Asymmetric Dimethylarginine, L-Arginine, and Endothelial Dysfunction in Essential Hypertension

Francesco Perticone, MD,* Angela Sciacqua, MD,* Raffaele Maio, MD,* Maria Perticone, MD,*
Renke Maas, MD,† Rainer H. Boger, MD,† Giuseppe Tripepi, STAT TECH,‡ Giorgio Sesti, MD,*
Carmine Zoccali, MD‡

Catanzaro and Reggio Calabria, Italy; and Hamburg–Eppendorf, Germany

OBJECTIVES	We investigated the relationship between ADMA plasma levels and endothelium-dependent vasodilation in 36 never-treated essential hypertensives and in 8 normotensive healthy subjects.
BACKGROUND	It has been demonstrated that endothelium-dependent vasodilatation is impaired in essential hypertension. The potential contribution of asymmetric dimethylarginine (ADMA) to endothelial dysfunction of hypertensive humans has received poor attention.
METHODS	Endothelial function was measured during intra-arterial infusion of acetylcholine (ACh), alone and during co-infusion of L-arginine, and sodium nitroprusside at increasing doses. Concentrations of ADMA and L-arginine in plasma were measured by high-performance liquid chromatography.
RESULTS	Hypertensive subjects had significantly higher ADMA and L-arginine plasma concentrations than normotensive healthy controls; ACh-stimulated forearm blood flow (FBF) was significantly reduced in hypertensive subjects in comparison to normotensive control subjects ($p < 0.0001$). Intra-arterial coinfusion of L-arginine induced a further significant enhancement in ACh-stimulated vasodilation in hypertensive patients. In these, ADMA was strongly and inversely associated with the peak increase in FBF. In a multivariate model, only ADMA and L-arginine were independent correlates, accounting for 33.9% and 8.9% of the variability in the peak FBF response to ACh ($p < 0.0001$), respectively.
CONCLUSIONS	The main finding in this study is that in essential hypertensives the L-arginine and endogenous inhibitor of nitric oxide synthase, ADMA, are inversely related to endothelial function. (J Am Coll Cardiol 2005;46:518–23) © 2005 by the American College of Cardiology Foundation

Anatomical and functional integrity of the vascular endothelium is fundamental for preventing the appearance and progression of both coronary and extracoronary atherosclerosis (1–3). Many vascular protective effects of the endothelium—including vasodilatation (1,4), inhibition of monocyte and leukocyte adhesion (2,3), inhibition of platelet aggregation (2,5), modulation of vascular smooth muscle cells, and fibroblasts proliferation (2,3,6)—are regulated by nitric oxide (NO), a short-lived molecule, produced by the endothelial enzyme nitric oxide synthase (e-NOS) from the amino acid L-arginine (7).

It is now well established that major risk factors for cardiovascular diseases (8–14) impact upon endothelial function by decreasing NO bioavailability. This condition, which occurs early in vascular disease, may be caused by various mechanisms including decreased NO synthesis, increased NO degradation due to oxidative stress, or to reduced sensitivity to NO (2,4,15,16). With regard to the first mechanism, the activity of e-NOS may be inhibited by endogenous analogues of L-arginine such as asymmetric dimethylarginine (ADMA) (17), which has been shown to be increased in patients with chronic renal diseases (18), in familial hypercholesterolemia, and in a variety of clinical situations (19–22) including essential hypertension (23,24).

It has been consistently demonstrated that endothelium-dependent vasodilatation is impaired in essential hypertension (8,9,14–16). This alteration has been attributed to reduced NO synthesis due to a specific defect in the phosphoinositide pathway leading to activation of e-NOS (25), increased NO breakdown due to an increased production of superoxide anions (8,15,16), and interaction with endothelium-derived factors (15). Even though the demonstration by Vallance that the vasoactive effects of NO inhibition by ADMA in man (18) dates back to the early 1990s, the potential contribution of ADMA to endothelial dysfunction of hypertensive humans has received poor attention.

