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Oral L-arginine supplementation in patients with mild arterial hypertension and its effect on plasma level of asymmetric dimethylarginine, L-citrulline, L-arginine and antioxidant status

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Abstract. – BACKGROUND: Potential role of L-arginine supplementation as a new effective strategy of improving endothelial function in patients with hypertension is recently under consideration.

OBJECTIVE: To evaluate influence of 28-day oral supplementation of L-arginine on plasma level of asymmetric dimethylarginine (ADMA), L-citrulline, L-arginine and total antioxidant status (TAS), in patients with mild arterial hypertension.

SUBJECTS AND METHODS: 54 participants (24 women and 30 men) were studied. Ambulatory blood pressure monitoring (ABPM) was used for allotting patients to either healthy control group (19 subjects) or hypertensive treatment group (35 patients). Patients were later randomized to either L-arginine (2 g tid or 4 g tid) or placebo. During 28 days of study on 5 consecutive visits TAS, plasma level of ADMA, L-citrulline, and L-arginine were measured.

RESULTS: In patients with mild hypertension treated with L-arginine significant increase in TAS and plasma level of arginine and citrulline was observed. Additionally plasma ADMA concentrations after 28 days of L-arginine supplementation significantly exceeded initial concentrations.

CONCLUSIONS: L-arginine supplementation increases plasma arginine, citrulline and TAS in patients with mild arterial hypertension. It confirms the thesis that augmented concentrations of L-arginine stimulate NO biosynthesis which leads to reduction of oxidative stress. Increase of ADMA plasma level after L-arginine supplementation confirms correlation between ADMA and L-arginine.

Key Words:
ADMA, L-citrulline, L-arginine, Supplementation, TAS.

Introduction

Endothelial dysfunction, which leads to decreased bioavailability of nitric oxide (NO)[1-3] is a disadvantageous prognostic factor for patients with arterial hypertension. This fact was revealed by Perticone et al, who proved that the risk of cardiovascular incident was fourfold greater in patients suffering endothelial dysfunction than those with correct function of endothelium[4]. The most essential elements influencing decreased bioavailability of NO are ADMA (asymmetric dimethylarginine) and increased production of O_2^{-}[5,6]. ADMA, the main endogenic NOS inhibitor, is created by methylation of arginine residues after protein hydrolysis. It is highly probable that ADMA normally is created constantly but its accumulation is prevented by activity of dimethylhydrolase of dimethylarginine (DDAH). Changes of DDAH activity may contribute to increased level of ADMA in various disease units[7]. It was proven that plasma levels of ADMA correlate with the value of arterial tension (patients with hypertension show significantly higher levels of ADMA than healthy individuals)[8,9].

One way to counteract negative effects of endogenic ADMA is the reversal of NOS competitive inhibition by application of exogenic L-arginine, which competes with endogenic ADMA. It was proven that supplementation with L-arginine corrected endothelial-dependent arterial functions in individuals suffering high levels of ADMA (decrease in generation of O_2^{-}; increase in NO synthesis) as well as it improved clinical status of patients with cardiovascular diseases[10-14].

Referring to some Authors, ADMA has become a marker of cardiovascular risk[15]. Current knowledge concerning relationships between ADMA, traditional risk factors and cardiovascular diseases may help to explain why some patients showing traditional risk factors have never experienced car-

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diovascular episodes while others without presence of such factors are exposed to these episodes.

Referring to Gokce[16], the mechanisms responsible for the role of L-arginine in arterial hypertension are: improvement of vasomotoric functions of endothelium, increased synthesis of NO in vessels, decreased activity of endothelin-1 and angiotensin II, improvement of L-arginine to ADMA ratio, modulation of hemodynamic changes in kidneys, lowering of oxidative stress and improved sensitivity to insulin.

On the other hand Loscalzo[17] shows some potential mechanisms of how L-arginine improves functions of endothelium. These mechanisms are: increased transport and intercellular level of arginine, competitive antagonism to ADMA, antioxidative function, stimulation of histamine release from mastocytes, decreased activity of noradrenaline, increased secretion of insulin and changes in intercellular pH and pH-dependent transfer.

These data determined the target of our research as evaluation of influence of 28-day oral supplementation of L-arginine on plasma level of ADMA, L-citrulline, L-arginine and total antioxidant status (TAS) in patients with mild arterial hypertension.

Subjects and Methods

The research protocol was approved by the local Bioethical Committee, Karol Marcinkowski University of Medical Sciences, Poznan (No 275/04). The study was carried out on a group of 54 people (30 men, 24 women). The whole group was divided into control group (19 healthy people; 10 men, 9 women; average age: 37.9 ± 8.03 years; average body mass:77.8 ± 16.1 kg) and group of patients with diagnosed mild arterial hypertension (35 people; 20 men; 15 women; average age: 39 ± 10.1 years; average body mass: 84.9 ± 14 kg).

