The effect of endurance training along with L-arginine supplementation on the levels of MMP-2 and MMP-9 in postmenopausal hypertensive women

Bita Bordbarazari[1], Mandana Gholami[1*], Khosrow Ebrahim[2], Hossein Abed Natanzi[1], Farshad Ghazalian[1]

1. Department of Physical Education and Sport Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Department of Physical Education and Sports Sciences, Shahid Beheshti University, Tehran, Iran

***Corresponding author:** Tel: +98 9121491868 Fax: +98 2144865179
Address: Department of Physical Education and Sport Sciences, Faculty of Literature, Humanities and Social Sciences, Science and Research Branch, Islamic Azad University, Daneshgah Blvd, Simon Bulivar Blvd, Tehran, Iran
E-mail: m.gholami@srbiau.ac.ir
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Abstract

Introduction: Matrix metalloproteinases (MMPs) including MMP2 and MMP9 play an important role in hypertension pathogenesis. The aim of present study was to identify the effect of endurance training along with L-arginine supplementation on the levels of MMP-2 and MMP-9 in postmenopausal hypertensive women.
Materials and methods: The 40 postmenopausal hypertensive women's (average age of 55.26 ± 2.56 years and BMI 28.61±1.18 kg/m^2) randomly divided in four groups including placebo, L-arginine, endurance training and endurance training + L-arginine groups. Exercise training program conducted for 12 weeks with 60-75 percent of maximum heart rate. L-arginine consumption considered 6 g daily. Blood sampling performed in pre and posttest (48 hours after last training session or L-arginine consumption) stages and MMP2 and MMP9 were measured by ELISA method.
Results: Present study findings indicated that L-arginine, training and training + L-arginine result in significant decrease of MMP-2, MMP-9 and systolic blood pressure ($P<0.05$). Moreover, the greatest reduction in MMP-2 and MMP-9 levels and systolic blood pressure were observed in training + L-arginine group.
Conclusion: It seems that, L-arginine supplementation increases the antihypertensive effect of endurance training that partly related to decrease in MMP-2 and MMP-9 levels.

Keywords: Matrix metalloproteinase, L-arginine, Hypertension, Postmenopausal

Introduction

Today, high blood pressure or hypertension has become the most common chronic disorder in developed countries and its prevalence among adults is about 25-30% (1). Although the pathogenesis of hypertension is multifaceted and highly complex, endothelial dysfunction is considered as a primary event in the pathogenesis of hypertension, which is associated with target tissue injury and the development of atherosclerosis (2). In patients with hypertension, endothelium-dependent vasodilation is impaired, which represent the endothelial dysfunction (3) that probably resulting from abnormal vascular remodeling and rearrangement of different parts of the vascular wall consist of extracellular matrix (ECM) proteins (4). Matrix metalloproteinases (MMPs) belong to family of zinc-dependent endopeptidases, which play important role in degradation of ECM components (5). Among the different members of MMPs family, special attention has been given to MMP-2 and MMP-9 due to their major role in the pathophysiology of cardiovascular changes associated with hypertension (6). In this regard, its reported that MMPs specially MMP-2 and MMP-9 affected many cardiovascular diseases such as hypertension and its related chronic complications (7). Although it has been reported that levels of MMP-2 and MMP-9 changed in hypertension status, the results are contradictory and increase (8) and decrease (9) of MMP-2 and MMP-9 have been observed in hypertensive patients. In addition, some researchers suggested that MMP-2 and MMP-9 levels remain unchanged in patients with hypertension (10, 11).

Inactive people are 30 to 50 percent more at risk for developing the hypertension (12). In contrast, researchers have shown the positive effect of different types of exercise training, especially endurance training in lowering the blood pressure of hypertensive individuals (13) and therefore, exercise training has been suggested as an antihypertensive treatment (14).

In addition to exercise training, L-arginine exert antihypertensive properties and play important role in management hypertension (15). L-Arginine is semi-essential amino acid and an important substrate for nitric oxide (NO) biosynthesis, which has a critical role in different physiological processes including vasodilation, neurotransmission, immunity and cytotoxicity in the human body (16). L-arginine is an important potential treatment for the improvement of various cardiovascular disorders (17). L-arginine decrease blood pressure by reducing peripheral vascular resistance (18) and researchers has shown that L-arginine supplementation improves the endothelium-dependent vasodilatation similar to exercise training (19). Its reported that L-arginine antihypertensive effect partly exert by decreasing levels of MMP-2 and MMP-9 in hypertensive patients (20).

