ELSEVIER

International Journal of Cardiology 75 (2000) 123–128

International Journal of Cardiology

www.elsevier.com/locate/ijcard

Review article

L-Arginine in coronary atherosclerosis

Costas Tentolouris MD[a], Dimitris Tousoulis MD Ph.D. FACC[a,*], George Goumas MD[a], Christodoulos Stefanadis MD[a], Graham Davies MD FRCP[b], Pavlos Toutouzas MD FACC[a]

[a]*Cardiology Unit, Hippokration Hospital, Athens University Medical School, Athens, Greece*
[b]*Cardiology Unit, Imperial College School of Medicine, Hammersmith Hospital, London, UK*

Received 18 January 2000; received in revised form 5 June 2000; accepted 13 June 2000

Abstract

Nitric oxide is formed from the N-guanido terminal of the amino acid L-arginine and from molecular oxygen by nitric oxide synthase enzymes. L-arginine administration improves the coronary blood flow response to acetylcholine in patients with normal coronary arteries and hypercholesterolemia, reverses the defective endothelium-dependent vasodilation associated with an elevated plasma low-density lipoprotein level or hypercholesterolemia, dilates coronary epicardial arteries and stenoses, enhances nitric oxide generation, and inhibits lesion formation after balloon angioplasty. Stimulation of endogenous nitric oxide production could inhibit atherogenesis, and therefore may be of benefit in patients with risk factors for atherosclerosis.

Keywords: Endothelium; Nitric oxide; Atherosclerosis; L-arginine

1. Introduction

The first proof that nitric oxide (NO) was at least one of the relaxing factors resulted from studies by Palmer et al. [1] who, using a chemiluminescence assay, demonstrated that cultured endothelial cells release nitric oxide when exposed to bradykinin.

NO is formed from the N-guanido terminal of the amino acid L-arginine and from molecular oxygen by NO synthase enzymes [2]. One of these enzymes is Ca^{2+}-dependent and is constitutive in various types of cells, including endothelial cells [3]. NO synthesized by constitutive NO synthase (cNOS), plays a primary role in the regulation of blood pressure [4]. Another type of NO synthase (iNOS) is Ca^{2+}-independent and inducible by immunological stimuli [5]. Endocardial and vascular endothelial cells express the constitutive form of NO synthase and this enzyme is known to modulate myocardial contraction and coronary tone [5]. The inducible form of NO synthase is located in myocytes, macrophages and endothelial cells [6] and may be involved in the depression of myocardial contractility in septic shock [7].

In the intact blood vessel wall, most of the NO is presumed to arise from the activity of endothelial cNOS [8]. There is continuous basal release of NO (constitutive) which represents a sizeable portion of the total NO-releasing capacity of native endothelial cells. The rate of NO formation under basal conditions seems to be substantially smaller in cultured endothelial cells, implying that native endothelial cells in vivo may be continuously exposed to stimuli, e.g. shear stress, which affect NO synthase expression [9]. Once the endothelium has been damaged, exposure of smooth muscle cells to cytokines and other stimulators of NOS induction may have im-

*Corresponding author. S. Karagiorga 69, 16675, Glifada, Athens, Greece. Tel.: +30-1-778-2446; fax: +30-1-778-4590.

0167-5273/00/$ – see front matter
PII: S0167-5273(00)00320-X

portant physiological consequences for the blood vessel. Medial smooth muscle cells exhibit a rapid proliferative response immediately after endothelial denudation, but this is followed by endothelial regrowth.

2. Effects of L-arginine in vascular atherosclerosis

The L-isomer of arginine is a substrate for both endothelial cell (constitutive) and inducible (in macrophages, foam cells and smooth muscle cells) isoforms of the enzyme nitric oxide synthase [10]. These enzymes convert L-arginine to citrulline and nitric oxide.

