



HAL
open science

Pulse-wave velocity in retinal arteries of healthy volunteers

Konstantin Kotliar, Marcus Baumann, Walthard Vilser, Ines M. Lanzl

► **To cite this version:**

Konstantin Kotliar, Marcus Baumann, Walthard Vilser, Ines M. Lanzl. Pulse-wave velocity in retinal arteries of healthy volunteers. *British Journal of Ophthalmology*, 2010, 95 (5), pp.675. 10.1136/bjo.2010.181263 . hal-00589717

HAL Id: hal-00589717

<https://hal.science/hal-00589717>

Submitted on 1 May 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Pulse-wave velocity in retinal arteries of healthy volunteers

Konstantin E. Kotliar^{1,2}, Marcus Baumann², Walthard Vilser^{3,4}, Ines M. Lanzl¹

¹Department of Ophthalmology, Klinikum rechts der Isar, Technische Universität München, Ismaninger Str. 22, 81675 Munich, Germany.

²Department of Nephrology, Klinikum rechts der Isar, Technische Universität München, Ismaninger Str. 22, 81675 Munich, Germany.

³Department of Biomedical Engineering, Ilmenau University of Technology, Gustaf Kirchhoff Str. 2, 98693 Ilmenau, Germany;

⁴ IMEDOS Systems UG, Am Naßtal 4, 07751 Jena, Germany.

Running title: Pulse wave velocity in retinal arteries

Word count: 2679

Corresponding author: Konstantin Kotliar, Department of Nephrology, Klinikum rechts der Isar, Technische Universität München, Ismaninger Str, 22, 81675 Munich, Germany.

E-mail: kotliar@lrz.tum.de Tel: + 49 (89) 4140-6890; Fax: +49 (89) 4140-4858

The Corresponding Author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive licence (or non exclusive for government employees) on a worldwide basis to the BMJ Publishing Group Ltd and its Licensees to permit this article (if accepted) to be published in BJO editions and any other BMJ PGL products to exploit all subsidiary rights, as set out in the licence of BMJ Publishing Group Ltd

Competing Interest: WV: director of Fa. IMEDOS Systems, which developed and commercializes Dynamic Vessel Analyser. KK, MB, IL: None to declare.

ABSTRACT

Background/aims: Pulse wave velocity (PWV) in large vessels has been extensively used in clinical practice as an indirect measure of arterial stiffness and an indicator of cardiovascular risk factors. Arterial stiffness increases with age and in coronary arterial disease. An in-vivo clinical method to characterize arterial stiffness of the central microcirculation was developed.

Methods: Time dependent alterations of retinal vessel diameter were examined by the Dynamic Vessel Analyzer in a randomly chosen eye of 10 young 26.0(23.5 – 27.0) years old [median(1.quartile – 3.quartile)] and 10 aged 67.0(61.3 – 69.5) years old healthy volunteers. Two segments of a retinal artery were measured simultaneously. The distance between those segments was measured using retinal photographs. The data was filtered and analyzed using methods of signal analysis in order to calculate PWV in the assessed retinal artery (rPWV).

Results: rPWV differed significantly between young: 21.5(17.9 – 34.6) mm/s and aged volunteers: 243.8(186.1 – 347.7) mm/s (P=0.0001, Mann-Whitney test with Bonferroni correction).

Conclusions: This study demonstrates higher retinal PWV in elderly. Therefore our measure resembles large artery PWV. This suggests that dynamic in-vivo imaging of the central microcirculation allows to measure local microvascular stiffness in a commercially available medical device.

Key words: retinal vessels, arterial stiffness, aging, arteriosclerosis

INTRODUCTION

The vascular system stiffens gradually because of the combined effects of ageing, high blood pressure, and other vascular risk factors.[1] Large artery stiffness is non-invasively measured by pulse wave velocity (PWV).[2] The aortic PWV appears to be of high prognostic relevance for cardiovascular events.[3] Population- and patient-based cohorts demonstrated a strong association between increased aortic PWV and age,[4] coronary artery disease,[5] myocardial infarction,[6] heart failure,[7] stroke,[8] and hypertension.[9]

By contrast, no microvascular measure of stiffness exists. O'Rourke and Safar [10] hypothesized that cerebral microvascular disease results from damaging forces of abnormal flow pulsations extending into small cerebral arteries as a consequence of arterial stiffening. The eye with its unique property of direct non-invasive assessment of small retinal arteries seems an ideal organ to gain insight in microvascular stiffness. Moreover, retinal arteries reflect the central microcirculation, thus mirror the cerebral vasculature and could be associated with stroke. Finally, structural changes in the retinal vasculature are clinical predictors of systemic vascular disease.[11]

The present study was undertaken to assess the possibility of measuring PWV in retinal arteries (rPWV). Since age is an important factor influencing large artery stiffness and hence PWV in large arteries increases with age,[1-2] we hypothesized in this study that PWV in retinal arteries increases with age similar to large artery PWV. We used a non invasive approach of imaging a sequence of retinal vascular diameter changes with an ophthalmoscope. Data image and signal analyses were applied to determine rPWV.

