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Functional Changes in Blood Microcirculation in the Skin of the Foot during Heating Tests in Patients with Diabetes Mellitus

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Abstract—The paper shows the possibility of using the laser Doppler flowmetry method for the assessment of system of blood microcirculation in the lower limbs of patients with diabetes mellitus. A series of experimental studies involving 76 patients with diabetes mellitus and 46 healthy volunteers was carried out. The state of peripheral blood flow was assessed during the local heating tests with different temperature regimes. The wavelet analysis of LDF-grams was used to evaluate the adaptive changes of microcirculation during the temperature tests. The data show that the proposed methodology in the form of heating tests and the use of the wavelet transform in the analysis of LDF-grams allows the evaluation of adaptation processes in the microcirculatory bed during thermal tests and the detection of the preclinical stage of trophic disorders.

Keywords: noninvasive diagnosis, diabetes mellitus, laser Doppler flowmetry, blood microcirculation, heating test

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The medical, social, and economic impact of diabetes mellitus (DM) is associated with that most patients develop complications, which disable the patients and decrease quality of life, as well as with the progressive rise of the incidence of the disease. According to data of the International Diabetes Federation (IDF), the number of patients with DM reached 415 million in 2015, and the number of patients with DM is predicted to be 642 million by 2040 [1]. One of the major causes of complications and mortality of the patients with DM is the development of macro- and microvascular disorders that provoke the development of retinopathy and nephropathy and lead to acceleration of atherosclerosis with injury of the cardiac, cerebral, and peripheral vessels. In patients with diabetic foot syndrome, macro- and microcirculatory disorders associated with the impaired neural trophism of tissues due to polyneuropathy contribute to the development of purulent-necrotic injuries and impaired wound repair [2–6].

Therefore, an urgent matter is the early detection of the changes in pathogenetic factors and diagnosis of complications both when the first sings of the disease appear and the pathological processes are still reversible and in the presence of impairments in order to prevent the aggravation of DM and select optimal correction of the treatment.

One of the challenging approaches in the study of the functional state of the microcirculatory bed and detection of associated complications in DM is the use of laser Doppler flowmetry (LDF) [7, 8]. This method is based on the probing of tissue with a laser beam and analysis of the light reflected and scattered by moving red blood cells. This method enables in vivo estimation of perfusion in the microcirculatory bed. Spectral analysis of the recorded signal provides further evaluation of the changes in blood microcirculation. The use of this mathematical device enables an analysis of different regulatory mechanisms within a wide frequency range (0.0095–1.6 Hz). Several main frequency ranges have currently been revealed according to the influence of different regulatory mechanisms: the endothelial (0.0095–0.02 Hz) to reflect NO-dependent influences [9–11], neurogenic (0.021–0.046 Hz) reflecting the influence of the neurogenic sympathetic vasomotor activity [12–14], common myogenic (0.047–0.145 Hz) associated with the activity of vascular smooth muscle cells [12, 14, 15], as well as cardiac (0.8–1.6 Hz) and respiratory (0.2–0.4 Hz) components reflecting the influence of cardiac con-

Table 1. Main clinical laboratory parameters of the examined patients with DM

No.	Parameter	Value
1	Gender (M/F)	22/48
2	Age, years	53.1 ± 12.9
3	Body mass index, kg/m^2	31.9 ± 6.4*
4	Type of diabetes, 1/2	16/54
5	The length of the disease, years	12.9 ± 8.7
6	Systolic blood pressure, mmHg	138.1 ± 17.7
7	Diastolic blood pressure, mmHg	83.4 ± 7.9
8	Fasting glucose, mmol/L	9.0 ± 4.5*
9	HbA1c, %	8.6 ± 1.4*
10	Total cholesterol, μmol/L	5.0 ± 1.2
11	ALT, U/L	33.2 ± 20.0
12	AST, U/L	29.8 ± 23.3
13	Urea, mmol/L	7.5 ± 6.1
14	Creatinine, μmol/L	81.2 ± 26.7

Reference values for the parameters: body mass index is less than 25 kg/m^2; HbA1c is 4.0–6.0%; total cholesterol is 3.5–5.0 mmol/L; ALT is 10–38 U/L; AST is 10–40 U/L; urea is 2.5–7.5 mmol/L; creatinine is 70–110 μmol/L. * Values that exceed the reference values.

tractions and respiration [16–18]. The common myogenic range includes oscillations of the sensory peptidergic origin 0.047–0.069 Hz and the true myogenic oscillations 0.07–0.145 Hz.