A prospective, randomized, double-blind design was applied. During 28 days of the study every patient had 5 visits. The visit 0 was the qualification and served to obtain initial surveys such as ambulatory blood pressure monitoring (ABPM), blood morphology, biochemical test, general urine test.

Based on the results of ABPM survey the patients were assigned to control or hypertension group and randomized to one of three subgroups: treated with 3 × 2 g of L-arginine per 24 hours, 3 × 4 g of L-arginine or placebo (see Table I). Moreover, 10 ml of blood was collected on every visit in order to monitor changes in studied parameters (TAS, ADMA, L-citrulline, and L-arginine). The results showing hypotensing effect of L-arginine supplementation (6 or 12 g daily) were presented in International Journal Medical Monitor Science[18].

The levels of ADMA, L-citrulline and L-arginine were determined using HPLC method, following procedure published by Zhang and Kaye[19] with small modifications. Total antioxidant status in plasma was determined with the use of Randox NX 2332 test (Randox Laboratories Ltd., Crumlin, UK).

Statistical Analysis

Statistical analysis was carried out using Microsoft Excel 2000 and Statistica 7.0. Basic statisti-

Table I. Clinical characteristic of participants divided into patients and healthy subgroups.

Group	n (M/W)	Age [years]	Body mass [kg]	Body height [cm]	BMI [kg/m^2]
All patients	35 (20/15)	39.0 ± 10.1	84.9 ± 14.0	176.0 ± 8.3	27.4 ± 3.5
All healthy	19 (10/9)	37.9 ± 8.0	77.8 ± 16.1	177.0 ± 8.6	24.6 ± 3.4
Patients 2 g	13 (9/4)	41.6 ± 12.2	87.5 ± 13.8	177.0 ± 8.6	27.8 ± 3.3
Patients 4 g	12 (5/7)	37.8 ± 10.0	85.0 ± 15.4	175 ± 8.4	27.6 ± 4.3
Patients placebo	10 (6/4)	36.7 ± 6.3	82.2 ± 13.1	175.0 ± 8.4	26.6 ± 2.4
Healthy 2 g	7 (4/3)	37.7 ± 10.3	73.3 ± 13.6	174.0 ± 5.9	23.9 ± 3.0
Healthy 4 g	6 (3/3)	40.7 ± 7.58	84.7 ± 16.0	178.0 ± 9.1	26.7 ± 3.4
Healthy placebo	6 (3/3)	35.3 ± 5.43	76.0 ± 19.1	180.0 ± 10.9	23.3 ± 3.5
Results of significance tests between groups of patients and healthy individuals					
Age			0.40		
Body mass			1.66		
Body Height			0.41		
BMI			2.80*		

*Differences statistically significant with $p < 0.05$).

cal parameters (arithmetical mean, standard deviation, variability range) were calculated. The equations of polynomial regression were also computed in order to draw curves illustrating changes of studied parameters. Mean values of analyzed parameters were compared between control and hypertension group on the beginning and at the end of the research to search for statistically significant differences. In order to compare non-related and independent parameters Fisher's *t*-test was used, while Wilcoxon signed-rank test for paired samples was used to compare mean values of the same parameter.

Results

Characteristics of studied parameters are illustrated in Figures 1-8. The results of the tests for statistical significance are summarized in Table II.

L-citrulline

There is a distinct increase of L-citrulline level in all patients taking L-arginine (Figures 1-2). Statistically significant increase was observed in subgroup of patients with hypertension treated with 3 × 2 g of L-arginine while in subgroup treated with 3 × 4 g of L-arginine the increase was close to statistical significance (Table II). The comparison of L-citrulline level between 0 and 4 visit revealed signs of statistical significance in subgroups treated with 3 × 2 g and 3 × 4 g of L-arginine (Table II).

L-arginine

There is a distinct increase of L-arginine level in all patients supplemented with this amino acid (Figures 3-4). Observed change in L-arginine level is statistically significant in both hypertensive and control groups treated with L-arginine (Table II).

ADMA

Obtained results are presented on Figures 5-6. The comparison of initial and final levels of ADMA in all subgroups revealed no signs of statistical significance (Table II). Changes in ADMA level between initial and final survey showed signs of increase, reaching the threshold of statistical significance in hypertension group treated with 3 × 2 g of L-arginine and control group treated with 3 × 4 g of L-arginine (Table II). In addition, initial levels of ADMA were significantly higher in hypertensive group compared to the control group (Table II).

