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Effects of asymmetric dimethylarginine (ADMA) infusion in humans

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Abstract Increased blood concentrations of the endogenous nitric oxide synthase (NOS) inhibitor asymmetric dimethylarginine (ADMA) can be found in patients with cardiovascular risk factors such as age, hypertension, diabetes, insulin resistance, hypercholesterolemia, hypertriglyceridemia, chronic kidney disease, and hyperhomocystinemia. ADMA has been shown to be a strong and independent predictor of cardiovascular and overall mortality in selected patient populations. Furthermore, in patients with chronic kidney disease, it is a strong and independent risk marker for decrease of renal function, progression to end-stage renal disease, and mortality. Infusion of exogenous ADMA in humans helped to elucidate its role in the pathogenesis of endothelial dysfunction. Pathophysiologically relevant concentrations of ADMA have been shown to decrease heart rate and cardiac output and to increase systemic vascular resistance and pulmonary vascular resistance. ADMA decreases effective renal plasma flow and increases renovascular resistance in a dose-related manner. Moreover, administration of ADMA causes significant sodium retention and blood pressure increase. These studies also revealed that ADMA is less potent than the synthetic NOS inhibitor N^G -nitro-L-arginine methyl ester (L-NAME) and behaves differently in respect to onset of action, which can be explained by the different routes of elimination as well as different cellular transport mechanisms. Collectively these

results document that ADMA has well-defined effects on cardiovascular and renal function in healthy subjects. It is therefore conceivable that ADMA causes sustained changes in vascular function through an intracellular action in endothelial cells at blood concentrations found in patients with cardiovascular pathology.

Keywords Asymmetric dimethylarginine · Infusion · Nitric oxide

Asymmetric dimethylarginine (ADMA)—a novel marker for cardiovascular disease

Dimethylarginines had been known to biochemists for decades [1, 2]. The medical community however started to gain interest in these substances, especially in the endogenous inhibitor of nitric oxide synthase, asymmetric dimethylarginine (ADMA), as late as 1992. In that year Vallance and coworkers published their landmark paper on the elevation of dimethylarginines in patients with end-stage renal disease [3]. They speculated that impaired NO synthesis due to accumulation of ADMA might contribute to the hypertension and immune dysfunction associated with chronic renal failure. Since then more than 350 publications on ADMA have been published. Elevated circulating ADMA levels in patients with end-stage renal disease were confirmed by many authors, although the absolute levels of ADMA differ up to one order of magnitude [4–8]. Early on it had been speculated that decreased renal function might not be the sole mechanism for the elevation of ADMA in this patient population, since hemodialysis patients with manifest atherosclerosis had higher ADMA plasma levels than hemodialysis patients without clinically manifest atherosclerosis [5]. Many studies showed this to be true. ADMA correlates with traditional and nontraditional risk factors for atherosclerosis such as hypertension [9, 10], age, mean arterial pressure and glucose tolerance [10], hypercholesterolemia [11], hyperhomocysteinemia [12–14], increased salt intake [15–17], insulin resistance [18], and plasma catecholamines [19].

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ADMA also correlates with carotid intima media thickness [10, 20], an established marker for the severity of atherosclerosis. Furthermore, elevated ADMA levels were found in patients with heart failure [21, 22] and in women with preeclampsia [23–25]. In senescent individuals ADMA seems to be involved in the decrease of renal perfusion and increase of blood pressure [9]. Also, prospective studies showed that ADMA is a strong and independent predictor of cardiovascular events and mortality in patients with end-stage renal disease [8], and that ADMA predicts acute coronary events in middle-aged nonsmoking men [26] and mortality in patients with idiopathic pulmonary arterial hypertension [27].

Pathophysiological importance of ADMA

Data from several experimental studies suggest that ADMA concentrations in a pathophysiological range (3–10 $\mu\text{mol/l}$) significantly inhibit vascular NO production [28–30]. ADMA given to rings of human middle cerebral artery at concentrations of 10–300 $\mu\text{mol/l}$ cause concentration- and endothelium-dependent contraction [31].

