated, the third reason for lower values than expected during hyperoxic breathing, stem from study subjects removing the oxygen facemask whilst the oximetry images were obtained, it is highly unlikely; First, the inspired and expired oxygen concentration had reach equilibrium (EtO_2 91±4%) before oximetry images were obtained. Second, the average time for oximetry imaging was fast, only about 30 seconds. Third, study subjects did not inhale during the imaging but exhaled slowly if needed.

Ultimately, the lower oxyhemoglobin saturation values obtained by the Oxymap T1 retinal oximeter than expected, give reason for its calibration to be reconsidered and to contemplate the countercurrent effects of the retinal circulation.

5.3 Retinal oximetry in COPD patients (Paper III)

The retinal oximetry study on patients with severe COPD was conducted to test if the retinal oximeter is sensitive to systemic hypoxemia. COPD is a systemic disease, characterized by hypoxia and inflammatory response that is known to negatively affect tissues and organ systems of the body. In patients with severe COPD the systemic hypoxemia is expected to be captured with retinal oximeter measurements. The daily supplemental oxygen therapy improves oxygenation and thus should be reflected in increased oxyhemoglobin saturation of the retinal vessels.

The retinal circulation offer direct non-invasive assessment of the central nervous circulation and thus, the systemic circulation. Spectrophotometric retinal oximetry is hypothetically at least as good indicator of the systemic

oxyhemoglobin saturation as invasive radial artery blood sample measurements and peripheral finger phlethysmography.

5.3.1 COPD subjects compared to healthy controls

Retinal oximetry captures systemic hypoxemia in both retinal arterioles and venules in people with severe COPD. During ambient air breathing the oxyhemoglobin is substantially lower in both retinal arterioles and venules than of healthy controls. When COPD subjects inspire their prescribed oxygen therapy the arterial oxyhemoglobin saturation shows trend toward that of the healthy controls. The tendency must however be interpreted with caution since the number of the study group is small (n=11). The AV-difference in COPD subjects is greater than in the control group and no considerable changes are observed when they are exposed to different breathing regimens during the experimental protocol. The mean vessel diameter of both retinal arterioles and venules is slightly narrower in COPD patients but statistically non-significant. The variance of vascular width between vessels segments (standard deviation) both within eyes and between eyes is similar to that healthy of controls.

5.3.2 Comparison with other studies

The improved arteriolar oxyhemoglobin saturation and unaltered AV-difference is in agreement with recent publication of similar patient group by Palkovits and associates (2013) using the Imedos retinal device (n=15). In their study the arteriolar oxyhemoglobin saturation during supplemental oxygen breathing was 92.2±4.6% and decreased by 2.1 percent points during ambient air breathing. The AV-difference was close to 25±5% under both breathing conditions. In contrast to our findings, they reported the venous oxyhemoglobin saturation of 67.6±7.8% during the supplemental oxygen breathing to be higher than in their healthy subject group. They also found a tendency (p=0.05) of decreased venous oxyhemoglobin saturation with cessation of oxygen breathing whereas we did not acquire statistical significance. It is important however, to keep in mind that both studies represent small groups of COPD subjects and therefore future studies should include more patients in order to enhance the capability to detect any latent changes therein.

Results on oxyhemoglobin saturation of retinal arterioles and venules in our COPD subjects match to the findings on patients with congenital system hypoxemia secondary to Eisenmenger syndrome (Traustason et al., 2011), using the same instrument but an older version. Those patients are shown to have abnormally low oxyhemoglobin saturation in retinal arterioles or 81±9% and in venules, or 44±12% when breathing ambient air. These values are

similar to our findings but information on measurements during supplemental oxygen breathing is not available.

5.3.3 COPD subjects under the experimental protocol

All COPD patients in this thesis were hemodynamically stable throughout the study period. In consonance with retinal oximeter measurements, all COPD participants suffered from moderate to severe hypoxemia during the ambient air breathing as manifested by the subnormal PO_2 arterial blood gas analysis. Two COPD patients were CO_2 retainers and all pH values were within normal limits. The bicarbonate level was mildly elevated in few of the cases, confirming the chronic hypoxia is compensated for. The unchanged AV-difference obtained by the retinal oximeter is indicative of the retinal tissue metabolic compensatory response to the chronic hypoxia.

5.3.4 Effects of chronic systemic hypoxemia on the inner retina

Systemic arterial hypoxemia is a limiting factor in the cellular metabolism of the inner retina as evident by the reduced venous oxyhemoglobin saturation. The reduction in mean arteriole and venules oxyhemoglobin saturation in patients with severe COPD inspiring ambient air indicates that they experience inadequate oxygen delivery to the inner retina and thus, hypoxemic conditions of the tissue.