Moreover, decrease in MMPs levels especially MMP2 by exercise training are associated with decreasing systolic and diastolic blood pressure (21). Interestingly, exercise training with L-arginine supplementation has a synergic effect and cause in further improvement in endothelial function in patients with hypertension (22). Despite the positive effects of exercise training, L-arginine and L-arginine with exercise training in controlling hypertension, its effectiveness mechanism is largely unclear and further studies are needed to identify its mechanisms. Therefore, the aim of present study was to identify the effect of endurance training along with L-arginine supplementation on the levels of MMP-2 and MMP-9 in postmenopausal hypertensive women.

Materials and methods

Subjects

Present study subjects consist of postmenopausal women with hypertension and among the available population, the researcher randomly selected 40 people as s present study subjects to conducting the research protocol.

Study design

The present study is registered with the Ethics ID of IR.IAU.SRB.REC.1398.009 in the iran national committee for ethics in biomedical research. In the first step, by informing and mentioning the necessary conditions for selecting the subjects, we tried to identify the volunteers and eligible subjects in order to carry out the present research. Among the patients referred, those who were not eligible were excluded from the study. After monitoring and selecting, the terms and conditions of the present research were explained for subjects to be aware from the potential benefits and disadvantages of the intended research intervention (endurance training, L-arginine or its combination) for hypertensive patients. Finally, based on the inclusion and exclusion criteria, the 40 subjects were selected to participate in the present study and informed consent form signed by all of subjects at the beginning of study. Subjects were randomly divided into four groups (each group consist of 10 subjects) including the placebo, L-arginine, endurance training and endurance training+L-arginine groups. The subjects were asked to refrain from any changes in their usual lifestyle during the 12-week study period. In addition, all subjects were required to make any change in the type or dose of the medication solely with the advice of the treating physician.

Some limitations were considered in order to entrance the volunteers subjects into the present study including: Menopause, hypertension (systolic blood pressure higher than 140 or diastolic blood pressure greater than 90 mmHg), lack of cardiovascular disease except hypertension, without malignancy (cancer), lack of Diabetes, non-participation in regular exercise training in the last years, no alcohol consumption, don’t receive any supplements, lack of physical limitations to attend the training program and signed a written informed consent. In some condition, subjects were excluded from the present study either before or during the 12-week research intervention which considered as exclusion criteria: Lack of hypertension, subject's disagreement with researcher's terms, absence in exercise training sessions, refusal to sign informed consent form, physical restriction to participate in exercise training, injury during exercise training period and subject's inability to continue the exercise program, other cardiovascular diseases except hypertension, don’t take part in pre-test and post-test blood sampling and prohibition of a physician to participate in exercise training.

Endurance training program

The endurance training program was performed for 12 weeks and three sessions per week by training and training+L-arginine groups. The endurance training program conducted on treadmill and consisted of three parts. At first, the subjects walked on the treadmill to warm up for 5 minutes at low intensity (40% of maximum heart rate). Immediately after warm-up, the main part of exercise training program started which consisted of 20 minutes walking-running on the treadmill with 60-75% of maximal heart rate intensity, and finally the cooling-down period including low-intensity walking (40% Maximum heart rate) on treadmill was performed for 5 minutes (23).

L-arginine supplementation

L-arginine supplement was taken 6 g daily (three servings of 2 g) by the subjects in L-arginine and training+L-arginine groups, and the placebo group was given the same amount of placebo (White flour capsule) (18).

Blood samples collection

Blood sampling was performed in two stages of pre-test and post-test by laboratory expert. For pre-test blood sampling, subjects were asked to visit the lab after about 12 hours of overnight fasting. A few days after pre-test blood sampling and baseline measurements, a 12-week research protocol was started and subjects were exposed to the independent variable (L-arginine, endurance training and endurance training+L-arginine) for 12 weeks. The 48 hours after the last training session or L-arginine supplementation, subjects were asked to blood sampling in post-test stage. Subjects were asked to refrain from any heavy exercise or physical activity in 48 before blood sampling. At each stage of blood sampling, 7 ml of blood in sitting position and after 30 minutes resting was obtained from subjects. Immediately after blood sampling, blood samples were poured into a Falcon tube and then centrifuged at 3000 rpm for 10 minutes and finally serum stored in the -80° C for subsequent experiments.

Biochemical analysis

Subjects' height and weight were measured by means of Seca scales and stadiometer, made in Germany. Body fat percentage was measured by Korean BOCA-X1 body composition analyzer. Systolic and diastolic blood pressure were also measured by a mercury barometer. Serum levels of MMP-2 (from bosterbio company, Catalog No: EK0459) and MMP-9 (from bosterbio company, Catalog No: EK0465) were measured by ELISA method. All assays were performed according to the kit manufacturer's instructions.