Despite numerous studies showing correlation between ADMA plasma levels and grade of disease and the data from *in vitro* studies, the possible role of ADMA in cardiovascular disease is discussed controversially [32]. One reason for this was the considerable variation in ADMA levels between laboratories which may be partly due to different analytical methods [32]. The ADMA levels measured in plasma are in some cases lower than the concentrations that are high enough to inhibit NO synthesis in experimental model systems, i.e., 3–10 $\mu\text{mol/l}$. The main reason, to question the role of ADMA, was the limited evidence supporting the pathophysiological role of ADMA *in vivo*.

In vivo evidence for the pathophysiological role of ADMA

Animal studies

Until today there are only five published studies on the acute effects of ADMA in animals (Table 1). Vallance et al. [3] described the effect of an ADMA infusion (3 mg/kg per h) to guinea pigs that led to an increase in systolic blood pressure by 15%. A bolus of ADMA (3–30 mg/kg) led to a dose-dependent increase in mean arterial blood pressure of up to 53 mmHg [3]. This dose-dependent pressor and bradycardiac effects of ADMA (1–100 mg/kg) in rats was confirmed by Gardiner and coworkers who showed renal, mesenteric, and hindquarter vasoconstrictions by ADMA [33]. Topical application of 10 and 100 μmol ADMA through cranial windows in anesthetized rats constricted the basilar artery by 9 ± 2 and $19\pm 1\%$, respectively [34]. In contrast to intravenous injection of ADMA that caused dose-dependent increases in mean arterial blood pressure, intracerebroventricular (i.c.v.) injection of ADMA decreased mean arterial blood pressure (by 39 ± 6 mmHg) and heart rate (by 50 ± 8 bpm) [35]. Investigating the diameter of rat mesenteric arterioles by intravital microscopy, ADMA (100 $\mu\text{mol/l}$) caused a maximal constriction to 68% of baseline diameter and a decrease in blood flow by 44% [36]. A recent study investigated the long-term effects of subcutaneous ADMA infusion (20, 40, and 60 mg/kg per day) for 4 weeks in mice [37]. In wild-type mice, long-term treatment with ADMA caused significant coronary microvascular lesions. Importantly, in endothelial NOS (eNOS) knockout mice, treatment with ADMA also caused coronary microvascular lesions the extent of which was comparable to that in wild-type mice. These vascular effects of ADMA in this model could not be prevented by supplementation of L-arginine. Compelling evidence for the impor-

Table 1 Summary of *in vivo* studies in animals on ADMA application and its effects

Author/reference	Animal	Application	Dose	Plasma concentration	Effect
Vallance et al. [3]	Guinea pigs ($n=3$)	i.v. infusion	3 mg/kg/h	9.8 $\mu\text{mol/l}$	Increase in systol. BP ^a by 15%
Vallance et al. [3]	Guinea pigs ($n=3$)	i.v. bolus	3–30 mg/kg	Not reported	Increase in BP by up to 53 mmHg
Gardiner et al. [33]	Rats ($n=8$)	i.v. bolus	1–100 mg/kg	Not reported	Dose-dependent increase in BP and fall in heart rate
Jin and D'Alecy [35]	Rats ($n=10$)	i.c.v.	1 mg	Not applicable	Decrease in mean arterial BP by 39 mmHg decrease in heart rate by 50 bpm
Jin and D'Alecy [35]	Rats ($n=10$)	i.v.	0.5–10 mg/kg	Not applicable	Increase in mean arterial BP by 37 mmHg
White et al. [36]	Rats ($n=6$)	Topical application	100 $\mu\text{mol/l}$	Not applicable	Constriction of mesenteric arterioles by 32% and decrease in blood flow by 44% BP, blood pressure
Faraci et al. [34]	Rats ($n=8$)	Topical application	10 and 100 $\mu\text{mol/l}$	Not applicable	Constriction of basilar by 9 and 19%, respectively
Faraci et al. [34]	Rabbits ($n=10$)	Topical application	300 $\mu\text{mol/l}$	Not applicable	Decrease in baseline diameter of cerebral arterioles by 11%

^aAt the end of the infusion

tance of ADMA comes from a transgenic mouse model in which the activity of dimethylarginine dimethylaminohydrolase (DDAH), i.e., the enzyme that metabolizes approximately 80% of the endogenously produced ADMA, is increased and leads to a decrease in plasma ADMA levels by 50%. The reduction in plasma ADMA levels is associated with a significant increase in NOS activity, as plasma and urinary nitrate levels are increased twofold. The increase in NOS activity translates into a 15-mmHg reduction in systolic blood pressure in the transgenic mouse [38].

