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Method of using laser doppler flowmetry in assessment of the state of blood microcirculation system

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ABSTRACT

The article describes the foundations of using method of using laser Doppler flowmetry in evaluation of the state of blood microcirculation system in students, future software engineers. By the method of laser Doppler flowmetry (LDF) individually-typological features of indicators of tissue blood flow in conventionally healthy adolescent students aged 17-20 years have been studied. Due to the results of research 3 types of LDF-grams have been defined: aperiodic, monotonous low amplitude, sinusoidal type with high perfusion. Among the examined students, future engineers-programmers different frequency of appearing microcirculatory types with predominance of normoemic type with characteristic "aperiodic" LDF-gram has been found.

Keywords: method of laser Doppler flowmetry, microcirculation, engineers-programmers

1. INTRODUCTION

In the process of vocational training of future Bachelors in Computer Sciences necessary conditions are the following: formation of specified levels of competence, professional culture of specialist, development of his needs in continuous professional self-improvement. The mentioned above conditions are basic for effective activity in the conditions of competitive environment[1,2].

One of the most important competencies for applicants for higher education is a health saving. During the last years the problem of saving health of educational process participants does not lose its relevance and takes a significant place in the fields of medical-biological and psychological-pedagogical researches[3]. The process of adaptation to students 'studying process is complicated, socio-psychological and is provided by a significant tension of compensatory-adaptive systems of the organism. Students, future engineers-programmers, because of the peculiarities of professional work, very quickly get used to the sedentary lifestyle.

Hypodynamia manifests in rapid fatigue, weakness, absolute unwanted physical strain that gets into the habit and form lifestyle without enough physical activity. Muscles become weaker, blood vessels lose their flexibility, metabolism processes violate due to the insufficient physical intension. Hypoxia of organs and tissues causes to the development of different chronic diseases, first of all, the diseases of the cardiovascular system. The system of blood microcirculation is a subsystem of the cardiovascular system that is why changes in the system of blood microcirculation correlate with shifts in central hemodynamics that allows using parameters of microcirculation as prognostic and diagnostic criteria in assessing general functional status and the level of students' health.

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Among the methods of researching tissue blood flow the method of laser Doppler flowmetry (LDF) has recently become very popular. The advantage of the LDF method is its possibility to measure invivo microcirculation and contactless that is very important in testing microhemodynamics, which changes its indices every time you try to connect the sensors to the capillaries[4]. Another important peculiarity of LDF is possibility to get big number of measurements (thousands per minute), their registration and processing in real time[5,6], which, in particular, allows for the creation of LDF monitoring systems. The last ones in prospects give an opportunity to analyze the whole spectrum of rhythmic processes in micro vessels from pulse to circadian. This method of examination of skin microcirculation is widespread abroad, but it is practically not used in Ukraine. That is why is necessary to systematize and summarize the literature on the LDF method for evaluation of tissue hemomicrocirculation. As shown in investigations [3,6,7], this method allows not only find the basic parameters of tissue blood flow, but also evaluate the state of functioning of microcirculatory control mechanisms.

In this regard, the purpose of this work was studying by LDF method individually-typological features of blood microcirculation in healthy adolescent students, future engineers-programmers.

2. RESEARCH RESULTS

Laser Doppler flowmetry (LDF) is the most currently available method for studying tissue blood flow, first proposed by M. Stern[4,6]. In 1977 the first LDF apparatus for clinical use by G. Holloway and P. Watkins was created. The object of investigation became skin of upper or lower limbs[7].

The study of reactions of skin blood flow to carrying out functional tests allows not only evaluating the nature of blood flow in the microcirculation, but also the reserve of capillary blood flow, and to do indirect evaluation of regulation of vessels of the microcirculation[6,8].

Some authors notice that skin vessels have ability quickly and variously respond to the effects of different stimuli, the basis of which are the vasoconstriction and vasodilatation phenomena caused by the vascular movement nerves[9]. To a greater extent, this applies to skin areas, where there is a large number of arterio-venular anastomoses (AVA);where there are none or little, vasodilatation and vasoconstriction are caused by the condition of the basal tone of the vessels.

