ORIGINAL ARTICLE

The Effect of Oral L-arginine Supplementation on Blood Pressure in Patients with Metabolic Syndrome: A Randomized Clinical Trial

Davood Bahrami[1], Hassan Mozaffari-Khosravi[1*]

1. Department of Nutrition, School of Public Health, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

***Correspondence:**
Hassan Mozaffari-Khosravi, Ph.D., Department of Nutrition, Faculty of Health, Shahid Sadoughi University of Medical Sciences, Bahonar Square, Central Building, Yazd, Iran
Tel: (98) 353 724 9333
Email: mozaffari.kh@gmail.com

Received: 07 January 2017

Accepted: 15 March 2018

Published in April 2018

Abstract

Objective: Hypertension is a common component of metabolic syndrome (MetS). Some studies showed that L-arginine reduced the blood pressure (BP) .Therefore this study was designed to evaluate the effect of L-arginine supplementation on BP in MetS.

Materials and Methods: In this randomized, double-blind, placebo-controlled trials, 60 patients with MetS were randomly divided into two groups: the L-arginine-supplemented group (AG), who received 5 gr of L-arginine daily and placebo group (PG). Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), pulse pressure (PP) were measured before and after the intervention and compared.

Results: At the end of the study in the AG significant reductions were observed in the SBP, DBP and MAP (*P*-value:0.001).The comparison of changes in variables between the AG and PG indicated that in the AG, patients had a significantly greater decrease in the SBP (-11.25±10.93 vs. -2.67±6.15 mmHg: *P*-value:0.001), DBP(-5.71±7.16 vs. -1.25±7.02 mmHg: p-value: 0.02) and MAP (-7.55±5.61 vs.-1.72±5.16 mmHg, *P*-value: 0.001). The PP decreased in both groups but a significant decrease compared with baseline in the AG was observed (-5.53±13.83 vs. -1.42±9.21 mmHg: *P*-value: 0.19).

Conclusion: The findings of the present study showed that 5 gr/d L- arginine supplementation for 12 weeks in MetS resulted in significant decrease in the SBP, DBP and MAP but had no significant effect on the PP.

Keywords: Metabolic syndrome, L-arginine, Blood pressure

Introduction

Metabolic syndrome (MetS) is a disorders including abdominal obesity, dyslipidemia, hyperglycemia and hypertension (1,2) which is considered a major challenge in health care across the world due to increasing urbanization, extra energy consumption, increasing obesity and sedentary lifestyle. (3) Also, MetS increases the risk of type 2 diabetes, cardiovascular disease, stroke and the risk of death from all causes.(4-6) There are several definitions of the MetS, such as the definition of Adult Treatment Panel III (ATP III), MetS is defined as having three or more of the following factors (7,8): waist circumference (WC) more than 102 cm in men and more than 88 cm in women, triglycerides (TG) ≥150 mg/dl, high density lipoprotein cholesterol (HDL-c) less than 40 mg/dl in men and less than 50 mg/dl in women, blood

pressure (BP) ≥ 85/130 mmHg, fasting blood glucose (FBS) ≥ 100 mg/dl.

Based on the meta-analysis conducted in 2015, according to the ATPIII criteria, the prevalence of MetS in Iranian adults was reported 39% that the prevalence of MetS was 15% lower in men than women.(9) In another study conducted in Iran the prevalence of MetS in primary school children was 5.3%.(10) Also the prevalence of the MetS in the United States increased from 32.9% to 34.7% during the 2003 – 2012.(11)

L-arginine is a semi-essential amino acid in the human diet. (12,13) This amino acid is relatively high in some foods such as seafood, meat and nuts. (14) This amino acid plays numerous functions in the body, such as participation in the manufacture of hormones, proteins, creatine and polyamines. (15-17) In several studies showed the effectiveness of L-arginine supplementation in the treatment and prevention of many diseases associated with endothelial dysfunction such as hyperlipidemia, arteriosclerosis and diabetes. (18,19). There is convincing evidence that L-arginine effects on the metabolism of energy substrates. (20) Also L-arginine as a substrate to make nitric oxide (NO) placed at the disposal of NO synthase. Arginine/NO pathway is a physiological mechanism of vasodilation in endothelial cells that impacts on the peripheral vascular resistance and thus can reduce the BP. (21) NO can effectively prevent the adhesion of thrombocytes, leukocytes, neutrophils and monocytes into the vessel wall. (22,23) Several studies were conducted on the effect of L-arginine supplementation on the BP. (24,25) and in some other L-arginine had no effect on the BP (26) In several studies in the early 1990s suggested that L-arginine/NO pathway plays key role in the pathogenesis of hypertension in pregnancy (27,28) and in animal models inhibition of NO production causes hypertension, proteinuria and fetal growth restriction. (27) Also L-arginine showed the beneficial effects on improving blood circulation maternal – fetal. (29) In this study we investigated the effect of 5 gr/daily L-arginine supplementation for 12 weeks on the BP in patients with MetS.

