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The Effect of L-arginine Supplementation on Blood Pressure in Patients with Type 2 Diabetes: A Double-Blind Randomized Clinical Trial

Sara Asadi; MSc[1,2], Hassan Mozaffari-Khosravi; PhD[3,4], Mohammad Mahdi Naghizade; MSc[2] & Azadeh Nadjarzadeh; PhD[*1,3]

[1] *Nutrition and Food Security Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
[2] *Fasa University of Medical Sciences and Health Services, Fasa, Iran.*
[3] *Department of Nutrition, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
[4] *Yazd Diabetic Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

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Corresponding author:
Department of Nutrition, School of Public Health, Shahid Sadoughi University of Medical Sciences, Shohaday Gomname BLV, Yazd, Iran.
azadehnajarzadeh@gmail.com

Postal code: 8915173160
Tel: +98 35 38209100-14

ABSTRACT

Background: The prevalence of hypertension in patients with type 2 diabetes (T2D) is approximately twice as much as healthy people. This study was designed to determine the effect of L-arginine supplementation on blood pressure in patients with T2D. **Methods**: In a double-blind randomized clinical trial, 75 T2D were randomly divided into three groups (3 g/d and 6g/d of L-arginine and placebo) for 3 months. Height, weight, waist circumference, dietary intake, and blood pressure (BP) were measured before and after intervention. **Results**: In patients who received 3g/d L-arginine, no significant difference was observed between BP before and after the intervention, however, subgroup analysis among patients with high BP showed significant reduction in systolic ($P = 0.036$) and diastolic BP ($P = 0.027$) after L-arginine supplementation. After 3 months of intervention, systolic and diastolic BP were significantly different compared to the baseline values and also with placebo value in patients receiving 6g/d of L-arginine ($P < 0.05$). **Conclusions**: The daily intake of 6g of L-arginine for 3 months in T2D may improve BP. Taking 3g/d of this supplement may help to improve BP only in patients with hypertension.

Keywords: *L-arginine; Diabetes; Blood pressure; Clinical trial*

Introduction

Type 2 diabetes (T2D) is a common metabolic disorder that its prevalence is increasing in the world.The prevalence of diabetes mellitus was reported to be 366 million people in the world in 2011 and it is predicted that this figure reaches 552 million

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by 2030 (Azimi-Nezhad et al., 2008). According to the another report in 2008, approximately 10% of Iranian adult population have diabetes(Esteghamati *et al.*, 2008). In this report, prevalence of diabetes in people over 30 y was over 14% (Delavari AR, 2004). Diabetes has been known as an important risk factor for cardiovascular diseases.The prevalence of hypertension in patients with T2Dwas about 71%, which is twice of its prevalence in other community members (Unit, 2005).

High blood pressure (BP) is a risk factor of cardiovascular diseases andincreases the development of diabetic complications. Prevention and control of high BP reduces morbidity and mortality (Chen and Reaven, 1997). In a study conducted by Adler and colleagues, it was demonstrated that every 10 mmHg decrease in systolic blood pressure (SBP) results in a 12% reduction in the risk of any of the diabetes complications and a 15% reduction in the risk of death caused by diabetes (Adler et al., 2000).

Despite numerous medications administered for control of BP, most of hypertensive patients are not able to regulate their BP. Therefore, currentprograms to reform the treatment of hypertension is essential (Rajapakse and Mattson, 2009).

L-arginine is an amino acid essential for normal development and multiple physiological processes in the body. It is considered as an essential amino acid for birds, carnivores, and young mammals, moreoverit is a conditional and essential amino acid for human adults, especially in trauma and patients (Visek, 1986, Wu and MORRIS, 1998).

As a result of L-arginine conversion to citrulline, Nitric oxide synthase (NOS) produces nitric oxide (NO) (Iyengar et al., 1987, Palmer et al., 1988). NO playsan important role as a molecule with diverse biological effects. In blood vessels, it produces endothelium-dependent dilation in response to stimulation caused by substances such as insulin (Steinberg et al., 1994), acetylcholine (Amezcua et al., 1988, Rees et al., 1989), and bradykinin (Palmer et al., 1987, Radomski et al., 1987). In the central nervous system and peripheral nervous tissues, NO acts as a neurotransmitter (Garthwaite and Boulton, 1995, Garthwaite et al., 1988, Vincent, 1994).