L-Arginine is first converted to N^G-hydroxy-L-arginine by eNOS [11] and alternative pathways for N-hydroxylation of L-arginine may increase the availability of this reaction intermediate, which could facilitate substrate turnover by the enzyme. In addition N^G-hydroxy-L-arginine inhibits arginase and may thereby increase intracellular steady-state levels of L-arginine [12]. L-arginine competes with other cationic amino acids for transport into cells, especially L-glutamine, and increased L-arginine availability may increase intracellular substrate concentration by competitively enhancing cellular uptake in this setting. A study indicates that NO synthesis by vascular smooth muscle is dependent on the presence of extracellular L-arginine [13]. In contrast NO synthesis by endothelial cells appears to be less dependent on extracellular L-arginine. It has been demonstrated that L-arginine transport in vascular cells is Na^+ independent and that inflammatory cytokines selectively stimulate Na^+ independent uptake [14].

In normal subjects L-arginine administration augments forearm vascular endothelium dependent dilation in response to acetylcholine [15], and significantly dilates proximal segments of epicardial coronary arteries [16]. However, intracoronary infusion of 50 μmol/min of L-arginine does not affect acetylcholine-induced vasomotion of large epicardial coronary arteries in control subjects [17].

L-Arginine administration improves the coronary blood flow response to acetylcholine in patients with normal coronary arteries and hypercholesterolemia [17,18], reverses the defective endothelium-dependent vasodilation associated with an elevated plasma low-density lipoprotein level or hypercholesterolemia [19–21], enhances nitric oxide generation, and inhibits lesion formation after balloon angioplasty [22–24]. L-arginine restores nitric oxide activity and inhibits monocyte accumulation after vascular injury in hypercholesterolemic rabbits [25]. Dietary L-arginine improves NO-dependent vasodilator function in cholesterol-fed rabbits and completely blocks the progression of plaques via restoration of NO synthase substrate availability and reduction of vascular oxidative stress in cholesterol-fed rabbits [26]. Administration of L-arginine to animals with preexisting intimal lesion augments vascular NO elaboration, reduces superoxide anion generation and is associated with a reduction in lesion surface area [27].

A recent study [28] showed vasodilation of coronary stenoses with intracoronary L-arginine and another [29] demonstrated that arginine produced non-stereospecific peripheral vasodilation. L-Arginine whether given intravenously or intra-arterially can reduce vascular tone [16,30,31]. Oral arginine has also been shown to improve brachial artery flow-mediated dilation in hypercholesterolemic patients, but not in normal individuals [32,33]. Quyyumi et al. [29] showed that parenteral arginine produced non-stereospecific peripheral vasodilation and improved endothelium-dependent vasodilation in patients with stable coronary artery disease by stimulation of insulin-dependent nitric oxide release or by non-enzymatic nitric oxide generation. In a recent study Blum et al. [34] using high resolution ultrasound for measurements in the brachial artery, showed that oral L-arginine administration does not improve NO bioavailability in patients with chronic stable angina on appropriate medical management. In contrast, other studies have shown that intravenous L-arginine, but not D-arginine, increased forearm dilation in hypercholesterolemic subjects in response to metacholine [35] and that intra-arterial L-arginine, but not D-arginine, increased the response to substance P in atherosclerotic patients [36].

The mechanism by which it exerts its vasodilator effects is controversial [29,36,37], but stimulation of the L-arginine-NO-synthase nitric oxide pathway appears to be of particular importance. However,

other possible mechanisms are volume expansion with increased atrial natriuretic peptide and insulin levels during L-arginine infusion and the hypertonicity of the L-arginine solution. Recently a circulating endogenous NO synthase inhibitor, asymmetric dimethylarginine (ADMA) has been detected in human plasma. Evidence suggests that the derangement of the NO synthase pathway plays a critical role in atherogenesis and that ADMA may participate in this endothelial dysfunction. Miyazaki et al. [38] suggests that plasma ADMA correlated with risk factors for atherosclerosis and ADMA level is significantly correlated with carotid intima-media thickness.

