Short-Term Retinal Vessel Diameter Variability in Relation to the History of Cold Extremities

Asan Kochkorov, Konstantin Gugleta, Claudia Zawinka, Robert Katamay, Josef Flammer, and Selim Orgul

PURPOSE. To test whether the regularity and short-term changes of retinal vessel diameter are related to the history of cold hands and feet and to nailfold circulatory response to cooling.

METHODS. In 13 vasospastic and 13 nonvasospastic young healthy women (based on their history of cold extremities and nailfold capillaroscopy) 20- to 30-second recordings of the ocular fundus was obtained with a retinal vessel analyzer. The spatial regularity of arterioles and venules was analyzed by means of the coefficient of variation of vessel diameter and by exploratory Fourier analysis of spatial frequencies. Temporal variability was analyzed as excursion amplitude of the vessel diameter, as a correlation of means and standard deviations of vessel diameter within a defined time period, and by Fourier analysis of temporal diameter change in the heartbeat frequency range.

RESULTS. Mean diameters of selected segments of arterioles (129.9 ± 15.3 and 124.4 ± 24.4 μm) and venules (150.8 ± 14.6 and 149.3 ± 19.6 μm) were not different between the vasospastic and nonvasospastic groups, respectively. Spatial variability: The coefficient of variation in arterioles was 8.8% ± 2.8% and 6.1% ± 1.7%, in venules 5.8% ± 1.4%, and 3.6% ± 0.9% in the vasospastic and nonvasospastic groups, respectively (difference by ANOVA, P = 0.017). Fourier analysis revealed differences between arterioles in the two groups, with relative Fourier power spectrum amplitudes of spatial frequencies higher in vasospastic eyes (Mann-Whitney P = 0.029). Temporal variability: The excursion amplitudes of vessel diameters were comparable in the two groups. Individual coefficients of correlation of successive means and standard deviations of the vessel diameter were 0.11 ± 0.23 and 0.09 ± 0.23 in the nonvasospastic group, and 0.25 ± 0.40 and 0.24 ± 0.22 in the vasospastic group, in the arterioles and venules, respectively (ANOVA: vasospastic versus nonvasospastic; P = 0.038). Fourier analysis in the heartbeat frequency range revealed differences in relative power spectrum amplitudes of temporal frequencies between arterioles in the two groups (higher in the vasospastic group, Mann-Whitney P = 0.029).

CONCLUSIONS. Retinal arterioles in healthy vasospastic women show higher spatial irregularity and an increased vessel diameter variation within the temporal frequency of the heartbeat than do arterioles in nonvasospastic women. (Invest Ophthalmol Vis Sci. 2006;47:4026 – 4033) DOI:10.1167/iovs.06-0177

Vasospasm is defined as inappropriate constriction or insufficient dilatation in the microcirculation, and because such vasoconstrictions are often combined with simultaneous arterial or venous dilatations in neighboring vessels or in other vascular beds, the term vascular dysregulation was introduced. Vascular dysregulation has been associated with several ocular diseases, including glaucoma, central vein thrombosis, nonarteritic anterior ischemic neuropathy, and central serous chorioretinopathy. Changes in retinal vessels have also been observed in patients with vascular dysregulation in other organs, such as the heart or the brain. Evaluating morphology of retinal vessels has been mostly limited to funduscopy, a method with poor reproducibility of measurements, or to static fundus photography. The retinal vessel analyzer offers high spatial vessel width resolution, high temporal resolution down to 40 milliseconds enables dynamic analysis of the vessel diameter changes. Mean retinal vessel diameter was statistically comparable between vasospastic and control subjects. However, dynamic recordings may offer relevant clinical details. To investigate a potential yield of information with such an approach, spatial properties and short-term changes in the vessel diameter of retinal arterioles and venules in vasospastic eyes were analyzed with a retinal vessel analyzer (Retinal Vessel Analyzer [RVA]; IMEDOS GmbH, Weimar, Germany).

