

# Age-Related Alterations in Retinal Tissue Perfusion and Volumetric Vessel Density

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**PURPOSE.** To determine age-related alterations in the retinal tissue perfusion (RTP) and volumetric vessel density (VVD) in healthy subjects.

**METHODS.** Total 148 healthy subjects (age 18 to 83 years) were enrolled and divided into four groups (G1, <35 years; G2, 35 ~ 49 years; G3, 50 ~ 64 years; and G4, ≥65 years). The RTP and VVD were measured at the macula. The RTP was calculated as the blood flow supplying the macular area ( $\phi$  2.5 mm) divided by the perfused tissue volume of the inner retina from the inner limiting membrane to the outer plexiform layer. The VVD of the macula ( $\phi$  2.5 mm) was calculated as the vessel density divided by the corresponding tissue volume.

**RESULTS.** The RTP and VVD of the retinal vascular network and deep vascular plexus (DVP) reached a peak in G2. Compared to G2, G4 had significantly lower RTP and VVD of DVP ( $P < 0.05$ ). After 35 years old, age was negatively related to the RTP ( $r = -0.26$ ,  $P = 0.02$ ) and VVD of the DVP ( $r = -0.47$ ,  $P < 0.001$ ). However, age was positively related to VVD of the superficial vascular plexus (SVP;  $r = 0.24$ ,  $P = 0.04$ ) in subjects aged more than 35 years. The RTP was correlated to VVD measurements ( $r = 0.23$ – $0.37$ ,  $P < 0.01$ ).

**CONCLUSIONS.** This is the first study to reveal the age-related alterations in the RTP and VVD during normal aging in a healthy population. Decreased RTP and VVD in the DVP along with increased VVD in the SVP may represent a characteristic pattern of normal aging in the healthy population.

**Keywords:** age, retina, retinal microcirculation, retinal tissue perfusion (RTP), volumetric vessel density (VVD)

Aging plays a role in alterations of the neuro-vascular-hemodynamic system and is associated with cardiovascular diseases and neurodegenerative disorders.<sup>1,2</sup> Aging increases the likelihood of neurodegeneration and altered hemodynamics such as hypoperfusion (lower blood supply) in the tissue, mostly affecting neural tissues (i.e., brain and retina) with high metabolic demand.<sup>3,4</sup> With advancing aging, cerebral blood flow (CBF) decreases in older adults.<sup>1,5</sup> In age-related neurodegenerative diseases such as Alzheimer's disease (AD), global and focal changes of the CBF are reported, indicating the presence of brain tissue hypoperfusion.<sup>6–8</sup> With the co-existence of neurodegeneration and vasculopathy in AD patients, the decrease of the CBF was more severe compared to the controls with similar age.<sup>6–8</sup>

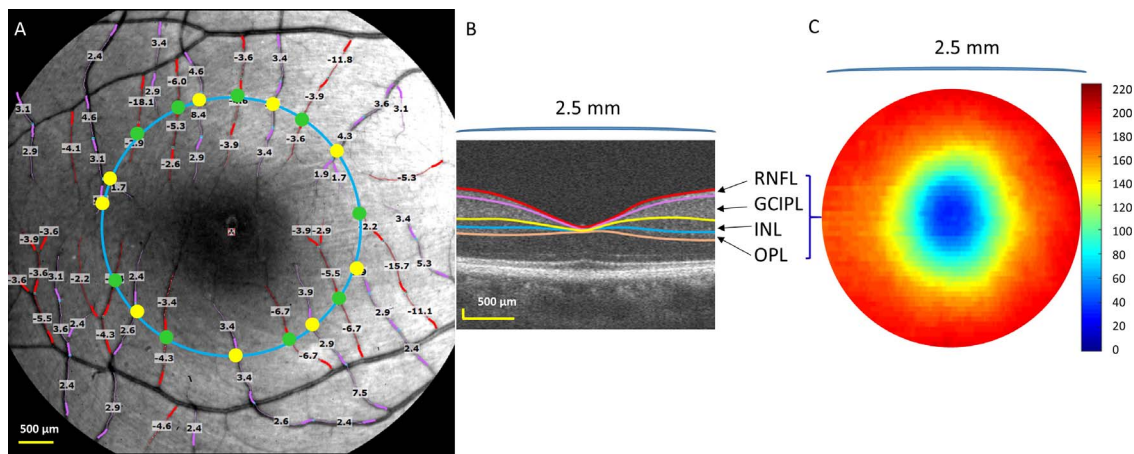
As a window to the brain, the retinal vasculature shows similar changes during aging, including loss of microvessels, thinning of the retinal nerve fiber layer (RNFL) and ganglion cell layer.<sup>9–11</sup> Due to the interaction between the vascular and nervous system, simultaneously studying the changes of these two components may provide a better understanding of normal aging and age-related neurodegenerative disorders. Wei et al.<sup>9</sup> demonstrated the age-related decline of retinal blood velocity, microvessel density, and intraretinal thickness and found the correlation between inner retinal thickness and microvascular

changes in a group of normal subjects. Similarly, Yu et al.<sup>11</sup> reported decreased macular vessel density and flow index with aging, measured using optical coherence tomography angiography (OCTA). However, whether the decline of the microvessel network is due to age-related vasculopathy or decreased metabolic demands secondary to age-related neurodegeneration remains uncertain.

Tissue perfusion is the passage of the blood flow through the circulatory system of certain tissue, usually referring to the delivery of blood to a capillary bed in the tissue.<sup>1</sup> Tissue perfusion is measured as the volume of blood per unit of time (blood flow) per unit of the tissue mass.<sup>1,12</sup> The delivery of oxygen and nutrients to the tissue relies on the tissue perfusion.<sup>1</sup> Although previous studies showed a decline of macular blood flow velocity (BFV)<sup>9,13</sup> and tissue loss of the inner retina (mainly containing nerve fiber and neurons)<sup>9,14,15</sup> during normal aging and in patients with AD compared to cognitively normal controls, it remains unknown whether retinal tissue hypoperfusion of the retina occurs during aging.