In this study we investigated the relationship between ADMA plasma levels and endothelium-dependent vasodilation in a well-selected group of never-treated essential hypertensives without cardiovascular complications and in a well-matched group of normotensive healthy subjects.

METHODS

Hypertensive subjects and healthy normotensive controls.
Thirty-six hypertensive subjects, consecutively recruited from the Department of Experimental and Clinical Medi-

---

Abbreviations and Acronyms

ACh   = acetylcholine
ADMA = asymmetric dimethylarginine
BMI   = body mass index
BP   = blood pressure
e-NOS = endothelial nitric oxide synthase
FBF   = forearm blood flow
HR   = heart rate
NO   = nitric oxide
SNP   = sodium nitroprusside
VR   = vascular resistance

---

cine of the University Hospital of Catanzaro, participated in this study. All subjects were Caucasian and underwent physical examination and review of their medical history. To be selected, patients had to have newly diagnosed essential hypertension with a serum creatinine <1.5 mg/dl, have an absence of proteinuria on the dipstick test, and have never received antihypertensive medications. Causes of secondary hypertension were excluded by appropriate clinical and biochemical tests. None of the patients had history or clinical evidence of coronary artery disease, valvular heart disease, diabetes, hyperlipidemia, peripheral vascular disease, coagulopathy, or any disease predisposing to vasculitis or Raynaud's phenomenon. The control group was formed by eight healthy normotensive volunteers well matched to hypertensive patients as for age, body mass index (BMI), and Framingham risk factors.

The study was approved by the institutional ethics committee, and informed written consent was obtained from each subject in accordance with principles of the Declaration of Helsinki.

Study protocol. Blood sampling and vascular function were performed at 9 AM after subjects had fasted overnight, with the subjects lying supine in a quiet, air-conditioned room (22°C to 24°C). Participants were instructed to continue their regular diet. Caffeine, alcohol, and smoking were stopped at least 24 h before the study. Readings of clinic blood pressure (BP) were obtained in the left arm of the supine patients, after 5 min of quiet rest, with a mercury sphygmomanometer. Three BP readings were taken on three separate occasions at least two weeks apart. Baseline BP values were the average of last two of the three consecutive measurements obtained at intervals of 3 min. Patients with a clinic BP ≥140 mm Hg systolic and/or 90 mm Hg diastolic were defined as hypertensive.

Hemodynamic studies. Forearm volume was determined by water displacement. Under local anesthesia and sterile conditions, a 20-gauge polyethylene catheter (Vasculon 2, BD, Franklin Lakes, New Jersey) was inserted into the brachial artery of the nondominant arm for evaluation of BP (Baxter Healthcare Corp., Deerfield, Illinois) and for drug's infusion. This arm was elevated above the level of the right atrium, and a mercury-filled silastic strain-gauge was placed on the widest part of the forearm. The strain-gauge was connected to a plethysmograph (model EC-4, D.E. Hokanson, Issaquah, Washington) calibrated to measure the percent change in volume; this was connected to a chart recorder to obtain the forearm blood flow (FBF) measurements. A cuff placed on the upper arm was inflated to 40 mm Hg with a rapid cuff inflator (model E-10 Hokanson) to exclude venous outflow from the extremity. A wrist cuff was inflated to BP values 1 min before each measurement to exclude the hand blood flow. The antecubital vein of the opposite arm was cannulated. The FBF was measured as the slope of the change in the forearm volume. The mean of at least three measurements was obtained at each time point. Forearm vascular resistance (VR), expressed in units, was calculated by dividing mean BP by FBF.