Infusion of ADMA in men

Part of the pioneer work by Vallance et al. [3] was a small study on the effect of local i.a. ADMA infusion on the forearm arteriolar bed of healthy volunteers (see Table 2). The i.a. application of 8 μmol ADMA into the brachial artery caused a decrease of forearm blood flow by $28\pm 8\%$ [3]. This was confirmed by Calver et al. [39] who showed that i.a. application of 8 μmol and 16 μmol of ADMA into the brachial artery led to a decrease of forearm blood flow by $29\pm 2\%$ and $44\pm 3\%$, respectively.

Controlled trials examining effects of ADMA on different vascular beds at pathophysiologically relevant plasma levels in humans had not been reported for a long time. The first study on the effect of systemic ADMA infusion in men was published in 2001. Unexpectedly, low doses of ADMA (i.e., 0.5 and 1.0 mg/kg per min) had no effects

on blood pressure or renal perfusion [40]. Therefore, we performed a dose-response study by infusing ADMA intravenously in escalating doses to healthy subjects and measured plasma concentrations as well as the effects on NO production and renal hemodynamics. We chose this experimental setting because the human (postglomerular) renal circulation is very sensitive to NOS inhibition and can be easily assessed with accurate invasive clearance techniques. Acute increases of plasma ADMA levels within the pathophysiologically relevant range, i.e., between 2 and 10 $\mu\text{mol/l}$, were achieved with ADMA doses of 0.5 and 1.0 mg/kg, with which a significant decrease in plasma cGMP concentrations was observed. A significant effect on effective renal plasma flow (ERPF) was documented with infusion of an ADMA dose of at least 3 mg/kg, whereas the glomerular filtration rate (GFR) remained unaffected [41]. In another study we investigated the effect of ADMA on systemic cardiovascular parameters, e.g., cardiac output and systemic vascular resistance, by placing a right heart catheter in healthy volunteers [41]. Infusion of ADMA to seven healthy subjects caused a significant and sustained decrease in cardiac output and a significant increase in systemic vascular resistance (SVR). In agreement with the response in the renal circulation, we observed an immediate effect of ADMA on systemic cardiovascular parameters. Interestingly, the action of ADMA lasted until 2 h after the end of the ADMA infusion. In addition, we observed a significant decrease in heart rate during ADMA infusion from 58 ± 7 to 54 ± 6 bpm; at the end of the postinfusion

Table 2 Summary of in vivo studies in human on systemic ADMA application and its effects

Author/reference	Subjects (n)	Application	Dose	Plasma concentration	Effect
Vallance et al. [3]	5	i.a. bolus	8 μmol	Not reported	Decrease in forearm blood flow by 28%
Calver et al. [39]	6	i.a.	8 μmol	Not reported	Decrease in forearm blood flow by 29%
			16 μmol	Not reported	Decrease in forearm blood flow by 44%
Kielstein et al. [41]	6	i.v. infusion over 40 min	0.5 mg/kg	4.2 $\mu\text{mol/l}^a$	Dose-dependent decrease in plasma cGMP of up to 36%
			1.0 mg/kg	8.1 $\mu\text{mol/l}$	Dose-dependent decrease in effective renal plasma flow of up to 11%
			1.5 mg/kg	11.8 $\mu\text{mol/l}$	
			3.0 mg/kg	15.7 $\mu\text{mol/l}$	
			6.0 mg/kg	32.9 $\mu\text{mol/l}$	
			10.0 mg/kg	42.1 $\mu\text{mol/l}$	
Achan et al. [42]	6	i.v.	4.0 mg/kg	23.0 $\mu\text{mol/l}^a$	Decrease in cardiac output by 14%, increase in systemic vascular resistance by 11%
			3.0 mg/kg	2.6 $\mu\text{mol/l}^b$	Decrease in heart rate by 9%
Kielstein et al. [43]	12	i.v. infusion over 40 min	0.8 mg/kg	Not reported	Decrease in cardiac output by 15%
					Increase in mean arterial blood pressure by 6%
					Increase in systemic vascular resistance by 24%
Kielstein et al. [27]	7	i.v. infusion over 40 min	0.1 mg/kg	Not reported	Decrease in cGMP by 30%
					Decrease in effective renal plasma flow by 10%
					Increase in filtration fraction by 17%
					Decrease in urinary sodium excretion by 16%
					Increase in pulmonary vascular resistance by 38 %