Total Antioxidant Status (TAS)

Figures 7-8 show changes in TAS level during the study. According to the data presented in Table II there is statistically significant difference in TAS value between hypertensive subgroup treated with 3 × 4 g of L-arginine and subgroup taking placebo. Moreover, significant difference in initial values of TAS was observed between control subgroups treated with 3 × 2 g and 3 × 4 g of L-arginine. In both hypertension subgroups (3 × 2 g and 3 × 4 g of L-arginine) statistically significant increase of TAS value between visit 0 and 4 was observed; such increase was not observed in control group (Table II).

Discussion

A prospective, randomized, double-blind de-

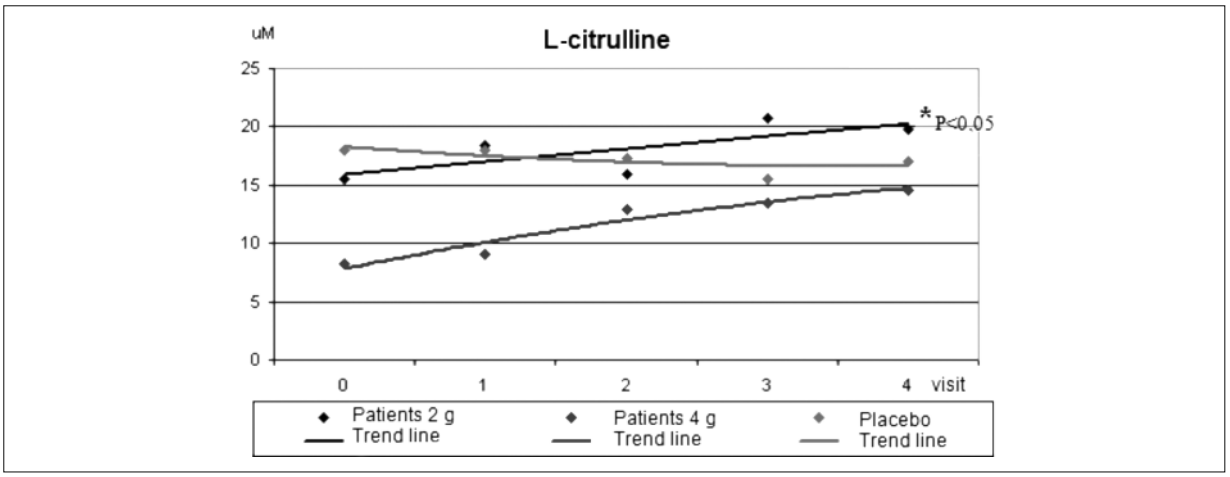

Figure 1. Graphic representation of average levels of L-citrulline in patients with hypertension (statistical significance with $p < 0.05$).

Table II. Summary of significance tests results.

	Fisher's t-test values						Wilcoxon signed-rank test values (for 0 and 4 visit)		
	2 g-4 g		2 g-placebo		4 g-placebo				
	Visit 0	Visit 4	Visit 0	Visit 4	Visit 0	Visit 4	2 g	4 g	Placebo
Patients									
L-citrulline	0.91	0.26	0.52	0.79	1.25	0.26	0.0117*	0.0367	0.4310
L-arginine	0.04	0.85	0.34	0.21	0.35	1.04	0.0069*	0.0069*	0.8927
ADMA	0.21	1.85	1.88	1.71	1.00	0.41	0.0218*	0.5751	0.1380
TAS	0.14	0.91	1.74	1.51	1.15	2.73*	0.0033*	0.0033*	0.7532
Healthy									
L-citrulline	2.55*	0.68	1.15	0.63	1.02	1.16	0.1730	0.5002	0.6121
L-arginine	0.42	0.06	0.76	1.09	0.37	0.90	0.0180*	0.0431*	0.2367
ADMA	0.62	1.13	0.13	0.07	0.66	1.04	0.0910	0.0431*	0.7353
TAS	2.08*	1.18	0.71	0.13	0.70	1.41	0.1282	0.4652	0.4690
ADMA Fisher's t-test value between the whole number of ill and healthy = 3.36*.									

*Differences statistically significant with $p < 0.05$).

sign was applied in order to perform this study. Oral supplementation of L-arginine was chosen due to the longer half-time compared to intravascular application[20]. Oral supplementation is preferred in a long-term treatment and applied doses allowed to increase consumption of L-arginine by 200%, compared to a standard diet containing 5.4 g of L-arginine[21].

