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A Device for Comprehensive Noninvasive Diagnostics of the Tissue Microcirculation System of Human Skin

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A device for simultaneous monitoring of blood and lymph microcirculation and oxidative metabolism in the tissue microcirculation system has been developed. The device implements the diagnostic methods of laser Doppler flowmetry (monitoring of blood and lymph microcirculation) and fluorescence spectroscopy (measurement of fluorescence of coenzyme biomarkers of oxidative metabolism). The device includes a unit for functional temperature tests of tissue adaptation used to assess the energetics of metabolism.

Introduction

Real-time diagnostic monitoring is of fundamental importance in in vivo studies of physiological disorders of skin because it allows detecting changes in the biophysical characteristics of skin associated with vital activities. The device described in this work provides comprehensive monitoring of the state of the tissue microcirculation system of human skin; namely, blood and lymph microcirculation and oxidative metabolism. The device combines the well-known method of laser Doppler flowmetry (LDF) [1] and fluorescence spectroscopy (FS). When tissue is exposed to laser radiation in the course of LDF, the reflected signal has components produced by radiation scattering on red blood cells and on lymph particles [1, 2]. Signals from blood and lymph flows are separated by frequency filtering for the corresponding ranges of velocities of red blood cells and lymph particles. Fluorescence of coenzyme biomarkers of metabolism (reduced nicotinamide adenine dinucleotide (NADH) and oxidized flavin adenine dinucleotide (FAD)) is measured to assess the oxidative metabolism [3-5]. The metabolic processes in the cellular structures of the tissue are energy dependent. A temperature test involving heating (enhancing metabolism) and cooling (slowing down metabolism) is performed to assess the adaptive capabilities and eliminate possible optical interference from tissue fluorophores in coenzyme fluorescence.

Methods, Engineering Implementation, and Results

The LAZMA ST device (Roszdravnadzor Certificate No. RZN 2017/5844, June 8, 2017) implements the comprehensive diagnostic technique described above. Diagnostics can be carried out at rest or during the functional temperature test involving cooling to 10 °C (slowed microcirculation and metabolism) and heating to 35 °C (enhanced microcirculation and metabolism). Special software provides presentation of the obtained results in graphical and numerical form. Figure 1 shows an example of simultaneously detected diagnostic parameters.

Complex index of oxidative metabolism is a measure of the functional state of the tissue microcirculation system. It is proportional to the microcirculation flow and inversely proportional to the sum of amplitudes of coenzyme fluorescence (see Section 2 below).

1. Laser Doppler flowmetry of blood and lymph microcirculation. The diagnostic technique is based on laser probing. An optical fiber probe delivers probing laser radiation and detects radiation backscattered from the tissue (Fig. 2).

The results of flowmetry can be presented as follows:

$$MI = C \cdot N \cdot V_{\text{mean}}, \quad (1)$$

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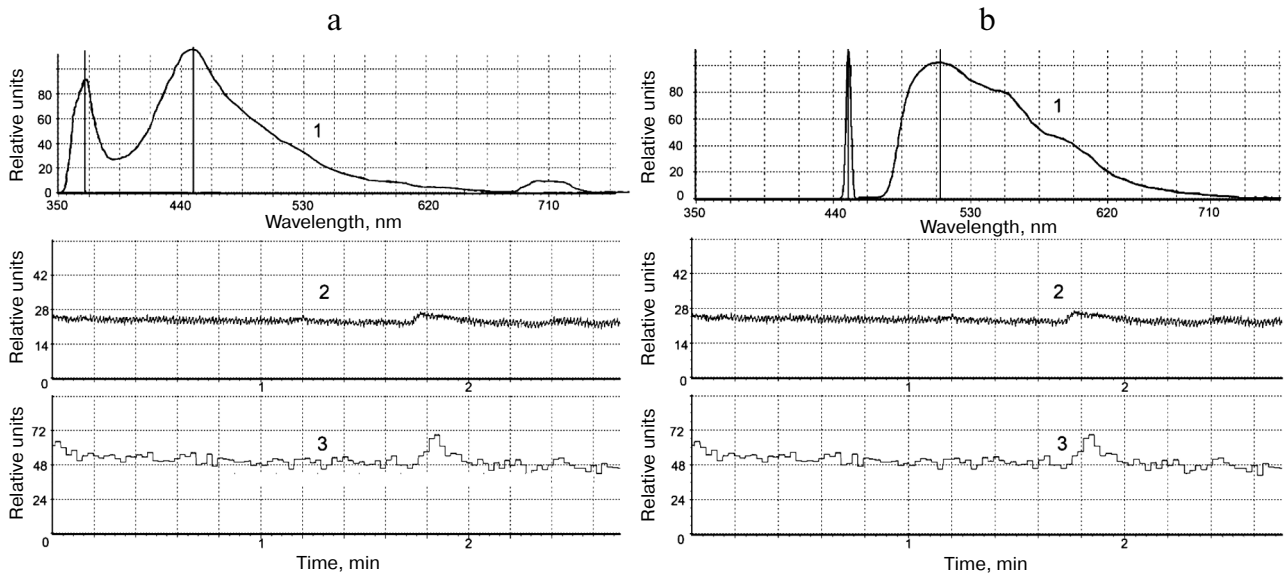