We have investigated the effects of intracoronary infusion of 50 and 150 μmol/min (each for 8 min) L-arginine, and we found that L-arginine significantly dilated atherosclerotic arteries in patients with risk factors and atherosclerotic coronary arteries [39]. These findings are consistent with the suggestion that diseased arteries may be relatively deficient in the substrate L-arginine [28,39–44]. Furthermore, we have shown that stenoses with a complex morphology show a greater dilation compared with those of smooth morphology, whether concentric or eccentric [45]. This may indicate a relative deficiency of L-arginine at the site of stenoses in diseased coronary arteries, particularly within stenoses with complex morphology. They are a further indication that complex morphology is a marker of increased functional activity and are consistent with enhanced nitric oxide activity. This could represent a natural compensatory mechanism to counteract the predisposition to constriction generated by atherosclerotic disease.

We have also shown that proximal segments dilate more than distal segments in response to L-arginine in patients with coronary artery disease [46]. This effect in proximal segments of 'normal arteries' and diseased arteries (including the site of stenosis) may suggest that it is either a physiological response or that it requires only minimal coronary disease which could be present in the proximal segments in the patients with normal coronary angiograms. An alternative explanation to the stimulation of nitric oxide synthase would be a physiological action on vascular smooth muscle which augments its responsiveness to nitric oxide or other endogenous vasodilator mechanisms.

3. The impact of L-arginine on risk factors for atherosclerosis

3.1. Smoking

Cigarette smoking is a well recognised coronary risk factor that induces endothelial dysfunction [47]. Cigarette smoking increases oxidative stress because of low circulating levels of oxygen-derived free radicals and lipid peroxides that degrade NO [48], enhance monocyte adhesion and increase the susceptibility of LDL to oxidation [49]. It has been shown [49,50] that antioxidant vitamins can reduce both smoking-induced lipid peroxidation and endothelial dysfunction. In contrast with a recent study [51] showing that acute administration of L-arginine reverses the abnormal myocardial blood flow response to cold pressor test in healthy long-term smokers, we did not find any difference in the vasomotor responses of epicardial coronary arteries to L-arginine between smokers and non-smokers [39]. However, because of the small number of the patients in our study we cannot exclude a possible relationship between smoking and the vasomotor effects of L-arginine [39].

3.2. Hypercholesterolemia

It has been shown that nitric oxide also has an anti-oxidant effect [52]. However, when it combines with equimolar amounts of superoxide, peroxynitrite is formed which is a strong oxidant. Inducible nitric oxide synthase can produce large amounts of nitric oxide and is present in human atherosclerotic lesions [53]. Apart from reducing nitric oxide production, substrate deficiency could lead to the generation of superoxide by both inducible and endothelial nitric oxide synthase [54]. In hypercholesterolaemic rabbits L-arginine administration restored cholinergic (nitric oxide dependent) relaxation of the thoracic aorta [40]. Clinical studies showed also correction of endothelial dysfunction by L-arginine in the coronary microcirculation of hypercholesterolaemic patients and in patients with chest pain and normal coronary arteries [17,18]. We showed that patients with cholesterol level $\leq$200 mg/dl were responsive to 50 μmol/min L-arginine, but those patients with cholesterol $>$200

mg/dl were only responsive to 150 μmol/min L-arginine [39].

3.3. Hypertension

In humans endothelium-dependent vasodilation to acetylcholine is reduced in essential hypertensive patients compared with normotensive control subjects [15] suggesting that endothelial function can be impaired in human hypertension. Endothelial function is impaired in human essential hypertension by the simultaneous presence of a defect in L-arginine–nitric oxide pathway and the production of a cyclooxygenase-dependent EDCF [15,55]. L-Arginine seems to normalise the response to endothelial agonists in offspring of essential hypertensive patients [56]. However, Panza et al. [15] demonstrated that intrabranchial infusion of L-arginine did not alter forearm vasodilation to acetylcholine in essential hypertensive patients whereas the amino acid increased vascular response to the muscarinic agonist in matched normotensive control subjects.

