Age-Related Decline of Retinal Oxygen Extraction in Healthy Subjects

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PURPOSE. To investigate the age-dependence of total retinal blood flow and total retinal oxygen extraction in healthy subjects and determine their possible correlations with structural optical coherence tomography (OCT) parameters.

METHODS. This observational cross-sectional study consisted of 68 healthy subjects (mean ± SD age, 45.6 ± 16.3 years; 47% female). Total retinal oxygen extraction was calculated based on measurement of total retinal blood flow using bi-directional Doppler OCT and measurement of oxygen saturation using spectroscopic reflectometry. Retinal nerve fiber layer thickness was measured using OCT, and the total number of retinal ganglion cells was estimated based on a previous published model. Correlation of these parameters with age was studied and the association between structural OCT parameters and hemodynamic vascular parameters was calculated.

RESULTS. Both structural and vascular parameters showed a significant decline with increasing age. The correlation coefficients were between r = −0.25 and r = −0.41. Furthermore, structural and vascular parameters were significantly correlated with each other. The strongest association was found between the level of total retinal oxygen extraction and the number of retinal ganglion cells (r = 0.75, P < 0.001).

CONCLUSIONS. We showed that there was an age-related decline of retinal oxygen extraction. Levels of retinal oxygen extraction are correlated to retinal nerve fiber layer thickness and number of retinal ganglion cells. Our data partially explain the wide inter-individual variability in retinal blood flow values in healthy subjects. Longitudinal studies are required to study the time course of vascular and neuronal loss in humans.

Keywords: oxygen metabolism, humans, loss of retinal neurons, aging, retinal blood flow

Aging is associated with a wide array of changes in the human body, including the eye. In the retina, several age-related changes—such as thinning of the retinal nerve fiber layer,1 changes in retinal oxygen saturation,2,3 or reduced blood flow to the posterior pole of the eye4—have been observed. Moreover, age is a major risk factor for a wide variety of neurodegenerative eye diseases.5–9

Different techniques were realized for the quantification of retinal and choroidal blood flow (such as laser Doppler flowmetry, laser Doppler velocimetry, laser speckle flowgraphy, color Doppler imaging, or the blue field entoptic phenomenon), but none of these techniques can provide absolute values of perfusion.10,11 In addition, the techniques have considerable limitations, and no system is currently used in clinical routine.11,12 Several solutions were proposed to quantify total retinal blood flow (Q) based on Doppler optical coherence tomography (OCT).13–19 Recently, a prototype instrument from a commercial company was introduced and good reproducibility was reported.20 We have developed a technique based on a two-beam approach15,21–23 that has been validated against invasive microsphere technology in non-human primates.24 By combining this technique with spectroscopic measurement of oxygen saturation in retinal vessels, we were able to show that total retinal oxygen extraction (extO2) can be calculated.25,26 This provides an important step toward metabolic imaging in the retina by indicating how much oxygen is consumed in the inner retina.

Vascular factors are important candidates for making the eyes more susceptible to ocular neurodegenerative disease with age. This is supported by a wide array of studies showing that systemic hypertension, diabetes, dyslipidemia, and atherosclerosis are risk factors for age-related eye disease.27 In the present study, we hypothesized that Q and extO2 may decline with age in healthy humans. We also examined whether these vascular parameters are related to structural OCT-derived parameters such as the retinal nerve fiber layer (RNFL) and the number of retinal ganglion cells as estimated from OCT measurements. This was done in an effort to understand whether reduced...
methods

subjects

the study was approved by the ethics committee of the medical university of vienna and followed the guidelines set forth in the declaration of helsinki. we included a total of 68 healthy subjects between 19 and 76 years after they signed written informed consent and the study procedures had been explained in detail. before the study, subjects passed a screening examination that included physical examination, assessment of visual acuity, slit lamp biomicroscopy, funduscopy, and measurement of intraocular pressure (iop) using goldmann applanation tonometry. inclusion criteria were visual acuity of 20/20 or better, iop <21 mm hg and normal findings from slit lamp biomicroscopy and funduscopy. exclusion criteria were ametropia ≥3 diopter, anisometropia ≥3 diopter, diabetes, glaucoma, amd, and other ocular abnormalities as well as any clinically relevant illness as judged by the investigators, blood donation or intake of any medication in the 3 weeks prior to the study. participants had to abstain from beverages containing alcohol or caffeine in the 12 hours before the study day.