Materials and Methods

This double-blinded randomized controlled clinical trial was conducted on 60 patients with MetS in Yazd, Iran during the January 2016 to March 2016. Inclusion criteria of the study were: agreement to participate in the study, age range of 20-60 years, history of diabetes less than 5 years and having 3 or more ATP III criteria and the exclusion criteria were: smoking, drinking alcohol, pregnancy and lactation, history of drug sensitivity or allergy, special diet, history of angina, stroke or heart disease in recent years, chronic kidney or liver disease, chronic inflammatory diseases and thyroid diseases during the past year and consumption any dietary supplement such as L-arginine during three months before the study. Patients were selected from Yazd Diabetes Research Center. All patients continued their routine course of treatment by an endocrinologist during the study.

Eligible patients were assigned to placebo group (PG) (n = 30) and L-arginine group (AG) (n = 30) based on random numbers generated by the computer and were studied for 3 months. The AG received sachets of L-arginine 2500 mg twice-daily and the PG received sachets of microcrystalline cellulose 2500 mg twice-daily after lunch and dinner for 3 months. Patients received their sachets every two weeks and were checked by phone once a week and patients who did not take more than 10 percent of their sachets during 3 months were excluded from the study. L-arginine sachets were prepared by Karen Company of drug and dietary supplement (Yazd, Iran). Also Placebo sachets were produced by the same factory, with the same size, shape, and color of L-arginine supplementation. To design a double-blind study, L-arginine and placebo sachets were placed in the similar boxes and were coded as A and B by a person unaware of the aims of the study. Participants were asked to not change their usual diet and

physical activity during the study and to be avoided from taking any supplements.

Measurements

Demographic questionnaire was completed by face to face interview. Anthropometric, dietary intake and physical activity were assessed at baseline and after 12 weeks of intervention by a dietitian. The weight of the participants was measured with a sensitivity of 0.1 kg without shoes and while they were wearing light clothing by using diagnostic scales, Omron BF 511 (made in Japan). Height was measured to the nearest 0.5 cm with a tape measure in standing position without shoes while shoulders were in normal condition. The Body mass index (BMI) was calculated by dividing weight in kilograms by the square of height in meters. Also WC was measured (between the lower margin of the last palpable rib and the top of the iliac crest) in a standing position, normal breathing position with a tape measure to the nearest 0.5 cm. At the beginning and at the end of the study, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by trained and experienced technicians unaware of the type of intervention with a sphygmomanometer (Rudolf Riester GmbH, Jungingen, Germany) with an accuracy of 2 mm Hg and with proper cuff on the right arm, in sitting position in a quiet room after a 10 minute rest. The mean arterial pressure (MAP) and pulse pressure (PP) patients were assessed by using the values for SBP and DBP and on the basis of suggested formulas: MAP = (SBP + 2 DBP) /3 and PP= SBP- DBP.(30) All patients were asked to refrain from exercising, eating, all drinks except water, smoking and the use of drugs that effect on the BP in at least one hour before measurement. In order to enhance the accuracy of the measurements the sphygmomanometer was calibrated before each measurement. Dietary intake was assessed by the 24-hour dietary recall questionnaire at the baseline and at the end of the study. These dietary recalls were evaluated based on estimated values in household measurements. Physical activity was evaluated with the completion of the International Physical Activity Questionnaire (IPAQ) at the beginning and at the end of the 12 weeks of intervention.

Statistical analysis

The normality of data distribution was determined using the Kolmogorov–Smirnov test. To compare qualitative variables between groups Fisher's exact test and the $\chi 2$-test Paired T-test and independent t-test were used. Dietary intake was evaluated by Nutritionist IV software modified for Iranian foods. All statistical analyses were conducted by using by SPSS software, version 20, and *P*-value> 0.05 was considered to be statistically significant.

Ethical considerations

This study was approved by Committee of Ethics in Research of Shahid Sadoughi University of Medical Sciences, Yazd, Iran with number of IR.SSU.SPH.REC. 1394.53, then was registered in the Iranian Clinical Trial Registration Center (www.irct.ir) with IRCT2015120425245N2 code. At the beginning of the study, all patients completed written consent after providing a full explanation of the objectives, advantages and methods of study.

Results

The intervention period in patients was showed in Figure 1. During the study, 2 patients were excluded from the AG (poor adherence (n = 1) and the discontinuation of the study (n = 1)) in addition two patients were excluded from the PG (poor adherence (n = 1) and gastrointestinal disorders (n = 1)) At the end of the study, 56 patients completed study (28 patients in the AG and 28 patients in the PG). So the study attrition rate was 6%.

Some features of clinical, demographic, and lifestyle of the participants were showed in Table 1. At the baseline, the mean ± SD age, weight and BMI were 50.07 ± 5.27 years, 78.15 ± 9.08 kg, 30 ± 3.25 kg/m2 respectively.