3.4. Diabetes mellitus

In patients with diabetes mellitus without detectable coronary atherosclerosis abnormal coronary responses to acetylcholine have been demonstrated, suggesting that the endothelium-derived nitric oxide system could be impaired before the development of overt atherosclerosis [57]. Such an impairment of endothelium-dependent dilation has also been demonstrated in extracardiac arterial and arteriolar vessels in insulin and non-insulin-dependent diabetes mellitus [58,59]. The administration of intravenous L-arginine in non-insulin-dependent diabetic patients with angiographically normal coronary arteries and no other risk factors does not improve the abnormal coronary artery responses to physiological stimuli [60].

4. Conclusions

Clinical and experimental studies show that L-arginine administration may have an impact in vascular atherosclerosis. There is evidence to suggest that at the site of stenosis the mechanism of nitric oxide production is intact and therefore stimulation of this pathway might provide therapeutic benefit in angina patients. Stimulation of endogenous nitric oxide production could inhibit atherogenesis or induce regression of pre-existing lesions. Patients with risk factors for atherosclerosis could benefit from L-arginine administration. However, the currently available data showing improvement in endothelial function is limited and long-term clinical benefit has not been clearly demonstrated because of the small number of patients involved in the clinical trials. The actions of L-arginine are complex and the responses of epicardial coronary arteries and brachial arteries to L-arginine may be different. Therefore, further studies are needed to elucidate a possible cause-and-effect relationship between L-arginine and atherosclerosis.

References

[1] Palmer RMJ, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature 1988;333:664–6.
[2] Chu A, Chambers DE, Lin C, Kuehl WD, Cobb FR. Nitric oxide modulates epicardial coronary basal vasomotor tone in awake dogs. Am J Physiol 1990;258:H1250–4.
[3] Nunokawa Y, Ishida N, Tanaka S. Cloning of inducible nitric oxide synthase in rat vascular smooth muscle cells. Biochem Biophys Res Commun 1993;191:89–94.
[4] Rees DD, Palmer RMJ, Hodson HF, Moncada S. A specific inhibitor of nitric oxide formation from L-arginine attenuates endothelium-dependent relaxation. Br J Pharmacol 1989;96:418–24.
[5] Schulz R, Nava E, Moncada S. Induction and potential biological relevance of a Ca^{2+}-independent nitric oxide synthase in the myocardium. Br J Pharmacol 1992;105:575–80.
[6] Waldman SA, Murad F. Biochemical mechanisms underlying vascular smooth muscle relaxation: the granulate cyclase-cyclic GMP system. J Cardiovasc Pharmacol 1988;12(suppl 5):S115–8.
[7] Nava E, Palmer RMJ, Moncada S. Inhibition of nitric oxide synthesis in septic shock: how much is beneficial? Lancet 1991;338:1555–7.
[8] Forstermann U, Schmidt HW, Pollock JS, Sheng H, Mitchell JA, Warner TD et al. Isoforms of nitric oxide synthase: characterisation and purification from different cell types. Biochem Pharmacol 1991;42:1849–57.
[9] Busse R, Mulsch A, Fleming I, Hecker M. Mechanisms of nitric release from the vascular endothelium. Circulation 1993;87(suppl V):V18–25.
[10] Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathology and pharmacology. Pharmacol Rev 1991;43:109–42.
[11] Zembowicz A, Hecker M, Macarthur H, Sessa WC, Vane JR. Nitric oxide and another potent vasodilator are formed by NG-hydroxy-L-arginine by cultured endothelial cells. Proc Natl Acad Sci USA 1991;88:11172–6.