MATERIALS AND METHODS

Subjects

Anamnestically healthy volunteers recruited at the Department of Ophthalmology at Munich University of Technology were enrolled in this study. One randomly chosen eye of each participant was measured. The volunteers were divided into two groups: 10 young subjects (6 female, 4 male, age: 26.0 (23.5 – 27.0) years [median(1.quartile – 3.quartile)]) and 10 old subjects (7 female, 3 male, age: 67.0 (61.3 – 69.5) years). History of smoking, drug or alcohol habits was recorded and an ophthalmic examination was performed in all subjects.

Inclusion criteria were: no history of systemic or ocular diseases; no systemic or topical medication including contraceptive pills at the time of the examination and no history of chronic medication; no history of smoking, drug or alcohol abuse; no cardiovascular events; visual acuity 0.8 or better; ametropia with spherical equivalent within -3.5 and 3.5 dpt; astigmatism 1 dpt or less; intraocular pressure less than 20 mmHg measured with Goldmann applanation tonometry; no pathologic findings in slit lamp examination.

Informed consent was obtained from all the subjects. The study protocol was reviewed by the ethics committee of Klinikum rechts der Isar (Munich University of Technology). All procedures adhered to the tenets of the Declaration of Helsinki.

Measurements

30 minutes after pupil dilation with 3 consecutive drops of topical tropicamide (Mydriaticum Stulln; Pharma Stulln Ltd., Germany) applied ten minutes apart, continuous measurement of retinal arterial diameter was performed for one minute using the Retinal Vessel

Analyzer (RVA, IMEDOS Systems, Jena, Germany). The fellow eye was covered during examination in order to improve fixation of the volunteer. Blood pressure and pulse rate were recorded before and immediately after the measurement.

Properties of RVA and its measurement principles have been previously described.[12] Briefly, the device allows noninvasive online assessment of vessel diameter, depending on times and locations along the vessel. For that purpose the RVA consists of a retinal camera (450 FF; Carl Zeiss, Jena, Germany), a CCD camera for electronic online imaging, and a personal computer for system control, analysis, and recording of the obtained data.

Off-line measurements

After the actual data acquisition measurements of the arterial reactions at two distinct measuring vessel segments were taken off-line from video tape recordings with the novel version of RVA named Dynamic Vessel Analyzer (DVA). In comparison to RVA, DVA possesses better measurement accuracy and allows simultaneous measurements of two vessel segments, which was crucial for the study. The peculiarities of DVA and its improvements compared to RVA were previously described.[12-13]

For off-line measurement a template window reference box was positioned at a bifurcation or at a vessel intersection in the inferior or superior temporal part of the fundus one to three optic disc diameter away from the rim of the optic disc. Two temporal arterial segments: a proximal (1) and a distal one (2) of approximately 300 MU in length (corresponding to 300 μm in an emmetropic eye) were chosen within the template window for the simultaneous assessment

fig 1

(fig 1A).

Distance measurement

Standard fundus photographs, showing the location of the measured vessel segments (fig 1A) were exported from DVA-program and then imported into VisualIS (IMEDOS Systems, Jena, Germany). The distance between midpoints of the measured segments was assessed in pixels and recalculated in μm .

Data evaluation

A template with corresponding macros in a spreadsheet (MS Excel 2000) was created to filter, process, and analyze the numerical data from the DVA and the VisualIS.

In order to filter and analyze the measured samples Fast Fourier Transform [14] (FFT) was used, an algorithm to compute the Discrete Fourier transform (DFT) and its inverse. The following common applications of FFT were used in the present study: frequency data filtering and cross-correlational analysis. A program based on FFT was created in MATLAB 7.0 for automated data processing.

From the whole temporal vessel diameter assessments lasting for 1 minute (fig 1B) data sequences were extracted for each subject for further analysis. Data sequences with no more than 0.5 s of discontinuity and less than 10% of whole data missing were analyzed. Discontinuities of less than 0.5 s were filled up by a macros program developed in MS Excel. Missed temporal points were linearly interpolated between the measured points.

