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Benefits of L-Arginine on Cardiovascular System

Emina Sudar-Milovanovic[1], Milan Obradovic[1], Aleksandra Jovanovic[1], Bozidarka Zaric[2], Sonja Zafirovic[1], Anastasija Panic[1], Djordje Radak[3] and Esma R. Isenovic[1,4,*]

[1]*Laboratory of Radiobiology and Molecular Genetics, Institute of Nuclear Sciences Vinca, University of Belgrade, Mike Petrovica Alasa 12-14, 11000 Belgrade, Serbia;* [2]*Department of Chemistry, ICTM, University of Belgrade, Belgrade, Serbia;* [3]*Department of Vascular Surgery, Dedinje Cardiovascular Institute, Belgrade University School of Medicine, Belgrade, Serbia;* [4]*The Pharmaceutical Research Institute at Albany, Albany College of Pharmacy and Health Sciences, New York, USA*

E.R. Isenovic

Abstract: The amino acid, L-Arginine (L-Arg) plays an important role in the cardiovascular system. Data from the literature show that L-Arg is the only substrate for the production of nitric oxide (NO), from which L-Arg develops its effects on the cardiovascular system. As a free radical, NO is synthesized in all mammalian cells by L-Arg with the activity of NO synthase (NOS). In states of hypertension, diabetes, hypercholesterolemia and vascular inflammation a disorder occurs in the metabolic pathway of the synthesis of NO from L-Arg which all together bring alterations of blood vessels. Experimental results obtained on animals, as well as clinical studies show that L-Arg has an effect on thrombocytes, on the process of coagulation and on the fibrolytic system. This mini review represents a summary of the latest scientific animal and human studies related to L-Arg and its mechanisms of actions with a focus on the role of L-Arg *via* NO pathway in cardiovascular disorders. Moreover, here we present data from recent animal and clinical studies suggesting that L-Arg could be one of the possible therapeutic molecules for improving the treatment of different cardiovascular disorders.

Keywords: Amino acid, Arginase, cardiovascular system, cardiovascular disorders, L-Arg, NO, NOS.

1. INTRODUCTION

L-Arginine (L-Arg) is an important amino acid present in the proteins of all life forms [1]. L-Arg is a precursor in the biosynthesis of various biologically important compounds like proteins, nitric oxide (NO), agmatine, creatine, urea, and polyamines. Thus, L-Arg has a crucial role in important cellular processes, such as cellular regeneration, wound healing, immunity, and protein turnover [2-4]. Normal plasma concentrations of L-Arg in humans are within the range 40-100 μmol/l [5], depending on the nutritional status and the development phase of a subject. Changes of L-Arg availability and also changes of the final products of various metabolic pathways of L-Arg in the body, may have significant physiological consequences [6].

L-Arg availability and its role in cardiovascular physiology and pathophysiology have been intensively investigated in the past couple of years, primarily because of its important role in the cardiovascular system (CVS) [6]. This amino acid is a precursor for synthesis of the NO molecule, a free radical synthetized in all mammalian cells by the activity of the NOS enzyme [7-10]. It has been shown that the only substrate for NO production is L-Arg. This amino acid has a crucial effect on the proper functioning of the CVS. Clinical studies on hypertensive and diabetic patients, and on healthy individuals, indicate that L-Arg can regulate vascular hemostasis [11-14]. Experimental results obtained on animals, and from *in vitro* experiments, suggest that L-Arg can exert effects on thrombocytes, coagulation, and on the fibrinolysis system [13-17]. This may indicate a new therapeutic potential of the amino acid L-Arg [11].

This mini review represents a summary of the latest scientific studies related to L-Arg and its mechanisms of action with a focus on the role of L-Arg via NO pathway in cardiovascular disorders. Moreover, here we have presented the data from recent studies conducted on animals, and clinical studies suggesting that L-Arg could be one of the possible therapeutic molecules for improving different cardiovascular disorders.

