The effect of L-arginine on microvascular reactivity in normotensive subjects with a family history of hypertension

Vpliv L-arginina na odzivnost mikrožilja pri osebah z normalnim krvnim tlakom, družinsko obremenjenih z arterijsko hipertenzijo

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Izvleček

Izhodišča: Eno od ključnih vlog pri nastanku, razvoju in napredovanju esencialne arterijske hipertenzije igra okvarjeno delovanje endotela zaradi zmanjšane razpoložljivosti dušikovega oksida. Z našo raziskavo smo želeli ugotoviti, ali lahko zaužitje L-arginina izboljša delovanje endotela in s tem delovanje mikrožilja pri osebah z normalnim tlakom, a družinsko obremenjenih z arterijsko hipertenzijo.

Metode: V obdobju naše prospektivne raziskave smo zdravim moškim z normalnim krvnim tlakom (N = 30), starim od 20 do 30 let, ki smo jih razdelili v dve skupini glede na družinsko obremenjenost s hipertenzijo, z napravo Task Force Monitor merili srčno-žilne parametre v mirovanju pred in po zaužitju 0,9 g L-arginina. Prav tako smo z lasersko dopplersko metodo merili pretoke v mikrožilju kože na podlakti pred in po zaužitju 0,9 g L-arginina. Od endotela odvisno vazodilatacijo smo ocenjevali z iontoforezo acetilholina, od endotela neodvisno vazodilatacijo pa z iontoforezo natrijevega nitroprusida.

Rezultati: Po zaužitju L-arginina sta se pri obeh skupinah preiskovancev statistično značilno zmanjšala srčna frekvenca in minutni volumen srca (parni t-test, $p < 0,05$), medtem ko se arterijski tlak ni statistično pomembno spremenil. Pri družinsko obremenjenih osebah z normalnim krvnim tlakom pa se je po zaužitju L-arginina akutno izboljšala od endotela odvisna vazodilatacija mikrožilja kože (parni t-test, $p < 0,05$), kar se ujema s predpostavko o že prisotni okvari delovanja endotela.

Zaključki: Pokazali smo, da se je po zaužitju L-arginina pri družinsko obremenjenih osebah z normalnim krvnim tlakom v primerjavi z družinsko neobremenjenimi osebami z normalnim krvnim tlakom izboljšala od endotela odvisna vazodilatacija. Rezultati raziskave govorijo v prid L-argininu kot sredstvu, ki izboljšuje delovanje endotela in s tem najverjetneje preprečuje ali vsaj upočasnjuje nastanek esencialne hipertenzije.

Abstract

Background: An increasing number of studies support the hypothesis that endothelial dysfunction due to reduced availability of nitric oxide plays a key role in initiation, development and progression of essential hypertension. The aim of our study was to determine whether the ingestion of L-arginine actually improves microvascular function in normotensive subjects with a family history of hypertension.

Methods: 30 normotensive healthy men, aged 20–30 years, were divided into two groups according to the family history of hypertension. We measured ECG, heart rate, systolic and diastolic arterial pressure, cardiac output, stroke volume, total peripheral resistance (Task Force Monitor) and laser Doppler (LD) flux in the microvessels of the skin on the forearm at rest, before and after the administration of 0.9 g L-arginine. The endothelium-dependent vasodilation was assessed by iontophoresis of acetylcholine and the endothelium-independent vasodilation by iontophoresis of sodium nitroprusside.

Results: After the ingestion of L-arginine the heart rate and the cardiac output statistically significantly decreased in both groups (paired t-test, $p < 0.05$). Arterial pressure did not change significantly. Stroke volume decreased and total peripheral resistance increased only in the group of subjects with a family history of hypertension (paired t-test, $p < 0.05$) The ingestion of L-arginine in predisposed normotensive subjects acutely improved the endothelium-dependent vasodilation (Dunnett's test, $p < 0.05$), which is consistent with the assumption that endothelial dysfunction is already present in these subjects.

Conclusions: In subjects with a family history of hypertension L-arginine improved endothelial function. This justifies L-arginine as a potential agent for the prevention and/or treatment of arterial hypertension.