Acetylcholine (ACh) and sodium nitroprusside (SNP) infusions. A standardized protocol, previously described by Panza et al. (8), and subsequently adopted by our group (13,14), was employed for the present study. All participants underwent measurement of FBF and BP during intra-arterial infusion of saline, ACh, and SNP at increasing doses. Acetylcholine (Sigma, Milan, Italy) was diluted with saline immediately before infusion. Sodium nitroprusside (Malesci, Florence, Italy) was diluted in 5% glucose solution immediately before each infusion and protected from light with aluminum foil. All participants rested 30 min after artery cannulation to reach a stable baseline before data collection; measurements of FBF and VR were repeated every 5 min until stable. Endothelium-dependent and endothelium-independent vasodilation were assessed by a dose-response curve to intra-arterial ACh infusions (7.5, 15, and 30 $\mu g/ml^{-1}/min^{-1}$, each for 5 min) and SNP infusions (0.8, 1.6, and 3.2 $\mu g/ml^{-1}/min^{-1}$, each for 5 min), respectively. The infusions of ACh and SNP were carried out in random order to avoid any bias related to the sequence of drug infusion. The drug infusion rate, adjusted for forearm volume of each subject, was 1 ml/min.

L-arginine infusion. After a stabilization period of 20 to 30 min, resting FBF was measured again, and a dose-response curve to intrabrachial ACh administration was performed during the co-infusion of L-arginine, the substrate for NO synthesis, at a constant dose of 200 $\mu mol/min$, starting 10 min before ACh administration and continuing throughout.

Determination of ADMA and L-arginine. Samples were stored in pre-chilled vacutainers containing edetic acid, placed immediately on ice, and centrifuged within 30 min at 4°C; plasma was stored at −80°C until required. Concentrations of ADMA and L-arginine in plasma were measured by high-performance liquid chromatography, by pre-column derivatization with o-phthalaldehyde, after removal of plasma samples with carboxylic acid solid-phase extraction cartridges (Varian, Harbor City, California). The coefficients of variation were 5.2% within-assay and 5.5% between-assay; the detection limit of the assay was 0.1 $\mu mol/l^{-1}$.

Statistical analysis. Differences for clinical and biological data were compared by using unpaired Student t test and chi-square test. The vasodilatory responses to ACh and SNP were compared by analysis of variance for repeated measurements and, when analysis was significant, the Tukey test was applied. Simple linear regression analysis was performed to assess the relationship between the peak percent increase in FBF in response to ACh infusion, ADMA, and L-arginine. The independent relationship between ADMA, L-arginine, and the hemodynamic response to ACh in hypertensive subjects was also tested by backward multiple regression analysis. This model retained just two variables as statistically significant and was therefore adequately powered (>10 subjects per covariate) to test the hypothesis. Parametric data are reported as mean ± SD. Significant differences were assumed to be at p < 0.05. All comparisons were performed using the statistical package SPSS 10.0 for Windows (SPSS Inc., Chicago, Illinois).

RESULTS

All participants completed the protocol. Baseline demographic, hemodynamic, and humoral characteristics are summarized in Table 1. There were no significant differences in age, total cholesterol, high-density lipoprotein cholesterol, glucose, triglycerides, BMI, and FBF between control subjects and hypertensive patients. All patients were normotolerant, and there were no smokers. Vascular resistance was higher (p < 0.05) in hypertensive subjects than normotensive healthy controls. According to inclusion criteria, systolic and diastolic BP values were significantly (p < 0.0001) lower in control subjects in comparison with hypertensive patients.

ADMA and L-arginine concentrations. Hypertensive subjects had significantly higher ADMA plasma concentration than normotensive healthy controls (0.59 ± 0.14 μmol/l vs. 0.40 ± 0.09 μmol/l, p < 0.0001). Plasma

