® Postepy Hig Med Dosw (online), 2018; 72: 89-100
e-ISSN 1732-2693

www.phmd.pl
Review

Received: 14.06.2017
Accepted: 17.11.2017
Published: 02.03.2018

Non-pharmacological modification of endothelial function: An important lesson for clinical practice

Niefarmakologiczna modyfikacja funkcji śródbłonka – ważna lekcja w praktyce klinicznej

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Summary

During the last few generations a rapid increase in the prevalence of cardiovascular diseases has been observed. This epidemiologic fact is in a wide range due to endothelial dysfunction. Endothelium, the inner layer of vessel wall, is a crucial structure in the proper functioning of the cardiovascular system. Next to classic cardiovascular risk factors such as diabetes, obesity, atherosclerosis and hypertension, endothelial dysfunction has become understood as one of the most important elements of cardiovascular pathologies, ranging from ischemic heart disease, stroke to carcinogenesis. This has given rise to a crucial need to develop new therapeutic directions aimed at ameliorating the endothelial function. A healthy lifestyle and proper well-balanced diet seem to be even more important than pharmacotherapy. Therefore, non-pharmacological interventions have become bullet points in a list of endothelial-targeted treatment strategies. Currently, several compounds have been studied as candidates for endothelial function improvement.

L-arginine supplementation is proved to reduce fat content. It also preferably modifies carbohydrates metabolism and the expression of genes responsible for increased cardiovascular risk. Spirulina increases the expression of endothelial nitric oxide synthase, which ameliorates nitrogen oxide production and leads to a decrease in blood pressure. The beneficial effect of green tea catechins is based mainly on the inactivation of reactive oxygen. Allicin present in garlic shows both antioxidative and anti-inflammatory effects. Probiotics prevent endothelial dysfunction in effect of improved vascular oxidative stress. Physical activity also demonstrates a number of mechanisms that ameliorate endothelial function.

The impact of endothelial function in the complex pathology of cardiovascular diseases reflects a number of scientific proofs showing favorable effects of non-pharmacological interventions in endothelial dysfunction treatment.

Keywords: **endothelium • cardiovascular risk • non-pharmacological treatment • L-arginine • spirulina**

GICID: 01.3001.0011.5963
DOI: 10.5604/01.3001.0011.5963
Word count: 9177
Tables: –
Figures: –
References: 103

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Abbreviations: **ACH –** acetylcholine, **AMP –** adenosine monophosphate, **ANP –** atrial natriuretic peptide, **AP-1 –** activator protein 1, **BMI –** body mass index, **BNP –** brain natriuretic peptide, **ChOx –** cholesterol oxides, **CNP –** type C natriuretic peptide, **COX** – cyclooxygenase, **CRP –** C-reactive protein, **CVD –** cardiovascular disease, **DPPH –** 1,1- diphenyl-2-picrylhydrazyl, **DVP –** digital volume pulse, **ED –** endothelial dysfunction, **EDCF –** endothelium-derived contracting factors, **EDHF –** endothelium-derived hyper-polarizing factors, **EDRF –** endothelium-derived relaxing factor, **EGCG –** epigallocatechin gallate, **eNOS –** endothelial nitric oxide synthase, **FMD –** flow-mediated dilatation, **GPx –** glutathione peroxidase, **Hcy** – homocysteine, **HHcy** – hyperhomocysteinemia, **HOMA-IR –** homeostatic model assessment- insulin resistance index, **ICAM-**1 **–** intercellular adhesion molecule-1, **IGF-I –** insulin-like growth factor I, **IκB –** nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, **IL-6 –** interleukin-6, **L-Arg –** L-arginine, **LDL –** low-density lipoproteins, **MAPK –** mitogen-activated protein kinase, **MP** – microparticles, **NADPH –** nicotinamide adenine dinucleotide phosphate, **NF-**κB **–** nuclear factor kappa-light-chain-enhancer of activated B cells, **NO –** nitric oxide, **NOS –** nitric oxide synthase, **oxLDL –** oxidized low-density lipoprotein, **PC** – phycocyanin, **PGC-1 –** peroxisome proliferator-activated receptor-gamma coactivator-1, **PI-3 –** kinase phosphatidylinositol 3-kinase, **PKB** (or **Akt**) **–** protein kinase, **PWA –** pulse wave analysis, **PWV –** pulse wave velocity, **RAAS –** renin-angiotensin-aldosterone system, **RH-PAT –** reactive hyperemia peripheral arterial tonometry, **RONS –** reactive oxygen and nitrogen species, **ROS –** reactive oxygen species, **SHR –** spontaneously hypertensive rat, **SHRSP –** spontaneously hypertensive stroke prone rats, **SOD –** superoxide dismutase, **TAS –** total antioxidant status, **TCH –** total cholesterol, **TG** – triglycerides, **TMAO –** trimethylamine N-oxide, **TNF –** tumor necrosis factor, **VCAM-1 –** vascular cell adhesion molecule 1.