[12] Buga GM, Singh R, Pervin S, Rogers NE, Schmitz DA, Jenkinson CP et al. Argininase activity in endothelial cells: inhibition by NG-hydroxy-L-arginine during high output NO production. Am J Physiol 1996;271:H1988–98.
[13] Busse R, Mulsch A. Induction of nitric oxide synthase by cytokine in vascular smooth muscle cells. FEBS Lett 1990;275:87–90.
[14] Durante W, Liao L, Iftikhar I, O'Brien WE, Schafer AI. Differential regulation of L-arginine transport and nitric oxide production by vascular smooth muscle and endothelium. Circ Res 1996;78:1075–82.
[15] Panza JA, Casino PR, Badar DM, Quyyumi AA. Effect of increased availability of endothelium-derived nitric oxide precursor on endothelium-dependent vascular relaxation in normal subjects and in patients with essential hypertension. Circulation 1993;87:1475–81.
[16] Tousoulis D, Tentolouris C, Crake T, Katsimaglis G, Stefanadis C, Toutouzas P et al. Effects of L- and D-arginine on the basal tone of human diseased coronary arteries and their responses to substance P. Heart 1999;81:505–11.
[17] Egashira K, Hirooka Y, Kuga T, Mohri M, Takeshita A. Effects of L-arginine supplementation on endothelium-dependent coronary vasodilation in patients with angina pectoris and normal coronary angiograms. Circulation 1996;94:130–4.
[18] Drexler H, Zeiher AM, Meinzer K, Just H. Correction of endothelial dysfunction in coronary microcirculation of hypercholesterolemic patients by L-arginine. Lancet 1991;338:1546–50.
[19] Maxwell AJ, Anderson B, Zapien MP, Cooke JP. Endothelial dysfunction in hypercholesterolemia is reversed by nutritional product designed to enhance nitric oxide activity. Cardiovasc Drugs Ther 2000;14:309–16.
[20] Imaizumi T, Hirooka Y, Masaki H, Harada S, Momohara M, Tagawa T et al. Effects of L-arginine on forearm vessels and responses to acetylcholine. Hypertension 1992;20:511–7.
[21] Jeremy RW, McCarron H, Sullivan D. Effects of dietary L-arginine on atherosclerosis and endothelium-dependent vasodilation in the hypercholesterolemic rabbit: response according to treatment duration, anatomic site, and sex. Circulation 1996;94:498–506.
[22] Girerd XJ, Hirsch AT, Cooke JP, Dzau VJ, Creager MA. L-arginine augments endothelium-dependent vasodilation in cholesterol-fed rabbits. Circ Res 1990;67:1301–8.
[23] Aji W, Ravalli S, Szabolcs M, Jiang XC, Sciacca RS, Michler RE et al. L-arginine prevents xanthoma development and inhibits atherosclerosis in LDL receptor knockout mice. Circulation 1997;95:430–7.
[24] Cooke JP, Singer AH, Tsao PS, Zera P, Rowan RA, Billingham ME. Anti-atherogenic effects of L-arginine in hypercholesterolemic rabbit. J Clin Invest 1992;90:1168–72.
[25] Wang BY, Candipan RC, Arjomandi M, Hsiun PT, Tsao PS, Cooke JP. Arginine restores nitric oxide activity and inhibits monocyte accumulation after vascular injury in hypercholesterolemic rabbits. J Am Coll Cardiol 1996;28:1573–9.
[26] Boger RH, Bode-Boger SM, Brandes RP, Phivthong-ngam L, Bohme M, Nafe R et al. Dietary L-arginine reduces the progression of atherosclerosis in cholesterol-fed rabbits: comparison with lovastatin. Circulation 1997;96:1282–90.
[27] Candipan RC, Wang BY, Buitrago R, Tsao P, Cooke J. Regression or progression. Dependency on vascular nitric oxide. Arterioscler Thromb Vasc Biol 1996;16:44–50.
[28] Tousoulis D, Davies G, Tentolouris C, Crake T, Toutouzas P. Coronary stenosis dilation induced by L-arginine. Lancet 1997;349:1812–3.