Initial levels of L-arginine were distinctly higher in hypertensive subgroups than in control group (consisted of healthy people). Similar phenomenon was described by Perticone et al[4]. Despite higher value of L-arginine in patients with hypertension its level was within the normal range.

Changes in the level of L-arginine observed during 28-day supplementation tend to increase in all analyzed subgroups. The increase of L-arginine level was statistically significant in all subjects (independently of the dose), which testifies that L-arginine was regularly taken by all patients. It is worth noticing that Chin-Dusting et al did not find any increase of L-arginine level in subjects taking 10 g of the amino acid[22].

Concentrations of L-arginine measured in our study varied from 30 μM to 75 μM. Other Authors found concentrations of L-arginine in a range of 40 to 236 μM[23-27]. Such differences might be caused by different methods of assessing the level of this amino acid.

Total antioxidant status (TAS) is the indicator of

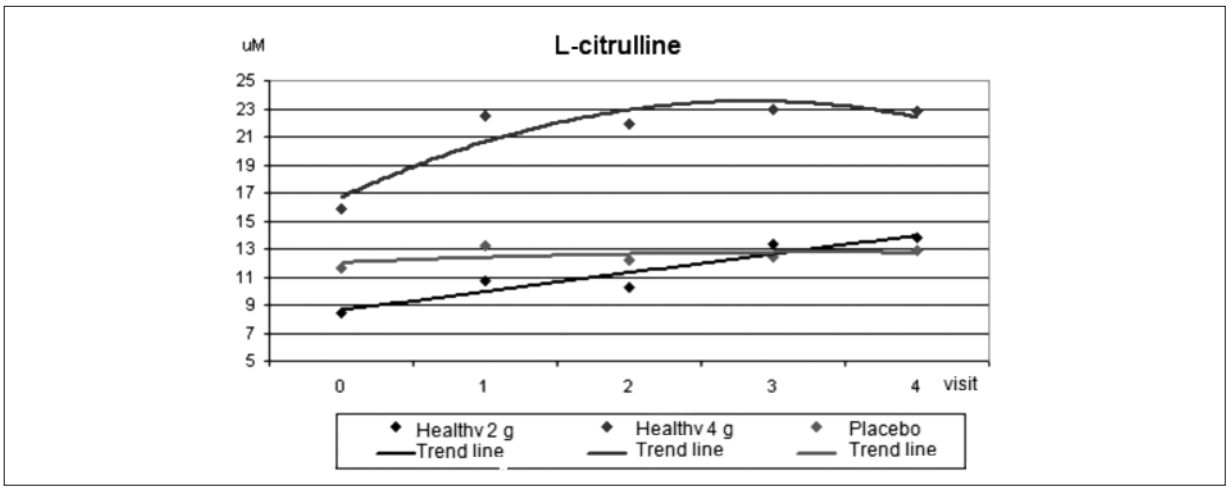

Figure 2. Graphic representation of average levels of L-citrulline in healthy individuals.

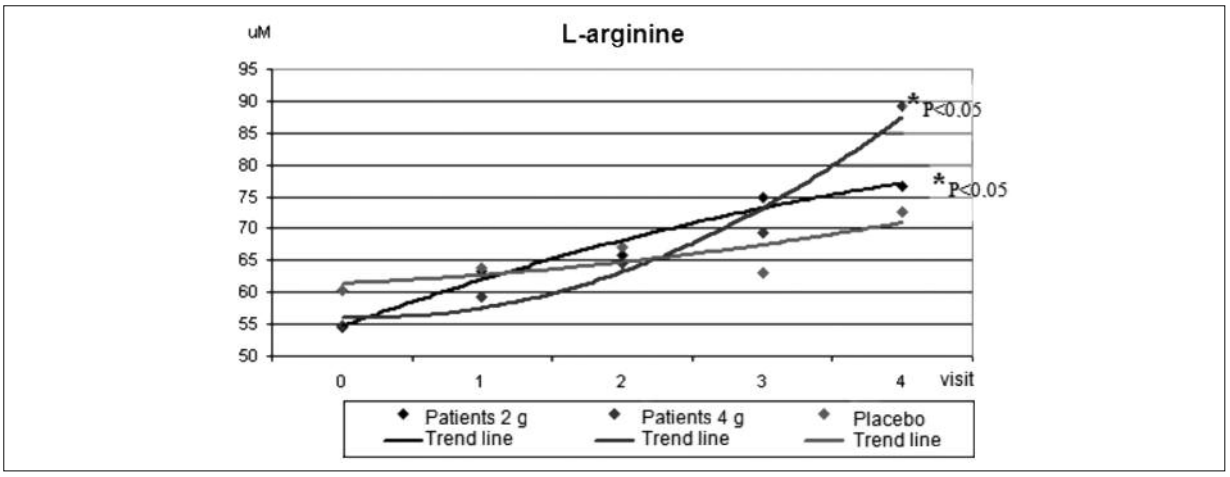

Figure 3. Graphic representation of average levels of L-arginine in patients with hypertension (statistical significance with $p < 0.05$).