There are conflicting evidences in animals and clinical studies about the effects of L-arginine and its derivative on NO and blood pressure (Ast *et al.*, 2011, Kelly *et al.*, 2001, Martina *et al.*, 2008, Mirfattahi *et al.*, 2012, Rytlewski *et al.*, 2005).

Since there has been no study on the effect of moderate doses of L-arginine on blood pressure, thisresearch was designed to determine the effect of a three-month of 3 and 6 g/d of L-arginine dietary supplementation on BP in T2D.

Materials and Methods

Study design and participants: This double-blind randomized clinical trial was conducted on 75 T2D in Fasa, Iran. By considering significant level of 1% and power of 80 and a standard deviation of 1.2 (Lucotti et al., 2006), a sample size of 25 patients in each group wasselected. Patients were selected and then randomly divided into three groups on the basis of inclusion and exclusion criteria from the study and medical records (using a random number table). Inclusion criteria consisted of: 1) history of T2D between 4 and 10 y, 2) fasting glucose range of 160-400 mg/dl, 3) age between 40-60 y. Exclusion criteria included renal, liver or gastrointestinal diseases, pregnancy, lactation, use of insulin, and having glycosylated hemoglobin A1c (HbA1c) greater than 7%. All patients continued their routine treatment prescribed byan endocrinologist. To participate in the study,after aninterview and explanation of theresearch objectivesvolunteers were asked to sign an informed consent statement. Then the participants were randomly divided into three groups of 25 members and were followed for 90 days. Hypertension is defined as BP equal or more than 140/90 mmHg (Herman *et al.*, 2004).

The intervention groups1 and 2 received daily 3 (3G) and 6 (6G) tablets of L-Arginine (1000 mg), respectively while the placebo group (PG) was given 3 g/d of placebo tablet. Patients were provided with tablets at the end of each month, they were asked if they had taken any tablets by themselves. Individuals, who had not taken more than 20% of thesupplements, were excluded from the analysis.

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L-arginine tablets with license No. 10302/11 were produced by Karen Company of Drug and Ddietary Supplement (Yazd, Iran). Placebo was also prepared bythe same factory, with the same size, shape, and color of arginine supplementation and was made from microcrystalline cellulose.

All participants in the study were asked to makeno changes in their lifestyle (diet, activity level, and smoking)as well asthe type and the dose of their medication until the end of study andif the need arose for changing the protocol,they were excluded from analysis.

Measurements: At the beginning and end of the study, weight, height, waist circumference, 24-hour dietary recall, and BP of all patients were measured. Participants' weights were calculated with light clothing and without shoes by a digital scale with sensitivity of 100 g. Height was measured by a tape meter with accuracy of 0.5 cm in a standing position without shoes, while the shoulders were in a normal state.Waist circumference (WC) was alsomeasured at the lower margin of the ribs and the iliac crest in a standing, normal breathing positionby a tape meter with accuracy of 0.5 cm. By dividing weight (kg) by the square of height (m) body mass index (BMI) was calculated. In order to eliminate individual errors, all measurements were performed by one person.

BP was measured by mercury sphygmomanometer (Model ALPK2; Tycosw, Arden, NC, USA). To record each participant's BP, the mean of three measurements was calculated.

Data analysis: Macronutrient intake, including energy, protein, fat, and carbohydrate intake in each patient were analyzed before and after the study using a 24-hour dietary recall and Nutritionist software version 4.The data were analyzed by SPSS.16 using Student *t*-test, its equivalent Mann-Whitney *test*, and ANOVA.

Ethical considerations: An informed consent was obtained from all of the participants. In addition; this clinical trial was registered in Iranian Registry of Clinical Trials with (www.irct.ir) IRCT2012071010240N1 code.

Results

From 75 T2D who were enrolled, 7 patients were excluded because of insulin injections, changing medications, and lack of willingness thus, 68 patients completed the study (23, 25, and 20 patients in the 3G, 6G, and PG, respectively) (**Figure 1**).