^aAt the end of the infusion

^bThirty minutes after injection

period mean heart rate was 56 ± 8 bpm. Mean plasma ADMA concentration increased from 0.95 ± 0.27 at baseline to 22.95 ± 4.91 $\mu\text{mol/l}$ at the end of the infusion period. Two hours after the end of the ADMA infusion, ADMA plasma concentration was 5.31 ± 1.43 $\mu\text{mol/l}$, which is within the pathophysiologically relevant range.

In general, these data are in accordance with data obtained with noninvasive techniques, i.e., bioimpedance cardiography [42]. In that study Achan and coworkers showed that a bolus of ADMA (3 mg/kg) reduced heart rate by $9.2 \pm 1.4\%$ and cardiac output by $14.8 \pm 1.2\%$. ADMA also increased mean blood pressure by $6.0 \pm 1.2\%$ and SVR by $23.7 \pm 2.1\%$. Interestingly, in the study by Achan et al. [42] the effect of ADMA on heart rate and cardiac output was not detectable 1 h after the bolus, in contrast to the data we obtained [41]. In the study by Achan et al. [42] it was shown that handgrip exercise increased cardiac output in control subjects by 96%, but in subjects given ADMA cardiac output increased by only 35%. ADMA plasma level 30 min after the infusion was 2.6 $\mu\text{mol/l}$, with baseline value not being provided [42].

Recently, we found that acute systemic administration of a subpressor dose of ADMA in healthy subjects reduced NO generation, renal perfusion, and sodium excretion without affecting the renin-angiotensin system and sympathetic activity [43]. The absence of changes in blood pressure and in cardiovascular hormones points to a direct effect of ADMA on renal function. Eventually, ADMA infusion in healthy volunteers increased pulmonary vascular resistance (68.9 ± 7.6 versus 95.6 ± 6.3 $\text{dyne} \cdot \text{s} \cdot \text{cm}^{-5}$; $P < 0.05$) and decreased stroke volume (101.1 ± 6.7 versus 95.6 ± 6.3 ml; $P < 0.05$) as measured by right heart catheterization [27].

The results obtained in a series of controlled clinical studies document that systemic administration of ADMA has definite effects on cardiovascular and renal function in healthy subjects. It is, therefore, conceivable that ADMA causes sustained changes in vascular function through an intracellular action in endothelial cells at blood concentrations found in patients with cardiovascular pathology.

ADMA and exogenous inhibitors of NO synthase

What is the rationale for infusing the endogenous NO synthase inhibitor ADMA?

First of all the pathophysiological importance of ADMA had to be proved at pathophysiologically relevant levels, i.e., at concentrations measured in selected patient populations with the same method in the same laboratory as the blood levels from the infusion studies, with ADMA plasma levels, however, not necessarily representing the intracellular level. In addition, the effect of ADMA has to be compared with that of synthetic inhibitors of NO synthase such as N^G -nitro-L-arginine methyl ester (L-NAME). Unlike ADMA, L-NAME is a prodrug and first has to be metabolized to its active form, i.e., N^G -nitro-L-arginine [44]. Furthermore, ADMA competes with L-arginine for transport by the inducible human cationic amino acid transporter

(hCAT-2B), whereas the transport of L-NAME does not depend on this transporter [45]. Recently, it has been shown that L-NAME is a weak inhibitor of L-arginine transport into platelets as compared with ADMA [46]. Due to these obvious differences we compared the infusion of L-NAME (120 $\mu\text{g/kg}$) and ADMA (10 mg/kg) in a double-blind, placebo-controlled study. Both substances caused a comparable decrease in ERPF and a significant increase in filtration fraction and renovascular resistance (RVR) [41]. Hence, on a molar basis L-NAME is by a factor of almost 80 more effective in inhibiting NOS than ADMA in vivo in humans. Another difference between ADMA and L-NAME is that the effect of ADMA on ERPF and RVR is observed immediately after the start of the infusion, whereas the effect of L-NAME occurs later, pointing to immediate NOS inhibition by ADMA. On the other hand, ADMA has, like L-NAME, a long duration of action. Two hours after discontinuation of the infusion mean ADMA blood concentration was below 10 $\mu\text{mol/l}$. Plasma cGMP concentration decreased significantly on administration of both NOS inhibitors, and was still 30% below baseline values 2 h after the infusion stopped. This is in accordance with the relatively long ADMA plasma half-life of several minutes as calculated from plasma decay curves in healthy subjects (see below).