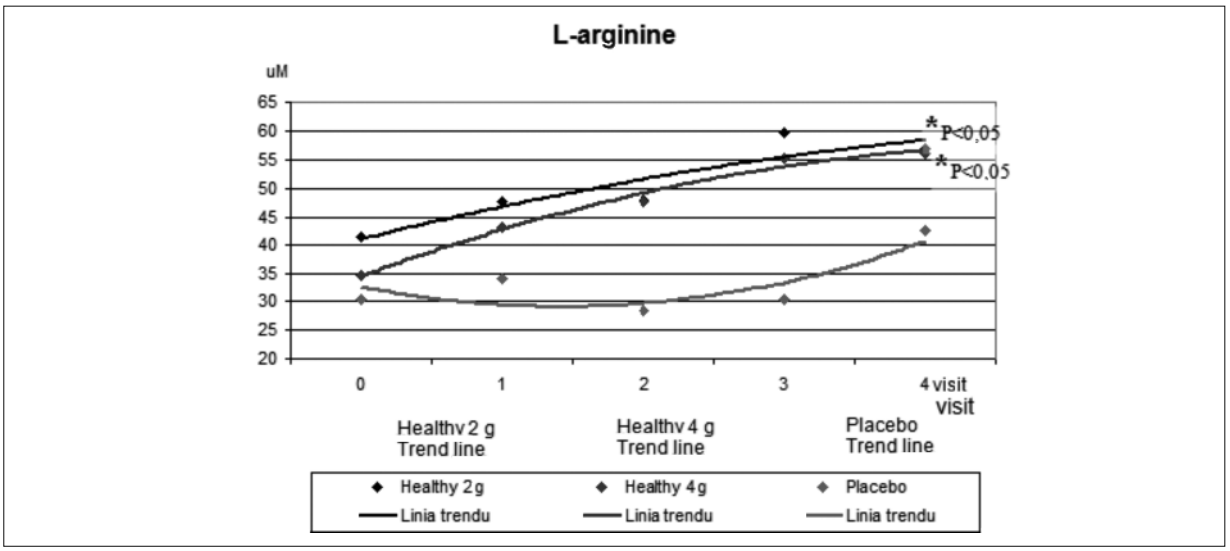

Figure 4. Graphic representation of average levels of L-arginine in healthy individuals (statistical significance with $p < 0.05$).

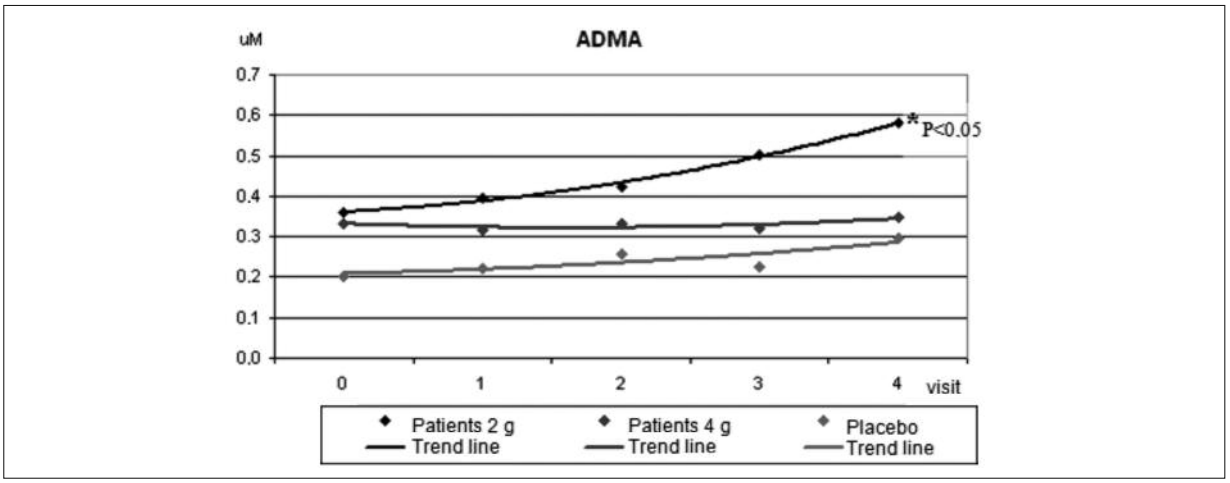

Figure 5. Graphic representation of average levels of ADMA in patients with hypertension (statistical significance with $p < 0.05$).

