Cardiovascular diseases are currently the most common cause of morbidity and mortality. Hypertension and ischemic heart disease often accompany obesity, diabetes, and hyperlipidemia. A significant number of patients suffering from these diseases develop myocardial infarction and ischemic cerebral incidents, which can lead to death or deterioration of the quality of life. However, it has been observed that restoration of normal blood pressure or glucose levels alone do not result in the expected prognostic improvement. Numerous reports have shown that there is one common factor linking all these diseases: vascular endothelial dysfunction.

The vascular endothelium, which lines the interior surface of blood vessels, is a dynamic organ with numerous properties which ensure normal vascular function. By the secretion of both vasoconstricting and vasodilating substances, it takes part in the regulation of vascular tone. Nitric oxide (NO) is the main vasodilating molecule which participates in the endothelial control of systemic blood pressure. As NO reveals mainly local action, its decisive role in vascular tone is attributed to its adequate amount, which may be synthesized only by normal endothelial cells. Moreover, it also exerts a protective effect on the vascular wall by preventing the oxygenation of lipids, mainly the LDL fraction, and decreasing the effect of free oxygen radicals. Due to these numerous beneficial effects on the vascular wall, nitric oxide has been considered an anti-atherosclerotic agent.
Normal vascular tone and, indirectly, normal blood pressure can be maintained due to the balance between endocrine and paracrine factors released by the endothelium. Disturbances in the balance are referred to as vascular endothelial dysfunction. Numerous reports have indicated that in the course of endothelial dysfunction, changes occur in the vascular walls which lead to thickening of the inner and middle layers and the formation of atherosclerotic plaque. The factors causing endothelial dysfunction include diabetes, hyperlipidemia, obesity, and smoking. A significant role has also been contributed to the increased activity of the rennin-angiotensin system and the sympathetic system.

The evaluation of endothelial function, as well as the possibility of improving it, has been the subject of numerous studies. Indirect methods applied in the evaluation of endothelial function include determination of the level of vasoconstricting molecules, or of NO, as the main vasodilating factor prior to and after termination of therapy. Endothelial function has also been evaluated by measuring vascular blood flow before and after application of a pharmacological agent. However, the absolute levels which might function as an endothelial dysfunction marker have not yet been determined.

**Vascular Endothelium**

The endothelium is not only a barrier separating the intravascular environment from the extravascular, but it also has a number of secretory and metabolic functions. Endothelial cells differ from each other depending on their location, which results in different reactions to stimuli. This may be exemplified by the lower level of prostacycline (PGI) synthesized by endothelial cells of the saphenous vein than by the cells of the thoracic artery, what has implications when using the vein as a coronary bypass.

The endothelium plays a significant role in maintaining homeostasis and the regulation of vascular function [17]. Other functions include its effect on vascular permeability, vascular tone, and the regulation of the properties of the vascular surface in homeostasis and inflammation. Vascular function depends on the balance between the endocrine and paracrine factors secreted by the endothelium. Numerous vasoactive molecules participate in the control of the above endothelial functions. The vasodilating agents produced by the endothelium include nitric oxide (NO) and prostacycline (PGI), while endothelin-1 (ET-1), thrombosan (TXA 2), and platelet activating factor (PAF) are vasoconstricting agents.

**Nitric Oxide**

Nitric oxide (NO), one of the most important endothelial molecules, is a potent vasodilator which plays a significant role in the functioning of the cardiovascular system. It is a lipophilic molecule with a short half-life of 5–7 s which easily diffuses through biological membranes. Its short half-life depends first of all on its reactions with peroxide ions and oxyhemoglobin. NO is synthesized by epithelial cells from arginine by various nitric oxide synthase (NOS) enzymes. There are three isoforms of NOS, two of which are present in the endothelium: the constitutive form eNOS (NOS III) and the inducible form nNOS (NOS II). The induction of NO synthesis by the epithelium occurs at three levels, i.e. by the effect of eNOS gene expression, by a change in eNOS enzyme activity, and by NO degradation. Disturbances in any of these mechanisms may lead to endothelial dysfunction.

**Nitric Oxide Synthase**

Constitutive NOS (NOS III) is a calcium-dependent isoform. It is produced by several types of cells. In humans the eNOS gene is located on chromosome 7. Clinical genetic examinations revealed the existence of gene polymorphisms [1]. The significance of these polymorphisms in the etiology of cardiovascular disease is controversial. Some reports have shown that the glutamate/asparagine conversion at site 298 of the eNOS gene has been more often associated with coronary atherosclerosis, increased processive response after the use of phenylephrine [2], lower basal blood flow through the coronary vessels with maintained response to adenosine [3], and vasoconstrictive angina pectoris [4]. Subsequent studies did not confirm such a direct association and the discrepancies were explained by ethnic differences in the study population and the role of genetic and environmental factors. A meta-analysis of clinical studies suggests that eNOS gene polymorphism is not associated with increased cardiovascular risk, but rather with an indirect genetic marker of atherosclerosis.

