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Plasma asymmetric dimethylarginine and retinal vessel diameters in middle-aged men

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Abstract

It has been suggested that asymmetric dimethylarginine (ADMA), an endogenous nitric oxide synthase inhibitor, is linked to hypertension and vascular reactivity. Retinal arteriolar narrowing has been observed to associate early with increased risk of hypertension. The objective of this study was to evaluate the role of ADMA as a biomarker for early vascular changes of retinal vessels and thus as a possible biomarker of hypertension risk. Thirty-five healthy white men aged 50.1 years (range, 45-55 years) were studied. Using digitized fundus photography, the following diameters of retinal arterioles and venules were measured 1 disc diameter from the optic disc edge: the mean arteriole width (MAW) and venule width (MVW), the sum of squares of widths of arterioles (SSWA) and venules (SSWV), and the central retinal artery equivalent (CRAE) and venous equivalent (CRVE). Arteriovenous ratio was determined using MAW/MVW, SSWA/SSWV, and CRAE/CRVE. Blood pressure was measured by 24-hour ambulatory recordings and also by resting measurements. Plasma ADMA was determined by a high-performance liquid chromatography tandem mass spectrometry. Plasma ADMA had a strong negative association with MAW, MVW, SSWA, SSWV, CRAE, and CRVE. Arteriovenous ratio measurements did not associate with plasma ADMA or with L-arginine to ADMA ratio, but arteriovenous ratios had a strong association with blood pressure. In a multivariate linear model, plasma ADMA concentration was the most significant predictor of arteriole and venule diameter measurements. These results suggest that plasma ADMA is associated with vascular phenomenon seen in early hypertension and that ADMA may be a potential biomarker candidate for development of hypertension.

1. Introduction

Retinal microvascular abnormalities may reflect the degree of arteriolar damage from hypertension or atherosclerosis [1] and are associated with increased cardiovascular risk [2,3]. Previously, it has also been demonstrated that generalized retinal arteriolar narrowing may precede the development of systemic hypertension [4]. Diminished bioavailability of nitric oxide (NO) impairs endothelium-dependent vasodilation and activates other mechanisms that may play an important role in the pathogenesis of endothelial dysfunction and atherosclerosis [5]. The role of NO in the maintenance of choroidal and retinal flow has been verified by demonstrating that the intravenously dosed NO synthase (NOS) inhibitor monomethylarginine (LMMA) impairs retinal capillary flow in normotensive [6] and in hypertensive patients [7] and that in experimental animal models,

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LMMA blocks vasodilation in isolated ocular blood vessels [8,9]. Asymmetric dimethylarginine (ADMA) is another endogenous competitive inhibitor of NOS that can modulate NO production and is suggested to be a marker of endothelial dysfunction [10,11]. Some studies have reported an association between ADMA and blood pressure (BP) [11,12]. Moreover, myocardial vasodilator capacity and impaired endothelium-dependent brachial artery vasodilation have been found to associate with plasma ADMA levels [13,14].

Generalized retinal arteriolar narrowing has been observed to associate with increased risk of hypertension [4]. Thus, this study was performed to clarify the role of plasma ADMA as a biomarker for retinal resistance vessel diameters and therefore also for increased risk of hypertension.

2. Materials and methods

2.1. Selection of subjects

At the beginning of the Tampere Ambulatory Hypertension Study, 97 healthy, untreated, white men were selected from routine health checkup carried out on 35-, 40-, and 45-year-old persons who are residents of the city of Tampere. Details of the study design have been presented earlier [15]. Ten years later, subjects with data on arginine derivatives, BP measurements, and retinal vessel measurements were included in the present analysis. Thus, 35 apparently healthy men without any medication were studied (Table 1). The study was approved by the ethics committee of Tampere University Hospital. All participants gave a written informed consent.