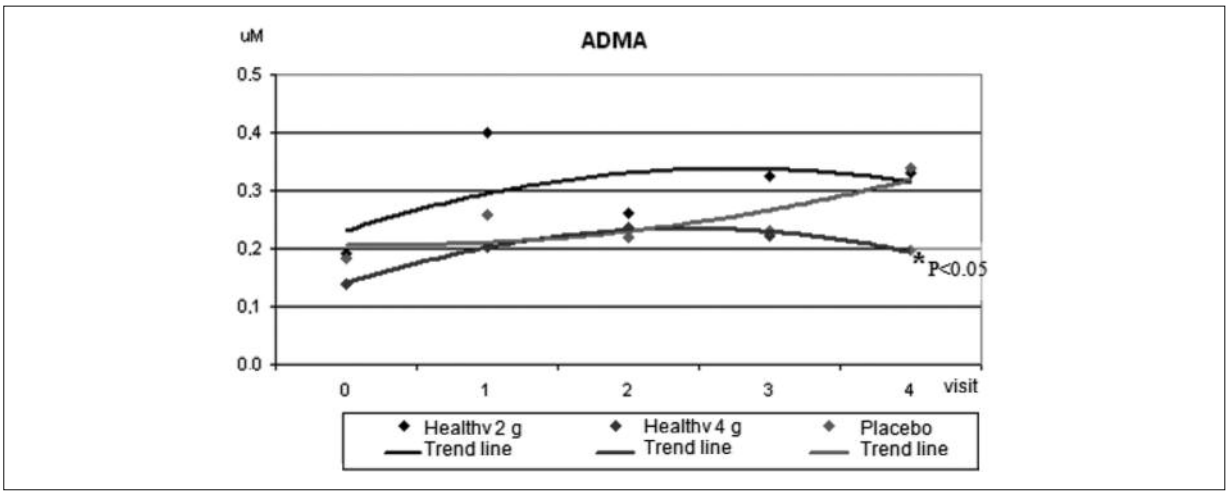

Figure 6. Graphic representation of average levels of ADMA in healthy individuals (statistical significance with $p < 0.05$).

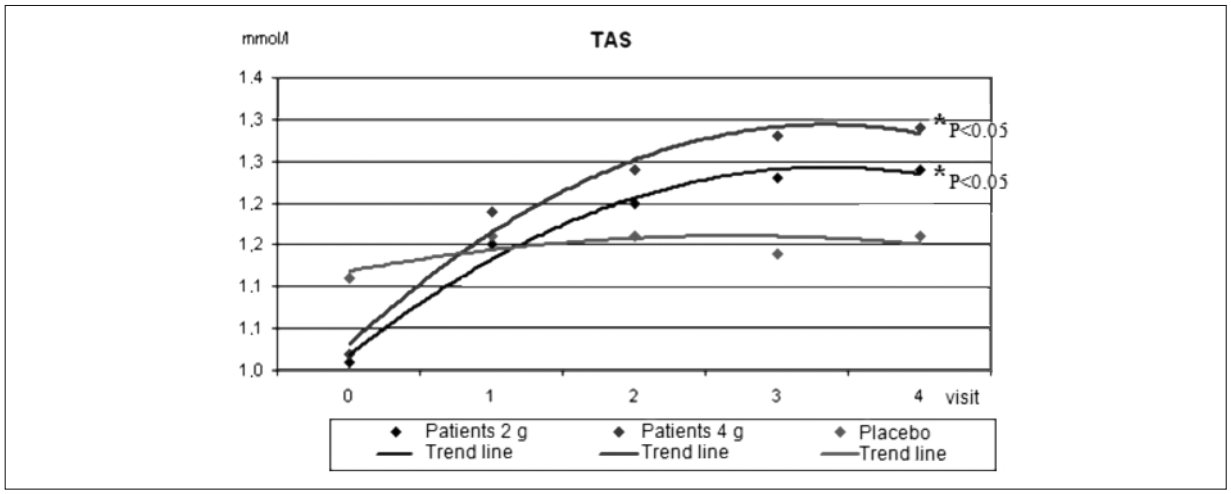

Figure 7. Graphic representation of average levels of TAS in patients with hypertension (statistical significance with $p < 0.05$).