The activity of constitutive NOS is affected by several factors: eNOS gene polymorphism, post-translational modification, the effect of other proteins, including caveolin, the presence of co-factors, e.g. tetrahydrobiopterin, activation by calcium, phosphorylation and dephosphorylation by protein kinases, availability of L-arginate substrate.

With no stimulation, eNOS is bound to the cell membrane within the membranous caveolae. The caveolae are hollows in the cell membrane which
contain the transmembrane protein caveolin, which plays an eNOS-inhibitory role thanks to interactions with this enzyme [5]. These structures were found both in the endothelial cells of blood vessels as well as in cardiomyocytes. Caveolae are the sites of sequestration of signaling proteins.

The activation of eNOS in response to the action of hormonal agonists, e.g. estradiol, bradykinin, or endothelial growth factor, occurs as an effect of increased intracellular level of calcium. The activation of eNOS in response to the effects of insulin, shear stress, or isometric shortening of vessels is different. In these cases the activation is independent of changes in the level of Ca²⁺. Shear stress activates eNOS due to the regulation of potassium channels with the participation of tyrosine kinase. Bradykinin is one of the most important eNOS activators in blood vessels. Apart from the previously discussed factors, eNOS activation is performed by acetylcholine, catecholamines, ADP, thrombin [6].

The activity of the constitutive form of eNOS depends on the level of intracellular calcium and the formation of Ca²⁺/calmodulin complexes. Short-lasting activation is the consequence of an increased level of intracellular calcium caused by the activation of receptors bound to G protein in endothelial cells and cardiomyocytes. Although eNOS is a constitutive enzyme, its level can still alter in relation to numerous factors, such as shear stress or the presence of atherosclerotic lesions in the blood vessels. eNOS-dependent secretion of NO is the main mechanism regulating the homeostasis of blood vessels. NO release is affected by mechanical stimulation of the blood stream and shear stress. Only laminar blood flow through blood vessels has a beneficial effect on NO synthesis. No expected eNOS stimulation was observed during disturbed blood flow [7].

The inducible form of the enzyme (jNOS) was found in monocytes, macrophages, and epithelial cells. The activity of this enzyme does not depend on the level of intracellular calcium. The induction of jNOS occurs by immunological stimulation and its regulation at the transcriptional level. Increased jNOS expression was observed in the walls of pathologically changed vessel and in atherosclerotic plaque [8]. jNOS stimulated by inflammatory cytokines or cellular immunological factors synthesizes NO. At this time, calmodulin is firmly bound to jNOS depending on the basal Ca level and, in contrast to constitutive eNOS, due to its prolonged activity it synthesizes a relatively large amount of NO. This overproduction of nitric oxide by jNOS may cause damage to the tissues, but in general it is potentially beneficial for the organism. It is assumed that jNOS may participate in the pathogenesis of numerous inflammatory reactions, asthma, transplant rejection, inflammatory bowel diseases, and septic shock. Studies have shown that jNOS activity is significantly increased in the area of myocardial infarction compared with the area of healthy myocardium [9]. The beneficial effects of jNOS were confirmed in studies on mice. Elimination of jNOS resulted in delayed wound healing, while reintroduction of the jNOS gene by means of adenovirus as a vehicle resulted in restoration of normal healing [10].

Nitric oxide synthase contains two catalytic subunits: a C-terminal reductase domain and an N-terminal domain of oxygenase. To function normally, both units require the presence of a necessary cofactor, as only then are they able to act simultaneously in synthesizing NO [11]. The mechanical stimulation of endothelial cells evoked by blood flow induces NO synthase, which occurs by way of its phosphorization. Nitric oxide synthase (NOS) catalyses the reaction in which L-arginine is synthesized to citrulline and NO, which requires the participation of calmodulin and tetrahydrobiopterine (BH4) as cofactors.

**The Nitric Oxide Synthase Cofactor Tetrahydrobiopterin**

Tetrahydrobiopterin (BH4) is a critical determinant of eNOS activity: if there is a limited availability of BH4, eNOS does not produce NO, but superoxide anions, even in the case of a sufficient amount of the substrate. This can be observed, for example, in various diseases in which oxygen degradation of BH4 or iNOS induction occur. This isoenzyme uses more cofactor and substrate for its activity [12].