Statistical analysis

Data analysis was performed using SPSS-24 software. Data distribution determined by Shapiro-Wilk test. distribution of data was normal ($P<0.05$) and therefore, between group changes were assessed by analysis of covariance (Ancova) test and Bonferroni post hoc test. Significance level for all stages of data analysis was considered $P<0.05$.

Results

Subjects physical characteristics including age, height, body weight and body mass index (BMI) in the different research groups have been shown in Table 1. Present study findings indicated that body fat percentage, BMI and diastolic blood pressure (DBP) were significantly decreased in the training and training + L-arginine groups compared to the placebo and L-arginine groups ($P<0.05$).

Table 1. Subjects physical characteristics.

Variables	Placebo	L-arginine	Training	Training + L-arginine
Age (years)	56.1±3.27	54.8±2.18	54.3±2.35	55.6±2.28
Height (cm)	157.1±4.08	156.8±5.24	159.4±5.82	158.7±5.40
Body weight (kg)	71.4±6.45	70.1±6.29	71.0±5.19	73.6±5.37
BMI (kg/m^2)	28.8±1.41	28.4±0.88	27.9±1.02	29.2±1.08

BMI, body mass index.

Systolic blood pressure (SBP) decreased significantly in L-arginine, training and training + L-arginine groups compared to placebo group ($P<0.001$). Moreover, decrease in SBP was also significant in the training + L-arginine group compared to training ($P=0.007$) and L-arginine ($P<0.001$) groups (Table 2).

Analysis of MMP-2 and MMP-9 levels by analysis of covariance (Ancova) test indicated significant difference between groups ($P<0.001$). Bonferroni post hoc test indicated significant decrease of MMP-2 levels in L-arginine ($P=0.042$), training ($P<0.001$) and training + L-arginine ($P<0.001$) groups compared to placebo group. In addition, MMP-2 levels significantly decreased in training+L-arginine group compared to training ($P=0.033$) and L-arginine ($P<0.001$) groups. Significant decrease of MMP-9 levels observed in L-arginine ($P=0.002$), training ($P<0.001$) and training+L-arginine ($P<0.001$) groups compared to placebo group and also in training+L-arginine group compared to L-arginine group ($P=0.020$). The results for BMI, body fat percentage, SBP, DBP, MMP-2 and MMP-9 levels in different groups reported in Table 2. All data expressed as mean ± standard deviation.

Discussion

Present study conducted aimed to investigate the effect of endurance training in combination with L-arginine supplementation on the serum levels of MMP-2 and MMP-9 in postmenopausal women with hypertension. The main finding of present study was that L-arginine ingestion increase the effectiveness of exercise training in decreasing blood pressure and simultaneously decreased serum levels of MMP-2 and MMP-9 in hypertensive subjects, which further decrease of MMP-2 and MMP-9 was observed in training + L-arginine group. The present study finding indicated that L-arginine consumption alone decrease systolic blood pressure and in combination with exercise training has a greater effect on its improvement.

Table 2. Levels of variables under study.

Variables	Groups	Pre test	Post test	t test P value	Ancova P value
BMI (kg/m^2)	Placebo	28.8±1.42	28.9±1.41	0.114	<0.001
	L-arginine	28.4±0.88	28.4±0.95	0.425	
	Training	27.9±1.02	27.2±1.05	<0.001	
	Training + L-arginine	29.2±1.08	28.6±1.06	<0.001	
Percent body fat	Placebo	35.7±3.81	36.0±3.63	0.082	<0.001
	L-arginine	34.6±2.68	34.7±3.07	0.544	
	Training	32.5±3.6	30.7±3.07	<0.001	
	Training + L-arginine	35.1±3.12	33.6±3.42	0.001	
SBP (mmHg)	Placebo	146.3±3.96	147.2±4.33	0.084	<0.001
	L-arginine	147.4±3.08	142.3±4.28	<0.001	
	Training	144.9±2.86	137.6±3.15	<0.001	
	Training + L-arginine	147.6±4.75	136.4±5.41	<0.001	
DBP (mmHg)	Placebo	93.4±3.39	93.2±3.71	0.506	<0.001
	L-arginine	91.8±2.31	91.0±1.87	0.054	
	Training	95.4±2.90	90.1±2.29	<0.001	
	Training + L-arginine	89.7±2.97	94.6±3.48	<0.001	
MMP-2 (ng/ml)	Placebo	61.4±9.57	62.1±9.68	0.205	<0.001
	L-arginine	57.3±8.78	55.2±8.42	<0.001	
	Training	63.2±10.44	58.3±10.50	0.001	
	Training + L-arginine	59.5±8.23	51.8±7.06	<0.001	
MMP-9 (ng/ml)	Placebo	48.9±5.12	48.3±4.94	<0.001	<0.001
	L-arginine	45.3±4.64	42.1±3.86	<0.001	
	Training	49.5±6.25	44.1±5.28	<0.001	
	Training + L-arginine	47.6±5.48	41.9±5.67	<0.001	

