

Subpressor Dose Asymmetric Dimethylarginine Modulates Renal Function in Humans through Nitric Oxide Synthase Inhibition

J.T. Kielstein^a S. Simmel^a S.M. Bode-Böger^b H.J. Roth^c
H. Schmidt-Gayk^c H. Haller^a D. Fliser^a

^aDepartment of Internal Medicine, Medical School, Hannover; ^bInstitute of Clinical Pharmacology, Otto von Guericke University, Magdeburg, and ^cLaboratory Limbach, Department of Endocrinology, Heidelberg, Germany

Key Words

Asymmetric dimethylarginine · Hypertension · Natriuresis · Nitric oxide · Renal function

Abstract

Increased blood concentrations of the endogenous nitric oxide (NO) synthase inhibitor asymmetric dimethylarginine (ADMA) have been linked to high blood pressure and to cardiovascular mortality. We evaluated the effects of a subpressor ADMA dose on NO production, renal hemodynamics, sodium handling and active renin and noradrenalin plasma concentrations in 12 healthy subjects (age 26 ± 1 year) using a double-blind placebo-controlled study design. Infusion of ADMA caused a significant decrease in plasma cyclic guanosine monophosphate (cGMP) levels, i.e. the second messenger of NO (from 6.1 ± 0.4 to 4.3 ± 0.3 pmol/l; $p < 0.05$). In parallel, effective renal plasma flow (ERPF) decreased while renovascular resistance (RVR) increased significantly (ERPF from 667 ± 9 to 603 ± 10 ml/min/1.73 m²; RVR from 79 ± 2 to 91 ± 2 ml/min/mm Hg; both $p < 0.05$ vs. baseline). Infusion of placebo did not cause significant changes in plasma cGMP levels, ERPF and RVR (cGMP from 5.7 ± 0.5 to 5.9 ± 0.6 pmol/l; ERPF from 665 ± 12 to $662 \pm$

11 ml/min/1.73 m²; RVR from 79 ± 2 to 78 ± 2 ml/min/mm Hg; all non-significant). Moreover, urinary sodium excretion was significantly lower with infusion of ADMA as compared with placebo infusion (128 ± 8 vs. 152 ± 7 μ mol/min; $p < 0.05$). In contrast, blood pressure, active renin and noradrenalin plasma concentrations did not change significantly with either infusion protocol. Acute infusion of a subpressor ADMA dose modulates several aspects of renal function in humans without affecting the activity of the renin-angiotensin and sympathetic system. Whether chronic (intrarenal) NO synthase inhibition in individuals with increased ADMA blood levels may cause persistent renal vasoconstriction and sodium retention must be evaluated.

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Introduction

Impaired endothelial-dependent vasodilatation due to reduced availability of nitric oxide (NO) is a hallmark of (incipient) vascular disease in patients with essential hypertension [1]. Several explanations for the impairment of endothelial-dependent vasodilatation in these patients have been proposed such as dyslipidemia, insulin resis-

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J.T. Kielstein, MD
Department of Internal Medicine, Division of Nephrology
Medical School Hannover, Carl-Neuberg-Strasse 1
DE-30625 Hannover (Germany)
Tel. +49 511 532 6319, Fax +49 511 55 23 66, E-Mail Kielstein@yahoo.com

tance and oxidative stress. Recently, increased blood levels of asymmetric dimethylarginine (ADMA), i.e. a naturally occurring inhibitor of NO synthase (NOS), have been linked to endothelial dysfunction, atherosclerosis and cardiovascular mortality in several high-risk populations [2–8]. Furthermore, a significant correlation between ADMA blood concentrations on the one hand and blood pressure levels on the other hand was found in both apparently healthy subjects and patients with essential hypertension [9, 10]. The reduced availability of NO due to endogenous NOS inhibition could therefore be causally involved in the pathogenesis of hypertension and even cause renal dysfunction in subjects in whom increased plasma ADMA levels are found.

Using a double-blind placebo-controlled study design, we explored the effects of a subpressor ADMA dose on NO production, renal hemodynamics and sodium handling, and on active renin and noradrenalin plasma concentrations in 12 healthy normotensive subjects. Renal hemodynamics were assessed using inulin and *p*-aminohippurate (PAH) clearance techniques.