The retina has a high oxygen demand and local blood vessel autoregulatory mechanisms maintain the oxygen concentration of the inner retinal tissue at relatively stable levels with a hypoxic vasodilatory threshold to decreased PaO_2 similar in both the retinal (Cheng et al., 2016) and cerebral circulations (Gupta et al., 1997; Kety & Schmidt, 1948). According to the Fick´s principle, the circulatory response to arterial hypoxemia is double; additional capillaries are recruited to enlarge the interface for oxygen exchange and shortening the diffusion distance. Secondly, the vascular resistance is reduced by arteriolar vasodilatation for enhanced oxygen delivery and tissue perfusion (Pittman, 2011a). The increase in vascular caliber is evident in both retinal arterioles and venules under induced acute systemic hypoxemia in healthy people (Palkovits, Lasta, et al., 2014). In our COPD study subjects however, the vasodilatatory response is seemingly lost. In fact the vessel lumen of both retinal arterioles and venules is slightly narrower as compared with the control group, both under the ambient air and supplemental oxygen breathing conditions.

5.3.5 Retinal adaption to chronic systemic hypoxemia

Absence of the inner retina vasodilatory response is most likely caused by long term retinal adaption to the system hypoxemic condition. Animal studies have shown the initial cerebral blood flow augmentation is attenuated by increased capillary density and oxygen carrying capacity of the blood (Boero, Ascher, Arregui, Rovainen, & Woolsey, 1999; Dunn et al., 2004) over time. Although studies on cerebral blood flow in COPD patients are lacking, most human studies on acclimatation to high altitude point toward unchanged blood flow over time, hypothetically by offset of the initially hypoxic vasodilatory response. Progressive ventilatory adjustment counteracts the initial rise in blood flow along with increased release of local factors and endothelium-derived vasoconstrictors and elevated sympathetic nervous system activity. These factors are likely to pertain to the unchanged cerebral blood flow that has been found in COPD patients (Ainslie & Ogoh, 2010) and hypothetically to the long term retinal circulatory adjustment as well.

It is known that the systemic inflammatory response of COPD involves aberration of the systemic vascular function although, the role of the endothelial-dependent and endothelium-independent mechanism for the impaired vasodilatation is of controversy (Eickhoff et al., 2008; Maclay et al., 2009). This vascular dysfunction including arterial stiffness (Maclay et al., 2009) is expected to be the cause for retinal vasoconstriction in people with severe COPD. However, retinal blood flow measurements and clarification on the mechanism behind the retinal circulatory hypoxic adjustment remains to be elucidated for future studies.

Some studies have supported evidence of both functional and structural changes of the retina in stable COPD patients. As already mentioned, the system inflammatory response and chronic hypoxemia are believed to provoke pathological changes on the ocular vasculature underlying those retinal changes. The average subfoveal choroidal thickness and peripapillary retinal nerve fiber layer (RNFL) thickness are found to be thinner than in healthy controls (Ozcimen et al., 2016; Ugurlu et al., 2016). Such degenerative tissue changes are indicative of the detrimental effects of the hypoxemia (Kergoat et al., 2006) on RNFL and consequent ganglion cells death. Seemingly, the retinal circulation is unable to compensate for the metabolic changes that underlie these structural damages (Kergoat et al., 2006; Ozcimen et al., 2016). Latencies of visual evoked potentials and amplitude anomalies have also been reported (Demir et al., 2012; Ozge et al., 2005) along with defect on the visual

field, which implies the neuropathological aspect of mild to moderate hypoxemia on the retina and the optic nerve itself (Demir et al., 2012).

5.3.6 Subnormal retinal venous oxyhemoglobin saturation

A hypoxic effect on the retinal tissue metabolism is demonstrated by the reduced average retinal venous oxyhemoglobin saturation which indicates increased oxygen extraction by the retinal tissue. The intra- and inter eye variability of those hypoxic effects are demonstrated by the standard deviation. Increased CO_2 production coupled with elevated H^+ production and reduced pH in the retinal tissue, shifts the oxyhemoglobin dissociation curve to the right which facilitates dissociation of the oxygen molecule from the hemoglobin binding site into the cell. Subsequently, a higher oxygen fraction is removed from retinal capillaries and the oxyhemoglobin saturation on the venous site becomes abnormally low in patients with severe COPD.