2.2. Measurements of blood vessels

Fundus photographs were taken with the Canon CF-60Z fundus camera (Canon, Tokyo, Japan) while both pupils of the subject dilated. Photographs were macula-centered, 60°-wide, color fundus photographs that were digitized at 5760 dots per inch resolution using the Imacon Flextight Precision II scanner (Imacon, Copenhagen, Denmark). The size of 1 pixel was estimated by using meta-analysis of the average size of the optic disc reported in 13 references (2774 eyes). Detailed description of the procedure has been published earlier [16]. According to those estimations, the size of 1 pixel was 2.81 × 2.81 μm and the average disc diameter was 1.83 mm (652 pixels). Measurements were made by one of the authors (VH) without knowledge of the subjects' clinical data, and the computer program Image-Pro Plus 4.0 (Media Cybernetics, Silver Spring, MD) was used. The optic disc edge was drawn as a circle 326 pixels from the center of the optic disc, and diameters of all arterioles and venules at least 7 pixels in diameter were measured from a circle 1 average disc diameter (1 DD) from the optic disc edge. Vessel borders were determined, and perpendicular vessel diameter was measured. Smokers discontinued smoking for 4 hours before the eye measurements were done.

Table 1
Clinical characteristics of the participants

Variable	Subjects (N = 35)
Age (y)	50.1 ± 4.3
BMI (kg/m^2)	27.4 ± 3.0
Tobacco smoking (no/yes)	17/18
Ambulatory SBP (mm Hg)	123 ± 14
Ambulatory DBP (mm Hg)	84 ± 9
Resting SBP (mm Hg)	140 ± 16
Resting DBP (mm Hg)	86 ± 11
TC (mmol/L)	5.48 ± 1.14
LDL cholesterol (mmol/L)	3.41 ± 1.05
HDL cholesterol (mmol/L)	1.37 ± 0.37
TGs (mmol/L)	1.64 ± 0.96
Creatinine clearance (mL/[s 1.73 m^2])	1.94 ± 0.36
Arginine (μmol/L)	139 ± 34
ADMA (μmol/L)	0.38 ± 0.12
Arginine-ADMA ratio	405.90 ± 168.00
SDMA (μmol/L)	0.79 ± 0.23

Values shown as mean ± SD. BMI indicates body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

2.3. Retinal blood vessel parameters and arteriovenous ratios

Measured arteriole and venule widths from 1 DD from the optic disc edge were used to form the following different retinal blood vessel parameters as described earlier [16]: mean arteriole width (MAW), mean venule width (MVW), sum of squares of widths of arterioles (SSWA), sum of squares of widths of venules (SSWV), central retinal artery equivalent (CRAE), and central retinal vein equivalent (CRVE). Arteriovenous (A/V) ratio is frequently used to represent generalized arteriolar narrowing [17]. The following A/V ratios were determined: MAW/MVW, SSWA/SSWV, and CRAE/CRVE. Measurements of both eyes were used if available (mean of right and left eye results); but if only one fundus picture measurement of the subject was available, then this was used. Detailed description and analyses of the A/V ratios were published earlier [16].

2.4. Blood pressure monitoring

Ambulatory BP monitoring was performed with the previously validated [18,19] DIASYS 200 device (Novacor, Rueil-Malmaison, France). Blood pressure was measured at 15-minute intervals between 6:00 AM and 10:00 PM and at 30-minute intervals between 10:00 PM and 6:00 AM. The 24-hour BP was calculated using hourly means. Only recordings with less than 10% missing or inappropriate values were accepted. The raw data were checked manually, and inappropriate readings [20] were excluded. Resting BP was measured with the participants in the sitting position after 10 minutes of rest using a calibrated aneroid barometer (Speidel and Keller, Jungingen, Germany). Systolic BP was read at the first Korotkoff sound and diastolic BP at the disappearance of the Korotkoff sounds (phase V). The deflation rate was 2 mm Hg/s. Blood pressure was recorded on 2 consecutive days:

before the ambulatory recording (3 measurements at least 1 minute apart) and after it (2 measurements at least 1 minute apart) The average of 5 readings was used for analysis.

