Effect of Aging on Retinal Artery Blood Column Diameter Measured along the Vessel Axis

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PURPOSE. To determine whether retinal branch arteries of healthy persons in different age groups show different longitudinal vessel profiles at baseline and during dynamic reaction to flicker stimulation.

METHODS. Thirty-three healthy subjects (age groups: 21–27 years, 40–59 years, and 60–85 years) were examined with the use of a retinal vessel analyzer. A monochromatic flicker of 12.5 Hz was applied for 60 seconds. Arterial diameters were measured in vessel segments of 1 mm in length to obtain the longitudinal vessel profile. Differences in amplitude and frequency of arterial width changes were characterized by the parameter spectral edge frequency (SEF).

RESULTS. SEF was significantly different between the young group and the senior group in each phase of the arterial reaction to flicker (baseline, dilation, constriction, relaxation; P < 0.05; Mann-Whitney U test). No significant difference within any age group was found in any phase of the arterial reaction. No significant difference between the middle-aged and either young or elderly subjects was found at baseline. However, after stimulation, the middle-aged group displayed a significant difference compared with the young group, with values resembling those of the elderly group.

CONCLUSIONS. In healthy elderly subjects, retinal arteries assumed a significantly less regular longitudinal vessel profile than those of young subjects. Middle-aged subjects assumed a more irregular profile only in the stimulated states of dilation, constriction, and relaxation. Early age-related changes in vessel profile are noted only after metabolic demand. These changes might be a cause for impaired blood flow and blood-vessel wall interaction. (Invest Ophthalmol Vis Sci. 2008;49:2094–2102) DOI:10.1167/iovs.07-0711

Atherosclerotic changes occur in blood vessels with age even in the absence of atherosclerotic risk factors.¹ ² The border between physiological aging and pathologic arteriosclerosis is not well defined. Both undergo similar biochemical processes and result in similar changes in the vasculature.³ ⁴ Arteriosclerosis involves the entire vascular system in a somewhat patchy appearance.⁵ Although typical arteriosclerotic lesions are confined to large conduit arteries, vessels of microcirculation are affected differently.⁶ In arteriosclerosis endothelial function seems to be attenuated, as demonstrated by altered vasodilatation to demand.⁷

Retinal vessels are part of the microvascular bed and have some special properties.⁸ ¹¹ The nonfenestrated endothelium creates the blood-retina barrier, similar to the blood-brain barrier of cerebral vessels. The difference between them is that retinal arteries have no adrenergic innervation.¹² ¹³ Transparency of the optic media allows for direct observation of this microvascular bed. It also enables us to use an elegant, noninvasive functional provocation of vessel dilation, namely light application, to the retina. Dilation of retinal vessels can be achieved by changing the flicker illumination of the retina.¹⁰ This effect is called neurovascular coupling and has been studied extensively in the ophthalmic community. The most effective stimulus for the human retina consists in the application of a rectangular flicker light with a frequency of 8 to 20 Hz.⁸ ¹⁰ ¹⁴ By using the retinal vessel analyzer (RVA; Imedos Ltd., Jena, Germany), it is possible to observe retinal vessels before, during, and after a defined monochromatic flicker stimulus application of 12.5 Hz for any chosen time.

Neurovascular coupling is also well known in the central nervous system (CNS). It is defined by neuronal activity evoking local change in blood flow. In the CNS, neurovascular coupling has been shown to be unaffected during normal aging.¹⁵ The effect is highly dependent on glial cell activity, as has been shown in mammalian retinal mounts.¹⁶

We supposed that age induces changes in the configuration of the arterial wall of vessels of the microcirculation. Our hypothesis was that in young persons the longitudinal profile is smoother and that this effect persists whether the vessel is constricting or dilating (Fig. 1).

To assess changes in microvasculature caused by age, we examined healthy subjects of different age groups and the behavior of their retinal vessels to flicker. From the obtained data of the blood column within the retinal vessels before, during, and after stimulation, a longitudinal vessel profile was determined, and vessel wall irregularities were calculated, thus defining a measure for vessel wall characteristics depending on age.

MATERIALS AND METHODS

Subjects

Eleven healthy subjects in each of three age groups—21 to 27 years (five men, six women; mean age, 24.9 ± 1.9 years); 40 to 59 years (three men, eight women; mean age, 50.2 ± 5.3 years); and 60 to 85 years (four men, seven women; mean age, 68.6 ± 7.6 years)—were entered into the prospective clinical study. They had not used regular medication for 3 months before the examination.