Introduction

Endothelial dysfunction plays an important role in the development of cardiovascular diseases.[1,2] The endothelium does not merely act as a barrier between the vessel lumen and the muscle layer of the vessel wall. It is a dynamic structure that affects vascular tone, participates in hemostasis, inflammation, and angiogenesis.[3] It plays an important role in local blood flow regulation, it releases vasoconstrictors (endothelin, thromboxane, prostaglandin H2, angiotensin II, superoxide anion)[4] and vasodilators (nitric oxide (NO), prostacyclin and the recently discovered endothelium-derived hyperpolarizing factor (EDHF)),[5] under the effect of mechanical or chemical stimuli. Since these vasodilators are produced by the endothelium and act on the vascular smooth muscle cells, this type of vasodilation is referred to as endothelium-dependent vasodilation.[6] Vasodilators such as nitroglycerin, sodium nitroprusside (SNP) and other nitrates, release a highly reactive free NO radical, which acts directly on the smooth muscle cells in the blood vessel walls, and produces endothelium-independent vasodilation.[7]

Endothelial dysfunction is defined as a systemic pathological process that progresses from early functional changes in the endothelium (impaired endothelium--dependent vasodilation and enhanced vasoconstriction) to a structurally modified microvasculature, which contributes to the vessel wall stiffness and further deterioration of the endothelial function.[8] The reduced availability of active NO plays one of key roles in endothelial dysfunction and consequently in the development of cardiovascular disease.[9,10] The NO molecule is produced from the amino acid L-arginine; the reaction is catalyzed by the enzyme endothelial NO synthase (eNOS).[11] NO is a highly potent vasodilator and is essential in regulating blood flow and arterial pressure.[10] L-arginine is the only substrate for NO synthesis and is thus essential for endothelium-dependent vasodilation.[12]

An increasing number of studies suggest that at least minimal inflammation of the vessel wall is present in essential arterial hypertension. L-arginine is metabolized in two different ways: 1) the enzyme eNOS metabolizes it to NO, and 2) the enzyme arginase converts the L-arginine into ornithine and urea. In cases of vascular disease and chronic inflammation the arginase activity is elevated.[13] An individual's ability to fight inflammation depends on the balance between these two reactions.[14] However, it remains unclear to what extent the inflammatory process effectively contributes to the development of essential hypertension.[15] Arginase-mediated L-arginine degradation reduces the availability of L-arginine, which leads to a reduced formation of NO molecules and an increased production of reactive oxygen species (ROS).[14] Arginase also increases the sensitivity of the endothelial cells to the endogenous eNOS inhibitor, asymmetric dimethyl-L-arginine (ADMA), the concentration of which is elevated in various pathological conditions associated with impaired endothelial function and is a powerful risk factor for the development of cardiovascular disease.[2,16,17]

It seems that under physiological conditions L-arginine is produced endogenously in sufficient quantities. Factors such as aging and various pathological conditions (inflammation, injury, starvation, stress) can lead to its deficiency.[13,14] L-arginine has been proven to be an effective antihypertensive agent by a number of studies on animal models of atherosclerosis, hypercholesterolemia and hypertension,[18,19,20] but its effects on the arterial pressure in humans were inconsistent. Lerman et al.,[21] Chin-Dusting et al.[22] and Adams et al.[23] showed no change in arterial pressure in healthy normotensive subjects while other researchers have found

that ingestion[24] or intravenous infusion of different concentrations of L-arginine reduced arterial pressure in healthy normotensive [25-27] as well as in hypertensive subjects.[27]

Most studies of L-arginine as antihypertensive agent were performed on conductive arteries. Regardless the mechanisms that initiate the increase in arterial pressure, resistant vessels (small resistant arteries, arterioles and capillaries) are key elements in the control of arterial pressure.[28] The aim of the present study was to determine the effect of L-arginine on the endothelium of resistant arteries. It would be worthy to know whether ingestion of L-arginine improves microvascular function in normotensive subjects with a family history of hypertension.