Methods

The study protocol was approved by the Ethics Committee of the Hannover Medical School and written informed consent was given by all participants. They were healthy normotensive male nonsmokers ($n = 12$; age 26 ± 1 years; body mass index 22.4 ± 0.5 kg/m²; serum creatinine concentration 1.0 ± 0.1 mg/dl) who took no medication and had a negative family history for hypertension or metabolic diseases. At entry into the study a thorough physical examination, routine chemistry and urine analysis were performed. Serum concentrations of low- and high-density lipoprotein cholesterol, total homocysteine and ADMA in our healthy subjects were 111 ± 7 mg/dl, 54 ± 2 mg/dl, 10.9 ± 1.0 , and 1.06 ± 0.11 μ mol/l, respectively. During the study all participants adhered to an isocaloric standardized diet and retained constant weight ($\pm 0.5\%$). Alcohol consumption was not allowed and physical activity was maintained at the usual level throughout.

Using a double-blind placebo-controlled study design, the volunteers were allocated in random order to infusion of placebo (50 ml of a 0.9% NaCl solution) or 0.2 mg ADMA/kg/min (N^N-asymmetric dimethylarginine, Sigma Chemicals, USA) dissolved in 50 ml of a 0.9% NaCl solution. This ADMA dose was found to be below the pressor threshold level of 0.25 mg ADMA/kg/min obtained in a previous dose-effect study [11]. ADMA was prepared by sterile filtration in our hospital pharmacy. All participants were admitted to the metabolic ward of our clinic at 8 p.m. on the day before the experiments in order to minimize environmental stress. On the morning of the next day blood pressure, glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were assessed in the supine position in a quiet room using steady-state inulin (C_{in}) and PAH (C_{PAH}) infusion techniques as described in detail elsewhere [12]. All subjects were fasted and voided urine before the start of the experiments. Then a

priming dose of 1,500 mg inulin/m² (Inutest, Laevosan, Austria) and 500 mg PAH/m² (Nephrotest, BAG GmbH, Germany) was given at 8 a.m. The bolus injection was followed by continuous infusions of inulin (10 mg/m²/min) and PAH (8 mg/m²/min) maintained with ultra-precise pumps (Fresenius VIAL Injectomat, Fresenius Hemo Care, Germany). After an appropriate equilibration period, blood samples for measurements of baseline GFR and ERPF were taken at 10-min intervals during a steady-state period of 50 min. Thereafter placebo or ADMA was infused for 40 min and hemodynamic parameters were assessed at regular time points during this period and another 100 min after discontinuation of the infusion. Mean arterial blood pressure (MAP) and heart rate were monitored oscillometrically at regular intervals throughout (Dinamap, Critikon, Colo., USA). Venous blood samples for measurements of *L*-arginine, cyclic guanine-monophosphate (cGMP), active renin and noradrenaline plasma levels were taken at the start and end of the infusion period. For this purpose blood samples were drawn in pre-chilled tubes, immediately centrifuged at 1,500 *g* at 4°C for 10 min, and the supernatants were stored in 1-ml aliquots at –80°C until further use. *L*-Arginine is the amino acid precursor of NO, and cGMP the main second messenger of NO in the cardiovascular system. In addition, timed urine specimens were obtained in all individuals on both study days. For this purpose the volunteers emptied their bladder immediately before the inulin and PAH bolus. Urine was collected thereafter and voided after completion of the clearance studies.

Inulin was measured enzymatically using inulinase and PAH photometrically. Inulin and PAH clearances were calculated from the delivered dose: $C = (I_r \times I_c) / S_c$; where C is the clearance, I_r is the infusion rate (ml/min), I_c is the concentration of the analyte in the infusion fluid (mg/ml), and S_c is the plasma concentration of the analyte (mg/ml). The filtration fraction (FF) was calculated as the ratio C_{in} / C_{PAH} , and renal vascular resistance (RVR) using the equation: $RVR = [(MAP - 12) \times 723 / ERPF]$. Fractional sodium excretion (FE_{Na}) was calculated as the ratio between urinary sodium excretion rate and filtered sodium load. Plasma *L*-arginine and ADMA levels were determined using HPLC with pre-column derivatization with *o*-phthalaldehyde as described previously [13]. The coefficients of variation of this method are 5.2% within assay and 5.5% between assay; the detection limit of the assay is 0.1 μ mol/l. Plasma noradrenaline levels were measured with HPLC as well. The normal range of the assay for healthy subject is 165–460 ng/l (supine position). The active renin plasma concentration was measured with an immunoradiometric assay using a highly sensitive and specific monoclonal antibody for renin (Renin III Generation, ERIA Diagnostics Pasteur, France). The normal range of the assay for healthy subject is 1.5–18.0 ng/l (supine position). The intra-assay and interassay coefficients of variation in healthy subjects are less than 5.2 and 7.4%, respectively. Plasma cGMP levels were measured using ELISA (R&D Systems, USA). All routine laboratory measurements were carried out using certified assay methods.

