

Skin Microhemodynamics and Mechanisms of Its Regulation in Type 2 Diabetes Mellitus

I. V. Tikhonova^a, A. A. Grinevich^a, A. V. Tankanag^a, and V. G. Safronova^{a,*}

^a Institute of Cell Biophysics, Russian Academy of Sciences,
Pushchino, Moscow oblast, 142290 Russia

* e-mail: safronova@icb.psn.ru

Received April 26, 2022; revised May 19, 2022; accepted May 20, 2022

Abstract—The review presents modern ideas about peripheral microhemodynamics, approaches to the analysis of skin blood flow oscillations and their diagnostic significance. Disorders of skin microhemodynamics in type 2 diabetes mellitus (DM) and the possibility of their interpretation from the standpoint of external and internal interactions between systems of skin blood flow regulation, based on a comparison of couplings in normal and pathological conditions, including models of pathologies on animals, are considered. The factors and mechanisms of vasomotor regulation, among them receptors and signaling events in endothelial and smooth muscle cells considered as models of microvessels are discussed. Attention was drawn to the disturbance of Ca²⁺-dependent regulation of coupling between vascular cells and NO-dependent regulation of vasodilation in diabetes mellitus. The main mechanisms of insulin resistance in type 2 DM are considered to be a defect in the number of insulin receptors and impaired signal transduction from the receptor to phosphatidylinositol-3-kinase and downstream targets. Reactive oxygen species plays an important role in vascular dysfunction in hyperglycemia. It is assumed that the considered molecular and cellular mechanisms of microhemodynamics regulation are involved in the formation of skin blood flow oscillations. Parameters of skin blood microcirculation can be used as diagnostic and prognostic markers for assessing the state of the body.

Keywords: microcirculation, type 2 diabetes mellitus, amplitude-frequency analysis, endothelium, vasoactive factor receptors, signaling

DOI: 10.1134/S0006350922040200

STUDY OF PERIPHERAL HEMODYNAMICS AND ANALYSIS OF SKIN BLOOD FLOW OSCILLATIONS

Currently, there are a large number of invasive and non-invasive techniques for the study of peripheral hemodynamics. Among the non-invasive techniques, video capillaroscopy, photoplethysmography (PPG), laser Doppler flowmetry (LDF), as well as laser Doppler imaging and laser contrast imaging of speckles have been widely used [1–8]. The state of microvascular bed of skin and mucous membranes reflects both as the organ-specific features of blood flow well as changes occurring in microvascular system of the whole organism [9–12]. Changes in skin microvascular system correlate with shifts in central hemodynamics, which makes it possible to use the parameters of blood microcirculation in skin as diagnostic and prognostic

markers to assess the health and general physical state of an organism [4, 11–14].

Peripheral blood flow is known not to be stable, but is subjected to temporal and spatial oscillations, reflecting the most important characteristic features of microcirculation: variability and adaptability to constantly changing hemodynamic conditions and to the need of tissues for blood perfusion [15, 16]. Therefore, in recent years, the main subject of microcirculation research has become mechanisms of its lability, since exactly the rhythmic oscillations of blood flow carry information about the state of peripheral microhemodynamics regulation systems.

Several non-overlapping frequency intervals of blood flow oscillations are considered to be identified in the frequency range from 0.005 to 2 Hz in skin microvascular bed in human [17]. There are characteristic frequency borders for each of the intervals, in which oscillations reflect the influence of heart rate, movement of thorax, myogenic activity of vascular smooth muscle cells, neurogenic regulation of vascular activity and endothelial vasomotor activity. In this case, low-frequency blood flow oscillations are the most inter-

Abbreviations: LDF; laser Doppler flowmetry, PPG; photoplethysmography; DM; diabetes mellitus, WPC; wavelet phase coherence; ROS; reactive oxygen species, TRP; Transient Receptor Potential; SOC; store-dependent calcium channel (Store Operated Channel); eNOS, endothelial NO synthase; NOX, NADPH Oxidase.

esting in the frequency range from 0.005 to 0.2 Hz, reflecting the influence of local regulatory processes of different origin: related to the activity of smooth muscle cells of vessel walls (0.06–0.2 Hz, the range of myogenic rhythm) [18, 19], neurogenic control (0.02–0.06 Hz, the range of neurogenic rhythm) [20] and endothelial activity (0.005–0.02 Hz, the range of endothelial rhythm) [17, 21].

Registration of skin blood flow oscillations with LDF methodology and further amplitude-frequency analysis of obtained LDF signals makes it possible to study myogenic, neurogenic and endothelial systems of skin microvasculature regulation both at rest and during functional tests (occlusion, thermal and ionophoretic tests). Functional tests are used to assess the state of peripheral blood flow regulation systems and identify their adaptive reserves to conditions of short-term ischemia [22–25], to change of skin temperature [4, 22, 26–29], in response to vasoactive substances (acetylcholine, nitroprusside, calcium blockers) [4, 11, 30–33] and to assess pathological changes in socially significant diseases including diabetes mellitus [34].

SKIN MICROHEMODYNAMIC DISTURBANCES IN TYPE 2 DIABETES MELLITUS

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease, manifested by impaired carbohydrate metabolism with the development of hyperglycemia due to insulin resistance, secretory dysfunction of beta-cells, and lipid metabolism disorders. This form covers individuals with relative (rather than absolute) insulin deficiency and peripheral insulin resistance. Although the specific etiology is unknown, there is no autoimmune destruction of beta-cells and there are no other known causes of diabetes in patients. Most patients with T2DM are overweight or obese [35]. One of the main causes of complications and mortality in T2DM patients is the development of macro- and microvascular disorders [36, 37]. Diabetes is accompanied by the progression of cardiovascular diseases and macrovascular complications such as atherosclerosis, coronary heart disease, and peripheral arterial disease, also it is a factor aggravating the course of coronavirus disease (COVID-19) [38–44].

The most commonly diagnosed complications in diabetic patients are associated with microcirculation disorders, which leads to foot ulcers, retinopathy, neuropathy, diabetic dermopathy and weakened wound healing [40, 41, 45]. These diabetes-related microvascular dysfunctions can ultimately lead to more severe complications. It indicates the need to identify microvascular dysfunctions both at an early stage of the disease, when pathological changes are still reversible, and at later stages to prevent the aggravation of the disease course and select an adequate treatment correction. Earlier the role of microvascular changes in diabetes pathogenesis was shown in relation to metabolism [46,

47], NO effect [48], changes in peripheral microvascularization [49], skin neurovascular dysfunction [50], microvascular dysfunction [51–54], and venous distension [55]. Microvascular disorders in diabetes occur in various organs, including kidneys, eyes and skin. Skin is the most available and convenient object to non-invasive research of microvascular disturbances. Disturbances of skin microvascular function were already shown by LDF method at an early stage of the disease [56]. Changes in blood flow and vascular properties were revealed in pre-diabetic and diabetic patients [57–62]. Local skin heating (thermal test) is one of the most frequently used and convenient functional tests for assessing the reactivity of skin microvessels in T2DM patients. It allows assessing the role of various mechanisms involved in the regulation of vasodilation and to reveal changes in adaptive capabilities of microvascular bed in diabetes [26, 40, 63–68]. However, in most studies of skin microhemodynamics by LDF method, only integral parameters characterizing skin blood perfusion were analyzed, and little attention was paid to the spectral composition of signals, allowing assessing regulatory systems of blood flow and its adaptive capabilities. In addition, the data obtained by different authors are sometimes contradictory. Spectral analysis of LDF signals recorded on the leg (ankle) showed that the relative spectral power in frequency intervals of endothelial, neurogenic, and myogenic activity was significantly lower in diabetic patients vs healthy subjects even at rest [69]. Whereas other authors found an increase in skin blood perfusion as well as a decrease in the amplitudes of blood flow oscillations in frequency ranges of endothelial and neurogenic activity in T1DM and T2DM patients vs healthy volunteers at rest [34]. Local heating also led to a decrease in the vasodilator response and the amplitudes of spectral components in endothelial and neurogenic intervals [34]. On the contrary, in another study there was an initial lower microvascular perfusion and a greater vasodilator reserve with local heating in patients with diabetes compared to the same in examinees from the control group [29]. Thus, despite the variety of studies in the field of microcirculation in patients with diabetes, it is difficult to compare the data of various studies with each other, as well as our own data with the results obtained by other authors. The reasons may be as follows: (1) most studies did not indicate the type of diabetes, or studies were carried out with a mixed group of patients with T1DM and T2DM, without dividing into types; (2) in most studies with LDF method the researchers evaluated only the integral parameters of skin blood flow without spectral analysis, which does not allow assessing changes in the functioning of regulatory systems of microhemodynamics in pathology; (3) registration of microcirculation parameters was conducted in different skin areas; (4) often the analysis of results was incorrect due to the formation of groups, when control group consisted of young aged healthy

volunteers (up to 35 yr old), while the average age of patients with diabetes was more than 50 yr; (5) the temperature, rate and duration of local heating varied greatly during heat test by different research groups. These reasons explain the inconsistency of the study results of skin microcirculation in human. The solution to the problem can be the correct planning and standardization of experimental protocols for the purpose of reproducibility and the possibility of comparing the results obtained by different groups.