Subjects and methods

Subjects

Thirty young, healthy, normotensive volunteers were recruited. All the subjects selected were males, in order to avoid the effect of fluctuations of sex hormones on the functioning of the cardiovascular system, which is typical for women during reproductive years.[29] Participants were 20 to 30 years old because at this age functional changes of the cardiovascular system are already present in subjects that will develop hypertension at a later stage.[30] All the subjects had a systolic arterial pressure (SAP) lower than 140 mmHg and a diastolic arterial pressure (DAP) lower than 90 mmHg. Arterial pressure was measured on the right upper arm every 2 minutes for approximately 2 hours using the automated biomedical device Task Force Monitor. For analysis we took the average value. Subjects were divided into two groups in accordance with their family history of arterial hypertension. The first group included 15 subjects (mean age 23.9 ± standard deviation 2.1 years), who had a family history of arterial hypertension, and a control group of 15 subjects (mean age 23.9 ± standard deviation 2.5 years) without a family history of arterial hypertension. The subjects were included into the first group if at least one parent had been receiving treatment for arterial hypertension from the age of 55 or earlier. Both groups had the same number of subjects and did not differ in their body mass index (BMI) and physical activity levels. All the subjects had abstained from smoking and drinking alcohol, coffee and tea for at least eight hours prior to the measurements and none of them were suffering from an acute illness. None of the subjects were on any kind of medication. The study was approved by the Medical Ethics Committee of the Republic of Slovenia. Each of the subjects was informed in detail on the examination and the potential risks involved and signed a Statement of Informed and Voluntary Consent for Participation in the Study.

Task Force Monitor

Using an automated biomedical device Task Force Monitor (CNSystems Medizintechnik, Austria), which is discussed in more detail elsewhere,[31] electrocardiograms, heart rate (HR), continuous (beat-to-beat) SAP, DAP and mean arterial pressure, stroke volume, cardiac output and total peripheral resistance at rest were recorded. Individual R-R intervals were used for spectral analysis of the 5-minute recordings at rest. The Autoregressive Transform method was employed. The results were expressed in Power Spectral Density, the squared amplitude calculated for each frequency. The area under the power spectrum curves of the high frequency band (HF 0.15–0.4 Hz) and the low frequency band (LF 0.04–0.15 Hz) was determined, the former being an indicator of parasympathetic nervous system activity[32-34] and the latter being particularly sensitive to cardiac sympathetic nerve activity. The coefficients LF/HF (sympathovagal balance), LF/(LF + HF) (primary sympathetic modulation of the HR),[35] and baroreflex sensitivity (BRS) during rest were calculated. BRS was determined by the sequence method, based on the computer identification in the time domain of spontaneously occurring sequences of four or more consecutive beats characterized by either a progressive rise in SAP and lengthening in R-R interval or by a progressive decrease in SAP and shortening in R-R interval.[36,37]

Laser Doppler flowmetry

The cutaneus microcirculatory flow was measured on the volar surface of the forearm with the Laser Doppler (LD) method, which is discussed in more detail elsewhere.[38] This method enables us to obtain semi-quantitative measurements of the cutaneous microcirculatory flow. The method is based on the reflection of laser light from moving red blood cells in the blood vessels, leading to the Doppler effect: a shift in the wavelength of light. The final value of the flow is proportional to the number and velocity of the red blood cells and is expressed in arbitrary perfusion units (PU). In our study, LD flux was measured by means of Periflux P4001 Master/4002 Satellite LD monitor (Perimed, Sweden). LD probes (PF481) with a patch and a pad for the substance that was added by iontophoresis, were attached to the volar surface of the left forearm. Placement of the LD probe on the left forearm between individual measurements varied. LD probe was attached to parts of the forearm without visible surface veins. The light with a wavelength of 780nm was used. The sampling rate was 500/sec. Analogue signals were converted to a digital form using an analog-to-digital converter and stored on a personal computer for further analysis.

Iontophoresis

Iontophoresis is a non-invasive method that enables the local introduction of charged molecules through the skin using a weak electrical current.[38,39] Endothelium--dependent vasodilation was assessed using acetylcholine (ACh), while endothelium--independent vasodilation was achieved with the use of sodium nitroprusside (SNP). Both substances were introduced into the skin by iontophoresis. Following ACh and SNP iontophoresis, vasodilation typically occurs and the effect of these vasodilators lasts for several minutes. The effect can be monitored by measuring the LD flow.[38] In our study, we used the Perimed's PeriIont PF 382 (Perimed Sweden) device. A 1 % ACh solution in deionized water (Merck, Germany) and a 1 % SNP solution in deionized water (Merck, Germany) was applied. The ACh and SNP iontophoresis protocol as described by Morris and Shore was used.[38] When introducing the positively charged ACh, the electrode used to deliver electrical current was an anode. ACh was introduced with a positive DC electric current of 0.1mA for 30s, repeated every 60s, at least 16 times, until a plateau was reached, when the flow was no longer increasing. The negatively charged SNP was introduced with a cathode. When introducing SNP, a negative DC current of 0.1mA for 30s was used. This was repeated every 120s, eight times.[38]