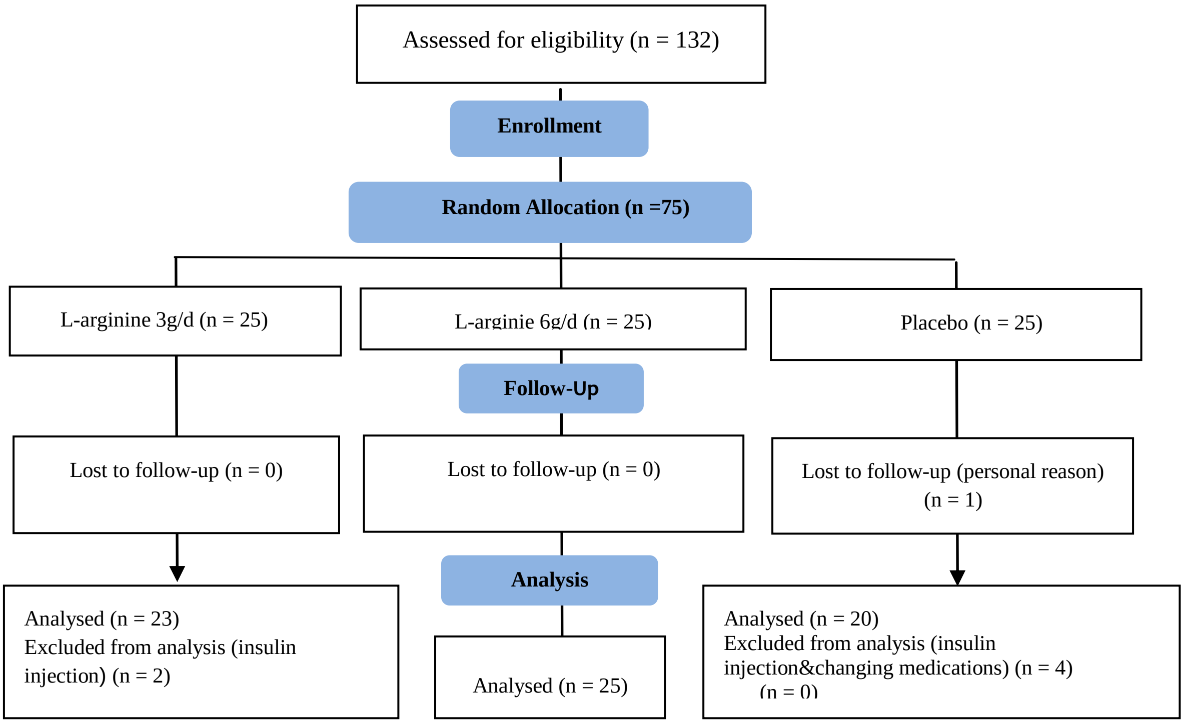

Figure 1. Flow chart of the study

According to **Table 1**, there was no significant difference between three groups of study in terms of age and anthropometric data. As it is shown in **Table 2**, there was no significant difference between groups regarding drugs and duration of diabetes, ($P > 0.05$).

Results related to dietary intake and their comparison within and between groups are represented in **Table 3**. No statistically significant difference was observed between the three groups at the beginning or end of the study in terms of nutrient intake (including daily energy, carbohydrate, protein, fat, saturated and unsaturated fatty acids, cholesterol, and arginine). Also, no significant difference was observed between the intervention and placebo groups regarding nutrient intake at the baseline and end of study.

As it is tabulated in **Table 4**, there was a significant difference at the end of the study compared with the baseline in the 6G for SBP ($P = 0.025$) and DBP ($P = 0.031$). ANOVA *test* showed a significant difference in SBP and DBP in this group in comparison with the PG ($P < 0.05$).

In the 3G who received 3g L-arginine per day, there was no significant difference between blood pressure before and after the intervention ($P > 0.05$), but subgroup analysis revealed a significant decrease in systolic ($P = 0.036$) and diastolic ($P = 0.027$) blood pressure in hypertensive participants (T**able 5**).