METHODS

Subjects

Forty healthy nonsmoking women were screened for the study, in compliance with the tenets of the Declaration of Helsinki. After approval by the ethics committee, we obtained informed consent from the subjects. A notification in the University Eye Clinic of Basel informed potential volunteers (collaborators, students, parents, and friends of patients) of the opportunity to participate in a scientific research project. Subjects were screened for ocular and systemic diseases. Detailed medical and ophthalmic histories were recorded, and all subjects completed an ophthalmic examination. Included were individuals with no history of ocular or systemic disease, no history of chronic or current systemic or topical medication, and no history of drug or alcohol abuse. Further inclusion criteria were a normal systolic (100–140 mm Hg) and diastolic (60–90 mm Hg) blood pressure (BP), a best-corrected visual acuity above 20/25 in both eyes, ametropia within –3.0 to +3.0 D of spherical equivalent and less than 1 D astigmatism in each eye, an intraocular pressure (IOP) lower than 20 mm Hg in each eye by (Goldmann) applanation tonometry, and no pathologic findings after slit lamp examination and indirect funduscopy. Subjects were classified as having vasospasm if they related a clear history of frequently cold hands (answering “yes” to the questions: “do you always have cold hands, even during the summer?” and “do other people tell you that you have cold hands?”) and as healthy subjects if they reported no such history. Vasospastic propensity or the absence of it had to be confirmed by nailfold capillaroscopy, the examiner being unaware of the history of cold hands. Subjects with contradictory findings in nailfold capillaroscopy and history or describ-
Nailfold Capillaroscopy

Nailfold capillaroscopy was performed in a room with a constant temperature of approximately 23°C (range, 21–25°C). Before the examination, the hands of the subjects were warmed in a water bath of 40°C. The skin of the nailfold was made transparent by a drop of oil, rendering the capillaries running parallel to the skin surface and the flow of cellular elements visible under a light microscope. A microscope coupled to a television monitor that was coupled to a video recorder, allowing the observed blood flow to be videotaped and to be analyzed offline was used. After baseline flow recording, the nailfold area was cooled down to 14°C to 15°C for 60 seconds by rapidly decompressing carbon dioxide (gas stream temperature, −15°C), and the occurrence and duration of blood flow standstill were recorded. A closure of one or more visible capillaries with an average stop time longer than 12 seconds was defined as a vasospastic reaction.19

Retinal Vessel Diameter Measurements

Retinal vessel diameter was measured with a retinal vessel analyzer (RVA ver. 4.150.0; IMEDOS GmbH).15,16 An essential part of the RVA device is the fundus camera (FF450; Carl Zeiss Meditec GmbH, Jena, Germany), which allows the examination and recordings of the ocular fundus. It incorporates the illumination and the observation optical pathway. After being reflected from the retina, the light is delivered through the observation pathway to the observation ocular and the charge-coupled device (CCD) chip of the video camera simultaneously. The standard video signal from the CCD then goes to the RVA control computer and to the SVHS recorder, which enables subsequent offline measurements of the recorded session later on. The measuring principle of the RVA is as follows: Inside the walls of the retinal blood vessels, there is a column of red blood cells, separated from the walls by the plasma edge stream. Red blood cells absorb one part of the light. The RVA measures the diameter of the column of the red blood cells. For measurements, the fundus camera is adjusted to the dilated pupil and a clear fundus image with good contrast and no reflection is obtained on the monitor. For the current experiment, temporal resolution was set at 40 ms, which translates to 25 captured video frames per second. RVA produces one vessel width measurement, expressed in units of measurement (UM), for each segment length of 12.5 UM. In an emmetropic person, 1 UM = 1 μm.

Most of the studies using RVA analyzed the temporal quadrant’s vessels, because they are among the largest in the retina and are readily accessible for the recording performed in the present study. In each subject, one spatially and temporally defined segment of the major temporal inferior branch of the arteriole and venule was analyzed. Several examples of chosen segments are shown in Figure 1. The chosen segment length was 500 UM (corresponding in an emmetropic eye to 500 μm), with 40 vessel width measurement along this segment length (40 × 12.5 UM = 500 μm), 1 to 2 disc diameters away from the optic disc. A time span of 10 seconds (between the 10th and the 20th second) was chosen, providing 10 × 25 = 250 video frames of a given segment, each containing 40 vessel width measurements along the segment length, as described.

A relatively short segment length (equivalent of 500 μm) was chosen because RVA recording is sensitive to eye movements. The shorter the segment, the fewer poorly recorded frames and missing data. In our sample, there was less than 5% of missing data. A short time span was chosen to capture and analyze temporal changes of vessel diameter related mostly to the cardiac cycle.

