Phase coherence of 0.1 Hz microvascular tone oscillations during the local heating

I A Mizeva
Institute of Continuous Media Mechanics UB RAS, 1, Acad. Korolev St. 1, Perm, 614013 Russia
E-mail: mizeva@icmm.ru

Abstract.
The origin of the mechanisms of blood flow oscillations at low frequencies is discussed. It is known that even isolated arteriole demonstrates oscillations with the frequency close to 0.1 Hz, which is caused by the synchronous activity of myocyte cells. On the other hand, oscillations with close frequency are found in the heart rate, which are associated with quite different mechanism. The main purpose of this work is to study phase coherence of the blood flow oscillations in the peripheral vessels under basal and perturbed conditions. Local heating which locally influences the microvascular tone, as one of currently elucidated in sufficient detail physiological test, was chosen. During such provocation blood flow thorough the small vessels significantly increases because of vasodilation induced by the local synthesis of nitric oxide. In the first part of the paper microvascular response to the local test is quantified in healthy and pathological conditions of diabetes mellitus type 1. It is obtained that regardless of the pathology, subjects with high basal perfusion had lower reserve for vasodilation, which can be caused by the low elasticity of microvascular structure. Further synchronization of pulsations of the heated and undisturbed skin was evaluated on the base of wavelet phase coherency analysis. Being highly synchronised in basal conditions 0.1 Hz pulsations became more independent during heating, especially during NO-mediated vasodilation.

1. Introduction
Spontaneous blood flow pulsations in microvessels have recently attracted significant interest in the context of biomedical research [1], [2], [3] and provided a basis for new diagnostic methods for clinical practice [4]. It is known that even isolated arteriole demonstrates spontaneous oscillations of the lumen diameter. The frequency of such oscillations is close to 0.1 Hz, and they arise due to the synchronous activity of myocyte cells [5]. In-vivo measurements of the vessel lumen is quite difficult that is why blood flow oscillations, which carry an information about vessel vasomotion, are usually monitored.

Skin, as one of the most accessible organs, is a model for the investigation of microvascular dysfunction [6]. There are a variety methods of indirect measurements of blood flow oscillations, such as photopletismography [7], laser speckle [8], temperature variations [32]. Among them laser Doppler flowmetry (LDF) is widely applied [12]. This method has become an attractive technique for the intravital studies of cutaneous blood flow. It enables simple real-time monitoring of the relative changes of red blood cell flux in a cutaneous microvascular bed, which consists of nutritive capillaries and deeper elements of the skin vascular tree. Individual differences in skin thickness and capillary density have a significant impact on the
person-to-person LDF signal variability. The mechanism of LDF signal formation is rather complicated and the problems concerning interpretation of such measurements are currently under discussion [9] [10].

In-vivo blood microcirculation system is affected by nerve, humoral and local factors, which influence both the average and oscillating components of the blood flow [11]. This leads to the presence of the fulfilled spectra of blood flow oscillations. Five assigned frequency bands fall in the range from 0.0095 to 1.5 Hz: cardiac [12], respiratory [13], myogenic [14], neurogenic [15] and endothelial [16]. Recently these mechanisms have been intensively studied [17], [18]. At the moment origin of blood flow variations at low frequencies are under discussion. In [17] it is assumed that the microvascular blood flow possesses local mechanisms [19] of generating low-frequency blood flow oscillations. On the other hand, it is found that central baroreflex mechanism [20] likely synchronizes low-frequency oscillations through the whole cardiovascular system. In the paper oscillations with the frequency close to 0.1 Hz are discussed. On the one hand these pulsations are associated with the synchronous action of myocyte cells and they can be not in phase in different locations. On the other hand, such oscillations are associated with central mechanisms [21]. In this case local action will not influence the oscillation synchronization.

To answer this question the local action, which can influence the synchronization of different oscillating system in the human body [22], is provided. In this study well studied local heating test is used.

The issue of changing skin perfusion in response to local heating is currently elucidated in sufficient detail [23], [24], [25], and it depends on the heating regime (rate, maximum value, duration of heating). The perfusion – time curve during this physiological test demonstrates several phases. First, fast increase of the blood flow for the first few minutes after heater turning on takes place and it is associated with the axon-reflex. During the prolonged heating with the same temperature the LDF signal has a local minimum that is accompanied by an increase of perfusion. This slow vasodilation is driven by another physiological mechanism – local synthesis of a vasodilator agent NO. The appearance of this perfusion – time curve differs in health and pathological subjects [26], [27], [28]. In this studies the initial LDF curve is smoothed and LDF pulsations are not taken into account.