Endothelial secretory function

Endothelium, the inner layer of blood vessels weighing approximately 1.5 kg with an area of four tennis courts [2], plays a crucial role in the functioning of the cardiovascular system. Results of a great number of recent studies allow us to identify endothelial functions. Regulation of vassal tonus and the influence of inflammation are the two best documented functions. However, many recent trials have shown the important endocrine activity of the endothelium. Increased scientific interest on endothelium is connected to the fact that beyond any doubt unfavorable modification of endothelial cells leading to endothelial dysfunction (ED) is a key mechanism of classic cardiovascular risk factors negative impact.

Endothelium is responsible for the production of a wide range of vasoconstriction factors called also endothelium-derived contracting factors (EDCF). Endothelial cells also synthesize cyclooxygenase-derived contracting factors. Endothelial factors are produced by enzymes, such as NADPH oxidases, cyclooxygenases, converting enzymes and epoxygenases. These substances are synthesized not only by endothelial cells, but also by vascular smooth muscle cells, inflammatory cells such as leukocytes, mesangial cells or adipocytes. The excessive production of endothelium-derived contracting factors caused by the hyperactivity of the abovementioned enzymes leads to vasoconstriction and vascular cell growth. The first identified EDCF were arachidonic acid-derived vasoconstrictors- prostanoids and a product of oxidative metabolism - superoxide anion. The vasoconstricting properties of the last one are mainly due to NO-inactivating properties [3]. The constricting abilities of reactive oxygen species produced by NADPH-oxidase and cytochrome P-450 expoxygenase were discovered in the 1990s [23,33]. An important peptide group of EDCF are endothelins, especially endothelin-1 (ET-1), which causes not only vasoconstriction, but also cell proliferation and endothelium-dependent contractions through thomboxane A-2 activation [3,5].

Endothelium is responsible for the production of endothelium-derived relaxing factor (EDRF) [3]. The most important vasodilator factor produced by endothelium is nitric oxide (NO). Apart from vasodilator properties, NO inhibits vascular smooth muscle cell growth and the adhesion of leukocytes to the endothelial surface, maintains normal blood flow, reduces platelets activity, limits vascular inflammatory reactions. Other endothelium-derived vasodilator factors are prostacyclins and endothelium-derived hyper-polarizing factor (EDHF) [8,38]. Except vessels tone regulation endothelium exerts an effect on coagulation and fibrinolysis. Nitric oxide inhibits platelet activity. Endothelium influences fibrinolysis producing tissue plasminogen activator and plasminogen activator inhibitor 1. What is more, it can be a source of von Willebrand factor and thrombomodulin, important coagulation factors [38]. The endothelium is metabolically active and plays an essential role in the maintenance of vascular homeostasis [70]. The condition of this complex organ serves as a major determinant of vascular physiology and pathophysiology [29].

Mechanisms of endothelial dysfunction

ED is characterized by reduced endothelium-mediated vasorelaxation, hemodynamic deregulation, impaired fibrinolytic ability, enhanced turnover, overproduction of growth factors, increased expression of adhesion molecules and inflammatory genes, excessive generation of reactive oxygen species, increased oxidative stress, and enhanced permeability of the cell layer [79].

Among several mechanisms regarding ED, the reduction in nitric oxide (NO) bioavailability is one of the most important. This is due to the fact that nitric oxide plays a major role regulating many physiological actions, such as vasodilation, anti-inflammation, antiplatelet, antiproliferation and antimigration [90]. Diminished production and release of NO in endothelium dysfunction cause the promotion of plaque formation, which underlays the pathogenesis of atherosclerosis [89]. The imbalance in nitric oxide bioavailability can be caused by oxidative stress, where reactive oxygen species damage the endothelium and leave it permeable, which allows cells that should remain in the blood pass through blood vessels. One of these compounds includes C-reactive protein (CRP), which causes inflammation [72]. Endothelium produces inflammatory and immune mediators (e.g. cytokines, adhesion molecules) and undergoes morphological modifications in response to inflammation. This process also increases endothelial permeability and promotes the adhesion of leukocytes to endothelial cells, which leads to transendothelial migration to inflammation sites [90].

Oxidative stress is harmful, as it increases vascular smooth muscle cells (which leads to the thickening of the vascular wall), endothelial cells apoptosis and increased expression and activity of matrix metalloproteinases. Reactive oxygen species react with NO and create reactive nitrogen species – peroxynitrite. This compound is toxic to the cells and damages DNA, lipids and proteins. Inflammation is associated with oxidative stress, which can increase vascular inflammation signaling pathways. At the same time, inflammatory cells release reactive oxygen species. There is also overexpression of inflammatory cytokines such as TNF-α (tumor necrosis factor α) and interleukin-1. These cytokines cause the release of adhesion molecules from endothelium cells and macrophages, which results in ED [62].

Another potential factor that leads to ED is the level of circulating microparticles (MP). MPs are a heterogeneous population of small membrane fragments shed from various cell types. As the endothelium is one of the primary targets of circulating MPs, these particles can trigger ED by disturbances in NO release, causing changes in vascular tone [53]. A high concentration of endothelial MPs negatively regulates the proliferation of endothelial cells leading to ED [43].

Because endothelium is a ubiquitous tissue, its dysfunction can be described as a systematic disorder [89]. ED is associated with the pathogenesis of many diseases, e.g. cardiovascular diseases, such as ischemic heart disease, stroke, limb ischemia or erectile dysfunction [37,84]. A better understanding of the mechanisms of ED could provide novel therapeutic targets of cardiovascular diseases [37].