In addition, the retinal vessel density is reported to decline during aging.<sup>9,11</sup> However, these previous studies did not count for the tissue volume which the vessels perfuse. To better describe the changes of the microvasculature in the tissue, the tissue volume of the intraretinal layers is needed for estimating





**FIGURE 1.** Macular blood flow and tissue volume. (A) Retinal BFV imaged using RFI. The field of view of 20° was used, and the BFV of the secondary and tertiary branches of the retinal vessels was measured. The arterioles are marked in red and overlaid with the measured blood flow velocities (mm/s). The venules and their respective velocities are marked in pink. A negative value indicates blood flow is moving away from the heart. In this case, the arteriolar flow moved toward the fovea. A positive value indicates blood flow is moving toward the heart. In this case, the vessels are venules. Vessel diameters were calculated at the location where the vessels crossed a circle ( $\phi$  2.5 mm) centered on the fovea and the vessel diameters were used to calculate the blood flow in the arterioles (green dots) and venules (yellow dots). The macular blood flow volume of the arterioles was the sum of the blood flow volumes in each arteriole crossing the circle. Similarly, the macular blood flow volume of the venules was the sum of the blood flow volumes in each venule crossing the circle. (B) The intraretinal layers were imaged using UHR-OCT and segmented using Orion software. (C) The thickness of the inner retina including RNFL, GCIPL, INL, and OPL in a disc ( $\phi$  2.5 mm).

the vessel density, here referred to as volumetric vessel density (VVD). The goal of the present study was to determine the retinal tissue perfusion (RTP) and VVD in intraretinal layers during normal aging in a healthy population.

## METHODS

### Subjects

The study was approved by the institutional review board of the University of Miami Miller School of Medicine. Written informed consent was provided by all participants, who were treated according to the Declaration of Helsinki.

One eye of 148 healthy normal subjects was imaged. The individuals with refractive error greater than  $-6$  diopters (D) or  $+3$  D, obvious ocular media opacity, macular degeneration, and glaucoma were excluded. Subjects with uncontrolled diabetes or hypertension, dementia, cerebrovascular diseases, cancer, systemic inflammatory or infectious diseases such as multiple sclerosis and infection of human immunodeficiency virus (HIV) were also excluded. Mini Mental Status Exam (MMSE) was done for elderly subjects ( $>60$  years old) to screen for dementia. Subjects with MMSE scores lower than 28/30 were excluded from the study.

The subjects were divided into four groups in intervals of 15 years, which is similar to a previous study.<sup>9</sup> Group 1 (G1) was aged  $<35$  years, group 2 (G2) was aged from 35 to 49 years, group 3 (G3) was aged from 50 to 64 years, and group 4 (G4) was aged  $\geq 65$  years. All subjects underwent comprehensive ophthalmic examinations including best corrected visual acuity, slit-lamp examination, intraocular pressure (IOP), diastolic blood pressure (DBP), and systolic blood pressure (SBP). The mean arterial pressure (MAP) was calculated as  $\text{DBP} + 1/3(\text{SBP} - \text{DBP})$ .

### Retinal Blood Flow (RBF) Volume Measured Using Retinal Functional Imager (RFI)

The RFI (RFI-3000, Optical Imaging, Rehovot, Israel) has been described in detail in previous publications.<sup>9,13,16</sup> The imaging

system is based on a fundus camera additionally equipped with a stroboscopic flashlight system, and an advanced high-speed digital camera. The hemoglobin in the red blood cells is used as an intrinsic contrast agent to track the motion of the erythrocytes. In the BFV operating mode, a green ("red-free") filter is used. The BFV is calculated by quantifying the motion of erythrocytes in eight consecutive fundus images acquired at an interflash interval of less than 20 ms. To reduce the effect of pulsation on blood flow measurement, image acquisition is synchronized with the subject's systolic pulse using a finger probe. Multiple image sessions are taken. During each session, at least four serial images centered on the fovea are required for the measurement of BFV using the RFI's built-in software. Both arteriolar and venular BFVs are acquired. In the present study, the field of view (FOV) was set to be 20 degrees, which had a calibrated field of view of  $4.3 \times 4.3$  mm.<sup>16</sup> One eye from each subject was imaged after pupillary dilation with 1% tropicamide. The first choice was the right eye. The left eye was chosen when the right eye did not meet the inclusion criteria such as high refraction or media opacity. Blood pressure and heart rate were measured before imaging the eye.

A BFV map (Fig. 1) of the arterioles and venules was determined in all measurable vessels, mainly including the secondary and tertiary branches of the retinal vessels.<sup>13</sup> To measure the blood flow supplying the foveal region, the blood flow volume was calculated within a 2.5-mm circle centered on the fovea, similar to the method used in previous studies.<sup>16-18</sup> The diameter of the vessels that crossed the 2.5-mm circle was determined by counting the pixels of the full width and half of the maximum in the intensity profile, which was perpendicular to the vessel. The width in the pixel was then converted to micrometers. Using measured velocities and corresponding vessel diameters, the blood flow volume of each vessel crossing the 2.5-mm circle was calculated. The blood flow volume was computed based on a previously published equation.<sup>13</sup> The total perifoveal blood flow volume (arteriolar and venular, separately) in the circled perifoveal zone was the sum of all measured blood flow in the circle. All of the blood flow volumes in the arterioles on this circle was added together to yield the arteriolar blood flow. Similarly, the blood

flow in all venules crossing the circle was added to yield the venular blood flow. Because the blood flow in the arterioles and venules was about the same as shown in previous publications,<sup>13,16</sup> the RBF averaged from the arteriolar, and venular blood flow volumes were used to calculate the RTP.<sup>18</sup>

### Retinal Tissue Volume Measured Using Ultra-High Resolution Optical Coherence Tomography (UHR-OCT)

The custom-built UHR-OCT has been described previously.<sup>9,19,20</sup> Briefly, a super-luminescent diode was used as the light source with a center wavelength of 840 nm and a bandwidth of 100 nm. A spectrometer was used with a line scan camera running at 24,000 A-scans per second. The axial resolution was  $\sim 3$   $\mu$ m in tissue. The macular cube was nominally 6  $\times$  6 mm and consisted of 128 B-scans at 512 A-scans per B-scan.