The method of laser Doppler flowmetry (LDF) is based on the optical (noninvasive) tissue sounding with a monochromatic signal (usually in the red area of the spectrum) and analysis of the frequency spectrum reflected from the moving erythrocytes signal[8]. The reflected from static (unmovable) tissue components, laser radiation does not change its frequency, and reflected from moving particles (erythrocytes) – has a Doppler frequency shift relative to the sounding signal. The changing part of reflected signal, proportional to the power of the Doppler shift spectrum, is defined by two factors: the concentration of red blood cells in the sounding volume and their speed. The signal registered at LDF characterizes blood flow in micro vessels in the amount of 1-1.5 mm3 of tissue. That is mean that in the human skin LDF gives integral information in a very huge number of erythrocytes, near 3,4 x 104, simultaneously located in the sounding tissue amount[9]. Further development of this method has shown that the most significant is not so much the measurement of erythrocyte movement speed as the temporal variability of red blood cells flow. Rhythmic fluctuations of blood flow and their changes allow receiving information about certain correlation of different mechanisms that determine the state of microcirculation.

The LDF method, despite the fact that it gives a semi-quantitative description of the state of microcirculation in the surface layers of tissues (skin, mucous membranes), is widely used in clinical practice. According to some authors, LDF is more characterized by periodic changes (fluctuations) of perfusion of blood tissues, which can occur with different frequency and amplitude[9,10]. As it has been turned out, blood flow at the microcirculatory level is not a completely stable phenomenon, but is disposed to temporal and spatial variations. Fluctuations in blood flow, also called oscillations or fluxmotions, periodically occur in tissues, reflecting the most important description of the process of their living: variability and adaptability of blood flow to constantly changing conditions of hemodynamics and needs of tissues in their perfusion by blood. The frequency and amplitude of blood flow oscillations at each given moment are variable, which reflects the LDF-grams. Variability of rhythmical characteristics of fluxmotions depends on many factors of individual blood flow changing, optical tissue properties, as well as the state of pre- and post-capillary resistance[8,9].

G. Schmid-Schonbeinet.al. develop conception, according to which fluctuations of tissue blood flow are the result of super positions of active and “passive” modulations of fluxmotions. Active modulations are due to both the myogenic mechanism, which is more characterized by the frequency of fluxmotions, and the neurogenic mechanism, which is characterized by aperiodic constrictor phases. The fallout of one or other rhythmic components of fluxmotions, treated as “spectral narrowing” of the LDF-gram, can serve as a diagnostic criterion for violations of the mechanisms of microcirculation regulation. The qualitative analysis of the LDF-grams, done by A. Scheffler certifies that their spectral narrowing corresponds to the progression of obliterating lesions of arterial vessels[9,11].

Among the fluctuations in tissue blood flow the significant should be considered, the so-called, low-frequency, high-frequency and pulse fluxmotions. Low-frequency fluctuations in blood flow (LF) from 4 to 10 fluc/min(0,05-0,15 Hz) are caused by spontaneous periodic activity of smooth myocytes in the arterioles wall, which causes periodic changes in their diameter; they are called vasomotions [10,11]. According to the conception of myogenic mechanism, proposed by B. Folkow, the spontaneous rhythmic contractions of smooth myocytes are caused by increasing transmural pressure[12]. The high-amplitude aperiodic fluxmotions about large amplitude, reflecting the influence of the sympathetic link of regulation are imposed onto the active modulations blood flow tissue with a help of vasomotor mechanism. The decrease in the amplitude of aperiodic low-frequency fluxmotions may indicate the suppression of the neurogenic vasomotor mechanism. As the mechanism of active modulation of tissue blood flow, the low-frequency fluxmotions are widely investigated using LDF in a variety of pathologies.

Among the low-frequency fluxmotions the oscillations with a very low frequency (VLF) less than 0.03 Hz (1 oscillation in 1-2 minutes) are distinguished. VLF- oscillations characterize influence of humoral and metabolic factors on the state of microcirculation and, probably, are related to the periodic contractions of endothelial cells due to the reduction of their cytoskeleton[4,6,8]. G. Schmid-Schönbeinet.al. think that fluctuations in tissue blood flow with a frequency of 0.01-0.03 Hz have got a myogenic nature[9].