EXTERNAL AND INTERNAL COUPLINGS BETWEEN THE REGULATORY SYSTEMS OF SKIN BLOOD FLOW IN NORM, IN HYPERGLYCEMIA, AND DIABETES MELLITUS

Currently, a number of studies are aimed at finding and assessing the external and internal couplings between the systems of skin blood flow regulation. The evaluation of external couplings both between regulatory systems and interactions between the processes of skin blood flow regulation in different parts of microvascular bed is performed using the wavelet phase coherence (WPC) function [70, 71], group WPC (gWPC) [72], or nonlinear modeling and Bayesian method [73, 74].

In low-frequency spectral range (neurogenic and myogenic intervals), the change in WPC between oscillations of temperature and skin blood flow was shown in healthy volunteers in response to cold stress [70, 75]. We revealed high reliable median WPC values for contralateral forearm skin blood flow in cardiac, respiratory and myogenic intervals in healthy volunteers at rest [71]. A change in external couplings between processes of skin hemodynamics regulation of upper and lower extremities was demonstrated in healthy volunteers under orthostasis [72]. In healthy subjects, orthostasis increased gWPC between skin blood flow oscillations on the foot and heart rate variability (HRV) or respiration, as well as between skin blood flow oscillations on the forearm and foot at a respiratory rate (~0.3 Hz).

Change in external couplings between the processes of peripheral microcirculation regulation are observed with age and under pathologies. In type 1 DM patients a weakening of phase interactions between respiratory activity and peripheral pulse was found, reconstructed using the multi-Gaussian modeling algorithm from experimental LDF signals [29]. The study of interactions between HRV, respiration, and skin microcirculation in healthy young and elderly volunteers and elderly patients with essential hypertension revealed the difference in WPC between the groups. The direction and functional dependence of the strength of couplings on the frequency of spectral components of registered signals was revealed: the strength of external couplings between regulatory systems of skin microhemodynamics decreased in aging,

and practically disappeared in patients with hypertension disease at a frequency of 0.1 Hz [76].

The activity of heart and respiratory excursions determine the central mechanism of skin microhemodynamics regulation which causes high-frequency oscillations (cardio- and respiratory rhythms). They are formed due to passive changes in blood pressure in microvessels during respiration and cardiac output, which leads to elastic stretching of vessel walls, including their muscle components. Passive stretching of smooth muscle cells promotes to increase in calcium concentration in the cytosol ($[Ca^{2+}]_i$) [77, 78]. Change in heart activity is characterized by oscillations of heart rate variability. These oscillations are formed by the autonomic and central nervous system, the activity of baro- and chemoreceptors, respiratory and endocrine regulation [79, 80]. It is known that the baroreflex and the direct interaction between the parasympathetic and sympathetic systems of regulation determine the proportions of low-frequency and high-frequency components in the spectrum of HRV oscillations characterizing the sympatho-vagal balance [81]. Disturbances of the sympatho-vagal balance can occur under pathologies: due to decreased baroreflex activity in patients with hypertension disease [82], due to autonomic neuropathy in type 2 DM patients [50, 83–85]. Targets of endothelial and neurogenic regulation are smooth muscle cells of microvessels [6, 13, 86–89]. There is also an interaction between endothelial cells and peripheral neurons via NO, calcitonin gene-related peptide (CGRP) and other mediators [86, 87, 90]. Moreover, smooth muscle cells have their own spontaneous activity, which determines the myogenic regulation of peripheral blood flow.

Analysis of literature showed that internal couplings were not earlier investigated, while external couplings were fragmentarily investigated in healthy volunteers, and they remain unstudied in type 2 DM patients. We have assessed for the first time the internal couplings characterizing the interaction between regulatory processes occurring in one part of microcirculatory bed in a small tissue volume [91]. Overlapping areas of adjacent frequencies with medium and strong internal couplings were found that characterized the interactions between regulatory mechanisms of skin blood flow: the central ones (respiratory and cardiac) and local (endothelial, neurogenic and myogenic). It was revealed that the number of internal couplings increased with age, both between local and between local and central mechanisms of regulation. We assume that changes caused by pathological disorders in the mechanisms described above will lead to a change in the number and strength of external and internal couplings between the processes regulating skin perfusion. The formation of ideas about the internal and external couplings between the processes of regulation of microcirculatory blood flow can provide a basis for their consideration as the reliable non-inva-

sive biomarkers of functional state of cardiovascular system under pathological changes in a human organism.

INVESTIGATION OF MICROCIRCULATION DISORDERS IN ANIMALS ON MODELS OF PATHOLOGIES (DIABETES MELLITUS AS AN EXAMPLE)

Currently, most of microcirculation studies using LDF are conducted with the participation of volunteers and patients with pathologies. However, such studies have a number of ethical and methodological limitations, so it becomes necessary to conduct research involving physiological and pathological models on animals. The studies of skin blood flow in laboratory animals, according to appropriate ethical protocols, allow confirming experimentally the hypotheses, verification of which is impossible in the studies with participation of volunteers. To date, there are investigations devoted to the study of skin microcirculation in animals, mainly rats. Particularly, the features of peripheral microhemodynamics in rats were studied under different conditions (aging, stress, acute and chronic hypoxia, hypothermia, ischemia/reperfusion syndrome) and on T2DM model [92–104]. A significant number of studies was aimed at investigation of the effect of physiologically active compounds and pharmacological substances on the processes occurring in skin microvascular bed in animals [105–110]. It should be noted that microcirculation changes under stimuli were estimated only by the total skin perfusion in most animal studies. There are only single studies of rhythmic components of peripheral skin microhemodynamics [102, 111]. This is probably a consequence of the fact that the need to immobilize the animal for measurements and functional tests using traditional methods of anesthesia can lead to changes in mechanisms of skin hemodynamics regulation. The use of combined injection-gas anesthesia “Zoletil–nitrous oxide” made it possible to adequately assess the parameters of microcirculation of animals in conditions close to normal sleep ones [112, 113]. Nowadays, the increase in the incidence of diabetes mellitus, mainly T2DM, which is dangerous with chronic complications, dictates the need for extensive research to find effective measures for its prevention and treatment [114]. Experimental models on animals that are the most sensitive to DM development using adequate methods of its induction will partially solve this problem. There are many ways to create animal models of diabetes, for example, using anti-insulin serum or antagonists of insulin receptor, pancreatectomy, glucose infusion, beta-cell cytotoxic agents and viruses; as well as diabetogenic nutritional and hormonal factors [115–117]. However, most of the currently used experimental models of diabetes (surgical, chemical, endocrine, immune, genetic) are not suitable for studying type 2 DM, since they are associated with or cause a signif-

icant destruction of beta-cells in the Langerhans islets of pancreas, which is type 1 DM characteristic.

The pathophysiology of type 2 DM is mainly based on a decrease in tissue sensitivity to insulin, which further leads to a decrease in insulin secretion. These processes cause hyperglycemia with subsequent damage to various organs. The creation of an animal model of type 2 DM, similar in etiology and course to type 2 DM in human, is difficult, but very important, as it will help to understand the complex pathophysiology of the disease, as well as give new ideas for research related to therapeutic procedures [118]. Analysis of modern literature showed that rodents (mainly mice) and minipigs are the most suitable animals for type 2 DM modeling. The following approaches are used: mono- or polygenic obesity, high-fat diet, non-obesity model and genetically induced model [115, 116]. In most of these models, obesity is provoked, which reflects the state of people in whom obesity is one of the main causes of type 2 DM [119]. In experimental type 2 DM models rodents were used: genetically modified animals (C57BL/KsJYLeprdb/+ mice, db/db mice, New Zealand Obese mice, TALLYHO/Jng mice, Obese Zucker Diabetic Fatty rats (OZDF), inbred and outbred rodent lines (C57BL/6 and BALB/c mice, Wistar rats) [115, 116, 120–126]. Since in the etiology of type 2 DM (as well as obesity) in human, excessive caloric intake is of primary importance, diet-induced models in various animal species seem to be the most adequate. The diet has a significant impact on the development of pathology. Thus, for the development of obesity and type 2 DM in C57BL/6 mice, a high-fat diet, including up to 60% fat, is required, and a faster and more pronounced effect is achieved when a large amount of sucrose or fructose is added to the diet [127, 128]. A diet with a high fructose content (35–60% kcal relied to fructose) was used to induce insulin resistance in Wistar rats [124, 129]. For the induction of type 2 DM in mini-pigs, it was also recommended a high-fat diet with addition of a large amount of sucrose [130].

Thus, the models induced by diets are the closest in etiology and development mechanisms to type 2 DM in human, among which high-fat and high-carbohydrate diets are the most effective. Some authors recommend additional using small, individually calculated doses of streptozotocin to accelerate the development of diet induced type 2 DM, so as not to completely destroy beta-cells of the pancreas, as it is the case with type 1 DM [104, 116, 131]. However, streptozotocin injection is an additional stress factor and can lead to increased severity and development of later progressive stages of the disease [132]. Besides, such models are extremely time-consuming and require constant monitoring of metabolic parameters in animals.