In the study it is included both average and pulsatile components of perfusion. In the first part of the paper we quantify the response of the microvascular blood flow measured by LDF to local heating in the zones under the heater and in its neighbourhood in two groups: control and patients with diabetes mellitus. In the second part phase coherence of 0.1 Hz pulsations of LDF signals collected at two points is discussed.

2. Materials and methods

2.1. Measurements

The study was carried out in control group of 12 healthy nonsmoking volunteers (CG) and 8 subjects with diabetes mellitus of type 1 without macrovascular complications (DM). All subjects voluntary signed an informed consent of participation in the study, which was proved by the local ethical committee of Perm Medical University. Subjects were all caffeine free for 12 hours before the test and under the controlled laboratory conditions for 15 minutes. Blood flow variations in the skin area were monitored by a two-channel laser Doppler flowmeter (Moor Instruments, UK). A skin heater unit (heat control accuracy ± 1°C) was mounted on the volar site of the forearm of the left hand of each volunteer using a double sided adhesive disk. A laser Doppler flowmeter sensor was installed into the heater through the hole. Measurements were recorded continuously. During the first 10 minutes the heating element was turned off, and the basal blood flow was recorded. In the eleventh minute, the heating element was turned on (heating up to 40°C during one minute was performed gradually - 1°C per 15 seconds). Then at 40°C the perfusion parameters were recorded during 40 minutes. 50 minutes after the
start of the experiment the heating element was turned off, and the restore process was recorded during ten minutes.

The second LDF probe was placed in the neighbourhood of the heater element at a distance of 3 cm from the geometric center of the heater. The imaginary line connected LDF probes was perpendicular to the blood flow in main arteries so that the probes were not positioned one after another in relation to the blood flow.

2.2. Data preprocessing and analysis

The first stage of the statistical analysis of average LDF variations involves a 5 second moving average. On the smooth curve illustrating the LDF signal, the maximum value lies within the axon-reflex phase and the mean value within the plateau phase.

Analysis of oscillating components and signal synchronization was carried out by the original algorithm based on wavelet decomposition. Firstly, data preprocessing was done: large scale trends and random peaks were removed from the signal. Secondly, the signal was decomposed using a wavelet transform [30]:

\[
W(\nu, \tau) = \nu \int_{-\infty}^{\infty} f(t) \psi^*(\nu(t - \tau)) dt,
\]

where * means complex conjugation. The Morlet wavelet written in the form

\[
\psi(t) = e^{2\pi it} e^{-t^2/\sigma}
\]

was used for the decomposition. Integrating the power over time gives the global wavelet spectrum

\[
M(\nu) = \frac{1}{T} \int_{0}^{T} |W(\nu, t)|^2 dt.
\]

Wavelet phase coherence is defined as

\[
C(\nu) = \sqrt{\langle \cos \Delta \phi(\nu, \tau) \rangle^2 + \langle \sin \Delta \phi(\nu, \tau) \rangle^2}.
\]

Function \(C(\nu)\) belongs to the interval \([0,1]\) and characterises the phase coherence of oscillations in two signals \(f_i(t)\) \((i = 1, 2)\) at the given frequency \(\nu\). Phase shift \(\Delta \phi(\nu, \tau)\) is defined for every frequency \(\nu\) as \(\Delta \phi(\nu, \tau) = \arg W_1(\nu, \tau)W_2^*(\nu, \tau)\), where \(W_i\) are wavelet coefficients for the \(f_i\).

The value of \(C(\nu)\) depends on the duration of the signal, short signals will demonstrate higher phase coherence, that’s why level of significance should be estimated prior to the research. In this study surrogate data obtained from the initial by amplitude-adjusted Fourier transform are used. We conserve spectral property of the real data and destroy temporal information. In current work 5000 pairs of surrogate data were produced, the duration of the surrogate signals was 15 minutes and significance level was defined as 2 standard deviations above the surrogate mean. The level of significance of the coherency depends on the frequency of pulsations, and higher at lower frequencies.

The Mann-Whitney test was used to compare the intragroup results and the Wilcoxon statistical test for intergroup variations. All data processing was implemented on Mathematica 8.0, Wolfram research.