Table 1. Comparison (mean ± SD) of baseline characteristics between two groups

Variables	Total (n=56)	L- arginine group (n=28)	Placebo group (n=28)	*P*-value[a]
Age (year)	50.07±5.27	50.32 ±5.02	49.82±5.58	0.72
Weight (kg)	78.15±9.08	78.4571±8.45	77.9179±9.8	0.82
Height (cm)	161.60±9.15	162.21±9.33	161±9.09	0.62
Body mass index (kg/m^2)	30±3.25	29.97±3.71	30.03±2.79	0.94
Waist circumference (cm)	99.07±5.48	98.39±5.16	99.75±5.80	0.35
Duration of diabetes (year)	3.72±1.36	3.6429± 1.44	3.8036 ±1.29	0.66
Gender	N (%)	N (%)		p [b]
Male	18(32.1)	10(35.7)	8(28.6)	
Female	38(67.9)	18(64.3)	20(71.4)	0.77
Marital status				
Married	51(91.1)	26(92.9)	25(89.3)	
Single or widowed	5(8.9)	2(7.1)	3(10.7)	0.63
Education level				
Illiterate	9(16.1)	4(14.3)	5(17.9)	
Elementary	19(33.9)	10(35.7)	9(32.1)	0.85
High school	18(32.1)	10(35.7)	8(28.6)	
University	10(17.9)	4(14.3)	6(21.4)	
Physical activity				
Low	10(17.9)	6(21.4)	4(14.3)	
Moderate	42(75)	20(71.4)	22(78.6)	0.78
High	4(7.1)	2(7.1)	2(7.1)	

[a] **independent Student's T-test,** [b] **Chi-square test**

Also at the baseline there were no significant differences between gender, marital status, education, physical activity, age, weight and body mass index, height, waist and duration of diabetes between two groups. Based on the analysis of dietary recalls (Table2) at the beginning and at the end of the intervention, there were not observed differences between energy intake and diet including: carbohydrates, fat, protein, fiber, monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), saturated fatty acids (SFA), cholesterol and vitamin D in both groups(*P*-value > 0.05).

The mean SBP, DBP, MAP and PP were presented in Table 3. At the end of the study the results showed that SBP was significantly lower in the AG than in the PG (137.14 ± 12.86 vs. 144.82 ± 11.26 mmHg, *P*-value: 0.02). Also the comparison within groups showed that SBP had significantly decreased in the AG and PG (*P*-value: 0.001 and *P*-value: 0.02, respectively). The two groups changes showed a significant SBP decrease in AG patients than PG (-11.25±10.93 vs. -2.67 ± 6.15 mmHg, *P*-value: 0.001). At the end of the intervention DBP was significantly lower in the AG than in the PG (88.92 ± 10.91 vs. 95.53±9.46 mmHg, *P*-value: 0.01) and within-group comparison showed that DBP had significantly decreased only in the AG (*P*-value= 0.001) also the comparison of changes between two groups showed that DBP had greater reduction in the AG (-5.71±7.16 vs. -1.25±7.02 mmHg, *P*-value: 0.02). At the end of the study MAP in the AG was significantly lower compared with the PG (105.00±9.97 and 111.96±9.02 mmHg: *P*-value =0.008) and within-group comparison showed that the MAP had a significant reduction in the AG (*P*-value= 0.001) and the mean change showed that MAP had a greater reduction in the AG (-7.55±5.61 vs. -1.72±5.16 mmHg: *P*-value=0.001). At the end of the study PP did not show a significant difference between the two groups. However within-group comparison showed the PP had significantly decreased only in the AG (*P*-value: 0.04). However, PP difference was not statistically significant between two groups (-5.53±13.83 and -1.42±9.21 mmHg, *P*-value: 0.19).

Discussion

We found that 5 g/d of L-arginine supplementation for 3 months in patients with MetS caused significant decrease in SBP, DBP and MAP. We did not find any significant effect of L-arginine supplementation on PP.

Table 2. Comparison (mean ± SD) of dietary intake between two groups

Dietary intake	Baseline	After	*P*-value[a]
Energy(kcal/d)			
Arginine Group	1983.32±156.40	1913.17±104.94	0.09
Placebo Group	1937.75±247.46	1863.75±113.08	0.11
***P*-value [b]**	0.41	0.09	
Carbohydrate(g/d)			
Arginine Group	260.73±15.36	251.23±23.54	0.10
Placebo Group	254.12±22.82	247.26±24.74	0.16
***P*-value**	0.20	0.54	
Fat(g/d)			
Arginine Group	64.31±6.74	61.49±6.48	0.07
Placebo Group	62.52±8.94	59.10±4.98	0.09
***P*-value**	0.40	0.12	
Protein (g/d)			
Arginine Group	72.75±11.20	68.78±5.76	0.10
Placebo Group	69.19±10.79	66.05±5.72	0.16
***P*-value**	0.23	0.08	
Dietary fiber			
Arginine Group	17.01±2.33	16.26±3.13	0.36
Placebo Group	16.79±3.00	17.30±3.09	0.49
***P*-value**	0.75	0.22	
SFAs (g/d)			
Arginine Group	18.22±4.28	16.68±2.54	0.09
Placebo Group	17.70±3.61	16.15±2.13	0.08
***P*-value**	0.62	0.42	
PUFAs (g/d)			
Arginine Group	25.82±3.74	24.36±3.39	0.07
Placebo Group	23.62±6.49	22.99±3.32	0.61
***P*-value**	0.12	0.13	
MUFAs (g/d)			
Arginine Group	18.49±5.06	16.28±3.01	0.06
Placebo Group	16.79±4.08	15.04±2.59	0.06
***P*-value**	0.17	0.10	
Cholesterol (mg/d)			
Arginine Group	201.95±94.01	197.28±62.15	0.82
Placebo Group	213.43±84.21	205.50±53.16	0.69
***P*-value**	0.63	0.59	
Vitamin D (µg/d)			
Arginine Group	6.90±1.84	7.15±1.90	0.64
Placebo Group	6.65±1.78	6.73±1.85	0.88
***P*-value**	0.60	0.40	