Table 1. Characteristics of the Study Population

	Hypertensives (n = 36)	Normotensives (n = 8)	p Value
Gender (males/females)	28/8	4/4	0.247
Age (yrs)	44.2 ± 8.8	44.5 ± 5.8	0.927
Body mass index (kg/m²)	27.2 ± 2.8	26.2 ± 4.0	0.404
Heart rate (beats/min)	74.5 ± 8.5	75.8 ± 11.2	0.714
Systolic BP (mm Hg)	155.9 ± 8.3	124.4 ± 4.1	0.000
Diastolic BP (mm Hg)	93.1 ± 7.3	69.1 ± 4.8	0.000
Pulse pressure (mm Hg)	62.8 ± 7.8	55.4 ± 6.0	0.016
Fasting glucose (mg/dl)	92.4 ± 8.4	90.4 ± 12.5	0.581
Total cholesterol (mg/dl)	194.6 ± 25.3	186.7 ± 29.0	0.444
LDL cholesterol (mg/dl)	126.4 ± 27.4	121.1 ± 31.4	0.632
HDL cholesterol (mg/dl)	48.0 ± 10.4	46.0 ± 8.3	0.614
Triglycerides (mg/dl)	100.6 ± 21.9	98.1 ± 35.4	0.797
Creatinine (mg/dl)	0.87 ± 0.10	0.83 ± 0.11	0.312
FBF (m · 100 ml⁻¹ of tissue · min⁻¹)	3.36 ± 0.28	3.49 ± 0.23	0.229
Vascular resistance (U)	33.2 ± 4.3	26.3 ± 2.5	0.000

BP = blood pressure; FBF = forearm blood flow; HDL = high-density lipoprotein; LDL = low-density lipoprotein.
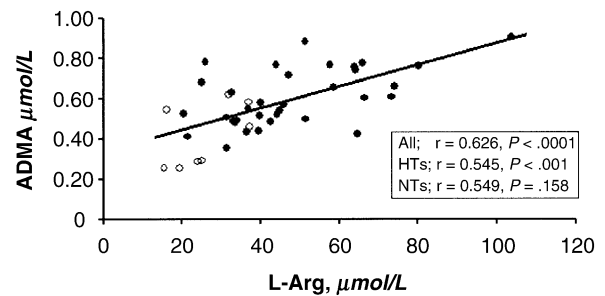

Figure 1. Relationship between L-arginine (L-Arg) and asymmetric dimethylarginine (ADMA) in both normotensive subjects (NTs) **(open circles)** and hypertensive patients (HTs) **(solid circles)**. In the whole population (r = 0.626; p < 0.0001) and in the HT group (r = 0.545; p < 0.001), the two covariates resulted linearly correlated; no relationship was found in the NT group.

L-arginine concentrations (μmol/l) were 47.4 ± 18.5 in the hypertensive group and 26.0 ± 8.1 in normotensive control subjects (p = 0.003). An increase in plasma ADMA resulted in a higher, but not significant, mean L-arginine/ADMA ratio in hypertensive than in normotensive subjects (79.4 ± 21.8 vs. 69.0 ± 25.5, p = 0.243). In Figure 1 we report the relationship between L-arginine and ADMA in the whole population.

Endothelium-dependent and -independent vasodilation. Baseline FBF did not differ in the two groups (Table 1). Intra-arterial infusion of ACh caused a significant dose-dependent increase in FBF and decrease in forearm VR in both groups. The FBF values at the three incremental doses of ACh were 7.5 ± 1.4, 11.5 ± 2.3, and 22.3 ± 5.9 ml/100 ml[1] of tissue/min[1] and 6.3 ± 1.9, 9.4 ± 2.5, and 15.3 ± 4.7 ml/100 ml[1] of tissue/min[1] for normotensive and hypertensive patients, respectively (Fig. 2). The VR values at the three incremental doses of ACh were 21.2 ± 7.4, 16.9 ±
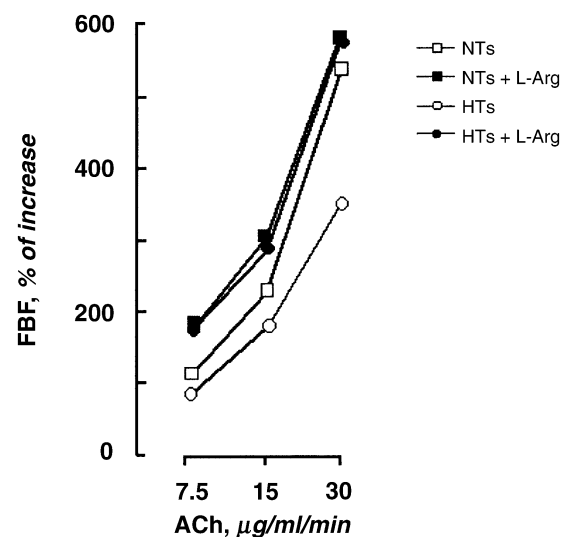