2. L-ARGININE

L-Arginine (L-Arg), an amino acid with the molecular formula: $C_6H_{14}N_4O_2$ (Fig. **1**) and molecular weight of 174.20 Da [1], was first isolated in 1886 by E. Schulze and E. Steiger [1]. Together with lysine and histidine, L-Arg belongs to the group of basic amino acids, marked as diamino monocarboxylic acids with one –COOH and two –NH_2 groups [18]. Other synonyms for L-Arg also are: 2-amino-5-guanidinopentanoic acid, (S)-2-amino-5-[(aminoiminomethyl)amino]pentanoic acid and 2-amino-5-guanidino-n-valeric acid. L-Arg contains a guanidino group

*Address correspondence to this author at the Institute of Nuclear Sciences Vinca, University of Belgrade, Laboratory of Radiobiology and Molecular Genetics, P.O. Box 522, 11000 Belgrade, Serbia; Tel/Fax: +38111-3408147; E-mail: isenovic@yahoo.com

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in the side chain, this part of the molecule is protonated and carries positive charge at physiological pH values [19].

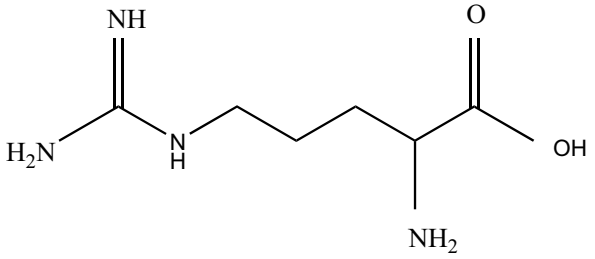

Fig. (1). Chemical structure of L-Arginine.

L-Arg is classified as a semi-essential or conditionally essential amino acid, and under normal circumstances the body can synthesize sufficient L-Arg to meet physiological demands [20, 21]. However, there are conditions that put an increased demand on the body for synthesis of L-Arg during children growth, pregnancy, lactating in women, intestinal resection or dysfunction, burns, renal dysfunction and wound healing [18, 22]. Under these conditions, L-Arg becomes essential, and additional ingestion of L-Arg must be provided through nutrition for optimum growth, [23] and for tissue regeneration [7, 24]. Nutritional sources of L-Arg are seafood, nuts, seeds, algae, meats, rice protein concentrate, and soy protein isolate [22, 25-27].

3. SEARCH STRATEGY

Data used for this review are obtained by searching the electronic database [PUBMED (1967 – August 2015)]. Results from national and international cardiovascular meetings were searched as well, and relevant authors were contacted to obtain further data. The main search keys were: L-Arg, L-Arg metabolism, L-Arg and NO, L-Arg and NO in CVS, L-Arg in animal studies, L-Arg in human studies, L-Arg and clinical trials.

4. METABOLISM OF L-ARGININ

To date, at least five enzymes are known that utilize L-Arg as the substrate for their activity. In addition to arginyl-tRNA synthetase, following four groups of enzymes in mammals use free L-Arg as the substrate: NO synthase (NOS) (at least three isoforms): arginase (2 isoforms), L-Arg-glycine amidinonotransferase and L-Arg decarboxylase [6]. Even though there are several pathways of L-Arg catabolism, there is only one pathway of L-Arg synthesis which leads from citrulline [6]. Citrulline is synthetized in the bowels of mammals from glutamine and proline, while the main sites for endogenous biosynthesis of L-Arg from citrulline are the kidneys [7, 28]. In addition to the kidneys, citrulline metabolizes to L-Arg in all tissues expressing the enzymes arginosuccinate synthetase (ASS) and arginosuccinate lyase (ASL), in the cycle known as the "citrulline-NO cycle" [6, 7, 29]. Large quantities of L-Arg are synthetized in the urea cycle in hepatocytes, and the synthetized L-Arg is immediately hydrolyzed to ornithine and urea, resulting in the fact that the urea cycle is not providing enough L-Arg for the entire body.