Mean values of cGMP blood levels, parameters of renal hemodynamics, blood pressure and vasoactive hormones were evaluated in the pre-infusion and post-infusion period using a Student's paired *t* test. In addition, intra-individual pre-infusion and post-infusion data with both infusion protocols were compared using a Student's paired *t* test. The significance levels were set at $p < 0.05$. Data are presented as mean \pm SEM.

Table 1. Effect of a subpressor dose of asymmetric dimethylarginine (ADMA) on plasma cGMP levels and cardiovascular hormones in 12 healthy subjects

	Placebo	ADMA
Plasma cGMP concentration, pmol/l		
Pre-infusion	5.7 ± 0.5	6.1 ± 0.4
Post-infusion	5.9 ± 0.6	4.3 ± 0.3*.#
Plasma active renin concentration, ng/l		
Pre-infusion	8.5 ± 0.7	8.1 ± 0.8
Post-infusion	8.0 ± 0.9	7.6 ± 1.0
Plasma noradrenaline concentration, ng/l		
Pre-infusion	185 ± 21	183 ± 19
Post-infusion	185 ± 27	172 ± 21

* p < 0.05: Comparison between ADMA and placebo infusion.

p < 0.05: Comparison between intra-individual pre- and post-infusion data.

Table 2. Effect of a subpressor dose of asymmetric dimethylarginine (ADMA) on systemic and renal hemodynamics and sodium handling in 12 healthy subjects

	Placebo	ADMA
Mean arterial blood pressure, mm Hg		
Pre-infusion	85 ± 2	85 ± 2
Post-infusion	86 ± 2	85 ± 1
Heart rate, beats/min		
Pre-infusion	57 ± 2	55 ± 3
Post-infusion	57 ± 2	57 ± 3
Glomerular filtration rate, ml/min/1.73 m ²		
Pre-infusion	122 ± 3	123 ± 3
Post-infusion	123 ± 3	125 ± 3
Effective renal plasma flow, ml/min/1.73 m ²		
Pre-infusion	665 ± 12	667 ± 9
Post-infusion	662 ± 11	603 ± 10*.#
Filtration fraction, ERPF/GFR		
Pre-infusion	0.18 ± 0.00	0.18 ± 0.00
Post-infusion	0.19 ± 0.00	0.21 ± 0.00*.#
Renovascular resistance, mm Hg/ml/min		
Pre-infusion	79 ± 2	79 ± 2
Post-infusion	78 ± 2	91 ± 2*.#

* p < 0.05: Comparison between ADMA and placebo infusion.

p < 0.05: Comparison between intra individual pre- and post-infusion data.