Following preliminary tests on rPWV calculations from data sequences of different length extracted from one minute assessments (128, 256, 512 and 1024 temporal points), a standard length of the analyzed samples of $2^8=256$ temporal points (≈ 10.2 s) was chosen for each subject, since FFT uses data samples of the length, which is a power of 2. This was a

compromise between a higher precision of the evaluation, necessity of continuous analyzed data sequences and the reduction of the influence of heart rate variability

fig 2

Both continuous analyzed samples from original temporal vessel diameter assessments of a proximal (1) and a distal (2) arterial segment (fig 1) were filtered in two steps using band pass frequency filters. Both filtered samples assessed in (1) and (2) were slightly shifted to each other (fig 1C). Cross-correlation function of these filtered signals was calculated. Its absolute maximum showed the temporal phase shift between the assessed samples (fig 2).

The sampling rate of DVA assessment of 40 ms was insufficient for most measured temporal phase shifts in the present study. For more precise assessment of the time shift from the cross-correlation function the latter was interpolated near the absolute maximum of the function with a polynomial [15] using MS Excel. The maximum of this fit was calculated and considered as the absolute maximum of the cross-correlation function with the corresponding temporal lag being the desired phase shift between vessel diameter assessments in segments 1 and 2 (fig 1).

Calculation of pulse wave velocity

Pulse wave velocity was calculated in mm/s for each individual case using the conventional formula:

rPWV = distance between analyzed segments / phase shift between vessel diameter assessments.

Since rPWV-value depends on absolute vessel diameter, we additionally calculated diameter independent normalized rPWV-values in 1/s as:

$$rPWV_n = 2 * rPWV / [vessel\ diameter(place1) + vessel\ diameter(place2)].$$

Statistical Analysis

Because it was impossible to prove normal distribution of measurement data, non-parametric statistics was applied for the evaluation. Values of measured and calculated parameters are represented in the following form: median (1.quartile; 3.quartile). Both groups were compared by Mann-Whitney test. Parameters within the groups were compared by Wilcoxon test. Due to the small number of subjects non parametric tests were applied on the level of significance of $p = 0.05$ for each evaluated parameter. Non parametric statistics were calculated by MS Excel, SPSS and by Primer of Biostatistics, v. 4.03 by Glantz.[16]

In order to avoid errors in multiple testing Bonferroni correction [16] was used to report significant differences between the groups.

RESULTS

Principal results of the study are shown in the table 1. P-values in the table 1 are represented as local values, as a result of group comparison for each parameter.

Circulation parameters

The mean systemic blood pressure and the pulse differ significantly between groups at the beginning of the examination ($P=0.00007$; table 1, $P=0.0009$ after Bonferroni correction). These circulation parameters did not change significantly within any group during the examination ($P=0.45$).

Pulse and cardiac period values calculated during mathematical analysis of DVA-assessments did not differ significantly between the groups ($P=0.105$; table 1). However, young volunteers showed higher variability of pulse and period within the group than the elderly.

Table 1. Principal results of the study.

	young volunteers (n=10)	elderly volunteers (n=10)	significance (local P-value)
	<i>median (1.quartile – 3.quartile)</i>		
age, years	26.0 (23.5 – 27.0)	67.0 (61.3 – 69.5)	0.00001
mean systemic blood pressure, mmHg	79.7 (74.1 – 82.9)	100.3 (93.4 – 112.7)	0.00007
systemic pulse rate, 1/min	59.0 (55.0 – 60.0)	68.5 (61.0 – 72.8)	0.105
arterial diameter, proximal segment, μm	105.5 (98.3 – 115.7)	109.8 (105.4 – 124.4)	0.290
arterial diameter, distal segment, μm	104.8 (98.6 – 116.5)	112.2 (100.9 – 126.5)	0.545
pulse rate (retinal arteries), Hz	0.98 (0.90 – 1.12)	1.07 (1.07 – 1.15)	0.096
period (retinal arteries), s	1.02 (0.90 – 1.11)	0.93 (0.87 – 0.93)	0.096
segmental distance, μm	6475 (5537 – 6919)	6671 (6141 – 7139)	0.496
segmental phase shift, s	0.30 (0.18 – 0.37)	0.03 (0.02 – 0.04)	0.00002
segmental phase shift, $^{\circ}$	134 (48 – 144)	11 (7 – 14)	0.00002
rPWV, mm/s	21.5 (17.9 – 34.6)	243.8 (186.1 – 347.7)	0.00001
normalized rPWV_n, 1/s	424 (312 – 709)	4380 (3431 – 5345)	0.00001

Assessment of retinal arterial parameters and retinal pulse wave velocity

Average diameters of both measured arterial segments differ neither between the proximal and distal location in both groups ($P=0.350$) nor between the groups ($P=0.290$; $P=0.545$; table 1). This fact substantiated the rationale to compare rPWV values between the groups.