By the activity of the enzyme arginase, L-Arg is converted to L-ornithine, the precursor in the synthesis of polyamines and urea, molecules essential for the urea cycle [7]. In addition, L-Arg is also the precursor for creatine (Fig. **2**), the compound that plays an important role in the metabolism of energy in muscles, nerves and testicles. Creatine also significantly contributes to the catabolism of L-Arg and the synthesis of agmatine and proteins [23, 29, 30]. Creatine is obtained through the diet, but also synthesized in the liver, kidney, and pancreas [31]. Enzyme arginine:glycine amidino-transferase (AGAT) catalyzes the reaction in which creatine is synthesized from glycine and L-Arg [31, 32]. The generation of creatine from L-Arg may reduce the pool of L-Arg available for NO generation [33]. However, creatine reduces the activity of AGAT by feedback inhibition, therefore augmenting dietary creatine concentrations has the potential to decrease conversion of L-Arg to creatine, which could increase the availability of L-Arg for NO synthesis [33-35].

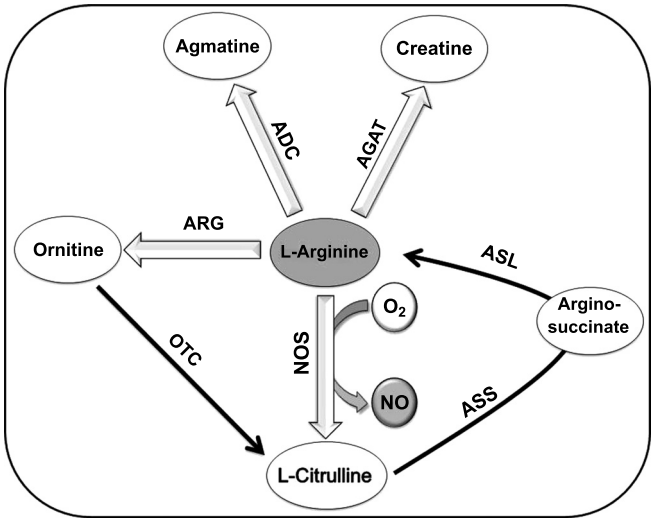

Fig. (2). Shematic overview of several pathways of L-Arginine catabolism, with highlighted pathways of NO synthesis. ADC - arginine decarboxylase, AGAT - arginine:glycine amidinotransferase, ARG - arginase, ASL - argininosuccinate lyase, ASS - argininosuccinate synthetase, DDAH - dimethylarginine dimethylaminohydrolase, NO - nitric oxide, NOS - nitric oxide synthase, O_2 - oxigen OTC - ornithine transcarbamylase.

Induction of the enzyme arginase results in increased catabolism of L-Arg to ornithine [36]. Since L-Arg is the limiting factor for NO synthesis [37], it can be expected that arginase activity is also included in induced NO synthesis. Based on the observation that inhibition of arginase activity leads to an increase of NO production in the endothelium, [38-43], arginase in cells of the endothelium most probably plays a role in regulating the availability of the substrate for NO synthesis [6].

In pathophysiological states, such as hypertension and ischemic reperfusion, the activity of endothelial arginase is increased and contributes to endothelial dysfunction, by further decreasing L-Arg concentration, this all together leading to NOS dysfunction (such as eNOS uncoupling) and

impaired NO production [6, 38, 40, 41, 43-45]. In addition, uncoupling of eNOS as a result of decreased intracellular availability of the substrate L-Arg is due to upregulation of arginase expression/activity or accumulation of endogenous methyl-arginines (e.g. asymmetric dimethylarginine (ADMA)) [46]. Evan endothelial cells have capacity to recycle L-citrulline to L-Arg, increased activity of arginase can lead to L-Arg deficiency and decrease in NO production [47]. The exact mechanism of eNOS uncoupling during endothelial dysfunction in pathophysiological conditions, such as hypercholesterolemia, diabetes mellitus or hypertension, are not completely understood [47-50]. Tetrahydrobiopterin (BH4) has been considered as a target molecule that can cause eNOS uncoupling [51]. However deficiency of L-Arg could be one of the mechanisms that can cause eNOS uncoupling [46]. Thus, increased L-Arg concentration may influence uncoupled eNOS, and promote binding. The activity of both enzymes, NOS and arginase, are significantly decreased in endothelial cells of diabetic rats, compared to controls [6, 52]. Arginase activity could be decreased or inhibited by N-hydroxyarginine, a mediator in the NOS signal pathway [53]. Thus, due to low arginase activity, more L-Arg is available as the substrate for the enzymes NOS whose activity is stimulated, resulting in increased NO production [54] and the level of arginase expression or activity could be a target in the treatment of certain cardiovascular disorders [6, 53].