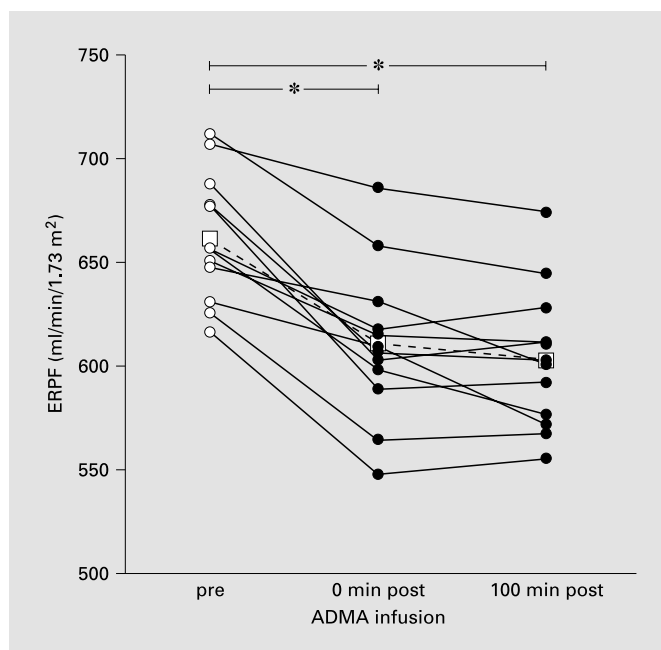


Fig. 1. Individual data on effective renal plasma flow (ERPF) with infusion of a subpressor dose of asymmetric dimethylarginine (ADMA) in 12 healthy subjects. ○ = Pre-infusion data; ● = post-infusion data immediately after discontinuation of ADMA infusion (0 min post) and 100 min thereafter (100 min post); □ = mean values. * p < 0.05: comparison between intra-individual pre-infusion and post-infusion data.

Results

Mean plasma cGMP levels decreased significantly with ADMA infusion (table 1), whereas active renin and noradrenaline concentrations remained unchanged. Infusion of placebo had no effect on plasma cGMP levels and on active renin and noradrenaline plasma concentrations. In addition, we found no significant changes in plasma *L*-arginine concentrations both with placebo (from 53.7 ± 1.6 to 55.0 ± 1.6 μmol/l; n.s.) and ADMA infusion (from 52.9 ± 2.0 to 54.6 ± 1.8 μmol/l; n.s.). The changes in plasma cGMP levels were accompanied by changes in renal hemodynamics, i.e. a significant decrease in ERPF and increase in RVR and FF (table 2), whereas GFR and MAP were not affected. Again, placebo infusion had no effect on renal hemodynamics and on blood pressure. Individual data on ERPF with ADMA infusion are shown in figure 1. Both urinary sodium excretion (152 ± 7 vs. 128 ± 8 μmol/min) and FE_{Na} (1.16 ± 0.14 vs. 0.94 ± 0.11%) were significantly (p < 0.05) lower with ADMA infusion as compared with placebo infusion, whereas

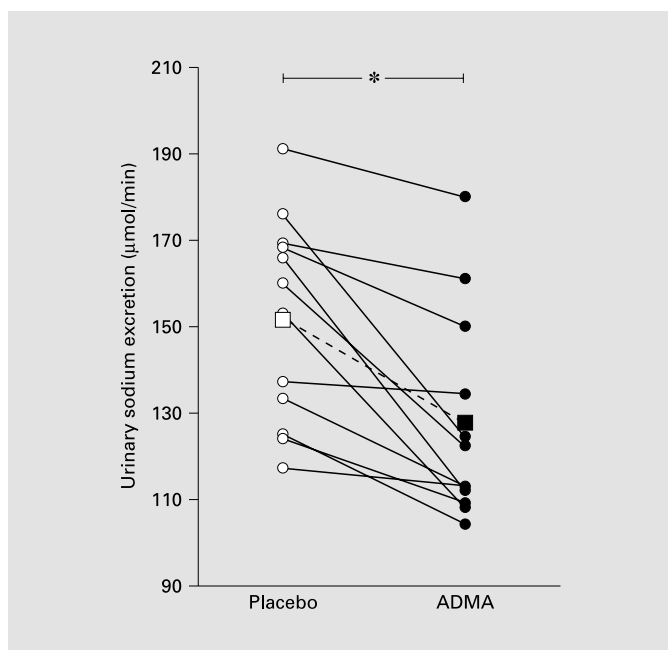


Fig. 2. Individual data on urinary sodium excretion (U_{Na}) with placebo infusion (○) and administration of a subpressor dose of asymmetric dimethylarginine (ADMA; ●) in 12 healthy subjects; □ = mean values. * $p < 0.05$: comparison between placebo and ADMA infusion.

urine flow rate did not change (1.07 ± 0.09 vs. 1.06 ± 0.14 ml/min; n.s.). Individual data on urinary sodium excretion are shown in figure 2.