The low-frequency fluxmotions of blood flow (HF) from 15 to 20 fluc/min(within ~ 0.25 Hz) firstly were described in patients with peripheral artery occlusion, although they can be observed in healthy examined ones[8]. Appearing of HF waves in the LDF-gram is due to the periodical changes of pressure in the venal department of the blood vessels caused by respiratory excursions[4,6,8]. This compensatory mechanism is usually observed in ischemic disorders of the skin blood flow, which, probably, should be considered as an additional mechanism of modulation of microcirculation unlike the active mechanism of vasomotions. However, it is possible that HF fluctuations in tissue blood flow associated with respiratory rhythm reflect the indirect (because of changes in heart contractions) influences of the parasympathetic link of regulation on the state of tissue blood flow.

The pulse waves (CF), which are different by low amplitude of fluctuations of fluxmotions and are caused by changes in intravascular pressure, which are more or less synchronized with the cardiorhythm, are considered as important component of oscillation in tissue blood flow should be considered. Pulse fluctuations in blood flow in micro vessels characterize the hemodynamic mechanism that determines the flow of blood in them. It should be considered as the main, basic mechanism of movement of erythrocytes in micro vessels; it is largely related to the influence of the autonomic nervous system on the regulation of the cardiovascular system, including microcirculation[8].

In conclusion, despite the widespread prevalence of LDF-metry in clinical practice, in the study of blood microcirculation in individuals with various pathologies, there is an acute deficiency in the characterization of normative parameters of LDF in healthy people, especially taking into account the age and sex differences of organism.

40 male volunteer students – future software engineers aged 17-20 years, relatively healthy, from Melitopol State Pedagogical University participated in the investigation.

In purpose of studying functional state and peculiarities of microcirculation of students’ organism the method of laser Doppler flowmetry (LDF), that allows to evaluate status of tissue blood flow and find the features of change in microcirculation under the influence of various factors, has been used.

LDF was performed by a “LAKK-01” Laser Blood Flow Analyzer (produced by SPE “Lasma”, Russia) with a laser radiation source at a wavelength of 0.63 μm (Fig. 1). Laser Analyzer is connected with personal computer at the base. The real-time LDF recording curve is displayed on the monitor screen. All recordings from LDF-grams are saved in the data base and, if necessary, transferred to paper. Microcirculation studies were performed in girls in sitting position. The head of the optical probe (instrument sensor) was fixed on the ventral surface of the 4th finger of the left hand; the arm was located at the level of the heart. The duration of standard recording was 4 minutes (Fig. 1).

All recording from LDF-gram,with the exception of the cases, when this was specifically pronounced, they were carried out in the morning from 10 to 12 hours. Recordings of LDF grams were carried out in accordance with the methodological recommendations: methods of laser Doppler flowmetry[8].

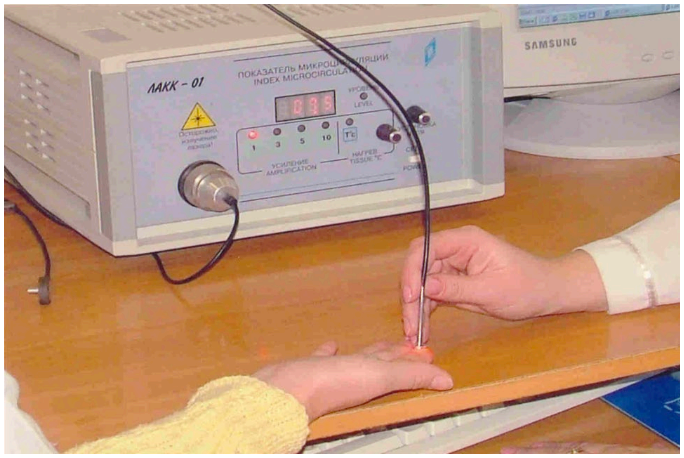


Figure 1. Photograph of the process of studying the microcirculation of blood in students using LDF.

A light probe, made of three monofilaments, is used as a sensor in the LAKK-01 blood flow analyzer. One filament can be used fordelivery of laser radiation from the device to the test tissue, the other two filaments are acceptable for diffused and reflected laser radiation. Delivered by filaments diffused radiation is detected by a two-channel photodetector. Electrical signals from photodetector go to the block of information processing of analyzer, where there is a selection in the registered signal of Doppler shift signal in frequency. The output signal is formed after analogical processing, which is proportional to the first moment of the power of the spectral solidity of the Doppler frequency shift, which corresponds to the product of the factors: the speed of movement of erythrocytes and their concentration in the probe amount of tissue[4,6] (Fig. 2). The amount of probed tissue area was about 1-1,5 mm3 in our study.

