The prevention of endothelial dysfunction through endothelial cell apoptosis inhibition in a hypercholesterolemic rabbit model: the effect of L-arginine supplementation

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Abstract

Background: The impact of L-arginine on atherogenesis and its ability to prevent endothelial dysfunction have been studied extensively during the past years. L-arginine is a substance for nitric oxide synthesis which involves in apoptosis. Hypercholesterolemia promotes endothelial dysfunction, and it is hypothesized that L-arginine prevents endothelial dysfunction through endothelial cells apoptosis inhibition. To test this hypothesis, thirty rabbits were assigned into two groups. The control group received 1% cholesterol diet for 4 weeks, and the L-arginine group received same diets plus 3% L-arginine in drinking water.

Results: No significant differences were observed in cholesterol level between two groups, but the nitrite concentration in L-arginine group was significantly higher than other group (control group: 11.8 ± 1; L-arginine group: 14.7 ± 0.5 μmol/l); (p < 0.05). The aorta score of fatty streak in control group was 0.875 ± 0.35, but no fatty streak lesion was detected in L-arginine group (p < 0.05). The number of intimal apoptotic cells/500 cells of aorta in two groups of experiment were statistically different (control group: 39.3 ± 7.6; L-arginine group: 21.5 ± 5.3) (p < 0.05).

Conclusion: The inhibition of endothelial cells apoptosis by L-arginine restores endothelial function in a model of hypercholesterolemia.

Background

The concept of programmed cell death was introduced to describe cell death during normal development [1], and apoptosis is the most common form of cell death. Apoptosis is characterized by cell shrinkage, nuclear fragmentation and membrane blabbing [2,3].

Atherosclerotic lesions develop in the tunica intima of the arteries, in which accumulation of cellular components, lipids, and extracellular matrix yields a fibro fatty plaque that focally thickens the artery wall [4]. Apoptosis is a feature of human atherosclerosis which is associated with development of the lesion necrotic core as well as instability of complex plaques [4-9].

The first evidence that endothelial cell (EC) apoptosis might contribute to the initiation of atherogenesis came from the observation that all classical risk factors known
To promote endothelial dysfunction (ED) and atherogenesis can induce vascular cell apoptosis [10]. However, there is some in vivo evidence for a pro-atherogenic effect of apoptosis. A study in monkeys revealed that vascular ED was present without evidence of atherosclerosis, which may be due to endothelial apoptosis [11]. Apoptotic vascular cells are also found in hypercholesterolemic pigs and mice [12]. On the other hand, shear stress leads to physiologic low concentrations of nitric oxide (NO) within ECs [13]. The continuous generation of NO can prevent ECs apoptosis, thereby protecting the endothelial monolayer from injury [14]. Intervention with NO donor; L-arginine, has induced beneficial effects on atherosclerosis [15]. These findings strongly support the current clinical concept that ED precedes plaque formation and disease progression in patients [16].

The role of L-arginine and NO in apoptosis have been studied in different conditions (17–30). NO has also been demonstrated to be involved in the regulation of apoptosis, and recent evidence indicates that NO is a potent modulator of homeostasis operationally preventing or inducing apoptosis [31,32]. It is also reported that in some cell types, NO can promote apoptosis, whereas in others it inhibits apoptosis [33]. L-arginine as a NO donor is a potent substance to reverse ED [34-37]. Otsuji et al. studied the relationship between L-arginine and the progression of atherosclerosis. They found that exogenous L-arginine reverses acetylcholine-induced vasoconstriction in human coronary arteries in the early stages of atherosclerosis [38]. In hypercholesterolaemic rabbits treated with L-arginine, platelet aggregation, myointimal cell proliferation and vascular monocyte accumulation were attenuated while endothelium dependent vasoreactivity was improved [39]. Therefore, it is a hypothesized that L-arginine prevents ED through EC apoptosis inhibition in a model of hypercholesterolemia, and this hypothesis was tested in this study.

