HUMAN MOVEMENT
2014, vol. 15 (2), 116–119

EFFECTS OF L-ARGININE SUPPLEMENTATION ON BLOOD PRESSURE REDUCTION PRE-, PERI-, AND POST-CARDIOVASCULAR STIMULUS IN HYPERTENSIVE SUBJECTS WITH DIFFERENT ANGIOTENSIN--CONVERTING-ENZYME GENOTYPES

doi: 10.2478/humo-2014-0012

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ABSTRACT
Purpose. The present study investigated whether L-arginine supplementation reduces blood pressure (BP) in hypertensive subjects with different ACE genotypes. **Methods.** Eight male hypertensive patients received L-arginine (2 or 4 g/day) or a placebo for a period of 4 days prior an exercise test. Statistical analysis consisted of one-way analysis of variance. **Results.** L-arginine supplementation induced a statistically significant ($p < 0.05$) reduction in systolic BP measured during rest (reductions of 7.8% and 12.3% with 2 and 4 g/day, respectively), exercise (reductions of 11.8% and 10.4% with 2 and 4 g/day, respectively), and recovery (reductions of 11.7% and 10.7% with 2 and 4 g/day, respectively). The observed magnitude of BP reduction suggests an association with ACE polymorphism; a larger effect was seen with the II and DI genotypes compared with the DD genotype (II: 121 mmHg and DI: 133 mmHg vs. DD: 144 mmHg). **Conclusions.** The results showed that L-arginine supplementation at low doses was efficient in reducing BP and that vasodilator actions that occurred through the secretion of nitric oxide might be ACE genotype dependent.

Key words: exercise, hypertension, nitric oxide, vasodilatation

Introduction

The synthesis of nitric oxide (NO) is the product of L-arginine metabolism through the NO synthase pathway. The accumulated knowledge on this pathway has opened a new avenue of study on L-arginine, the so-called 'conditionally' essential amino acid present in many food items, as it plays a central role in a number of major metabolic pathways [1]. The average consumption of L-arginine in a diet is 5.4 g/day, assuming a total daily protein intake of 100 g/day. L-arginine supplementation is posited to cause vasodilatation and reduce inflammation when metabolized by nitric oxide synthases (NOS) into nitric oxide and L-citrulline [2].

The insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme (ACE) gene has been recently identified as a potential risk factor for several cardiovascular disorders. The presence of ACE gene polymorphisms in humans has been postulated from segregation analysis of plasma ACE levels in several families [3]. These polymorphic variations have been correlated with different plasma and cellular ACE levels, which probably modulate gene expression. ACE gene insertion/deletion polymorphism is characterized by the presence (insertion – allele I) or absence (deletion – allele D) of a 287-bp fragment in intron 16 of this gene [4], where the presence of the D allele is associated with higher ACE levels and vice versa [5]. In itself, ACE is a key enzyme involved in the presence of angiotensin II-induced vasoconstriction and cardiovascular disease [6].

Therefore, the purpose of this study was to determine whether different doses of oral L-arginine supplementation induce a reduction in blood pressure before, during, and after a cardiovascular stimulus and whether correlations between blood pressure reduction and ACE polymorphism can be discerned in individuals with hypertension.

Material and methods

The study group consisted of eight male hypertensive patients with a medical diagnosis of hypertension in accordance with the JNC [7]; clinical characteristics are presented in Table 1. The participants received L-arginine (2 g/day or 4 g/day) or a placebo for 4 days prior to the testing day when an exercise test was administered. All patients signed an informed consent form prior to their participation in the study in accordance with the Resolution of the National Council of Health. The procedures described in the present study were

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Table 1. Clinical characteristics of patients (N = 8)