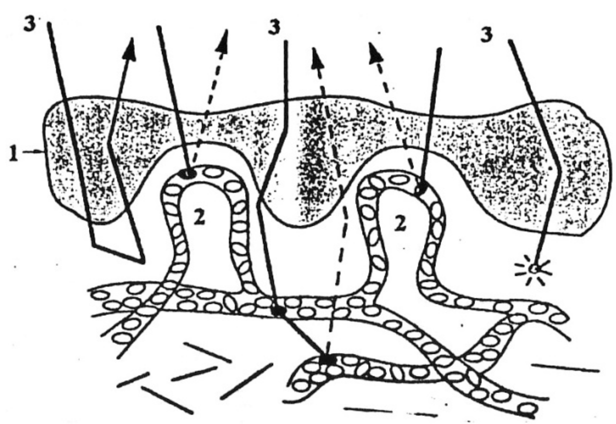


Figure 2. Optical probing of a skin surface by laser radiation at LDF (Note: 1 –skin surface, 2 – capillaries, 3 – ways of radiation).

The results of the LDF-testing are registered in the relative perfusion units – perf. units, which reflect the degree of perfusion, mainly by erythrocyte fraction, units of tissue volume per time unit and allow to trace its dynamics in the reaction of blood flow to various influences[4,6,8].

Due to the connection of LAKK-01 to the computer, LDF registration was performed in the monitoring mode that allowed observation on monitor screen real-time blood flow changes in the process of researches (Fig. 3).

As the studies showed, the integral characteristics of blood flow, which was registered by LDF, is a microcirculation index (*MI*), which is a function of the average speed of red blood cell movement (*Vavg*.),index of capillary hematocrit (*Htk)* and number of functioning capillaries in measured volume of tissues (*Nk*) (formula 1):

$$MI = Vavg. \times Htk \times Nk \qquad (1)$$

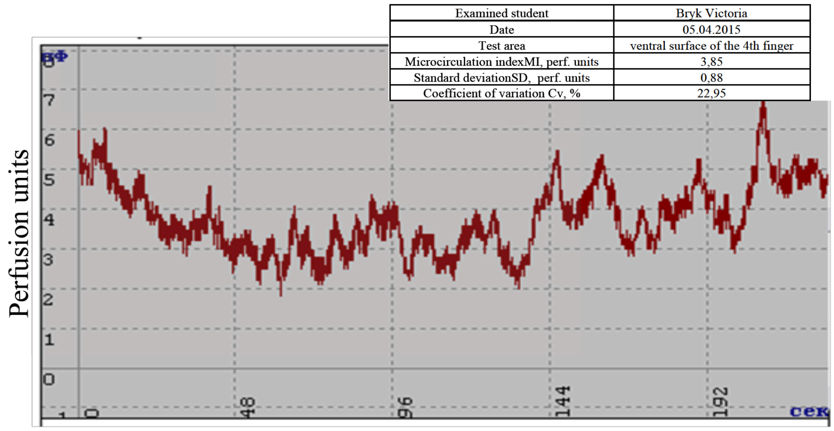


Figure 3. LDF-gramat 4 minutes of recording (ventral surface of the 4th finger of the wrist).

Computer program for processing LDF-gram allows to identify such features of microcirculation: *MI*– microcirculation index. This parameter is numerically equal to the arithmetic mean value of the Doppler signal and characterizes the average amount of perfusion of the probed tissue with blood per unit time in relative units; SD – standard deviation of registered Doppler signals from the average value, this value characterizes the variability of the flow value over time (in the LDF-metric literature this value is referred to as the Flux level); C_v– coefficient of variation, measured in % (formula 2):

$$C_v = \frac{SD}{MI} \cdot 100\% \tag{2}$$

An important stage of LDF-metric is analysis of the amplitude-frequency spectrum (AFS) of hemodynamic rhythms fluctuations in tissue blood flow. AFS analysisis carried out using a special computer program.As a result of the spectral decomposition of the LDF-gram into harmonic components, it becomes possible to judge the degree of expressiveness or dominance of various fluctuations of tissue blood flow in tissue hemodynamics. The software is based on the spectraldecomposition of LDF-gram using a Fourier mathematical apparatus. In this case, the amplitude of each harmonic is automatically determined in the frequency range from 0.01 to 1.2 Hz (Fig. 4).