Experimental Procedures

After evaluation of inclusion criteria and the nailfold capillaroscopy, an RVA measurement was scheduled. The investigator performing RVA measurements was masked to the history and nailfold capillaroscopy results. Retinal vessel diameters were measured in one randomly chosen eye, in the morning after the subject had fasted for 12 hours overnight. Participants were instructed to abstain from a large meal, alcohol consumption (including alcohol-containing products and drugs), and physical exercise for 24 hours before the measurements. On the day of the experiments, the subjects were seated for 30 minutes in the laboratory, and local tropicamide was applied in one eye three times every 5 minutes for pupil dilation. Blood pressure was measured every 10 minutes for at least 30 minutes. After stabilization of BP, retinal vessel diameter was assessed. The fellow eye was covered to improve fixation during imaging with the fundus camera. The image of the retina was adjusted on the screen of the real-time monitor, and a recording was taken of the inferior temporal quadrant. The measurement time was 20 to 30 seconds. BP and pulse rate readings were taken during the measurement. The recordings were stored on a videotape and evaluated offline, outlining a region of interest on the desired site of measurement along the major temporal inferior branch arteriole and venule, as will be described. After retinal vessel assessment, intraocular pressure (IOP) was measured by Goldmann applanation tonometry.

Evaluation of Spatial Regularity of Retinal Vessels

To test a spatial variability of vessel diameter in the two groups, we calculated the coefficient of variation. In each of the 250 video frames containing 40 individual data points as described, the mean, the standard deviation, and the coefficient of variation (CV of diameter values along the analyzed segment for a single frame = SD/mean × 100%) were calculated. Thus, CV is expressed as a percentage of the mean diameter of that frame. The obtained 250 CVs were then averaged, producing one mean CV per vessel. Each video frame was captured within 40 ms and contained the whole vessel segment. As each frame provided its own CV value, this method does not artificially decrease spatial variability by averaging. Overall mean CVs, one for each arteriole and a venule, were obtained.

To analyze spatial frequencies in the retinal vessel of the two groups with an oscillating function, we performed a Fourier analysis of spatial frequencies in a selected vessel segment. As mentioned, one video frame produces a spatial series of 40 measurements: one diameter measurement every 12.5 UM of segment length. There were 250 such video frames. The following method was applied: Two groups of five consecutive video frames were sampled from data for each vessel and averaged across time, providing two spatial series of vessel diameters. In an attempt to sample data from different phases of the heart cycle, these two video frame groups were separated from each other with six video frames that were left out of averaging. The two averaged spatial series underwent the Fourier analysis separately. Not knowing the frequency range of interest in advance, we inspected each Fourier power spectrum curve and identified the spatial frequency with the highest relative amplitude (in other words, the spatial frequency with the highest statistical likelihood to be found in the spectrum of frequencies). Means of the two amplitudes and two frequencies are reported.

Evaluation of Temporal Vessel Diameter Changes

Changes in the time span of 10 seconds mostly reflect pulsatile changes that occur during the cardiac cycle. The present study was not de-
signed to assess long-term retinal vasomotion; hence, the time span of 10 seconds was chosen, to minimize other sources of temporal vessel diameter variability. Short-term retinal vessel diameter changes were analyzed in three ways.

First, to test whether the amplitude of vessel wall movements are different in the two groups, **excursion amplitude** (EA) was calculated. EA is a quantitative measure of the excursion of the vessel diameter during the defined time of 10 seconds. The mean diameter in each of the 250 frames in the same time-space tables from the previous two analyses was calculated, producing a data series of 250 mean diameters for 10 seconds. These were sorted in ascending order, and the mean of the lower and upper 25 values (corresponding approximately to the 5 th and 95 th percentiles) were used to calculate the EA of each vessel, according to the formula: 

\[
EA = \frac{(\text{mean of highest 25 values} - \text{mean of lowest 25 values})}{(\text{mean of highest 25 values} + \text{mean of lowest 25 values})/2} \cdot 100\%
\]

Thus, EA was expressed as a percentage of the mean diameter. EAs, one for each arteriole and venule, were tested in a two-way ANOVA model, with vasospastic propensity as one factor (difference between the two groups), and the difference between vessels (arteriole/venule) as the other.