All results are expressed as mean ± SD, [a] paired samples student's t-test, [b] independent samples student's T-test MUFAs: Monounsaturated fatty acids, PUFAs: Polyunsaturated fatty acids, SFAs: Saturated fatty acids

MetS components play a key role in the pathogenesis of stiffness and thickness of the arteries. (31) Several studies showed a relationship between the high BP and cardiovascular disease mortality. (32,33) There are several variables that determine a person's BP, including SBP, DBP, MAP and PP. SBP and DBP guarantee perfusion of most organs in the body and PP reflects hardness of the aorta and large arteries and pulse wave velocity. (34,35) PP is the difference between SBP and DBP. The SBP and DBP increased with age up to sixth decade of life.(31) Then SBP continuously enhance while DBP remains constant or declines and results in an increase in PP. On the other hand high PP especially in the elderly is a marker of arterial stiffness and makes extensive atherosclerosis with adverse cardiac implications, such as congestive heart failure and atrial fibrillation.(36,37)

The MAP is a major determinant of tissue perfusion regardless of the PP and influences heart function and properties of the vessel. High levels of MAP is associated with cardiovascular disease, organ damage while its low levels in unstable hemodynamic condition is harmful.(38) Numerous studies were conducted on the effect of L-arginine

Table 3. Changes in blood pressure values among placebo and L-arginine groups (mean ± SD)

Blood pressure	Before	After	*P*-value [b]	Differences
Systolic blood pressure (mmHg)				
L- Arginine Group	148.39±12.62	137.14±12.86	0.001	-11.25±10.93
Placebo Group	147.50±13.01	144.82±11.26	0.02	-2.67±6.15
***P*-value [a]**	0.79	0.02		0.001
Diastolic blood pressure (mmHg)				
L- Arginine Group	94.64±9.32	88.92±10.91	0.001	-5.71±7.16
Placebo Group	96.78±9.64	95.53±9.46	0.35	-1.25±7.02
***P*-value [a]**	0.40	0.01		0.02
Mean arterial blood pressure (mmHg)				
L- Arginine Group	112.55±9.32	105.00±9.97	0.001	-7.55±5.61
Placebo Group	113.69±9.69	111.96±9.02	0.08	-1.72±5.16
***P*-value [a]**	0.65	0.008		0.001
Pulse pressure (mmHg)				
L- Arginine Group	53.75±10.41	48.21±12.56	0.04	-5.53±13.83
Placebo Group	50.71±10.51	49.28±9.59	0.41	-1.42±9.21
***P*-value [a]**	0.28	0.72		0.19

[a] Student T-test, [b] paired T-test

supplementation on the BP in different diseases. Facchinetti et al (39) showed the intake of L-arginine 20 g/500ml intravenously daily for 5 days followed by 4g/d orally for 2 weeks resulted in the significant decrease in SBP and DBP in patients with gestational hypertension.

West et al (40) showed the intake of 12 g/d L-arginine supplementation for three weeks reduced SBP and DBP in men with hypercholesterolemia. Lucotti et al (41) in a study on the obese people with type 2 diabetes (T2D) found that the consumption of 8.3 g/d L-arginine supplementation for 21 days resulted in a significant reduction in SBP and DBP. Asadi et al (42) in a study among patients with T2D showed that intake of 3 g/d L-arginine for three months did not show significant effect on the BP but 6 g/day L-arginine reduced SBP and DBP in these patients. Battaglia et al (43) also observed that 8 g/d L-arginine for 6 months resulted in a significant reduction in SBP and DBP in patients with polycystic ovarian syndrome. Siani et al (25) found that an L-arginine-enriched diet such as dried beans and nuts (10 g/d) and oral L-arginine supplementation (10 g/d) compared to low L-arginine diet (3.5- 4.0 g/d) led to a significant decrease in the SBP and DBP. But in some studies despite the use of different doses of L-arginine, did not show significant change in the BP. Bogdanski et al (44,45) showed that 9 g/d L-arginine for 3 and 6 months had no significant effect on the SBP and DBP. In another study Lucotti et al (46) examined intake of 6.4 g/d L-arginine for 6 months and showed that L-arginine had no effect on the SBP but led to a significant decrease in the DBP. Neri et al (47) also found that 4 g/d L-arginine supplementation for 10 to 12 weeks did not lead to decrease in the BP in pregnant women with chronic hypertension. Ast et al (48) concluded in a study that 6 g/d and 12 g/d L-arginine supplementation for 4-weeks did not result in a significant reduction in SBP and DBP in healthy subjects and only showed a significant reduction in the SBP during the night. In general review of previous studies indicates that the studies on the effect of L-arginine on the BP show conflicting results. This is due to the difference in the target group, the sample size, duration of intervention, dose of L-arginine as well as type of supplements (oral or intravenous).