The main criteria for DM development and the effectiveness of the therapeutic and preventive measures used are the content of glucose, insulin and glycosylated hemoglobin in blood plasma, the number of beta-cells

in the pancreatic islets, the result of a glucose tolerance test, the index of insulin resistance, as well as the results of histology and histochemistry of the pancreas and other organs [35].

FACTORS AND MECHANISMS OF VASOMOTOR REGULATION

Endogenous and exogenous vasoactive agents act on blood vessels, causing vasoconstriction or vasodilatation. Many factors are vasoactive: hormones, peptides, lipid metabolism products, proteases, extracellular vesicles, low molecular weight substances (for example, nitrogen oxide – NO, hydrogen peroxide – H₂O₂), internal and external environmental factors (temperature, shear stress, pressure, pH, partial pressure of oxygen or CO₂). Their action is mediated by the activation of certain receptors, signaling pathways or by the effect on intracellular components in endothelial and smooth muscle cells [4, 13, 133–139].

Ion channels and membrane receptors participate in the regulation of vasoactivity: large-conductance Ca²⁺-activated K⁺-channel, voltage-dependent K⁺-channel, mechanosensitive ion channels, receptors—ion channels (purine, isoforms of transient receptor potential channel, nicotinic acetylcholine receptors), receptors coupled to heterotrimeric GTP-binding proteins (adrenoceptors, adenosine, serotonin, vasopressin, endothelin receptors and other), receptors—tyrosine kinases (insulin receptor, Tie1-2, growth factor receptors) [140–142], receptor-type protein tyrosine phosphatases [143–145]. The following signal transduction sequences can serve as examples of principle schemes of intracellular and intercellular communication components activation in the action of vasoactive factors: (1) phospholipase A2 → cytochrome P450 → eicosanoids → large-conductance Ca²⁺-activated K⁺-channel, the activation of which leads to hyperpolarization of the membrane of smooth muscle cells and subsequent relaxation of the vessel (for example, in shear stress); (2) endothelial NO-synthase → NO release → smooth muscle cells → soluble guanylate cyclase → cGMP → protein kinase G → change of Ca²⁺ concentration in the cytosol → large-conductance Ca²⁺-activated K⁺-channel; (3) oxidases (NADPH-oxidase, xanthine oxidase and others) → reactive oxygen species (ROS) → decreased NO and/or increased hydrogen peroxide levels. The given schemes can be detailed by transduction of signals from certain receptors of vasoactive agents, involved in the formation of external or internal couplings, to intracellular effector molecules. Each of the activation schemes is not complete and rather conventional, since it is a generalization of the results obtained in studies of blood vessels from different organs and under functional tests in human or animals, while it is known that blood flow regulation in microvascular bed is species and tissue specific [146–

148]. In some cases, inhibitors and activators of certain signaling components were applied by iontophoretic method, in which agents can influence surrounding tissues [13, 149]. In addition, the schemes include some results obtained on large vessels therefore their application to microvascular bed requires further research. It should be noted that modern views do not explain mechanisms of the formation of oscillations in peripheral blood flow, which characterize the state of microvascular bed more fully and significantly change in pathologies including T2DM [150–153].

CHANGING Ca²⁺-DEPENDENT REGULATION OF COUPLING BETWEEN ENDOTHELIAL AND MUSCLE VASCULAR CELLS IN DIABETES MELLITUS

The main regulatory mechanisms of smooth muscle contractility are considered changes of membrane potential and Ca²⁺-metabolism. NO, Ca²⁺, metabolites of arachidonic acid, ROS, and redox and potential sensitivity of regulatory partners should be especially noted among regulatory factors. Ca²⁺-signaling in endothelial and smooth muscle cells of microvessels needs to be clarified in norm and in T2DM. So, the above-mentioned large-conductance Ca²⁺-activated K⁺-channel, included in endothelial-muscle communication, was studied mainly on large vessels in human or animals [135, 154, 155]. The following players are involved in calcium metabolism in endothelial cells of different tissues: inositol 1,4,5-trisphosphate receptors responsible for Ca²⁺-release from the endoplasmic reticulum, isoforms of transient receptor potential channel, members of the ORAI family of calcium channels, store-operated calcium channels (SOC), endothelial T-type voltage gated calcium channels, endoplasmic reticulum Ca²⁺-ATPase 2a (SERCA2a), plasma membrane Ca²⁺-ATPases (PMCA1 and PMCA4), Na⁺/Ca²⁺-exchanger [156–161]. In smooth muscle and endothelial cells as well as in microvascular pericytes, ion channels make an important in the regulation of cell-cell interactions and microvascular function by generating intracellular Ca²⁺ and membrane potential signals [162–164]. Ca²⁺-homeostasis and mechanisms of regulation of Ca²⁺ concentration in the cytosol of blood vessel endotheliocytes may be altered in DM. Thus, in the aorta endothelium of the OZDF (Obese Zucker Diabetic Fatty) rats, a decreased SOC-dependent Ca²⁺ entry was revealed, whereas no differences were found in the pool of Ca²⁺ released from the endoplasmic reticulum in response to inositol-1,4,5-triphosphate [165]. It has been shown that channels with transient receptor potential (channels of the TRP family: TRPC, canonical; TRPV, vanilloid; TRPM, melastatin; TRPML, mucolipin) are involved in changes and vascular dysfunction in diabetes and obesity or are potentially associated with vascular dysfunction [166]. Hyperglycemia and diabetes decrease

the expression of channels of the vanilloid receptor TRPV4 and their functional activity in endothelial cells of retinal microvessels [167]. A decrease in the expression of Ca^{2+} -sensitive receptor on endothelial and smooth muscle cells of the aorta in obese mice with T2DM was shown, which may be involved in the development of diabetic vasculopathy [168]. Changes in the activity of voltage-dependent channels of cardiomyocytes, observed in diabetic cardiomyopathy accompanying T2DM, lead to changes in heart rate and heart contractile function [169]. We expect that the consequence will be a change in the profile of heart rate variability, which will affect the spectral characteristics as well as the external and internal couplings. Besides, Ca^{2+} -dependent mechanisms of NADPH oxidase activation are impaired in T2DM that associated with impaired actin polymerization and Ca^{2+} transport [170, 171]. There is an increase in the baseline level of $[\text{Ca}^{2+}]_i$ and a decrease in the peak of Ca^{2+} responses to stimuli in neutrophils of T2DM patients [172]. It is assumed that this is due to impairments of either the Ca^{2+} entry, or its release from intracellular stores, or both processes. The mentioned disturbances of Ca^{2+} regulation in T2DM were studied mainly on the cells of large vessels. It remains unknown how Ca^{2+} regulation is involved in the formation of rhythmic processes in microvasculature.

NO-DEPENDENT REGULATION OF VASODILATION AND ITS ABNORMALITIES IN DIABETES MELLITUS

NO is one of the universal transmitters involved in blood vessel vasodilation. It is produced from L-arginine by NO-synthases (NOS) in the presence of cofactors, including tetrahydrobiopterin, FAD, CaMK and others [173]. The activity of endothelial NOS (eNOS) is regulated at the transcriptional, post-transcriptional and post-translational levels [90, 174]. One of the main mechanisms of eNOS post-translational modification is phosphorylation, which can be carried out by protein kinase C, protein kinase B, Ca^{2+} /calmodulin-dependent protein kinase, protein kinase A, PI3K/protein kinase B, AMP-activated protein kinase initiated through receptors—tyrosine kinases, G-protein coupled receptors or by shear stress [135, 174]. The activation of eNOS can occur in Ca^{2+} -dependent or Ca^{2+} -independent manner, for example, by Ca^{2+} /calmodulin-dependent protein kinase or shear stress, respectively [173]. Ca^{2+} -dependent NO release initiated by arachidonic acid was shown in cultivated endothelial cells of human cerebral microvessels [175].

The role of signal components mentioned above has been studied fragmentarily in microvasculature oscillatory processes. So, in spontaneously hypertensive rats at the age of 8 to 18 weeks, spectral analysis using wavelet transform revealed a significant decrease in the amplitudes of pancreatic microcirculation oscil-

lations in the endothelial range, which can be partially explained by an increase in plasma levels of nitrite/nitrate, endothelin-1, malonic dialdehyde and interleukin-6 and a decrease in the level of superoxide dismutase in the blood [176]. A comprehensive study of the spectral characteristics of murine skin microhemodynamics oscillations and oscillations of Ca^{2+} and NO concentrations in the cytoplasm of cultured murine microvascular endotheliocytes showed the presence of low-frequency oscillations of both Ca^{2+} and NO concentrations in the frequency interval coinciding with the interval of NO-independent endothelial activity of skin microcirculation [177]. In smooth muscle cells NO can initiate a cGMP-dependent decrease of the frequency of agonist-induced Ca^{2+} oscillations due to a decrease of Ca^{2+} mobilization from intracellular stores [178]. Thus, oscillatory intercellular signals from endothelial cells can interact with oscillations in smooth muscle cells. It is assumed that such an interaction of oscillators may be reflected on a change of strength of external and internal couplings in microvascular bed.