Second, to test whether variability of the vessel diameter in the selected vessel segment is stationary or is subject to short-term changes, the pattern of temporal vessel diameter change was calculated as a correlation between the means and standard deviations of the vessel diameter. Each video frame provided one mean and one SD of the vessel diameters along the segment. Pearson’s linear correlation coefficient between this mean and the corresponding SD was obtained in 250 successive frames for each vessel. In this model, a positive correlation would mean an increased vessel “waviness” (higher spatial irregularity) in the moment of largest average vessel diameter, no correlation would indicate a uniform diameter increase in the whole vessel segment, and “smoothening” of the vessel segment in the moment of largest average vessel diameter would result in a negative correlation.

Third, to test whether an oscillating function can detect differences in the temporal course of the vessel diameter between the two groups, **Fourier analysis of the temporal course of mean vessel diameter** was performed on the temporal series of 250 successive mean vessel diameters. Unlike in the Fourier analysis of spatial frequencies, which was more exploratory in character, in this case, an analysis of the power spectrum for the frequency range of 50 to 90 beats per minute was performed for each vessel. A mean relative Fourier power spectrum amplitude (the statistical likelihood of the given frequency to be found in the spectrum of frequencies) was calculated for the 50- to 90-bpm frequency range. This frequency range was based on the measured pulse rate (see the Results section).

**Statistical Analysis**

All obtained parameters were tested with the Kolmogorov-Smirnov test for normality of distribution. If normally distributed, the parameter was tested in a two-way ANOVA model, with vasospastic propensity as one
factor (difference between the two groups) and the difference between vessels (arteriole/venule) as the other. If not, the Mann-Whitney test was used separately in the arterioles and venules.

RESULTS

Twenty-six subjects entered the analysis, 13 with a positive history of cold hands and feet and a positive nailfold capillaroscopy result, and 13 with a negative history and negative capillaroscopy result. Average age was 22.3 ± 2.7 and 24.0 ± 4.0 years (t-test for independent samples, P = 0.35), IOP was 13.2 ± 1.9 and 12.3 ± 2.2 mm Hg (P = 0.30), systolic BP 113.1 ± 9.8 and 113.8 ± 13.2 mm Hg (P = 0.87), diastolic BP 72.3 ± 9.5 and 72.7 ± 8.9 mm Hg (P = 0.92), pulse rate 67.7 ± 8.6 (range, 56–85) and 69.3 ± 9.1 (range, 55–88) beats per minute (P = 0.65), ocular perfusion pressure 44.1 ± 5.2 and 45.3 ± 5.6 mm Hg (P = 0.58), spherical equivalent in the experimental eye –2.4 ± 1.5 (0 to –3.0) and –1.3 ± 1.2 D (+0.5 to –3.0; P = 0.14) in the nonvasospastic and vasospastic groups, respectively. Both groups were also comparable in terms of contraceptive pill use (9 positive/4 negative vs. 10 positive/3 negative in the nonvasospastic and vasospastic groups, respectively; χ² P = 0.66). Mean diameters of selected segments of arterioles (129.9 ± 15.3 and 124.4 ± 24.4 μm; unpaired t-test P = 0.50) and venules (150.8 ± 14.6 and 149.3 ± 19.6 μm; unpaired t-test, P = 0.82) were not different between the vasospastic and nonvasospastic groups, respectively.

Kolmogorov-Smirnov testing revealed normal distribution of the following parameters: coefficient of variation, EA, and coefficient of correlation between mean and SD of vessel diameter. Spatial and temporal frequency amplitudes, were not normally distributed. In contrast, the corresponding spatial frequencies with the highest amplitude in the Fourier analysis were normally distributed.

Coefficient of variation in arterioles and venules, EAs of arterioles and venules, and coefficient of correlation between the mean and SD of vessel diameter are reported in Table 1.

Spatial Variability Analysis

The two-way ANOVA model for the coefficient of variation (Table 1, Fig. 2) disclosed a larger coefficient in vasospastic subjects (P = 0.017) in general. However, arterioles and venules seem to behave differently in this respect (significant interaction, P = 0.0097). Indeed, planned comparison testing revealed that the difference in coefficient of variation between the vasospastic and nonvasospastic groups is present only in arterioles (P = 0.007).