The use of different functional tests in addition to the LDF technique raises the information capacity of diagnosis [19]. This expands the diagnostic potential of the proposed method and makes it possible to evaluate both the overall functional state of the microcirculatory bed and its reserve and adaptive capacities. A local heating test provides other options to evaluate the functional state of the microcirculatory bed in DM and identify factors that promote the development of complications, since vasodilation during this test is associated with the activation of the unmyelinated nociceptive C-fibers (under moderate heating to 34–35°C) and NO release from the endothelium upon further heating to 41–42°C [8, 20, 21]. The functioning of these regulatory mechanisms is impaired significantly in DM.

The NO-dependent vasodilation under the influence of physical load was examined in a study in [21], foot skin was heated locally to 44°C; the influence of successive heating on the perfusion change was not studied. Our study offers a successive application of local temperature tests under different regimes, at 25°C, 35°C, and 42°C. This innovative method of successive local temperature stimulation enables a differentiated study of the vascular responses in relation to different regulatory mechanisms of blood flow and evaluation of different mechanisms of heat vasodilation in DM.

The aim of this study was to analyze the changes in blood microcirculation indices under different regimes of foot skin local heating in patients with DM.

MATERIALS AND METHODS

This study involved 70 patients from the Endocrinology Department of Orel Regional Clinical Hospital (Orel, Russia) diagnosed with diabetes mellitus. The exclusion criteria were the history of the diabetic foot syndrome; an acute period of diseases of the cardiovascular, bronchopulmonary, gastrointestinal tract, liver, kidneys, and blood that could influence the result of diagnosis; and a history of alcoholism or drug addiction. The major clinical laboratory parameters were measured according to standard laboratory procedures. Blood pressure was measured after a 5-min rest of a patient in the sitting position. The major clinical laboratory parameters of the examined patients from the Endocrinology Department are given in Table 1.

The control group included 47 apparently healthy volunteers (22 women, 25 men) with the mean age of 46.6 ± 13 years who had not been diagnosed with diseases of the circulation, musculoskeletal system, or connective tissue according to questionnaire data.

Experimental studies were conducted according to a specially developed study protocol approved by the Ethics Committee of the Orel State University named after I.S. Turgenev of November 3, 2015 (protocol of the Ethics Committee meeting no. 7). All the participants of the experimental studies were acquainted with the contents of the study and signed an informed consent form with indication of their readiness to participate in the measurements.

Hemodynamic parameters were measured using a channel of the LAZMA-MC diagnostic LDF system (SPE LAZMA, Moscow, Russia) [22]. In the LDF channel used, the tissue was probed at 1064 nm wavelength, the average sample volume was 1–3 mm^3. Heating tests were performed using the LAZMA-TEST device (NPP LAZMA, Moscow, Russia) [22]. During experimental studies the optical probe was installed on the dorsal surface of the foot at the plateau point between the first and second metatarsals. The positioning of the optical probe of the LAZMA-MC complex and the heat probe of LAZMA-TEST during experimental studies is shown in Fig. 1a.

The frequency analysis of different regulatory mechanisms: passive mechanisms, which are formed beyond the microcirculatory bed and enter microvessels with blood flow, and active mechanisms, which are formed in the microcirculatory bed and reflect dynamic changes in the mechanisms of tone formation, was performed using the LDF3.0.2.384 software (NPP LAZMA). The software performs continuous