In T2DM, eNOS regulation is impaired at least due to two factors: insulin resistance and hyperglycemia. Insulin plays an important role in the functioning of microvessels, increasing the expression of eNOS and vascular endothelial growth factor VEGF genes in endothelial cells, increasing the density of the capillary network and enhancing NO-mediated vasodilation [4]. Insulin resistance is usually defined as a decrease in insulin sensitivity or the response rate to the metabolic action of insulin. In the different cells of healthy organism, binding of insulin to the insulin receptor causes its autophosphorylation on tyrosine residues and subsequent phosphorylation of the insulin receptor substrate-1 (IRS-1) then activating the Ca^{2+} -independent signaling pathway phosphatidylinositol-3-kinase/phosphoinositide-dependent kinase-1/protein kinase B. Further events are activation of eNOS in endothelial cells, translocation of the glucose transporter GLUT4 into the plasma membrane, regulation of glycogen, protein and lipid synthesis, and glucose consumption [179–181]. There are features of the insulin action, regulation of glucose transport and metabolism in cells of different tissues. It should be noted that phosphatidylinositol-3-kinase/protein kinase B signaling pathway provides vasodilation and pleiotropic effects of insulin on metabolism in the endothelium. Insulin activates the production of endothelin-1, adhesion molecules (VCAM-1 and E-selectin) through signaling pathway Sos/Ras/Raf/MAPK (Sos, adapter protein (Son of sevenless), Ras, small GTPase from proto-oncogene family Ras (Retrovirus associated DNA sequences), Raf, serin/threonine protein kinase of the proto-oncogene family Raf (Rapidly accelerated fibrosarcoma), MAPK, mitogen activated protein kinase), which leads to vasoconstriction, regulates gene expression, cell proliferation and growth [179–181]. Hypertension and vascular dysfunction were

observed in mice on a high-fat diet, not as a result of defective signaling from the insulin receptor via protein kinase B, but due to a decrease in the level of eNOS phosphorylation mediated by free fatty acids.

Endothelial insulin resistance is usually accompanied by suppression of phosphatidylinositol-3-kinase/NO signaling pathway and enhancement or preservation of the intact MAPK/endothelin-1 signaling [182, 183]. So, in the endothelial cells of subcutaneous microvessels obtained from small biopsies of T2DM patients, increased levels of ERK1/2 phosphorylation and endothelin-1 mRNA expression were revealed, at that the excessive MAPK (ERK1/2) activation in patients can be explained by the action of endothelin-1 [184].

PATHOPHYSIOLOGICAL FACTORS OF INSULIN RESISTANCE DEVELOPMENT

It is believed that the reasons of the development of insulin resistance are: lipotoxicity, glucose toxicity, inflammatory status, mitochondrial dysfunction, endoplasmic reticulum stress, hyperinsulinemia in the early stages of type 2 DM, and in some cases a decrease in the expression of insulin receptor or mutations [185–188]. The main mechanisms of insulin resistance in different tissues include a defect in the insulin receptor number and a disturbance of signal transduction from the receptor to PI3K and downstream targets [189]. In addition, the cause may be a disturbance in phosphorylation of insulin receptor and signaling components due to the activation of Ser/Thr protein kinases [190]. “Diabetogenic” factors, especially free fatty acids, inflammatory cytokines, ROS, hyperinsulinemia, increase excessively the activity of various serine protein kinases regulating glucose homeostasis, including c-Jun N-terminal kinase, protein kinase C isoforms, Rho-associated kinase [188, 191, 192]. However, insulin resistance was shown to be a heterogeneous pathological sign that occurs for various reasons in different tissues, but the primary mechanism of this effect includes a single pathway of insulin resistance [193]. This mechanism remains poorly understood in microvessels, and its role in establishing external and internal couplings between the systems of skin blood flow regulation has not been studied. Whereas vascular complications are the main reason of morbidity and mortality in patients with DM, while microvascular complications, such as neuropathy, nephropathy and retinopathy, contribute to a significant decrease in the quality of life in type 2 DM [183]. Blood vessels suffer both from progressive damage of the sympathetic and parasympathetic ganglia, and from direct damage of endothelial cells. The latter is the result of a decrease in NO bioavailability due to the damaging effect of hyperglycemia on eNOS, or on TRPV-4 type calcium channels, or a decrease in the bioavailability of L-arginine, the substrate of eNOS. Oxidative stress resulting from excessive ROS produc-

tion by both endothelial and circulating immune cells is also considered as the reason. The resulting peroxynitrite serves as an additional damage factor.

THE ROLE OF REACTIVE OXYGEN SPECIES IN VASCULAR DYSFUNCTION IN HYPERGLYCEMIA

Sources of ROS in endothelial cells are NADPH oxidases, mitochondrial respiratory chain, NOS, xanthine oxidases, peroxidases, cyclooxygenases, and lipoxygenases [194–196]. NADPH oxidase which catalyzes the production of superoxide anion radical, a precursor of other ROS, is a main player. In normal conditions, superoxide anion radical and hydrogen peroxide, product of its dismutation, regulate endothelium-dependent relaxation and contractility of smooth muscles of blood vessel. In pathologies, the increased expression and activity of NADPH oxidase lead to decrease of NO level, weakening endothelial functions, and a significant increase of contractility [196]. The inclusion of a generalized NADPH oxidase (NOX) image in signaling schemes requires clarification, since NOX1, NOX2, NOX4, and NOX5 (supplied with Ca²⁺ binding sites) isoforms with a high degree of homology, which differ in activation mechanisms, are currently defined in the endothelium [196–201]. For example, cytoplasmic components do not participate in NOX4 activation, it has a high constitutive activity and provides the basal ROS production in vessels, and NOX5 is absent in rodents. The active NOX2-based NADPH oxidase complex includes the membrane subunit p22phox and the cytoplasmic subunits p47phox, p67phox, p40phox, and the small GTP-binding protein Rac1. Polymorphonuclear neutrophil granulocytes (neutrophils) produce superoxide radicals most intensively when NOX2 activated. Also they generate secondary oxidants due to the activity of myeloperoxidase. In DM, neutrophils, the most numerous and extremely reactive population of blood cells, play a significant role in the disturbance of the redox balance leading to oxidative stress [202].

Oxidative stress plays an important role in the development of type 2 DM: ROS level in the blood is an important pathogenetic factor contributing to vascular endothelial and smooth muscle cell dysfunction [42]. The formation of ROS that modify the state of blood vessels in various organs is the result of the activity of many enzymes and biochemical pathways both in the cells themselves and in blood cells. The main sources of ROS in hyperglycemia and diabetes are glucose autoxidation, the polyol pathway of glucose metabolism, the formation of advanced glycation end products, NADPH oxidases, mitochondrial electron transport chain, uncoupled eNOS activity, and xanthine oxidase [203–205]. Potentiation of the endogenous antioxidant system and the use of therapeutic drugs that decrease ROS level can improve the state of the vascular system in hyperglycemia [206]. In particular,