Discussion

The results of the present study document that acute systemic administration of a subpressor dose of the (endogenous) NOS inhibitor ADMA in healthy subjects reduces NO generation, renal perfusion and sodium excretion without affecting the renin-angiotensin system and sympathetic activity (the small decrease in active renin concentration with ADMA infusion might be the consequence of a feedback mechanisms because of sodium retention). The absence of changes in blood pressure and in cardiovascular hormones points to a direct effect of ADMA on renal function. These findings confirm evidence accumulated from experimental studies that ADMA is a potent (intrarenal) NOS inhibitor [2, 11, 14]. As a consequence, chronically elevated plasma levels of this endogenously occurring substance may be of relevance not only in human vascular pathology but also in

the pathophysiology of hypertension and, in parallel, in the development of renal damage [10, 15, 16]. Reduced renal NO availability is thought to be a major contributor to abnormal pressure-natriuresis and salt-sensitive hypertension in animal models of human essential hypertension [17]. In addition, results from clinical studies documenting a significant relationship between increased ADMA levels on the one hand and high blood pressure on the other hand support this notion [9, 10].

We have studied the effect of ADMA on renal hemodynamics because the renal vasculature is very sensitive to NOS inhibition; this has been documented in numerous animal experiments and in human studies as well [14, 15, 18–20]. With the dose of ADMA chosen, we have achieved a significant decrease in ERPF and an increase in RVR by about 10%. The effects of ADMA on NO production and renal perfusion are comparable with the action of other NOS inhibitors such as *L*-NAME or *L*-NMMA [11, 18–20]. In contrast to ADMA, *L*-NAME is a synthetic pro-drug which has to be metabolized in its active form first. In addition, *L*-NAME is not metabolized by dimethylarginine dimethylaminohydrolase, i.e. the enzyme that metabolizes ADMA. This could be the reason that ADMA must be given in much higher doses than *L*-NAME in order to achieve similar effects on renal perfusion [11]. Further, the concentration of the endogenous NOS inhibitor *L*-NMMA in the circulation is about 10 times smaller than that of ADMA [2, 21], and compared to the long-lasting action of ADMA the effects of *L*-NMMA on NO production, renal hemodynamics and blood pressure are of relatively short duration [20]. Thus ADMA is a biologically relevant NOS inhibitor, the blood concentrations of which are found to be significantly elevated in renal patients and in patients with various cardiovascular diseases [2, 8, 22].

Acute infusion of ADMA had no effect on plasma noradrenaline concentrations in healthy subjects. This does not exclude a significant interaction between ADMA and the sympathetic system in patients with cardiovascular pathology, however. As a matter of fact, in a recent epidemiological study from Italy a highly significant association between ADMA and noradrenaline blood levels, and cardiovascular mortality was found in patients with terminal renal failure [23]. In this respect we have to admit that the individuals we examined differ from patients with renal disease in several aspects such as age, presence of hypertension, etc. Thus, the effect of ADMA might be different in patients in whom cardiovascular comorbidity is present.

In conclusion, acute systemic infusion of a subpressor dose of the endogenous NOS inhibitor ADMA modulates renal function in humans without affecting the activity of the renin-angiotensin system and the sympathetic system. Thus, further studies are warranted to elucidate whether chronic (intrarenal) NOS inhibition in individuals with increased ADMA blood levels causes persistent renal vasoconstriction and sodium retention, and contributes to the development of high blood pressure.