Endothelium and cardiovascular risk

ED is found to be an independent factor of cardiovascular risk. Changes associated with the deterioration of endothelial function are responsible for vascular remodeling and the activation of the inflammatory and thrombotic process and are early markers of atherosclerosis development [76]. Detailed knowledge about pathologic mechanisms leading to ED in arterial hypertension, obesity, dyslipidemia is a crucial point of studies on new therapeutic methods of cardioprotective properties. Risk factors leading to ED are the following: hypercholesterolemia, homocysteinemia, hyperglycemia, hypertension, smoking, inflammation and aging [90].

Obesity

Obesity, a global epidemic affecting 700 million people worldwide, is an important factor disrupting endothelial function. ED in obesity has a genetic and non-genetic basis [86]. In obese patients, enhanced vasoconstriction is observed mainly due to endothelin and cyclooxygenase hyperactivity [81]. Also, endothelial prostanoids hyper-production is present. The main sources of these factors in obesity are COX-dependent pathways. Also, prostacyclin is responsible for vasoconstriction in obesity determining progenitor cells development in adipocytes [3]. Increased body mass leads to NO inactivation by superoxide anion ($O2^-$) and the formation of peroxynitrite. This results in the limitation of endothelium vasodilatory properties [24,75]. Other free radicals affecting the endothelium produced in increased amount in obesity are hydroxyl radicals [63]. Obesity promotes a low-intense inflammatory state leading to the over-secretion of inflammation mediators such as TNF-α or interleukin-6 (IL-6), significantly affecting endothelium function [3]. There are also some peptide factors connecting obesity with ED. Neuropeptide Y involved in appetite regulation takes part in obesity development by stimulating adipogenesis. Neuropeptide Y leads to NO-dependent dilation, endothelial cell growth stimulation and affects endothelial cell permeability. Atrial natriuretic peptides such as ANP (atrial natriuretic peptide), BNP (brain natriuretic peptide), and CNP (type C natriuretic peptide), formed and metabolized in endothelial cells, are involved in lipids mobilization in obesity [3,14]. It has been observed that obesity leads to angiotensin-1 receptors hyper-production, which can be the basis of hypertension in overweight subjects [63]. It has been widely documented that visceral obesity significantly aggravates ED. ED is known as a main indicator of atherosclerosis progression. Changes related to endothelial damage are: increased inflamma-

tion state in the wall of the vessel, the increased oxidation of lipoproteins, smooth muscle cell proliferation, extracellular matrix deposition, increased cell adhesion and prothrombotic activity. These changes have been proven to occur in obesity [10,34]. Individuals with abdominal obesity were characterized by a reactive oxygen and nitrogen species (RONS) overproduction and an antioxidant system capacity impairment. In homeostasis RONS play an important role as mediators and regulators in growth, a differentiation and a proliferation processes, and they provide an adaptation of cells to changing conditions [87]. However, oxidant/antioxidant imbalance, which is common in obese people, can lead to cellular structures damage and the activation of pathological transcription factors, among them NF-κB or AP-1. This can, in turn, lead to the dysfunction of cells [19]. It has been proven that high cholesterol blood levels affect endothelium function. High serum concentration of low-density lipoproteins (LDL) and triglycerides (TG) induced by high-cholesterol diet led to a decrease in superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity and an increase in NADPH oxidase activity. Moreover, an elevated serum cholesterol level brings disturbances in xanthine oxidase and myeloperoxidase function. A high-cholesterol diet is responsible for ACH (acetylcholine) -induced vasodilation disturbances and an increased production of superoxide anions causing ED [32]. Elevated oxidative stress caused by hypercholesterolemia leads to pro-inflammatory state, which results in alterations in the bioavailability of vascular nitric oxide [88]. This unbalanced oxidative condition is observed particularly early in microvessels and is one of the major factors of atherosclerosis, the main reason for cardiac ischemic disease and stroke [32].

Hypertension

Arterial hypertension, crucial cardiovascular risk factor, is strictly related to endothelial function. Endothelial cells are responsible for the production of angiotensin-II, a vasoconstrictor factor being a part of the renin-angiotensin-aldosterone system (RAAS). Angiotensin-II regulates endothelin production, thus RAAS hyper-activity in hypertension leads to increased serum concentration of endothelin. This RAAS and endothelin interaction, observed especially in overweight subjects, functions as positive feedback loop impairing the function of endothelium. What is more, endothelin-1 is known to be a significant atherogenic factor increasing the endothelium damage caused by high arterial pressure [3,4]. Endothelium-dependent vasodilation in hypertension is also reduced by increased level of serum cholesterol oxides (ChOx), which shows a strict connection between hypertension and hypercholesterolemia in endothelium dysfunction [61].