Automatic retinal segmentation software (Orion, Voxeleron LLC, Pleasanton, CA, USA) was used to segment the thickness maps of the intraretinal layers (Fig. 1).<sup>9,20</sup> The macular cube was automatically segmented to obtain the volume of each layer, including the retinal nerve fiber layer (RNFL), ganglion cell-inner plexiform layer (GCIPL), inner nuclear layer (INL) and outer plexiform layer (OPL) in a circular area ( $\phi$  2.5 mm) centered on the fovea. Tissue volume of the inner retina perfused by central retinal vessels included the volumes of RNFL, GCIPL, INL, and OPL, which were used to calculate the RTP (Fig. 1).

### Retinal Microvasculature Imaged Using OCTA

The retinal vessels were imaged using Zeiss HD-OCT with an Angioplex OCTA device (Carl Zeiss Meditec, Dublin, CA, USA). In the present study, the 3  $\times$  3 mm scan was acquired.<sup>9</sup> Angiographic images of the total retinal vascular network (RVN), superficial vascular plexus (SVP), and deep vascular plexus (DVP) were exported for further processing and fractal analysis. To measure the vessel density, the OCTA images were resampled to 1024  $\times$  1024 pixels for vessel segmentation by a custom software program in Matlab (The MathWorks, Inc., Natick, MA, USA). A series of image processing procedures, including inverting, equalizing, and removing nonvessel structures and background noise, was used to create a binary image of the vessels. In the binary image, the large vessels were defined as any vessel with a diameter of  $\geq 25$   $\mu$ m and were extracted from the OCTA images. The remaining vessels were defined as the small vessels. The small vessels of RVN, SVP, and DVP were analyzed. The foveal avascular zone (FAZ) was detected based on the intensity gradient of the image. The intensity gradient is a directional change of the intensity in a gray scale image and the dark areas indicate lower value. The intensity gradient is one of the common image processing for edge detection.<sup>21</sup> In the present study, the intensity gradient method was used to detect the edge of the FAZ then located the geometric center of the foveal center. The annulus from 0.6 to 2.5 mm was analyzed. Using the fractal analysis toolbox (TruSoft Benoit Pro 2.0, TruSoft International, Inc., St. Petersburg, FL, USA), the box-counting method was used to calculate the fractal dimension (Dbox) in the annulus, which represents vessel density.

### RTP and VVD Calculation

The RTP was calculated by dividing the blood flow entering a circular area of the macula centered on the fovea ( $\phi$  2.5 mm) by the tissue volume of the inner retina from the inner limiting membrane to the OPL.<sup>18</sup> VVD was calculated as the vessel

density (measured as fractal dimension Dbox) divided by the corresponding tissue volume in the same area ( $\phi$  2.5 mm, Fig. 2). VVD in the RVN (VVD<sub>r</sub>) was the Dbox of RVN divided by the tissue volume of RNFL, GCIPL, INL, and OPL. VVD in the SVP (VVD<sub>s</sub>) was the Dbox of SVP divided by the tissue volume of RNFL and GCIPL. Similarly, VVD in the DVP (VVD<sub>d</sub>) was the Dbox of DVP divided by the tissue volume of INL and OPL.

### Statistical Analysis

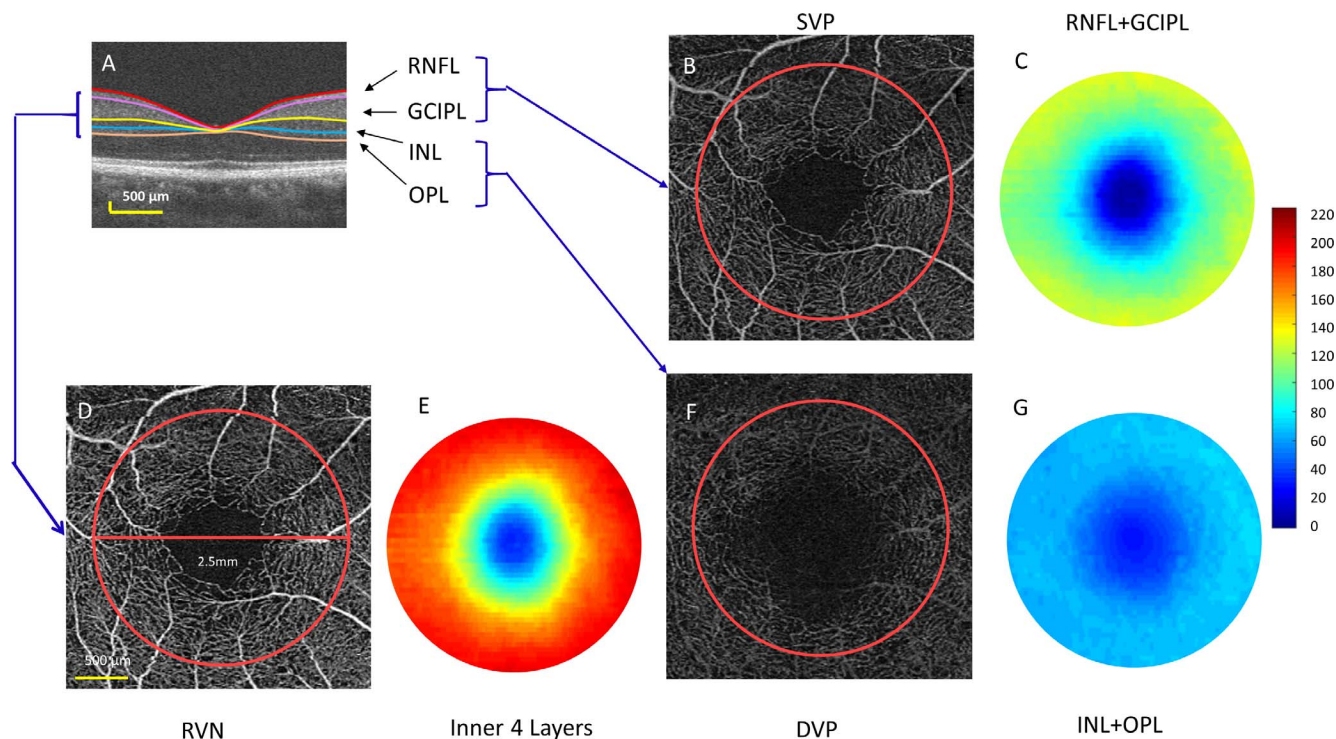
Statistical analyses were performed using a statistical software package (STATISTICA, StatSoft, Inc., Tulsa, OK, USA). One-way analysis of variance (ANOVA) was used to test the differences between groups, and post hoc tests were performed to test pair-wise differences between groups. Polynomial regression with the quadratic model was used to determine the relations between the measurements and age and the turning points of the measurements. Pearson correlation coefficients were used to determine the associations between measurements. In addition, generalized linear regression was used to validate the relations of the vascular measurements with age with control of confounding factors (sex, controlled diabetes, controlled hypertension, and refraction). The refraction was calculated as the spherical equivalent (the sphere and the  $\frac{1}{2}$  cylinder, in diopter). All data are presented as the mean  $\pm$  standard deviation, and a *P*-value of less than 0.05 was considered statistically significant.