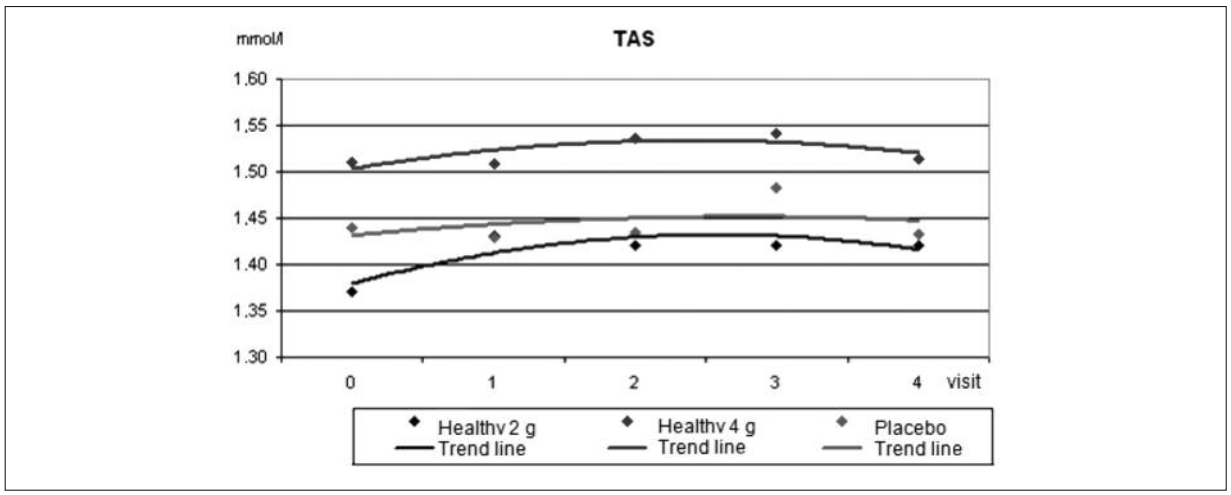

Figure 8. Graphic representation of average levels of TAS in healthy individuals.

antioxidant mechanisms effectiveness in patients with arterial hypertension. The level of TAS is mainly influenced by non-enzymatic low-molecular antioxidants[28]. According to Rendax Company data, normal range of TAS for European population is 1.30-1.77 mmol/l. However, it is advisable to define the range for each laboratory due to some local differences that may occur (for example cased by genetic factors). TAS level measured in our research was normal in control group while being significantly lowered in patients with hypertension. Statistically significant increase in TAS level was observed in subgroups of patients with hypertension treated with L-arginine, reaching the lower range of normal level at the end of treatment. Moreover, in subgroup treated with 3 × 4 g dose the increase was significantly higher than in patients taking placebo. Beneficial influence of L-arginine on the oxidative stress indices was described by Jabłecka et al[29] in a research focusing on detecting NO and TAS levels in patients with sclerotic ischaemia of lower limbs, treated with 3 × 2 g or 3 × 4 g of L-arginine. After 28 days of supplementation statistically significant correction of the whole antioxidant status was noticed, which was interpreted as a proof of indirect antioxidative effect of L-arginine.

Böger et al[30] were one of the first who found antioxidative effect of L-arginine. The Authors revealed restraining effect of this amino acid on O_2^- release in *in vivo* material. Moreover, while decreasing production of O_2^-, L-arginine increased amount of available antioxidants which were protecting native forms of LDL from oxidation.

Lantos et al[31] revealed the influence of hypotensive treatment on TAS level in patients with arterial hypertension. 47 patients with initial TAS values below the norm (1.11 ± 0.15 mM) after treatment showed increased level of TAS.

It appears that the influence of L-arginine on TAS not only results from lowering the arterial tension but mainly from correction of NO bioavailability. Supplementation with L-arginine significantly increases the concentration of nitric oxide, which can take part in inhibition of ROS without affecting the main function of NO (vasorelaxation)[32].

It cannot be excluded that L-arginine, lowering oxidative stress, probably restrains indirectly the increase in concentration of vasoconstrictors secreted under influence of ROS, such as ET-1[33].

Changes in plasma concentration of L-citrulline confirmed the increased synthesis of NO in examined group. L-citrulline is a co-product of biosynthesis of NO catalysed by NOS and its levels are closely related to the dynamics of nitric oxide synthesis[34]. Such interdependence is widely used in studies aiming to reveal increased synthesis of NO from L-arginine[35-37]. Kerry et al[38] point out that supplementation with L-arginine leads to increase of L-citrulline plasma concentration and decrease of blood pressure and peripheral vessels resistance. Oral supplementation with L-arginine that does not lead to increase of L-citrulline concentration does not affect blood pressure[39].

Initial levels of L-citrulline measured in presented research were slightly lower than in other available laboratories[19]. These discrepancies might result from population differences and varied methods of measure.