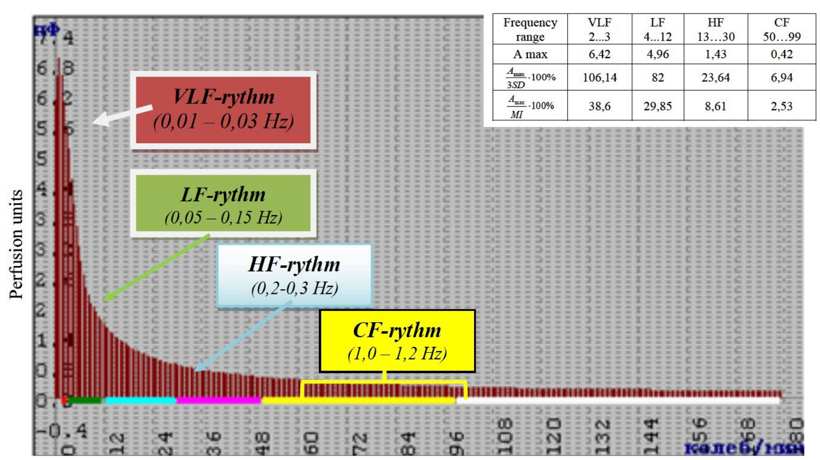


Figure 4. Amplitude-frequency spectrum of blood flow fluctuations (ventral surface of the 4th finger) Note: A – amplitude, VLF – metabolic fluctuations, LF – vasomotor fluctuations, HF – respiratory fluctuations, CF – pulse waves.

Among the fluctuations of blood flow, reflected in the LDF-gram and its amplitude-frequency spectrum, the most important physiologically are very low frequency oscillations VLF (0.01-0.03 Hz), which characterize the influence of humoral-metabolic factors on the state of microcirculation [4,6,8]. They, probably, are related to the periodic contractions of endothelial cells that are caused by contractions of their cytoskeleton[8,9]. However, G. Schmid-Schonbein et. al. believe that these oscillations can have a myogenic nature[9].

The low frequency LF-oscillations (0.05-0.15 Hz) are caused by a spontaneous periodical activity of smooth myocytes in the wall of arterioles, which causes periodic changes in their diameter, they are called vasomotions[4,6]. Due to the conception of myogenic mechanism, proposed by B. Folkow, spontaneous rhythmical reductions of smooth myocytes, caused by increased transmural pressure[4,6,8,9]. The high-amplitude aperiodic fluxmotions regarding to a large amplitude, which reflects, according to V.I. Kozlov et al., impact of sympathetic regulation link, are superimposed on active modulations of tissue blood flow with the help of a vasomotor mechanism[8]. Decrease in the amplitude of aperiodic law frequency oscillations may indicate inhibition of the neurogenic vasomotor mechanism. High frequency HF-oscillations (0.2-0.3 Hz) are caused by periodical changes in pressure in the venous section of the microsirculation caused by respiratory excursions. The severity of these fluctuations increases in conditions of pathology, especially when there is a decrease in active myogenic vasomotions. Pulse oscillations of skin blood flow CF (1.0-1.2 Hz) differ by small amplitude of fluxmotions' fluctuations and are caused by changes in intravascular pressure, which are more or less synchronized with cardio rhythm[4,6,8].