### RESULTS

Demographic information of the four subgroups is listed in Tables 1 and 2. The RTP reached a peak in G2 and therefore G2 was used as the reference group for comparisons among groups (Figs. 3, 4). Compared to G2, G4 had significantly lower RTP and RBF (*P* < 0.05).

The VVD<sub>r</sub> and VVD<sub>d</sub> also showed the same pattern with the peak in G2 in the RVN and DVP, while the VVD<sub>s</sub> showed the peak in G3 (Fig. 4). Compared to G1, G2 showed a significant increase in the VVD<sub>r</sub> (*P* < 0.05; Fig. 4). The trend of the VVD<sub>r</sub> was similar to the trend of the RTP with the G2 (35~49 years) at the peak of perfusion. G2, G3, and G4 showed an increase in the VVD<sub>s</sub> (*P* < 0.05) compared to G1. Compared to G1 and G2, G4 showed a significant decrease in the VVD<sub>d</sub>; compared to G2, G3 also showed a significant decrease (*P* < 0.05). Compared to G2, VVD<sub>r</sub> in G1 and VVD<sub>d</sub> in G3 and G4 were significantly lower (*P* < 0.05). In contrast, VVD<sub>s</sub> in G2 to G4 was higher than G1 (*P* < 0.01). The vessel density (without considering the tissue volume) in G4 was significantly lower compared to all other groups in RVN, SVP, and DVP (*P* < 0.05). However, the tissue volume of the inner retina in G1 was higher than G2 (*P* < 0.05). The tissue volume of RNFL and GCIPL in G1 was higher than all other three groups (*P* < 0.05). In contrast, the tissue volume of INL and OPL in G3 and G4 were higher than G2 (*P* < 0.05).

Polynomial regression with the second order (quadratic model) fitted the data set of all 148 cases (Fig. 5). There were turning points in all measurements except for VVD<sub>s</sub> (D) and tissue volume of RNFL and GCIPL. The turning points were 42 years for RTP, 37 years for RBF, 32 years for VVD<sub>d</sub>, and 33 years for tissue volume of INL+OPL, with a median of 35 years, which was used as a cut off value for analysis of linear regression in subjects with age  $\geq 35$  years (i.e., G2-G4). After 35 years old, age was negatively related to the RTP (*r* = -0.26, *P* = 0.02; Fig. 5; Table 3) and RBF (*r* = -0.24, *P* = 0.03). However, age was not related to VVD<sub>r</sub> (*r* = -0.15, *P* = 0.21). Age was positively related to VVD<sub>s</sub> (*r* = 0.24, *P* = 0.04) and negatively related to VVD<sub>d</sub> (*r* = -0.47, *P* < 0.001), respectively.



**FIGURE 2.** Tissue volumes and vessel densities of the intraretinal layers. (A) Intraretinal layers were imaged using UHR-OCT and segmented using the Orion software. (B) The SVP of a scan of  $3 \times 3$  mm imaged using OCTA with the analyzed area of a 2.5-mm disc (red circle). (C) The thickness map of the RNFL + GCIPL in a circular area ( $\phi$  2.5 mm). The VVD of the SVP (VVDs) was the vessel density of the SVP (analyzed as fractal dimension Dbox) divided by the tissue volume of the RNFL and GCIPL in the disc ( $\phi$  2.5 mm). (D) The RVN. (E) The thickness map of the inner retina including RNFL, GCIPL, INL, and OPL. The VVD<sub>r</sub> was the vessel density of the RVN (analyzed as fractal dimension Dbox) divided by the tissue volume of the inner retina. (F) The DVP. (G) The thickness map of the INL and OPL in a disc ( $\phi$  2.5 mm). The VVD<sub>d</sub> was the vessel density of the DVP (analyzed as fractal dimension Dbox) divided by the tissue volume of the INL and OPL.

In contrast, age was not related to the tissue volume of the inner retina ( $r = 0.11$ ,  $P = 0.35$ ), while age was negatively related to the tissue volume of RNFL+GCIPL ( $r = -0.29$ ,  $P < 0.001$ ), and positively related to the tissue volume of INL+OPL ( $r = 0.40$ ,  $P < 0.001$ ). Generalized linear regression showed age had a significant effect on RTP ( $P = 0.046$ ), VVDs ( $P < 0.001$ ), and VVD<sub>d</sub> ( $P < 0.001$ ). In all cases, the RTP was strongly related to VVD<sub>r</sub> ( $r = 0.37$ ,  $P < 0.001$ ; Fig. 6), VVDs ( $r = 0.30$ ,  $P < 0.001$ ), and VVD<sub>d</sub> ( $r = 0.23$ ,  $P = 0.005$ ).

**DISCUSSION**

Normal aging is associated with modifications in the biomechanical properties of blood vessels, which may result in anatomical and functional alterations in the tissue and potentially lead to hypoperfusion or neurodegeneration.<sup>1</sup> To the best of our knowledge, this is the first study to directly measure the changes in the RTP and VVD with advancing age.