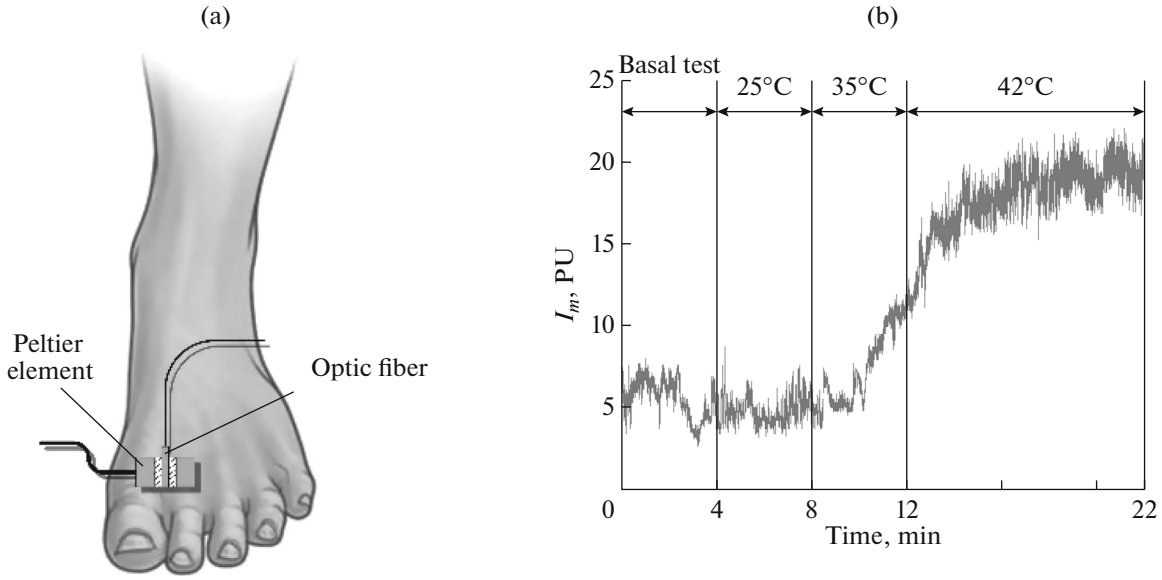

Fig. 1. Schematic diagrams of (a) an optic probe positioning on the foot of patients during experimental examinations and (b) experimental examinations at the example of LDF-gram recording of an apparently healthy volunteer.

wavelet transform using complex-valued Morlet wavelet as the analyzing wavelet [17, 23] [15].

Each study included four stages: a 4-min basal test, cooling to 25°C for 4 min, and local heating tests at temperatures of 35°C and 42°C for 4 and 10 min, respectively. Thus, the total measurement on one foot lasted for 22 min; both feet were analyzed (the scheme of the study is given on Fig. 1b). The tissue was cooled to 25°C because of the differences in the initial temperature in the studied area and the need to adjust the skin temperature in the area under study in all the participants to the same initial conditions.

All the examinations were performed in the lying position, 2 h after taking a meal, at physical and psychological rest, and after preliminary adaptation of the participants to the room temperature of 21–24°C for at least 10 min.

During experimental studies at all the stages, the index of blood microcirculation was recorded (I_m, perfusion units, PU). Afterwards, the amplitude–frequency spectra of the recorded LDF-grams, including oscillation amplitudes of the endothelial (A_e, PU), neurogenic (A_n, PU), myogenic (A_m, PU), respiratory (A_r, PU), and cardiac (A_c, PU) origins, were analyzed according to the standard technique [24]. In addition to the listed parameters, we analyzed the contribution of oscillation amplitudes of the measured frequency range relative to the moderate modulation of blood flow for the endothelial (A_e/σ, arbitrary units), neurogenic (A_n/σ, arbitrary units), and myogenic (A_m/σ arbitrary units) ranges of tone formation.

The contribution of different blood flow pathways to the common blood flow was evaluated from estimation of the bypass index (BI, arb. units) and the value of nutritive blood flow (I_{mn}, PU) using a technique [25] that analyzes the amplitude-frequency range and takes into account the almost complete absence of arteriovenous anastomoses in the skin at the back side of feet.