The unchanged AV-difference during cessation and reapplication of the supplemental oxygen therapy suggests an unaltered mitochondrial consumption for ATP production of the hypoxic inner retinal tissue in patients with severe COPD. Since the autoregulatory response of the choroidal circulation to changes in PaO_2 is minimal, the oxygen influx from choriocapillaries declines linearly across the outer retinal tissue under systemic hypoxemia (Pournaras et al., 2008). Under normal conditions, increased photoreceptors metabolic activity already demands some additional oxygen flux from the retinal circulation (Linsenmeier & Braun, 1992) to the retinal outer segments. In people with severe COPD, the chronic systemic hypoxemia of the outer segments most likely constantly demands the inner retinal circulation to contribute oxygen for the energy consuming photoreceptor activity. This will exceed the capacity of the already hypoxemic retinal circulation for oxygen contribution as manifested by low venous oxyhemoglobin saturation and increased AV-difference from normal, eventually leading to functional and structural changes of the retina in stable COPD patients that was mentioned above.

5.3.7 Effects of prescribed oxygen on retina in COPD

Supplemental oxygen therapy improves global oxygen delivery and consequently the oxyhemoglobin saturation of retinal arterioles and venules as shown in the COPD subjects group. A high FiO_2 augments the oxygen influx from choriocapillaries, not only to the outer retina but to the inner retina as well as is shown in the hyperoxia study of this thesis (Olafsdottir, Eliasdottir, Kristjansdottir, Hardarson, & Stefánsson, 2015). Although the effect of

supplemental oxygen on retinal venous oxyhemoglobin saturation is not statistically significant, it shows improvement. Inference of posteriori power analysis implicates that the lack of significance may be owed to inadequate power to detect a difference of 5.5% in oxyhemoglobin saturation (power 90%, p=0.05). Nonetheless, retinal oximetry implicates local metabolic changes associated with systemic hypoxemia in people with COPD. Those metabolic changes are probably causative for the retinal neuropathological changes that have started to evolve around the systemic effects on the retina in COPD.

5.3.8 Retinal oximetry compared with radial artery blood and finger pulse oximetry

Despite of some intra-individual variations, retinal arteriole oxyhemoglobin saturation values were in general lower than those measured from radial artery blood samples and with finger pulse oximetry. The Bland Altman plots illustrate the tendency of retinal oximetry to produce lower oxyhemoglobin saturation measures but show a fair agreement with the other two modalities. Bland Altman plot of retinal oximetry and arterial blood sample oxyhemoglobin values indicate a degree of bias with three retinal oximetry outliers contributing substantially to the width of the limits of agreement. Nevertheless, the calculated variability implicates fair agreement between the radial artery blood sample and retinal oximetry measurements of oxyhemoglobin saturation. It should be kept in mind that estimated oxyhemoglobin saturation in arterial blood is quantified from normal values of PaO_2 and pH and standard oxyhemoglobin dissociation curve based on probable oxygen-hemoglobin affinity and 2,3 DPG concentration. Therefore the radial artery oxyhemoglobin saturation should be interpreted with care in the presence of pathology (Haymond, 2006).

A Bland Altman plot of the finger and the retinal oximetry oxyhemoglobin saturation values also show some difference between the two modalities. The plot illustrates the tendency of retinal oximetry to measure lower oxyhemoglobin saturation values but, like with radial artery blood sample, demonstrated fair agreement between those two techniques.

The lower retinal arteriole values acquired by retinal oximetry could simply be due to calibration of the device. If the difference is real however, a likely reason for lower retinal arterial oxyhemoglobin saturation is that the central retinal artery and vein lie adjacent to each other (about 1cm) within the optic nerve where the utmost oxygen countercurrent exchange may occur between the artery and vein. This could result in somewhat lower oxyhemoglobin saturation in retinal arterioles compared with the larger arteries. In addition,

one possible reason is that, the fairly small retinal arterioles that are measured, loose more oxygen through their vessel walls by diffusion than arteries measured in the finger or the wrist. Retinal measurements are made on vessel segments that stretch for some length into the retina whereas measurements in the finger and the wrist are confined. Moreover, the retinal oximetry calculation is based on average measurements of multiple vessel segments of very metabolically active tissue of the eye. Peripheral radial artery and finger calculation however, represent single measure of oxygen delivery to a tissue that is not that metabolically active.