Peak relative spatial frequency amplitudes in the Fourier analysis were 1224 ± 1209 and 424 ± 503 in arterioles, and 190 ± 168 and 210 ± 222 in venules, in the vasospastic and nonvasospastic groups, respectively. The Mann-Whitney test revealed a difference between groups in arterioles (P = 0.029) but not in venules (P = 0.90). The corresponding spatial frequencies were 0.042 ± 0.015 spacial interval⁻¹ and 0.049 ± 0.017 spacial interval⁻¹ in arterioles and 0.052 ± 0.015 and 0.054 ± 0.024 in venules (1 spacial interval corresponds to 12.5 μM = 12.5 μm in an emmetropic eye), in the vasospastic and nonvasospastic groups, respectively. Being normally distributed, this parameter was subjected to two-way ANOVA: vasospastic versus nonvasospastic P = 0.36, arterioles versus venules P = 0.14, interaction P = 0.61.

Temporal Variability Analysis

The EA (Table 1; Fig. 3) was significantly higher in arterioles than in venules (P = 0.0002), but it was not different between the vasospastic and nonvasospastic groups. In the analysis of the pattern of short-term vessel diameter change, the correlation of coefficients between successive means and standard deviations of the vessel diameters (Table 1; Fig. 4) were significantly different between nonvasospastic and vasospastic subjects (P = 0.038). The observed positive correlation and testing in the ANOVA model indicated an increased vessel waviness at the average vessel diameter peak in vasospastic subjects, both in arterioles and venules. (Fig. 5).

Table 1. Coefficient of Variation in Arterioles and Venules

<table>
<thead>
<tr>
<th>Vessel Parameter</th>
<th>Vasospastic</th>
<th>Nonvasospastic</th>
<th>Two-Way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coefficient of variation arterioles (%)</td>
<td>8.8 ± 2.8</td>
<td>6.1 ± 1.7</td>
<td>Factor 1: P = 0.017</td>
</tr>
<tr>
<td>Coefficient of variation venules (%)</td>
<td>3.8 ± 1.4</td>
<td>3.6 ± 0.9</td>
<td>Factor 2: P &lt; 0.0001</td>
</tr>
<tr>
<td>Excursion amplitude arterioles (%)</td>
<td>11.4 ± 5.9</td>
<td>9.3 ± 2.1</td>
<td>Factor 1: P = 0.29</td>
</tr>
<tr>
<td>Excursion amplitude venules (%)</td>
<td>6.1 ± 2.3</td>
<td>6.1 ± 1.7</td>
<td>Factor 2: P = 0.0002</td>
</tr>
<tr>
<td>Mean/SD correlation coefficients arterioles</td>
<td>0.25 ± 0.40</td>
<td>0.11 ± 0.23</td>
<td>Factor 1: P = 0.038</td>
</tr>
<tr>
<td>Mean/SD correlation coefficients venules</td>
<td>0.24 ± 0.22</td>
<td>0.09 ± 0.23</td>
<td>Factor 2: P = 0.77</td>
</tr>
</tbody>
</table>

Data are the measure of spatial irregularity (%), excursion amplitude of arterioles and venules (%), and correlation coefficients mean vs. SD of arterioles and venules, in vasospastic and nonvasospastic subjects. Probabilities of two-way ANOVA, with vasospastic propensity (difference between groups) as factor 1 and difference between vessels (arteriole/venule) as factor 2.
The mean relative amplitude of temporal frequencies in the heartbeat frequency range in the Fourier analysis of mean temporal change in vessel diameter were 173 ± 205 and 68 ± 46 in arterioles, 93 ± 77 and 109 ± 116 in venules, in vasospastic and nonvasospastic group, respectively. The Mann-Whitney test revealed a difference between groups in arterioles and nonvasospastic group, respectively. The Mann-Whitney test revealed a difference between groups in arterioles and venules.28,29 However, the origin of increased vessel waviness in patients with diabetic retinopathy and retinopathy of prematurity is probably different from our group of otherwise healthy subjects with vascular dysregulation. A suggested underlying cause of vascular dysregulation is endotheliopathy,1,2 and the observed phenomenon may be an indicator of endotheliopathy.