Insulin sensitivity

Endothelium is proven to be insulin-responsive, it produces insulin-like growth factor I (IGF-I) and the hybrid insulin/IGF-I receptors. Based on these facts, hyperglycemia-caused ED is indisputable. The physiological basis of insulin influence on the endothelium is increased NO production through phosphatidylinositol 3-kinase (PI-3 kinase)/protein kinase B (PKB or Akt) pathway activation, leading to endothelial nitric oxide synthase (eNOS) activation and mitogen-activated protein kinase (MAPK) pathway regulation responsible for cell proliferation and the production of endothelin-1 and adhesion molecules. Diabetes and hyperinsulinemia are associated with a wide range of disturbances leading to endothelium damage, such as abnormal vascular reactivity, increased production of reactive oxygen species, decreased NO bioavailability [52]. In diabetes and co-existing insulin-resistance insulin responses through the PI3-kinase/Akt/eNOS pathway are attenuated. At the same time its action via the MAPK pathway is unimpaired or even elevated. In effect, the production of adhesion molecules and ET-1 is increased and the production and bioavailability of NO decreases, leading to atherogenic and vasoconstrictive response [20]. Many recent studies clearly show that the endothelium is a target organ of insulin, and chronic hyperinsulinemia and hyperglycemia are the significant reasons of such diabetes complications as cardiovascular diseases and organ damage [52].

Homocysteine concentration

Elevated homocysteine (Hcy) serum level (mild: 15 to 30 mmol/L, moderate: 30 to 100 mmol/L) present in 5% to 10% of the general population is an independent risk factor for such cardiovascular diseases as stroke, silent brain infarct and dementia. Sever HHcy contributes to tissue ischemia. Hyperhomocysteinemia (HHcy) significantly impairs NO-signaling pathway and increases nitrative stress in endothelial cells leading to the opening of the blood-brain barrier [57]. A direct consequence of HHcy is cerebral small vessel disease [35]. HHcy affects superoxides and peroxynitrite production. In the central nervous system, endothelial damage caused by HHcy leads to neurovascular diseases, such as stroke and cognitive impairment [57]. Hcy is associated with elevated carotid intima-media thickness and a decreased number of colony-forming unit-endothelial cells in patients with arterial hypertension. The underlying mechanism is Hcy-induced oxidative stress [7]. Recently, it has been shown that HHcy impairs EDHF-mediated vascular relaxation in microvessels, mesenteric arteries and aorta mainly due to small-conductance and intermediate-conductance potassium channel impairment [57].

Assessment of endothelial function in clinical practice

Understanding the importance of ED in the pathology of cardiovascular diseases has led to the development of measurement methods of the grade of this dysfunction. Commonly used methods of ED examination are biochemical measurements and non-invasive functional tests. Most widespread biochemical measurements are: nitrogen oxide, endothelial nitric oxide synthase, vas-

cular growth factors, cell adhesion molecules, markers of oxidation and inflammation status, chemotactive proteins, metallopeptidases, homocysteine, lipoproteins and apolipoproteins A, B and E.

Flow-mediated dilatation

In the flow-mediated dilatation (FMD) method, the degree of broadening of the brachial artery provoked by increased blood flow after a prior total occlusion of the proximal arm or forearm artery is measured. The observed increase in the flow is a result of decreased tension of the artery, which leads to an increase in shear force. Short-term effects of shear forces triggers cellular processes, leading to the activation of the endothelial eNOS, thereby to the release of NO. The brachial artery is imaged in the longitudinal projection 2D, 4-5 cm above the elbow using Doppler ultrasound 7-12 MHz. In the first stage of examination the diameter of the brachial artery and blood flow velocity is measured. Then the cuff of sphingmometer placed in the proximal part of arm or distal part of forearm is filled for 5 minutes to the value exceeding 50 mmHg systolic blood pressure of the patient. Maximal blood flow is estimated at 15s and the maximal vessel diameter at 60s after the release of artery compression. Brachial artery dilatation after ischemia is defined as the percentage change in the diameter of the brachial artery at the time of congestion in relation to the diameter of the vessel before ischemia. FMD values for healthy people are within 7-10%, while in patients witch coronary artery disease they are smaller, in extreme cases reaching zero. FMD method has a predictive value with respect to the future cardiovascular events [22,67].

Pulse wave analysis

Another non-invasive method of endothelial function assessment is pulse wave analysis (PWA), which allows us to estimate central pressure. In this method a pulse wave recording in the radial artery by a tonometer is used. The technique allows us to assess arterial stiffness [46,67]. The next method of endothelium examination based on applanation tonometry allows us to evaluate pulse wave velocity (PWV). The registration of pulse wave is performed in two different points, the most common are carotid and femoral or carotid and radial artery. The value of the PWV index is inversely proportional to the stiffness of the arterial wall. The predictive value of this method grows rapidly [17,46,67].

Digital volume pulse

The cardiovascular risk can be assessed using digital volume pulse (DVP) method allowing for the measurement of the stiffness index (SI). In this technique the pulse wave is measured. Its systolic component is created by pressure carried from the aorta to the fingers. The diastolic component is created by pressure carried from the left ventricle to the lower parts of the body, where it is reflected along the aorta to the fingers. The measurement allows for the assessment of DVP curve, whose shape depends on arterial stiffness and vascular tone. SI is defined as the ratio of the patient's height and the propagation time of the reflected pulse wave [17].