[29] Quyyumi A. Does acute improvement of endothelial dysfunction in coronary artery disease improve myocardial ischemia? A double-blind comparison of parenteral D- and L-arginine. J Am Coll Cardiol 1998;32:904–11.
[30] Drexler H, Fischell TA, Pinto F, Chenzbraun A, Botas J, Cooke J et al. Effect of L-arginine on coronary endothelial function in cardiac transplant recipients. Relation to vessel wall morphology. Circulation 1994;89:1615–23.
[31] Quyyumi A, Dakak N, Diodati J, Gilligan D, Panza J, Cannon RO. Effect of L-arginine on human coronary endothelial-dependent and physiologic vasodilation. J Am Coll Cardiol 1997;30:1220–7.
[32] Clarkson P, Adams MR, Powe AJ, Donald AE, McCredie R, Robinson J et al. Oral L-arginine improves endothelium-dependent dilation in hypercholesterolaemic young adults. J Clin Invest 1996;97:1989–94.
[33] Tsao PS, McEvoy LM, Drexler H, Butcher EC, Cooke JP. Enhanced endothelial adhesiveness in hypercholesterolemia is attenuated by L-arginine. Circulation 1994;89:2176–82.
[34] Blum A, Hathaway L, Mincemoyer R et al. Oral L-arginine in patients with coronary disease on medical managment. Circulation 2000;101:2160–4.
[35] Hirooka Y, Egashira K, Imaizumi T, Tagawa T, Hisashi K, Sugimashi M et al. Effect of L-arginine on acetylcholine-induced endothelium-dependent vasodilation differs between the coronary and forearm vasculatures in humans. J Am Coll Cardiol 1994;24:948–55.
[36] Jeremy RW, McCarron H, Sullivan D. Effects of dietary L-arginine on atherosclerosis and endothelium-dependent vasodilation in the hypercholesterolemic rabbit: response according to treatment duration, anatomic site, and sex. Circulation 1996;94:498–506.
[37] Creager MA, Gallagher SM, Girerd XJ, Dzau VJ, Cooke JP. L-arginine improves endothelium-dependent vasodilation in hypercholesterolemic humans. J Clin Invest 1992;90:1248–53.
[38] Miyazaki H, Matsuoka H, Cooke JP, Usui M, Ueda S, Okuda S et al. Endogenous nitric oxide synthase inhibitor. A novel marker of atherosclerosis. Circulation 1999;99:1141–6.
[39] Tentolouris C, Tousoulis D, Davies GJ, Stefanadis C, Toutouzas P. Serum cholesterol level smoking and vasomotor responses to L-arginine in diseased epicardial coronary arteries. Am J Cardiol 2000;85:500–3.
[40] Cooke JP, Andon NA, Girerd XJ, Hirsch AT, Creager MA. Arginine restores cholinergic relaxation of hypercholesterolemic rabbit thoracic aorta. Circulation 1991;83:1057–62.
[41] Boger R, Bode-Boger SM, Thiele W, Creutzig A, Alexander K, Frolich JC. Restoring vascular nitric oxide formation by L-arginine improves the symptoms of intermittent claudication in patients with peripheral arterial occlusive disease. J Am Coll Cardiol 1998;32:13336–44.
[42] Calver A, Collier J, Vallance P. Dilator actions of arginine in human peripheral vasculature. Clin Sci 1991;81:695–700.
[43] Tentolouris C, Tousoulis D, Davies G, Toutouzas P. Effects of acute administration of L-arginine in coronary atherosclerosis. Circulation 1999;99:1646–9.
[44] Dubois-Rande JL, Zelinsky R, Rovoot F, Chabriet PE, Castaingne A, Geschwind H et al. Effects of infusion of L-arginine into the left anterior descending coronary artery on acetylcholine-induced vasoconstriction of human atheromatous coronary arteries. Am J Cardiol 1992;70:1269–75.
[45] Tentolouris C, Tousoulis D, Crake T, Katsimaglis G, Trikas A, Stefanadis C et al. L-arginine administration enhances the endothelium-dependent dilation in atherosclerotic arteries and stenoses. Eur Heart J 1998;19(suppl):379.