Figure 2. Forearm blood flow (FBF) increase during infusion of acetylcholine (ACh). As shown, ACh-stimulated FBF was significantly reduced in hypertensive subjects (HTs) in comparison to normotensive control (NTs) subjects. Intra-arterial coinfusion of L-arginine (L-Arg) induced a further significant enhancement in ACh-stimulated vasodilation in HT patients.

5.5, and 8.7 ± 2.6 U and 26.5 ± 8.1, 19.7 ± 6.9, and 11.2 ± 2.5 U for normotensive subjects and hypertensive patients, respectively. Thus, ACh-stimulated FBF was significantly reduced in hypertensive subjects in comparison to normotensive control subjects ($p < 0.0001$). In both groups BP and heart rate (HR) remained unchanged during intra-arterial infusion of ACh.

Incremental doses of intra-arterial infusion of SNP induced a significant increase in FBF as well as a decrease in forearm VR in both normotensive subjects and hypertensive patients. At the highest dose of SNP, the FBF percent increments ($17.7 ± 2.3$ vs. $17.3 ± 4.1$ ml/100 ml^{-1} of tissue/min^{-1}) as well as the VR ($4.9 ± 1.6$ vs. $6.6 ± 1.6$ U) decrements from baseline did not differ between the two groups. In both groups intra-arterial infusion of SNP did not cause any significant change in BP or HR.

Effects of L-arginine on endothelial function. As shown in Figure 2, intra-arterial coinfusion of L-arginine induced a further significant enhancement—above resting levels—in ACh-stimulated vasodilation in hypertensive patients (area under the curve = $664 ± 70$ vs. $402 ± 151$; $p < 0.0001$). A slight, but significant, increase was observed also in normotensive subjects (area under the curve = $688 ± 84$ vs. $556 ± 108$; p= 0.016). Comparison of area under the curve in normotensive and hypertensive subjects was not significant ($p = 0.402$).

The L-arginine coinfusion did not change arterial BP or HR in both hypertensives and control subjects.

Correlation analyses. As shown in Figure 3, in hypertensive subjects ADMA was strongly and inversely associated with the peak increase in ACh-stimulated FBF. Of note, also L-arginine was inversely related with the same hemodynamic response (Table 2). L-arginine and ADMA were directly related ($r = 0.545$, $p < 0.001$) (Fig. 1). No such relationships were found in normotensive subjects (p values from 0.06 to 0.260).

To further analyze the independent contribution of ADMA and L-arginine to the peak FBF response to ACh, we constructed a backward multivariate model testing ADMA, L-arginine, and Framingham risk factors listed in Table 1. This multivariate model retained only ADMA and L-arginine as independent correlates of the outcome mea-
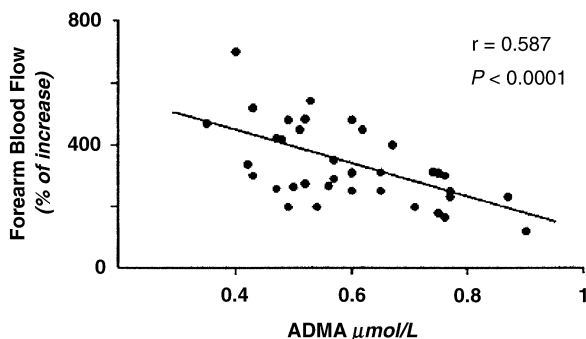
Table 2. Correlational Analysis Between Forearm Blood Flow as Dependent Variable, ADMA, L-Arginine, and L-Arginine/ADMA