3. Results

3.1. Average perfusion analysis

The characteristic LDF records collected in two regions of the hand of healthy volunteer are presented in figure 1. The LDF-time curve has a pronounced peak after the heater is switched.
on, which corresponds to axon-reflex vasodilation. After that, perfusion decreases and then slowly increases up to the NO mediated plateau stage. The form of the curve conforms to that described in [23]. In this example, the local heating induces blood flow variation not only directly under the heater but also in the neighboring region, which coincides with the results obtained in [22]. Note that the behaviour of this kind was not observed in all experiments.

Local vasodilation during the prolonged heating is caused by local synthesis of NO, and this effect is slightly observed at the distance of 3 cm, that’s why it is assumed that NO concentration is smaller in the undisturbed skin in comparison with heating and input of NO-related mechanisms of vascular tone regulation is smaller in the undisturbed skin.

Averaged over all experiments values of the perfusion are summarized in the Table 1. The difference in the baseline perfusion ((9.5 ± 0.75) p.u. and (7.5 ± 1.4) p.u) is not statistically meaningful. An increase in the blood flow due to the axon reflex is lower in the DM (110±50) p.u. than in CG (190±90) p.u., but the difference is not significant (p=0.06). The mean value of the blood flow caused by the NO dependent vasodilation for CG (250±100) p.u. is much higher than that of the DM (140±70) p.u., p=0.03. The relative variations of perfusion caused by the axon-reflex response to the baseline and perfusion during the NO dependent vasodilation obtained in two groups differed only slightly. A linear correlation analysis ($R_{m,n}$) was carried out to study the mean values of perfusion within three time intervals, which are marked as $m, n =$”Basal”, ”Axon-reflex”, ”NO”, 4-6 lines of the Table 1. The results indicated a moderate anticorrelation of perfusion under basal conditions and at time of axon-reflex mediated vasodilation (-0.44 for CG and -0.40 for DM). In addition, perfusion at the stage of NO mediated vasodilation is anti-correlated with perfusion observed under basal conditions (-0.65 for CG and -0.46 for DM subjects). In both groups, there was a significant correlation (0.96) in vasodilation between the axon-peak and NO-mediated plateau mechanisms.

A negative correlation of the blood flow during vasodilation and under basal conditions with
Table 1. Statistics of LDF variations

| Parameter                                      | CG            | DM            | p  |
|------------------------------------------------|---------------|---------------|----|
| Baseline perfusion, p.u.                      | 9.5 ± 0.75    | 7.5 ± 1.4     | 0.3|
| Axon reflex perfusion, p.u.                   | 190 ± 90      | 110 ± 50      | 0.06|
| NO dependent vasodilation perfusion, p.u.    | 250 ± 100     | 140 ± 70      | 0.03|
| $R_{Basal, Axon-reflex}$                      | -0.44         | -0.40         |    |
| $R_{Basal, NO}$                               | -0.65         | -0.46         |    |
| $R_{Axon-reflex, NO}$                         | 0.96          | 0.96          |    |

Figure 2. Averaged spectra of LDF signals in CG (blue lines) and DM (gray lines) in basal state (solid) and during axon reflex vasodilation stage (dashed). Spectra of LDF signals collected under the heater (left panel) and in the neighborhood (right panel).

Close coefficients at both stages (axon-reflex mediated and NO vasodilation) demonstrates a lower reserve for the vasodilation of microvessels in subjects with high basal perfusion. This is independent of the mechanism responsible for vasodilation ($R_{A, NO}$ is high in both groups). In general, the second stage of microvascular system response to heating leads to a higher vasodilation difference ($p < 0.05$) between the control and DM groups.

3.2. Blood flow oscillations

In the figures 2, 3 averaged spectra in two time intervals corresponds to different stages of vasodilation are presented. In every plot spectra in basal conditions for reference are shown. Spectra of LDF pulsation in basal conditions had no significant difference in CG and DM. During the axon-mediated vasodilation amplitudes of pulsations increased in both groups, spectral slope changed, thus high frequency pulsations had higher impact in the LDF signals. No significant difference between CG and DM was founded.