Significant increase in L-citrulline level was observed in group of patients treated with L-arginine. The concentration of L-citrulline increased (not significantly) also in healthy individuals supplemented with L-arginine. Such dependences confirm the mechanism of L-arginine acting by restoring biodiversity of NO in case of the competitive inhibition of NOS by increased ADMA levels in patients with hypertension[40,41].

ADMA has been considered by some Authors a new cardiovascular risk factor. Cooke[15] called ADMA, "an Uber marker" i.e. a marker including the influence of traditional and some of new risk factors.

The main mechanism underlying biological influence of ADMA is the competitive inhibition of NO synthases. Vallance et al[42] were the first to notice the endogenic inhibition of NOS as a result of ADMA accumulation in dialyzed patients. Dialysis was lowering ADMA level, restoring normal function of endothelium.

The experimental data suggest that high concentrations of ADMA may disturb the creation of active eNOS dimmer, which consequently leads to decrease of NO production. Moreover, the NO synthase begins to act as a reductor creating free radicals which intensify the oxidative stress[43].

It was proven that high concentrations of ADMA restrain the activity of L-arginine y^+ transporters[44,45]. It is also known that ADMA level *in vitro* antagonizing L-arginine are much higher than observed *in vivo*. *In situ* concentration of ADMA in the neighbourhood of endothelial cells was not recognized so far but it is presumed that intercellular levels of ADMA are 8 to 10 fold greater than the plasma level[46,47]. It points out that the migration of L-arginine may be hampered in patients suffering some cardiovascular

diseases including arterial hypertension[48,49]. The literature indicates approximately two times higher level of ADMA in patients with arterial hypertension than in healthy people[9,50,51].

There are still controversies associated with the establishment of norm for ADMA. One of first such trial was the study performed by Schulze et al[52] on 500 healthy people. The examination revealed reference levels of ADMA in a range of 0.36 to 1.17 μM (0.69 μM on average). Horowitz and Heresztyn[53] analyzed the methods of estimation of ADMA level revealing too great dispersion of ADMA values (some of which significantly overvalued compared to most extensive laboratories). Most of observed differences results undoubtedly from different methods of estimation (HPLC, MS, ELISA). The Authors conclude that average ADMA level in healthy population is within the range of 0.4 to 0.6 μM and increases with age. It is also noted that in many researches relatively small increases of ADMA levels are associated with significant influence on assumed end points.

ADMA levels observed in this study are lower or within normal range. Twofold increase of initial ADMA levels was observed in group of patients with hypertension.

Some kind of surprise was increase of ADMA levels observed during our study which was statistically significant in both healthy individuals and patients with hypertension treated with L-arginine. L-arginine is considered a substance decreasing the risk generated by increased levels of ADMA (for example by competitive displacing ADMA from eNOS or reducing oxidative stress). Böger et al[54] discovered decreased levels of ADMA after supplementation with 3 g of L-arginine per 24 hours. Similarly, Lucotti et al[55] observed that ADMA levels decreased by 27 % after 6 months of L-arginine supplementation (6.4 g per 24 hours) in patients with CAD (coronary artery disease). Other Authors[56] observed the increase of L-arginine to ADMA ratio in patients with hypertension and microvascular angina after 28 days of L-arginine supplementation (6 g per 24 hours). Strong positive correlation between ADMA and L-arginine levels was also observed by Perticone et al[57] and Moss et al[49]. Decrease in L-arginine level was observed in researches concerning substitute hormonal therapy which seemed to reduce level of ADMA[58,59].

In presented study statistically significant increase of L-arginine levels was observed in groups taking this amino acid. Obtained results may reflect the strong correlation between ADMA and L-arginine levels, which needs further research and explanation. It cannot be excluded that the increase of ADMA level was caused by displacing this substance from NOS by L-arginine. Perticone et al[6] observed that ADMA and L-arginine level may increase parallel in patients with essential hypertension. The Authors also found insulin resistance (measured by homeostasis model assessment – HOMA) as a strong determinant of endothelial dysfunction in hypertension and showed that association between ADMA and insulin resistance may underlie a possible mechanism of ADMA-induced vascular damage. Such hypothesis, however, needs further research.

Conclusions

Oral supplementation with L-arginine increases the level of arginine, citrulline and TAS in patients with mild arterial hypertension. It confirms that increased concentrations of this amino acid lead to reduction of oxidative stress by stimulating NO biosynthesis.

Increase in ADMA level in plasma after 28 days of L-arginine supplementation was observed in patients with mild arterial hypertension. Despite various interpretations it can be assumed that this result confirms the correlation between ADMA and L-arginine.

Acknowledgements

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