EFFECT OF L-ARGININE SUPPLEMENTATION ON THE CONCENTRATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN A CHOLESTEROL-RICH DIET WITHDRAWAL MODEL

Shaghayegh Haghjooyjavanmard[(1)], Mehdi Nematbakhsh[(2)]
Alireza Monajemi[(3)], Masoud Soleimani[(4)]

Abstract

INTRODUCTION: Increased serum level of vascular endothelial growth factor (VEGF) is well-documented in hypercholesterolemia and atherosclerosis. It is associated with atherosclerotic lesions and is considered as a marker for endothelial dysfunction and injury. In the present study, experiments were designed to examine the combined effects of dietary lipid withdrawal and L-arginine supplementation on serum VEGF concentration.

METHODS: After 4 weeks on a high-cholesterol diet, white male rabbits (n=22) were randomly assigned to 2 groups. The diet withdrawal (DW) group (n=11) was fed normal diet and the L-arginine group was fed normal diet and 3% L-arginine in drinking water for another 4 weeks. The serum levels of lipids, VEGF and L-arginine were measured before and after 4 and 8 weeks of experiment.

RESULTS: The cholesterol-rich diet induced a significant increase in total cholesterol and LDL-cholesterol in all animals. There was no significant difference between the groups ($P>0.05$). After 4 weeks of cholesterol-rich diet withdrawal, animals of the DW and the L-arginine group had similar levels of total cholesterol and LDL-cholesterol. L-arginine supplementation resulted in a significantly higher serum level of L arginine in the L-arginine group than in the DW group ($P<0.05$). After 4 weeks, no significant difference was found between the serum level of VEGF of the two groups. By the end of study, hypercholesterolemic diet withdrawal had apparently led to decreases in VEGF in both groups, but the serum level of VEGF was significantly lower in the group treated with L-arginine ($P<0.05$).

CONCLUSIONS: This study showed the synergistic effect of two endothelial protective factors, lipid lowering by diet withdrawal and L-arginine supplementation, on VEGF production.

Keywords: Endothelial dysfunction, atherosclerosis, vascular endothelial growth factor (VEGF), L-arginine, diet withdrawal.

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Introduction

Vascular endothelial growth factor (VEGF), a heparin-binding growth factor, elicits an array of biological effects on endothelial cells in vivo and in vitro; these include survival, proliferation and migration, nitric oxide (NO) production, and increased vascular permeability.[1] The best known role of VEGF is its participation in normal and pathological angiogenesis.[1,2] As a cytokine, VEGF increases permeability and is a chemotactic factor for macrophage and vascular smooth muscle cells.[3,4] It induces the synthesis of metalloproteinase and adhesion molecules.[5] Hence, it has an important role in atherosclerosis lesion formation. It is shown that VEGF synthesis is regulated by hypoxia, oxidized low-density lipoprotein (Ox-LDL) and some other cytokines such as transforming growth factor-beta-1 (TGF-beta-1), interleukin-1 (IL-1) and interleukin-6 (IL-6).[6-8] Recently, several studies have documented increased levels of plasma VEGF in hypercholesterolemia and atherosclerosis in.[5,9-11] Increased VEGF is associated with atherosclerotic lesions and is recognized as a marker of endothelial dysfunction and injury.[12-14] Endothelial dysfunction (ED) is an early event in atherosclerosis and has a pivotal role in atherogenesis.[15] ED is characterized by reduced bioavailability of nitric oxide (NO).[16]

(1) Shaghayegh Haghjooyjavanmard M.D. Applied Physiology Research Center, Isfahan University of Medical Sciences, Isfahan, Iran. Phone: +98 (311) 7922295, Email: haghjooyejavanmard@resident.mui.ac.ir
(2) Mehdi Nematbakhsh Ph.D., Professor, Applied Physiology Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
(3) Alireza Monajemi M.D., Applied Physiology Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
(4) Masoud Soleimani Ph.D., Haematology Department, School of Medicine, Tarbiat Modares University, Tehran, Iran

Corresponding author: Shaghayegh Haghjooyjavanmard

NO is a potent antiatherosclerotic molecule and any intervention that enhances NO bioavailability might be a promising strategy for the prevention and treatment of atherosclerosis.[15] One straightforward approach towards increasing NO bioavailability is to provide additional substrate for nitric oxide synthase.[17] L-Arginine is the substrate of endothelial nitric oxide synthase (eNOS) and the main precursor of NO in the vascular endothelium. Findings of numerous studies suggest that L-arginine supplementation restores endothelial function in several disease states associated with ED, such as hypercholesterolemia.[18-22]

In the present study, experiments were designed to examine the combined effects of dietary lipid withdrawal and L-arginine supplementation on serum VEGF concentration.