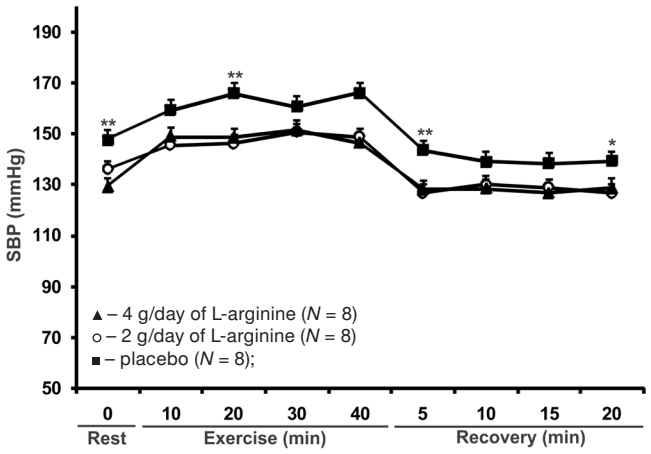
Variable	Mean ± SD
Age (years)	50.8 ± 9.4
Body fat (%)	40.5 ± 5.2
VO_{2max} (ml · kg^{-1} · min^{-1})	24.4 ± 8.5
Fasting glucose (mg/dl)	103.6 ± 24.4
Triglycerides (mg/dl)	159.5 ± 78.6
Cholesterol (mg/dl)	202.6 ± 43.6
Systolic blood pressure (mmHg)	144.6 ± 3.7
Diastolic blood pressure (mmHg)	85.8 ± 8.2
Heart rate (beats · min^{-1})	77.8 ± 12.2

performed in compliance with the Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects. The study was approved by the local institutional Ethics Committee of the Midwest State University of Parana (Nr. 101.700/2012).

On the day of testing, the Mile test protocol [8], an indirect measure of VO_{2max}, was administered to generate a cardiovascular stimulus. The participants did not use their antihypertensive medication for 12 hours prior to the test, where antihypertensive medication was allowed for consumption only 2 hours post-test. The exercise test was preceded by a 10 min rest period. Participants then ran on a treadmill ergometer (model RT250 Pro, Movement, Brazil) at 40% heart rate (HR) reserve for 40 min followed by passive recovery for 20 min. The speed of the treadmill was individually adjusted to achieve a workload of 40% of HR reserve for each patient. Blood pressure and heart rate were measured using a mercury sphygmomanometer and Polar T61 monitor (Polar, USA), respectively, every 2 min during the rest, exercise, and recovery periods.

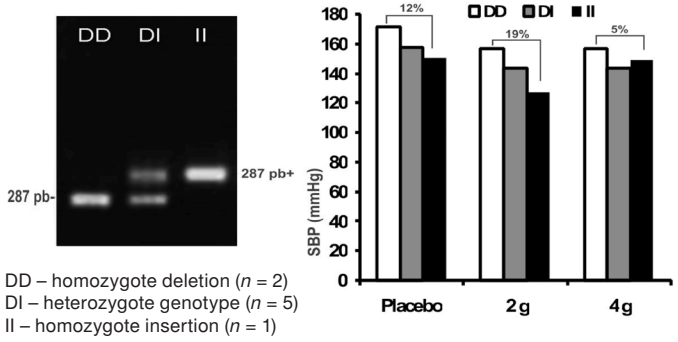
Patients' ACE genotype was determined using standard methods. ACE gene fragment began with extracting DNA using a commercial kit following the manufacturer's instructions (Axygen, USA). A 287-bp fragment was amplified from the intron 16 of the ACE gene by PCR using ACE-1F and ACE-1R primers, used previously in other studies [9]. The reaction was made up with the following reagents: 1x *Taq* DNA Polymerase buffer (200 mM Tris [pH 8.4] and 500 mM of KCl), 50 mM $MgCl_2$, 2 µM dNTP mix, 10 µm of primers ACE-1F 5'-GCC CTG CAG GTG TCT GCA GCA TGT-3' and ACE-1R 5'-GGA TGG CTC TCC CCG CCT TGT CTC-3', 1 U of *Taq* DNA Polymerase (Invitrogen, USA), and 10 ng of template DNA. The cycling conditions were: 1 cycle of 5 minutes at 94°C; 35 cycles of 1 minute at 94°C, 45 s at 56°C, and 1 min at 72°C; and 1 cycle of 7 minutes at 72°C.

Means and standard deviations were calculated for all data. Statistical analysis consisted of one-way analysis of variance (ANOVA) and only significant ($p < 0.05$) F values are presented. Post-hoc analysis using Tukey's range test was performed when appropriate.