Young healthy volunteers showed a significantly larger phase shift between filtered oscillations of retinal arterial diameter than old volunteers (table 1, fig 1C, $P=0.0003$ after Bonferroni correction). Thus, in retinal arteries of young volunteers the pulse wave needs more time to propagate between the measured segments than in arteries of old volunteers. Locations of assessed vessel segments and distances between them were determined only by the structure of the vessel tree and did therefore not differ between the groups ($P=0.496$; table 1).

fig 3

rPWV values calculated from the phase shift and the distance between measured vessel segments differ tenfold between the groups ($P=0.0001$ after Bonferroni correction) and amounted to 21.5 (17.9 – 34.6) mm/s in young volunteers and to 243.8 (186.1 – 347.7) mm/s in old volunteers (table 1, fig 3, left panel). Normalized rPWV_n amounted to 423 (312 – 709) 1/s in young and to 4380 (3431 – 5345) 1/s in old volunteers ($P=0.0001$ after Bonferroni correction).

DISCUSSION

This is the first report on quantitative values of retinal artery PWV using a non-invasive, commercially available device. We demonstrated that rPWV is low in young and high in old volunteers potentially reflecting age-related vascular stiffness as described for large arteries.[2]

Historically the assessment of vascular pulsations in the eye has been limited to the choroidal circulation.[17-18] Assessment of pulsations of the blood flow in a single location for example in the optic nerve head, retinal, and choroidal circulation, is possible using the method described by Petrig et al.[19] An actual pulse-wave propagation from the heart to the ophthalmic artery and choroidal circulation has been estimated at 4.08 m/s in a study of healthy subjects by Michelson et al.[20] Indirect measurements of retinal vessel pulse are complicated by the fact that the choroidal circulation constitutes most of the ocular blood flow [21] and determines most of the pulse amplitude.[18]

Pulse wave velocity in the microvessels was observed to be two orders of magnitude slower than in the aorta.[22] These experimental findings were shown to be consonant with linear pulse wave transmission theory in a branching system of vessels.[23] Thus the rPWV values reported in the present study seem to be consistent with contemporary vascular physiology.

In 2000 Otto reported quantitative data on PWV in retinal arteries by analysis of motion compensated angiography image sequences.[15] Otto reported rPWV values of 24-26 mm/s in retinal arteries of a healthy 30-years volunteer; 240 mm/s in a healthy 63 years volunteer and

>300 mm/s in a 45-years patient with arterial hypertension.[15] These estimated rPWV values are in accordance with our measurements.

Main limitations of the method by Otto were its invasiveness and the necessity of correction to the sampling rate of the laser scanning system. By contrast, our model is non-invasive and reproducible based on a commercially available DVA-system.[12]

Using DVA-system Gugleta et al. recently analyzed phase delay between retinal arterioles and venules and used it to calculate choroid-to-retina pulse delay.[13] In their study healthy young vasospastic subjects demonstrated an altered phase delay between the arterioles and venules, presumably indicating faster pulse-wave propagation in the retinal arteriolar tree. However, rPWV was not calculated.

Considerations of the technical peculiarities and limitations of our method must be elucidated to enable better interpretation of our reported results.

Limitations in image resolution of DVA and corresponding sampling rate of 25 measurements a second restrict retinal PWV assessment to a limited range. Assuming the distances between two measured arterial segments in the range of 4.5÷9.5 mm, as in the present study, one can derive the maximal measurable rPWV-value being in the range of 112.5÷237.5 mm/s. Additionally inaccuracy of rPWV measurements from raw DVA data increases with the measured value. Calculating with a standard formula for indirect measurements for the distance of 5 mm between the measured arterial segments the inaccuracy of rPWV values would amount to: ± 0.8 mm/s for rPWV=10 mm/s (i.e. $\pm 8\%$), ± 20 mm/s for rPWV=50mm/s ($\pm 40\%$) and ± 80 mm/s for rPWV=100mm/s ($\pm 80\%$). In the present study we used mathematical signal processing of raw DVA data. This allows to measure rPWV-values up to 5000 mm/s and to attain higher

measurement accuracy, which amounts to approx. $\pm 4.0\%$ for $rPWV=20\text{mm/s}$, $\pm 4.5\%$ for $rPWV=100\text{mm/s}$ and $\pm 10.8\%$ for $rPWV=500\text{mm/s}$.