Patients with any eye disease or with any of the following were excluded: acute myocardial infarction, diabetes mellitus, blood pressure higher than 150/95 mm Hg, heart disease state III or IV (New York Heart Association classification).

At the beginning of every test, a clinical ophthalmic examination consisting of measurement of visual acuity, objective refraction, kera-
the most common feature of the device. For this feature, changes have been described. Briefly, the device allows noninvasive online assessment of vessel diameter, depending on times and locations along the vessel (Fig. 2, upper panel). Before, during, and after stimulation, arterial and venous diameters could be assessed. For that purpose the RVA consists of a retinal camera (450 FF; Carl Zeiss, Jena, Germany), a charge-coupled device camera for electronic online imaging, and a personal computer for system control, analysis, and recording of the obtained data.

For each examination, a segment (approximately 1 mm) of a retinal vessel 1 to 3 disc diameters away from the optic disc was investigated. The vessel longitudinal section within the region of interest was scanned 25 times per second. The automated algorithm allows compensation for eye movements and obtains data along the vessel over time, thus creating a three-dimensional matrix of values (Fig. 2, upper panel) obtained during functional measurement. The RVA measures vessel diameters in measurement units (MUs). These arbitrary units are equal to 12.5 μm if the measured object corresponds to the normal eye of Gullstrand.

Assessment of Longitudinal Vessel Profiles
Temporal assessment of retinal vessel behavior in response to stimuli is the most common feature of the device. For this feature, changes in vessel segment diameter mean over time are traced by the intelligent algorithm (Fig. 2, upper panel, white line). The obtained data also allow the observation of spatial changes in vessel diameter along a chosen segment and thus of a longitudinal profile of the vessel segment at chosen time intervals (Fig. 2, upper panel, green line; middle panel). Through this feature it was possible to assess in vivo noninvasively dynamic variations in longitudinal vessel profile in humans during different states of stimulation. The method of data acquisition for local vessel analysis with RVA has been explained in detail. Differences in diameters along the vessel segment during a defined time period (e.g., time interval between the two dashed lines in the upper panel of Fig. 2) can be assessed. For each pixel (point of the segment), the mean of all measurements in this location during the chosen time interval was calculated. We termed the result longitudinal vessel profile. It reflects the configuration of the vessel–blood interface in the longitudinal vessel section when assuming the vessel to be axially symmetrical (Fig. 2, middle panel). Profiles obtained at different time intervals can be compared (Fig. 2, bottom panel).

To describe the longitudinal profile of a vessel and its caliber changes, we must characterize the frequency in those changes. Waves along a curve (temporal or spatial) can be defined by their frequency and amplitude. Applying these principles to the longitudinal retinal vessel profile, waves of different frequency can be determined, namely high-frequency waves (HFW), including waves in the longitudinal vessel profiles with 10 to 20 oscillations per 1 mm of the vessel segment and a magnitude between 1.5 μm and 15 μm, and low-frequency waves (LFW), defined as 0.2 to 5 oscillations per 1 mm of vessel segment and a magnitude of 15 μm to 40 μm.

Stimulus
During examination, retinal vessels were stimulated with flickering light relying on the principles of neurovascular coupling. Briefly, an optoelectronic shutter is inserted in the retina camera in place of an additional optical filter. The shutter interrupts the observation light (530–600 nm) with irradiance at the fundus of approximately $1.96 \times 10^{-4}$ W/cm² over the entire 30° illuminated field of the retinal camera. The chosen frequency of 12.5 Hz rectangular light interruption provides a sequence of one normal illuminated and one dark single frame at a video frequency of 25 Hz.

Measurement of the baseline vessel diameter for 100 seconds was followed by two cycles of 60-second flicker provocation and 150-second observation. Total duration of the measurements, including baseline and observation between flicker provocations, amounted to 9 minutes.

Pulse and Blood Pressure
During the examination time, Riva-Rocci (RR) blood pressure measurements were taken once a minute. From those data, we calculated the mean systemic arterial blood pressure as follows: mean RR = RR diastole + $\frac{1}{3}$ × (RR systole − RR diastole) mm Hg.
Data Evaluation and Statistical Analysis

Time courses of vessel diameter changes in all subgroups were plotted. The ensuing slope of the temporal response was consistent in all subjects and corresponded to results in healthy subjects reported by other authors. This time response was not the subject of the present study. Given that it represents the basis for the definition of time segments in our study design, however, it is briefly described.