protocol

only the right eye was examined after it was dilated with a drop of tropicamide (mydriatikum; agepha, vienna, austria). this was followed by a resting period of at least 20 minutes to ensure full development of mydriasis and stabilization of blood pressure. thereafter, retinal blood flow was measured using the custom-built dual-beam bidirectional Doppler fourier-domain oct. fundus photographs were taken using the retinal vessel analyser (rva; imedos systems ug, jena, germany) to quantify the retinal oxygen saturation. blood pressure and pulse rate were measured non-invasively.

measurement of retinal blood flow and oxygen saturation

for the measurement of retinal blood flow and retinal oxygen saturation, we used a custom-built dual-beam Doppler fourier-domain oct system coupled to a fundus camera that was previously described in detail.15 with this system, we illuminated the vessels with two orthogonally polarized laser beams that stem from the same superluminescent diode under an angle 𝛼 to overcome the problem of angle-ambiguity. as such, the system also consists of two spectrometers that collect the spectral information of the two orthogonally polarized laser beams and two oct images formed under two different angles of incidence. the phase changes in these channels are different with different doppler angles. the central wavelength of the laser is at 𝜆0 = 838.8 nm with a spectral bandwidth of 54.0 nm. this results in an axial resolution of approximately 6 μm. the lateral resolution of the system is approximately 20 μm. we use a bi-directional oct because in doppler oct, knowledge on the angle between the incident light beam and the vessel is required to calculate the absolute blood velocity.28 when two light beams are used, however, knowledge of the Doppler angle is not necessary as long as Δ 𝛼 is small. as such, our system is insensitive to motion artefacts during the measurements and knowledge of the doppler angle is not required.25 the doppler shifts 𝜙1 and 𝜙2 are measured for each channel separately by calculating the difference of the phase of the fourier transform of subsequent a-line recordings. the phase difference 𝛿 𝜙 = 𝜙1 − 𝜙2 between the two beams is then calculated and used to obtain the absolute flow velocity

\[ v = \frac{\Delta \phi}{4 \Delta \nu \sin \beta \Delta \alpha} \]  

in equation (1), 𝜈 = 1.37 is the refractive index of blood and β is the angle of the vessel’s blood flow velocity vector with respect to the plane spanned by the two probe beams. the angle β can easily be obtained from either the fundus photographs or from the enface oct images. the angle Δα is dependent on the eye length of the subjects. as such it was calculated for each subject’s eye individually, taking into account the separation of the two probe beams at the pupil plane (in our case, 6 mm), the individual eye length, and the individual refraction value. all phase images were corrected for bulk-motion and phase wrapping, as described previously.25 in the present study, we used a rectangular scanning pattern to ensure that all vessels entering the optic nerve head (onh) were captured (fig. 1). this scanning pattern was specifically customized for each subject based on a fundus photograph. the specific angioarchitecture was considered and care was taken that the scanning did not include bifurcations and was hit under an angle β > 45 degrees. the typical size of such a scanning pattern is shown in figure 1. the mean velocities in all the retinal arteries (qa) leaving the onh and retinal veins (qv) entering the onh are measured for each subject. to allow for averaging over several pulse periods, the measurement time per single location was set to approximately 5 seconds. using the mean velocity 𝑣 from the oct recordings and the vessel diameter d extracted from the phase images,29 blood flow q in each individual vessel entering or leaving the onh can be calculated. by summing up the blood flow values qa in all measured arteries, one can determine the total arterial blood flow qa toi in all measured veins, it is possible to calculate the total venous blood flow qa toi. since the retina is an end organ qa toi needs to equal qv toi, and q was calculated as the

figure 1. exemplary fundus image showing the individualized oct scanning pattern around the optic nerve head. the scanning pattern was customized to ensure that all vessels entering the optic nerve head were scanned. doppler oct are shown for each of these scans, vessels appear either blue or red/yellow, depending on the angle of incidence and the direction of blood flow.
Retinal Oxygen Metabolism Decreases With Age

The OCT setup was integrated into the optical setup of the Dynamic Vessel Analyzer (DVA), which consists of a modified fundus camera (FF450plus; Carl Zeiss Meditec AG, Jena, Germany) and a Charge Coupled Device (CCD) chip. This allows for the measurement of retinal oxygen saturation from fundus photographs based on reflectance spectroscopy with a two-wavelength method.\(^{30}\) The oxygen saturation was measured in all retinal arteries (\(\text{SaO}_2\_A\)) and all retinal veins (\(\text{SaO}_2\_V\)) at exactly the same positions where the velocity measurements were done.