Coefficient of spatial variation was higher in the arterioles of vasospastic subjects. This result was supported by the oscillation function analysis. Fourier analysis of spatial frequencies of retinal vessel diameters in the present study revealed highest amplitudes at the frequencies between 0.042 and 0.054 spatial interval, which in the present setting translates to a repetitive oscillating spatial sequence between 231.5 μm in an emmetropic eye (1/0.054 × 12.5 μm) and 297.6 μm (1/0.042 × 12.5 μm). In a vessel segment of 500 μm there are 10 to 20 endothelial cells, which were 25 to 50 μm wide, and elongated in the direction of blood flow. This segment is covered by 5 to 20 smooth muscle cells 25 to 80 μm long.30,31 The RVA length resolution of 12.5 μM (equivalent to micrometers in an emmetropic eye) would theoretically be able to detect a contribution of a single endothelial and/or smooth muscle cell to the vessel diameter variability. In this case, however, an expected length of repetitive spatial sequences would correspond to the length of a single cell (minimum 25 to maximum 80 μm), which is 3 to 10 times shorter than observed in our data. A putative unit of vessel spatial variability (graphically presented in Fig. 5) seems to be longer than a single endothelial or smooth muscle cell in both arterioles and venules. One possible explanation is that endothelin-1, which seems to be produced more in vasospastic subjects,32 diffuses abuminally and simultaneously affects several smooth muscle cells. Another possible explanation is that extravascular factors, such as the retinal nerve fiber layer in which the vessels are embedded, the state of vitreous attachment to the vessels, or small retinal vessels that closely cross the measured vessel may have contributed to the spatial vessel diameter variability.

Vessel diameter is not stationary. In fact, Fourier analysis of the temporal course of mean arteriolar diameter was able to detect a significant difference between the two examined groups, with arterioles in the vasospastic eyes pulsating more in phase with the heartbeat frequencies. This finding fits with the results of other analyses. In general, in two oscillating curves with the same area under the curve—that is, with overall mean value, with the same frequency, and with the same minima and maxima—the oscillating curve with a more quadrangular shape, with steeper ascending and descending

**DISCUSSION**

In the present study, we tested whether spatial and short-term temporal features of the retinal vessels, analyzed by means of the RVA, differ between healthy vasospastic and nonvasospastic subjects. Arterioles in vasospastic subjects are less smooth than in nonvasospastic subjects. EA was not different in the two groups; however, an altered pattern of short-term change in vessel diameter was observed in vasospastic subjects.

Female gender has an overwhelming tendency toward vasospasm.20-22 To eliminate an effect of gender, we recruited only female subjects. One negative aspect of such an approach is that the relevance of the study findings for men is unclear.

Attempting optimal separation between the vasospastic and the nonvasospastic group, only subjects who had a clear positive history of cold hands confirmed by a positive nailfold capillaroscopy result were included in the vasospastic group and vice versa. In contrast, groups were balanced in terms of age, BP, IOP, refraction, contraceptive pills history, and menstrual cycle.

An acute hypertension can cause spatial irregularities in small vessels, a phenomenon termed “sausage on a string.”25 There was no difference in BP or IOP between our two cohorts, still we observed an increased “waviness” (spatial irregularity) in arterioles of vasospastic eyes. Digital image analysis has been used in the past to analyze the tortuosities of retinal vessels in retinopathy of prematurity.24-27 Venous beading in diabetic retinopathy has been analyzed by means of Fourier transformation of the data from digitized fundus images.28,29 However, the origin of increased vessel waviness in patients with diabetic retinopathy and retinopathy of prematurity is probably different from our group of otherwise healthy subjects with vascular dysregulation. A suggested underlying cause of vascular dysregulation is endotheliopathy,1,2 and the observed phenomenon may be an indicator of endotheliopathy.

![Figure 3](image-url)

**Figure 3.** EA of arterioles and venules (percentage mean ± SD) in vasospastic (V) and nonvasospastic (NV) women. *Significant difference.

The mean relative amplitude of temporal frequencies in the heartbeat frequency range in the Fourier analysis of mean temporal change in vessel diameter were 173 ± 205 and 68 ± 46 in arterioles, 93 ± 77 and 109 ± 116 in venules, in vasospastic and nonvasospastic group, respectively. The Mann-Whitney test revealed a difference between groups in arterioles and nonvasospastic group, respectively. The Mann-Whitney test revealed a difference between groups in arterioles and venules.28,29 However, the origin of increased vessel waviness in patients with diabetic retinopathy and retinopathy of prematurity is probably different from our group of otherwise healthy subjects with vascular dysregulation. A suggested underlying cause of vascular dysregulation is endotheliopathy,1,2 and the observed phenomenon may be an indicator of endotheliopathy.