Results

Cholesterol and Nitrite Concentrations

The data for the cholesterol and nitrite concentrations are tabulated in table 1. The statistical analyses indicate that no significant difference was observed between the cholesterol levels of two groups, but the nitrite concentration in L-arginine group was significantly higher than control group (p < 0.05).

Fatty streak formation

The score of aorta fatty streak in first group was 0.875 ± 0.35, but no fatty streak lesion was detected in L-arginine group. The statistical analysis indicates that fatty streak formation is significantly lower in L-arginine group (p < 0.05).

The number of intimal apoptotic cells

The number of intimal apoptotic cells/500 cells in rabbit’s aortas is demonstrated in figure 1 (control group: 39.3 ± 7.6, L-arginine group: 21.5 ± 5.3) (p < 0.05). In situ detection of apoptotic cells indicate that in aorta section from control group, the apoptosis of intimal cells is a prominent feature of atherosclerotic lesions, but less apoptosis cells were observed in L-arginine group (figure 2).

Discussion

The role of L-arginine in ED prevention through EC apoptosis inhibition was the main objective of this study. There was no fatty streak in the aorta of L-arginine treated group while significant fatty streak lesions were found in control group. Subsequently, L-arginine supplementation successfully prevented atherosclerosis process, and this is in agreement with the results of several other studies in which L-arginine supplementation restores endothelial function [40-47].

Among the NO metabolites, nitrite is a major oxidative metabolite, which was implicated to be both an indicator for NO synthase (NOS) activity [48,49] and a circulating NO donor [50]. It has been shown that up to 70–90% of plasma nitrite derived from eNOS activity in fasted humans and other mammals [49,50].

The assumption of L-arginine efficacy theoretically has been based on eNOS activation and nitrite production enhancement. Our results were in line of this assumption, in which L-arginine supplementation led to significantly higher plasma nitrite concentration. Other studies have reported NO metabolites increasing in hypercholesterolemic animals [51-53]. Although decreased NO bioactivity (stems from flow mediated dilation studies) has been

| group         | cholesterol (mg/dl) before | cholesterol (mg/dl) after | nitrite (μmol/l) before | nitrite (μmol/l) after |
|---------------|----------------------------|---------------------------|-------------------------|------------------------|
| Control (n = 16) | 111.7 ± 14.1               | 2129.1 ± 176.2            | 10 ± 0.7                | 11.8 ± 1               |
| L-arginine (n = 14) | 125.4 ± 14.5               | 2109.1 ± 166.9            | 11.6 ± 0.5              | 14.7 ± 0.5             |
| p             | >0.05                      | >0.05                     | >0.05                   | <0.05                  |
attributed to ED [54], increased plasma level of nitrite has been reported in hypercholesterolemic patients too [55]. It has been suggested that enhanced NO synthesis might be a defense mechanism to compensate continuous inactivation of NO and to protect from damaging factors such as hypercholesterolemia [56,57]. Another proposed mechanism for the elevation of nitrite may be NO production by other isoforms of NOS enzymes [57,58]. Although decreased activity of eNOS has been indicated in atherosclerosis but NO may be produced by iNOS in macrophages and other cell types in the atherosclerosis [57,58].

NO is also an essential signaling molecule for endothelial integrity and growth [59]. A moderate basal NO production can protect ECs from damaging effects of risk factor [49]. We hypothesized that if intrinsic protective mechanisms could be activated by moderate NO production in ECs, these cells could be better prepared to the ensuing risk factor (hypercholesterolemia) assault. Our results corroborate the protective role as the L-arginine group had significantly lower apoptotic cells in aorta intimal layer after 4 weeks of diet consumption. The precise mechanism responsible for inhibition of apoptosis by NO is not clear. Several possibilities exist that may explain the antiapoptotic effects of NO. NO has been shown to increase Bcl-2, thioredoxin, and heat-shock protein-70 and -32 expression, and therefore it inhibits the release of mitochondrial cytochrome c and apoptosis inducing factors [33,60,61]. The activation of cGMP and cGMP-dependent protein kinase by NO increases a major intracellular antiapoptotic protein, both directly and indirectly [62].