Elimination kinetics of ADMA from the circulation

The elimination half-life of ADMA was determined in healthy humans. The study protocol was approved by the

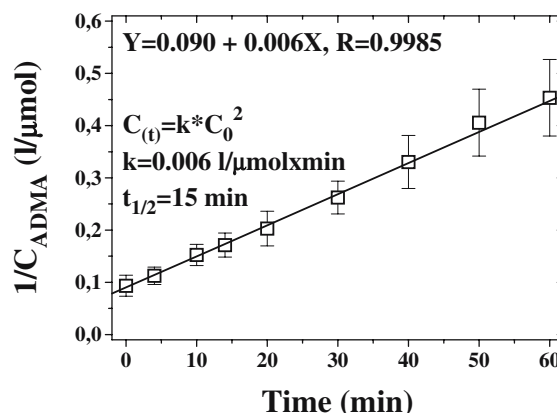


Fig. 1 Application of the integral method to analyze the elimination kinetics and to determine the plasma half-life of ADMA in healthy humans ($n=6$) who received i.v. infusion of ADMA (1.5 mg/kg over 40 min). Data were taken from the study by Kielstein et al. [41] and are shown as mean \pm standard deviation. The finding that plotting of $1/C$ versus time yields a straight line suggests that ADMA follows an elimination kinetics of second order with respect to ADMA plasma concentration. The y-axis intercept provides the mean initial ADMA concentration (C_0) which prevails after the stop of the infusion, i.e., 11.1 $\mu\text{mol/l}$. The slope of the straight line provides the mean reaction rate k which is determined to be 0.006 $\text{l}/\mu\text{mol} \times \text{min}$. From these data the mean elimination half-life $t_{1/2}$ [$t_{1/2} = 1/(C_0 \times k)$] of ADMA is calculated to be 15 min. Separate analysis of the data obtained from each volunteer offers values of 0.0069 ± 0.0024 $\text{l}/\mu\text{mol} \times \text{min}$ for k and 16.3 ± 6.4 min for $t_{1/2}$

Ethics Committee of the Hannover Medical School. Written informed consent was given by all participants, who were healthy nonsmoking male volunteers. During the study, all participants adhered to an isocaloric standardized diet. After a 40-min infusion of 0.025 mg ADMA/kg per min ($n=6$) blood samples for measurement of ADMA plasma levels were taken at 2, 4, 6, 8, 10, 15, 20, 30, 40, 50, and 60 min after discontinuation of the infusion. The elimination half-life of ADMA was determined by analyzing the plasma concentration-time profile of all subjects by the integral method. Plotting of the reciprocal ADMA concentration, i.e., $1/C$, versus time yielded a straight line (Fig. 1), suggesting that the decay of ADMA concentration in plasma follows a second-order kinetics. Mean plasma ADMA half-life was calculated to be 15 min (Fig. 1). Presumably, the plasma half-life of ADMA is longer in conditions such as in hypercholesterolemia and diabetes mellitus [18, 47], in which the main pathway of ADMA degradation, i.e., the hydrolysis of ADMA to L-citrulline and dimethylamine by DDAH, may be impaired.

Conclusion

Systemic infusion of ADMA in animals and in men yielding pathophysiologically relevant ADMA blood concentrations causes a significant and sustained decrease in cardiac output, while increasing systemic vascular resistance and blood pressure. ADMA infusion also decreases effective renal plasma flow in a dose-related manner. Thus, chronically elevated ADMA blood levels may contribute to progression of vascular disease via endothelial damage. This effect seems to involve more than just reduced NO availability secondary to NOS inhibition.