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References

- 1 Taddei S, Virdis A, Ghiadoni L, Sudano I, Salvetti A: Endothelial dysfunction in hypertension. *J Cardiovasc Pharmacol* 2001;38(suppl 2):S11–S14.
- 2 Cooke JP: Does ADMA cause endothelial dysfunction? *Arterioscler Thromb Vasc Biol* 2000;20:2032–2037.
- 3 Boger RH, Bode-Boger SM, Thiele W, Junker W, Alexander K, Frolich JC: Biochemical evidence for impaired nitric oxide synthesis in patients with peripheral arterial occlusive disease. *Circulation* 1997;95:2068–2074.
- 4 Kielstein JT, Boger RH, Bode-Boger SM, Schaffer J, Barbey M, Koch KM, Frolich JC: Asymmetric dimethylarginine plasma concentrations differ in patients with end-stage renal disease: Relationship to treatment method and atherosclerotic disease. *J Am Soc Nephrol* 1999;10:594–600.
- 5 Yoo J, Lee S: Elevated levels of plasma homocysteine and asymmetric dimethylarginine in elderly patients with stroke. *Atherosclerosis* 2001;158:425–430.
- 6 Zoccali C, Bode-Boger S, Mallamaci F, Benedetto F, Tripepi G, Malatino L, Cataliotti A, Bellanuova I, Fermo I, Frolich J, Boger R: Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: A prospective study. *Lancet* 2001;358:2113–2117.
- 7 Valkonen VP, Päivä H, Salonen JT, Lakka TA, Lehtimäki T, Laakso J, Laaksonen R: Risk of acute coronary events and serum concentration of asymmetric dimethylarginine. *Lancet* 2001;358:2127–2128.
- 8 Vallance P: Importance of asymmetrical dimethylarginine in cardiovascular risk. *Lancet* 2001;358:2096–2097.
- 9 Miyazaki H, Matsuoka H, Cooke JP, Usui M, Ueda S, Okuda S, Imaizumi T: Endogenous nitric oxide synthase inhibitor: A novel marker of atherosclerosis. *Circulation* 1999;99:1141–1146.
- 10 Kielstein JT, Bode-Böger SM, Frölich C, Ritz E, Haller H, Fliser D: Asymmetric dimethylarginine (ADMA), blood pressure, and renal perfusion in elderly subjects. *Circulation* 2003;107:1891–1895.
- 11 Kielstein JT, Impraïm B, Simmel S, Bode-Boger SM, Tsikas R, Hoepfer MM, Frolich JC, Haller H, Fliser D: Cardiovascular effects of systemic NO synthase inhibition with asymmetric dimethylarginine in humans. *Circulation* 2004;109:172–177.
- 12 Fliser D, Zeier M, Nowack R, Ritz E: Renal functional reserve in healthy elderly subjects. *J Am Soc Nephrol* 1993;3:1371–1377.
- 13 Bode-Boger SM, Boger RH, Kienke S, Junker W, Frolich JC: Elevated *L*-arginine/dimethylarginine ratio contributes to enhanced systemic NO production by dietary *L*-arginine in hypercholesterolemic rabbits. *Biochem Biophys Res Commun* 1996;219:598–603.
- 14 Gardiner SM, Kemp PA, Bennett T, Palmer RM, Moncada S: Regional and cardiac haemodynamic effects of NG, NG, dimethyl-*L*-arginine and their reversibility by vasodilators in conscious rats. *Br J Pharmacol* 1993;110:1457–1464.
- 15 Baylis C, Mitruka B, Deng A: Chronic blockade of nitric oxide synthesis in the rat produces systemic hypertension and glomerular damage. *J Clin Invest* 1992;90:278–281.
- 16 Sander M, Chavoshan B, Victor RG: A large blood pressure-raising effect of nitric oxide synthase inhibition in humans. *Hypertension* 1999;33:937–942.
- 17 Granger JP, Alexander BT: Abnormal pressure-natriuresis in hypertension: Role of nitric oxide. *Acta Physiol Scand* 2000;168:161–168.
- 18 Lahera V, Salom MG, Miranda-Guardiola F, Moncada S, Romero JC: Effects of NG-nitro-*L*-arginine methyl ester on renal function and blood pressure. *Am J Physiol* 1991;261:F1033–F1037.
- 19 Broere A, Van Den Meiracker AH, Boomsma F, Derckx FH, Veld AJ, Schalekamp MA: Human renal and systemic hemodynamic, natriuretic, and neurohumoral responses to different doses of *L*-NAME. *Am J Physiol* 1998;275:F870–F877.
- 20 Bech JN, Nielsen CB, Pedersen EB: Effects of systemic NO synthesis inhibition on RPF, GFR, UNa, and vasoactive hormones in healthy humans. *Am J Physiol* 1996;270:F845–F851.
- 21 Avontuur JA, Buijk SL, Bruining HA: Distribution and metabolism of N(G)-nitro-*L*-arginine methyl ester in patients with septic shock. *Eur J Clin Pharmacol* 1998;54:627–631.
- 22 Kielstein JT, Böger RH, Bode-Böger SM, Frölich C, Haller H, Ritz E, Fliser D: Marked increase of asymmetric dimethylarginine in patients with incipient primary chronic renal disease. *J Am Soc Nephrol* 2002;13:170–176.
- 23 Mallamaci F, Tripepi G, Maas R, Malatino R, Boger R, Zoccali C: Analysis of the relationship between norepinephrine and asymmetric dimethyl arginine among patients with end-stage renal disease. *J Am Soc Nephrol* 2004;15:435–441.