Average arterial diameter over time demonstrated a diameter increase during provocation; a reactive vessel constriction was observed after release of the stimulus, with an ensuing return to initial baseline values (Fig. 3). These temporal phases were observed in all groups. Four time intervals for examination were defined as shown in Figure 3.

The start of time segments II and III (Fig. 3) was assigned individually. For each subject, an individual time interval included the point of maximal dilation or constriction and was chosen so that changes in vessel diameter during the interval were minimal.

Longitudinal vessel profiles during selected time segments were assessed (Fig. 3) at defined time intervals and were compared with each other within one subject (Figs. 2 [bottom panel], 4).

To characterize the longitudinal vessel profile, we used a fast Fourier transform (FFT), an algorithm to compute the discrete Fou-
The discrete Fourier transform (DFT) and its inverse, DFT, is widely used in signal processing and related fields to analyze the frequencies contained in a sampled signal, which is expressed in terms of a sum of sinusoidal components. DFT determines amplitude, frequency, and phase of each component. One of the results of FFT application, the distribution of frequencies in the analyzed signal, can be represented in the form of a power spectrum.

Power spectra of longitudinal vessel profiles of all subjects in each phase of vessel reaction were obtained by FFT. Each power spectrum was reduced by dividing each value in the frequency distribution by the whole area of the power spectrum, as described in detail elsewhere. For each type of spatial curves and for each age group, average power spectra were derived from these reduced individual power spectra by calculation of the median value in the group for each point of frequency distribution, as suggested by other authors for brain vessels.

The parameter spectral edge frequency (SEF) was introduced and calculated for each subject for the following time intervals: I, local baseline; II, dilation; III, constriction; IV, relaxation. SEF is a quantitative parameter to describe the presence of high frequencies and to characterize high frequencies. It is derived from the power spectrum of the vessel profile, created with FFT. SEF divides the whole area under the power spectrum into two nonequal parts, 95% and 5%. In general, high SEF values describe a more wavy curve (with more frequent peaks and troughs) and low SEF values describe a less wavy curve. SEF may reflect variations of HFW for a subject at different time intervals and variability in HFW between subjects (Fig. 5).

A program was created (MATLAB 6.1; The MathWorks, Natick, MA) to plot power spectra and to evaluate the chosen parameter. Vessel segment lengths were measured in the same relative units (RUs) used to evaluate vessel diameters. Data transfer to a spreadsheet (Excel; Microsoft, Redmond, WA) for evaluation was performed in pixels: 1 pixel = 12.5 μm in a normal Gullstrand eye.

The spatial frequency parameter SEF is represented in reciprocal measurement units: MU⁻¹. To simplify matters, we also termed this unit Hz because it represented an analogue of temporal frequency for spatial curves, which is usually measured in Hz. One spatial Hz corresponds to one oscillation in an MU. Consequently, for example, the value of 0.1 spatial Hz corresponds to one whole oscillation of a vessel profile in 10 MU.

A template with corresponding macros in a spreadsheet (Excel; Microsoft) was created for each subject to filter, process, and analyze...
the numerical data from the RVA. Because it was impossible to prove
the normal distribution of measurement data, the Mann–Whitney
U test for independent samples was used to assess statistical differences
of the evaluated characteristics. Since comparing three groups of
subjects regarding one parameter, necessary adjustment for multiple
comparisons were considered by the Dunnett method24 with a coef-
ficient of 3. Because of the small number of subjects, the nonparamet-
ric tests were applied on the level of significance of $P < 0.05$ for each
evaluated parameter. Nonparametric statistics were calculated (SPSS,
Chicago, IL) 11.0 for Windows.

RESULTS

Circulation Parameters

Mean systemic blood pressure did not vary significantly be-
tween groups at the beginning of the examination and did not
change significantly within any group during the entire exam-
ination period.

Measured Retinal Vessel Reactions

Average power spectra for the three age groups are repre-
sented in Figure 5. The sequence of phases was chosen accord-
ing to increases in diameter of the vessel from left to right.

In Figure 6, HFW expressed by SEF is demonstrated accord-
ing to the phase of arterial reaction. SEF was significantly
different between the young group and the senior group in
each phase ($P < 0.05$; Dunnett test). SEF was also significantly
different between the young group and the middle-aged group
in dilation, constriction, and relaxation ($P < 0.05$). No signifi-
cant difference could be found between the young group and
the middle-aged group for baseline tonus.

The statistically significant increase of HFW with age in
arteries is demonstrated for each phase of the vessel reaction in
Figure 7.