L-arginine as a substrate for the production of NO, may indicate activity lower the BP by producing and increasing the bioavailability of NO in the vascular smooth muscle cells which is essential for maintaining vascular homeostasis. (49,50).In a meta-analysis study in 2009 showed that short-term use of the L-arginine supplementation improves vascular

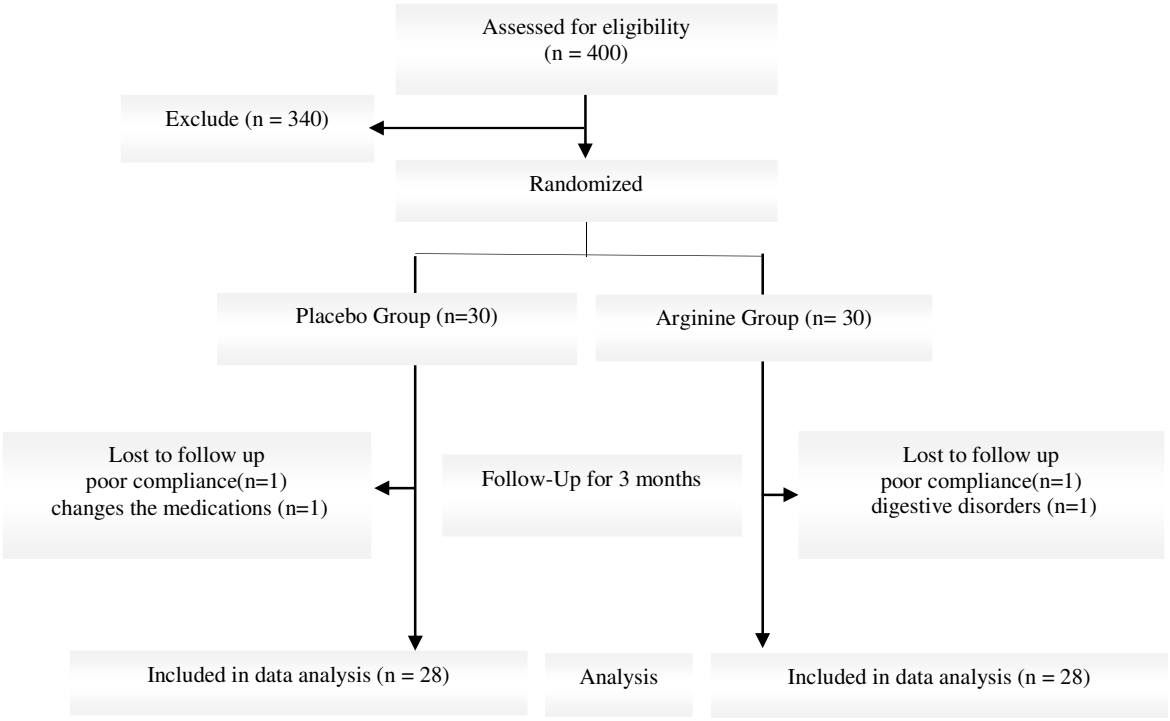

Figure 1. Flow chart of study

endothelial function when the baseline flow-mediated dilation (FMD) is low. (51)

Additionally, L-arginine can improve insulin resistance (52) that insulin resistance plays an important role in high BP.(53) A systematic review study in 2014 (54) showed that L-arginine supplementation may cause the prevention and treatment of preeclampsia which is related to asymmetric dimethylarginine (ADMA). ADMA is a NO synthase inhibitor (55) and is considered as a marker of endothelial dysfunction and a prognostic indicator of cardiovascular disease risk (56) also high concentration of ADMA is found in endothelial dysfunction and atherosclerosis. (57) Reducing the activity of ADMA is underlie the beneficial effects of L-arginine supplementation in decreasing BP. (58) NO as a gas releases from endothelial cells and stimulates soluble guanylate cyclase in vascular smooth muscle underlying the endothelium, increasing the concentration of C-GMP leads to activation of C-GMP dependent kinases that promote relaxation (59) by changing in the activity of K^+ and Ca^{2+} channels results in cell hyper polarization and reduction of the intracellular Ca^{2+}. Protein kinase G (PKG) can activate myosin light chain (MLC) phosphatase and increase dephosphorylation of the myosin chains thus leads to vascular relaxation. (60) On the other hand L-arginine is an inhibitor of angiotensin converting enzyme (ACE) that reduces Ang-II and the renin-angiotensin system and consequently reduces the BP. (61) There were also some limitations in present study including more women participants than men so that women constituted 67.9% of individuals who completed the intervention. Also we could not measure the concentration of L-arginine or NO. For future research, we suggest that double-blind studies with larger sample size and longer intervention time are required to evaluate the effect of L-arginine supplementation on the BP in patients with MetS.