Protocol

We performed a total of four measurements in each subject. The entire protocol was completed in approximately 2.5 hours. For the first measurement, we focused on the endothelium-dependent vasodilation by iontophoresis of ACh (150µl, 1 % solution), and for the second measurement, the endothelium independent vasodilation by SNP (150µl, 1 % solution) was examined. The subject then ingested a solution of 0.9 g arginine hydrochloride dissolved in 200mL of water. This was followed by a 30-minute break, during which the level of the C-reactive protein (CRP) in each subject was measured by taking a drop of blood from the tip of the finger , in order to exclude infection. It was hypothesized that L-arginine had been already absorbed from the gastrointestinal tract 30 minutes after ingestion. The third measurement was taken to determine the effect of L-arginine on the endothelium-dependent vasodilation and the fourth to determine the effect of L-arginine on the endothelium-independent vasodilation.

Data evaluation and statistical analysis

The digital signals of the LD flow were analyzed via the computer package 'LDDA acquisition system' (Nevrokard Kiauta Slovenia). The LD flow at rest was expressed as the average value of the measurements during a 5-minute recording. The LD flow values during iontophoresis were expressed as a percentage of the basal flow for each subject. The LD flow responses to the

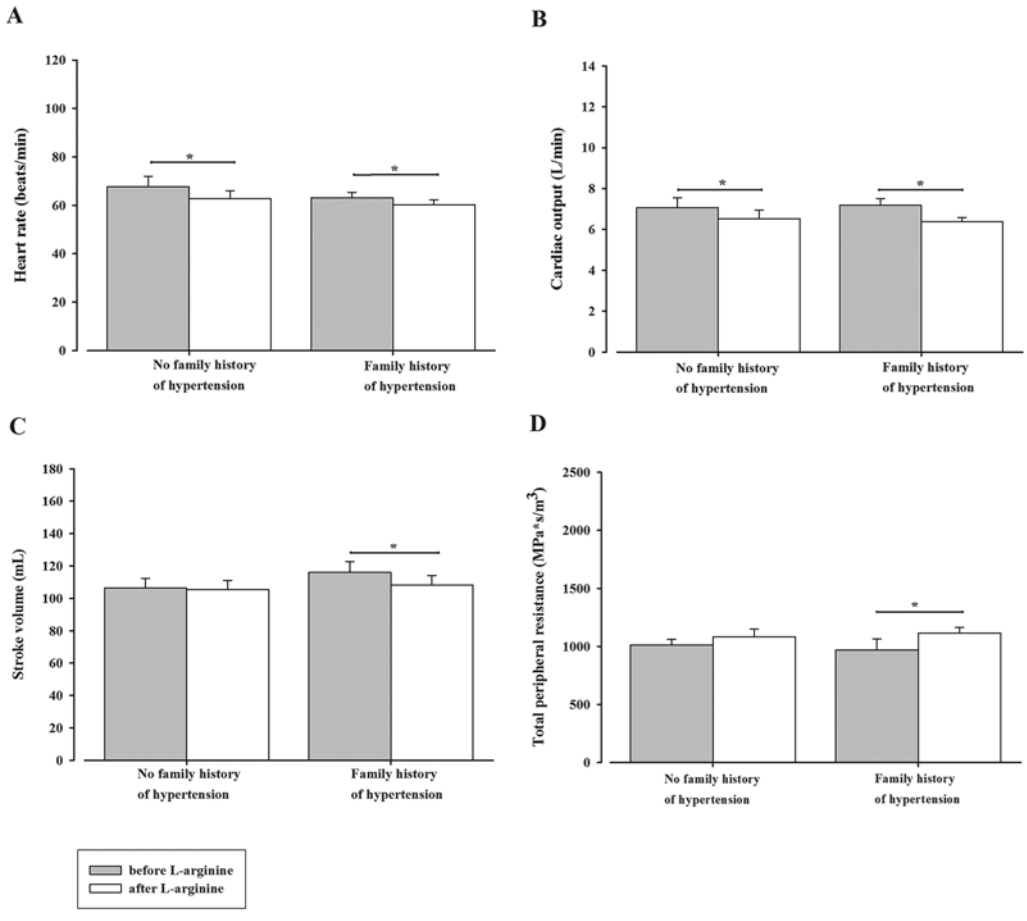

Figure 1: A–Heart rate, B–cardiac output, C–stroke volume and D–total peripheral resistance–before and after the administration of L-arginine in subjects without a family history of hypertension and those with a family history of hypertension. (Data are given as mean values ± SE, *statistically significant difference before and after ingestion of L-arginine at $p < 0.05$).