Spectra of LDF pulsations for NO-mediated vasodilation plateau are presented in the figure 3. Energy of pulsations in all frequency bands were significantly higher in the heated tissue in comparison with basal state. High frequency oscillations demonstrated higher increment then lower ones. Significant difference between CG and DM was found in the 0.07-0.12 Hz frequency band. Note, that controls demonstrated higher energy of pulsations. Perfusion of the unheated skin also had higher pulsations in both groups, which indicates indirect influence of heating. Significant difference between groups in this time interval was found in the frequency band, which corresponds to the respiratory activity.

To study oscillations phase coherency, first, surrogate data were tested. Note that for reliable statistic of slow oscillations longer records duration is needed, and significant coherence of 0.01
Figure 3. Averaged spectra of LDF signals in CG (blue lines) and DM (gray lines) in basal state (solid) and during NO-mediated vasodilation stage (dashed). Spectra of LDF signals, collected under the heater (left panel) and in the neighborhood (right panel). Filling show the frequency band, where difference between CG and DM, estimated by Mann-Whitney test, was significant.

Figure 4. Box-whisker diagram for the coherence between two LDF records in different points for CG (blue rectangles) and DM (gray rectangles in five time intervals 1- basal state, 2- axon reflex-mediated vasodilation stage, transition process to the NO-mediated plateau, 4- NO-mediated plateau, 5 - restore processes after the heating switching off). Level of significance is shown by dashed lines. In the left panel synchronization of 0.1 Hz pulsations and 1 Hz - in the right panel are demonstrated.

Hz pulsations for the 15 minutes record is higher as 0.7. In this study we didn’t get such high values of coherence, that’s why only the frequency band 0.05-2 Hz was studied. Oscillations associated with cardiac activity and close to 0.1 Hz oscillations were analysed. The dynamics of phase coherence of pulsations in perfusion in two points of measurements on this two frequencies was traced (figure 4).

In the basal conditions 0.1 Hz LDF pulsations in two measurement points were coherent ($C = 0.7$). With the heating, phase coherence coefficient decreased, difference between basal conditions and heating conditions became significant in the NO-mediated vasodilation stage ($p < 0.05$). After the heater switched off, $C$ restored up to the level in basal state. The dynamic is quite the same for healthy and diabetic subjects.

Using the same technique 1 Hz pulsation associated with cardiac activity were analysed (right panel of the figure 4). The level of significance for such pulsations are lower than for 0.1 Hz, and it is shown by the dashed line on the right panel of the figure 4. 1 Hz pulsations are statistically
meaningful during the whole measurement interval, no significantly variation were obtained. Intergroup statistics also did not reveal any significant difference. Thus we don’t see rapture of synchronization of oscillations associated with cardiac activity, this support the technique adequateness.

4. Conclusion

In the paper, the variations of the blood flow in the microvascular system caused by local heating were studied. It is found, that NO-mediated vasodilation is impaired in diabetic patients, which indicates an endothelial dysfunction [32] or weaker vasodilation response in pathological conditions. These results coincides with founded earlier impaired vasodilation caused by decreased production of (NO) [29], [31].

It has been established that the high basal perfusion measured by means of LDF leads to a lower reserve for microvessel vasodilation, and this effect does not depend on both the mechanism of vasodilation and on the group under consideration. The limit of tissue saturation is supposed to exist, and therefore the higher saturated tissue is able to get the lower volume of blood than the less saturated one. Another possible reason is the variation of the dynamic permeability range from subject to subject. The lower dynamic permeability range means that the system is less elastic. Note that this conclusion is correct for both groups, but mostly diabetes patients revealed lower dynamic range for vasodilation. Summing up, these characteristics are correct for both vasodilation mechanisms, and the NO mediated vasodilation is found to be more effective than one induced by the axon reflex.

The synchronisation of pulsations at two measurement points spaced 3 cm apart was studied at frequencies 1 Hz and 0.1 Hz. It has been found that the phase coherence of blood flow oscillations, associated with cardiac activity was not influenced by the local heating. At the same time 0.1 Hz were synchronous in the basal state and remained synchronous during the axon reflex mediated vasodilation. The situation was different over the time period of NO mediated vasodilation, namely, the index of phase coherence $C$ significantly decreases in $CG$, as well as in $DM$. This fact indicates local NO associated vasodilation influence on the phase of 0.1 Hz vascular tone pulsations. The 0.1 Hz LDF pulsations associated with the myogenic vascular tone regulation were assumed to be locally originated, and therefore the local disturbance can change their characteristics.