2.5. Blood sampling

Blood samples were drawn into EDTA tubes in ice after participants had fasted for 12 hours. Smokers discontinued smoking for 4 hours before the blood samplings were done. Plasma was separated by low-speed ultracentrifugation and stored at −80°C until analyzed. Plasma total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides (TGs), arginine, ADMA, and symmetric dimethylarginine (SDMA) concentrations were determined.

2.6. Determinations of plasma lipids and arginine derivatives

The concentrations of plasma TG, TC, and HDL cholesterol were determined using Cobas Integra 700 automatic analyzer with reagents and calibrators as recommended by the manufacturer (Roche Diagnostics, Basel, Switzerland). The LDL concentration was calculated by using the Friedewald formula because plasma TG levels did not exceed 4.0 mmol/L [21]. The interassay coefficients of variation were 1.4% for the TC, 1.0% for the TGs, and 3.7% for the HDL cholesterol assessments.

Arginine, ADMA, and SDMA were determined by using a high-performance liquid chromatography (HPLC) tandem mass spectrometry. A 200-μL aliquot of plasma was diluted and applied on solid-phase, straight-phase silica column (100 mg Bond Elut; Varian, Palo Alto, CA). After washing with methanol arginine, its methylated derivatives were eluted into 4 mol/L NH_4OH in 50% acetonitrile; and eluent was evaporated with a stream of N_2. Before analysis, dry residue was dissolved in HPLC mobile phase. Tandem mass spectrometry (API3000, Applied Biosystems/Sciex, Foster City, CA) was used to detect arginine and its metabolites after separation on short C18 HPLC column. The mass spectra observed were similar to those found by Vishwanathan et al [22]. Transitions yielding the best signal-to-noise ratios on the API3000 instrument were m/z 203 → 70 for ADMA and SDMA, m/z 189 → 70 for LMMA, and m/z 175 → 60 for arginine. Vishwanathan et al [21] used the Micromeritics (Norcross, GA) tandem mass spectrometry equipment and found m/z 203 → 158 for ADMA and m/z 203 → 172 for SDMA to give the best response. All samples were carried out from the beginning, that is, also through the sample pretreatments, in parallel with and without SDMA spike. Normalized difference in SDMA peaks of samples run with and without added SDMA was calculated. That is the peak height of arginine, and other peaks except SDMA were adjusted to exactly the same level in the 2 samples. The procedure allowed the normalized difference of SDMA between samples containing endogenous SDMA and those containing endogenous plus added SDMA to be calculated. The difference thus gave a response for a known SDMA supplement in each of the samples, allowing calculation of SDMA concentration in the sample containing only endogenous SDMA; that is, SDMA calibration was performed by the method of standard additions for each sample. For other analytes, external standard curves were established by using the difference in normalized SDMA peak heights as an internal standard. For ADMA, total coefficient of variation was 10.3%; and respective intra- and interday component coefficients of variation were 7.0% and 7.6%, respectively, obtained by analysis of a pooled serum sample. Arginine and its methylated metabolites used for calibration were obtained from Sigma Chemicals (St Louis, MO).

2.7. Statistical methods

The data were analyzed with the Statistica for Windows statistics program (StatSoft, Tulsa, OK). The normality of each variable was studied by the Kolmogorov-Smirnov test. Univariate correlation analysis was carried out with the Pearson correlation test for normally distributed variables. Linear regression analysis was used to find out the determinants of retinal arteriole and venule diameter measurements. Data are presented as mean ± SD unless otherwise stated. A P value of less than 0.05 was considered statistically significant.

3. Results

3.1. Clinical characteristics

The clinical characteristics of study subjects are given in Table 1. Study subjects consisted of 35 middle-aged (range, 45-55 years) white men without symptoms of any disease and who had no medication. They had normal to moderately elevated BP and lipids, and normal renal function. Data of retinal vessel measurements were based on the mean of the measurements of 2 eyes in 25 subjects and of 1 eye in 10 subjects.