**TABLE 1.** The Demography of the Healthy Subjects

	Mean ± SD	Range
No. of subject	148	-
Male:female	61:87	-
Eye, OD:OS	133:15	-
Age, y	38.1 ± 15.5	18–83
SBP, mm Hg	119.8 ± 15.3	89–166
DBP, mm Hg	76.6 ± 9.8	49–107
MAP, mm Hg	91.0 ± 10.4	65–126
Heart rate, per min	70.7 ± 11.1	45–102

The key findings were the decline of the RTP and VVD<sub>d</sub>, and an increase of VVDs during aging in subjects older than 35 years. Quantitative analysis of the retinal blood supply and vessels responsible for blood flow distribution with the consideration of the perfused tissue volume may provide a better understanding of the natural course of aging affecting the neuro-vascular-hemodynamic system. Sufficient blood flow and rich capillary network are crucial for maintaining the normal metabolic activities and integrity of the nerve tissues in the brain<sup>1,5</sup> and retina.<sup>9,16–18</sup>

The RTP decreased during aging after 35 years old, which appeared to be a primary event. The RTP decreased with aging after 35 years and reached the significant level in subjects with age more than 65 years old (G4). The change in the RTP appeared to be predominantly due to the changes in the RBE, rather than due to the variation of the volume of the inner retina. Previous studies demonstrated the decline of retinal BFV,<sup>9,10</sup> implying changes of the RBE. The present study provided the direct evidence that the retinal hypoperfusion occurred at the later life during normal aging.

The retinal hypoperfusion found in the present study is also in support of the cerebral hypoperfusion, which occurs during

**TABLE 2.** Grouping Information

Age Groups	G1	G2	G3	G4
Range, y	<35	35–49	50–64	≥ 65
Mean ± SD, y	25.9 ± 3.7	39.7 ± 4.0	56.4 ± 4.1	71.9 ± 7.1
Number	73	41	21	13
Male:female	31:42	15:26	8:13	6:7

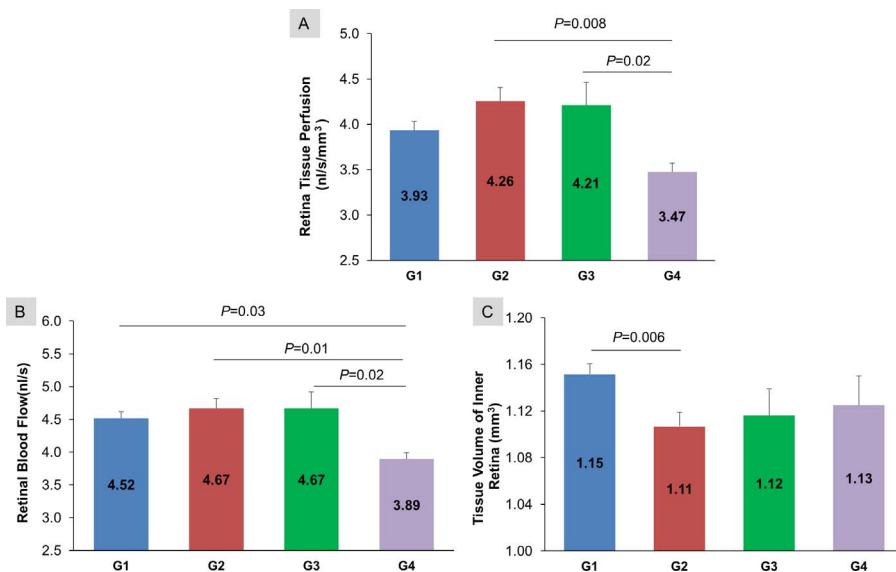


FIGURE 3. The RTP, blood flow, and tissue volume of the inner retina. Among groups, there were significant differences in the RTP (A), RBF (B), and tissue volume (C). Bars = standard error.

normal aging (Table 4).<sup>1,5,11,22</sup> Chen et al.<sup>1</sup> studied the age-related reduction of CBF during normal aging in a cohort of normal subjects and found that the CBF as an indirect measurement of brain perfusion declined at a rate of  $-0.38\%$  per year when the entire cortex was examined. Meanwhile, the cortical grey matter volume decreased at a rate of  $-0.85\%$  per year, which exceeds the reductions in CBF.<sup>1</sup> Leenders et al.<sup>5</sup> reported that the CBF, cerebral blood volume, and cerebral metabolic rates of oxygen decline at a rate of  $-0.5\%$  per year during normal aging. The changing rate of the RTP ( $-0.47\%$  per

year), RBF ( $-0.41\%$  per year), and VVDd ( $-0.44\%$  per year) found in the present study well matches the CBF change rates reported by Chen et al.<sup>1</sup> and Leenders et al.,<sup>5</sup> indicating that the RTP, RBF, and VVDd may be good candidates for imaging markers of the age-related retinal changes during normal aging. As both tissues have the highest metabolic activities in the retina and brain,<sup>3,4</sup> similar rates of the changes with advancing age are expected. Yu et al.<sup>11</sup> also reported the similar changing rate per year in the RBF index and vessel density, although the tissue volume was not considered. The parafoveal flow index