	Hypertensives (n = 36)	p Value	Normotensives (n = 8)	p Value
ADMA	−0.583	0.007	0.601	0.057
L-arginine	−0.567	0.000	0.018	0.483
L-arginine/ ADMA	−0.281	0.049	−0.268	0.260

ADMA = asymmetric dimethylarginine.

sure accounting for 33.9% and 8.9% of the variability in the peak FBF response to ACh ($p < 0.0001$), respectively. In particular, ACh-stimulated FBF decreases by 2.4% for every μmol of L-arginine, and by 35.8% for every 0.1 μmol of ADMA.

DISCUSSION

The main finding in this study is that in essential hypertensives the endogenous inhibitor of e-NOS ADMA is inversely related to endothelial function as measured by the peak hemodynamic response to ACh. Such relationship occurs in a range of ADMA values within the boundaries of the normal range. A companion, unexpected, finding in this study is that circulating L-arginine is directly related to plasma ADMA and, like plasma ADMA, it is inversely related to endothelial function.

The importance of ADMA as an endogenous inhibitor of e-NOS is now well established (26–28). Elegant studies in healthy volunteers convincingly demonstrated that intravenous ADMA infusion at a dose resulting in pathophysiological concentrations augments peripheral and renovascular resistance and arterial pressure (22). High plasma ADMA concentration was observed in the presence of traditional or emerging cardiovascular risk factors (e.g., hyperhomocysteinemia) (18–21,29), inducing endothelial dysfunction in some of these conditions (30–33).

The relationship between ADMA and essential hypertension has been scarcely explored. Plasma ADMA levels were measured only in four studies and coherently found to be higher in hypertensive patients (23,24) than in normotensive healthy subjects (22), particularly in salt-sensitive individuals (34). However, in none of these studies the relationship between ADMA and endothelial function was tested. In one study, urinary nitrate excretion was reduced concomitantly with elevated ADMA plasma levels in patients with essential hypertension, suggesting that systemic NO production was impaired in these patients (24).

Remarkably, we found that plasma ADMA, though within the limits of the physiologic concentration, was higher in hypertensives than in normotensive subjects and inversely related with ACh-stimulated FBF. This relationship was independent of potential confounders because, in a multivariate model, ADMA, but not other risk factors, retained an independent association with such a response. Thus, our study provides the first demonstration that in

Figure 3. In this figure the correlation between asymmetric dimethylarginine (ADMA) levels and the peak increase in acetylcholine-stimulated forearm blood flow is reported in hypertensive patients.

essential hypertension relatively higher ADMA plasma levels impair endothelium-dependent vasodilation.

The cause of high plasma ADMA concentration in essential hypertension is presently unknown. Increased shear stress triggers ADMA synthesis, and high ADMA in hypertension may therefore be an epiphenomenon of high BP (35). Alternatively, high ADMA may result from reduced catabolic rate secondary to dimethylaminohydrolase inhibition brought about by oxidative stress, a well-known feature of human hypertension (15,26). Independently of the mechanism responsible for the ADMA increase, our data suggest that this increase is causally involved in endothelial dysfunction; in keeping with this, impaired FBF response to ACh in hypertensive subjects reverts to normal during coinfusion of L-arginine, an amino acid that competes with ADMA at level of catalytic sites of e-NOS.