* statistical difference between interventions, $p < 0.05$

Figure 1. Effects of L-arginine supplementation on systolic blood pressure before, during, and after the exercise test



DD – homozygote deletion (n = 2)
DI – heterozygote genotype (n = 5)
II – homozygote insertion (n = 1)

Figure 2. Effect of L-arginine supplementation on systolic blood pressure associated with ACE polymorphism

Results

As presented in Figure 1, L-arginine supplementation promoted a significant systolic BP reduction ($p < 0.05$) in the tested stages, i.e. during rest (reductions of 7.8% and 12.3% with 2 and 4 g/day, respectively), exercise (reductions of 11.8% and 10.4% with 2 and 4 g/day, respectively), and recovery (reductions of 11.7% and 10.7% with 2 and 4 g/day, respectively).

Despite the small number of subjects in this study, the results suggest that the observed magnitude of BP reduction, induced by L-arginine supplementation during exercise, may be associated with the ACE gene polymorphism. The largest effect on BP was observed in patients carrying the II and DI genotypes compared with the DD genotype carriers (DD: 144 mmHg vs. II: 121 mmHg and DI: 133 mmHg, 15 % and 8%, respectively). A smaller reduction in BP was observed when the placebo was used with the same genotypes (DD: 171 mmHg vs. II: 150 mmHg and DI: 157 mmHg, 12% and 8% reduction, respectively; Fig. 2).

No significant differences were observed in diastolic BP and heart rate values between the different conditions used in the protocol of supplementation and physical exercise.

Discussion

The potential relationships between the effects of L-arginine supplementation on reducing blood pressure and ACE polymorphism were investigated in hypertensive patients. ACE is a key enzyme involved in the presence of angiotensin II-induced vasoconstriction and cardiovascular disease [6], where polymorphisms of the ACE gene have been correlated with different plasma and cellular ACE levels [10].

Previous studies using animals suggested that an exercise training program improves NO-dependent vascular function and upregulates endothelial constitutive NO-synthase expression [11]. Additionally, exercise was found to also improve the endothelium-dependent function in adults with cardiac failure [12, 13] and coronary disease [14, 15].

It is well known that the balance between Ang II and NO is important in the regulation of vascular tone [16, 17]. Ang II increases vascular superoxide production by membrane activation associated with nicotinamide adenine dinucleotide diaphorase/nicotinamide adenine dinucleotide phosphate oxidase, resulting in NO inactivation and toxic production of peroxynitrite. Therefore, exercise can increase NO by inhibiting the production of Ang II [18].

He et al. [19] recently reported that plasma levels of nitrite and nitrate (NOx) decreased with an increased number of D alleles in a group of hypertensive subjects. In the present study, plasma levels of NOx in individuals with genotype DD were significantly lower than those with genotype II, suggesting that polymorphism has an impact on NO production in hypertensive subjects. Hence, hypertensive patients who carry the D allele and suffer from insufficient NO production, which leads to impaired endothelium-dependent vasodilatation, might benefit from oral L-arginine supplementation.

The present study showed that significant doses of L-arginine supplementation (2 and 4 g/day) were not as effective in the reduction of blood pressure of participants with the DD genotype compared with carriers of the DI and II genotypes. Thus, the reduction in blood pressure during exercise was more evident in subjects who received supplementation than in those who received a placebo. Moreover, among the subjects who received L-arginine, this reduction was more evident in those with the DI and II genotypes than those with the DD genotype.

For medical reasons, interest in this amino acid has been stimulated by the observation that supplementation with L-arginine could attenuate endothelial dysfunction and coronary heart disease [1]. The potential role of L-arginine supplementation as a new effective strategy to improve endothelial function in patients with hypertension is recently under consideration. Jabecka et al. [20] demonstrated that L-arginine supplementation increases plasma arginine, citrulline, and TAS levels in patients with mild arterial hypertension, supporting the hypothesis that augmented concentrations of L-arginine stimulate NO biosynthesis and leads to a reduction in oxidative stress. The increase in ADMA plasma levels after L-arginine supplementation confirms a correlation between ADMA and L-arginine.

Conclusions

The results in this study show that supplementation with L-arginine at low doses was efficient to attenuate BP before, during, and after a cardiovascular stimulus in hypertensive patients, suggesting that L-arginine can promote improved BP control during exercise. Additionally, the vasodilation that results from nitric oxide secretion and BP reduction during and after exercise may be ACE genotype dependent.

Acknowledgments
This study was supported by the following the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; National Council for Science and Technology) and the Fundação Araucária (FA; Araucária Foundation) from the State of Paraná. The authors declare that there are no conflicts of interest.

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Paper received by the Editors: April 8, 2013
Paper accepted for publication: April 23, 2014

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