Nitric Oxide, Cardiovascular Disease and Physical Exercise

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Arterial hypertension (AH) has a high incidence in the world’s population and its etiology is multifactorial, involving genetic, environmental and psychological factors. Its prevalence has progressively increased, especially among women, African descendants and the elderly. More than 50% of the subjects between 60 and 69 years of age and approximately ¼ of the population older than 70 years are affected by AH. Additionally, there is a positive and direct correlation between the elevated arterial pressure levels and the risk of cardiovascular diseases, regardless of gender, age range and ethnicity. Hence, the understanding of the cell and molecular mechanisms involved in the genesis of AH is fundamental for the attainment of preventive and/or therapeutic measures, with the objective of controlling pressure levels and consequently, reduce the cardiovascular risks associated with AH. Therefore, this review aims at discussing the mechanisms involved in the arterial pressure control, focusing on the role of the vascular endothelium and the importance of physical exercise in the regulation of nitric oxide production by endothelial cells.

Regulation of the arterial blood pressure

The arterial pressure (AP) can be essentially controlled through two mechanisms: the neural regulation, which is primarily carried out by the autonomous nervous system (associated to baroreceptors and chemoreceptors) and the humoral regulation that is performed by a variety of substances released by different cell types, such as the endothelial and the juxtaglomerular cells. Alterations in one or both mechanisms of AP control (neural and/or humoral) can result in an elevation of pressure levels, leading to AH.

The autonomous nervous system comprises the sympathetic and the parasympathetic nervous systems, and has a crucial role in AP control; both have been largely studied, aiming for understanding their participation in the genesis and/or maintenance of the hypertensive state. Baroreceptors, the primary mediators of the autonomous nervous system in the control of AP and cardiac frequency, are nerve endings that respond to the deformation or stretching of the vessel walls where they are located. The carotid sinuses and the aortic arch are the sites with the highest concentration of these afferences and highest physiological expression. The carotid sinuses are dilations of the internal carotid arteries that have a thinner wall and higher amount of elastic tissue than smooth muscle tissue, compared to other portions of the arterial wall. This area’s sensory innervation is provided by branches of the glossopharyngeal and vagus nerves, whereas the innervation of the aortic baroreceptors is provided by the aortic nerve. The baroreceptor afferences end in the nucleus of the solitary tract (NST), controlling sympathetic and parasympathetic tone.

The increase in AP promotes the stimulation of the aortic and carotid baroreceptors, triggering by reflex the inhibition of sympathetic overflow, whereas the AP decrease produces the opposing effect. The chemoreceptors are located in the aorta and carotid and respond to chemical stimuli of the partial pressure of oxygen (O₂), partial pressure of carbon dioxide (CO₂), and pH, playing important role in the anoxia and/or hypoxia states.

The humoral control is attained by different cell types. The juxtaglomerular cells present in the kidneys release renin, which, once in the bloodstream, will act on a plasmatic protein called angiotensinogen, forming angiotensin I. Angiotensin I is then converted into angiotensin II, with this conversion occurring almost entirely in the small vessels of the lung, catalyzed by the angiotensin-converting enzyme present in the endothelium of the pulmonary vessels. Angiotensin II is a potent vasoconstrictor, and its connection with the AT₁ receptors, present in the vascular smooth musculature, activates G protein with the consequent activation of phospholipase C-β and the formation of 1,4,5-triphosphate and diacylglycerol, which induces the increase of intracellular calcium concentration and activation of protein-C kinase, promoting vasoconstriction and consequently, the elevation of AP. It is noteworthy that the response to angiotensin II is biphasic in most part of the vascular beds, as there is a slight vasodilation triggered by the activation of the AT₁ receptors present in the endothelial cells, followed by potent vasoconstriction through the activation of the AT₂ receptors present in the vascular smooth cells. Angiotensin II also promotes left ventricular hypertrophy, renal vasoconstriction, mesangial cell contraction, increase of the Na⁺/H⁺ pump activity, proximal and distal to the renal tubules, renin secretion inhibition and aldosterone release.

The endothelial cells have a crucial role in the control of cardiovascular tonus, regulating vasomotricity, vascular permeability, the metabolism of endogenous and exogenous substances, and platelet and leukocyte activity. Furchgott & Zawadski were the first researchers to demonstrate the importance of the endothelium in the control
of vascular tonus. The authors reported that the vasodilation induced by acetylcholine depended on the presence of an intact endothelium, and that the endothelial cells released a relaxing factor, called endothelium-derived relaxing factor (EDRF).