5. THE ROLE OF L-ARGININE AND NO IN THE CARDIOVASCULAR SYSTEM

In cardiovascular physiology and pathophysiology, the effect of NO plays a key role on the vascular endothelium [6]. Previous studies revealed that NOS inhibition, and thereby decrease in NO production, causes hypertension and cardiac hypertrophy [55, 56]. These findings led to identification of NO as essential factor involved in development of atherosclerosis and other cardiovascular disorders. The amino acid L-Arg is the only precursor for NO synthesis, and L-Arg catabolic enzymes, that exert the most effects on the CVS by their action are NOS and arginase [6, 54, 57, 58]. L-Arg is the substrate for the NOS enzyme, which converts it to the amino acid L-citrulline with the release of NO (Fig. **2**). From the formed citrulline, L-Arg, can be recycled by the enzymes ASS and ASL [6, 29], but can also be degraded by arginase (Fig. **2**).

Nitric oxide is generated by the three isomorphic forms of NOS: neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS), which are widely expressed in virtually all vascular cell types [59-61]. NOS isoforms have similar enzymatic mechanisms for production of NO, and for proper function all NOS isoforms require cofactors, such as (BH4), nicotinamide-adeninedinucleotide phosphate (NADPH), flavin adenine dinucleotide, and flavin mononucleotide. NO influences a number of metabolic, biosynthetic, signalling and membrane transport processes [60, 62], and one of the crucial role is its regulation of vascular tone and structure [60, 63]. Other molecules such as acetylcholine, bradykinin, thrombin, adenosine diphosphate (ADP), phosphodiesterase type 5 (PDE5) inhibitors and nitrovasodilators among others, also stimulates NO production which leads to a relaxation of vascular smooth muscle cell (VSMC) [60, 64-66]. NO reacts with various molecules, but the main mechanism of its action includes activation of soluble guanylate cyclase and amplifying the production of cyclic guanosine monophosphate [60, 67-69]. Reduced bioavailability of NO either as a result of decreased production by NOS or increased breakdown by reactive oxygen species (ROS) is implicated in the development of various vascular disorders [70-72], including vasoconstriction, platelet aggregation, thrombus formation, leukocyte adhesion and VSMC proliferation and migration [60, 73-75]. Besides, endothelial dysfunction that occurs early in the development of atherosclerosis is characterized by an impaired NO bioavailability [60, 65, 76]. Numerous factors inhibit biological activity of NO, such are decreased L-Arg uptake, decreased NOS cofactors (Ca^{2+}, calmodulin, BH4), inhibition of electron flow (NADPH, flavins), inhibition of NOS expression [60, 73, 77]. In addition, NO may react with ROS and increased ROS concentrations (e.g. superoxide anion) and reduce the amount of bioactive NO, and form active intermediate, such as toxic peroxynitrite [70, 78], and on that way NO contributes to vascular oxidative stress [60, 79-81].