Conclusions

Our findings indicated that the daily intake of 5 g/d of L-arginine for 3 months in patients with MetS led to a significant decrease in the

SBP, DBP and MAP but had no significant effect on the PP so; this supplement can be useful in these patients.

Acknowledgments

We would like to thanks all patients and organizations for their assistance. We would also like to express my appreciation to Yazd Diabetes Research Centre, Shahid Sadoughi University of Medical Sciences, Yazd, Iran for their cooperation with researchers.

References

1. Expert Panel on Detection E. Executive summary of the Third Report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). Jama 2001;285:2486.
2. Pitsavos C, Panagiotakos D, Weinem M, Stefanadis C. Diet, exercise and the metabolic syndrome. Rev Diabet Stud 2006;3:118-126.
3. Kaur J. A comprehensive review on metabolic syndrome. Cardiology research and practice 2014;2014.
4. Alberti K, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome. Circulation 2009;120:1640-5.
5. Alberti KGM, Zimmet P, Shaw J. The metabolic syndrome-a new worldwide definition. The Lancet 2005;366:1059.
6. Katakami N, Kaneto H, Funahashi T, Shimomura I. Type 2 diabetes and atherosclerosis: focusing on metabolic syndrome. Diabetology international 2013;4:143-8.
7. Pourfarzam M, Zadhoush F, Sadeghi M. The difference in correlation between insulin resistance index and chronic inflammation in type 2 diabetes with and without metabolic syndrome. Advanced Biomedical Research 2016;5.
8. Rezaianzadeh A, Namayandeh SM, Sadr SM. National Cholesterol Education Program Adult Treatment Panel III Versus International Diabetic Federation Definition of Metabolic Syndrome, Which One Is Associated to Diabetes Mellitus and Coronary Artery Disease? International journal of preventive medicine 2012;3.
9. Amirkalali B, Fakhrzadeh H, Sharifi F, Kelishadi R, Zamani F, Asayesh H, et al. Prevalence of Metabolic Syndrome and Its Components in the Iranian Adult Population: A Systematic Review and Meta-Analysis. Iranian Red Crescent Medical Journal 2015;17.
10. Zardast M, Namakin K, Chahkandi T, Taheri F, Kazemi T, Bijari B. Prevalence of metabolic syndrome in elementary school children in east of Iran. Journal of cardiovascular and thoracic research 2015;7:158.
11. Aguilar M, Bhuket T, Torres S, Liu B, Wong RJ. Prevalence of the metabolic syndrome in the United States, 2003-2012. Jama 2015;313:1973-4.
12. Nascimento M, Higa E, de Mello M, Tufik S, Oyama L, Santos R, et al. Effects of short-term l-arginine supplementation on lipid profile and inflammatory proteins after acute resistance exercise in overweight men. e-SPEN Journal 2014,9:141-5.
13. Flynn N, Meininger C, Haynes T, Wu G. The metabolic basis of arginine nutrition and pharmacotherapy. Biomedicine & Pharmacotherapy 2002;56:427-38.
14. Wu G, Bazer FW, Davis TA, Kim SW, Li P, Rhoads JM, et al. Arginine metabolism and nutrition in growth, health and disease. Amino acids 2009;37:153-68.
15. Morris SM. Arginine: beyond protein. The American journal of clinical nutrition 2006;83:508S-12S.
16. Morris SM. Arginine metabolism: boundaries of our knowledge. The Journal of nutrition 2007;137:1602-9.
17. Wu G. Amino acids: metabolism, functions, and nutrition. Amino acids 2009;37:1-17.
18. ElMissiry M, Othman A, Amer M. l-Arginine ameliorates oxidative stress in alloxan-induced experimental diabetes mellitus. Journal of Applied Toxicology 2004;24:93-7.
19. Howell K, Costello CM, Sands M, Dooley I, McLoughlin P. L-Arginine promotes angiogenesis in the chronically hypoxic lung: a novel mechanism ameliorating pulmonary hypertension. American Journal of Physiology-Lung Cellular and Molecular Physiology 2009;296:1042-50.
20. McKnight JR, Satterfield MC, Jobgen WS, Smith SB, Spencer TE, Meininger CJ,et al. Beneficial effects of L-arginine on reducing obesity: potential mechanisms and important implications for human health. Amino acids 2010;39:349-57.
21. Jablecka A, Bogdanski P, Balcer N, Cieslewicz A, Skoluda A, Musialik K. The effect of oral L-arginine supplementation on fasting glucose, HbA1c, nitric oxide and total antioxidant status in diabetic patients with atherosclerotic peripheral arterial disease of lower extremities. Eur Rev Med Pharmacol Sci 2012;16:342-50.