In the amplitude-frequency analysis of LDF-gram were calculated: amplitude (A) of myogenic metabolic oscillations in the frequency range from 0.01 to 0.03 Hz (1-2 oscillations per minute) (AVLF), vasomotor oscillations in the frequency range from 0.05 to 0.15 Hz (4-8 oscillations per minute) (AVLF), respiratory oscillations in the frequency range from 0.2 to 0.3 Hz (AVHF) and pulse waves (ACF).The contribution of the various rhythmic components (P) was evaluated by their power as a percentage of the total power of fluxmotions (formula3):

$$\text{P} = P = \frac{A_{LF}^2}{A_{VLF}^2 + A_{LF}^2 + A_{HF}^2 + A_{CF}^2} \cdot 100\% \quad (3)$$

Correlation of active modulations in skin blood flow, caused by myogenic and neurogenic mechanisms, and additional parasympathetic affects at it was calculated as the index of the effectiveness of fluxmotions (formula4):

$$IFM = \frac{A_{LF}}{A_{HF} + A_{CF}} . \quad (4)$$

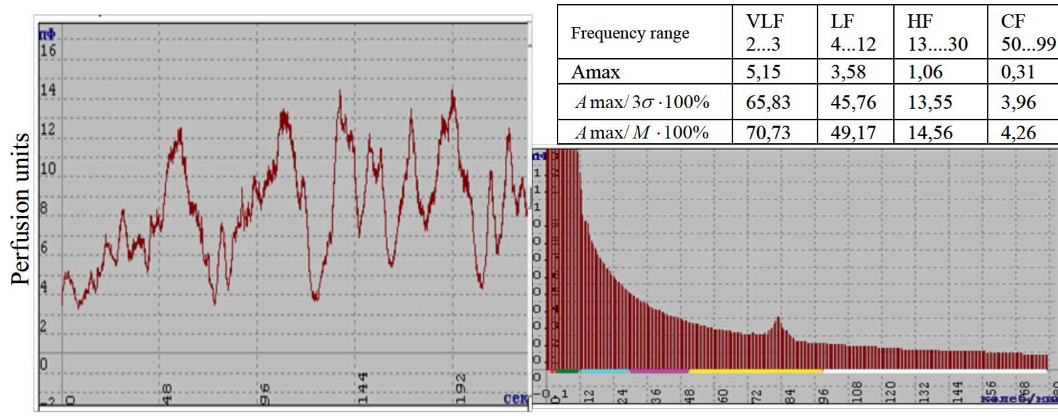
The evaluation of reliability of difference between the data obtained from the study was estimated using the Student's t-test. As the result of research microcirculation in adolescent students have been defined the following indicators: parameter of microcirculation M in the fingers skin of the wrist ranged from 4.03 to 26.8 perf. units; on average it was 15.35 ± 0.68 perf. units. The level of fluctuations in tissue blood flow – SD varied from 0.5 to 4.72 perf. units, on average accounting 2.05 ± 0.05 perf. units. The coefficient of variation (Kv) varied in the range from 3.22 to 35.17% and in average became 15.8 ± 0.35%. Study of different individual peculiarities of microcirculation in the examined students with the LDF method allowed finding out three types of LDF-gram, which reflect the state of blood microcirculation (Table1).

Table 1. Indicators of microcirculation in examined students (M ± m).

Group number /Averagevalues	**Type of microcirculation**	**Parameters of microcirculation, M, perf.units**	**Flux level, SD, perf.units**	**Coefficient of variation,Cv, %**
1	Aperiodic type (n=22)	15,12±0,19	2,87±0,32	21,16±2,43
2	Monotypic type with low perfusion (n=18)	9,24±0,46	1,48±0,46 p1≤0,001	20,43±0,73 p1≤0,001
3	Monotypic type with high perfusion (n=6)	19,88±0,42 p1≤0,001;	1,66±0,12 p1≤0,001;	8,61±0,64 p1≤0,001

Note: p1 –reliability of difference in relation to the aperiodic type by Student's t-test

The type I aperiodic LDF-gram was characterized by occasional fluctuations in tissue blood flow with relatively high amplitude. This type of LDF-gram is typical for a balanced state of the mechanisms, "active" (related to sympathetic influences) and "passive" (related to parasympathetic influences) regulation of fluctuations in tissue blood flow (Fig. 5).