5.3.9 Validity of retinal oximetry in systemic hypoxemia

In humans, noninvasive Spectrophotometric retinal oximetry has shown sensitivity to alteration in oxyhemoglobin saturation in people with systemic hypoxemia secondary to Eisenmenger syndrome (Traustason et al., 2011) and severe COPD (Palkovits et al., 2013). These retinal measures significantly correlated with earlobe capillary blood (Palkovits et al., 2013), femoral artery (Traustason et al., 2011) and finger pulse oximeter measurements (Palkovits et al., 2013; Traustason et al., 2011). Significant correlation has also been revealed between retinal vessel oxyhemoglobin saturation and finger pulse oximetry during induced hypoxemia in healthy people (Palkovits, Told, Schmidl, et al., 2014). Moreover, the sensitivity of the Oxymap retinal oximeter has been confirmed in pigs exposed to acute hypoxemia with a good correlation with the intra-vitreal and femoral artery oxygen content (Traustason et al., 2013). In addition, Denninghoff and associates (1998) used noninvasive low power scanning laser eye oximeter to estimate the sensitivity of retinal venous oxyhemoglobin saturation for early hemorrhage and resuscitations in swine. They reported a good correlation of retinal venous oxyhemoglobin saturation with mixed venous saturation, cardiac output, blood volume (Denninghoff et al., 2003) and the rate of blood loss in which retina demonstrated higher sensitivity for blood loss than conventional vital signs, i.e. blood pressure and heart rate (Denninghoff, Smith, Hillman, Redden, & Rue, 1998). These animal studies of exsanguinations and resuscitation demonstrate the potential of retinal oximetry monitoring during acute hemorrhage whilst the compensatory hemodynamic response is still intact.

5.3.10 Limitations on retinal oximetry in COPD patients

The study on hypoxemia in patients with COPD has some limitation and some have already been discussed previously such as the small number of subjects, which may have resulted in the wide confidence interval. Invasive radial artery

blood gas sample was only obtained after cessation of the prescribed oxygen breathing. Preferably, a radial artery blood sample would also have been drawn for oxyhemoglobin saturation measurement at baseline, when COPD patients were still on their prescribed oxygen therapy. It was however deemed unjustifiable due to its invasive nature including complication risks and local discomfort. As already argued, the calibration factor for the oximeter is based on laboratory values for healthy subject which are lower than the reference normal values in the systemic circulation and call for reassessment on calibration constants of the device. The subjects of this study are older than this reference group and suffer from systemic hypoxemia that probable cause bias on calculated values based on those calibrational constants.

A number of experimental spectrophotometric measurements of retinal arteriolar and venous oxyhemoglobin saturation in human subjects have been carried out. Despite of that, no “normal” gold standard values exist because of the invasive nature of the procedure needed to acquire the essential parameters to establish such normal values *in vivo*. This obstacle is also held accountable for the lack of an absolute margin for retinal vessel hypoxemia. Nevertheless, retinal oximeter measurements give relative values (not absolute) and can give important information with respect to relative and trend oxyhemoglobin saturation in retinal arterioles and venules.

At the present there is little known about the effects of chronic systemic hypoxemia on the central circulation in patients with severe COPD. Most case control studies are conducted on subjects that have less severe health conditions. Additional studies are needed to investigate the impact of acute and chronic systemic hypoxemia on the central circulation in real life situations and retinal oximetry might be a valuable tool in these investigations.

5.4 Retinal oximetry in neonates with SLO (Paper IV)

The retinal oximetry study on neonates was performed to test whether retinal oximetry can be applied to infants by combining SLO with retinal oximetry. At the present retinal oximetry is only performed in adult persons. Extending the technique to pediatrics could be a vital step for managing neonates and preterm babies at the neonates’ intensive care units in the future.

The software algorithm allows for assessment of oxyhemoglobin saturation in retinal vessels by evaluating the relationship between oxyhemoglobin and deoxyhemoglobin in retinal arterioles and venules. Since this is a new technology in children, the initial step is to test whether the method can be

used in neonates and to estimate the optical density ratio for retinal oximetry analysis and future estimation of normal oxyhemoglobin saturation.

Normative data was obtained on the ODR for arterioles and venules in healthy fullterm neonates. The combined SLO and retinal oximetry technique is sensitivity to oxyhemoglobin and deoxyhemoglobin content of the retinal circulation, as indicated by the statistically significant difference between the ODR of arterioles and venules. This is a novel technique on children and the first study to conduct retinal oximetry analysis on neonates. Only one study has been published on the combined SLO and retinal oximetry method. Kristjansdottir and associates measured the ODR of arterioles (0.210) and venules (0.351) in healthy adults and fitted together with the calibrated oxyhemoglobin saturation values obtained in vitro (Schweitzer et al., 1999) and has previously been described in this thesis. By using those mean values of 92.2% for arterioles and 57.9% they defined the calibration constants as: a = -2.4733 and b=1.4388. Subsequently they calculated the mean oxyhemoglobin saturation of the study subjects to be 92±13% for arterioles and 57±12% for venules. The repeatability of the measures as demonstrated by standard deviation of 3.5% for arterioles 4.4% for venules was considered adequate bearing in mind the early development of this technique. The sensitivity of the instrument was confirmed under induced hyperoxic condition (oxygen 100%) and local hypoxia secondary to retinal vein occlusion in the eyes (Kristjansdottir et al., 2014).