**L-arginine**

The amino acid L-arginine, necessary for the synthesis of NO, is synthesized in the urea cycle in the degeneration of endo- and exogenous proteins, as an effect of recycling, from citrulline produced by NOS synthesis in the citrullin/NO cycle [13]. In humans, the serum level of L-arginine is stable and does not change in diseases associated with endothelial dysfunction. L-arginine is concentrated in endothelial and other cells in the process of specific transport. Although the L-arginine level in the cells is relatively high, some authors have demonstrated that the administration of high doses of arginine to patients with hypercholesterolemia
potentiates the endothelium-dependent dilatation effect of NO. This phenomenon is known as the “arginine paradox”. The potential mechanism of this phenomenon includes a change in the eNOS affinity to the substrate, L-arginine, by elevated arginine level in the epithelial cells, among others under pathological conditions associated with endothelial dysfunction [13]. Apart from its participation in NO synthesis, L-arginine may indirectly cause elevation of NO’s biologically active in the vascular system by, for example, increasing the secretion of insulin, which facilitates relaxation of the blood vessels, or increasing histamine secretion, which also results in vascular dilatation [14]. Other studies have shown that the administration of arginine analogues results in the inhibition of NO synthesis, which leads to an increase in peripheral resistance in the blood vessels and hypertension. Recent findings suggest an alternative hypothesis, according to which NG-monomethyl-L-arginine and asymmetric dimethylarginine (ADMA) structurally resemble L-arginine and are compensatory antagonists of eNOS [15]. NG-monomethyl-L-arginine causes endothelial dysfunction both in humans and in animals.

**Asymmetric Dimethylarginine**

In humans, the serum level of asymmetric dimethylarginine (ADMA) is 10 times higher than that of NG-monomethyl-L-arginine. The level of ADMA is elevated in patients with cholesterolemia, homocysteinemia, insulin resistance, kidney diseases, diabetes, and coronary X syndrome [6, 16]. Elevated levels of ADMA in serum are associated with vasodilation-dependent impaired blood flow in the brachial artery in patients with dyslipidemia and homocysteinemia, which was confirmed by some authors [6, 16]. However, other studies on men with stable angina pectoris demonstrated that the L-arginine/ADMA ratio did not correlate with endothelial function, which was evaluated by measuring blood flow through the brachial artery after administration of acetylcholine.

**The Biological Role of Nitric Oxide**

Most of the nitric oxide synthesized by epithelial cells diffuses to the lumen of the blood vessel and the rest to the surrounding tissues. Thanks to this, its action involves not only NO-synthesizing endothelial cells, but also smooth muscle cells of the blood vessels and morphotic blood cells. Nitric oxide activates guanylyl cyclase, which leads to the synthesis of cyclic guanosinomonophosphate (cGMP), which, in turn, activates potassium channels, resulting in hyperpolarization of vascular smooth muscle cells and, consequently, their relaxation. NO is involved in the regulation of systemic blood pressure, vascular smooth muscle tone, and angiogenesis [18]. It participates in the development of cardiac muscle tissue, protects cardiomyocytes against apoptosis, and affects the electrophysiological properties of the heart [19]. NO strongly affects the contractility of the heart muscle by inhibiting the L-type calcium current. This occurs by way of cGMP-dependent and cGMP-independent mechanisms. Inhibition of the L-type calcium channel results in the protection of cardiomyocytes against calcium overload, which occurs in tachycardia. NO deficiency may lead to calcium overload in cells in tachyarrhythmia [19]. Nitric oxide diminishes the effect of the sympathetic system on atrioventricular node conductivity and sinus node automatism, augmenting the activity of the parasympathetic system [20]. Nitric oxide plays a significant role in decreasing the adhesion of thrombocytes and leukocytes and also in inhibiting the proliferation of vascular smooth muscle cells. Additionally, it plays the role of an anti-atherosclerotic, anti-inflammatory, and anti-proliferative factor and shows antioxidative properties. The protective effect of NO on the vascular wall results from its prevention of lipid oxidation and reduction of the effect of free oxygen radicals. As may be expected, inhibition of NO synthesis by administration of an NOS inhibitor or by inactivation of the NOS gene leads to hyperplasia of the neointima and acceleration of the development of atherosclerotic lesions [21, 22].

The vasodilating effect of NO on blood vessels occurs by virtue of smooth muscle relaxation. Nitric oxide contributes to the activation of guanylyl cyclase, and thus to the formation and accumulation of cyclic adenosinomonophosphate (cGMP).

Shear stress, by mediating in phosphorization, regulates the activity of eNOS; however, the mechanism of eNOS activation in this case is not yet well understood [23]. The regulation of vascular activity may also occur on the neuronal pathway as a result of catecholamine release induced by NO inhibition on adrenergic endings, which was demonstrated in animal and human studies [24].