DISCUSSION

The present study investigated whether changes in vascular
configuration of retinal vessels can be observed in different age
groups. We used a noninvasive functional method measuring
the blood column within a vessel segment. The functional
stimulus applied here was flicker light, using the effect of
neurovascular coupling and observing dilation and constriction
of the vessel after stimulus application.

Our main finding is that the blood column diameters of
retinal arteries show a significantly higher degree of irregularity
in older subjects than in younger subjects. The irregularities
are likely the result of microirregularities of the inner arterial
wall. Such age-dependent findings have been reported in larger
conduit arteries such as the carotid artery for the intima media
thickness.25 The technology to investigate this phenomenon in
much smaller arteries in the microcirculation has not existed
thus far. Because retinal vessels are easily observable and are
part of the microvasculature, they can be used as a substitute
for the assessment of small vessel disease.

The primary functional characteristic of vessels is lumen
diameter. The diameter determines vessel resistance. The di-
ameter, in turn, is determined by the active properties and
structural properties of the vessel. Effectors for displaying the
active properties of a vessel are the smooth muscle cells, their
number, their arrangement, and their state of contraction. The
latter is massively influenced by the endothelial cells and their
mediators.
Functional endothelial changes are seen in atherosclerosis, arterial hypertension,26–28 diabetes mellitus,29 ischemia,30,31 and cardiovascular disease.30 Several studies indicate that aging and endothelial dysfunction progress in parallel.4,32,33 Vascular tone depends on a balance between vasodilators (e.g., endothelium-derived nitric oxide) and vasoconstrictors (e.g., endothelin), with reduced formation of vasodilators resulting in vasoconstriction. Endothelial cells play an important role in modulating the microvascular tone and autoregulation. In neurovascular coupling, vessel dilation is caused by a local increase of endothelium-derived nitric oxide triggered by the application of light to the retina. The effect can be blocked by N\textsuperscript{G}-monomethyl-L-arginine,34 which highlights the importance of nitric oxide and regular endothelial function for a normal neurovascular coupling effect.

In their review on aging, Ferrari et al.3 describe the migration or proliferation of vascular smooth muscle cells infiltrating the subendothelial space, increased deposition of collagen, elastin, and proteoglycans, and increased blood cells. Lundberg and Crow35 show impaired signal transduction pathways in vascular smooth muscle cells when examining the ability of older cells to respond to inhibitors. If these changes were nonuniformly distributed along an artery, these pathologic processes could cause irregularities in the longitudinal vessel profile.

In our study, we found a statistically significant difference in the arteriolar longitudinal vessel profile between the young group and the senior group regardless of the dilation state. In older healthy persons, greater numbers of small undulations were found. The younger group demonstrated lower frequency diameter changes. Interestingly, this pattern was undisturbed by stimulation and was kept constant in the dilation and constriction states caused by neurovascular coupling.

It is known that the relationship between arteriolar and venous diameter changes with age.36 In older age, arteries have smaller diameters in relation to the retinal veins independently of any other arteriosclerotic risk factors. This could be an effect in addition to the more irregular structure of the vascular profile, caused by the same pathophysiology.

The middle-aged group also showed interesting findings. At baseline, longitudinal vessel profiles amounted to values between those of the young and senior groups without reaching statistical significance. However, during all functional stimulated states (dilation, constriction, and relaxation), the middle-aged group differed significantly from the young group and was almost identical with the senior group (Fig. 6). We did not find significant differences in arteriolar longitudinal vessel profiles during stimulation in the middle-aged group, indicating that in the unstimulated state, the vessel wall in middle-aged persons is still similar to that in young persons. However, when metabolic demand by stimulation through mediators causes a muscular reaction, this pattern changes to the one encountered in older age. Therefore, in middle age, there is already some functional impairment in vascular reaction after flicker light stimulation. Previously, we investigated longitudinal arterial profiles in glaucoma using another type of functional provocation and found a similar phenomenon. In a group of glaucoma patients, baseline SEF values were not different from those of age-matched controls, but during dilation there was a significant change in SEF with even more irregular values.11 This underscores the fact that early age-related and pathologic changes in longitudinal arterial structure might be discovered only in the dynamic vessel behavior after provocation.