![Figure 4](image-url)

**Figure 4.** Correlation coefficients between the mean and SD of the vessel diameter (mean ± SE) in vasospastic and nonvasospastic subjects. *Significant difference.
Expression of temporal variability – pulsatile changes of vessel diameter: subsegments of vessel more responsive to pressure variations

slopes produces higher Fourier power spectrum amplitudes. In the present case, an overall mean would correspond to the overall mean vessel diameter, frequency to the heart rate, and minima-maxima to the excursion amplitude. There was no difference between the groups in any of these regards, indicating more rapid vessel response (steeper ascending and descending slopes of the temporal mean vessel diameter curve) to transmural pressure gradient changes in the vasospastic groups. It is important to understand that a vessel’s shape and diameter variability along the vessel segment are also not stationary in time. Indeed, analysis of the pattern of short-term diameter changes revealed different vessel behavior between the vasospastic and nonvasospastic group, as suggested in Figures 4 and 5. One could portray a vessel like a long balloon held under a series of tight crossing wires (smooth muscle cells in various degrees of contraction). The present findings taken together indicate that in the vasospastic group, some subsegments of vessels are more responsive to pressure variations. Movement of a vessel wall during a pulse cycle depends on the intraluminal/extraluminal pressure gradient and on the vessel wall characteristics. An origin of pulsations in arterioles and venules is different, arterioles transmit the pulse wave coming from large arteries, whereas pulsation of venules is caused by IOP pulsations and thus by the choroidal circulation. An intraluminal–extraluminal pressure gradient is much lower in venules than in arterioles. Moreover, composition of a vessel wall is different in arterioles and venules, the former having a stronger smooth muscle layer. Still, this pulsation pattern was similar between arterioles and venules and significantly different between the vasospastic and control group. Endothelial cells and the disturbance thereof could be one explanation for the common behavior of arterioles and venules. Extravas-
cular factors, as discussed earlier, could also be implicated. Elucidating a cause of different spatial and temporal behavior of retinal vessel diameter between vasospastic and nonvasospastic subjects requires further studies.

It is likely that the observed vascular changes have little bearing on the retinal hemodynamic at baseline, because bulk flow at baseline in vasospastic subjects is no different from controls in the optic nerve head
57 and in the choroid,20 and only after a hemodynamic challenge does altered response become obvious. This may be clinically relevant, because systemic vascular dysregulation can contribute to ocular disease, such as in central serous chorioretinopathy,59 central vein thrombosis, nonarteritic anterior ischemic neuropathy,5 and glaucoma. Present data lend further support to previous observations that there is a strong association between peripheral and ocular circulation. Vascular dysregulation in the eye itself has been elusive and insufficiently characterized. A proper definition of vascular dysregulation in the eye would in the clinical setting translate to the possibility of its objective assessment and treatment. The results of the present study contribute to our understanding of the manifestations of vascular dysregulation in retinal vessels.

It seems that the ocular pulse amplitude is unaltered in vasospasm,3 so it is unlikely that a pulse amplitude (IOP pulsation) could have had any influence on the observed difference between the vasospastic and nonvasospastic subjects. No correction of for magnification effect was performed in the present study. However, the two groups were not different in refractive error. More important, beside vessel diameters (expressed as UM: 1 UM in refractive error. More important, beside vessel diameters expressed as UM: 1 UM in an emmetropic eye) for which results confirmed recently published findings, all other analyzed parameters were relative numbers, calculated within the same subject.

The position of the measured vessel segments, their spatial length and measurement duration were standardized in all subjects, and the differences between the vasospastic and nonvasospastic group were found. However, because only the inferior temporal segments 1 to 2 disc diameters away from the disc were sampled, it is possible that retinal vessels in other regions would have demonstrated different behavior.

RVA measures the diameter of the red blood cell column in a vessel and not a vessel lumen or a vessel wall itself. It is not clear how accurately dynamic changes of the blood cell column reflect the wall’s vasomotion. Additional factors may be relevant (e.g., the altered blood viscosity and rheological properties in persons with Raynaud phenomenon).59-41 However, none of our subjects reported classic symptoms of Raynaud (tricolor phenomenon).

In conclusion, retinal arterioles show larger spatial irregularity in vasospastic subjects. Short-term vessel diameter changes show an altered pattern in otherwise healthy vasospastic female subjects compared with the nonvasospastic control.

References