Also, it has been shown that NO inhibits the caspases-3 and 8 activations in L-arginine treated ECs and consequently inhibits apoptosis, which is consistent with our findings [63-65]. It should be further acknowledged, however, that the protective effect of L-arginine could also be mediated through non-eNOS-dependent pathways, since L-arginine has anti oxidant effects too [66,67]. Of course more studies are warranted in this field, and for future researches, using different doses of L-arginine and cholesterol diet in acute and chronic models of hypercholesterolemia are suggested.

Conclusion
L-arginine attenuates the number of apoptosis cells in the aorta of a model of hypercholesterolemia. The inhibition of EC apoptosis may be the underlying mechanism of restore endothelial function by L-arginine.

Methods
Animals and Experimental design
This study was reviewed and approved by the Ethics Committee of Isfahan University of Medical Sciences. Thirty white male rabbits weighing 1.95 ± 0.25 kg were obtained from the Pasteur Institute of Iran. All animals were housed three per cage with free access to food and water. After 1-week acclimation period and an overnight fasting, blood samples were taken as pre-experimental sampling to obtain baseline data. Collected blood samples were centrifuged (10,000 _ g), and the resulting serum was stored at -70°C until measurements. The animals were then randomly assigned to 2 groups. The rabbits were fed rabbit chow supplemented with 1% cholesterol (hypercholesterolemic diet; control group, n = 16) or high-cholesterol diet with oral L-arginine (3% in drinking water) (L-arginine group, n = 14) for 4 weeks. Pure cholesterol and L-arginine were obtained from Scharlau Chemie (Barcelona, Spain) and Ajinomoto Co (Japan) respectively. At the end of experiment, fasting blood samplings were
obtained, and half of the animals of each group randomly were selected and euthanized by an overdose of sodium pentobarbital (50 mg/kg) and ex-sanguinated. The animal's aortas were harvested for pathological investigation. The serum levels of cholesterol, and nitrite were measured. The fatty streak formation and the number of apoptotic cells also were determined as previously described [53,68,2,3].

**Serum cholesterol and nitrite measurements**

Total cholesterol level was measured using standard enzymatic kit (Pars Azmoon Co, Iran). The serum level of nitrite (stable NO metabolite) was measured using a colorimetric assay kit (R&D Systems, Minneapolis, USA) that involves the Griess reaction. Briefly, serums were added into wells (96-well enzymatic assay plate). A sulphanilamide solution was added to all experimental samples, and after incubation, N-1-naphthylethenediamine dihydrochloride solution was added. Then, absorbance was measured by a microreader in 540 nm wavelength. The samples nitrite concentration was determined by comparison to nitrite standard reference curve. The detection limit was 0.25 μM nitrite.

**Fatty streak determination**

The abdominal aortas were subjected to pathological investigation to verify fatty dot or fatty streak lesions formation. The entire aorta, from the aortic arch to the external iliac arteries, was dissected out and cleaned of excess adventitial tissue. The aortas were fixed in buffered 10% formalin for 24 h, and then embedded in paraffin. The paraffin-embedded specimens were sectioned at 5 μm (20 sections in succession) and stained with haematoxylin and eosin, and examined by light microscopy to measure fatty streak by two pathologists in a double-blinded manner.

Fatty streak lesions were graded as zero for no fatty streak, 1 for existence of fatty streak in 1–4 sections, 2 for existence of fatty streak in 5–9 sections, 3 for existence of fatty streak in 10–14 sections and 4 for existence of fatty streak in 15 to all 20 sections of vessels.