In addition to acetylcholine, it was later observed that other agonists, such as histamine, bradykinin, ATP, thrombin, noradrenaline, angiotensin and serotonin were also able to release EDRF. Studies have shown that endothelial cells are able to synthesize several vasoactive substances, which were classified as relaxing factors and contractile factors. The endothelium-derived relaxing factors are nitric oxide (NO), prostacyclin (PGI2) and the endothelium-derived hyperpolarizing factor (EDHF). The endothelium-derived contractile factors are endothelin and thromboxane (TXA2).

The NO produced by the endothelial cells has a particularly important role in cardiovascular control, in the peripheral vascular resistance as well as platelet aggregation control. NO is a potent vasodilator and thus its role in AP control is extremely relevant. Additionally, the NO inhibits platelet aggregation, preventing thrombus formation, and consequently preventing the thrombosis and atherothrombotic disease processes.

Nitric oxide synthesis

NO biosynthesis comprehends one of the most important functions of L-arginine metabolism in the body. NO is formed from the nitrogen in the guanidine present in L-arginine, under the catalytic action of the enzyme NO synthase (NOS), generating equimolar concentrations of L-citrulline. The process of NO formation is shown in Figure 1.

Fig. 1 - Illustrative scheme of nitric oxide formation from the arginine metabolism through the activation of the Nitric Oxide Synthase enzyme (adapted from LEHNINGER, 2002).

The NO synthesis depends on the activation of an enzyme, triggering the process of NO formation. Thus, NO synthesis only occurs from the activation of NOS, which exists as two isoforms: the constitutive and the inducible isoforms.

The constitutive isoforms (cNOS) are originally found in the endothelium and neurons, being then called eNOS (endothelial NOS) and nNOS (neuronal NOS), respectively. The nNOS isoform, commonly called isoform I or bNOS (brain NOS), is found in the brain, spinal cord, sympathetic ganglia, adrenal glands, nitricergic neurons and in other structures such as epithelial cells of the lung, uterus and stomach, macula densa cells of the kidney, pancreas islet cells and skeletal muscle cells. The eNOS isoform, also called isoform III, is related to the endothelial cell membrane, regulating the tonus of the vascular smooth muscle cell, as well as platelet adhesion and aggregation. The eNOS isoform can also be found in syncytiotrophoblasts, tubular epithelial cells of the kidney, interstitial colon and hippocampus cells. Both isoforms are present in the cells and are stimulated by a complex signaling pathway that are dependent or independent of calcium ions (Ca2+). Both eNOS and nNOS require an electron donor, nicotinamide adenine dinucleotide phosphate (NADPH) and co-factors such as flavine adenine dinucleotide (FAD), flavine mononucleotide (FMN), and tetrahydrobiopterin (BH4). Humans, and presumably, in most of the other species, these isozymes are codified by three different genes located in three diverse chromosomes.

The inducible isoform (iNOS) is activated from some pathological stimuli, such as bacterial lipopolysaccharide (LPS), cytokines, including interleukin-1, endotoxins and tumor necrosis factor, which are Ca2+-independent. This isoform can be expressed in a large variety of cell types, including macrophages, lymphocytes, neutrophils, eosinophils, Kupffer cells, hepatocytes and epithelial cells. One of the main differences between the constitutive NOS and the inducible type is that the iNOS is able to release large amounts of NO for relatively long periods of time, and this can generate some exaggerated effects, producing toxic responses to the body, whereas cNOS produces small amounts of NO for a shorter period of time. Despite the differences between the NOS isoforms, all of them act as catalysts in the oxidation of the terminal nitrogen atom of the guanidine group of the L-arginine, forming equimolar amounts of NO and L-citrulline, as shown in Figure 1.

The activation of NOS and the consequent synthesis of NO by the endothelial cells occur from stimuli that can be chemical or physical. The chemical stimuli originate from the interaction of endogenous/exogenous agonists with specific receptors present in the endothelial cells, such as acetylcholine, ATP and bradykinin. The agonist-receptor interaction in the endothelial cell promotes the formation of inositol triphosphate (IP3), which, in turn, induces the release of Ca2+ ions from the endoplasmic reticulum, elevating the intracellular Ca2+ levels, forming the calcium-calmodulin complex, activating the NOS enzyme, which will act on L-arginine, generating the formation of NO by the endothelium.