22. Roberts W, Riba R, Homer-Vanniasinkam S, Farndale R, Naseem K: Nitric oxide specifically inhibits integrin-mediated platelet adhesion and spreading on collagen. Journal of Thrombosis and Haemostasis 2008;6:2175-85.
23. de Haro Miralles J, Martínez-Aguilar E, Florez A, Varela C, Bleda S, Acin F. Nitric oxide: link between endothelial dysfunction and inflammation in patients with peripheral arterial disease of the lower limbs. Interactive cardiovascular and thoracic surgery 2009;9:107-12.
24. Rector TS, Bank AJ, Mullen KA, Tschumperlin LK, Sih R, Pillai K, et al. Randomized, double-blind, placebo-controlled study of supplemental oral L-arginine in patients with heart failure. Circulation 1996;93:2135-41.
25. Siani A, Pagano E, Iacone R, Iacoviello L, Scopacasa F, Strazzullo P. Blood pressure and metabolic changes during dietary L-arginine supplementation in humans. American journal of hypertension 2000;13:547-51.
26. Lekakis JP, Papathanassiou S, Papaioannou TG, Papamichael CM, Zakopoulos N, Kotsis V, et al. Oral L-arginine improves endothelial dysfunction in patients with essential hypertension. International journal of cardiology 2002;86:317-323.
27. Yallampalli C, Garfield RE. Inhibition of nitric oxide synthesis in rats during pregnancy produces signs similar to those of preeclampsia. American journal of obstetrics and gynecology 1993;169:1316-20.
28. Buhimschi I, Yallampalli C, Chwalisz K, Garfield RE. Pre-eclampsia-like conditions produced by nitric oxide inhibition: effects of L-arginine, D-arginine and steroid hormones. Human Reproduction 1995;10:2723-30.
29. Germain AM, Valdes G, Romanik MC, Reyes MS. Evidence Supporting a Beneficial Role for Long-Term L-Arginine Supplementation in High-Risk Pregnancies. Hypertension 2004;44:1.
30. Kodama S, Horikawa C, Fujihara K, Yoshizawa S, Yachi Y, Tanaka S, et al. Meta-analysis of the quantitative relation between pulse pressure and mean arterial pressure and cardiovascular risk in patients with diabetes mellitus. The American journal of cardiology 2014;113:1058-65.
31. Kwon Y-J, Chung T-H, Shim J-Y, Lee Y-J. The association of pulse pressure with metabolic syndrome in Korean elderly: A nationwide population-based study. Diabetes Research and Clinical Practice 2017;123:75-81.
32. Lawes CM, Vander Hoorn S, Law MR, Elliott P, MacMahon S, Rodgers A. Blood pressure and the global burden of disease 2000. Part II: estimates of attributable burden. Journal of hypertension 2006;24:423-30.
33. Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. The Lancet 2006;367:1747-57.
34. Dart AM, Kingwell BA: Pulse pressure—a review of mechanisms and clinical relevance. Journal of the American College of Cardiology 2001, 37:975-984.
35. Assmann G, Cullen P, Evers T, Petzinna D, Schulte H. Importance of arterial pulse pressure as a predictor of coronary heart disease risk in PROCAM. European heart journal 2005;26:2120-6.
36. Cremer A, Lainé M, Papaioannou G, Yeim S, Gosse P. Increased arterial stiffness is an independent predictor of atrial fibrillation in hypertensive patients. Journal of hypertension 2015;33:2150-5.
37. Haider AW, Larson MG, Franklin SS, Levy D. Systolic blood pressure, diastolic blood pressure, and pulse pressure as predictors of risk for congestive heart failure in the Framingham Heart Study. Annals of Internal Medicine 2003;138:10-6.
38. Papaioannou TG, Protogerou AD, Vrachatis D, Konstantonis G, Aissopou E, Argyris A, et al. Mean arterial pressure values calculated using seven different methods and their associations with target organ deterioration in a single-center study of 1878 individuals. Hypertension Research 2016;39:640-7.
39. Facchinetti F, Saade GR, Neri I, Pizzi C, Longo M, Volpe A. L-arginine supplementation in patients with gestational hypertension: a pilot study. Hypertension in pregnancy 2007;26:121-30.
40. West SG, Likos-Krick A, Brown P, Mariotti F. Oral L-arginine improves hemodynamic responses to stress and reduces plasma homocysteine in hypercholesterolemic men. The Journal of nutrition 2005;135:212-7.
41. Lucotti P, Setola E, Monti LD, Galluccio E, Costa S, Sandoli EP, et al. Beneficial effects of a long-term oral L-arginine treatment added to a hypocaloric diet and exercise training program in obese, insulin-resistant type 2 diabetic patients. American Journal of Physiology-Endocrinology and Metabolism 2006;291:906-12.
42. Asadi S, Mozaffari-Khosravi H, Naghizade MM, Nadjarzadeh A. The Effect of L-arginine Supplementation on Blood Pressure in Patients with Type 2 Diabetes: a Double-Blind Randomized Clinical Trial. Journal of Nutrition and Food Security 2016;1:17-27.
43. Battaglia C, Mancini F, Battaglia B, Facchinetti F, Artini PG, Venturoli S. L-arginine plus drospirenone-ethinyl estradiol in the treatment of patients with PCOS: a prospective, placebo controlled, randomised, pilot study. Gynecological Endocrinology 2010;26:861-8.