**In situ detection of apoptotic cells by TUNEL method**

The Terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling (TUNEL) method was used for in situ detection of apoptotic cells by in situ cell death detection kit (Roche Applied Science, Indianapolis, IN, USA) as the manufacturer's instructions. Briefly, after dewaxation of formalin-fixed tissue sections; the slides were placed in a plastic jar containing 200 ml 0.1 M citrate buffer, pH 6.0, and were heated applying 350 W microwave irradiation for 5 min. After rinsing the slide with PBS (20°–25°C), they were immersed in a blocking solution containing 0.1 M Tris-HCl, 3% BSA, and 20% normal bovine serum, pH 7.5 for 30 min at room temperature, and washed again with PBS. Then TUNEL reaction mixture was added and incubated for 60 min at 37°C in a humidified chamber. The slides were washed, and anti fluorescein conjugated with alkaline phosphatase were added, and incubated again for 30 min. After rinsing in PBS, BCIP-NBT substrate solution was added and incubated for 15 min. The slides were subjected to wash extensively in tap water and were counterstained with hematoxylin. For apoptotic cells enumeration, at least 500 intimal cells were counted and the number of apoptotic cells was determined per 500 cells using light microscope.

**Statistical Analysis**

The data are reported as the mean ± SEM. A statistical software package, SPSS (version 13), was used to perform statistical analysis. The data were tested for normality and homogeneity of variance. Otherwise, unpaired Student's t-test (equal or unequal variance assumed accordingly) was used to assess the significance of any change between groups. Statistical significance was accepted at p < 0.05.

**Abbreviations**

EC: Endothelial Cell; ED: Endothelial Dysfunction; NO: Nitric Oxide; NOS: Nitric Oxide Synthase; eNOS: Endothelial Nitric Oxide Synthase; TUNEL: Terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

MN carried out the design and coordinated the study, participated in most of the experiments and prepared the manuscript. SH provide assistance in the design of the study, coordinated and carried out all the experiments and participated in manuscript preparation. FM and ALM were selected and euthanized by an overdose of sodium pentobarbital (50 mg/kg) and ex-sanguinated. The animal's aortas were harvested for pathological investigation. The serum levels of cholesterol, and nitrite were measured. The fatty streak formation and the number of apoptotic cells also were determined as previously described [53,68,2,3].

**References**

1. Thompson CB: Apoptosis in the pathogenesis and treatment of disease. Science 1995, 267(5203):1456-62.
2. Wyllie AH, Morris RG, Smith A, Dunlop D: Chromatin cleavage in apoptosis: association with condensed chromatin morphology and dependence on macromolecular synthesis. J Pathol 1984, 142:67-77.
3. Kockx MM: Apoptosis in the atherosclerotic plaque: quantitative and qualitative aspects. Arterioscler Thromb Vasc Biol 1998, 18(10):1519-22.
4. Isner JM, Kearney M, Bortman S, Passeri J: Apoptosis in human atherosclerosis and restenOSis. Circulation 1995, 91:2703-2711.
5. Hegyi L, Skepper JN, Cary NR, Mitchinson MJ: Foam cell apoptosis and the development of the lipid core of human atheroscle-
   rotic plaques. J Pathol 1996, 180:423-9.

6. Kockx MM, De Meyer GR, Muhring J, Bult H, Bultinck J, Herman A: Distribution of cell replication and apoptosis in atheroscle-
   rotic plaques of cholesterol-fed rabbits. Atherosclerosis 1996, 120(1):115-124.

7. Cheng YJ, Libby P: Evidence for apoptosis in advanced human atheroma: colocalization with interleukin 1β converting enzyme. Am J Pathol 1995, 147:251-66.

8. Han DK, Haudenschild CC, Hong MK, Tinkle BT, Leon MB, Liu G: Evidence for apoptosis in human atherogenesis and in a rat 
   vascular injury model. Am J Pathol 1995, 147:267-77.

9. Björkerud S, Björkerud B: Apoptosis is abundant in human atherosclerotic lesions, especially in inflammatory cells (macrophages and T cells), and may contribute to the accumu-
   lation of gruel and plaque instability. Am J Pathol 1996, 147:1-14.

10. Stefaneanu T: Endothelial apoptosis: could it have a role in the pathogenesis and treatment of disease? Curr 2000, 117(3):841-54.

11. Asai K, Kudej RK, Shen YT, Yang GP, Takagi G, Kudej AB, Geng YJ, Saito N, Nakanishi JN, Van Der Meulen F, Bishop SP, Vatner DE, Natividad F, Bishop SP, Vatner SF: Nitric oxide inhibition 
   mediated by nitric oxide. Circulation 2000, 101:1518-24.