Table 1. Age and anthropometric data in the interventions and placebo groups

Variables/Groups	3 g/d L-arginine (n = 23)	6 g/d L-arginine (n = 25)	Placebo (n = 20)	P-value
Age (year)	49.86 ± 5.30	52.04 ± 6.80	51.92 ± 7.12	0.13[a] 0.74[b] 0.43[c]
Height (cm)	164.9 ± 9.58	165.19 ± 10.12	164.17 ± 9.06	0.84 0.61 0.47
Weight (kg)				
Before	79.17 ± 11.06	76.83 ± 9.72	76.96 ± 11.82	0.55
After	79.23 ± 11.12	76.80 ± 9.68	77.03 ± 11.32	0.42
P-value[d]	0.43	0.71	0.54	0.64
Waist circumference (cm)				0.44
Before	100.20 ± 12.01	101.36 ± 11.25	99.30 ± 10.11	0.37
After	100.28 ± 12.14	101.92 ± 11.84	99.61 ± 9.01	0.86
P-value	0.15	0.29	0.62	
Body mass index (kg/m2)				0.38
Before	29.19 ± 5.43	28.15 ± 4.38	28.96 ± 4.20	0.61
After	29.21 ± 5.51	28.02 ± 4.43	29.02 ± 4.16	0.28
P-value	0.81	0.37	0.66	

[a]: Comparison between placebo and 3g/d L-arginine (ANOVA, Tukey Post hoc); [b]: Comparison between placebo and 6g/d L-arginine (ANOVA, Tukey Post hoc); [c]: Comparison between 3g/d L-arginineand 6g/d L-arginine (ANOVA, Tukey Post hoc); [d]::Paired *t*-test

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Table 2. Drugs consumption and diabetes duration in the interventions and placebo groups

Variables/ Groups	3 g/d L-arginine n = 23	6 g/d L-arginine n = 25	Placebo n = 20	P-value
Duration of diabetes (year)	6.24 ± 1.14	7.53 ± 1.03	6.85 ± 0.98	0.13[a] 0.74[b] 0.43[c]
Hypoglycemic drugs consumption	23(100)[e]	25(100)	20(100)	1
Lipid-lowering drugs consumption	10(38)	9 (61)	8(28)	0.61
Antihypertensive drugs consumption	12(44)	11(37)	7(86)	0.44

[a]: Comparison between placebo and 3g/d L-arginine (Student *t*-test); [b]: Comparison between placebo and 6g/d L-arginine (Student *t*-test) ; [c]: Comparison between 3g/d L-arginine and 6/d L-arginine (Student *t*-test); [e]: Number (%)

Table 3. Mean (SD) of daily dietary intake of participants in the interventions and placebo groups

Variables/Groups	3 g/d L-arginine (n = 23)	6 g/d L-rginine (n = 25)	Placebo (n = 20)	P-value
Energy (kcal)				
Before	2124 ± 687	2168 ± 549	1998 ± 864	0.87[a]
After	2179 ± 546	2106 ± 672	2123 ± 591	0.76[b]
P-value[d]	0.19	0.09	0.37	0.80[c]
Carbohydrate (g)				
Before	308 ± 93	325 ± 71	289 ± 101	0.38
After	329 ± 84	298 ± 84	302 ± 92	0.54
P-value	0.74	0.23	0.16	0.23
Protein (g)				
Before	19.80 ± 74.40	21.30 ± 86.72	17.50 ± 74.95	0.13
After	71.68 ± 30.83	69.28 ± 25.32	71.56 ± 15.35	0.61
P-value	0.11	0.37	0.53	0.64
Fat (g)				
Before	66.08 ± 24.00	57.81 ± 18.00	59.94 ± 21.00	0.16
After	71.36 ± 25.73	64.33 ± 23.12	53.96 ± 21.07	0.45
P-value	0.35	0.27	0.41	0.09
Saturated fatty acids (g)				
Before	5.93 ± 21.12	8.30 ± 17.98	8.56 ± 17.92	0.31
After	22.69 ± 6.72	21.08 ± 7.98	19.37 ± 6.96	0.43
P-value	0.32	0.71	0.81	0.19
Mono unsaturated fatty acids (g)				
Before	23.76 ± 10.31	20.88 ± 08.10	18.80 ± 7.80	0.24
After	19.53 ± 8.90	20.81 ± 11.17	21.73 ± 8.75	0.92
P-value	0.65	0.28	0.18	0.38
Poly unsaturated fatty acids (g)				
Before	15.18 ±7.24	13.92 ± 6.12	15.60 ± 7.36	0.57
After	18.56 ± 8.94	13.09 ± 9.36	14.46 ± 6.36	0.30
P-value	0.32	0.37	0.67	0.49
Cholesterol (mg)				
Before	195.1 3 ± 74	202.84 ± 101	189.67 ± 93	0.47
After	184.35 ± 65	197.04 ± 94	201.37 ± 87	0.79