The physical stimulation is performed by the force that the blood applies to the artery walls, called shear stress. The mechanism by which shear stress promotes the formation of NO is yet to be clarified. It is known that endothelial cells have mechanoreceptors, which can directly activate G proteins, the ionic channels and enzymes from the protein-kinase and phosphatase group that will promote the formation of second messengers, triggering a series of chemical reactions leading to vasodilation.

Thus, endothelial cells behave dynamically, and respond to the alterations of physical stimuli (shear stress) as well as chemical ones, promoting the synthesis and release of vasoactive substances.
Once released, the NO is rapidly dispersed from the generating cell to the target cell, or more particularly, from the endothelial cells to the blood vessel smooth muscle. In the smooth muscle cell, the NO will activate a catalytic enzyme, the soluble guanylate cyclase (sGC). This activation is attained by the coupling of NO with the heme group of this enzyme (receptor site), which will, in turn, form the cyclic guanosine monophosphate (cGMP) from the breakdown of guanosine triphosphate (GTP). The formation of cGMP promotes the activation of the calcium pump within the smooth muscle cell, decreasing the concentrations of intracellular calcium, which will reduce the vascular tonus.

Other mechanisms by which the NO/cGMP pathway will induce vasodilation include the inhibition of IP3 generation (in smooth musculature), dephosphorylation of the myosin light chain, inhibition of the Ca^{2+} inflow, protein kinase activation, stimulation of the membrane Ca^{2+}-ATPase and opening of K+ channels.

Therefore, in blood vessels, the intracellular increase of cGMP induces the relaxation of the vascular smooth muscle and, consequently, vasodilation. In platelets, the formation of cGMP will inhibit platelet aggregation. In the kidney, this will trigger an increase in the renal excretion of sodium, and the consequent loss of water and decrease in blood volume.

Figure 2 illustrates the synthesis, release and action of NO and the role of eNOS in its production.

![Figure 2](image-url)

**Fig. 2** - Illustrative scheme of NO synthesis, release and action and the role of eNOS in its production.

As shown in Figure 2, the Ca^{2+} ions have an important role in the control of vascular tonus. As the smooth musculature does not have troponin, a regulating protein present in skeletal muscle, which is activated by the Ca^{2+} ions to promote muscular contraction, the contraction of the smooth musculature occurs due to the combination between calcium and calmodulin. This combination activates a phosphorylative enzyme, myosin-kinase, which phosphorylates the myosin light chains, acquiring the capacity of attaching itself to the actin filament and performing the muscular contraction. Hence, the decrease in Ca^{2+} concentration would prevent the calcium/calmodulin combination, generating a relaxation of the vascular smooth musculature and the consequent vasodilation.

**The role of NO in cardiovascular diseases**

The role of NO in the cardiovascular system has been extensively studied, with a protective role being attributed to NO in AH, atherosclerosis, coronary artery disease and thromboembolic diseases.

Thus, the endothelial dysfunction, characterized by a lower production and/or bioavailability of NO, is one of the factors that contribute to the genesis of cardiovascular diseases.

**Dyslipidemias and atherosclerosis**

The dyslipidemias comprehend hypertriglyceridemia and hypercholesterolemia. Hypertriglyceridemia is defined as levels of triglycerides > 200 mg/dL, whereas hypercholesterolemia is defined as the elevation of plasma concentrations of cholesterol > 240 mg/dL. In hypercholesterolemia, the amount of low-density lipoprotein (LDL) is high and its elevated concentration in circulation promotes its oxidation (LDL-ox). The process of atherosclerosis is closely related to plasma levels of LDL-cholesterol, especially after it and it is believed that the presence of LDL-ox is the first phase in the process of atherosclerosis development. An elevated plasma concentration of LDL-cholesterol promotes a saturation of its clearance system, resulting in an increase of its permanence time in circulation and, consequently, its oxidation. The LDL molecule can be oxidized by calcium ions, lipoxygenases (produced by endothelial cells), myeloperoxidases (secreted by phagocytes) and oxygen reactive species, triggering the entire atherosclerotic process.