We have previously formulated a model on how to extract extO\(_2\) based on these measurements.\(^{25}\) Currently, we are not able to calculate local oxygen extraction because it is unknown which specific vein is draining the blood that is delivered via a specific artery. As such, we formulated a model that estimates oxygen content (cO\(_2\)) in the central retinal artery (CRA) and in the central retinal vein (CRV). This model takes into account the loss of oxygen from the measurement site to the center of the ONH.\(^{25}\) Obviously blood flow in the CRA equals \(Q_{\text{A,Tot}}\) and blood flow in the CRV equals \(Q_{\text{V,Tot}}\).

Multiplying the difference of oxygen content in CRA and CRV by Q allows for obtaining total retinal oxygen extraction:

\[
\text{extO}_2 = (cO_2\_CRA - cO_2\_CRV) \cdot Q. \tag{3}
\]

In comparison to our previous model,\(^{25}\) we have simplified the approach by omitting the oxygen that is not bound to hemoglobin (as quantified by oxygen tension). The error that is introduced by this approach is, however, less than 1%.

**Measurement of RNFL Thickness**

Structural OCTs were recorded using a commercially available spectral domain OCT system (SD-OCT, Heidelberg Spectralis OCT, SPECTRALIS software version 5.3.3.0, Eye Explorer Software 1.6.4.0; Heidelberg Engineering, Heidelberg, Germany). A scan with a 3.46 mm-diameter circle centered on the optic disc is performed and RNFL thickness values are provided for four quadrants, six sectors, and an overall average over the entire 360 degrees. These average RNFL thickness measurements derived from SPECTRALIS RNFL analysis report were used for the present study.

**Estimation of Total Number of Retinal Ganglion Cells**

We calculated the estimates of the total number of retinal ganglion cells based on the model proposed by Harwerth and co-workers.\(^{31}\) Briefly, they examined both structural and functional data from OCT-derived RNFL thickness measurements and histology-derived number of retinal ganglion cells using a non-human primates model. The model included age-related loss of axonal density as well as the change in the relationship between the neuronal and non-neuronal components contributing to RNFL thickness as measured by OCT with increasing disease severity.

**Measurement of Intraocular Pressure, Blood Pressure, and Pulse Rate**

Intraocular pressure was measured using a Goldman appplanation tonometer. Systolic, diastolic, and mean arterial blood pressures (SBP, DBP, MAP) were measured on the upper arm using an automated oscillometric device (Infinity Delta; Dräger, Vienna, Austria). The same device was used to record pulse rate. Ocular perfusion pressure (OPP) in the sitting position was calculated as OPP = 2/3 * MAP − IOP.\(^{32}\)

**Measurement of Axial Eye Length**

Axial eye length was measured using a commercially available IOL Master 500 (Zeiss, Jena, Germany).

**Data Analysis**

In addition to Q and extO\(_2\), we also calculated these parameters separately for the vessels temporal to the ONH and the vessels nasal to the ONH. For this purpose, we drew a vertical line through the ONH, and all larger vessels nasal to this line and temporal to this line were analyzed separately. This approach has some limitations because larger vessels nasally to the optic nerve head can still supply temporal areas and vice versa, but there is currently no non-invasive approach to visualize which capillaries are supplied by which arterioles. All outcome parameters were checked for normality using the Shapiro–Wilk test. Pearson product-moment correlation analysis was performed to investigate the association of Q and extO\(_2\) with age, systemic hemodynamic variables, ocular perfusion pressure, RNFL thickness, and number of retinal ganglion cells. Sex differences between Q and extO\(_2\) values were assessed with \(t\)-tests for independent samples. In addition, we performed a multivariate model looking into the associations between age, sex, systemic hemodynamic variables, ocular perfusion pressure, RNFL thickness, and number of retinal ganglion cells. Sex differences between Q and extO\(_2\) values were assessed with \(t\)-tests for independent samples. In addition, we performed a multivariate model looking into the associations between age, sex, systemic hemodynamic variables, ocular perfusion pressure, RNFL thickness, and number of retinal ganglion cells (independent variables) and Q and extO\(_2\) (dependent variables). Variables with \(P\) values <0.1 in the univariate model were included in the multivariate model. Data are presented as means ± SD. A \(P\) value <0.05 was considered the level of significance. Statistical analysis was carried out using CSS Statistica for Windows (Version 6.0; Statsoft, Inc., Tulsa, CA, USA).