The bypass coefficient was calculated as follows:

$$\mathrm{BI}(I_m) = \mathrm{BI}(I_m)_1 + \mathrm{BI}(I_m)_2. \quad (1)$$

$\mathrm{BI}(I_m)_1$ was calculated using the following equation:

$$\mathrm{BI}(I_m)_1 = A(I_m)_{\mathrm{act}}/A(I_m)_{\mathrm{m}}, \quad (2)$$

where $A(I_m)_{\mathrm{act}}$ is the maximum amplitude of blood flow oscillations associated with the active mechanisms of regulation (the oscillations of the endothelial, neurogenic, and myogenic origins) $A(I_m)_{\mathrm{m}}$ is the amplitude of blood flow oscillations within the myogenic range.

BI $(I_m)_2$ was calculated as follows:

$$\mathrm{BI}(I_m)_2 = A(I_m)_{\mathrm{pass}}/A(I_m)_{\mathrm{m}}, \quad (3)$$

where $A(I_m)_{\mathrm{pass}}$ is the maximum amplitude of blood flow oscillations associated with the passive regulation mechanisms (the oscillations of the cardiac or respiratory origin).

$\mathrm{BI}(I_m)_2$ was used in the estimation of the bypass coefficient if $\mathrm{BI}(I_m)_2 \geq 1$ [26].

The nutritive blood flow was calculated using the following equation:

$$I_{mn} = I_m/\mathrm{BI}(I_m). \quad (4)$$

The experimental data were analyzed statistically. All the data were checked for normality of distribution

Table 2. Results of estimation of the main hemodynamic parameters

Parameter	Stage 1 basal test		Stage 2 cooling to 25°C		Stage 3 heating to 35°C		Stage 4 heating to 42°C	
	4 min		4 min		4 min		10 min	
	healthy	patients	healthy	patients	healthy	patients	healthy	patients
I_m, PU	5.28 ± 1.91	5.05 ± 1.97	4.76 ± 1.79	4.73 ± 2.08	9.44 ± 3.28	6.74 ± 2.70*	20.12 ± 4.35	11.89 ± 3.71*
A_e, PU	0.49 ± 0.41	0.27 ± 0.16*	0.45 ± 0.36	0.32 ± 0.17*	0.60 ± 0.31	1.00 ± 0.48*	0.63 ± 0.42	0.46 ± 0.29*
A_n, PU	0.54 ± 0.42	0.37 ± 0.19*	0.49 ± 0.37	0.39 ± 0.23*	0.61 ± 0. 33	0.40 ± 0.24*	0.51 ± 0.46	0.36 ± 0.18*
A_m, PU	0.31 ± 0.26	0.23 ± 0.13*	0.27 ± 0.19	0.20 ± 0.12*	0.50 ± 0.24	0.38 ± 0.17*	0.46 ± 0.34	0.26 ± 0.16*
A_r, PU	0.18 ± 0.13	0.19 ± 0.08	0.19 ± 0.16	0.20 ± 0.09	0.24 ± 0.15	0.29 ± 0.17	0.34 ± 0.15	0.24 ± 0.20*
A_c, PU	0.56 ± 0.24	0.48 ± 0.21*	0.50 ± 0.22	0.48 ± 0.23	0.65 ± 0.22	0.54 ± 0.26*	1.18 ± 0.47	0.86 ± 0.41*
A_e/σ, arb. units	0.48 ± 0.30	0.34 ± 0.14*	0.46 ± 0.14	0.44 ± 0.37	0.41 ± 0.25	0.46 ± 0.37	0.33 ± 0.28	0.30 ± 0.14
A_n/σ, arb. units	0.54 ± 0.37	0.46 ± 0.19	0.49 ± 0.15	0.54 ± 0.36	0.40 ± 0.26	0.43 ± 0.26	0.27 ± 0.30	0.25 ± 0.10
A_m/σ, arb. units	0.33 ± 0.26	0.30 ± 0.15	0.28 ± 0.12	0.28 ± 0.21	0.34 ± 0.22	0.33 ± 0.32	0.24 ± 0.22	0.18 ± 0.08*
BC, arb. units	3.54 ± 2.78	3.68 ± 3.00	3.66 ± 3.30	4.35 ± 2.79*	2.52 ± 1.25	4.05 ± 2.83*	3.92 ± 2.09	5.06 ± 3.56*
I_{mn}, PU	1.49 ± 1.50	1.37 ± 1.79*	1.3 ± 1.15	1.08 ± 0.97*	3.74 ± 3.00	1.66 ± 2.16*	5.13 ± 3.19	2.34 ± 2.06*