12. Boulanger CM, Scoazec A, Ebrahimian T, Henry P, Mathieu E, Tedgui A, Mallat Z: Circulating microparticles from patients with myocardial infarction cause endothelial dysfunction. Circula-
   tion 2001, 104:2649-52.

13. Dimmelser S, Assmus B, Hermann C, Haendeler J, Zeiher AM: Fluid shear stress stimulates phosphorylation of Akt in human endothelial 
   cells: involvement in suppression of apoptosis. Circ Res 1998, 83:334-41.

14. Tricot O, Mallat Z, Heymes C, Belmin J, Lesèche G, Tedgui A: Relation between endothelial cell apoptosis and blood flow direction 
   in human atherosclerotic plaques. Circulation 2000, 102:1-8.

15. Napoli C, Ignarro LJ: Nitric oxide-releasing drugs. Annu Rev Pha-
   rmacol Toxicol 2003, 43:97-123.

16. Schachinger V, Britten MB, Zeiher AM: Prognostic impact of coro-
   nary vasodilator dysfunction on adverse longterm outcome of coronary heart disease. Circulation 2000, 101:1889-906.

17. Wu CT, Ren YF, Liu JF, Zhang JH, Lei ST: L-arginine reduces intes-
   tinal epithelial cell apoptosis in rats with severe abdominal infection. Nan Fang Yi Ke Da Xue Xue Bao 2007, 27(1):1728-30.

18. Sumou IK, Du JB, Wei B, Zhang CY, Qi JG, Tang CS: Nitric oxide induces DNA 
   apoptosis and DNA damage in rats. Jpn J Cancer Res 2008, 99(1):27-31.

19. Tan X, Pan JQ, Li JC, Liu YJ, Li J, McCarthy SN, Keech A, Celermajer DS, Deanfield JE: Oral L-
   arginine improves endothelium-dependent dilation in hypercholesterolemic young adults. J Clin Invest 1996, 97:1989-94.

20. Hirooka Y, Egashira K, Imaizumi T, Tagawa T, Kai H, Sugimachi M, Takeshita A: Effect of L-arginine on acetylcholine-induced 
   endothelium-dependent vasodilation differs between coronary and forearm vasculatures in humans. J Am Coll Cardiol 2001, 24(4):948-55.

21. Creager MA, Gallagher SJ, Girerd XJ, Coleman SM, Dzau VJ, Cooke JP: L-arginine improves endothelium dependent vasodilation 
   in hypercholesterolemic monkeys. J Clin Invest 1992, 90:128-33.

22. Drexler H, Zeiher AM, Meinerz K, Just H: Correlation of endothe-
   lium dysfunction in coronary microcirculation of hypercholesterolemic 
   patients by L-arginine. Lancet 1991, 338:546-50.

23. Otsuji S, Nakajima O, Waku S, Kojima S, Hosokawa H, Kinoshita I, Oiobu T, Tamoto S, Takada K, Ishihara T: Attenuation of acetyl-
   choline-induced vasoconstriction by L-arginine is related to the progression of atherosclerosis. Am J Hypertens 1998, 11(3):300-8.

24. Fisman EZ, Tenenbaum A, Shapira I, Pines A, Motro M: The nitric oxide pathway: is L-arginine a gate to the new millennium? A 
   meta-analysis of L-arginine effects. J Cardiovasc Pharmacol 1999, 33(4):413-48.

25. Siasos G, Tousoulis D, Antoniades C, Stefanadis C, Stefanadis C: Mechanisms of disease: L-arginine in coronary 
   atherosclerosis – a clinical perspective. Nat Clin Pract Cardiovasc Med 2007, 4(5):274-83.

26. Siasos G, Siasos T, Antoniades C, Stefanadis C, Stefanadis C: L-
   arginine, the substrate for NO synthesis: an alternative pathway to NO in the atherosclerotic process. Curr Med Chem 2007, 14(15):1519-24.