5.1. L-Arginine Paradox

Acute and chronic administration of L-Arg improves endothelial function in animal models of hypercholesterolemia and atherosclerosis [82]. Impairment of the NO bioavailability in CVS, which occurs due to reduced activity of eNOS, can be caused by L-Arg analogs that are substituted at the guanidine nitrogen atom, such as N^G-monomethyl-L-arginine (L-NMMA) or N^G-nitro-L-arginine [83]. Excess L-Arg overcomes inhibitory action of these analogues [83], indicating that there is competition for enzyme binding between L-Arg and its inhibitory analogues [5]. Increased plasma concentration of L-Arg leads to increased production of both vascular and systemic NO [29, 53, 84-87]. Studies on VSMC have shown that the limiting factor for increasing the possibility of NO synthesis by aid of iNOS is in fact the possibility of L-Arg recycling [6, 88]. Schott *et al*. have demonstrated that iNOS activity depends on the concentration of extracellular L-Arg [89]. The possibility of the existence of an intracellular pool of L-Arg not accessible to the iNOS enzyme, so that extracellular L-Arg is necessary for iNOS activity and the resulting NO production, also speaks in favor of this paradox [77, 90, 91]. Therefore, the term "L-arginine paradox" pertains to the specific situation when L-Arg supplementation stimulates NOS activity and NO production, even though the plasma level of L-Arg is within physiological limits [92]. "L-arginine paradox" may be caused by several possible reasons. First, L-Arg may be compartmentalized in the cytoplasm, so that local concentrations in the vicinity of eNOS may be lower than expected. There is recent evidence that L-Arg transporter (CAT-1) and eNOS are colocalized in caveolae which are associated with membrane [5, 81, 84]. In this case, increase of NO concentration can be achieved by eNOS using extracellular L-Arg within microenvironment of caveolae [5]. Another explanation for the "L-arginine paradox" could be the competitive inhibition of eNOS by endogenous inhibitors such as ADMA, which arises as a product of metabolism in the body [80], but in

pathophysiological conditions, level of ADMA is elevated [93]. ADMA competes with L-Arg for binding to NOS thus leading to an annulment of the eNOS activity and to a reduced production of NO as well as endothelial dysfunction [94, 95]. Inhibition of eNOS activity may be overcome by excess substrate giving rise to the hypothesis that L-Arg may be beneficial in patients with elevated ADMA with no effects on NO-dependent mechanisms in subjects with low or normal ADMA levels [96, 97]. Moreover, a possible cause of "L-arginine paradox" could also be the interaction of L-Arg with L-glutamine (L-Gln). When NO production is sustained, the endothelial cells can recycle L-Arg to L-citrulline [98-100]. Hecker *et al*. [98] and Sessa *et al*.[99] reported that formation of L-Arg from L-citrulline is inhibited by L-Gln when its concentrations are below those encountered in either tissue culture media or in plasma [98-100]. The same authors found that L-Gln inhibited release of endothelium-derived NO in response to ADP [100]. Furthermore, Arnal *et al*. [100] show that L-Gln, in concentrations circulating *in vivo*, affects endothelial release of NO and registered that L-Arg may enhance NO release *via* reversal of the inhibitory effect of L-Gln, independently of enhancing NOS substrate [100]. This effect seemed to involve a complex interplay with L-Gln and receptor-mediated activation of NOS, which was independent of intracellular L-Arg levels [100].

Endocrine mechanisms may also contribute to vasodilatation induced by L-Arg. High intravenous (IV) doses of L-Arg stimulate pituitary growth hormone (GH) secretion [97, 101]. GH and insulin, whose release is also stimulated by L-Arg [102], can induce vasodilatation [5]. This endocrine effect of L-Arg was blocked, and vasodilator effect partly abolished by coinfusion of somatostatin [97, 103]. Release of GH after IV L-Arg [104] was antagonized by octreotide pretreatment and restored by coadministration of recombinant GH with L-Arg [97]. Böger and Bone-Böger in their study show that chronic administration of recombinant GH increases NO production in GH-deficient patients [5, 105] and in patients with dilated cardiomyopathy [5, 106]. Furthermore, it has been suggested that some unspecific effects may be involved in the vasodilatory action of L-Arg. Calver *et al*. [107] reported that IV infusion of either L-Arg or D-Arg in human subjects induced vasodilatation, suggesting that this vasodilator effect was related to osmolality or pH effects, and not to enhanced endothelial NO formation. All these effects have been significant only at high, micromolar to millimolar, plasma concentrations of L-Arg [97].