One potential limitation is that one randomly chosen retinal artery was chosen to assess $rPWV$. Since the whole retinal vasculature undergoes age related changes of vessel structure and biomechanics we assumed that $rPWV$ values in all large retinal vessels of a healthy subject are of the same order and depend on the vessel diameter and other factors. We tested this assumption in some eyes (see an example in fig 4) and concluded that it is reasonable. However the reliability of the assumption needs to be elucidated in future studies.

fig 4

As shown in figure 4, some retinal artery segments are almost straight, whereas others are not. Arterial tortuosity may influence retinal PWV and needs to be elucidated in future studies, in particular as vascular disease is often associated with arterial tortuosity.

Usually aortic PWV increases by 30-50% from 20 to 70 years of age.[2, 24] In the present study an almost tenfold increase of retinal PWV for the similar age range was found. There is no direct information in the literature up to date which can confirm or reject this measured tenfold age-related increase of $rPWV$. Since small arteries are shown to be much more elastic than large arteries [23], which predict corresponding $rPWVs$ being in the range of cm/s , one cannot exclude, that aging process in small arteries differs from this process in large arteries.

In this first report on $rPWV$ assessment in retinal arteries using RVA we tested the method in a small cohort of anamnestic healthy volunteers in order to reveal possible aging effect on the retinal PWV values and to estimate their normal range. The broad range of retinal PWV in the examined elderly subjects may reflect a broad band of cardiovascular risk factors in this group compared to the examined young group, rather than the decrease of measurement

accuracy of the method at high rPWV-values. However, in this pilot study neither intersession nor interobserver variability was measured. Thus, the broad range of rPWV values in elderly subjects may reflect the case of measurements variability.

Further studies with a more detailed description of the cardiovascular risk factors of the examined participants need to be performed in order to give a perspective to the clinical usage of the method and to reveal its potential value for differentiating healthy from pathological values of retinal PWV.

In summary, we proposed a method to investigate retinal PWV (rPWV). Our method is based on the phase delay between vessel pulsations at different places along a measured vessel, which is similar to common approaches of rPWV measurements in large vessels.[24] Our results are in accordance to theoretical considerations of PWV in microvessels and in accordance with invasive measures of retinal PWV. Finally, we show an age-related increase of retinal PWV suggesting that aging of retinal arteries is associated with vascular stiffness similar to large arteries. Whether retinal PWV mirrors the cerebral vasculature and is associated with cerebrovascular disease needs to be examined in further studies.

REFERENCES

- 1 Benetos A, Waeber B, Izzo J, et al. Influence of age, risk factors, and cardiovascular and renal disease on arterial stiffness: clinical applications. *Am J Hypertens* 2002;15(12):1101-8.
- 2 Asmar R, Benetos A, Topouchian J, et al. Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies. *Hypertension* 1995;26(3):485-90.
- 3 Laurent S, Cockcroft J, Van Bortel L, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J* 2006;27(21):2588-605.
- 4 Nichols WW, O'Rourke MF, Avolio AP, et al. Effects of age on ventricular-vascular coupling. *Am J Cardiol* 1985;55(9):1179-84.
- 5 Weber T, Auer J, O'Rourke MF, et al. Arterial stiffness, wave reflections, and the risk of coronary artery disease. *Circulation* 2004;109(2):184-9.
- 6 Hirai T, Sasayama S, Kawasaki T, et al. Stiffness of systemic arteries in patients with myocardial infarction. A noninvasive method to predict severity of coronary atherosclerosis. *Circulation* 1989;80(1):78-86.
- 7 Nichols WW, Pepine CJ. Ventricular/vascular interaction in health and heart failure. *Compr Ther* 1992;18(7):12-9.
- 8 Laurent S, Katsahian S, Fassot C, et al. Aortic stiffness is an independent predictor of fatal stroke in essential hypertension. *Stroke* 2003;34(5):1203-6.
- 9 Laurent S, Boutouyrie P, Asmar R, et al. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension* 2001;37(5):1236-41.