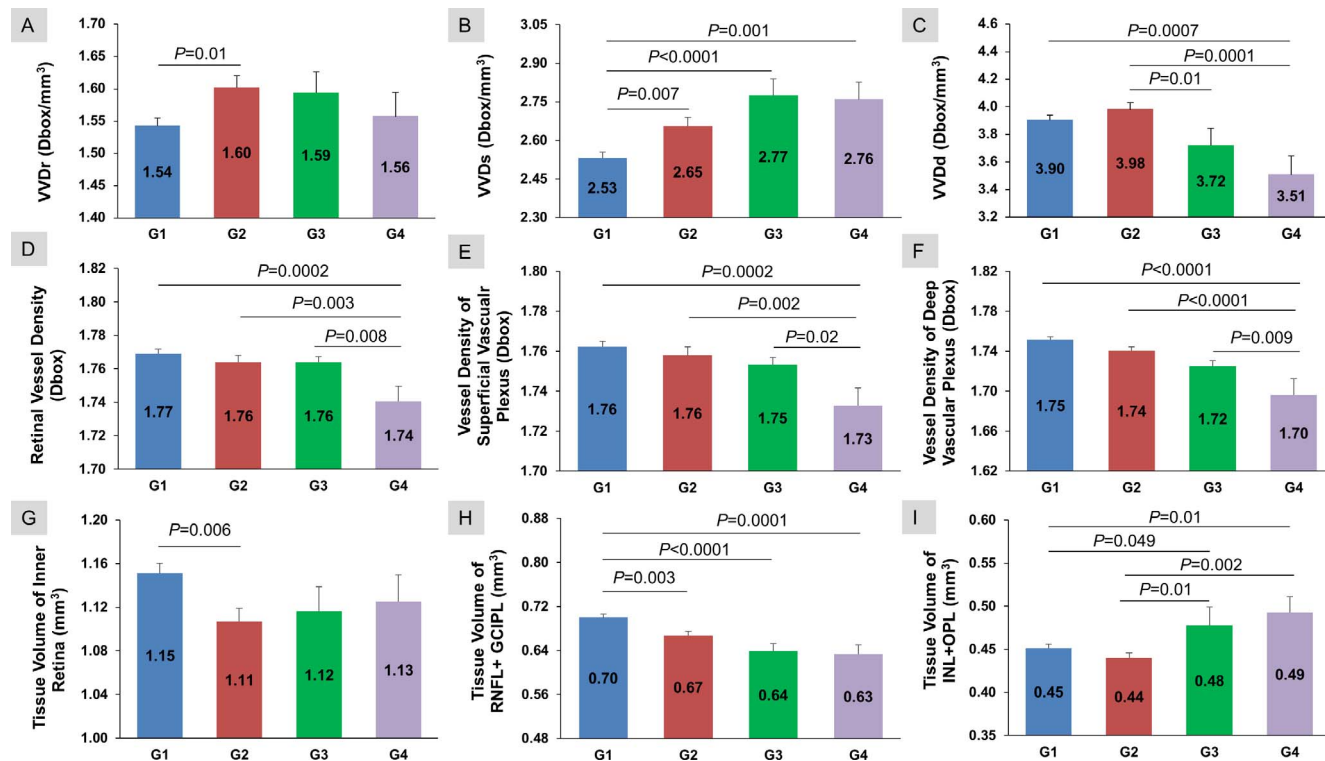
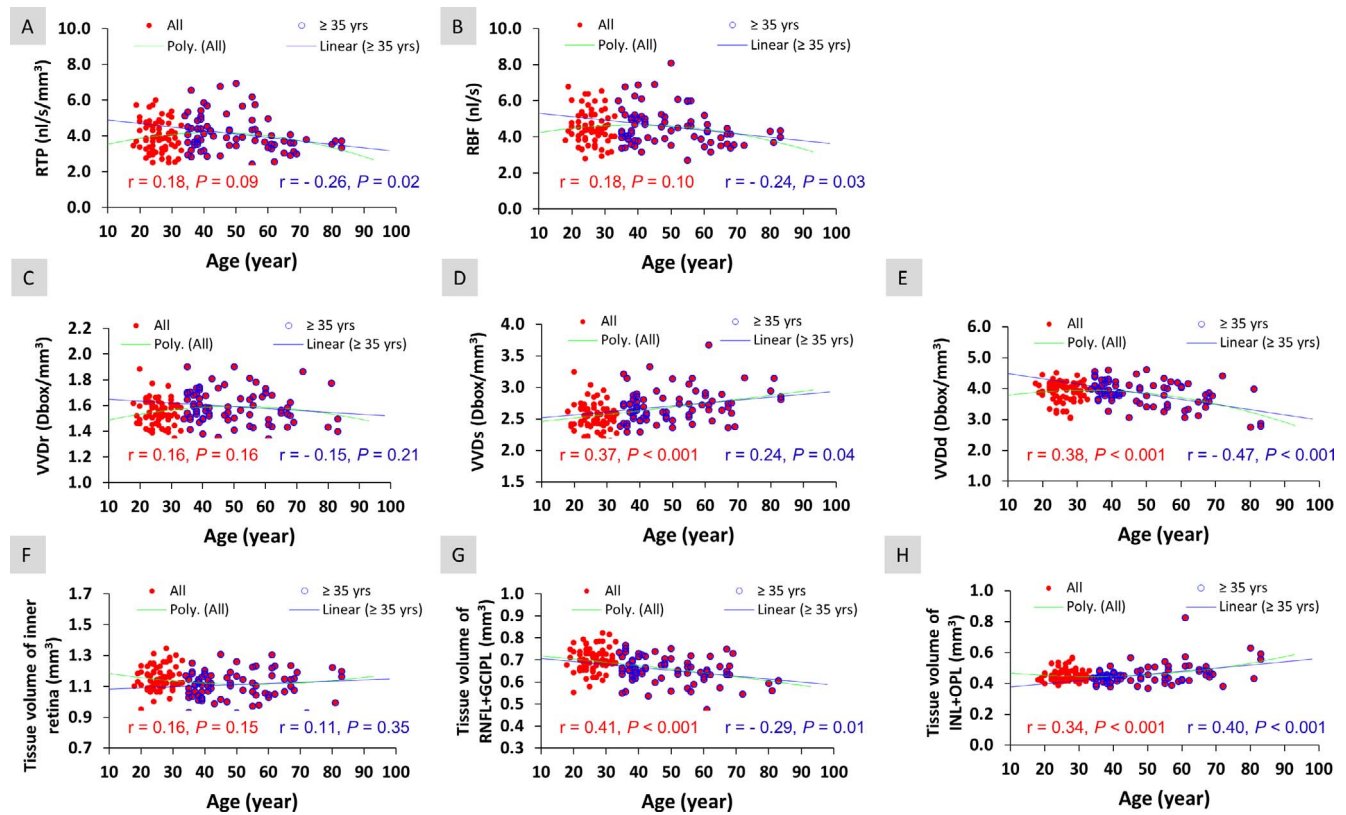


FIGURE 4. The VVD, retinal vessel density, and retinal volume. Among groups, there were differences in the VVD (A, B, C), vessel density (D, E, F), and tissue volume (G, H, I). VVD measurements showed differences among group (A, B, C). Bars = standard error.