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References

1. Kakimoto Y, Akazawa S (1970) Isolation and identification of N^G, N^G - and N^G, N^G -dimethyl- arginine, N^E -mono-, di-, and trimethyllysine, and glucosylgalactosyl- and galactosyl- δ -hydroxylysine from human urine. *J Biol Chem* 245:5751–5758
2. McDermott JR (1976) Studies on the catabolism of N^G -methylarginine, N^G, N^G -dimethylarginine and N^G, N^G -dimethyl- arginine in the rabbit. *Biochem J* 154:179–184
3. Vallance P, Leone A, Calver A, Collier J, Moncada S (1992) Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* 339:572–575
4. Anderstam B, Katzarski K, Bergström J (1997) Serum levels of N^G, N^G -dimethyl-L-arginine, a potential endogenous nitric oxide inhibitor in dialysis patients. *J Am Soc Nephrol* 8: 1437–1442
5. Kielstein JT, Böger RH, Bode-Böger SM, Schäffer J, Barbey M, Koch KM, Frölich JC (1999) Asymmetric dimethylarginine plasma concentrations differ in patients with end-stage renal disease: relationship to treatment method and atherosclerotic disease. *J Am Soc Nephrol* 10:594–600

6. Kielstein JT, Böger RH, Bode-Böger SM, Martens-Lobenhoffer J, Lonnemann G, Frölich JC, Haller H, Fliser D (2004) Low dialysance of asymmetric dimethylarginine (ADMA)—in vivo and in vitro evidence of significant protein binding. *Clin Nephrol* 62:295–300
7. Schmidt RJ, Domico J, Samsell LS, Yokota S, Tracy TS, Sorkin MI, Engels K, Baylis C (1999) Indices of activity of the nitric oxide system in hemodialysis patients. *Am J Kidney Dis* 34: 228–234
8. Zoccali C, Bode-Böger SM, Mallamaci F, Benedetto F, Tripepi G, Malatino L, Cataliotti A, Bellanuova I, Fermo I, Frölich JC, Böger RH (2001) Plasma concentration of asymmetrical di- methylarginine and mortality in patients with end-stage renal disease: a prospective study. *Lancet* 358:2113–2117
9. Kielstein JT, Bode-Böger SM, Frölich JC, Ritz E, Haller H, Fliser D (2003) Asymmetric dimethylarginine, blood pres- sure, and renal perfusion in elderly subjects. *Circulation* 107: 1891–1895
10. Miyazaki H, Matsuoka H, Cooke JP, Usui M, Ueda S, Okuda S, Imaizumi T (1999) Endogenous nitric oxide synthase inhibitor: a novel marker of atherosclerosis. *Circulation* 99:1141–1146
11. Böger RH, Bode-Böger SM, Szuba A, Tsao PS, Chan JR, Tangphao O, Blaschke TF, Cooke JP (1998) Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. *Circulation* 98: 1842–1847
12. Böger RH, Lentz SR, Bode-Böger SM, Knapp HR, Haynes WG (2001) Elevation of asymmetrical dimethylarginine may mediate endothelial dysfunction during experimental hyperho- mocyst(e)inaemia in humans. *Clin Sci* 100:161–167
13. Stuhlinger MC, Tsao PS, Her JH, Kimoto M, Balint RF, Cooke JP (2001) Homocysteine impairs the nitric oxide synthase pathway: role of asymmetric dimethylarginine. *Circulation* 104: 2569–2575
14. Stuhlinger MC, Oka RK, Graf EE, Schmolzer I, Upson BM, Kapoor O, Szuba A, Malinow MR, Wascher TC, Pachinger O, Cooke JP (2003) Endothelial dysfunction induced by hyperho- mocyst(e)inemia: role of asymmetric dimethylarginine. *Circu- lation* 108:933–938
15. Fujiwara N, Osanai T, Kamada T, Katoh T, Takahashi K, Okumura K (2000) Study on the relationship between plasma nitrite and nitrate level and salt sensitivity in human hyperten- sion: modulation of nitric oxide synthesis by salt intake. *Circu- lation* 101:856–861
16. Osanai T, Fujiwara N, Saitoh M, Sasaki S, Tomita H, Nakamura M, Osawa H, Yamabe H, Okumura K (2002) Relationship between salt intake, nitric oxide and asymmetric dimethylargi- nine and its relevance to patients with end-stage renal disease. *Blood Purif* 20:466–468
17. Scuteri A, Stuehlinger MC, Cooke JP, Wright JG, Lakatta EG, Anderson DE, Fleg JL (2003) Nitric oxide inhibition as a mechanism for blood pressure increase during salt loading in normotensive postmenopausal women. *J Hypertens* 21: 1339–1346
18. Stuhlinger MC, Abbasi F, Chu JW, Lamendola C, McLaughlin TL, Cooke JP, Reaven GM, Tsao PS (2002) Relationship be- tween insulin resistance and an endogenous nitric oxide syn- thase inhibitor. *JAMA* 287:1420–1426
19. Mallamaci F, Tripepi G, Maas R, Malatino L, Böger RH, Zoccali C (2004) Analysis of the relationship between norepi- nephine and asymmetric dimethyl arginine levels among patients with end-stage renal disease. *J Am Soc Nephrol* 15: 435–441
20. Zoccali C, Benedetto FA, Maas R, Mallamaci F, Tripepi G, Salvatore ML, Böger RH (2002) Asymmetric dimethylarginine, C-reactive protein, and carotid intima-media thickness in end- stage renal disease. *J Am Soc Nephrol* 13:490–496
21. Kielstein JT, Bode-Böger SM, Klein G, Graf S, Haller H, Fliser D (2003) Endogenous nitric oxide synthase inhibitors and renal perfusion in patients with heart failure. *Eur J Clin Invest* 33: 370–375