**RESULTS**

A total of 36 males and 32 females with a mean age of 45.6 ± 16.3 years were included for analysis. The Shapiro–Wilk test showed none of the parameters to be significant. Patient characteristics are presented in Table 1. Retinal blood flow in the temporal area was higher than in the nasal area (\(P < 0.001\)). Likewise, retinal oxygen extraction in the temporal part of the retina was also higher than in the nasal part of the retina (\(P < 0.001\)). Blood flow values as obtained from arteries (\(Q_{\text{A,Tot}} = 39.9 ± 7.3 \mu l/min\)) and veins (\(Q_{\text{V,Tot}} = 39.5 ± 7.2 \mu l/min\)) were almost similar (paired \(t\)-test between values obtained in arteries and veins \(P = 0.721\), correlation coefficient \(r = 0.96\), \(P < 0.001\)). None of the outcome parameters revealed any sex differences (data not shown).

The correlation graphs between the outcome parameters and age are shown in Figure 2. Both retinal vascular and structural parameters declined with age. Correlation coefficients were slightly higher for extO\(_2\) and number of retinal ganglion cells than for Q and RNFL thickness. The association between vascular and structural parameters is depicted in Figure 3. Both Q and extO\(_2\) were correlated with structural parameters. The highest degree of association was found between extO\(_2\) and the number of retinal ganglion cells. For retinal blood flow, the intercept of the regression lines with the \(y\)-axis occurred at 13.4 \(\mu l/min\) when correlated with RNFL thickness and at 13.2 \(\mu l/min\) when correlated with number of retinal ganglion cells. For correlation analysis with extO\(_2\), the values were 0.91 \(\mu lO_2/min\) when RNFL thickness reached 0...
and 1.0 μlO2/min when number of retinal ganglion cells reached 0. Temporal retinal oxygen extraction was correlated with temporal RNFL thickness ($r = 0.49$, $P < 0.001$) as well as temporal number of retinal ganglion cells ($r = 0.72$, $P < 0.001$).

The correlation of nasal retinal oxygen extraction with nasal RNFL thickness ($r = 0.24$, $P = 0.047$) and nasal number of retinal ganglion cells ($r = 0.26$, $P = 0.036$) was still significant, but the association was weaker. None of the outcome parameters were associated with systemic blood pressure, ocular perfusion pressure, or pulse rate (data not shown).

The results of the multivariate model are presented in Tables 2 and 3. Results show that both $Q$ and extO2 are positively associated with RNFL thickness and number of retinal ganglion cells and negatively associated with age.

**DISCUSSION**

To the best of our knowledge, this is the first study showing an age-related decline of retinal oxygen extraction in humans. The present study also confirms previous reports that retinal blood flow and RNFL thickness decreases with age. Correlation analysis revealed that there is an association between reduced retinal oxygen extraction and RNFL thickness. It has previously been reported that $Q$ shows considerable inter-subject variability. The present study indicates that this may be partially explained by the number of retinal ganglion cells and their axons, which are nourished via the
retinal circulation. The data are compatible with previous reports that show an association between RNFL thickness and retinal vessel diameters as well as vessels density measured using OCT angiography. With the present approach, we measure retinal oxygen extraction, which closely reflects total inner retinal consumption. The retina is oxygenated via two distinct vascular beds. The retinal circulation oxygenates the inner

![Figure 3. Correlation between retinal hemodynamic parameters and structural parameters (n = 68). The regression line and the 95% confidence interval are shown. Equations for correlation lines: RBF = 15.27 + 0.256 * RNFL thickness; RBF = 13.34 + 21.93 * number of RGCs (×10^6); extO2 = 0.91 + 0.0090 * RNFL thickness; extO2 = 1.01 + 0.75 * number of RGCs (×10^6).]