provocation tests described were evaluated with an analysis of the variance for repeated measurements (RM-ANOVA). The values obtained through the measurements taken with the Task Force Monitor were compared between the two groups with a t-test. In each group, the values obtained before and after the administration of L-arginine, were compared with a paired t-test. All the results are given as mean values and standard errors of means (± SE). The criterion of significance was $p < 0.05$.

Results

Before ingestion of L-arginine, there were no statistically significant differences between the two groups in the mean values of HR, SAP, DAP and mean arterial pressure, stroke volume, cardiac output, total peripheral resistance and LD flow. We noticed that the SAP measured on the upper arm of individuals with a family history of hypertension were slightly, but not significantly, higher than in control individuals. All subjects had a level of CRP below 8 mg/L. By measurement of CRP major acute inflammatory processes were excluded.

Overall cardiovascular response to L-arginine ingestion

We noticed a statistically significant decrease in HR and cardiac output following the administration of L-arginine in healthy subjects without a predisposition as well as in normotensive subjects with a family history of hypertension (paired t-test, $p < 0.05$), as shown in Figures 1A and B. Besides, in normotensive subjects with a family history of hypertension we noticed a statistically significant decrease in stroke volume (paired t-test, $p < 0.05$) and increase in total peripheral resistance (paired t-test, $p < 0.05$), as shown in Figures 1C and D.

There were no statistically significant differences in SAP, DAP and mean arterial pressure following the administration of L--arginine either in individuals with a family history of hypertension or in the group of

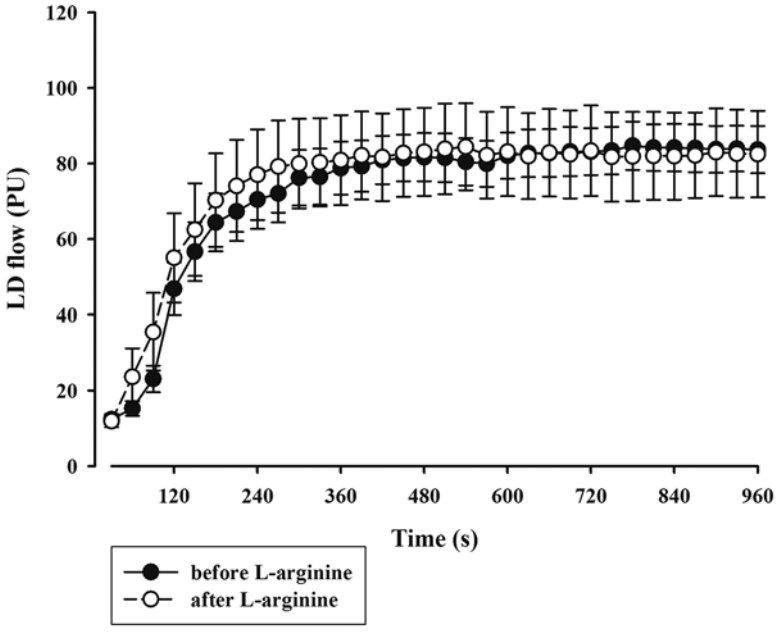

Figure 2: Endothelium-dependent vasodilation in the skin microvasculature in normotensive subjects without a family history of hypertension, before and after the ingestion of L-arginine. (Data are given as mean values ± SE.)

subjects without a family history of hypertension. There were also no significant differences between both groups of subjects following the administration of L-arginine in the LD flow values at rest and in the ratio between sympathetic and parasympathetic tonic activity as determined by HR variability analysis. The differences in the LF (representing mainly sympathetic activity) and HF (representing parasympathetic activity) part of the spectrum of HR variability and their ratio before and after administration of L-arginine were not statistically significant between the two groups. There were also no statistically significant differences in BRS.