The NO produced by the endothelial cells has an important protective role in atherosclerosis, inhibiting the oxidation of LDL-cholesterol molecules and preventing platelet aggregation. The mechanism by which NO prevents the formation of LDL-ox molecule is carried out through its antioxidant action (which is concentration-dependent), preventing the formation of superoxide anions (free radicals) that promote the oxidation of the LDL-cholesterol molecule. The anti-aggregation action of the NO is due to its coupling to the molecule of guanylyl cyclase or GC, which induces the formation of cyclic guanylyl monophosphate that promotes vasodilation and reduces the vascular tonus inside the platelet, inhibiting its activation and aggregation.

The formation of LDL-ox promotes cellular events of leukocyte recruiting for the affected vascular region, which will produce deleterious substances for the endothelial cells (interleukins), reducing NO production and/or its bioavailability. Clinical studies show that subjects with hypercholesterolemia present a decrease in the production of endothelium-derived relaxing factors. On the other hand, other studies have shown that the NO production is not affected by the elevated levels of circulating cholesterol, but the production of reactive species of oxygen, such as the superoxide anions (ONOO), react with the produced NO molecule, decreasing its availability for the cells and favoring the production of reactive species of oxygen.
the thromboembolic events. This would happen due to the fact that the hypercholesterolemia is associated with the increase of NAD/NADPH oxidase activity, which is crucial for the formation of superoxide anions.38,45,46.

The NO reduction promotes platelet aggregation, hyperplasia and hypertrophy of smooth muscle cells, resulting in a significant reduction of vessel lumen and, consequently, tissue ischemia. In the heart, the reduced blood flow in the coronary vessels results in myocardial performance deficiency.47 Laboratory animal studies showed that the impairment of the endothelium-dependent relaxation process in coronary vessels can cause chronic heart failure.42,43,49,68.

The improvement of the lipid profile restores the production and/or bioavailability of NO. Thus, the reduction of plasma LDL-cholesterol levels and/or increase of the HDL-cholesterol fraction, which performs the reverse LDL-cholesterol molecule transport, are extremely beneficial for the prevention and/or treatment of atherosclerosis.37.

Therefore to reduce circulating LDL-cholesterol levels is attained through the regular practice of physical exercises. Several studies have shown that physically active subjects have a lipid profile within the normal range, when compared to sedentary ones.38,40,49. Hence, the regular practice of physical exercises is an important prevention and/or treatment intervention in dyslipidemias, of which mechanisms involve the restoration of endothelial cell function and lipid profile improvement.49.

**Arterial hypertension**

AH is a disease with a multifactorial etiology that causes injuries to several organs, such as the heart, brain, vessels, kidneys and retina, being considered one of the most important risk factors for the development of cardiovascular complications such as 40% of deaths due to stroke and 25% of coronary diseases. The diagnosis of AH must take into account not only the pressure levels, but also risk factors, target-organ injuries and associated comorbidities. AH is basically an asymptomatic disease; however, it can sometimes present symptoms such as headache, usually in the morning and in the occipital region, dizziness, scotoma, tinnitus, palpitations and precordial discomfort.

The effect of regular physical exercises on AH has been extensively studied in humans as well as in laboratory animals. Experimental data show that the reduction of AP after exercising is higher in hypertensive subjects than in normotensive ones. The decrease of systolic and diastolic AP levels due to physical activity varies from 18-20 mmHg and 7-9 mmHg, respectively, in humans with mild or moderate hypertension and in normotensive subjects, the decrease is 8-10 mmHg and 3-5 mmHg, respectively.49.

The beneficial effects of physical exercise on AP control have been approached in two aspects: preventive and therapeutic ones. The preventive aspects involve health promotion and prevention of the hypertensive state in subjects at higher risk of developing the disease. The therapeutic aspects involve, in addition to the reduction of pressure levels and lipid profile alteration, the decrease of mortality, even when there is no decrease of pressure levels. Studies have demonstrated that variations in exercise intensity have different effects on the AP of spontaneously hypertensive rats (SHR) and humans.41,52. Aerobic or dynamic exercises such as cycling, swimming, stepping, treadmill running or walking are more effective as an alternative therapy (or concomitantly with drug therapy) in the treatment of mild or moderate hypertension.53-57. The programs of physical conditioning for hypertensive subjects recommend that the exercises should be performed at least three times a week for a minimum of 30 min per session. Low to moderate intensity exercises (40-70% VO2 max) produce better results in PA reduction than higher-intensity exercises (>75% VO2 max)58. Most studies show that PA reduction in essentially hypertensive subjects occurs from 3 weeks to 3 months after the physical training start, and that this reduction is maintained as long as the training continues.40,56.