22. Usui M, Matsuoka H, Miyazaki H, Ueda S, Okuda S, Imaizumi T (1998) Increased endogenous nitric oxide synthase inhibitor in patients with congestive heart failure. *Life Sci* 62:2425–2430
23. Fickling SA, Williams D, Vallance P, Nussey SS, Whitley GS (1993) Plasma concentrations of endogenous inhibitor of nitric oxide synthesis in normal pregnancy and pre-eclampsia. *Lancet* 342:242–243
24. Holden DP, Fickling SA, Whitley GS, Nussey SS (1998) Plasma concentrations of asymmetric dimethylarginine, a natural inhibitor of nitric oxide synthase, in normal pregnancy and preeclampsia. *Am J Obstet Gynecol* 178:551–556
25. Pettersson A, Hedner T, Milsom I (1998) Increased circulating concentrations of asymmetric dimethyl arginine (ADMA), an endogenous inhibitor of nitric oxide synthesis, in preeclampsia. *Acta Obstet Gynecol Scand* 77:808–813
26. Valkonen VP, Paiva H, Salonen JT, Lakka TA, Lehtimäki T, Laakso J, Laaksonen R (2001) Risk of acute coronary events and serum concentration of asymmetrical dimethylarginine. *Lancet* 358:2127–2128
27. Kielstein JT, Bode-Böger SM, Hesse G, Martens-Lobenhoffer J, Takacs A, Fliser D, Hoepfer MM (2005) Asymmetrical dimethylarginine in idiopathic pulmonary arterial hypertension. *Arterioscler Thromb Vasc Biol* 25:1414–1418
28. Kurose I, Wolf R, Grisham MB, Granger DN (1995) Effects of an endogenous inhibitor of nitric oxide synthesis on postcapillary venules. *Am J Physiol* 268:H2224–H2231
29. MacAllister RJ, Whitley GS, Vallance P (1994) Effects of guanidino and uremic compounds on nitric oxide pathways. *Kidney Int* 45:737–742
30. Segarra G, Medina P, Vila JM, Chuan P, Domenech C, Torondel B, Lluch A (2001) Inhibition of nitric oxide activity by arginine analogs in human renal arteries. *Am J Hypertens* 14:1142–1148
31. Segarra G, Medina P, Ballester RM, Lluch P, Aldasoro M, Vila JM, Lluch S, Pelligrino DA (1999) Effects of some guanidino compounds on human cerebral arteries. *Stroke* 30:2206–2210
32. Kielstein JT, Zoccali C (2005) Asymmetric dimethylarginine: a cardiovascular risk factor and a uremic toxin coming of age? *Am J Kidney Dis* 46:186–202
33. Gardiner SM, Kemp PA, Bennett T, Palmer RM, Moncada S (1993) Regional and cardiac haemodynamic effects of N^G , N^G -dimethyl-L-arginine and their reversibility by vasodilators in conscious rats. *Br J Pharmacol* 110:1457–1464
34. Faraci FM, Brian JE Jr, Heistad DD (1995) Response of cerebral blood vessels to an endogenous inhibitor of nitric oxide synthase. *Am J Physiol* 269:H1522–H1527
35. Jin JS, D'Alecy LG (1996) Central and peripheral effects of asymmetric dimethylarginine, an endogenous nitric oxide synthetase inhibitor. *J Cardiovasc Pharmacol* 28:439–446
36. White R, Barefield D, Ram S, Work J (1995) Peritoneal dialysis solutions reverse the hemodynamic effects of nitric oxide synthesis inhibitors. *Kidney Int* 48:1986–1993