**FIGURE 5.** Relations between age and the RTP and VVD. To describe the relations between the measurements as dependent variables and age as the independent variable, polynomial regression with the second order (quadratic model) was used to fit the data set of all 148 cases (red dots, green regression lines, and red  $r$  and  $P$  values, A–H). There were turning points in all measurements except for VVDs (D) and tissue volume of RNFL and GCIPL (G). The turning points were 42 years for RTP (A), 37 years for RBF (B), 32 years for VVDd (E), and 33 years for tissue volume of INL+OPL (H), with a median of 35 years, which was used as a cut off value for further analysis using linear regression (blue dots, blue lines, and blue  $r$  and  $P$  values, A–H). After 35 years old, age was negatively related to the RTP (A) and RBF (B). Age was not related to VVDr (C). Age was positively related to VVDs (D) and negatively related to the VVDd (E), respectively. Age was not related to the tissue volume of the inner retina (F). Age was negatively related to the tissue volume of RNFL+GCIPL (G). Age was positively related to tissue volume of INL+ OPL (H). VVDs, VVD of the SVP.

and vessel density decreased by 0.6% and 0.4% yearly, respectively. Interestingly, the choroidal volume also declined 0.73% per year.<sup>11</sup> The brain and retina have the same embryological origin, and their microvasculature has similar anatomical and physiological features. Retinal aging could present or mimic the cerebral aging. Imaging the microcirculation in the retina may also assist in establishing easy access to inexpensive biomarkers of neurodegenerative disorders that could be used in the evaluation of treatments to prevent or slow the disease progression.<sup>16,18</sup>

In contrast, the changing rate of volume of the inner retina is different between the retina and brain tissue. Almost no change of the inner retinal volume found in the present study is mostly due to the changes in the opposite directions of the RNFL + GCIPL (decreasing) and INL + OPL (increasing). The thinning of the RNFL and GCIPL layers had been reported in the prior studies.<sup>9,23–25</sup> Moreno et al.<sup>26</sup> observed a positive correlation between age and OPL, which is in agreement with the OPL finding in the present study. Therefore, simultaneously analyzing the changes in tissue volume and vascular param-

**TABLE 3.** Age-Related Changes in RTP and Vessel Densities in Subjects (Age  $\geq 35$  y)

Parameters	Unit	Mean $\pm$ SD	Relation, $r$	Change per Decade	Change per Year, %
RTP	nl/s/mm <sup>3</sup>	4.11 $\pm$ 0.98	<b>-0.26</b>	-0.19	-0.47
RBF	nl/s	4.54 $\pm$ 1.02	<b>-0.24</b>	-0.19	-0.41
Tissue volume of inner retina	mm <sup>3</sup>	1.11 $\pm$ 0.09	0.11	0.01	0.06
Tissue volume of RNFL+GCIPL	mm <sup>3</sup>	0.65 $\pm$ 0.06	<b>-0.29</b>	-0.01	-0.20
Tissue volume of INL+OPL	mm <sup>3</sup>	0.46 $\pm$ 0.07	<b>0.40</b>	0.02	0.44
VVDr	Dbox/mm <sup>3</sup>	1.59 $\pm$ 0.13	-0.15	-0.01	-0.09
VVDs	Dbox/mm <sup>3</sup>	2.71 $\pm$ 0.26	<b>0.24</b>	0.05	0.17
VVDd	Dbox/mm <sup>3</sup>	3.82 $\pm$ 0.47	<b>-0.47</b>	-0.17	-0.44
VD of RVN	Fractal dimension (Dbox)	1.76 $\pm$ 0.03	<b>-0.30</b>	-0.01	-0.03
VD of SVP	Fractal dimension (Dbox)	1.75 $\pm$ 0.03	<b>-0.30</b>	-0.01	-0.03
VD of DVP	Fractal dimension (Dbox)	1.73 $\pm$ 0.04	<b>-0.47</b>	-0.01	-0.08

Bold font denotes  $P < 0.05$ . VVDs, VVD of the SVP; VD, vessel density.

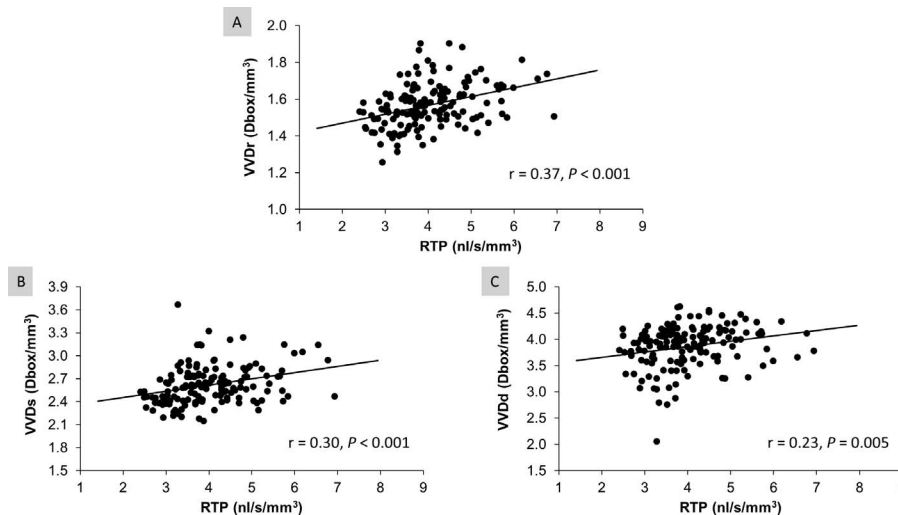


FIGURE 6. The relationship between RTP and VVD. The RTP was related to VVD<sub>r</sub> (A), VVD<sub>s</sub> (B), and VVD<sub>d</sub> (C). VVD<sub>s</sub>, VVD of the SVP.

ters (blood flow and vessel density) is essential and may provide further information about the age-related vascular changes.

The metabolic activities of the neuronal tissue require adequate blood flow, which relies on the rich capillary network for the blood passage (perfusion). Decreased blood vessel network may result in alterations in the metabolic activity of the neurons.<sup>27</sup> Growth hormone (GH) and insulin-like growth factor 1 (IGF-1) are responsible for stimulating endothelial cell proliferation, tube formation, and angiogenesis, which are essential for maintaining sufficient capillary network for adequate tissue perfusion.<sup>28</sup> Therefore, the alteration of these hormones may in part explain the findings in the present study. Sonntag et al.<sup>28</sup> reported that GH and IGF-1 decreased with age, suggesting their important role in vascular maintenance and remodeling. A close relationship was also established between type 1 IGF receptors and the decline in vessel density and synaptic density in the cortex of the animal models. It can be speculated that the changes of these hormones with advancing age lead a slow decline of vessel density of the tissue, which resulted in the loss of vessel density with the alterations of the intraretinal layers. However, since this study did not measure the GH and IGF-1 values,

further studies will be needed to establish the link between these hormones and retinal vasculature.