* The significance of difference between the values was confirmed with $p < 0.05$ using one-way ANOVA.

using the Kolmogorov–Smirnov test and homogeneity of variance using the Levene's test. Difference significance of the samples was estimated using one-way analysis of variance (ANOVA). The difference was considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Analysis of the experimental findings has shown that heat exposure leads to a significant change in the hemodynamic parameters of the microcirculatory bed. Figure 2 shows typical examples of the recorded LDF-grams of a patient (Fig. 2a) and an apparently healthy volunteer (Fig. 2b), as well as their amplitude-frequency spectra before (Figs. 2c, 2d) and after (Figs. 2e, 2f) local heating to 35°C for the patient and healthy volunteer, respectively.

As seen in Fig. 2, adaptive changes of microcirculation during heating test differ between a healthy volunteer and patient. Upon tissue heating to 35°C, healthy volunteers exhibit rearrangement of oscillations within the active range, as well as an increase in the peak in the region of 0.06 Hz and the appearance of a peak at a frequency of 0.12 Hz due to the activation of the sensory peptidergic fibers and neurotransmitter release from their nerve endings at initial vasodilation (around 0.06 Hz) and the influence of myogenic oscillations (around 0.12 Hz).

Different processes occur in the patient with DM. Upon local heating to 35°C, the oscillations within the active frequency range do not change, while the amplitudes of the respiratory (0.2–0.4 Hz) and cardiac (0.8–1.6 Hz) oscillations increase. This response points to lack of the response to heating by the sensory nerve fibers and the activation of arterial and venular hyperemia processes.

Table 2 presents the results of estimation of the major hemodynamic parameters for all stages of the study and their statistical analysis; the estimated values of the mean and mean square deviation of the study parameters are given.

Figure 3 shows the results of statistical data processing presented as box plots for nutritive blood flow (Fig. 3a) and myogenic rhythms (Fig. 3b). In the plots, the central line is the median and the edges of the box are the low and upper quartiles (the 25th and the 75th).

On the basis of the findings, some estimated parameters under local heat stimulation (values of the bypass index and nutritive blood flow) were found to differ in patients with DM and the control group. Since heating led to the changes mostly in the perfusion indices of the large vessels than of the microcirculatory bed (A_c and A_r), as well as in the ratio of the bypass pathway to the nutritive pathway of blood flow, it is noted that the studied participants without diabetic foot symptoms retained in general the microves-