An unexpected and intriguing finding in this study is that, again within the limits of the normal range, plasma L-arginine was higher in essential hypertensives than in normotensive subjects. This alteration has been very recently noted in another study and attributed to an inhibition of L-arginine transport via system y+, a phenomenon that may also limit NO synthesis (36). Furthermore, we observed that plasma L-arginine concentration was directly related to plasma ADMA concentration. L-arginine metabolism is complex and highly regulated (37). This amino acid is synthesized from citrulline by sequential action of cytosolic enzymes arginosuccinate synthetase and lyase (38). Arginase activity may be another determinant of L-arginine plasma concentrations (39). Animal studies indeed suggest that expression or activity of arginases, which degrade L-arginine, may be altered in hypertension as well (40,41). In this perspective it can be speculated that that relatively higher L-arginine in essential hypertensives is a counter-regulatory response aimed at compensating NO inhibition by ADMA, a possibility supported by the direct relationship linking plasma L-arginine and ADMA. Although the two interpretations are not mutually exclusive, the inverse relationship between plasma L-arginine and maximal response to ACh, obtained by us, would support the hypothesis that L-arginine transport or metabolism is altered in essential hypertension. On the other hand, the fact that intra-arterial infusion of L-arginine restores endothelial function clearly indicates that disturbed transport of this amino acid into the endothelial cell is completely surmounted at high infusion rates.

In conclusion, there is a subtle increase in plasma ADMA in essential hypertensives. Such an increase seems functionally relevant because relatively higher plasma ADMA levels underlie endothelial dysfunction. Plasma L-arginine concentration in hypertensive patients parallels plasma ADMA concentration, and relatively higher L-arginine levels are associated with compromised endothelial function, a phenomenon in keeping with the hypothesis that in essential hypertension the transport of this amino acid is altered.

Reprint requests and correspondence: Dr. Francesco Perticone, Department of Medicina Sperimentale e Clinica, Policlinico Mater Domini, Via Tommaso Campanella, 88100 Catanzaro, Italy. E-mail: perticone@unicz.it.

REFERENCES

1. Vane JR, Auggard EE, Botting RM. Regulatory functions of the vascular endothelium. N Engl J Med 1990;323:27–36.
2. Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease. N Engl J Med 1992;326:242–50.
3. Ross R. Atherosclerosis: an inflammatory disease. N Engl J Med 1999;340:115–26.
4. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980;288:373–6.
5. de Graaf JC, Banga JD, Moncada S, Palmer RM, de Groot PG, Sixma JJ. Nitric oxide inhibitor of platelet adhesion under flow conditions. Circulation 1992;85:2284–90.
6. Jeremy JY, Rowe D, Emsley AM, Newby AC. Nitric oxide and the proliferation of vascular smooth muscle cells. Cardiovasc Res 1999;43:580–94.
7. Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987;327:524–6.
8. Panza JA, Quyyumi AA, Brush JE Jr., Epstein SE. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. N Engl J Med 1990;323:22–7.
9. Taddei S, Virdis A, Mattei P, Salvetti A. Vasodilation to acetylcholine in primary and secondary forms of human hypertension. Hypertension 1993;21:929–33.
10. Zeiher AM, Schächinger V, Minners J. Long-term cigarette smoking impairs endothelium-dependent coronary arterial vasodilation function. Circulation 1995;92:1094–100.
11. Johnstone MT, Creager SJ, Scales KM, Cusco JA, Lee BK, Creager MA. Impaired endothelium-dependent coronary arterial vasodilation in patients with insulin-dependent diabetes mellitus. Circulation 1993;88:2510–6.
12. Creager MA, Cooke JP, Mendelsohn ME, et al. Impaired vasodilation of forearm resistance vessel in hypercholesterolemic humans. J Clin Invest 1990;86:228–34.
13. Perticone F, Ceravolo R, Candigliota M, et al. Obesity and body fat distribution induces endothelial dysfunction by oxidative stress. Protective effect of vitamin C. Diabetes 2001;501:159–65.
14. Perticone F, Ceravolo R, Pujia A, et al. Prognostic significance of endothelial dysfunction in hypertensive patients. Circulation 2001;104:191–6.
15. Lüscher TF, Vanhoutte PM. The Endothelium: Modulator of Cardiovascular Function. Boca Raton, FL: CRC Press, 1990.
16. Quyyumi AA. Endothelial function in health and disease: new insights into the genesis of cardiovascular disease. Am J Med 1998;105:32–9S.
17. MacAllister RJ, Fickling SA, Whitley GS, Vallance P. Metabolism of methylarginines by human vasculature: implications for the regulation of nitric oxide synthesis. Br J Pharmacol 1994;112:43–8.
18. Vallance P, Leone A, Calver A, Collier J, Moncada S. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. Lancet 1992;339:572–5.
19. Lu TM, Ding YA, Leu HB, Yin WH, Sheu WH, Chu KM. Effect of rosuvastatin on plasma levels of asymmetric dimethylarginine in patients with hypercholesterolemia. Am J Cardiol 2004;94:157–61.
20. Cooke JP, Dzan VJ. Derangements of the nitric oxide synthase pathway, L-arginine, and cardiovascular diseases. Circulation 1997;96:379–82.
21. Boger RH, Bode-Boger SM, Thiele W, Junker W, Alexander K, Frolich JC. Biochemical evidence for impaired nitric oxide synthase in patients with peripheral arterial occlusive diseases. Circulation 1997;95:2068–74.
22. Calver A, Collier J, Leone A, Moncada S, Vallance P. Effect of local intra-arterial asymmetric dimethylarginine (ADMA) on the forearm arteriolar bed of healthy volunteers. J Hum Hypertens 1993;7:193–4.
23. Palloshi A, Fragasso G, Piatti P, et al. Effect of oral L-arginine on blood pressure and symptoms and endothelial function in patients with