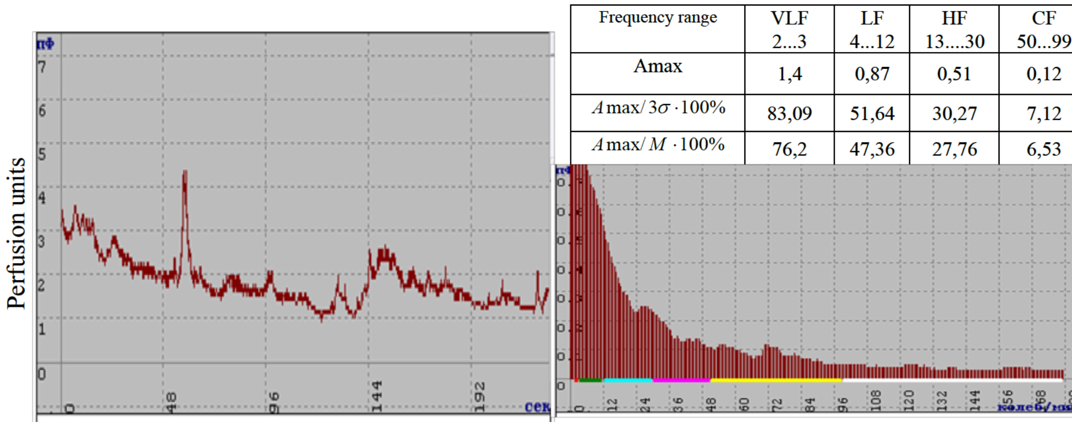
Frequency range	VLF 2...3	LF 4...12	HF 13....30	CF 50...99
Amax	5,15	3,58	1,06	0,31
$A\max/3\sigma\cdot100\%$	65,83	45,76	13,55	3,96
$A\max/M\cdot100\%$	70,73	49,17	14,56	4,26

Figure 5. AperiodicLDF-gramtypeI Note: A – amplitude of oscillation: VLF – metabolic, LF – vasomotor, HF – respiratory, CF – pulse.

In this type thestandard deviation is relatively high – 2.87 ± 0.32 perf. units. The coefficient of variation (Cv) had value of 21.16 ± 2.43%. There were identified 22 persons,accounting for 48% of the total number of the examined young men.

Spectral analysis of the amplitude-frequency spectrum of aperiodic LDF-grams showed that for this type the maximum contribution to the total power of the spectrum is observed by the VLF oscillations – 48 % and LF oscillations – 34 %. High frequency HF oscillations 13 % and pulse CF oscillations 5 % take a small part of the total spectrum. The IEM indicator, which describes effectiveness of fluxmotions, in average, was 1.76 ± 0.2. This type of LDF-grams corresponds to the “normoemic” type of microcirculation[8,13,14].

Among the examined men 18 people (39 %) have got second type of LDF-gram, which was characterized by relatively low parametersM 9.24 ± 0.46 perf. units and relatively monotonous nature of fluxmotions (Fig. 6). The LDF-grams of that type are characterized by significantly low values of SD 1.48 ± 0.46 perf. units, which is significantly lower than in examined students of the aperiodic type. This type of LDF-gram corresponds to a condition in which there is a relative increase in sympathetic influences in the regulation of tissue blood flow. This microcirculatory type is designated by us as hypoemic one.

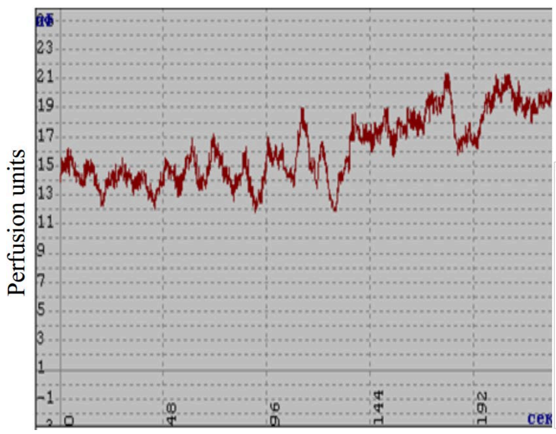


Frequency range	VLF 2...3	LF 4...12	HF 13....30	CF 50...99
Amax	1,4	0,87	0,51	0,12
$A\max/3\sigma\cdot100\%$	83,09	51,64	30,27	7,12
$A\max/M\cdot100\%$	76,2	47,36	27,76	6,53

Figure 6. Monotone LDF-gram with low indexofMI(typeIII) Note: A – amplitude of oscillation: VLF – metabolic, LF – vasomotor, HF – respiratory, CF – pulse.