3.2. Arginine derivatives, BP, and other characteristics

The average plasma L-arginine, ADMA, and SDMA concentrations were 139 ± 34, 0.38 ± 0.12, and 0.79 ± 0.23 μmol/L, respectively. Plasma ADMA concentrations or L-arginine to ADMA ratios were not associated with systolic or diastolic BP values. Plasma arginine derivatives did not associate with age, body mass index, creatinine clearance, or lipids. Concentrations of plasma arginine derivatives were comparable in smokers and nonsmokers.

3.3. Arginine derivatives, retinal arteriole-venule measurements, and smoking

In 1 DD, the MAW and the MVW were 54.1 ± 10.0 and 73.5 ± 12.9 μm, respectively. The average SSWA and SSWV were 38 353.7 ± 11 788.3 and 67 024.8 ± 21 994.8 μm^2, respectively. The mean CRAE and CRVE were 146.0 ± 22.9 and 203.7 ± 31.2 μm, respectively. Retinal vessel diameters

Table 2
Associations between ADMA, ambulatory blood pressure, and retinal vessel diameters

A/V measurements (N = 35)	ADMA		SBP		DBP	
	R	*P*[a]	*R*	*P*[a]	*R*	*P*[a]
MAW	−0.40	0.02	−0.068	0.71	−0.11	0.54
MVW	−0.43	0.009	0.20	0.26	0.040	0.82
MAW/MVW	0.059	0.73	−0.35	0.04	−0.34	0.04
SSWA	−0.48	0.004	−0.069	0.69	−0.17	0.32
SSWV	−0.43	0.01	0.29	0.09	0.14	0.44
SSWA/SSWV	−0.0041	0.98	−0.53	0.001	−0.39	0.02
CRAE	−0.46	0.006	−0.034	0.85	−0.064	0.72
CRVE	−0.43	0.009	0.26	0.13	0.089	0.61
CRAE/CRVE	−0.030	0.87	−0.40	0.02	−0.21	0.23

[a] Pearson correlation analysis.

in general seemed to be slightly wider in smokers than those in nonsmokers. However, a borderline significant difference was observed only for SSWV (74 081.3 ± 22 115.5 vs 59 553.1 ± 19 824.8 μm^2, $P = .05$) and CRAE (153.2 ± 25.6 vs 138.4 ± 17.2 μm, $P = .05$). Smoking intensity (pack years) was partly associated with retinal vessel calibers (SSWV: $r = 0.39$, $P = .03$ and CRVE: $r = 0.37$, $P = .04$, respectively) but not with the arginine derivatives.

Plasma ADMA had a strong negative association with MAW and MVW, SSWA and SSWV, and CRAE and CRVE (Table 2). The A/V ratio measurements (MAW/MVW, SSWA/SSWV and CRAE/CRVE) did not associate with plasma ADMA, arginine, or L-arginine to ADMA ratio; but A/V ratios had a significant association with BP.

In a multivariate linear model, plasma ADMA concentration and, partly, age were the significant predictors of arteriole and venule diameters (Table 3). The other explanatory variables in the model were arginine and smoking.

4. Discussion

The results of this study show that plasma ADMA has a significant negative association between retinal arteriole and venule diameters. In addition, retinal arteriole and venule diameter measurements associate negatively with BP. However, plasma ADMA do not associate with BP in subjects with normal or slightly elevated BP levels. These data suggest that although plasma ADMA does not directly associate with systemic BP, it may have a role in regulating retinal vessel diameter and be a potential biomarker candidate for development of hypertension.