- 10 O'Rourke MF, Safar ME. Relationship between aortic stiffening and microvascular disease in brain and kidney: cause and logic of therapy. *Hypertension* 2005;46(1):200-4.
- 11 Walsh JB. Hypertensive retinopathy. Description, classification, and prognosis. *Ophthalmology* 1982;89(10):1127-31.
- 12 Garhofer G, Bek T, Boehm AG, et al. Use of the retinal vessel analyzer in ocular blood flow research. *Acta Ophthalmol*. Published Online First: 14 August 2009. doi: 10.1111/j.1755-3768.2009.01587.x
- 13 Gugleta K, Kochkorov A, Katamay R, et al. On pulse-wave propagation in the ocular circulation. *Invest Ophthalmol Vis Sci* 2006;47(9):4019-25.
- 14 Brenner N, Rader C. A New Principle for Fast Fourier Transformation. *IEEE Acoustics, Speech & Signal Processing* 1976;24:264-266.
- 15 Otto TP. Modellierung des retinalen Blutflusses durch Auswertung bewegungskorrigierter Angiographie Bildfolgen. PhD-Thesis. Heidelberg: Ruprecht-Karl-Universität 2000: 135.
- 16 Glantz SA. *Primer of biostatistics*. New York, St. Louis, San Francisco: McGraw-Hill; 1999.
- 17 Mittag TW, Serle J, Schumer R, et al. Studies of the ocular pulse in primates. *Surv Ophthalmol* 1994;38 Suppl:S183-90.
- 18 Silver D, Farrell R, Langham M. Estimation of pulsatile ocular blood flow from intraocular pressure. *Acta Ophthalmologica* 1989; 191(Suppl):25-29.
- 19 Petrig BL, Riva CE, Hayreh SS. Laser Doppler flowmetry and optic nerve head blood flow. *Am J Ophthalmol* 1999;127(4):413-25.
- 20 Michelson G, Grundler A, Steinmeier R, et al. Simultaneous measurement of ocular micro- and macrocirculation, intraocular pressure, and systemic functions. *Ger J Ophthalmol* 1994;3(1):48-53.

- 21 Cioffi GA, Granstam E, Alm A. Ocular circulation. In: Kaufmann PL, Alm A, editors. Adler's physiology of the eye. St. Louis, London: Mosby 2003: 747-784.
- 22 Intaglietta M, Pawula RF, Tompkins WR. Pressure measurements in the mammalian microvasculature. *Microvasc Res* 1970;2(2):212-20.
- 23 Salotto AG, Muscarella LF, Melbin J, et al. Pressure pulse transmission into vascular beds. *Microvasc Res* 1986;32(2):152-63.
- 24 Busse R. *Kreislaufphysiologie*. Stuttgart, New York: Thieme 1982: 264.