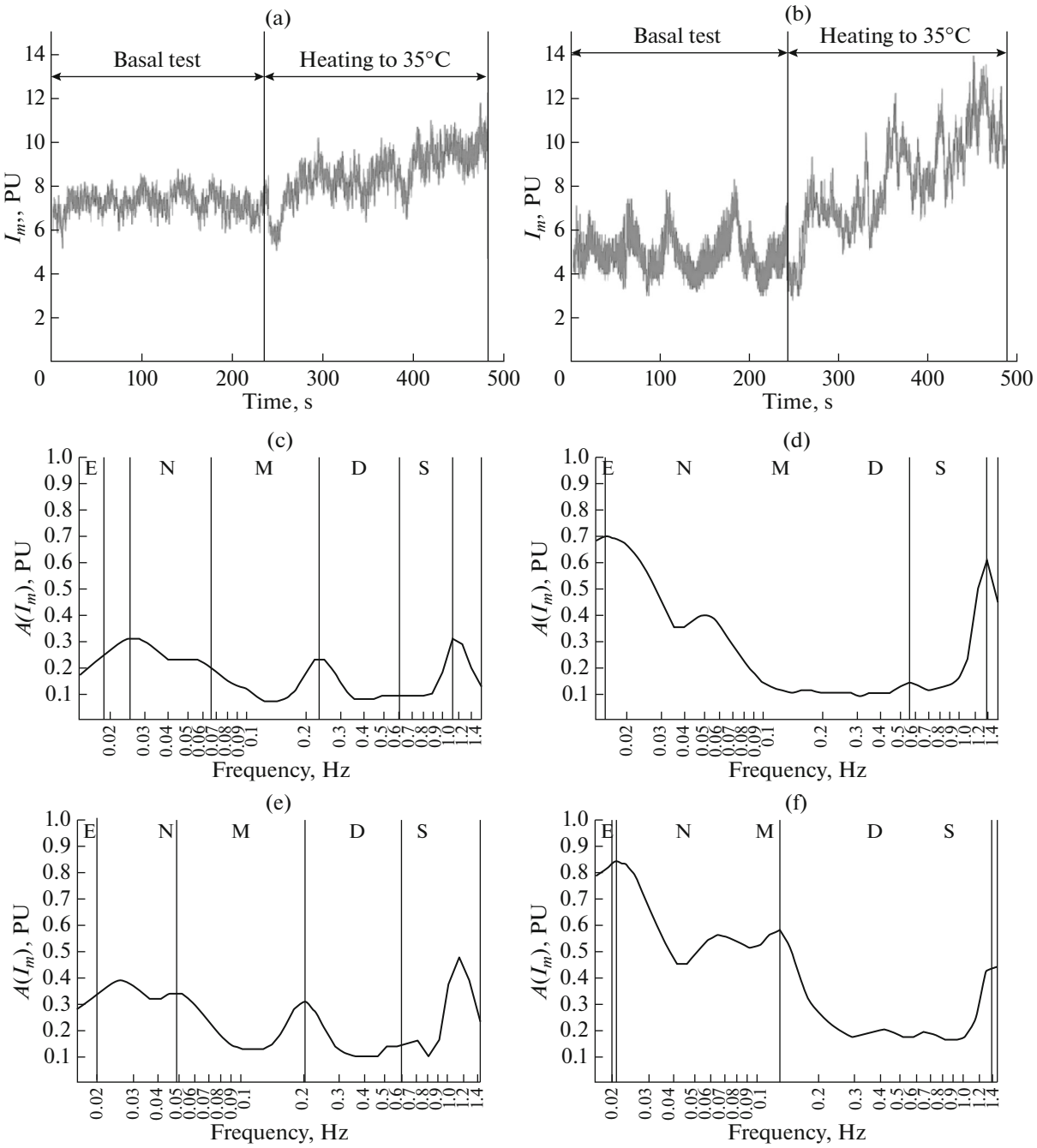

Fig. 2. An example of typical LDF-grams of (a) a patient and (b) an apparently healthy volunteer and their amplitude–frequency spectra (c, d) before (for a patient and a healthy volunteer, respectively) and (e, f) after local heating to 35°C. When using wavelet analysis of the LDF-grams in heat tests, we estimated the fragments of records without regions with intermediate processes caused by local biological tissue heating.

sel regulation, except for the deficient myogenic mechanisms under high heating temperature.

A low initial endothelial activity and a decreased blood flow to microvessels (according to cardiac rhythm) were observed in patients with DM at rest before testing. Upon heating to 35°C, the only lower amplitude was the one of the cardiac rhythm (blood inflow) in patients with DM, the rest values of the spectrum did not differ. After heating to 42°C, low pressure indices of blood inflow and blood outflow (according to cardiac and respiratory rhythms), and, therefore, a significantly low myogenic activity were observed.