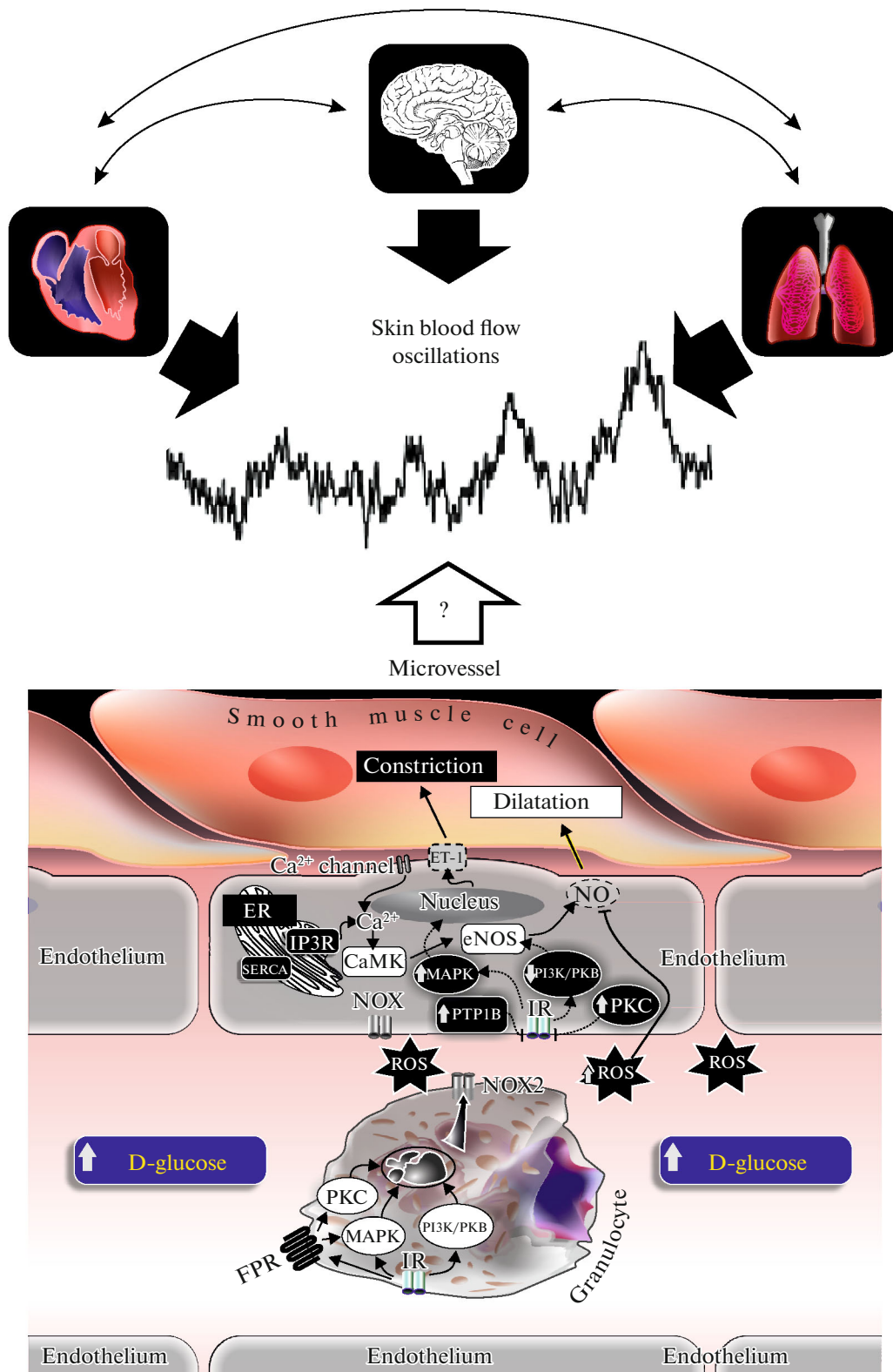


Fig. 1. The scheme of couplings in the regulation of skin blood flow oscillations in humans and animals. White arrows indicate changes in the activity of enzymes or the number of metabolites in type 2 diabetes mellitus; black arrows indicate the direction of transduction of the regulatory signal.

betaine, which promotes the production of glutathione, inhibits pathological neovascularization of the cornea of the eye [207]. Metformin also has a beneficial effect on the state of blood vessels. It has multiple therapeutic effects in type 2 diabetes. In particular, as an antioxidant it directly captures the hydroxyl radical, enhancing the activity of glutathione reductase, catalase and superoxide dismutase and suppressing NADPH oxidase activity [208]. NADPH oxidase is a target of hyperglycemia, which causes disorders in the mechanisms of enzyme activation associated with phagocytosis and the state of intracellular signaling systems, including those dependent on Ca^{2+} [171]. The direct oxidation of sulfhydryl side chains is the main mechanism of protein modification by ROS. The targets are channels, receptor proteins, small G-proteins, enzymes. So, H_2O_2 activates TRP channels in the cultivated endothelial cells of human and mouse pulmonary microvessels [209]. Besides, in hyperglycemia condition in type 2 diabetes, DNA damage and gene transcription changes are observed against the background of increased ROS levels and metabolic depletion of antioxidant reserves. The complex relationship between metabolism and epigenetic changes can provide a conceptual basis for understanding how pathological stimuli operate the development of complications in type 2 DM [210]. Studies of the ROS role in the type 2 DM etiology is promising to a large extent for the development of pharmaceutical agents based on NOX inhibitors [211]. The interaction of signaling pathways, including the calcium metabolism activation and ROS production can be realized in synergy of calcium responses to vasoactive agents in endothelial cells [212]. In addition, ROS generated in vascular smooth muscle cells with participation of NOX4 and NOX5 can create feedback [196, 213, 214].

CONCLUSIONS

Type 2 diabetes mellitus is accompanied by the development of macro- and microvascular complications [36, 37]. Hyperglycemia, a key feature of diabetes, is considered to be the main mechanism that creates conditions for the occurrence of microvascular disorders which are the main cause of the development of retinopathy, nephropathy, diabetic cardiomyopathy and peripheral neuropathy. Mechanisms contributing to the development of diabetic microangiopathy include oxidative stress, impaired regulation of vascular regeneration and vascular cell functions (endothelial, smooth muscle and stromal cells, pericytes), as well as immune cells interacting with the endothelium. Endothelial dysfunction in DM is characterized by a weakening of endothelium-dependent vasodilation and a relative predominance of vasoconstriction, hypercoagulation and an increase in vascular wall permeability. All these circumstances lead to blood flow disturbances in microvascular system and the develop-

ment of diabetic complications. Therefore, the search for effective markers of the functional state of microvessels for early diagnosis of vascular disorders is currently relevant. Analysis of the characteristic frequency modes of skin microcirculatory blood flow oscillations, formed under the influence of the nervous system, lungs, heart and vascular endothelium (Fig. 1), can make a significant contribution to solving this problem. Sympatho-vagal balance with the participation of endogenous vasoactive agents determines the central and local mechanisms of skin blood flow regulation and affects the interrelationships of regulatory systems. But, the couplings between integral characteristics of microcirculatory blood flow and processes of their regulation, including characteristic oscillatory modes of concentrations of extracellular and intracellular signaling mediators (Ca^{2+} , NO, ROS, and others), remain poorly investigated both in normal and in pathologies. The components of the main signaling pathways in endothelial cells under the regulatory influence of blood leukocytes make contribution to the change in the balance of external and internal couplings between the processes of skin microhemodynamics regulation in norm and type 2 DM. Though signaling in endothelial cells themselves and in their interaction with smooth muscle cells has been studied in some detail, it is noteworthy that the schemes of signaling events were constructed with the inclusion of results obtained mainly on large vessels. However, great care is required to extrapolate patterns obtained on endothelial cells of macrovessels to microvessels of various tissues and organs. The limitation is imposed by the fact that, despite the common mesodermal origin of the vessels, they have phenotypic, structural and functional heterogeneity [215–218], and the expression of genes and their products may differ in cells adapted to cultivation and in native endothelial cells due to different local microenvironment and epigenetic modification [159, 217, 218]. Besides, there is no unambiguous understanding of the correspondence of molecular and cellular mechanisms to the integral parameters of microcirculatory blood flow. Comprehensive studies are needed on the whole organism and on cultured cells to create a basis for understanding the molecular and cellular mechanisms of regulation of rhythmic processes of blood flow in skin microvascular bed in human, as well as to assess the role of intracellular targets of vasoactive impacts in the formation of peripheral blood flow oscillations and its modulation in type 2 DM. The development of ideas about internal and external couplings between the processes of regulation of microcirculatory blood flow can give basis for their consideration as reliable non-invasive biomarkers of the functional state of the cardiovascular system in pathological changes in the human body.

FUNDING

The work was supported by the Russian Science Foundation (grant no. 22-15-00215).

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflicts of interest.

Statement on the welfare of humans or animals. This article does not contain any studies involving animals or human subjects performed by any of the authors.