Reactive hyperemia peripheral arterial tonometry

Reactive hyperemia peripheral arterial tonometry (RH-PAT) is a well-validated method used to examine the endothelial function. It is an automatic and non-invasive method, which uses a plethysmograph sensor applied to the patient's finger. This allows the registration of finger volume changes following the pulsatile blood flow. The test is performed on both upper limbs placed on an equal level. The cuff of sphygmomanometer is put on one arm – the tested arm, while the second is the control arm. The RH-PAT sensors are placed on the symmetrical fingers of both hands. The fingers are separated from each other by soft sponge rings. The pulsating volume changes of the two fingers are registered. After 10 minutes required to obtain a sustainable record, the cuff is filled up to a value exceeding 60 mmHg systolic blood pressure of a patient for 5 min. Then the pressure of sphygmomanometer is released to induce reactive hyperemia. Meanwhile, the data from plethysmograph sensor are registered. After 10 min, the patient is administered a single dose of nitroglycerin (0.4 mg, sublingual) to assess the endothelial-independent response and after subsequent 10 min the data registration is completed. The collected data is analyzed by a computer. The data obtained from the tested arm is normalized in relation to the control arm in order to compensate possible systemic changes. This new method is completely non-invasive, easy to perform and reproducible [46,67].

Non-pharmacological interventions against endothelial dysfunction

In the twentieth and twenty-first century a rapid increase in cardiovascular risk factors prevalence has been observed. Understanding the importance of ED in the pathology of cardiovascular diseases gives the possibility to investigate and modify endothelial function. Non-pharmacological interventions in endothelial function are widely available, cheap and devoid of side effects and attractive. An increasing number of scientific proofs show its favorable cardioprotective impact, thus further studies on this topic are needed. In recent years, this field has become of special interest to our research team. In many studies, including both animal models and clinical studies, we and others have investigated the influence on the endothelial function of such non-pharmacological interventions as L-arginine, spirulina, green tea, garlic, probiotics and physical activity.

L-arginine

L-Arginine (L-Arg), an essential amino acid, is a substrate of NO by the nitric oxide synthase (NOS). L-Arg

supplementation is confirmed to improve endothelial function, even despite the fact that its intracellular content is greater than required for NOS activity. What is more, L-Arg decreases blood pressure, attenuates atherosclerosis and enhances NO synthesis. This 'L-Arg paradox' is explained by compartmentalization of NOS and a local deficiency of this amino acid. The beneficial effects of L-Arg and vitamin C co-supplementation have been recently documented in some studies [8].

There are several mechanisms connecting L-Arg supplementation with endothelial function improvement. The first one is a decrease in fat content leading to the extenuation of sub-clinical inflammatory state. Fuet et al., in a 10-week study on Zucker Diabetic Fatty Rats on low fat diet supplemented with L-Arg, show absolute and proportional weight reduction of the abdominal adipose tissue [26]. In a similar study on Male Sprague–Dawley rats on high-fat diet supplemented with L-Arg or L-Alanine for 12 weeks a significantly lower relative weights of major white fat pads was observed only in L-Arg supplemented group [40]. Supplementation of L-Arg leads to the modifications of gene expression. L-Arg added to a corn- and soybean-meal-based diet caused the up-regulation of lipid metabolism associated with genes in white adipose tissue and skeletal muscle in pigs. Also, the expression of genes involved in fat accretion in retroperitoneal tissue was down-regulated in response to a 12-week high-fat diet with addition of L-Arg in rats [8]. The transcript level of hepatic 3-hydroxyl-3-methylglutaryl-CoA reductase was reduced after L-Arg supplementation in broiler chickens, leading to the reduction of TG, LDL and total cholesterol (TCH) plasma concentration. Also, coconut water rich with L-Arg gives a hypolipemic effect in rats on a high-cholesterol diet [8]. Moreover, supplementation of L-Arg decreases serum insulin concentration in rats [8]. Hcy plasma level was also diminished after L-Arg consumption both in rats on high fat diet and in Zucker Diabetic fatty rats fed standard diet [8]. L-Arg preferably modifies carbohydrate metabolism. Supplementation of L-Arg in obese patients lowered elevated HOMA-IR (homeostatic model assessment- insulin resistance index) [9]. The improvement of insulin sensitivity after L-Arg supplementation is related to an increase in adiponectin concentration [59]. High blood level of insulin in pregnant women with excess body mass was reduced after infusion of L-Arg [71]. In the elderly, the supply of L-Arg leads to a decrease in insulin resistance along with the moderation of oxidative stress and atherosclerotic changes [8]. Moreover, L-Arg improves lipid profile. It has been shown that the administration of L-Arg lowers serum total cholesterol and total thiols in the case of myocardial infarction or acute angina [93]. L-Arg modifies oxidative stress and Hcy concentration. It has been demonstrated that L-Arg decreases liver protein oxidative damage. What is more, supplementation of L-Arg lowers the concentration of carbonyls in the liver and elevates plasma TAS (total antioxidant status) level. Moreover, Hcy plasma level was diminished after L-Arg consumption in men with hypercholesterolemia and this effect was dose-dependent [101]. The effect of L-Arg on Hcy is caused by significant up-regulation of genes encoding NOS-1, heme oxygenase-3, AMP-activated protein kinase and peroxisome proliferator-activated receptor gamma coactivator-1alpha, NOS-1, and PGC-1 in adipose tissue (reviewed in [68]). Systemic low grade inflammation in the case of excessive body mass and diabetes is caused by a deficiency of L-Arg. Supplementation of L-Arg leads to inflammation decrease due to the reduction of TNF-α level in rats [8].