An explanation for our findings and the reduced arteriovenous relation in older age could be a functional one, with smooth muscle cells exhibiting more pronounced constriction during baseline with increasing age. This may be attributed to endothelial dysfunction and may have several effects. Dilation may be defective even though the endothelium is intact, which may contribute to increases in vasoconstrictor response.37 Vasodilation may be attenuated or reversed to vasoconstriction in response to vasoactive products released by activated plate-
Impaired endothelium-dependent relaxation may contribute to augmented vasoconstriction by serotonin released by platelet aggregation. Increased destruction of nitric oxide may play an important role in the impairment of endothelium-dependent relaxation; thus, the formation of nitric oxide may be normal or even increased, but the increased degradation of nitric oxide may result in impaired vasodilatation.

Impaired production or impaired reaction to mediators could explain a more irregular appearance of the wall, depending on local expression of the changes.

Another explanation for our findings of more high-frequency and more irregular longitudinal vessel profiles with age could be a structural one implying that in older age, similar to the state in hypertension, a smaller lumen exists with normalized and functioning smooth muscle cells. Because the diameter of the lumen is reduced, even small irregularities in smooth muscle cell status are reflected in a more pronounced way. Gerontology research by Ferrari et al. demonstrated that although arteries of healthy elderly subjects have no endothelial lesions or discontinuities, endothelial cells can be irregularly shaped and have increased height. This fact may also be part of the age-related irregularity we found in the longitudinal arterial profile.

As mentioned, the pattern of wall irregularities did not change during stimulation in the young and the senior groups. Because the middle-aged group demonstrated a functional difference in the stimulated states, we think the feedback mechanism (endothelial cell–smooth muscle cell) in a healthy population is different in different age groups. Whether it is able to react ideally to physiologic stimuli in an adequate way even in older age seems questionable.

The question remains whether more irregular and higher frequency vessel wall diameter changes have any implication. We have learned from fluid mechanical simulations in Newtonian fluids that the irregular rough structure of the internal vessel wall leads to increased resistance to flow (Kotliar KE et al. IOVS 2006;47:ARVO E-Abstract 469), which primarily will lead to decreases in blood flow. Consistent peripheral demand could in consequence lead to an increase in blood pressure to supply the need. Our findings might therefore be an explanation for the increase in blood pressure in older populations. In addition, the adherence of cellular blood components might be increased by more irregular structures.

There is one further point to consider in the interpretation of our data: RVA data acquisition was performed by assessing gray values in an image. RVA, therefore, measures what it detects optically. In our case, it was not the inner diameter of a vessel but the thickness of the red blood cell (RBC) column without the blood plasma layer. The irregularity of measured RBC–column profiles might be related to the microirregularities of the inner arterial wall, as described. The low frequent waviness of the vessel longitudinal profile is obviously a property of the vessel wall configuration. For high-frequency waves, a fluid mechanical effect might also be considered. A mathematical model for non-Newtonian media, by Khantuleva et al., describes self-organization and self-regulation effects in open systems. It explains systems adapting to varying conditions and models the changes during transitions caused by the
interacting underlying mechanisms. For blood flow in small vessels, it includes the possibility of a random local formation of small vortex pulsations near the vessel wall (at the border of the plasma layer), dependent on flow parameters, rheological conditions, and geometrical configuration of the vessel. Consequently, the effects on microirregularities of the RBC-column found in our clinical experiments could also be attributed to these self-regulated, non-Newtonian, hemodynamic effects in the blood flow and not only to changes in the vessel wall. This must be validated in further experimental and numerical studies.

Assessment of microirregularities of the vessel lumen applying contemporary methods of image analysis is now widely performed in clinical medicine. Characterization of microirregularities of the arterial inner wall using mathematical methods, including frequency analysis, has been recently published by several groups analyzing intima media configuration in the carotid artery.\textsuperscript{42–44} Labropoulos\textsuperscript{42} report that “with increased age and number of risk factors present, the wall/blood interface in the arteria carotis became more irregular.” However, those attempts have been mostly limited to large vessels using parameters such as, for example, intima media thickness, or pulse wave velocity. Microcirculation plays an important role in tissue metabolism. Vascular characteristics of the microcirculation thus far are difficult to examine noninvasively in vivo. The retina is unique in the body. Its vasculature resembles that of the CNS and is easily accessible by noninvasive optical methods. Our findings in retinal arteries represent the central microcirculation and complement the knowledge acquired for microirregularities in large vessels.

In summary, in the present study, the evaluation of functional retinal arterial vessel reactions was possible, the characterization of microirregularities was feasible, and a difference between older and younger healthy subjects was seen. The validity of the longitudinal vessel profile measurement and the clinical implications of our findings remain to be evaluated. A study in a hypertensive population would be helpful for a greater understanding of the value of irregular vessel wall formation.

References