Systemic or oral administration of L-Arg improves cardiovascular function and decreases cardiac ischemia in patients suffering from coronary arteries disease [7, 84, 108], and also leads to decrease of blood pressure and vascular resistance in the kidneys of hypertensive patients with normal or impaired renal function [7, 109]. Even though plasma concentration of L-Arg in patients with hypercholesterolemia is unchanged oral or IV administration of L-Arg can restore endothelial functions in these patients [110]. Since the majority of endogenous L-Arg is synthesized in the kidneys, renal dysfunction could also contribute to a decrease of the plasma level of L-Arg [6]. In patients suffering from the short gut syndrome citrulline synthesis is reduced, leading also to a reduced L-Arg level in plasma [6, 111-113], therefore, L-Arg is regarded as an essential amino acid in nutrition in patients with a weakened renal or intestinal function [6]. There is the opinion that atherosclerosis is caused by a lack of L-Arg, most probably due to a disturbed relationship between lysine and L-Arg concentrations [114], and to the interaction between estrogen and L-Arg metabolism [7, 115].

5.2. Evidence from Animal Studies

In the 1990-s, researchers' attention was focused on the influence of L-Arg supplementation in different animal models of hypercholesterolemia [116-118]. Namely, Aji *et al.* studied effects of L-Arg on CVS in a mouse model of familial hypercholesterolemia [116], and demonstrated that oral L-Arg supplementation for 10 weeks in LDL-receptor-deficient mice, fed a high-cholesterol diet, prevent xanthoma formation and reduce atherosclerosis [116]. In the study by Cooke *et al.* white New Zealand rabbits were either fed a high-cholesterol diet or high-cholesterol diet supplemented with L-Arg [117], after 10 weeks, endothelium dependent relaxation was impaired in cholesterol fed rabbits, while the L-Arg group showed improved endothelial function [117]. Boger *et al.* showed that hypercholesterolemia is associated with vascular monocyte accumulation and increased myointimal proliferation in rabbits [118]. Moreover, they reported that administration of L-NMMA in cholesterol fed rabbits further enhances myointimal proliferation and entirely abrogate endothelium-dependent relaxation, while L-Arg supplementation decreases intimal plaque area and accumulation of monocytes in the vascular wall [118]. Beside animal models of hypercholesterolemia effects of L-Arg supplementation on CVS were studied extensively in animal models of hypertension [119-122], too. Susic *et al.* reported that combined therapy with L-Arg and angiotensin-converting-enzyme inhibitor decreases systolic pressure and left ventricular mass in old Wistar-Kyoto rats, with developed systolic hypertension [119]. Prolonged administration of L-Arg decreases arterial pressure and total peripheral resistance in old spontaneously hypertensive rats (SHR) [120]. Increasing L-Arg availability reduces peripheral sympathetic hyperactivity in the SHR, and this is at least in part mediated by increasing the L-Arg/NO/cGMP signalling [122]. Development of hypertension is associated with increased endothelin-1 (ET-1) production in blood vessels [123], while NO supresses its local production and attenuates its biological effects, thereby maintains blood pressure homeostasis [124]. Dumont *et a*l. examined the effects on L-Arg supplementation on blood pressure and ET-1 production in hypertensive uraemic rats [121] and showed that adding L-Arg in drinking water for 5 weeks prevents further increase in systolic blood pressure in already hypertensive rats [121]. In addition, ET-1 clearance and NO metabolites NO_2/NO_3 were increased in L-Arg treated rats. The authors suggest that increasing dietary L-Arg, and thereafter level of NO, attenuates development of hypertension, probably due to reduction of ET-1 production [121].