Spirulina

Many dietary supplements exert a positive effect on endothelial function. It has been proven that flavonoid consumption leads to the improvement in arterial stiffness. Spirulina maxima (*Arthrospira maxima*), a cyanobacterium (*Bluegreen alga*), is rich source of protein, vitamins, minerals, carotenoids, and phycocyanins. Its lack of toxicity has been shown in many studies and nowadays it is a valuable diet supplement. Spirulina has been demonstrated to have beneficial effects against hyperglycemia, oxidative stress and elevated blood cholesterol level. Much evidence shows its role in the therapy of diabetes, arterial hypertension, bronchial asthma and cancer [45,58,60,69]. Animal studies on rats have shown that spirulina increased the expression of aortic endothelial nitric oxide synthase. Rats with metabolic syndrome showed amelioration of endothelial NO production and a decrease in both systolic and diastolic blood pressure after spirulina supply [39]. The mechanisms of endothelial function improvement after spirulina supply in humans have not yet been well investigated. The arterial stiffness can be positively affected after spirulina consumption due to its beneficial influence on oxidative stress status and inflammatory processes. Spirulina has been demonstrated to have antihypertensive potential, especially in reference to systolic pressure. This effect is caused by a presence of phycocyanin (PC), a blue dye with antioxidant properties. PC leads to the enhancement of eNOS expression in the aorta after adiponectin stimulation [39,60]. Amelioration of endothelial NO production and a decrease in both systolic and diastolic blood pressure were recorded in patients with type 2 diabetes mellitus after a 12-week spirulina supplementation, along with body mass reduction. Weight loss after spirulina was observed also in patients with ischemic heart disease and arterial hypertension [60,73]. Body mass reduction and consequent amelioration of endothelial function is caused by reduced infiltration of visceral fat by macrophages and diminished liver lipid accumulation [27].

Green tea

The beneficial effect of green tea on endothelial function is caused mainly by the content of catechins, a group of polyphenols and antioxidants. The antioxidant activity of green tea depends on the quantity and qual-

ity of polyphenolic compounds in it. Green tea, due to catechins reaching levels of 78% of polyphenol fraction shows very high antioxidant activity in an assay with DPPH reagent (1,1-diphenyl-2- picrylhydrazyl) of all kinds of tea [77]. Green tea catechins reduce the amount of free radicals, the main factor in the pathogenesis of ED. Catechins contained in green tea are able to prevent the development of hypertension and atherosclerosis, and decrease the cardiovascular mortality [92]. Experimental studies [92] showed the positive effect of supplementation of green tea on blood pressure in animals. In SHR (Spontaneously Hypertensive Rats) the effect of green tea supply on blood pressure was comparable with enalapril. Similar results were found in rats with spontaneous hypertension prone to stroke (SHRSP, Spontaneously Hypertensive Stroke Prone Rats) and in rats with metabolic syndrome. The hypotensive effect of green tea is due to the influence on the production of vasoconstrictive substances including angiotensin II, prostaglandins, endothelin-1 as well as vasodilating substances such as prostacyclins [6]. Anti-inflammatory effect of green tea supplementation results from the reduction of TNF-α mRNA level observed in Wistar rats. Green tea catechins play a protective role in oxidative damage. The antioxidant activity of green tea was confirmed in many experimental models, i.e. in metabolic syndrome in rats. Green tea significantly reversed the effect of ethanol intoxication in rats expressed by TAS reduction in erythrocytes. It has been shown that green tea protects the DNA from oxidative damage [92]. Many human studies revealed the favorable effect of antioxidants in patients with high cardiovascular risk and ED due to oxidative stress reduction, NO synthesis enhancement, LDL oxidation decrease, the growth of smooth muscle cells of blood vessels inhibition and blood pressure reduction (reviewed in [31]). Experimental and clinical observations demonstrated anti-inflammatory effects of green tea in humans. EGCG (epigallocatechin gallate) present in green tea extract inhibits the production of CRP induced by angiotensin II and IL-6, even in smokers. Catechins modulate the pro-inflammatory signaling pathways. EGCG can decrease damage of the endothelial cells by inhibiting the action of transcription factors AP-1 and NF-κB and decreasing production of IL-6 and TNF-α. Effects of EGCG on NF-κB and cells is based on many mechanisms including the inhibition of IκB kinase, IκB phosphorylation, p65NFκB acetylation and decrease in the binding activity of NF-κB to DNA [99]. Drinking green tea enhances antioxidative capacity of saliva even by 42% [65]. The mechanism of antioxidant action of catechins is based on: inactivation of reactive oxygen and nitrogen, the regeneration of antioxidants, i.e. β-carotene or α-tocopherol, the chelation of transition metals, inhibition of pro-oxidant enzymes [16]. It has been proven that green tea reduces the concentration of lipid peroxidation products markers, the level of oxidative DNA damage in smokers, the endothelial NADPH oxidase activity and the production of superoxide anion $O2^{-}$. What is more, polyphenols present in green tea reduce the production of ROS by increased expression of catalase [78].