44. Bogdanski P, Suliburska J, Grabanska K, Musialik K, Cieslewicz A, Skoluda A, et al. Effect of 3-month L-arginine supplementation on insulin resistance and tumor necrosis factor activity in patients with visceral obesity. Eur Rev Med Pharmacol Sci 2012, 16:816-23.
45. Bogdanski P, Szulinska M, Suliburska J, Pupek-Musialik D, Jablecka A, Witmanowski H: Supplementation with L-arginine favorably influences plasminogen activator inhibitor type 1 concentration in obese patients. A randomized, double blind trial. Journal of endocrinological investigation 2013;36:221-6.
46. Lucotti P, Monti L, Setola E, La Canna G, Castiglioni A, Rossodivita A, et al. Oral L-arginine supplementation improves endothelial function and ameliorates insulin sensitivity and inflammation in cardiopathic nondiabetic patients after an aortocoronary bypass. Metabolism 2009;58:1270-6.
47. Neri I, Monari F, Sgarbi L, Berardi A, Masellis G, Facchinetti F. L-arginine supplementation in women with chronic hypertension: impact on blood pressure and maternal and neonatal complications. The Journal of Maternal-Fetal & Neonatal Medicine 2010;23:1456-60.
48. Ast J, Cieslewicz A, Korzeniowska K, Bogdanski P, Kazmierczak E, Olszewski J, et al. Supplementation with L-arginine does not influence arterial blood pressure in healthy people: a randomized, double blind, trial. Eur Rev Med Pharmacol Sci 2011;15:1375-84.
49. Thomas GD, Zhang W, Victor RG. Nitric oxide deficiency as a cause of clinical hypertension: promising new drug targets for refractory hypertension. Jama 2001;285:2055-7.
50. Augustyniak RA, Thomas GD, Victor RG, Zhang W. Nitric oxide pathway as new drug targets for refractory hypertension. Current pharmaceutical design 2005;11:3307-15.
51. Bai Y, Sun L, Yang T, Sun K, Chen J, Hui R. Increase in fasting vascular endothelial function after short-term oral L-arginine is effective when baseline flow-mediated dilation is low: a meta-analysis of randomized controlled trials. The American journal of clinical nutrition 2009;89:77-84.
52. Piatti P, Monti LD, Valsecchi G, Magni F, Setola E, Marchesi F, et al. Long-term oral L-arginine administration improves peripheral and hepatic insulin sensitivity in type 2 diabetic patients. Diabetes care 2001;24:875-80.
53. Dong J-Y, Qin L-Q, Zhang Z, Zhao Y, Wang J, Arigoni F, et al. Effect of oral L-arginine supplementation on blood pressure: a meta-analysis of randomized, double-blind, placebo-controlled trials. American heart journal 2011;162:959-65.
54. Dorniak-Wall T, Grivell R, Dekker G, Hague W, Dodd J. The role of L-arginine in the prevention and treatment of pre-eclampsia: a systematic review of randomised trials. Journal of human hypertension 2014;28:230-5.
55. Böger RH, Diemert A, Schwedhelm E, Lüneburg N, Maas R, Hecher K: The role of nitric oxide synthase inhibition by asymmetric dimethylarginine in the pathophysiology of preeclampsia. Gynecologic and obstetric investigation 2009;69:1-13.
56. Heitzer T, Schlinzig T, Krohn K, Meinertz T, Münzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. Circulation 2001;104:2673-8.
57. Miyazaki H, Matsuoka H, Cooke JP, Usui M, Ueda S, Okuda S, et al. Endogenous nitric oxide synthase inhibitor. Circulation 1999;99:1141-6.
58. Rajapakse NW, Mattson DL. Role of L-arginine in nitric oxide production in health and hypertension. Clinical and Experimental Pharmacology and Physiology 2009;36:249-55.
59. Cannon RO. Role of nitric oxide in cardiovascular disease: focus on the endothelium. Clinical chemistry 1998;44:1809-19.
60. Newsholme P, De Bittencourt PIH, O'Hagan C, De Vito G, Murphy C, Krause MS. Exercise and possible molecular mechanisms of protection from vascular disease and diabetes: the central role of ROS and nitric oxide. Clinical Science 2010;118:341-9.
61. Higashi Y, Oshima T, Ono N, Hiraga H, Yoshimura M, Watanabe M, et al. Intravenous administration of L-arginine inhibits angiotensin-converting enzyme in humans. The Journal of Clinical Endocrinology & Metabolism 1995;80:2198-202.