REFERENCES

- C. Puissant, P. Abraham, S. Durand, et al., *PLoS One* **8**, e61320 (2013).
- P. Tafner, F. K. Chen, R. F. Rabello, et al., *Rev. Bras. Ter. Intensiva* **29**, 238 (2017).
- R. T. Junejo, C. J. Ray, and J. M. Marshall, *Eur. J. Appl. Physiol.* **119**, 665 (2019).
- J. L. Cracowski and M. Roustit, *Compr. Physiol.* **10**, 1105 (2020).
- D. A. Low, H. Jones, N. T. Cable, et al., *Eur. J. Appl. Physiol.* **120**, 1 (2020).
- A. Dara, A. Arvanitaki, M. Theodorakopoulou, et al., *Mediterr. J. Rheumatol.* **32**, 6 (2021).
- Y. P. Wang, R. H. Cheng, Y. He, et al., *Front. Bioeng. Biotechnol.* **9**, 786615 (2021).
- A. Lazaridis, A. Triantafyllou, K. Dipla, et al., *Hypertens. Res.* **45**, 445 (2022).
- V. I. Kozlov, in *Proceedings of the III All-Russian Symposium "Application of Laser Doppler Flowmetry in Medical Practice"* (Moskva, 2000), pp. 5–15.
- R. G. Ijzerman, R. T. de Jongh, M. A. Beijk, et al., *Eur. J. Clin. Invest.* **33**, 536 (2003).
- L. A. Holowatz, C. S. Thompson, C. T. Minson, et al., *J. Physiol.* **563**, 965 (2005).
- L. A. Holowatz, C. Thompson-Torgerson, and W. L. Kenney, *Front. Biosci.* **15**, 718 (2010).
- M. Roustit and J. L. Cracowski, *Trends Pharmacol. Sci.* **34**, 373 (2013).
- Y. K. Jan, *Diagnostics (Basel)* **12**, 620 (2022).
- J. A. Schmidt, P. Borgstrom, G. P. Firestone, et al., *J. Vasc. Surg.* **18**, 207 (1993).
- A. J. Chipperfield, M. Thanaj, E. Scorletti, et al., *Microcirculation* **26**, e12538 (2019).
- A. Stefanovska, M. Bracic, and H. D. Kvernmo, *IEEE Trans. Biomed. Engineer.* **46**, 1230 (1999).
- W. Funk, B. Endrich, K. Messmer, et al., *Int. J. Microcirc.: Clin. Exp.* **2**, 11 (1983).
- J. Kastrup, J. Bulow, and N. A. Lassen, *Int. J. Microcirc.* **8**, 205 (1989).
- M. E. Muck-Weymann, H. P. Albrecht, D. Hager, et al., *Microvasc. Res.* **52**, 69 (1996).
- H. D. Kvernmo, A. Stefanovska, K. A. Kirkeboen, et al., *Microvasc. Res.* **57**, 298 (1999).
- Functional Diagnostics of the State of Microcirculatory Tissue Systems. Fluctuations, Information, Nonlinearity: A Guide for Physicians*, Ed. by A. I. Krupatkin and V. V. Sidorov (Librokom, Moscow, 2013).
- M. Rossi, S. Bertuglia, M. Varanini, et al., *Biomed. Pharmacother.* **59**, 233 (2005).
- G. B. Yvonne-Tee, A. H. Rasool, A. S. Halim, et al., *J. Pharmacol. Toxicol. Methods* **52**, 286 (2005).
- J. L. Cracowski, C. T. Minson, M. Salvat-Melis, et al., *Trends Pharmacol. Sci.* **27**, 503 (2006).
- C. T. Minson, *J. Appl. Physiol.* **109**, 1239 (2010).
- V. E. Brunt and C. T. Minson, *J. Appl. Physiol.* **111** (1), 5 (2011).
- G. A. Tew, M. Klonizakis, J. Moss, et al., *Microvasc. Res.* **81** (2), 177 (2011).
- M. Sorelli, P. Francia, L. Bocchi, et al., *Microvasc. Res.* **124**, 91 (2019).
- M. Rossi, S. Taddei, A. Fabbri, et al., *J. Cardiovasc. Pharmacol.* **29**, 406 (1997).
- S. Blaise, M. Hellmann, M. Roustit, et al., *Br. J. Pharmacol.* **160**, 1128 (2010).
- R. S. Bruning, L. Santhanam, A. E. Stanhewicz, et al., *J. Appl. Physiol.* **112**, 2019 (2012).
- A. Jekell, M. Kalani, and T. Kahan, *Heart Vessels* **34**, 484 (2019).
- I. Mizeva, E. Zharkikh, V. Dremin, et al., *Microvasc. Res.* **120**, 13 (2018).
- American Diabetes Association, *Diabetes Care* **44** (Suppl. 1), S15 (2021).
- R. Madonna, C. R. Balistreri, Y. J. Geng, et al., *Vasc. Pharmacol.* **90**, 1 (2017).
- R. Madonna, D. Pieragostino, C. R. Balistreri, et al., *Vasc. Pharmacol.* **107**, 27 (2018).
- M. V. Shestakova, O. K. Vikulova, M. A. Isakov, et al., *Probl. Endokrinol.* **66**, 35 (2020).
- A. Al-Awar, K. Kupai, M. Veszelka, et al., *J. Diabetes Res.* **2016**, 9051426 (2016).
- D. Fuchs, P. P. Dupon, L. A. Schaap, et al., *Cardiovasc. Diabetol.* **16**, 11 (2017).
- A. Carrizzo, C. Izzo, M. Olivetti, et al., *Int. J. Mol. Sci.* **19**, 2968 (2018).
- W. D. Strain and P. M. Paldanius, *Cardiovasc. Diabetol.* **17**, 57 (2018).
- J. M. Gregory, J. C. Slaughter, S. H. Duffus, et al., *Diabetes Care* **44**, 526 (2021).
- A. Yonekawa and N. Shimono, *Biology (Basel)* **11**, 400 (2022).
- H. M. Murphy-Lavoie, A. Ramsey, M. Nguyen, et al., in *StatPearls* (Stat-Pearls, Treasure Island, 2021).
- N. E. Cameron, S. E. Eaton, M. A. Cotter, et al., *Diabetologia* **44**, 1973 (2001).
- R. L. Greenman, S. Panasyuk, X. Wang, et al., *Lancet* **366** (9498), 1711 (2005).
- L. A. Sokolnicki, S. K. Roberts, B. W. Wilkins, et al., *Am. J. Physiol. Endocrinol.* **292**, E314 (2007).
- J. E. Tooke, *Cardiovasc. Res.* **32**, 764 (1996).
- A. I. Vinik, T. Erbas, T. S. Park, et al., *Diabetes Care* **24**, 1468 (2001).
- I. Kozlov, E. Zharebtsov, G. Masalygina, et al., *Diagnostics (Basel)* **11**, 267 (2021).

52. R. A. S. Sancho and G. M. Pastore, *Food Res. Int.* **46**, 378 (2012).
53. S. C. Sanka, D. C. Bennett, J. D. Rojas, et al., *Proc. SPIE* **3924**, 56 (2000).
54. M. Kalani, *Vasc. Health Risk Manage.* **4**, 1061 (2008).
55. J. D. Ward, A. J. Boulton, J. M. Simms, et al., *J. Roy. Soc. Med.* **76**, 1011 (1983).
56. A. E. Caballero, S. Arora, R. Saouaf, et al., *Diabetes* **48**, 1856 (1999).
57. K. M. Azadzoi and I. Saenz de Tejada, *J. Urol.* **148**, 1587 (1992).
58. L. A. Suzuki, M. Poot, R. G. Gerrity, et al., *Diabetes* **50**, 851 (2001).
59. V. Urbancic-Rovan, A. Stefanovska, A. Bernjak, et al., *J. Vasc. Res.* **41**, 535 (2004).
60. J. Turner, J. J. Belch, and F. Khan, *Trends Cardiovasc. Med.* **18**, 109 (2008).
61. P. C. Sun, C. S. Chen, C. D. Kuo, et al., *Microvasc. Res.* **83**, 243 (2012).
62. H. F. Hu, H. Hsiu, C. J. Sung, et al., *Lasers Med. Sci.* **32**, 327 (2017).
63. T. Forst, A. Caduff, M. Talary, et al., *Diabetes Technol. Ther.* **8**, 94 (2006).
64. D. L. Kellogg, Jr., J. L. Zhao, Y. Wu, et al., *J. Appl. Physiol.* **110**, 1406 (2011).
65. J. Petrofsky, D. Paluso, D. Anderson, et al., *Diabetes Technol. Ther.* **13**, 365 (2011).
66. M. Roustit and J. L. Cracowski, *Microcirculation* **19**, 47 (2012).
67. J. S. Petrofsky, F. Alshammari, G. S. Bains, et al., *Med. Sci. Monitor* **19**, 257 (2013).
68. D. Lowry, M. Saeed, P. Narendran, et al., *J. Diabetes Sci. Technol.* **11**, 914 (2017).
69. H. Hsiu, H. F. Hu, and H. C. Tsai, *Microvasc. Res.* **115**, 1 (2018).
70. A. Bandrivskyy, A. Bernjak, P. McClintock, et al., *Cardiovasc. Eng.* **4**, 89 (2004).
71. A. V. Tankanag, A. A. Grinevich, T. V. Kirilina, et al., *Microvasc. Res.* **95**, 53 (2014).
72. I. V. Tikhonova, A. A. Grinevich, I. E. Guseva, et al., *Microcirculation* **28**, e12655 (2021).
73. Y. Shiogai, A. Stefanovska, and P. V. McClintock, *Phys. Rep.* **488** (2–3), 51 (2010).
74. V. Ticcinielli, T. Stankovski, P. V. McClintock, et al., *Annu. Int. Conf. IEEE Eng. Med. Biol. Soc.* **2015**, 7366 (2015).
75. M. Elstad, I. Zilakos, and T. K. Bergersen, *Physiol. Meas.* **38**, 848 (2017).
76. V. Ticcinielli, T. Stankovski, D. Iatsenko, et al., *Front. Physiol.* **8**, 749 (2017).
77. G. Gilbert, T. Ducret, R. Marthan, et al., *Cardiovasc. Res.* **103**, 313 (2014).
78. G. Ji, R. J. Barsotti, M. E. Feldman, et al., *J. Gen. Physiol.* **119**, 533 (2002).
79. F. Shaffer and J. P. Ginsberg, *Front. Public Health* **5**, 258 (2017).
80. F. Shaffer, R. McCraty, and C. L. Zerr, *Front. Psychol.* **5**, 1040 (2014).
81. Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology, *Circulation* **93**, 1043 (1996).
82. T. E. Lohmeier and J. E. Hall, *Circ. Res.* **124**, 1071 (2019).
83. A. I. Vinik, R. Freeman and T. Erbas, *Semin. Neurol.* **23**, 365 (2003).
84. A. I. Vinik, T. Erbas, and C. M. Casellini, *J. Diabetes Invest.* **4**, 4 (2013).
85. V. L. Fisher and A. A. Tahrani, *Diabetes Metab. Syndr. Obes.* **10**, 419 (2017).
86. M. Shibasaki, D. A. Low, S. L. Davis, et al., *J. Appl. Physiol.* **105**, 1504 (2008).
87. S. J. Smillie and S. D. Brain, *Neuropeptides* **45**, 93 (2011).
88. A. G. Goodwill and J. C. Frisbee, *Vasc. Pharmacol.* **57** (5–6), 150 (2012).
89. J. L. Cracowski and M. Roustit, *Microcirculation* **23**, 337 (2016).
90. N. Tran, T. Garcia, M. Aniq, et al., *Am. J. Biomed. Sci. Res.* **15**, 153 (2022).
91. A. Grinevich, A. Tankanag, I. Tikhonova, et al., *Microvasc. Res.* **126**, 103889 (2019).
92. N. A. Lycheva, D. A. Makushina, and A. V. Sedov, *Sovrem. Probl. Nauki Obraz. No. 5*, (2017). <http://www.science-education.ru/ru/article/view?id=26992>. Cited 18 April, 2022.
93. S. Inoue, Y. Asai, and Y. Kawai, *Ann. Plast. Surg.* **50**, 64 (2003).
94. G. J. Hodges, D. N. Jackson, L. Mattar, et al., *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **297** (3), R546 (2009).
95. F. Polito, A. Bitto, M. Galeano, et al., *J. Vasc. Surg.* **55**, 479 (2012).
96. J. Decorps, J. L. Saumet, P. Sommer, et al., *Ageing Res. Rev.* **13**, 90 (2014).
97. S. Gohin, J. Decorps, D. Sigaudou-Roussel, et al., *Microvasc. Res.* **101**, 103 (2015).
98. Y. Q. Liu, Y. F. Liu, X. M. Ma, et al., *J. Plast. Reconstr. Aesthet. Surg.* **68**, e147 (2015).
99. K. X. Song, M. Z. Zhang, J. Q. Hu, et al., *BMC Surg.* **15**, 92 (2015).
100. Y. D. Xiao, Y. Q. Liu, J. L. Li, et al., *J. Surg. Res.* **199**, 732 (2015).
101. S. Hu, Z. L. Lin, Z. K. Zhao, et al., *J. Parenter. Enteral Nutr.* **40**, 924 (2016).
102. X. Yuan, Q. Wu, F. Shang, et al., *Clin. Exp. Hypertens.* **41**, 342 (2019).
103. J. S. Tan, C. C. Lin, and G. S. Chen, *BMJ Open Diabetes Res. Care* **8**, e001004 (2020).
104. Y. Wei, H. Chen, Q. Chi, et al., *Med. Biol. Eng. Comput.* **59**, 1151 (2021).
105. M. Amoroso, O. Ozkan, O. Ozkan, et al., *Plast. Reconstr. Surg.* **136**, 512 (2015).
106. M. Aral, S. Tuncer, A. Sencan, et al., *J. Reconstr. Microsurg.* **31**, 487 (2015).
107. B. Kaya, C. Çerkez, S. E. Işılğan, et al., *J. Plast. Surg. Hand Surg.* **49**, 358 (2015).