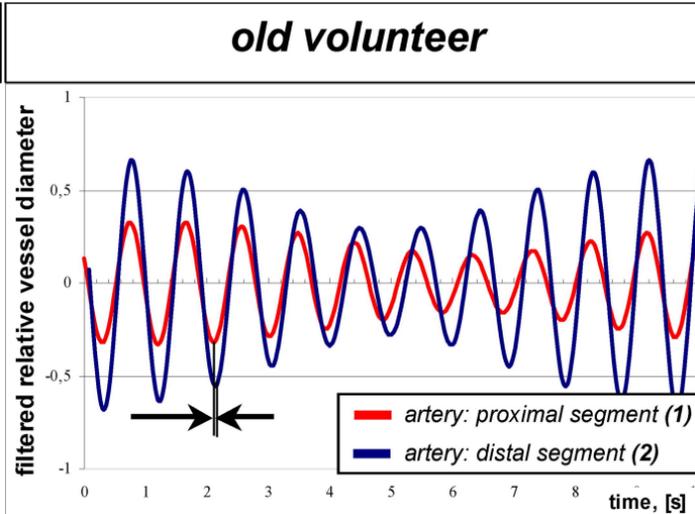
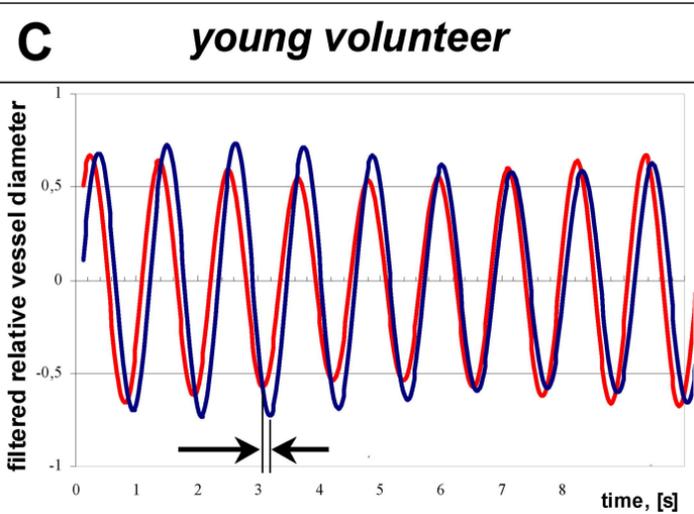
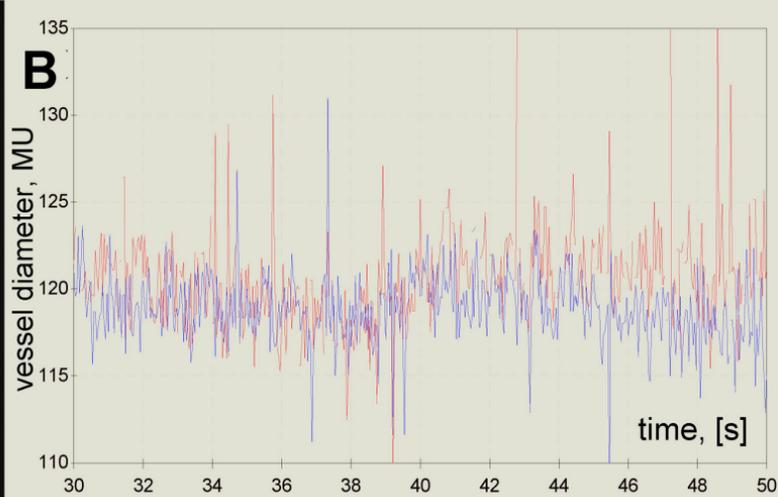
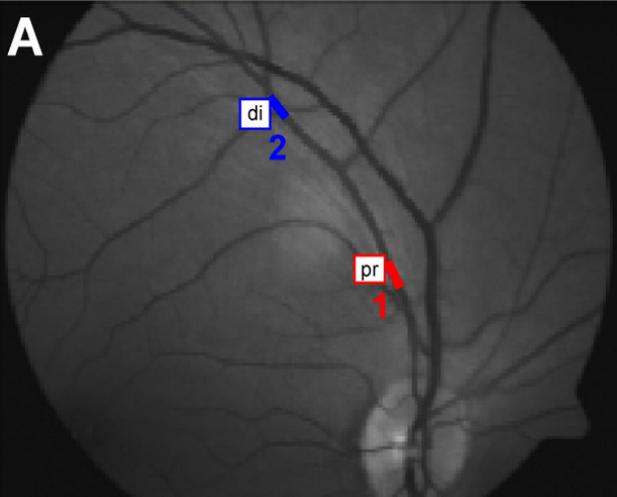
FIGURE LEGENDS