5.3. Evidence from Human Studies

There is considerable number of human clinical studies investigating effects of L-Arg on CVS [125-131]. Bocchi *et al.* studied the effects of NO inhalation and IV infusion of L-Arg on hemodynamics and left ventricular mechanics in patients with congestive heart failure (CHF) [125]. In their study seven patients with chronic heart failure inhaled NO for 10 minutes, followed by 30 minutes without drug, and afterwards received IV infusion of L-Arg, Results show that NO inhalation decreases pulmonary vascular resistance, without systemic effects, while IV applied L-Arg decreases heart rate, mean systemic arterial pressure and systemic vascular resistance [125]. Similarly, in the study by Koifman *et al.* 12 patients with CHF received IV infusion of L-Arg [126], and L-Arg increased stroke volume, cardiac output and NO production and decreased mean arterial blood pressure and systemic vascular resistance [126]. The authors hypothesize that L-Arg stimulated NOS activity and therefore increased NO production which led to decrease in arterial blood pressure and vascular resistance [126]. Adams *et al.* are the first to report the effect of L-Arg on endothelial physiology in humans with coronary artery disease (CAD) [127]. In their study, they demonstrated that oral administration of L-Arg at a dose of 21g/day, for 3 days improves endothelium-dependent vasodilation in patients with CAD [127]. Ast J *et al*. conducted a prospective randomized double blind study to show direct association between L-Arg supplementation and blood pressure reduction. Study was performed on 54 hypertensive or normotensive patients, randomized to receive three times a day for four weeks either 2 or 4 g of L-Arg or placebo. At the end of the study, blood pressure was measured again and results show that hypertensive patients who received 12 g/day L-Arg had lower both systolic and diastolic blood pressure [132]. Moreover, dietary supplementation with L-Arg for 4 weeks at a dose of 21g/day was found to significantly improve endothelium-dependent vasodilation in young hypercholesterolemic subjects, and this could favorably influence the atherogenic process [130]. Parallelly with this, Adams *et al.* measured monocyte adhesion to human umbilical vein endothelial cells with or without L-Arg supplementation [127]. L-Arg supplementation reduced monocyte/endothelial cells adhesion, and this effect was reversed by the addition of L-NMMA, an inhibitor of all NOS isoforms [127]. Furthermore, Bode-Boger *et al.* examined the effects of L-Arg vascular endothelial function in healthy old participants, and found that administration of L-Arg at a dose of 16g/day, for 2 weeks, improves endothelium-dependent vasodilation [131]. Bogdanski *et al*. [133] show that L-Arg supplementation 9 g/day in obese patients after 6 months significantly increased NO level, insulin sensitivity, total antioxidant status, and significantly decreased level of plasminogen activator inhibitor type 1. However, some researchers failed to report the effects of L-Arg administration on CVS. Schenke *et al.* show that L-Arg therapy does not improve NO bioavailability in CAD patients [128]. In their study, patients with CAD received either L-Arg at a dose of 9g/day or identical placebo capsules for 1 month. There was no significant difference in NO level, flow-mediated dilation of the brachial artery, cell adhesion molecules E-selectin, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 between patients who received L-Arg and those who received placebo, after 1 month [128]. Similarly, Schulman *et al.* concluded that oral supplementation with L-Arg at a dose of 9g/day, for 6 months has no effect on the vascular stiffness measurements and left ventricular function [129]. All these findings suggest that L-Arg supplementation may have important effects in improving different cardiovascular disorders.

5.4. Possible Harmful Effects of L-Arginine Supplementation and Therapeutic Approach

In most clinical trials administration of L-Arg improved the symptoms of cardiovascular diseases (CVD) [132, 134-136], while in some, long-term studies administration of L-Arg is not useful for treatment of CVD, such as peripheral arterial disease [137] or postinfarction therapy [129]. To our knowledge, there is no data in the literature which indicate direct causation between L-Arg supplementation in patients and toxic effects of high level of nitrite and nitrate. However, results from animal studies indicate that L-Arg supplementation may have detrimental effects [138-141]. It has been reported that early L-Arg supplementation can inhibit cardiomyocyte apoptosis, only if L-Arg is given during the late phase of reperfusion it promotes apoptosis in rats [139]. Similarly, Wang *et al.* show that L-Arg supplementation reverses the impaired cardiac function and decreases infarct size in the early stage of reperfusion partially, while treatment during the late stage of reperfusion increases infarct size and increases peroxynitrite ($ONOO^-$) formation [141]. Thus, uncontrolled production of NO in an oxidative environment consequently leads to $ONOO^-$ formation [142, 143], which causes different damage of cellular components, including lipid peroxidation, damage of enzymes, structural proteins and DNA [144]. In addition, $ONOO^-$ can induce lipid peroxidation, oxidize LDL, and oxidize BH4, which causes "uncoupling" of NOS enzymes, leading to further production of O_2^- [145].