Many of the hemodynamic features associated with essential hypertension can be accounted for by alterations in the structure of resistance vessels [23,24]. Retinal vessels

Table 3
Predictors of retinal vessel diameters, based on a standard univariate and multiregression model

Arteriole measurements

Explanatory variable in	Retinal vessel parameters											
	MAW				SSWA				CRAE			
	B	SE	*β*	*P*	*B*	SE	*β*	*P*	*B*	SE	*β*	*P*
Univariate model												
ADMA (μmol/L)	−34.38	13.56	−0.40	.02	−47 919.6	15 395.67	−0.48	.004	−88.95	30.21	−0.46	.006
Multivariate model												
ADMA (μmol/L)	−33.77	12.93	−0.40	.01	−47 399.6	14 517.11	−0.47	.003	−84.60	27.22	−0.44	.004
Arginine (μmol/L)	−0.079	0.043	−0.27	.08	−87.6	48.75	−0.26	.08	−0.19	0.091	−0.27	.06
Age (y)	0.60	0.35	0.26	.10	760.1	393.12	0.22	.06	1.54	0.74	0.29	.05
Smoking (yes/no)	3.74	2.99	0.19	.22	4307.3	3359.31	0.19	.21	12.35	6.30	0.27	.06
Full model	$R^2 = 0.26$			.01	$R^2 = 0.33$			.003	$R^2 = 0.37$			.001

Venule measurements

Explanatory variable in	Retinal vessel parameters											
	MVW				SSWV				CRVE			
	B	SE	*β*	*P*	*B*	SE	*β*	*P*	*B*	SE	*β*	*P*
Univariate model												
ADMA (μmol/L)	−47.87	17.30	−0.43	.009	−80 975.7	29 473.63	−0.43	0.01	−115.47	41.75	−0.43	.009
Multivariate model												
ADMA (μmol/L)	−45.23	18.15	−0.41	.02	−75 037.8	28 307.22	−0.40	0.01	−107.79	40.73	−0.40	.01
Arginine (μmol/L)	−0.019	0.061	−0.049	.76	−15.4	95.05	−0.024	0.87	−0.030	0.14	−0.031	.83
Age (y)	0.39	0.49	0.13	.44	1670.9	766.55	0.33	0.04	2.21	1.10	0.30	.05
Smoking (yes/no)	4.34	4.20	0.17	.31	12 216.0	6550.39	0.28	0.07	16.11	9.42	0.26	.10
Full model	$R^2 = 0.13$			.08	$R^2 = 0.27$			0.009	$R^2 = 0.25$			.01

B indicates unstandardized regression coefficient; *β*, standardized regression coefficient; SE, standard error; R^2, adjusted coefficients of determination.

represent resistance vessels, and abnormalities of the retinal vasculature may reflect the microvascular damage due to hypertension [25]. It has also been demonstrated that generalized retinal arteriolar narrowing may precede the development of systemic hypertension [4]. Furthermore, persons with familial predisposition had arteriolar narrowing in the prehypertensive stage [26]. Taken together, it is still uncertain whether generalized arteriolar narrowing antedates high BP, occurs as a secondary adaptation, or both.

The results of the prospective studies suggest that plasma ADMA may be a significant predictor of vascular disease [27,28]. In the present study, we measured retinal vessel diameters using digitized fundus photography, which has been demonstrated to be a reliable and highly repeatable method in evaluating retinal microvascular changes [1]. The present study demonstrated a strong negative association between plasma ADMA and retinal arteriolar and venule diameter. We also considered a previously reported finding [29,30] that nonsmokers have slightly narrower retinal vessel calibers than smokers. Thus, smoking was included in the vessel diameter–predicting multivariate model together with arginine, which is competing with ADMA for the same NOS binding site. Despite adding these potential confounding factors to the multiregression model, plasma ADMA concentration was the most important determinant for diameters, suggesting that plasma ADMA may truly modulate retinal vessel diameter.

The ratio of retinal arteriolar to venule (A/V ratio) diameters is often used as a marker of generalized arteriolar narrowing [1,16]. In the present study, we determined A/V ratios using 3 different formulas and found that A/V ratios correlate significantly with systemic BP in agreement with an earlier study [1]. However, A/V ratios were not associated with plasma ADMA. Because ADMA associated with both arteriolar and venule diameters, its effects on A/V ratio values are not anticipated to be profound.