Fig. 1. An example of simultaneously detected diagnostic parameters: a) NADH (FS; curve 1), blood microcirculation (LDF; curve 2), and lymph microcirculation (LDF; curve 3); b) FAD (1), blood microcirculation (2), and lymph microcirculation (3).

where MI is the microcirculation index (signal amplitude, V), C is the proportionality coefficient (calibration-dependent), N is the number of scatterers in the exposed tissue volume, and V_{mean} is the mean velocity of scatterers in the exposed tissue. Laser radiation in blood microvessels is mainly scattered by red blood cells; in lymph microvessels, by interstitial scatterers entering lymph microvessels in the process of lymphopoiesis.

LDF-signals have constant and time-variable components. Thus, the microcirculation index can be described by the following expression:

$$MI(t) = M + \delta MI(t), \qquad (2)$$

where M is the constant flow component and $\delta MI(t)$ is its time-variable component depending on changes in the cross sections and diameters of the vessels and the flow velocity (which, in turn, is determined by microcirculation regulatory factors).

The amplitude of the signal proportional to the product $C \cdot N \cdot V_{mean}$ is assessed in relative units.

The diagnostic approach described above makes it possible to simultaneously monitor the blood and lymph flows as physiologically related components of the human tissue microcirculation system.

2. Fluorescence spectroscopy of oxidative coenzymes. Fluorescence of the coenzymes NADH and FAD [6, 7] was excited using a Nichia NVSU233B-D4 LED with a wavelength of 365 nm (NADH fluorescence wavelength is in the range of 460-470 nm) and an OSRAM PLT5 450B laser diode with a wavelength of 450 nm (FAD fluorescence maximum is in the range of 510-520 nm).

The temperature test was used to study the tissue response to cooling and heating. The more pronounced the changes in the NADH and FAD concentrations during the temperature test compared with the control, the less substrate and coenzymes are utilized in the initial state of the tissue and the more pronounced is the decrease in the oxidative metabolism. The oxidative metabolism is normalized as the utilization of the sub-

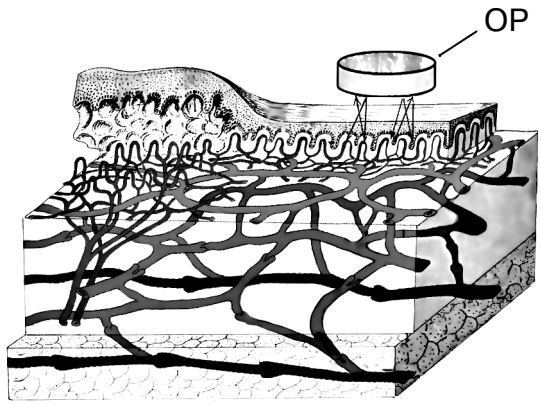

Fig. 2. Laser Doppler flowmetry of tissue (OP is the optical probe).

strate and coenzymes is restored to its normal level. Control values of the coenzyme fluorescence amplitudes depend on the purpose of the study and the participant's age. Under otherwise identical conditions, control values increase with age due to the age-related decrease in metabolic activity.

It was proposed to assess the oxidative metabolism from changes in the fluorescence responses during a temperature functional test. This allows the optical interference due to the fluorescence of fluorophores at the coenzyme fluorescence wavelengths to be reduced. The fluorescence of collagen (one of the dominant skin fluorophores) has no effect on the increase (with cooling) or decrease (with heating) in the fluorescence amplitudes of coenzymes. Heating to 35 °C and cooling to 10 °C do not change the collagen content in the tissue. Thus, in calculating the difference in the fluorescence amplitudes (A) for NADHH (ΔA_n) and FAD (ΔA_f), the constant contribution of the collagen fluorescence is eliminated. The heating temperature of 35 °C was selected for two reasons. The first reason is that proteins, including collagen, do not denaturate at 35 °C; the second is that this temperature is close enough to the temperature range in which the highest rate of enzymatic reactions is attained (38-40 °C) [8]. In addition, the activity of local mechanisms of regulation of cutaneous blood flow increases at 35 °C, leading to an increase in the microcirculation capillaries.