Microvascular response to ACh iontophoresis

ACh iontophoresis was used to assess the endothelium-dependent vasodilation of the skin microvasculature via endothelial synthesis of the vasodilator NO. In normotensive subjects without a family history of hypertension, there was no statistically significant difference in skin LD flow after ingestion of L-arginine (Figure 2), while there was a statistically significant increase in the LD flow in normotensive subjects with a family history of hypertension (Figure 3) (paired t-test, $p < 0.05$).

Microvascular response to SNP iontophoresis

The iontophoresis of SNP, which releases NO, was used to assess the endothelium-independent vasodilation of the skin microvasculature. There were no significant differences between the two groups in the skin microvasculature LD flow before and after the ingestion of L-arginine.

Discussion

Systemic effect of L-arginine

In both groups of subjects, there were no changes in the SAP, DAP and mean arterial pressure after ingestion of L-arginine. Both groups also showed a statistically significant decrease in HR and cardiac output. In addition, a statistically significant decrease in stroke volume and increase in total peripheral resistance was observed in normotensive subjects with a family history of hypertension. The most likely explanation is that L-arginine slightly dilated arterioles and so reduced venous return. This was followed by the fall of HR and cardiac output. Decreased cardiac output resulted in decreased mean arterial pressure, which was compensated by arteriolar vasoconstriction via baroreflex, except in areas where the need for blood flow was high. The overall result was redistribution of blood, unloading of the heart and a small increase in total peripheral resistance.

L-arginine was proven to be an effective antihypertensive agent by a number of studies on animal models,[18-20] but its effects on the arterial pressure in humans varied. Present study showed no change in arterial pressure both in healthy normotensive subjects and those with a family history of hypertension, after the administration of L-arginine. Similar results were obtained by Lerman et al.[21], Chin-Dusting et al.[22], Adams et al.[23], while other researchers have found that ingestion[24] or intravenous infusion of different concentrations of L-arginine reduced arterial pressure in healthy normotensive subjects[25-27] as well as in hypertensive subjects.[27] The difference in the obtained results may be explained by different me-

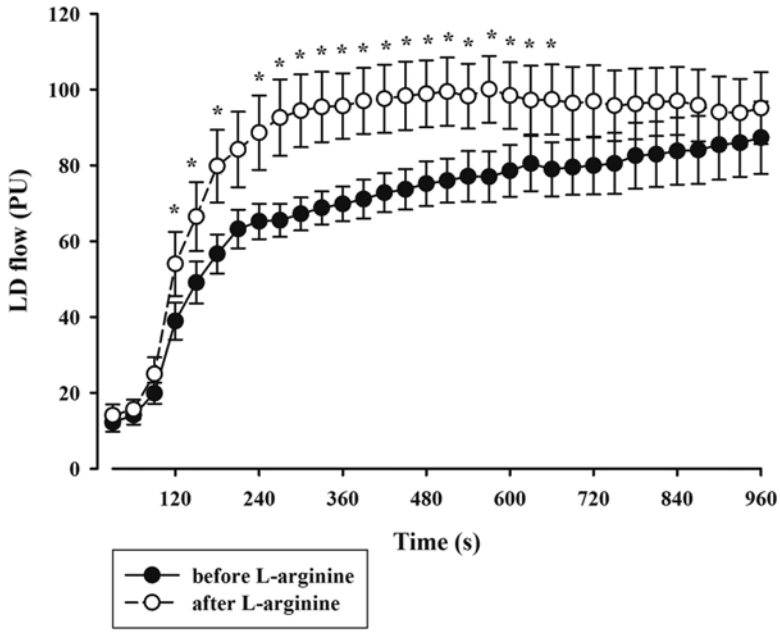

Figure 3: Endothelium-dependent vasodilation in the skin microvasculature in normotensive subjects with a family history of hypertension before and after the administration of L-arginine. (Data are given as mean values ± SE, *statistically significant difference before and after ingestion of L-arginine at $p < 0.05$).

thods of application and/or different doses used.