Previously, an association between ADMA and BP has been reported in some studies [11,12], but not in all [31-33]. In this study, there was no association between plasma ADMA and BP. We emphasize that the participants in the present study were normotensive or only mildly hypertensive subjects whose BP values had a narrow range, making it difficult to find correlations. Whereas in a recent study we could confirm a well-known [34] association between systemic vascular resistance and BP, we did not find an association between plasma ADMA and systemic vascular resistance or BP [28]. This study examined the associations in a small group of voluntary subjects. In addition, they all were men. Therefore, some selection bias may be present. Furthermore, it is unknown whether the results can be extrapolated to female subjects.

Taken together, plasma ADMA associates strongly with retinal arteriolar narrowing that in previous studies has preceded the development of systemic hypertension. Thus, plasma ADMA may potentially serve as an early biomarker for hypertension. However, large-scale studies are needed to validate the present findings before ADMA measurements can be offered to physicians as a risk estimation tool.

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References

[1] Hubbard LD, Brothers BS, King WN, Clegg LX, et al. Methods for evaluating of retinal microvascular abnormalities associated with hypertension/sclerosis in the Atherosclerosis Risk in Communities Study. Ophthalmology 1999;106:2269-80.

[2] Klein R, Sharrett AR, Klein BE, et al. Are retinal arteriolar abnormalities related to atherosclerosis? The Atherosclerosis Risk in Communities Study. Arterioscler Thromb Vasc Biol 2000;20:1644-50.

[3] Wong TY, Klein R, Nieto FJ, et al. Retinal microvascular abnormalities and ten-year cardiovascular mortality. A population-based case-control study. Ophthalmology 2003;110:933-40.

[4] Ikram MK, Witteman JCM, Vingerling JR, et al. Retinal vessel diameters and risk of hypertension. The Rotterdam Study. Hypertension 2006;47:189-94.

[5] Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1992;362:801-9.

[6] Delles C, Michelson G, Harazny J, et al. Impaired endothelial function of the retinal vasculature in hypertensive patients. Stroke 2004;35:1289-93.

[7] Michelson G, Warntges S, Harazny J, et al. Effect of NOS inhibition on retinal arterial and capillary circulation in early arterial hypertension. Retina 2006;26:437-44.

[8] Haefliger IO, Flammer J, Luscher TF. Heterogeneity of endothelium-dependent regulation in ophthalmic and ciliary arteries. Invest Ophthalmol Vis Sci 1993;34:1722-30.

[9] Toda N, Toda M, Ayajiki K, et al. Cholinergic nerve function in monkey ciliary arteries innervated by nitroxidergic nerve. Am J Physiol Heart Circ Physiol 1998;274:H1582-9.

[10] Leiper J, Vallance P. Biological significance of endogenous methylarginines that inhibit nitric oxide synthase. Cardiovasc Res 1999;43:542-8.

[11] Miyazaki H, Matsuoka H, Cooke JP, et al. Endogenous nitric oxide synthase inhibitor, a novel marker of atherosclerosis. Circulation 1999;99:1141-6.

[12] Surdacki A, Nowicki M, Sandmann J, et al. Reduced urinary excretion of nitric oxide metabolites and increased plasma levels of asymmetric dimethylarginine in men with essential hypertension. J Cardiovasc Pharmacol 1999;33:652-8.

[13] Böger RH, Bode-Böger SM, Szuba A, et al. Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. Circulation 1998;98:1842-7.

[14] Päivä H, Laakso J, Laine H, et al. Plasma asymmetric dimethylarginine and hyperemic myocardial blood flow in young subjects with borderline hypertension or familial hypercholesterolemia. J Am Coll Cardiol 2002;40:1241-7.

[15] Majahalme S, Turjanmaa V, Weder AB, et al. Blood pressure level and variability in the prediction of blood pressure after 5-year follow-up. Hypertension 1996:725-31.