A low increase in perfusion at both stages of heating (3 and 4 stages, respectively) in patients compared to controls points to the insufficient regulation of blood

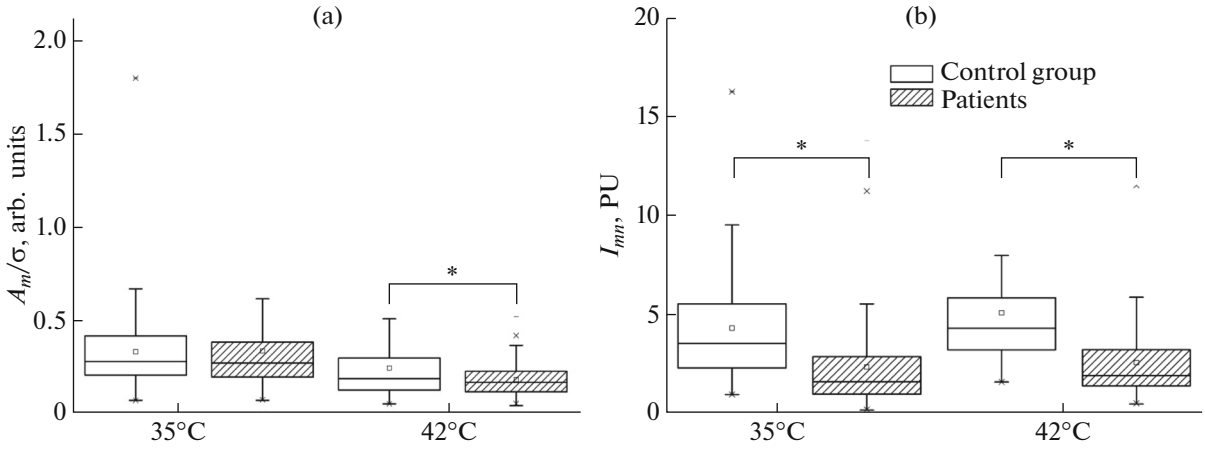

Fig. 3. Box plots for (a) normalized myogenic rhythms and (b) nutritive blood flow. * Difference significance was confirmed with $p < 0.05$ using one-way ANOVA.

microcirculation by mechanisms responsible for vasodilation. In particular, decreased perfusion values in patients at 35°C can be considered as a criterion of dysfunction of the sensory nerve fibers, and this may indirectly indicate the presence of polyneuropathy complications. A lower increase in perfusion at 42°C indicates deficient endothelium-dependent vasodilation resulting from balance shifting in the endothelial synthesis of vasodilators (nitric oxide, NO) and vasoconstrictors (endothelin-1) towards endothelin-1 dominance and development of endothelial dysfunction. This response of the microcirculatory bed appears during the interaction of multiple pathological factors, namely, hyperglycemia, insulin resistance, oxidative stress, dyslipidemia, and as shown in [27–29], the development of hyperglycemia and accumulation of glycation end products play a key role in the suppression of NO synthesis in endothelial cells in diabetes mellitus.

In addition, we observed a decrease in the amplitudes of the respiratory and cardiac oscillations in the group of patients during heating to 42°C compared to the control group because of the decreased microcirculation pressure and perfusion decrease.

The observed lower value of the amplitude of myogenic oscillations normalized to σ upon heating to 42°C in patients with DM compared to controls indicates a decrease in the vasomotor activity of precapillary sphincters and an increase in their tone leading to reduced nutritive blood flow.

The low values of the nutritive blood flow in patients at both stages of heating compared to the control group, as well as an increase in the bypass index, indicate redistribution of blood flow to the side of the bypass component and a decrease in the capillary perfusion.

CONCLUSIONS

Thus, the use of laser Doppler flowmetry with wavelet analysis of the blood flow oscillations of the microcirculatory bed enables one to analyze perfusion disorders and blood flow regulation mechanisms in microvessels at the early stages of DM and to estimate the nutritive blood flow. The regulatory mechanisms of the microcirculatory bed of the endothelial and myogenic origins are impaired in the patients with DM, and the function of the fine unmyelinated sympathetic and sensory nerve fibers is affected. This leads to a decrease in blood flow reserves during heating test, dysfunction of precapillary sphincters, redistribution of blood flow in microvessels with dominance of the bypass component and a decrease in the nutritive perfusion. The revealed functional changes in the microcirculatory bed can cause tissue hypoxia in DM and decreased tissue viability with the development of trophic ulcers.

The estimation of the functional state of the microcirculatory bed in DM can be advantageous for early preclinical diagnosis of trophic complications and improve quality of life of the patients.

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