Several animal models have shown that the inhibition of NOS by analogs of L-arginine, such as L-NAME, results in a dose-dependent decrease in NO production and elevation of AH.59-61. The daily administration of L-NAME (20 mg/rat/day) to rats leads to a chronic inhibition of NO synthesis, producing AP elevation, which is followed by kidney functional and heart structural alterations. In the heart, the cardiac abnormalities include mainly ventricular hypertrophy, and focus of necrosis and fibrosis.60,61. The cardiac injuries seem to be the result of the intense coronary vasoconstriction caused by L-NAME, which leads to a lower oxygen and nutrient supply to cardiomyocytes, and consequently, their death. Myocyte lesions promote formation of fibrotic conjunctive tissue, which can cause relatively severe impairment to the cardiac system.

**Nitric oxide and physical activity**

Up to the 90s, several studies showed the importance of the regular practice of physical exercises in the prevention and treatment of several diseases, and especially the cardiovascular diseases. However, what or which factors were involved in the beneficial effects of physical exercise was not known. From the discovery of the NO molecule, several studies were carried out, evaluating the effect of physical exercise on the endothelial cells, the production of relaxing factors, and the correlation with the beneficial effects produced by physical exercise.27,28,40,69,70.

Studies in humans and laboratory animals have shown that the shear stress induced by physical exercise is a powerful stimulant for the release of vasorelaxing factors produced by the vascular endothelium, such as NO and the endothelium-derived hyperpolarizing factor (EDHF), resulting in a decrease of AP levels.40,71. It was demonstrated that moderate physical training increases the relaxation of the vascular and non-vascular smooth musculature, and that this increased relaxation would be due to a higher production of EDHF and NO by the endothelial cells in response to physical exercise.48,72-74. Additionally, it was observed that the shear stress induced by the physical exercise increased the expression of endothelial and neuronal NO.27,70,75.
Thus, the beneficial effects of the regular practice of exercises on cardiovascular diseases have been mainly associated to a higher production of endothelium-derived vasodilating agents (NO and EDHF), with a consequent reduction of the peripheral vascular resistance, decrease of LDL cholesterol levels and inhibition of platelet aggregation. This effect would generate an improvement in pressure levels and consequently, a lower incidence of AH.

It is worth mentioning that physical exercise seems to have a protective effect in the endothelial integrity, either by increasing the NO production in vessels with preserved endothelium, or by repairing the endothelial dysfunction. Higashi et al. confirmed this fact by comparing the vascular responsiveness between hypertensive and normotensive subjects submitted to aerobic physical exercise. The authors observed that normotensive volunteers presented a significantly higher relaxation of the blood vessels than the hypertensive group after the physical exercise session, suggesting the existence of endothelial dysfunction in the hypertensive group. Also, when comparing the control hypertensive group with the hypertensive group that underwent a 12-week program of aerobic activity, the vascular responsiveness was increased in the group that practiced exercises, i.e., the physical activity contributed to restore the endothelial function. Thus, the role of physical activity becomes essential for cardiovascular control, not only for the benefits regarding AP control but also as inhibitor of platelet aggregation and formation of LDL-ox, acting preventively and/or therapeutically in several pathologies such as atherosclerosis, AH and dyslipidemias.

Final conclusions
The role of NO in cardiovascular diseases has been of note in all areas of knowledge. The beneficial action of NO on AP thromboembolic diseases and dyslipidemias is well established. The endothelial integrity is crucial for NO production and he physical exercise has been employed as an extremely important tool to maintain endothelial function and/or restore it. Thus, the regular practice of physical exercises is vital for the health maintenance and prevention and/or treatment of cardiovascular diseases, among others. Brazil has a high number of sedentary subjects, being necessary to establish public policies directed at investments in the areas of sports and leisure. Additionally, the professional qualification of the physical education professional, within the healthcare area, is of great relevance as an educational strategy in the reduction of cardiovascular disease prevalence in the Brazilian population.

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Potential Conflict of Interest
No potential conflict of interest relevant to this article was reported.

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