27. Creager MA, Gallagher SJ, Girerd XJ, Coleman SM, Dzau VJ, Cooke JP: L-arginine improves endothelium dependent vasodilation 
   in hypercholesterolemic monkeys. J Clin Invest 1992, 90:128-33.

28. Drexler H, Zeiher AM, Meinerz K, Just H: Correlation of endothe-
   lium dysfunction in coronary microcirculation of hypercholesterolemic 
   patients by L-arginine. Lancet 1991, 338:546-50.

29. Otsuji S, Nakajima O, Waku S, Kojima S, Hosokawa H, Kinoshita I, Oiobu T, Tamoto S, Takada K, Ishihara T: Attenuation of acetyl-
   choline-induced vasoconstriction by L-arginine is related to the progression of atherosclerosis. Am J Hypertens 1998, 11(3):300-8.

30. Fisman EZ, Tenenbaum A, Shapira I, Pines A, Motro M: The nitric oxide pathway: is L-arginine a gate to the new millennium? A 
   meta-analysis of L-arginine effects. J Cardiovasc Pharmacol 1999, 33(4):413-48.

31. Siasos G, Tousoulis D, Antoniades C, Stefanadis C, Stefanadis C: L-
   arginine, the substrate for NO synthesis: an alternative pathway to NO in the atherosclerotic process. Curr Med Chem 2007, 14(15):1519-24.

32. Creager MA, Gallagher SJ, Girerd XJ, Coleman SM, Dzau VJ, Cooke JP: L-arginine improves endothelium dependent vasodilation 
   in hypercholesterolemic monkeys. J Clin Invest 1992, 90:128-33.

33. Drexler H, Zeiher AM, Meinerz K, Just H: Correlation of endothe-
   lium dysfunction in coronary microcirculation of hypercholesterolemic 
   patients by L-arginine. Lancet 1991, 338:546-50.

34. Otsuji S, Nakajima O, Waku S, Kojima S, Hosokawa H, Kinoshita I, Oiobu T, Tamoto S, Takada K, Ishihara T: Attenuation of acetyl-
   choline-induced vasoconstriction by L-arginine is related to the progression of atherosclerosis. Am J Hypertens 1998, 11(3):300-8.

35. Fisman EZ, Tenenbaum A, Shapira I, Pines A, Motro M: The nitric oxide pathway: is L-arginine a gate to the new millennium? A 
   meta-analysis of L-arginine effects. J Cardiovasc Pharmacol 1999, 33(4):413-48.
in hypercholesterolemic rabbits: inhibition with L-arginine. Circulation 1998, 98:1776-82.

47. Kleinbongard P, Dejam A, Lauer T, Rassaf T, Schindler A, Picker O, Bryan NS, Feilisch M, Kelm M: Plasma nitrite concentrations reflect the degree of endothelial dysfunction in humans. Free Radic Biol Med 2006, 40(2):295-302.

48. Kleinbongard P, Dejam A, Lauer T, Rassaf T, Schindler A, Picker O, Scheeren T, Gödecke A, Schrader J, Schulz R, Heusch G, Schaub GA, Bryan NS, Feilisch M, Kelm M: Plasma nitrite reflects constitutive nitric oxide synthase activity in mammals. Free Radic Biol Med 2003, 35(7):790-6.

49. Kleinbongard P, Dejam A, Lauer T, Rassaf T, Schindler A, Picker O, Bryan NS, Feilisch M, Kelm M: Nitrite in nitric oxide biology: cause or consequence? A systems-based review. Free Radic Biol Med 2006, 41(5):691-701.

50. Minor RL Jr, Myers PR, Guerra R Jr, Bates JN, Harrison DG: Diet-induced atherosclerosis increases the release of nitrogen oxides from rabbit aorta. J Clin Invest 1990, 86(6):2109-16.

51. Minor RL Jr, Myers PR, Guerra R Jr, Bates JN, Harrison DG: Comparison of endothelial function in the carotid artery between normal and short-term hypercholesterolemic rabbits. Comp Biochem Physiol C Toxicol Pharmacol 2006, 144(2):197-203.