Garlic

Garlic (*Allium sativum*) consists of many bioactive components. This perennial was used by ancient civilizations and is widely considered as a remedy for many human diseases. Current studies confirm that garlic and its bioactive components have medical properties, including a positive effect on cardiovascular system [80]. Sulfur compound such as allicin, diallyl trisulfide, allyl methyl trisulfide, diallyl disulphide and others, show anticancer, antioxidation, anti-inflammation, immunomodulatory, antimicrobial and hypoglycemic properties [97]. There are several mechanisms in which garlic is involved in the functioning of endothelium. Oxidative stress is one of them. Reactive oxygen species play a critical role in regulating endothelial function and vascular tone [94]. S-allyl cysteine is one of the most plentiful organosulfur compounds in aged garlic and it is known to have antioxidant properties [56]. Several studies in animal models demonstrated that garlic may reduce oxidative stress, and thus be a useful tool in preventing ED [13,51,54,56]. Koseoglu et al. demonstrated that garlic used as dietary supplementation may be beneficial in increasing antioxidant capacity in healthy subjects [44]. It is well known that bioavailability of nitric oxide is strictly connected with oxidative stress. A reduction in NO bioavailability may induce oxidative stress and therefore lead to ED [80]. Khatua et al. demonstrated that in isopreterenol-induced oxidative stress in mice, garlic provided a cardioprotective function through its ability to increase NO levels [41]. Other studies confirmed that the antioxidant properties of garlic extract increase the bioavailability of NO and may improve endothelial function [42,100]. Because NO is known to induce vasodilation and has blood pressure lowering properties, it is reasonable to propose that increased NO production as a mechanism by which garlic causes antihypertensive effects [80]. Human studies have demonstrated that garlic shows both antioxidative and anti-inflammatory effects. Allicin from garlic has a protective role in the endothelial cell injury and the most probable mechanism is decreasing inflammation and oxidative stress via the inhibition of ERK1/2pathway [98]. Other studies demonstrated that garlic may improve endothelial function through enhancing the expression of antioxidant enzymes via the Nrf2-ARE signaling pathway and exerts potential anti-inflammatory action via attenuating vascular cell adhesion molecule 1 (VCAM-1) expression [36]. When endothelium encounters proinflammatory cytokines, the expression of adhesion molecules is promoted, which causes the migration of leukocytes across the vascular endothelium [49]. One of these proinflammatory compounds is TNF-α. Van Doorn et al. were investigating the influence of garlic powder in overweight smokers on biomarkers of inflammation and endothelial function. Interestingly, there was no effect of garlic powder on plasma CRP or TNF-α levels in comparison to placebo [96]. Lau et al. demonstrated that daily allium vegetable intake (including garlic) is an independent predictor of endothelial func-

tion in patients with ischemic stroke and therefore may play a role in the secondary prevention of atherosclerotic events [47]. Another study investigating the influence of garlic extract confirmed its protective role on endothelial function. Williams et al. were testing the effect of aged garlic extract on endothelial function. The obtained results suggest that short-term administration of garlic extract may improve impaired endothelial function in men with coronary artery disease [102]. Although many studies demonstrated the positive effect of garlic on cardiovascular health and endothelial function, not all of them confirmed their beneficial properties. Turner et al. did not observe any significant changes in arterial stiffness after 12-week treatment with garlic powder and Legnani et al. did not observe any relation between garlic administration and plasminogen activity inhibitor 1 (PAI-1). Both studies were conducted on healthy subjects [48,95]. Since the prevalence of current studies demonstrate the positive effects of garlic on endothelial function, some result are inconsistent and there is a great need for further research, particularly those performed as double-blind placebo-controlled randomized clinical trials. Differences in results may derive from the fact that various doses were used in particular studies and there might be differences in the bioavailability of the active components of garlic supplements.