[46] Tousoulis D, Davies G, Tentolouris C, Crake T, Katsimaglis G, Stefanadis C et al. Effects of changing the availability of the substrate for nitric oxide synthase by L-arginine on coronary vasomotor tone in angina patients with angiographically narrowed and in patients with angiographically normal coronary arteries. Am J Cardiol 1998;82:1110–3.

[47] Zeiher AM, Schaechinger V, Minners J. Long-term cigarette smoking impairs endothelium-dependent coronary artery vasodilator function. Circulation 1995;92:1094–100.

[48] Sanderson KJ, van Rij AM, Wade CR, Sutherland WHF. Lipid peroxidation of circulating low density lipoproteins with age, smoking and in peripheral vascular disease. Atherosclerosis 1995;118:45–51.

[49] Weber C, Erl W, Weber K, Weber PC. Increased adhesiveness of isolated monocytes to endothelium is prevented by vitamin C intake in smokers. Circulation 1996;93:1488–92.

[50] Heitzer T, Hanjorg J, Munzel T. Antioxidant vitamin C improves endothelial dysfunction in chronic smokers. Circulation 1996;94:6–9.

[51] Camprisi R, Czernin J, Schoder H, Sayre JW, Schelbert RH. L-arginine normalizes coronary vasomotion in long-term smokers. Circulation 1999;99:491–7.

[52] Clancy RM, Leszczynska P, Piziak J, Abramson SB. Nitric oxide, an endothelial cell relaxation factor, inhibits neutrophil superoxide anion production via a direct action on NADPH oxidase. J Clin Invest 1992;90:1116–21.

[53] Buttery LDK, Springall DR, Chester AH, Evans TJ, Standfield N, Parums DV et al. Inducible NO synthase is present within human atherosclerotic lesions and promotes the formation and activity of peroxynitrite. Lab Invest 1996;75:77–85.

[54] Hogg N, Darley-Usman VM, Wilson MT, Moncada S. Production of hydroxyl radicals from the simultaneous generation of superoxide and nitric oxide. Biochem J 1992;281:419–24.

[55] Taddei S, Mattei P, Virdis A, Sudano I, Ghiadoni L, Salvetti A. Effect of potassium on vasodilation to acetylcholine in essential hypertension. Hypertension 1994;23:485–90.

[56] Taddei S, Virdis A, Mattei P, Ghiadoni L, Sudano I, Salvetti A. Defective L-arginine-nitric oxide pathway in offspring of essential hypertensive patients. Circulation 1996;94:1298–303.

[57] Nitenberg A, Valensi P, Sachs R, Dali M, Aptecar E, Attali JR. Impairment of coronary vascular reserve and Ach-induced coronary vasodilation in diabetic patients with angiographically normal coronary arteries and normal left ventricular systolic function. Diabetes 1993;42:1017–25.

[58] Saenz De Tejada I, Goldstein I, Azadzoi K, Krane RJ, Cohen RA. Impaired neurogenic and endothelium-mediated relaxation of penile smooth muscle from diabetes men with impotence. N Engl J Med 1989;320:1025–30.

[59] Calver A, Collier J, Vallance P. Inhibition and stimulation of nitric oxide synthesis in the human forearm arterial bed of patients with insulin-dependent diabetes. J Clin Invest 1992;90:2548–54.

[60] Nitenberg A, Paycha F, Ledoux S, Sachs R, Attali JR, Valensi P. Coronary artery responses to physiolocal stimuli are improved by deferoxamine but not by L-arginine in non-insulin-dependent diabetic patients with angiographically normal coronary arteries and no other risk factors. Circulation 1998;97:736–43.