Cooling leads to vasodilation. This makes unambiguous measurement of metabolic slowdown impossible due to an increase in blood flow leading to additional tissue heating. The cooling temperature of 10 °C was selected to facilitate assessment of the tissue response: at this temperature the onset of cold vasodilation is delayed by about 1 min, during which time it is possible to correctly measure the drop in the utilization of the substrate and coenzymes.

The LAZMA ST device consists of the LAZMA-D analyzer for monitoring peripheral blood and lymph flow and coenzyme markers of oxidative metabolism and the LAZMA-TEST unit for functional temperature tests (Fig. 3a). The optical fiber probe of the LAZMA-D analyzer, which delivers laser radiation to the tissue and detects radiation backscattered from it, is combined with the temperature probe of the LAZMA-TEST unit. The combined probe is fixed to the tissue area under examination; for example, to the big toe (Fig. 3b).

The index of oxidative metabolism (*IOM*) is calculated as:

$$IOM = M_{nutr}/(A_n + A_f),$$

where M_{nutr} is the nutritive blood flow; $M_{nutr} = A_m/(A_{neur} + A_h)$, where A_{neur}, A_m, and A_h are the blood flow oscillation amplitudes in the neurogenic, myogenic, and cardiogenic ranges, respectively. The obtained results are compared to control.

Cooling activates the reserves of the tissue microcirculation system. If the adaptation coefficient $Ca^{-}) = [1 - (IOM_{in} - IOM_{10})/ IOM_{in}]$ (where IOM_{in} and IOM_{10} are *IOM* values in the initial state and upon cooling to 10 °C, respectively) is in the control range of 0.8-1.0, the adaptive capabilities are considered as normal. If $Ca^{-})$ is below the control range, the system reserves are reduced. The heating test allows the additional energy supply required

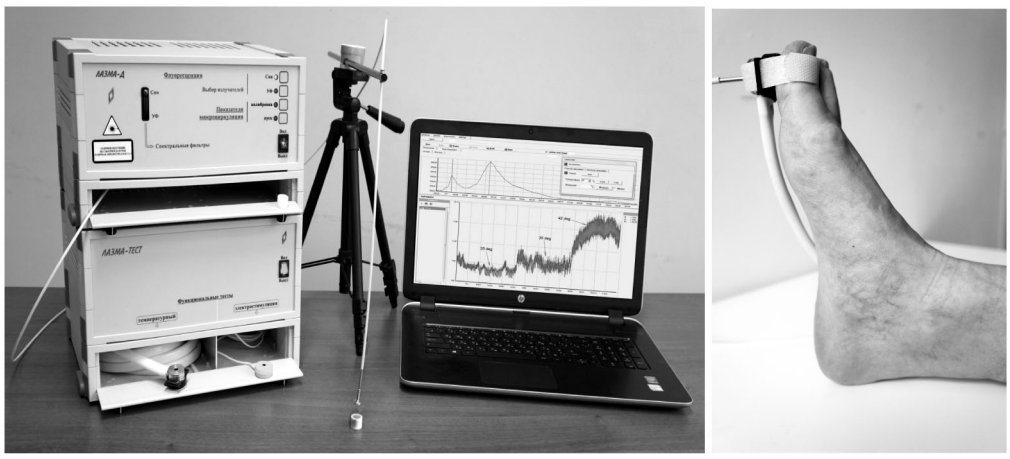

Fig. 3. a) LAZMA ST device and a laptop with recording and processing software; (b) an example of mounting of the optical fiber probe.

for normal functioning of the tissue microcirculation system to be determined. If heating to 35 °C leads to the coefficient $Ca^{+)} = [1 - (IOM_{35} - IOM_{in})/IOM_{in}]$ decreasing below the specified control range, the tissue demands more energy to reach its initial functional state.

The diagnostic method considered in this work can be used, for example, for noninvasive early diagnosis of diabetes mellitus and for choosing the optimal therapy [9].

Discussion and Conclusions

The developed device and techniques for noninvasive monitoring can be used to detect disorders of the tissue microcirculation system of human skin. The developed techniques are based on general physiological processes in the tissue, which makes them applicable for solving various diagnostic problems. In particular, they can be used in healthy subjects; for example, for testing athletes during exercise and in tests for adaptation to extreme conditions. They can also be used for diagnosis of various pathologies. The device and techniques described in this work make it possible to detect treatment-related improvements in the energetics of metabolism.

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