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P-value	0.09	0.35	0.49	0.24
Fiber (g)				
Before	14.3 ± 6.1	17.1 ± 9.3	15.1 ± 8.9	0.81
After	16.1 ± 6.9	16.1 ± 8.7	15.9 ± 7.4	0.22
P-value	0.58	0.51	0.17	0.38
Arginine (mg)				
Before	712.2 ± 301	846.1 ± 287.3	801.7 ± 246.9	0.30
After	699.3 ± 283	783.2 ± 243.9	803.7 ± 272.8	0.26
P-value	0.72	0.36	0.12	0.71

[a]: Comparison between placebo and 3g/d L-arginine (ANOVA, Tukey Post hoc); [b]: Comparison between placebo and 6g/d L-arginine (ANOVA, Tukey Post hoc); [c]: Comparison between 3g/d L-arginine and 6g/d L-arginine (ANOVA, Tukey Post hoc); [d]::Paired *t*-test

Table 4. Comparison of mean (SD) of blood pressurebetween and within groups

Variables/Groups	Before	After	P-value[d]
Systolic blood pressure (mmHg)			
3g/d L-arginine (n = 23)	138.4 ± 13.3	130.9 ± 12.3	0.074
6g/d L-arginine (n = 12)	133.4 ± 14.6	117.0 ±16.4	0.025
Placebo (n = 20)	128.8 ± 15.3	124.8 ± 13.9	0.403
P-value[a]	0.732	0.130	
P-value[b]	0.435	0.042	
P-value[c]	0.362	0.251	
Diastolic blood pressure (mmHg)			
3g/d L-arginine (n = 23)	84.9 ± 9.5	79.8 ± 10.3	0.088
6g/d L-arginine (n = 12)	86.3 ± 10.2	71.3 ± 9.8	0.031
Placebo (n = 20)	80.6 ± 10.3	78.9 ± 9.1	0.805
P-value	0.491	0.148	
P-value	0.294	0.039	
P-value	0.707	0.128	

[a]: Comparison between placebo and 3g/d L-arginine (ANOVA, Tukey Post hoc); [b]: Comparison between placebo and 6g/d L-arginine (ANOVA, Tukey Post hoc); [c]: Comparison between 3g/d L-arginine and 6g/d L-arginine (ANOVA, Tukey Post hoc); [d]::Paired *t*-test

Table 5. Comparison of mean (SD) of blood pressure in3g/d L-arginine stratified by normotensive or hypertensive status and placebo group

Variables	Before	After	P-value[d]
Systolic blood pressure (mmHg)			
Hypertensive (n = 23)	138.4 ± 13.3	130.9 ± 12.3	0.074
Normotensive (n = 2)	149.3 ± 14.2	133.3 ±13.6	0.036
Placebo (n = 20)	128.8 ± 15.3	124.8 ± 13.9	0.403
P-value[a]	0.732	0.130	
P-value[b]	0.416	0.064	
P-value[c]	0.389	0.129	

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Diastolic blood pressure (mmHg)			
Hypertensive (n = 23)	84.9 ± 9.5	79.2 ± 10.3	0.088
Normotensive (n = 12)	99.7 ± 8.3	87.9 ± 8.5	0.027
Placebo (n = 20)	80.6 ± 10.3	78.9 ± 9.1	0.805
P-value	0.491	0.148	
P-value	0.294	0.039	
P-value	0.707	0.128	

[a]: Comparison between placebo and hypertensive individuals (ANOVA, Tukey Post hoc); [b]: Comparison between placebo and normotensive individuals (ANOVA, Tukey Post hoc); [c]: Comparison between hypertensive and normotensive individuals (ANOVA, Tukey Post hoc); [d]::Paired *t*-test

Discussion

The aim of this study was to investigate the effect of receiving 3 months of 3g/d and 6g/d of L-arginine on BP in T2D. In this study receiving 3 g/d L-arginine had no effect on blood pressure in all T2D, although it could improve blood pressure in hypertensive subgroup. But receiving 6 g/d of the supplement reduced SBP and DBP.

Evans et al. studied healthy individuals' response to different doses of L-arginine.Twelve healthyparticipantsreceived doses of 3, 9, 21, and 30 g/d L-arginine for one week. They concluded that 9 g/d of L-arginine, with minimal side effects, is sufficient to increase circulating concentrations of L-arginine (Evans et al., 2004). Due to lack of L-arginine in our country and the fact that its powder is unsavory with a bad taste maximum dose of 6 g/d can be used. Furthermore, because of the high number of drugs used in diabetic patients a dose of 3 g/d was considered in this study.