BMI, body mass index. SBP, systolic blood pressure. DBP, diastolic blood pressure. MMP, matrix metalloproteinase. Data shown as mean ± standard deviation.

This finding represents that adding L-arginine to exercise training has a synergic effect on decreasing blood pressure. Consistent with these findings, previous studies have also reported a significant decrease in systolic blood pressure following eight weeks of L-arginine (3 g/day or 6 g /day) consumption and the researchers attributed decrease blood pressure to improvement lipid levels as well as BMI decrease (24). Bahrami et al (2018) also reported that L-arginine supplementation for 12 weeks (5 g daily) significantly decrease SBP and DBP in patients with metabolic syndrome (25). The researchers suggested that decrease blood pressure with L-arginine supplementation are associated with decreased oxidative stress and improve the endothelial function in hypertensive diabetic patients (26) and it seem that anti-hypertensive effect of L-arginine largely exert by increase the NO bioavailability which in turn result in improve vasodilation and finally decrease blood pressure (27).

In addition to L-arginine consumption, exercise training plays important role in management hypertension as approved in present study. Exercise training role in management and control hypertension is exerted through a different mechanism including the improve in endothelial function, change in arterial compliance, sympathetic activity, heart rate variability, as well as change in arterial elasticity (28). The effect of exercise training on hypertension may vary depending on the type of exercise training (endurance or resistance training), especially duration of the training period, duration of each session, training frequency and intensity of exercise training (29). Although, Simultaneous effect of exercise training and L-Arginine Supplementation on blood pressure has received less attention, Lucotti et al (2006) reported that exercise training with L-arginine are associated with decreasing blood pressure that probably related to decreased in endothelin-1 and increased NO levels (22). However, present study indicated that improvement in blood pressure by endurance exercise training+L-arginine is due to decrease of MMP-2 and MMP-9 levels and further decrease in levels of MMP-2 and MMP-9 were observed in training+L-arginine group.

The results about changes in levels of MMPs including MMP-2 and MMP-9 is controversial and decrease, increase and non-changes of MMP-2 and MMP-9 levels in hypertensive patients (7). Consistent with the present findings, Garcia et al (2016) indicated that exogenous L-arginine in patients with hypertension are associated with significant decrease of MMP-2 and MMP-9 levels and simultaneously restoring the oxidative stress balance were observed that is consequence of antioxidant properties of L-arginine (20). Regarding to effect of exercise training, Donley et al (2014) found that endurance training reduces arterial stiffness and blood pressure in metabolic syndrome patients and significant decrease of MMP-2 after exercise training was observed only in metabolic syndrome patients not in control group (30). Interestingly, moderate intensity and high intensity exercise training in hypertensive patients with metabolic syndrome decrease the MMP-2 and MMP-9 levels that can result in significant decrease of systolic and diastolic blood pressure (31).

In addition to hypertension, exercise training decrease levels of MMP-2 and MMP-9 in other condition such as diabetes and suggested that exercise-induced beneficial effects on inflammation and glucose regulation may have mediated such changes, which even in these patients also reduced MMP-2 and MMP-9 levels is associated with lowering systolic blood pressure (21). Mentioned researches represent that up regulation the MMP-2 and MMP-9 in different pathological condition like hypertension, metabolic syndrome, diabetes and etc, play important role in endothelial dysfunction and hypertension. In contrast, exercise training or L-arginine alone or in combination ameliorates these effects by down regulation of some factors such as MMP-2 and MMP-9. However, respect to little sample size in the present study and few researches that conducted regarding effect of exercise training along with L-arginine supplementation on hypertension and its possible mechanisms, future researches needed to identify different aspects of training+L-arginine effectiveness in hypertension.

Conclusion

According to present study findings, it can be concluded that adding L-arginine supplement to exercise training program increase the antihypertensive effect of endurance training and has a synergic effect. Present study indicated that probably this synergic effect is related to further decrease in MMP-2 and MMP-9 levels that play important role in development of hypertension and endothelial dysfunction.

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Conflict of interest

The authors declare that no conflict of interest exists.

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