Still 1984, Mizunuma *et al.* [146] investigated the effect of L-Arg on different tissues in rats, and showed that high doses of L-Arg injected intraperitoneally induced acute pancreatitis. Necrosis was also observed in adipose tissues around the pancreas [146]. Today, L-Arg-induced acute pancreatitis presents a favorable experimental model for studying the mechanisms of acute necrotizing pancreatitis [147, 148]. Although the exact mechanism by which L-Arg causes pancreatitis is not fully understood, evidence suggests that proinflammatory cytokines, oxygen free radicals and NO, all have a role in this negative effects of L-Arg [149-151]. In clinical study there is no evidence that L-Arg supplementation has negative effect on pancreas morphology and physiology. In only one case study [152], it has been reported that in 16-year-old male patient, supplementation with L-Arg 500 mg/day and zinc 10 mg/day over five months (for the purpose of body building), caused acute pancreatitis.

L-Arg supplementation could become toxic through increases of NO and $ONOO^-$ formation in patients with CVD, since most of these pathological states are accompanied

with increased ROS production in the vasculature. A possible therapeutic approach should be administration of L-Arg in combination with antioxidants or other compounds to prevent or reduce $ONOO^-$ production [47, 153-155]. Nitro-L-Arg (an inhibitor of $ONOO^-$ biosynthesis) and urate (a scavenger of $ONOO^-$) were shown to produce a recovery of cardiac function, left ventricular developed pressure, and efficiency of O_2^- utilization in isolated rat heart subjected to ischemia/reperfusion [154]. Infusions of vitamin C or folic acid can be used to reduce oxidative stress, or restore eNOS functionality and increase level of BH4 [47]. In addition, N-acetylcisteine (NAC) is a potent antioxidant which neutralizes ROS and reduction of the oxidative stress leads to increase in NO bioavailability [156]. Valentino *et al.* showed that combined administration of NAC and L-Arg improves NO bioavailability via reduction of oxidative stress and increase of NO production, and thereby improves endothelial function in hypertensive patients with diabetes mellitus type 2 [153].

6. CONCLUSIONS

L-Arg a conditionally essential amino acid is of high functional priority in NO production and consequently in cardiovascular physiology [157, 158]. Mechanism of L-Arg action may be particularly important when the extracellular supply of L-Arg is limited [159]. Furthermore, the regulation of NO bioavailability after employing L-Arg drugs is critical for developing new strategies for treatment of cardiovascular disorders, since NO has both beneficial and detrimental effects. Further investigations in animals and humans are necessary to elucidate the effect of L-Arg deficiency in pathophysiology of cardiovascular disorders and also to find the best way to supplement L-Arg and to enhance the L-Arg/NO pathway in cardiovascular disorders. In addition, we expect that the future larger clinical trials using drugs based on L-Arg will be introduced in clinical practice.

In this review we have summarized recent literature data from animal and human studies, related to L-Arg as one of the possible therapeutic molecules for improving various cardiovascular disorders. Data from literature [7, 11], together with our published data [54, 58, 160, 161], and our preliminary results related to the roles of L-Arg in the CVS, suggest that L-Arg could be one of the important therapeutic molecules for improving the treatment of cardiovascular disorders.

LIST OF ABBREVIATIONS

ADMA = Asymmetric dimethylarginine

ADP = Adenosine diphosphate

ASL = Arginosuccinate lyase

ASS = Arginosuccinate synthetase

BH4 = Tetrahydrobiopterin

CAD = Coronary artery disease

CHF = Congestive heart failure

CVD = Cardiovascular diseases

CVS = Cardiovascular system

ET-1 = Endothelin-1

GH = Growth hormone

IV = Intravenous

L-Arg = L-arginine

L-Gln = L-glutamine

L-NMMA = N^G-Monomethyl-L-arginine

NAC = N-acetylcisteine

NADPH = Nicotinamide-adeninedinucleotide phosphate

NO = Nitric oxide

NOS = NO synthase

eNOS = Endothelial NOS

iNOS = Inducible NOS

nNOS = Neuronal NOS

PDE5 = Phosphodiesterase type 5

ROS = Reactive oxygen species

SHR = Spontaneously hypertensive rats

VSMC = Vascular smooth muscle cell

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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