Materials and methods

This study was approved by the Ethics Committee of Isfahan University of Medical Sciences. Twenty-two white male rabbits weighing 2±0.2 kg were obtained from Razi Institute, Iran. After a 1-week acclimation period, the rabbits were fed rabbit chow supplemented with 1% cholesterol for 4 weeks; then the high-cholesterol diet was stopped and the animals were randomly assigned to 2 groups, namely the diet withdrawal group (DW, n=11) which was given a standard diet for another 4 weeks, and the L-arginine group (n=11) which was given a standard diet with oral L-arginine (3% in drinking water) for another 4 weeks. At the end of 4 and 8 weeks, blood samples were taken and serum was stored at -70 ˚C until measurement.

Total and LDL- cholesterol levels were measured using a standard enzymatic kit according to the manufacture's instructions (Pars Azmoon Co, Iran). To measure L-arginine, the plasma was deproteinized with sulfosalicylic acid (30%) containing 1 mmol/l ß-(2-thienyl) (±) alanine as an internal standard. The samples were stored at 4 ˚C for 30 minutes and centrifuged at 12000 g for 5 minutes. The supernatant was analyzed for L-arginine by HPLC. After dilution of plasma extract in membrane-filtered water (Waters Millipore) and 2-minute derivatization with o-phthaldialdehyde, a 10 µl sample was injected onto the column by an autosampler (Spark Triathlon). Separation of the derivatized amino acids was achieved using a HPLC (Beckman) equipped with a 3 mm particle size and 125×4.6 mm ODS Hypersil column (Bischoff) with a two-buffer-system gradient elution. L-arginine was measured in Masoud Medical laboratory Tehran, Iran.

Serum VEGF concentration was measured using enzyme-linked immunosorbent assay(ELISA) using available reagents and recombinant standards (R & D Systems, Minneapolis, MN) according to the manufacture's instructions. The VEGF assay has a minimum sensitivity of 3.0 pg/ml.

SPSS statistical software version 13.0 (SPSS, Inc. Chicago, IL) was used to perform statistical analyses. Data were tested for normality and homogeneity of variance. The data are reported as the mean ± standard error of mean (SEM). Independent-sample Student's t-test was used to assess the significance of any difference between the groups. Statistical significance was set at $P<0.05$.

Results

The cholesterol-rich diet was associated with a significant increase in total- and LDL-cholesterol in all animals studied. There was no significant difference between groups (Table 1). After 4 weeks of cholesterol-rich diet withdrawal, animals of the DW and the L-arginine group had similar levels of total- and LDL-cholesterol (Table 1).

Supplementation with 3% L-arginine for 4 weeks was associated with a significantly higher serum level of L-arginine in the L-arginine group than in the DW group ($P<0.05$) (Table 1).

After 4 weeks of hypercholesterolemic diet, no significant difference was seen in serum levels of VEGF of the two groups. By the end of the study, hypercholesterolemic diet withdrawal had apparently led to decreases in VEGF in both groups (259.89±132.1 vs. 111.14±55.0 pg/ml in the DW group, 192.25±87.3 vs. 10.74±2.8 pg/ml in the L-arginine group).

TABLE 1. The serum concentration of L-arginine, total-and LDL- cholesterol after induction of hypercholesterolemia (4 weeks) and after diet withdrawal in the two groups of experiment .

	L-arginine supplementation	Diet withdrawal
L-arginine (µmol/L) after 4 weeks	101.00±8.0	79.50±16.4
L-arginine (µmol/L) after 8 weeks	139.75±9.9	94.50±16.4
Total cholesterol (mg/dl) after 4 weeks	2291.68±277.8	2018.36±223.1
Total cholesterol (mg/dl) after 8weeks	1433.40±161.3	1222.19±198.9
LDL-C (mg/dl) after 4 weeks	1419.89±189.6	1418.08±293.3
LDL-C (mg/dl) after 8 weeks	635.09±3.7	649.27±190.0