### Table 2. Multivariate Analysis of Factors Associated With Total Retinal Blood Flow (Q)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Univariate Analysis</th>
<th>P Value</th>
<th>Multivariate Analysis</th>
<th>P Value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Coefficients (95%CI)</td>
<td></td>
<td>Coefficients (95%CI)</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>0.31 (−0.21, 0.87)</td>
<td>0.595</td>
<td>3.23 (1.52, 5.07)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>0.13 (−0.32, 0.67)</td>
<td>0.732</td>
<td>3.76 (1.98, 5.56)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean blood pressure (mm Hg)</td>
<td>0.17 (−0.27, 0.75)</td>
<td>0.678</td>
<td>3.12 (1.43, 4.66)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intraocular pressure (mm Hg)</td>
<td>−0.45 (−1.21, 0.37)</td>
<td>0.473</td>
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<td></td>
</tr>
<tr>
<td>Ocular perfusion pressure (mm Hg)</td>
<td>0.34 (−0.31, 0.97)</td>
<td>0.553</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulse rate (beats/min)</td>
<td>−0.39 (−1.81, 1.53)</td>
<td>0.812</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retinal nerve fiber layer thickness (µm)</td>
<td>5.67 (2.87, 8.76)</td>
<td>&lt;0.001</td>
<td>3.23 (1.52, 5.07)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total number of retinal ganglion cells (∗10^6)</td>
<td>6.13 (3.19, 8.98)</td>
<td>&lt;0.001</td>
<td>3.76 (1.98, 5.56)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>−4.76 (−7.37, −2.55)</td>
<td>&lt;0.001</td>
<td>−3.12 (−1.43, −4.66)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex</td>
<td>Male Reference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female −0.53 (−1.73, 1.21)</td>
<td>0.789</td>
<td></td>
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</tbody>
</table>
Retinal nerve fiber layer thickness (μm) 6.63 (3.66, 9.72) <0.001 3.77 (1.98, 5.61) <0.001
Number of retinal ganglion cells (×10^6) 8.25 (5.29, 10.96) <0.001 5.65 (2.87, 8.31) <0.001
Age (years) −5.12 (−7.66, −2.31) <0.001 −3.43 (−1.78, −5.03) <0.001
Sex
Male Reference
Female −0.64 (−1.63, 0.67) 0.582

In conclusion, this study shows an age-related decline of extO2. The combination of measurement of retinal oxygen extraction with OCT angiography may be an optimal approach to characterize inner retinal oxygen metabolism. The present study has a few limitations. It is a cross-sectional study; therefore, we are unable to answer the question regarding which event comes first: the loss of retinal ganglion cells or the reduction of retinal blood flow and retinal oxygen extraction. The objective yet non-invasive nature of the technique is suitable for longitudinal studies to help us determine this causal inter-relationship. In addition, we did not perform macular OCT scans, which may provide a more reliable estimate of RGC counts. Finally, we need to consider the errors of Doppler OCT and spectroscopic reflectometry measurements. Doppler OCT has been validated against invasive microsphere technology in primates, where eye motion can be eliminated. In humans, validation is difficult due to the absence of a gold standard technology, and the error of measurements is difficult to estimate. We did, however, show previously that there is good correlation with laser Doppler velocimetry, that retinal blood flow shows the expected decrease with breathing of 100% oxygen, and that the law of mass conservation is fulfilled at retinal bifurcations. Moreover, our previous data—as well as the data from the present study—have shown excellent agreement between retinal arterial and retinal venous blood flow values. Spectroscopic reflectometry validation is even more difficult because of the lack of a gold standard technique. Microelectrode measurements provide local values of retinal oxygen tension only. Phorescence quenching measures retinal oxygen tension in retinal vessels, and direct comparison with oxygen saturation measurements as obtained in the present study may be difficult. However, there are several studies indicating that the technique provides sufficiently valid measurements. Oxygen saturation in retina is reduced in patients with systemic diseases (such as chronic obstructive pulmonary disease, or Eisenmenger’s syndrome), and the values correlate with the degree of systemic hypoxia. The degree of systemic hypoxia is also well reflected when healthy subjects inhale mixtures with low oxygen content. Another limitation is that the method of measuring SaO2 is currently not good enough to measure the loss in arterial oxygen saturation along a single vessel. Importantly, this change in SaO2 along the vessel is very small as also indicated from our estimation of oxygen flux that is based on invasive measurements in animal models.

In conclusion, this study shows an age-related decline of extO2. In addition, extO2 is correlated to RNFL thickness and
estimated number of retinal ganglion cells. These results are compatible with two hypotheses: age-related retinal vascular changes trigger ganglion cell loss or else reduced oxygen extraction is due to reduced metabolic demand as a consequence of neuronal loss. The non-invasive nature of the techniques mentioned here can be used in longitudinal studies to help clarify the mechanistic question of cell death and vascular decline in eye diseases.

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