108. D. W. Lee, H. J. Hong, H. Roh, et al., *Ann. Plast. Surg.* **75**, 84 (2015).
109. Y. H. Qin, H. S. Jiao, A. S. Li, et al., *J. Plast. Surg. Hand Surg.* **49**, 319 (2015).
110. W. Feng, S. Liu, C. Zhang, et al., *Theranostics* **9**, 5854 (2019).
111. J. M. Newman, R. M. Dwyer, P. St-Pierre, et al., *J. Physiol.* **587**, 2579 (2009).
112. M. E. Astashev, D. A. Serov, and A. V. Tankanag, *Biofizika* **63**, 159 (2018).
113. M. E. Astashev, D. A. Serov and A. V. Tankanag, *Skin Res. Technol.* **25**, 40 (2019).
114. I. I. Dedov, M. V. Shestakova, O. K. Vikulova, et al., *Sakharnyi Diabet* **24**, 204 (2021).
115. S. Pandey and M. C. Dvorakova, *Endocr. Metab. Immune Disord. Drug Targets* **20**, 25 (2020).
116. C. P. D. Kottaisamy, D. S. Raj, V. Prasanth Kumar, et al., *Lab. Anim. Res.* **37**, 23 (2021).
117. B. Proniewski, A. Bar, A. Kieronska-Rudek, et al., *Cells* **10**, 1448 (2021).
118. F. Beguinot and C. Nigro, *Methods Mol. Biol.* **933**, 219 (2012).
119. J. H. Kim and A. M. Saxton, *Methods Mol. Biol.* **933**, 75 (2012).
120. V. K. Mazo, Yu. S. Sidorova, and A. A. Kochetkova, *Vopr. Pitan.* **84**, 63 (2015).
121. O. I. Stepanova, V. N. Karkishchenko, O. V. Baranova, et al., *Biomeditsina* **2**, 28 (2009).
122. J. Molnar, S. Yu, N. Mzhavia, et al., *Circ. Res.* **96**, 1178 (2005).
123. K. K. Wu and Y. M. Huan, *Atherosclerosis* **191**, 241 (2007).
124. H. Poudyal, S. Panchal, and L. Brown, *Br. J. Nutr.* **104**, 1322 (2010).
125. H.-G. Joost, H. Al-Hasani, and A. Schurmann, *Methods Mol. Biol.* **933**, 325 (2012).
126. V. R. Konda, A. Desai, G. Darland, et al., *PLoS One* **9**, e87848 (2014).
127. M.N. Makarov and V. G. Makarov, *Lab. Zhivotn. Nauchn. Issled.*, No. 3, (2018). <https://labanimals-journal.ru/ru/node/763>. Cited April 18, 2022.
128. R. Kohen-Avramoglu, M. A. Laplante, K. Le Quang, et al., *Can. J. Diabetes* **37**, 351 (2013).
129. A. Tominaga, N. Ishizaki, Y. Naruse, et al., *Acupunct. Med.* **29**, 276 (2011).
130. S. Xi, W. Yin, Z. Wang, et al., *Int. J. Exp. Pathol.* **85** (4), 223 (2004).
131. A. K. Azemi, S. S. Mokhtar, L. J. Hou, et al., *Biotech. Histochem.* **96**, 498 (2021).
132. A. Heydemann, *J. Diabetes Res.* **2016**, 2902351 (2016).
133. V. I. Evlakhov and I. Z. Poyasov, *Angiol. Sosudistaya Khir.* **25**, 1 (2019).
134. A. N. Ivanov, E. B. Popykhova, and N. E. Tereshkina, *Usp. Fiziol. Nauk* **51**, 82 (2020).
135. C. R. Triggle, S. M. Samuel, S. Ravishankar, et al., *Can. J. Physiol. Pharmacol.* **90**, 713 (2012).
136. W. C. Cole, G. R. Gordon, and A. P. Braun, *Adv. Exp. Med. Biol.* **1124**, 297 (2019).
137. L. Jaimes, R. Vinet, M. Knox, et al., *Animals (Basel)* **9**, 288 (2019).
138. N. Méndez-Barbero, C. Gutiérrez-Muñoz, and L. M. Blanco-Colio, *Int. J. Mol. Sci.* **22**, 7284 (2021).
139. J. L. Martin-Ventura, C. Roncal, J. Orbe, et al., *Front. Cell Dev. Biol.* **10**, 813885 (2022).
140. K. V. Woo and H. S. Baldwin, *Trends Cardiovasc. Med.* **21**, 118 (2011).
141. K. J. Bubb, R. H. Ritchie, and G. A. Figtree, *Microcirculation* **26**, e12501 (2019).
142. S. N. Baldwin, E. A. Forrester, L. McEwan, et al., *Br. J. Pharmacol.* **179**, 1338 (2022).
143. E. Boedtkjer, K. B. Hansen, D. M. B. Boedtkjer, et al., *J. Cereb. Blood Flow Metab.* **36**, 965 (2016).
144. K. Takahashi, R. Kim, C. Lauhan, et al., *PLoS One* **12**, e0177192 (2017).
145. A. F. O. Justo and P. P. L. Afonso, *J. Cell Commun. Signal.* **15**, 467 (2021).
146. H. G. Augustin and G. Y. Koh, *Science* **357** (6353), eaal2379 (2017).
147. T. Minami, M. Muramatsu, and T. Kume, *Biol. Pharm. Bull.* **42**, 1609 (2019).
148. L. Gifre-Renom, M. Daems, A. Luttun, et al., *Int. J. Mol. Sci.* **23**, 1477 (2022).
149. Z. Melik, P. Zaletel, T. Virtic, et al., *Clin. Hemorheol. Microcirc.* **65**, 205 (2017).
150. I. V. Tikhonova, A. V. Tankanag, N. I. Kosyakova, et al., *Pul'monologiya*, No. 1, 57 (2008).
151. I. V. Tikhonova, A. V. Tankanag, N. I. Kosyakova, et al., *Fundam. Issled.* **7**, 364 (2014).
152. I. V. Tikhonova, N. I. Kosyakova, A. V. Tankanag, et al., *Vestn. Ross. Akad. Med. Nauk* **71**, 233 (2016).
153. I. V. Tikhonova, N. I. Kosyakova, A. V. Tankanag, et al., *Microcirculation* **23**, 33 (2016).
154. J. M. Hughes, M. A. Riddle, M. L. Paffett, et al., *Am. J. Physiol. Heart Circ. Physiol.* **299** (5), H1439 (2010).
155. Z. G. Wu, L. Gu, Y. K. Si, et al., *Endocr. Metab. Immune* **21**, 1270 (2021).
156. M. L. Paffett, M. A. Riddle, N. L. Kanagy, et al., *J. Pharmacol. Exp. Ther.* **334** (3), 753 (2010).
157. A. Filippini, A. D'Amore, and A. D'Alessio, *Int. J. Mol. Sci.* **20**, 4525 (2019).
158. F. Moccia, S. Negri, M. Shekha, et al., *Int. J. Mol. Sci.* **20**, 3962 (2019).
159. P. Thakore and S. Earley, *Compr. Physiol.* **9**, 1249 (2019).
160. K. S. Hong and M. G. Lee, *Korean J. Physiol. Pharmacol.* **24**, 287 (2020).
161. S. Negri, P. Faris and F. Moccia, *Int. J. Mol. Sci.* **22**, 9821 (2021).
162. W. F. Jackson, *Curr. Top. Membr.* **85**, 1 (2020).
163. L. Liu, M. Guo, X. Lv, et al., *Front. Mol. Biosci.* **8**, 677661 (2021).
164. W. F. Jackson, *Front. Physiol.* **13**, 805149 (2022).
165. R. Berra-Romani, A. Guzman-Silva, A. Vargaz-Guadarrama, et al., *Int. J. Mol. Sci.* **21**, 250 (2020).
166. R. D. A. Moraes, R. C. Webb, and D. F. Silva, *Front. Physiol.* **12**, 645109 (2021).