While the RTP can be regarded as the measurement of blood supply to the tissue that is perfused by the retinal vascular system, the VVD can be regarded as the blood flow distribution in the intraretinal layers and may provide more information on details of the age-related changes. As the vessel densities of the RVN, SVP, and DVP decreased with advancing age, they reached the significant lowest level in subjects older than 65 years, which is similar to our previous report.<sup>9</sup> However, due to the changes in the opposite directions, the tissue of RNFL and GCIPL showed a decrease (i.e., neurodegeneration) during aging, while the tissue of INL and OPL showed an increase with advancing age. These changes lead to the changes of the VVDs and VVD<sub>d</sub> in opposite directions. The increased VVDs indicates more vessels per cube tissue with advancing age, although the loss of vessels occurs in the older people compared to the younger people. This may be a compensation mechanism in an attempt to maintain the highest metabolic activities in these remaining neural tissues.

Alternatively, this phenomenon may be explained by the predominant change in the RNFL and GCIPL, which exceeds the reduction of the SVP during aging. In contrast, a decrease

TABLE 4. Age-Related Vascular Changes in the Eye and Brain

Study	Parameter	Sample	Age, y	Method	Correlation Coefficient, <i>r</i>	Change per Year, %
Present study	RBF	75 subjects	35–83	RFI	−0.24	−0.41
	RTP	75 eyes		RFI/UHR	−0.26	−0.47
	VVD <sub>d</sub>			OCTA/UHR	−0.47	−0.44
	VVD <sub>s</sub>			OCTA/UHR	0.24	0.17
Yu et al. 2015 <sup>11</sup>	Parafoveal flow index	45 subjects	24–59	OCTA		−0.60
	Vessel area density	76 eyes				−0.40
Barteselli et al. 2012 <sup>22</sup>	Choroidal volume	114 subjects	14–89	EDI SD-OCT	−0.39	−0.73
		176 eyes				
Chen et al. 2011 <sup>1</sup>	CBF	86 subjects	23–88	MRI		−0.38
Leenders et al. 1990 <sup>5</sup>	CBF	34 subjects	22–82	PET	−0.35	−0.47
	CBV				−0.47	−0.47
	CMRO <sub>2</sub>				−0.52	−0.53

VVD<sub>d</sub>, VVD of DVP; VVD<sub>s</sub>, VVD of SVP; CBV, cerebral blood volume; CMRO<sub>2</sub>, cerebral metabolic rates of oxygen; RFI, retinal function imager; EDI SD-OCT, enhanced depth imaging spectral domain optical coherence tomography; MRI, magnetic resonance imaging; PET, positron emission tomography.

of vessel density over an increased tissue volume occurred in the DVP, leading to the age-related decline of the VVDd, sharing the same trend as the RTP. Although OCTA does not directly measure the tissue perfusion, the blood flow in the vessels is detected on the angiography, often referred to as capillary perfusion.<sup>29</sup> Indeed, the angiography could represent the blood flow in the tissue regardless of the flow in the arterioles, capillaries, or venules. Therefore, the vessel density in the intraretinal layers could be regarded as the blood flow distribution and the VVD could be interpreted as the blood flow distribution. Our results of the VVD would then indicate that redistribution of the blood flow may result from occurring with advancing age. As such, the already reduced blood flow may result from re-allocating more flow to the RNFL and GCIPL for maintaining the integrity and function of these nervous layers. Further studies are needed to validate this concept.

Although positive and negative relations with age were found in the RTP and VVD measurements, positive relations between the RTP and VVD measurements were found. With the same denominator (tissue volume), the relation between the RTP and VVD<sub>r</sub> was as expected. Interestingly, with the different denominators, the RTP and VVDs/VVDd were still positively related, indicating that the blood supply to the tissue influences the overall blood flow in the perfused tissues, regardless of the changes during aging. This may represent a characteristic pattern in normal aging. Further studies in the diseased conditions may use this finding as for the control for further validation.

Some limitations of the present study should be noted. First, although a relatively large sample size of 148 cases was enrolled, the sample size of the old subjects is still limited. Second, we did not perform a longitudinal study, and therefore we could not assess the temporal trends of these changes during aging. Instead, we used the group comparisons to establish the age-related alterations. Third, the large vessels in the SVP projected a shadow on DVP and resulted in shadow-graphic projection artifacts. We were not able to access the inherited algorithms to remove the artifacts. Instead, we used image processing to remove the large vessels in DVP.<sup>9,30</sup> As there are no large vessels in the DVP, this may have led to some degrees of inaccurate measurements of DVP. Fourth, although the cut-off age for linear regression was justified by polynomial regression, our grouping setting was the same as we did in a previous study.<sup>9</sup> The arbitrary grouping for group analysis of the differences may be debatable. However, our polynomial and linear regression may provide a full picture of the trend in age-related changes. Fifth, the FOV of these imaging devices varies for each depending on the axial length. Technically, the FOV needs to be corrected in each subject. Although we excluded subjects with refraction out of the range of  $-6 \sim 3$  D, the measurement errors induced by the FOV could still be introduced. Finally, data acquired using OCTA and UHR-OCT were automatically processed, whereas vessels in the RFI images were manually drawn then the measurements were automatically processed.<sup>9,13,16,17</sup> This manual procedure may have a measurement bias although the low measurement variability (7.5%) was reported.<sup>10</sup>

In conclusion, this study revealed age-related alterations in the RTP and VVD during normal aging in a healthy population. Decreased RTP and VVD in the DVP along with increased VVD in the SVP may represent a characteristic pattern of normal aging in the healthy population. These measures may be good candidates which can be developed as imaging markers for age-related changes in the microvasculature, microcirculation, and microstructure. Our findings may lead to further validation for utilizing the retinal microvascular changes in clinical research aimed at the detection and monitoring of systemic and brain

vascular diseases and the effect of various classes of vascular therapies.

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