Studies that have examined intravenous L-arginine have shown that this amino acid improves blood pressure (Bode-Boger et al., 1994, Bode-Böger et al., 1996, Böger et al., 1997, Giugliano et al., 1997a, Giugliano et al., 1997b, Marietta et al., 1997, Mimran et al., 1995). Effects of oral intake of L-arginine on blood pressure vary. Daily doses from 1 to 30 g/d for 2 days to 3 months in different diseases such as heart failure (Rector et al., 1996), hypercholesterolemia (West et al., 2005), T2D (Facchinetti et al., 2007, Huynh and Tayek, 2002, Martina et al., 2008), preeclampcia (Rytlewski et al., 2005), chronic renal failure(Kelly et al., 2001), and prediabetes (Mirfattahi et al., 2012) caused a significant decrease in SBP and DBP which was consistent with the present study. But, there are some other studies that did not demonstrate any significant effect on blood pressure (Adams et al., 1995, Adams et al., 1997, Ast et al., 2011, Chin-Dusting et al., 1996a, Chin-Dusting et al., 1996b, Evans et al., 2004, Lerman et al., 1998, Zhang et al., 2001). Doses of 50 mg for 1 day (Lechin et al., 2006) and 2.1g per day for 1 week (Miller, 2006) in healthy individuals reduced DBP but had no effect on SBP. Clinical trials conducted in this field showed conflicting results that might be due to differences in the target group, the characteristics of the study population, sample size, duration of treatment, and the rout of intake.Most studies have investigated very high or very low doses of L-arginine. Therefore, whether L-arginine is effective in improving blood pressure in T2D or not still remains unclear thus, drawing any conclusion on this issue requires further clinical trials.

Amino acid L-arginine is essential for normal development and multiple physiological processes in the body. This amino acid is not only used as a precursor in the synthesis of proteins but also plays a role in the production of NO, urea, polyamines, and agmatine (Burke et al., 1999). NO is a productof NOS which causes oxidation of L-arginine and L-citrulline (Palmer et al., 1988). It is a key molecule involved in a wide range of physiological functions throughout the body (Chan and Vallance, 2002).In the vascular system, NO regulates vascular tone and blood flow by activating guanylatcyclase in vascular smooth muscle. Also, it is essential in leukocyte adhesion and platelet aggregation and controls mitochondrial oxygen consumption by inhibition

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of cytochromeC oxidase. Impairment in vascular NO production and transfer causes problem in endothelial function along with pathological conditions such as hypertension, atherosclerosis, and vascular irregularities related to vascular regeneration (Luiking et al., 2010). NO increases cGMP of platelet that has inhibitory effect on adhesion and aggregation of platelets. There are endothelial receptors for a number of vasoconstrictors, such as serotonin, norepinephrine, and endothel vasopressin (Cocks and Angus, 1983, Katusic et al., 1984). When the endothelial receptors are occupied by these agonists, NO is released, thereby attenuating the vasoconstriction to these agents. The endothelium tends to maintain vascular patency by halting the response to vasoconstrictors and by inhibiting platelet adherence and aggregation. In the presence of the endothelium, therefore, these vasoconstrictors cause mild vasoconstriction or even vasodilation (Katusic et al., 1984).

Different effects of 3g/d dose L-arginine in reducing blood pressure of hypertensive and normotensive diabetic patients may be explained by this approach in which the amount of NO in hypertensive patients is less thannormotensive ones.So, dose of 3g of L-arginine isable to compensate for this deficiency and affects blood pressure.

Due to some limitations in this study, we could not measurethe concentration of L-arginine or NO in blood samples. For future studies, it is recommended to measure plasma L-arginine,NO, pro-inflammatory markers, and factors influencing endothelial function, which affect cardiovascular function.

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Author contributions

Mozaffari-Khosravi H and Nadjarzadeh A participated to conception and design of study, managing the project and drafting the manuscript. Asadi A and Naghizade MM participated to acquisition of data, data analysis and drafting the manuscript. All authors read manuscript and they finally verified it.

Conflicts of Interest

There is no conflict of interest.

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