37. Suda O, Tsutsui M, Morishita T, Tasaki H, Ueno S, Nakata S, Tsujimoto T, Toyohira Y, Hayashida Y, Sasaguri Y, Ueta Y, Nakashima Y, Yanagihara N (2004) Asymmetric dimethylarginine produces vascular lesions in endothelial nitric oxide synthase-deficient mice: involvement of renin-angiotensin system and oxidative stress. *Arterioscler Thromb Vasc Biol* 24:1682–1688
38. Dayoub H, Achan V, Adimoolam S, Jacobi J, Stuhlinger MC, Wang BY, Tsao PS, Kimoto M, Vallance P, Patterson AJ, Cooke JP (2003) Dimethylarginine dimethylaminohydrolase regulates nitric oxide synthesis: genetic and physiological evidence. *Circulation* 108:3042–3047
39. Calver A, Collier J, Leone A, Moncada S, Vallance P (1993) Effect of local intra-arterial asymmetric dimethylarginine (ADMA) on the forearm arteriolar bed of healthy volunteers. *J Hum Hypertens* 7:193–194
40. Kielstein JT, Impraïm B, Bode-Böger SM, Frölich JC, Haller H, Fliser D (2001) Acute administration of the endogenous NO synthase inhibitor asymmetric dimethylarginine does not affect blood pressure and renal perfusion in man. *J Am Soc Nephrol* 12:513 A
41. Kielstein JT, Impraïm B, Simmel S, Bode-Böger SM, Tsikas D, Frölich JC, Hoepfer MM, Haller H, Fliser D (2004) Cardiovascular effects of systemic nitric oxide synthase inhibition with asymmetrical dimethylarginine in humans. *Circulation* 109:172–177
42. Achan V, Broadhead M, Malaki M, Whitley G, Leiper J, MacAllister R, Vallance P (2003) Asymmetric dimethylarginine causes hypertension and cardiac dysfunction in humans and is actively metabolized by dimethylarginine dimethylaminohydrolase. *Arterioscler Thromb Vasc Biol* 23:1455–1459
43. Kielstein JT, Simmel S, Bode-Böger SM, Roth HJ, Schmidt-Gayk H, Haller H, Fliser D (2004) Subpressor dose asymmetric dimethylarginine modulates renal function in humans through nitric oxide synthase inhibition. *Kidney Blood Press Res* 27:143–147
44. Avontuur JA, Buijk SL, Bruining HA (1998) Distribution and metabolism of NG-nitro-L-arginine methyl ester in patients with septic shock. *Eur J Clin Pharmacol* 54:627–631
45. Closs EI, Basha FZ, Habermeier A, Förstermann U (1997) Interference of L-arginine analogues with L-arginine transport mediated by the y^+ carrier hCAT-2B. *Nitric Oxide* 1:65–73
46. Brunini T, Moss M, Siqueira M, Meirelles L, Rozental A, Mann G, Ellory J, Soares dM, Mendes-Ribeiro A (2004) Inhibition of L-arginine transport in platelets by asymmetric dimethylarginine and N-monomethyl-L-arginine: effects of arterial hypertension. *Clin Exp Pharmacol Physiol* 31:738–740
47. Ito A, Tsao PS, Adimoolam S, Kimoto M, Ogawa T, Cooke JP (1999) Novel mechanism for endothelial dysfunction: dysregulation of dimethylarginine dimethylaminohydrolase. *Circulation* 99:3092–3095