NO exerts a bimodal effect on the heart muscle. When the level of NO is normal, a positive isotropic and lusitropic effect is observed. High NO levels produce a negative isotropic effect. Evaluation of the level is difficult in both cases, and the amount of bioactive NO includes exoge-
nous (external) and endogenous sources. The anti-thrombotic effect of NO is produced, among others, by decreasing the tissue factor expression induced by cytokines and endotoxines, which is a critical determinant of thrombin synthesis [24].

**Modification of Nitric Oxide Synthesis**

Numerous diseases, including hypertension, diabetes, and atherosclerosis, cause endothelial dysfunction, which results in decreased synthesis and bioavailability of nitric oxide. The oldest method, known for decades, of increasing the bioavailability of NO in the cardiovascular system relies on the administration of short-acting or long-acting nitrates. However, this is merely a supplementation therapy used only in selected cases. It seems that improvement of vascular endothelial function is the crucial method leading to an enhancement of nitric oxide synthesis and bioavailability.

There are observations which demonstrate that some drugs applied in therapy reveal, apart from the basic therapeutic effect, an additional effect on the endothelium, increasing the bioavailability of nitric oxide. Some of these drugs are statins and angiotensin convertase inhibitors. Endothelial cells contain an angiotensin convertase enzyme (ACE), which leads to the conversion of angiotensin I (Ang I) to angiotensin II (Ang II) on the tissue level. Nitric oxide may decrease the synthesis of ACE in the endothelium, which contains type 1 receptors for angiotensin I (AT 1) in the smooth muscle cells and thus potentially decrease the synthesis and activity of angiotensin II [25]. The peptide angiotensin II (Ang II) stimulates the growth and migration of smooth muscle cells. It has an antithrombotic effect by increasing the adhesion and aggregation of blood platelets and stimulating the synthesis of the plasminogen activator inhibitor PAI-1. It enhances the synthesis of endothelin-1, which reveals mitogenic and pressive properties.

Increased bioavailability of nitric oxide, which reveals antagonistic action to Ang II, as well as excessive activity of Ang II increase endothelial dysfunction. ACE inhibitors, which inhibit the synthesis of Ang II, prevent the above-mentioned unfavorable effects of this factor. Additionally, ACE inhibitors, apart from decreasing angiotensin I synthesis, prevent the degradation of bradykinin, one of the most important molecules participating in NO release [26]. Despite the common mechanism of ACE inhibitor activity, there is still controversy whether this is a group effect or rather an individual effect revealed only by some of them.

Statins show a pleiotropic effect. Apart from their basic hypolipemic action, they have anti-inflammatory and antithrombotic effects and improve vascular relaxation. The ability of statins to increase the expression and activation of eNOS may be an important mechanism due to which statins may improve endothelial function. Increasing the expression of eNOS occurs by virtue of a mechanism which is independent of cholesterol, with the participation of small GTPases. Studies have shown that the effect occurs after administration of all statins (group effect).

The effect of specific PPAR-α agonists, such as fenofibrate, on eNOS regulation in the endothelial cells depends on their specificity. The less specific PPAR-α agonist fenofibrate revealed a beneficial effect on eNOS, while the highly specific PPAR-γ agonist rosiglitazone did not [27].

Physical effort seems to be the non-pharmacological method of increasing NO bioavailability. It has been suggested that regular physical exercise contributes to arteriogenesis dependent on the arising shear stress. NO release may be the adaptive response to the increase in transmural pressure and tone of the wall [28]. It has been reported that physical effort increases the expression of the eNOS gene [29]. The amount of NO released in response to physical exercise probably depends on the size of the blood vessel; larger vessels are exposed to higher shear stress and reveal an increased ability to synthesize NO [30].

The presented data indicate that the problem of endothelial dysfunction may concern patients with primary metabolic diseases and cardiovascular diseases. Such patients are candidates for immediate implementation of pharmacological and non-pharmacological therapy aimed at improving vascular endothelial function, regardless of the treatment of the primary disease. Early identification of patients without evident metabolic or cardiovascular disease with the risk of endothelial dysfunction already in the initial stage of the disease seems mandatory in order to institute appropriate therapy. At present this is difficult, as objective methods enabling a direct evaluation of endothelial function are unknown. Currently, treatment is given first of all to patients with manifested disease in whom the unfavorable effect on the endothelium has been well documented, with the aim to postpone the dysfunction and prevent its consequences. Endothelial dysfunction therapy involves elimination of risk factors, such as smoking or obesity, and increases physical activity. Effective pharmacological therapy of the primary disease, which may potentially damage the endothelium is also necessary, and should include drugs which improve its function, such as statins and ACE inhibitors.
References


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