Probiotics

The term "probiotic" derives from Greek, meaning "for life". It can be defined as "a live microbial feed supplement which beneficially affects the host by improving its intestinal balance". Probiotics are considered to be alternative supplements, which can modulate composition and the function of gut microbiota. Improper diet and sedentary lifestyle lead to obesity, which results in chronic inflammatory response. Sub-clinical inflammation is a reason of dysbiosis and increased permeability of the intestinal wall. Due to this phenomenon bacterial lipopolysaccharide penetrates into the bloodstream and causes increased production of trimethylamine N-oxide (TMAO) and decreased TAS (Total Antioxidant Status). Entirety of these processes lead to ED. Proper diet supplemented with probiotics causes increased NO synthesis and elevates TAS, limiting the inflammatory response and ameliorating endothelial function [28]. It is estimated that the human organism is inhabited by more than 10^14 microbial cells. Most of them are not harmful for the host and can even provide benefits through symbiotic relationships. The main residents of the human gut are such phyla as *Bacteroidetes*, *Firmicutes, Proteobacteria, Actinobacteria* and *Verrucomicrobia* [11,55]. These bacteria perform a multitude of functions and have an impact on human health, including cardiovascular system [21,66]. Probiotics ameliorates human cardiovascular health by reducing the negative impact of ROS. Findings of the study by Friques et al. showed the beneficial effect of kefir on the imbalance between ROS production and NO bioavailability and the recruitment of endothelial progenitor cells to repair the damage to the endothelial surface layer. Chronic administration of kefir resulted in higher NO bioavailability and a decrease in the production of ROS [25]. Many factors such as antibiotic use, age, host genetic and dietary patterns can alter the gut microbiota [11]. Thanks to current studies, it is known that probiotics provide not only a healthy digestive system, but can affect the whole cardiovascular system by influencing endothelium. Many *in vitro* researches suggest that probiotics supplementation may have a preventative and therapeutic effect on cardiovascular disease by influencing many different mechanisms, for example atherosclerosis (reviewed in [103]). Supplementation of probiotics may have a therapeutic effect on cardiovascular diseases (CVD) by reducing total serum cholesterol, LDL-cholesterol and inflammation [103]. The change in the gut microbiota composition may lead to increased vulnerability to infections, immune disorders, inflammation, oxidative stress and insulin resistance. These events are caused by metabolic endotoxemia, which proceed from exposure to harmful intestinal products [12]. One of them is lipopolysaccharide, a component of the gram-negative bacteria's cell walls. It has an impact on endothelial cells by binding to toll-like receptor-4 (TLR4). This reaction initiates an inflammatory response and oxidative stress and can lead to ED and damage [103]. The study on the effect of soy milk fermented with selected probiotics in cell model systems revealed that probiotics caused amelioration in the imbalance between ROS production and NO bioavailability, leading to favorable changes in oxidative processes [15]. Several animal studies were investigating the influence of probiotics on endothelial cells. Rashid et al. demonstrated that probiotics may prevent ED of mesenteric artery of rats and this effect is associated with an improved vascular oxidative stress most likely by reducing bacterial translocation and the local angiotensin system [74]. Spontaneously hypertensive rats receiving specific probiotics showed amelioration in imbalance between ROS production and NO bioavailability [30]. The study on atherosclerotic rabbits demonstrated that probiotics protect the endothelium by reducing VCAM-1 and intercellular adhesion molecule-1 (ICAM-1) [64].

Physical activity

It has been demonstrated that physical activity favorably modifies endothelial function and reduces cardiovascular risk, both in healthy people and in patients suffering from diabetes, metabolic syndrome, hypertension and ischemic cardiac disease. Physical activity increases the production of NO and SOD. This effect was best proven in coronary arteries, where physical effort leads to an increase in microcirculation efficiency, a reduction in atherosclerotic plaque, formation of collaterals, a decrease in endothelial viscosity and an increase in diastolic perfusion. These mechanisms can lead to the cessation of endothelial remodeling in cardiac ischemic disease. In people performing regular physical activity arterial walls are more susceptible to vasodilatory factors such as NO than in people having a sedentary

lifestyle [83]. Also, the bioavailability of NO in people performing regular exercise is higher than in sedentary lifestyle [18]. This effect is present in healthy people and in patients with heart failure [50]. Physical activity plays a protective role against endothelial aging processes by maintaining NO production and lowering oxidative stress [83]. The beneficial effect of physical activity was shown not only in coronary, but also in pulmonary arteries. Physical exercise increases the pulmonary arterial wall susceptibility to vasodilatory factors such as acetylcholine and NO and decreases the synthesis of vasoconstrictor factors [83]. Regularity is the key factor of the influence of physical activity on the endothelium. Allen et al. compared the endothelial function in a group of healthy young men and in a group of people with high cardiovascular risk. It was demonstrated that only regular physical activity ameliorates endothelial function in a group of high-risk patients bringing it to the level of healthy young men [1]. Physical activity plays an important role in endothelial function in obesity. It decreases systolic and diastolic blood pressure, both resting and exercise, BMI and waist circumference, one of the main cardiovascular risk factors [82]. Moreover, it prevents obesity-caused kidney and liver organ damage, in which vascular dysfunction is one of the underlying mechanisms [85,91]. The beneficial role of regular physical activity on endothelial function is especially evident in obese children. It leads to a decrease in ROS production, an increase in NO synthesis, a decrease in endothelial adhesive properties and a decrease in atherosclerotic plaques instability. These effects were most strongly expressed when regular physical exercise was implemented before the age of 20 [83]. What is more, regular physical activity leads to a decrease in insulin resistance negatively affecting the endothelium and which is increased in obesity [18]. Physical exercise leads to a decrease in blood pressure and total and LDL cholesterol serum concentration. However, these effects are not permanent and decline about month after cessation of regular sport. Not only regularity but also the level of exercise is a key factor of exercise role in endothelial function. It has been demonstrated that the beneficial influence of sport on endothelium diminishes after exceeding the anaerobic threshold [83].

Conclusion

Understanding the importance of ED in the complex pathology of cardiovascular diseases, knowledge about mechanisms responsible for ED progress and the development of methods of ED control and prevention brings new therapeutic possibilities for people with elevated cardiovascular risk. A rising number of scientific proofs show the favorable effects of nutritional interventions and physical activity on endothelial function. Further double blind, prospective, randomized trials are crucial due to confirm the clinical benefits of described interventions.

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The authors have no potential conflicts of interest to declare.