Acknowledgment

The work is supported by the Russian foundation for basic research RFBR-ra under projects 17-41-590560 17-44-590755. The author kindly thank prof. E. Smirnova and E. Loran for the clinical base.

References

[1] Stefanovska A, Bracic M and Kvernmo H D 1999 IEEE transactions on bio-medical engineering 46 1230–1239
[2] Rossi M, Carpi A, Galetta F, Franzoni F and Santoro G 2008 Biomed. and Pharmacother. 62 541–545
[3] Schmidt J, Breit G, Bostrom P and Intaglietta M 1995 Inter. J. Microcirc. 15 28–36
[4] Martini R, Ticcinielli V and Bagno A 2014 Clin. Hemorheol. Micro. 4 347–358
[5] Aalkjer C, Boedtkjer D and Matchkov V. 2011 Acta Physiologica 202 253–269
[6] Stirban A 2014 Cur. Diabet. Rep. 14 1–9
[7] Allen J 2007 Phys. Meas. 28 R1-39
[8] Ansari M, Kang E, Manole M, Dreier J, Humeau-Heurtier A 2017 Microvasc. Res. 111 49–59
[9] Obeid A N 1993 Med. Biol. Eng. Comput. 31 3–52
[10] Mizeva I, Frick P and Podtavev S 2016 J. Biomed. Opt. 21 085002
[11] Krupatkin A 2007 Human Physiology 33 595–602
[12] Stefanovska A 1999 Contemp. Phys. 40 31–55
[13] Bollinger A, Yanar A, Hoffmann U and Franzcek U K 1993 Prog. Appl. Microcirc. 20 52–58
[14] Bertuglia S, Colantuoni A and Intaglietta M 1994 Microvasc. Res. 48 68–84
[15] Kvandal P, Landsverk S A, Bernjak A, Stefanovska A, Kvernmo H D and Kirkebøn K A 2006 Microvasc. Res. 72 120-127
[16] Bernjak A and Stefanovska A 2007 Conference proceedings : ... Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Conference 2007 4064–4067
[17] Tankanag A, Grinevich T, Kirilina T, Krasnikov G, Piskunova G and Chemeris N 2014 Microvasc. Res. 95 53–59
[18] Sheppard L W, Stefanovska A, McClintock P V E 2011 Phys. Rev. E. 85 046205
[19] Salerud E, Tenland T, Nilsson G and Oberg P 1983 Inter. J. Microcirc. 2 91–102
[20] Heistad D, Abboud F, Mark A and Schmid P 1973 J. Appl. Physiol. 35 581–586
[21] Shvartz V, Karavaev A, Borovkova E, Mironov S, Ponomarenko V, Prokhorov M, Ishbulatov Y, Lapsheva E, Gridnev V and Kiselev A 2016 Russian Open Medical Journal 5
[22] Liao F and Jan Y-K 2012 Med. Bio. Eng. Comput. 50 1059–1070
[23] Johnson J and Kellogg D 2010 J. Appl. Physiol 109 1229–1238
[24] Sorelli M, Stoynova Z, Mizeva I and Bocci L 2017 Phys. Meas. in press
[25] Parshakov A, Zubareva N, Podtaev S and Frick P 2016 Microcirc. 23 406–415
[26] Bandini A, Orlandi S, Manfredi C, Evangelisti A, Barrella M, Bevilacqua M and Bocchi L 2013 J. of healthcare engineering 4 541–554
[27] Fredriksson I, Larsson M, Nyström F H, Länne T, Östgren C J and Strömberg T 2010 Diabetes 59 1578–1584
[28] Arora S, Smakowski P, Frykberg R G, Simeone L R, Freeman R, LoGerfo F W and Veves A 1998 Diabet. Care. 21 1339–1344
[29] Stevens M, Dananberg J, Feldman E, Lattimer S, Kamijo M, Thomas T, Shindo H, Sima A and Greene D 1994 J. Clin. Invest. 94 853–859
[30] Frick P, Mizeva I and Podtaev S 2015 Bio. Sign. Proc. and Cont. 21 1–7
[31] Stevens M, Feldman E and Greene D 1995 Diabet. Med. 12 566–579
[32] Smirnova E and Podtave S and Mizeva I and Lorin E 2013 Diabet. Vasc. Dis. Res 10 489–497