[16] Hemminki V, Kähönen M, Tuomisto MT, et al. Determination of retinal blood vessel diameters and arteriovenous ratios in systemic

hypertension: comparison of different calculation formulas. Graefes Arch Clin Exp Ophthalmol 2007;245:8-17.
[17] Wong TY, Klein R, Klein BEK, et al. Retinal microvascular abnormalities and their relationship with hypertension, cardiovascular disease, and mortality. Surv Ophthalmol 2001;46:59-80.
[18] O'Brien E, Mee F, Atkins N, et al. Accuracy of the SpaceLabs 90207, Novacor Diasys 200, Del Mar Avionics Pressurometer IV and Takeda TM-2420 ambulatory systems according to British and American criteria. J Hypertens 1991;9:S332-3.
[19] O'Brien E, O'Malley K, Mee F, et al. Ambulatory blood pressure measurements in the diagnosis and management of hypertension. J Hum Hypertens 1991;5:23-30.
[20] Devereux RB, James GD, Pickering TG. What is normal blood pressure? Comparison of ambulatory blood pressure level and variability in patients with normal or abnormal left ventricular geometry. Am J Hypertens 1993;6:S211-5.
[21] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.
[22] Vishwanathan K, Tackett RL, Stewart JT, et al. Determination of arginine and methylated arginines in human plasma by liquid chromatography–tandem mass spectrometry. J Chromatogr B Biomed Sci Appl 2000;748:157-66.
[23] Folkow B. Structural, myogenic, humoral and nervous factors controlling peripheral resistance. In: Harington M, editor. Hypotensive drugs. London: Pergamon; 1956. p. 163-74.
[24] Baumbach GL, Heistad DD. Remodeling of cerebral arterioles in chronic hypertension. Hypertension 1989;13:968-72.
[25] Sharrett AR, Hubbard LD, Cooper LS, et al. Retinal arteriolar diameters and elevated blood pressure: the Atherosclerosis Risk in Communities Study. Am J Epidemiol 1999;150:263-70.
[26] Noon JP, Walker BR, Webb DJ, et al. Impaired microvascular dilation and capillary rarefaction in young adults with predisposition to high blood pressure. J Clin Invest 1997;15:1873-9.
[27] Zoccali C, Bode-Böger S, Mallamaci F, et al. Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: a prospective study. Lancet 2001;358: 2113-7.
[28] Valkonen VP, Päivä H, Salonen JT, et al. Risk of acute coronary events and serum concentration of asymmetrical dimethylarginine. Lancet 2001;358:2127-8.
[29] Wong TY, Kaminemi A, Klein R, et al. Quantitative retinal venular caliber and risk of cardiovascular disease in older persons: the cardiovascular health study. Arch Intern Med 2006;166:2388-94.
[30] Wong TY, Islam FM, Klein R, et al. Retinal vascular caliber, cardiovascular risk factors, and inflammation: the multi-ethnic study of atherosclerosis (MESA). Invest Ophthalmol Vis Sci 2006;47: 2341-50.
[31] Delles C, Schneider MP, John S, et al. Angiotensin converting enzyme inhibition and angiotensin II AT 1-receptor blockade reduce the levels of asymmetrical N(g), N(G)-dimethylarginine in human essential hypertension. Am J Hypertens 2002;15:590-3.
[32] Päivä H, Laakso J, Kähönen M, et al. Asymmetric dimethylarginine and hemodynamic regulation in middle-aged men. Metabolism 2006;55:771-7.
[33] Päivä H, Lehtimäki T, Laakso J, et al. Plasma concentrations of asymmetric-dimethyl-arginine in type 2 diabetes associate with glycemic control and glomerular filtration rate but not with risk factors of vasculopathy. Metabolism 2003;52:303-7.
[34] Shepherd JT. Franz Volhard lecture. Increased systemic vascular resistance and primary hypertension: the expanding complexity. J Hypertens 1990;8(Suppl):S15-S27 [Review].