In neonates, the ODR was quite variable with the optical density ranging from 0.150 to 0.337 for the arterioles and more pronounced in venules or 0.268 to 0.626. Up to date, there is no background work on appropriate calibration factors for infants and adult calibration constants are considered inapplicable to neonates given the disparity between the ODR of these age groups. More work on calibration is needed before a step can be taken to estimate the oxyhemoglobin saturation of central nervous system circulation in fullterm neonates in the future.

5.4.1 Limitation on the oximetry study in neonates with SLO

Given the fact that retinal oximetry is a novel technique the study on neonates has several limits some of which have already been discussed above. First, additional studies are needed for determination of precise calibrational constants for calculation of oxyhemoglobin saturation values to base on. Secondly, technological advantages are warrant to reduce the inconsistency in measurements of the optical density. That could possibly be achieved by

modification of the software and improving the laser imaging as the existing system is optimized for color images. Currently, the bulk size of the instrument is also a limiting factor for its use whereby the infant is needed to be held in a flying baby position in order to adjust the eye next to the device.

6 Conclusions

This thesis demonstrates the spectrophotometric retinal oximetry is sensitive to local and systemic changes in oxyhemoglobin saturation. The Oxymap T1 retinal oximeter identifies retinal tissue hypoxia and variability of the local hypoxia in people with central retinal vein occlusion. The method is able to detect systemic hyperoxia in healthy subjects and confines the recovery process back to the pre-experimental baseline values. The instrument captures system hypoxemia in patients with severe COPD and is sensitive to changes of their inspired oxygen concentration. Measured retinal oxyhemoglobin saturation values are however shown to be slightly lower than finger pulse oximetry and radial artery blood values. These discrepancies are expected to originate in a countercurrent exchange between the central retinal artery and vein within the optic nerve, where they lie closely together for a centimeter. The countercurrent exchange would lower oxyhemoglobin saturation in retinal arterioles compared with aorta and other central arteries. Calibration issues however, cannot be excluded as a contributing factor to this difference. The reason for the differences in oxyhemoglobin saturation of retinal arterioles and peripheral vascular beds need to be clarified. Reconsideration of the instrument calibration and further studies on larger groups of healthy subjects and patients suffering from systemic hypoxic condition are necessary to address the matter of absolute arterial oxyhemoglobin saturation and retinal arteriolar relative values.

The thesis indicates the ability of retinal oximetry to detect hypoxic metabolic changes of the inner retinal tissue. Retinal oximetry is the only system that allows for non-invasive venous oxyhemoglobin saturation measures. Up to date such measures stipulate invasive catheterization for central- or mixed venous oxygen monitoring in patients who are critically ill.

The study on combined scanning laser ophthalmoscope and retinal oximetry demonstrates the feasibility of that technique in newly born babies. This is the first study on retinal oximetry in neonates and validates its sensitivity to oxyhemoglobin and deoxyhemoglobin in fullterm neonates. The primary task with this novel technique was to obtain optical density ratio on fullterm healthy neonates for the purpose of further oximetry analysis. Next steps need to aim at technological advantages to improve consistency in optical density measurements. More studies are required for furthering the precision and determination of calibration factors for the verification of the calculations of

oxyhemoglobin saturation values. Extending the retinal oximetry technique to the neonatal population is feasible and relevant for the refinement of the method and future application studies.

6.1 Future perspectives

This thesis suggests the oxyhemoglobin saturation of the systemic circulation can be measured through the retinal circulation. Following calibration upgrade and technological improvement, experimental verification retinal oximetry applied to critically ill and anesthesia care patients may be considered in order to test the feasibility of the technique for non-invasive monitoring in the future.

One of the significant barriers to the application of this technology in clinical settings is the sheer size and bulk of the oximeter equipment. Currently, study subjects are required to sit in front of the fundus camera after mydriasis for on time retinal imaging. Handheld version without pupil dilation is a consequent plan for furthering this technique. The instrument has already been miniaturized and the prototype is currently being tested on healthy subjects. The next step is to re-evaluate the calibration constants before bringing the miniaturized version of the device to the bedside of patients for retinal oximetry analysis studies of their central nervous system vessels and hence, the systemic circulation.

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Original publications

Paper I

Paper II

Paper III

Paper IV

Appendix