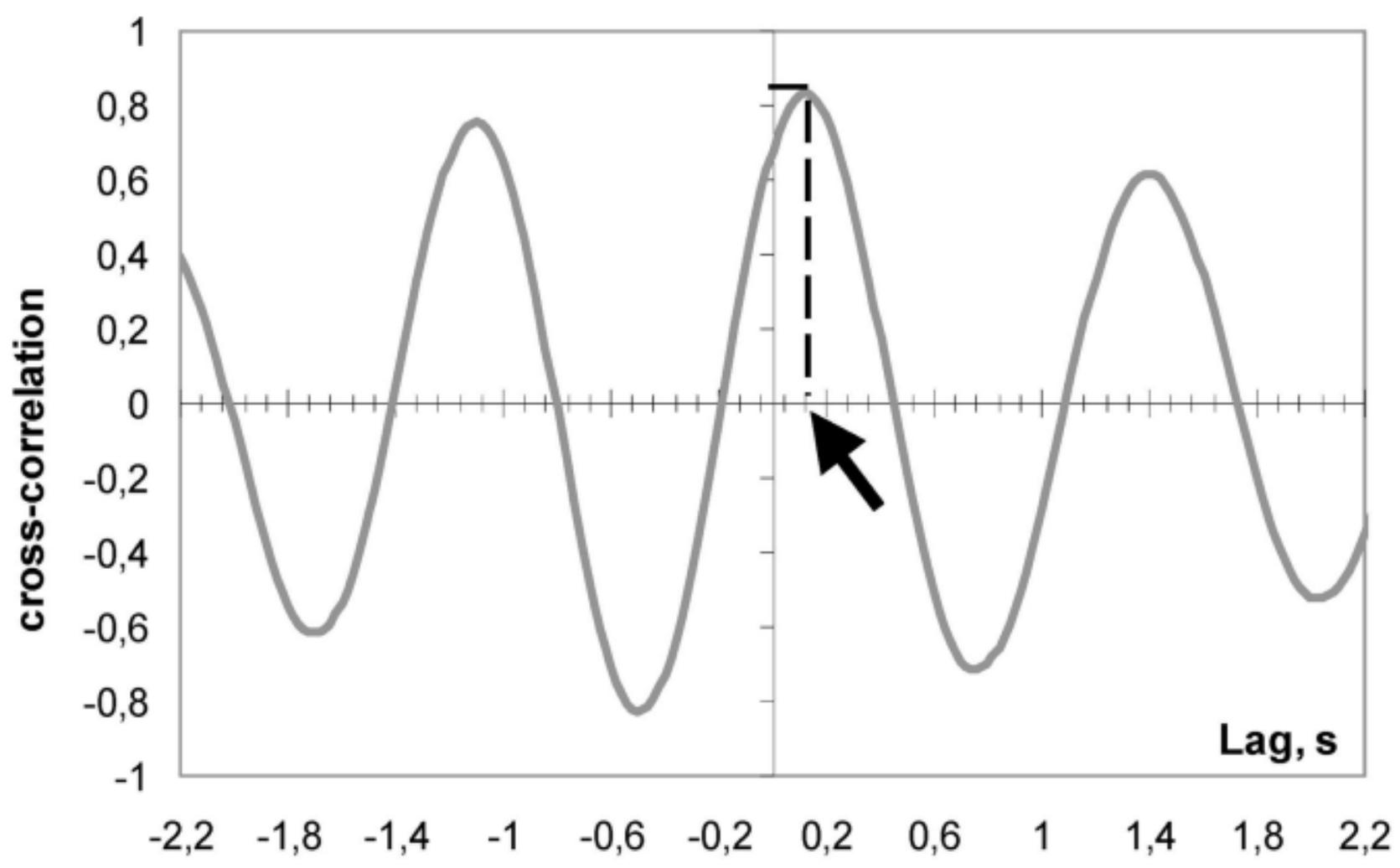
Figure 1. Continuous assessment and filtering of temporal vessel diameter changes in two retinal arterial segments: (1) proximal (red) and (2) distal (blue). **A:** Location of measured segments in the fundus: the pulse wave propagates from 1 to 2. **B:** Simultaneous DVA assessment in segments 1 and 2: vessel diameters are measured in relative units, which correspond to 1 μm in the Gullstrand's eye. Chaotic high frequency peaks of 5-20 MU in the time course due to eye blinks are eliminated using signal analysis. **C:** Characteristic examples of filtered time courses like in **B**. The red curve corresponds to the proximal arterial segment (1), the blue curve pertains to the distal one (2). Since the pulse wave propagates from the proximal to the distal location, the blue curve is delayed compared to the red one. This time delay is larger in a young volunteer than in a senior volunteer: a pulse wave needs more time for its propagation in an elastic retinal artery of a young volunteer than in a stiffer artery of an old volunteer. Thus the velocity of such propagation is lower in the young volunteer.

Figure 2. Cross-correlation function of filtered time courses in fig 1C, left panel. A lag of the maximal point of the cross-correlation function (black arrow) equals 0.12 s and corresponds to the phase shift between both filtered time courses. The lag is positive, since the first signal from the proximally located arterial segment has been assessed before the second one from the distally located segment.

Figure 3. The value of the calculated parameter retinal pulse wave velocity (rPWV) differs significantly between both groups: $P=0.0001$, Mann-Whitney Test with Bonferroni correction. These results show increased retinal arterial stiffness in old healthy volunteers compared to the young ones.

Figure 4. Pulse wave velocity values in different arterial segments of both eyes of a young healthy volunteer are of the same magnitude. Since the rPWV values are dependent on the absolute vessel diameter, the corresponding rPWV_n values show even more stable results. rPWV_n in this example amount to: 308; 293; 286; 330 and 312 1/s (counterclockwise, starting from the left upper corner correspondingly) with coefficient of variation of 5.6%.





pulse-wave velocity, mm/s

800
700
600
500
400
300
200
100
0

youths

seniors