No change in arterial pressure in response to ingestion of L-arginine in the present study is in agreement with unchanged BRS, sympathetic and parasympathetic activity. One of the basic characteristics of the developed hypertension is the increased sympathetic compared to the parasympathetic system activity.[40] This was also proven in patients with borderline hypertension and some normotensive subjects with a family history of hypertension.[41] Our results of spectral analysis of HR variability in subjects with a family history of hypertension suggest there is no increase in the ratio between sympathetic and parasympathetic tonic activity on the SA node. It is still not entirely clear whether the reduction in BRS is one of the pathogenetic mechanisms in the development of primary arterial hypertension or the reduction in BRS is a consequence of high arterial pressure. Most researchers lean toward the latter hypothesis,[42] which is in agreement with the findings of our study.

The effect of L-arginine on the vasodilator capacity of microcirculation

In subjects with a family history of hypertension, the ingestion of L-arginine resulted in a statistically significant improvement in the endothelium-dependent vasodilation and an increased cutaneous microvascular LD flow, which was not observed in subjects without a family history of hypertension. The endothelium-independent vasodilation of skin microvasculature was comparable in both groups and did not change after the ingestion of L-arginine. Thus, we were able to show a specific effect of L-arginine on endothelium-dependent vasodilation at the level of microcirculation.

King et al.[43] and Gupta et al.[44] tried to determine, whether inflammation of the vessel wall is already present in subjects in the pre-hypertensive stage of arterial hypertension (defined as SAP between 120 and 139 mmHg and DAP between 80 and 89 mmHg)[43] and in subjects with already developed hypertension.[44] The high sensitivity CRP (Hs-CRP) value in the serum which can detect very low CRP levels was used as an indicator of inflammation in the vessel wall. The results showed significantly elevated Hs-CRP levels in both groups of subjects. In our study none of the subjects had CRP level higher than 8 mg/L., Unfortunately, our method for detection was not sensitive enough to detect CRP levels under 8 mg/L, therefore minimal inflammation cannot be excluded.

NO deficiency may be due to an irregularities in the activity of the eNOS enzyme, a deficiency of eNOS cofactors, L-arginine deficiency or a reduced cellular uptake of L-arginine, which is the sole substrate for NO synthesis, disruptions of the insulin signaling pathways (impaired insulin effect on the cellular uptake of L-arginine and on arterial pressure regulation), increased endogenous eNOS inhibitors (e.g. ADMA) or increased degradation of L-arginine with arginase and concurrent greater production of ROS, which occur abundantly in a dysfunctional endothelium.[12]

Panza et al.[45] showed reduced endothelial NO synthesis in patients with essential hypertension, compared with a control group of healthy normotensive subjects. Taddei et al.[46] and McAllister et al.[47] tried to answer the question whether endothelial dysfunction due to the reduced availability of NO plays an important role in the evolution and progression of essential hypertension, or

whether it is merely the result of increased arterial pressure. They found that endothelial dysfunction is present in individuals with a family history of hypertension using intrabrachial infusion or ingestion of L-arginine and measuring forearm blood flow response or flow-mediated dilation of brachial artery, which is consistent with the results of our study. The results of these studies might indicate that the endothelial dysfunction is not merely a result of high arterial pressure, but a possible cause for the development of essential hypertension.[46,47]

L-arginine as a dietary supplement increases the levels of plasma L-arginine,[12] improves insulin resistance, [12,48] inhibits the activity of the renin-angiotensin system[49] and reduces oxidative stress.[44,46] It was found that L-arginine is essential for vasodilation supplied via NO,[12,46,50-52] which is also in line with our findings. The effects of L-arginine as mentioned above may result in a lower arterial pressure and reduced complications associated with hypertension, which justifies the use of L-arginine as a therapeutic agent for the treatment of arterial hypertension.[12] Finding of the present study that L-arginine improves endothelium-dependent vasodilation in subjects with a family history of hypertension argues in favour of the fact that L-arginine may prevent onset of hypertension. Additional prospective studies are required to determine the long--term effects of L-arginine on the endothelial function and its use for clinical purposes in normotensive subjects with a family history of hypertension and in hypertensive patients.

Conclusions

Our results indicate that the ingestion of L-arginine increases the endothelium-dependent vasodilation of the microvasculature in the group of healthy normotensive subjects with a family history of hypertension. This justifies the use of L-arginine as a potential therapeutic agent for preventing or at least delaying the onset and treatment of arterial hypertension. The ingestion of L-arginine in low doses facilitates easy and accurate dosing, which is accessible to everyone.

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