52. Nematbakhsh M, Hayat-Davoodi P, Rajabi P, Samarian SH: The effect of estrogen on endothelial permeability of aorta and the level of serum nitrite concentration in cholesterol-fed ovariectomized rabbit. Iran Biomed J 2002, 6(2-3):77-82.

53. Yeboah J, Crouse JR, Hsu FC, Burke GL, Harrington DM: Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: the Cardiovascular Health Study. Circulation 2007, 115:2390-7.

54. Ferlito S, Gallina M, Catassi S, Bisicchia A, Di Salvo MM: Nitrite plasma levels in normolipemic and hypercholesterolemic patients with peripheral occlusive arteriopathy. Panminerva Med 1999, 41(4):307-9.

55. Cai H, Harrison DG: Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res 2000, 87(10):840-4.

56. Hayashi T, Matsuura-Hira H, Fukatsu A, Sumi D, Kano-Hayashi H, Rani PJA, Iguchi A: Selective iNOS inhibitor, ONO1714 successfully retards the development of high-cholesterol diet induced atherosclerosis by novel mechanism. Atherosclerosis 2006, 187(2):316-24.

57. Nachtigal P, Kopecky M, Solichova D, Zdansky P, Semecky V: The changes in the endothelial expression of cell adhesion molecules and iNOS in the vessel wall after the short-term administration of simvastatin in rabbit model of atherosclerosis. J Pharm Pharmacol 2005, 57(2):197-203.

58. Ignarro LJ, Napoli C: Novel features of nitric oxide, endothelial nitric oxide synthase, and atherosclerosis. Curr Diab Rep 2005, 5(1):17-23.

59. Oral L-arginine improves endothelial dependent dilation and reduces monocyte adhesion to endothelial cells in young men with coronary disease. Atherosclerosis 1997, 129:261-9.

60. Li J, Billiar TR, Talianian RV, Kim YM: Nitric oxide reversibly inhibits seven members of the caspase family via S-nitrosylation. Biochem. Biophys. Res Commun 1997, 240:19-24.

61. Kim YM, Chung HT, Simmons RL, Billiar TR: Cellular non-heme iron content is a determinant of nitric oxide-mediated apoptosis, necrosis, and caspase inhibition. J Biol Chem 2000, 275:10954-61.

62. Razavi HM, Hamilton JA, Feng Q: Inhibition of apoptosis by nitric oxide: implications in myocardial ischemia and heart failure. Pharmacol Ther 2005, 106(2):147-62.

63. Li CQ, Wogan GN: Nitric oxide as a modulator of apoptosis. Cancer Lett 2005, 226(1):1-15.

64. Li J, Billiar TR, Talianian RV, Kim YM: Nitric oxide reversibly inhibits seven members of the caspase family via S-nitrosylation. Biochem. Biophys. Res Commun 1997, 240:19-24.

65. Kim YM, Chung HT, Simmons RL, Billiar TR: Cellular non-heme iron content is a determinant of nitric oxide-mediated apoptosis, necrosis, and caspase inhibition. J Biol Chem 2000, 275:10954-61.

66. Jablonska A, Checinski P, Krauss H, Micker M, Ast J: The influence of two different doses of L-arginine oral supplementation on nitric oxide (NO) concentration and total antioxidant status (TAS) in atherosclerotic patients. Med Sci Monit 2004, 10(1):CR29-32.

67. Suastik Blücher A, Lass A, Mayer B, Brunner F: Antioxidative and myocardial protective effects of L-arginine in oxygen radical-induced injury of isolated perfused rat hearts. Naunyn Schmiedebers Arch Pharmacol 2002, 365(4):269-76.

68. Nematbakhsh M, Ali-Hemmatti A, Dashi G, Rajabi P: Estrogen attenuates the accumulation of fatty streaks in coronary arteries of ovariectomized high cholesterol-fed rabbits. Atherosclerosis 2002, 6(1):13-6.