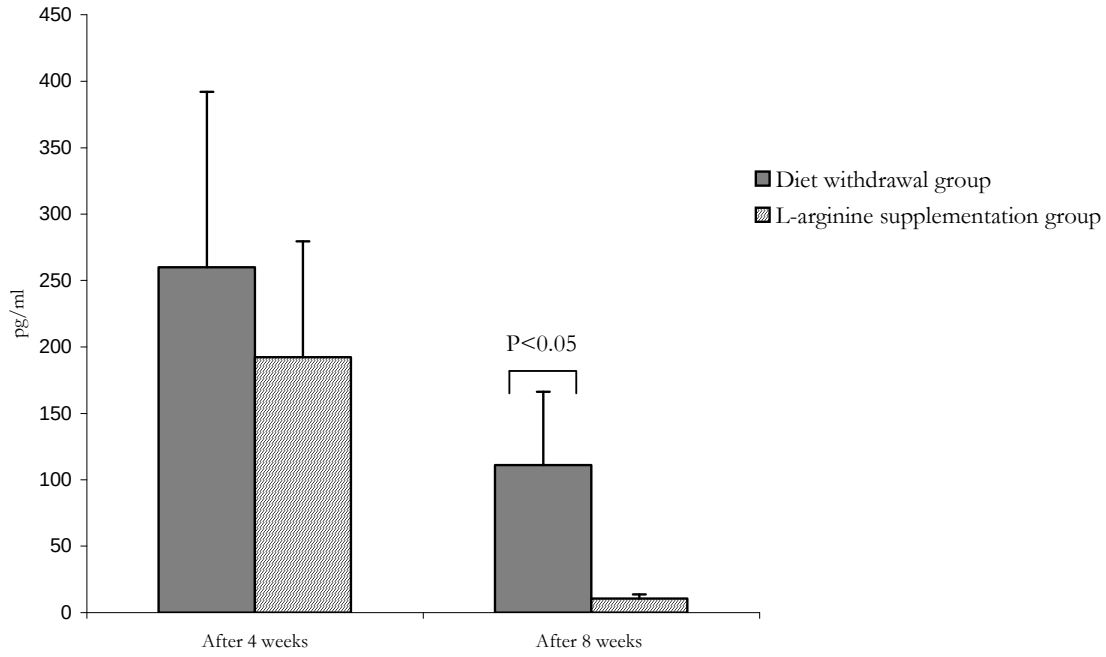

FIGURE 1: Serum VEGF concentration (pg/ml) after 4 weeks of hypercholesterolemic diet and after hypercholesterolemic diet withdrawal (the 8th week) in two groups of experiment. Serum VEGF concentration in the L-arginine-supplemented group was significantly lower than in the diet-withdrawal group (P<0.05).

The serum level of VEGF was significantly lower in the L-arginine treated group (P<0.05) (Figure 1).

Discussion

We assessed changes in VEGF following withdrawal of a cholesterol diet and L-arginine supplementation. VEGF significantly decreased following the withdrawal of a hypercholesterolemic diet. This is in agreement with the results of studies reporting decreased VEGF concentration in hypercholesterolemic patients treated with lipid-lowering agents, i.e.Simvastatin.[10] Ox-LDL is thought to induce VEGF production in smooth muscle cells, macrophages, and endothelial cells.[10,23,24] The increase in VEGF in hypercholesterolemia, and the decrease in VEGF seen when lowering lipids may be related to the effect of LDL-C. Another potential mechanism leading to increased VEGF secretion by endothelial cells might be endothelial dysfunction induced by hypercholesterolemia and high LDL concentration in hypercholesterolemic animals. Decreased production of NO in the early stages of atherosclerosis reduces oxygen supply to the arterial wall. Subsequently, local ischemia is followed by activation of hypoxia-inducible factor (HIF), a transcription factor that regulates the expression of VEGF.[24] This could explain the increased serum VEGF levels in hypercholesterolemia. It seems rational to conclude that decreasing serum lipids leads to decreased VEGF levels.

VEGF, a cytokine with an important role in atherosclerosis lesion formation, has been noticed as a biomarker of ED.[25] It is assumed that any intervention that restores the endothelial function might decrease the VEGF concentration. Several studies have shown the protective role of risk factor management on endothelial function.[26-28] L-arginine supplementation has been frequently cited as a factor that can restore endothelial integrity through increasing NO production. In this study, withdrawal of a hypercholesterolemic diet was associated with decreased VEGF in both groups .The serum level of VEGF was significantly lower in the L-arginine treated group, showing the synergistic effect of two endothelial protective factors i.e. lipid lowering by diet withdrawal and L-arginine supplementation on VEGF production.

Furthermore, there is some evidence showing that higher levels of VEGF in hypercholesterolemia can lead to angiogenesis in atherosclerotic plaques, and may cause plaque instability and serious associated complications .[29] As in studies demonstrating the adverse effects of high VEGF levels on the endothelium, this study supports the idea that

endothelial protective mechanisms might diminish the level of VEGF and restore endothelial function.

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