167. K. Monaghan, J. McNaughten, M. K. McGahon, et al., *PLoS One* **10**, e0128359 (2015).
168. Y. Guo, X. Yang, J. He, et al., *Life Sci.* **209**, 217 (2018).
169. N. Ozturk, S. Uslu, and S. Ozdemir, *World J. Diabetes* **12**, 1 (2021).
170. A. Advani, S. M. Marshall, and T. H. Thomas, *Diabetologia* **45**, 719 (2002).
171. I. V. Tikhonova, A. A. Grinevich, I. E. Guseva, et al., *Free Radic. Biol. Med.* **159**, 76 (2020).
172. S. S. Kappala, J. Espino, J. A. Pariente, et al., *Mol. Cell. Biochem.* **387**, 251 (2014).
173. Y. Zhao, P. M. Vanhoutte, and S. W. Leung, *J. Pharmacol. Sci.* **129**, 83 (2015).
174. J. Qian and D. Fulton, *Front. Physiol.* **4**, 347 (2013).
175. R. Berra-Romani, P. Faris, S. Negri, et al., *Cells* **8**, 689 (2019).
176. M. Liu, X. Song, B. Wang, et al., *Am. J. Hypertens.* **34**, 100 (2021).
177. D. Serov, A. Tankanag, and M. Astashev, *Cell Biol. Int.* **46**, 427 (2022).
178. J. F. Perez-Zoghbi, Y. Bai, and M. J. Sanderson, *J. Gen. Physiol.* **135**, 247 (2010).
179. J. A. Kim, M. Montagnani, K. K. Koh, et al., *Circulation* **113**, 1888 (2006).
180. H. Kolb, K. Kempf, M. Rohling, et al., *BMC Med.* **18**, 224 (2020).
181. B. A. Lazar, G. Jancso, and P. Santha, *Int. J. Mol. Sci.* **21**, 2507 (2020).
182. R. Muniyappa and J. R. Sowers, *Rev. Endocr. Metab. Disord.* **14**, 5 (2013).
183. T. Maruhashi and Y. Higashi, *Antioxidants (Basel)* **10**, 1306 (2021).
184. S. Gogg, U. Smith, and P. A. Jansson, *Diabetes* **58**, 2238 (2009).
185. J. Boucher, A. Kleinriders, and C. R. Kahn, *Cold Spring Harbor Perspect. Biol.* **6**, a009191 (2014).
186. I. S. Stafeev, A. V. Vorotnikov, E. I. Ratner, et al., *Int. J. Endocrinol.* **2017**, 5076732 (2017).
187. A. V. Vorotnikov, I. S. Stafeev, M. Y. Menshikov, et al., *Biochemistry (Moscow)* **84**, 1329 (2019).
188. M. Khalid, J. Alkaabi, M. A. B. Khan, et al., *Int. J. Mol. Sci.* **22**, 8590 (2021).
189. M. C. Petersen and G. I. Shulman, *Physiol. Rev.* **98**, 2133 (2018).
190. G. L. King, K. Park, and Q. Li, *Diabetes* **65**, 1462 (2016).
191. P. Geraldès and G. L. King, *Circ. Res.* **106**, 1319 (2010).
192. K. C. Nandipati, S. Subramanian, and D. K. Agrawal, *Mol. Cell. Biochem.* **426**, 27 (2017).
193. D. E. James, J. Stockli, and M. J. Birnbaum, *Nat. Rev. Mol. Cell. Biol.* **22**, 751 (2021).
194. N. V. Goncharov, A. D. Nadeev, R. O. Jenkins, et al., *Oxid. Med. Cell Longevity* **2017**, 9759735 (2017).
195. A. O. Kadlec and D. D. Gutterman, *Compr. Physiol.* **10**, 229 (2019).
196. G. A. Knock, *Free Radical Biol. Med.* **145**, 385 (2019).
197. A. C. Montezano, D. Burger, G. S. Ceravolo, et al., *Clin. Sci. (London)* **120**, 131 (2011).
198. B. K. Rodino-Janeiro, B. Paradela-Dobarro, M. I. Castineiras-Landeira, et al., *Vasc. Health Risk Manage.* **9**, 401 (2013).
199. A. Konior, A. Schramm, M. Czesnikiewicz-Guzik, et al., *Antioxid. Redox Signal.* **20**, 2794 (2014).
200. M. Santillo, A. Colantuoni, P. Mondola, et al., *Front. Physiol.* **6**, 194 (2015).
201. A. Vermot, I. Petit-Hartlein, S. M. E. Smith, et al., *Antioxidants (Basel)* **10**, 890 (2021).
202. J. Johnson, R. M. Jagers, G. Sreejit, et al., *Antioxid. Redox Signal.* **36**, 652 (2021).
203. S. W. Schaffer, C. J. Jong, and M. Mozaffari, *Vasc. Pharmacol.* **57** (5–6), 139 (2012).
204. P. Newsholme, V. F. Cruzat, K. N. Keane, et al., *Biochem. J.* **473**, 4527 (2016).
205. O. O. Oguntibeju, *Int. J. Physiol. Pathophysiol. Pharmacol.* **11**, 45 (2019).
206. J. B. de Haan and M. E. Cooper, *Front. Biosci.* **3** (2), 709 (2011).
207. S. W. Park, H. O. Jun, E. Kwon, et al., *Vasc. Pharmacol.* **90**, 19 (2017).
208. N. Apostolova, F. Iannantuoni, A. Gruevska, et al., *Redox Biol.* **34**, 101517 (2020).
209. K. Suresh, L. Servinsky, J. Reyes, et al., *Am. J. Physiol.: Lung Cell. Mol. Physiol.* **309**, L1467 (2015).
210. G. W. Davison, R. E. Irwin, and C. P. Walsh, *Free Radic. Biol. Med.* **170**, 194 (2021).
211. S. Altenhofer, K. A. Radermacher, P. W. Kleikers, et al., *Antioxid. Redox Signal.* **23**, 406 (2015).
212. P. V. Avdonin, A. D. Nadeev, G. Y. Mironova, et al., *Oxid. Med. Cell Longevity* **2019**, 1701478 (2019).
213. M. Billaud, R. Marthan, J. P. Savineau, et al., *PLoS One* **4**, e6432 (2009).
214. L. L. Camargo, A. C. Montezano, M. Hussain, et al., *Cardiovasc. Res.* **118**, 1359 (2022).
215. K. Choi, M. Kennedy, A. Kazarov, et al., *Development* **125**, 725 (1998).
216. W. C. Aird, *Circ. Res.* **100**, 158 (2007).
217. W. C. Aird, *Circ. Res.* **100**, 174 (2007).
218. W. C. Aird, *Cold Spring Harbor Perspect. Med.* **2**, a006429 (2012).