In the LDF-grams of type II relating to type I the spectral power index changes slightly VLF (46%) and LFoscillations (39%), while among the high frequency waves there is a slight decrease in power of HF-waves to 12 % and abrupt increase in power of CF-oscillations to 8%. The main vasomotor rhythm in the frequency range from 4 to 12 oscillations / min has an amplitude of 2.74 ± 0.1 perf. units, a significantly lower than in LDF-grams of type I. The predominance of the sympathetic link in the regulation of tissue blood flow is typical for this type of microcirculation.

The type III of the LDF-grams, observed in 6 boys (13% of cases), on the contrary, was characterized by high value of perfusion of 19.88 ± 0.42 perf. units, than in examined two previous groups with predominance in the LDF-gram of a clearly expressed “sinusoidal” rhythm (Fig. 7).



Frequency range	VLF 2...3	LF 4...12	HF 13....30	CF 50...99
Amax	4,29	2,11	0,42	0,47
$A\max/3\sigma\cdot100\%$	61,39	30,2	11,45	6,73
$A\max/M\cdot100\%$	27,98	13,76	5,22	3,07

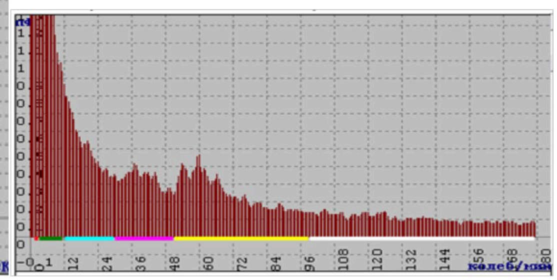


Figure 7. Monotone LDF-gram with high index of MI (type II) Note: A – amplitude of oscillation: VLF – metabolic, LF – vasomotor, HF – respiratory, CF – pulse.

The standard deviation (SD) for this type of LDF-grams was 1.66 ± 0.12 perf. units, which is higher than in type II. The maximum contribution was noted by VLF oscillations of 46% and LF oscillations of 35%. Contribution to the power of the amplitude-frequency spectrum of HF oscillations was 13%, CF oscillations – 6%. This type of LDF-gram corresponds to the hyperemic state observed in the case of increase in blood flow to the microcirculation system related to some dilation of microvessels[8,14–17] due to the relative attenuation in the regulation of tissue blood flow of sympathetic influences.

Thus, significant fluctuations of the main indicators of microcirculation in the three types of the LDF-grams allowed to identify three types of microcirculation among the examined young men:normoemic, in which there is an “aperiodic” LDF-gram; hyperemic, which corresponds to a “sinusoidal” LDF-gram with a high index of microcirculation; and hypoemic type, in which a “monotonically low-amplitude” LDF-gram with a low microcirculation index is detected.

Analyzing the contributions to the power of the frequency-amplitude spectrum of different rhythmic components of blood flow oscillations, it should be noted that for all three types, the maximum contribution is noted by the VLF oscillations.Maximum VLF contribution detected in boys with normoemic type of microcirculation (corresponding type I of the LDF gram) – 48 %, for guys with hypoemic and hyperemic types of microcirculation (types II and II of the LDF-gram in accordance) this indicator was 46 %. The maximum contribution of LF oscillations to the power of the frequency-amplitude spectrum is registered in boys with nornoemic type – 35%. The contribution of HF oscillations had the maximum value in guys with nornoemic type and had no significant differences – 13%.

3. CONCLUSIONS

Method of laser Doppler flowmetry provides a detail analysis of the system blood circulation state, based on the selection of rhythmical components of hemodynamic flows in the tissues of the human organism. The LDF method allows investigation and evaluation of mechanisms of active and passive regulation in tissue blood flow. Scholars study also another factors affecting state of human health[16]. Differentiated analysis of individually-typological features of blood microcirculation by the LDF method showed that three types of blood microcirculation are characteristic of the young men of Melitopol State Pedagogical University examined by us: normoemic (48%), hypoemic (39%), hyperemic (13%). The prospects of further investigations will be directed on using method of laser Doppler flowmetry with further analysis of amplitude-frequency spectrum of components of tissue blood flow in the study of reserve capabilities of the blood microcirculation system in future software engineers using physical samples.

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