systemic hypertension, positive exercise test, and normal coronary arteries. Am J Cardiol 2004;93:930–5.

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

25. Zeng G, Nystrom FH, Ravichandran LV, et al. Roles for insulin receptor, PI3-kinase, and Akt in insulin-signaling pathways related to production of nitric oxide in human vascular endothelial cells. Circulation 2000;101:1539–45.

26. Achan V, Broadhead M, Malaki M, et al. Asymmetric dimethylarginine causes hypertension and cardiac dysfunction in humans and is actively metabolized by dimethylarginine dimethylaminohydrolase. Arterioscler Thromb Vasc Biol 2003;23:1455–9.

27. Cooke JP. Asymmetrical dimethylarginine: the Uber marker? Circulation 2004;109:1813–8.

28. Böger RH. The emerging role of asymmetric dimethylarginine as a novel cardiovascular risk factor. Cardiovasc Res 2003;59:824–33.

29. Stuhlinger MC, Oka RK, Graf EE, et al. Endothelial dysfunction induced by hyperhomocyst(e)inemia: role of asymmetric dimethylarginine. Circulation 2003;108:933–8.

30. Thorne S, Mullen MJ, Clarkson P, Donald AE, Deanfield JE. Early endothelial dysfunction in adults at risk from atherosclerosis: different responses to L-arginine. J Am Coll Cardiol 1998;32:110–6.

31. Fard A, Tuck CH, Donis JA, et al. Acute elevations of plasma asymmetric dimethylarginine and impaired endothelial function in response to a high-fat meal in patients with type 2 diabetes. Arterioscler Thromb Vasc Biol 2000;20:2039–44.

32. Chao CL, Kuo TL, Lee YT. Effects of methionine-induced hyperhomocysteinemia on endothelium-dependent vasodilation and oxidative status in healthy adults. Circulation 2000;101:485–90.

33. Böger RH, Lentz SR, Bode-Boger SM, Knapp HR, Haynes WG. Elevation of asymmetrical dimethylarginine may mediate endothelial dysfunction during experimental hyperhomocyst(e)inaemia in humans. Clin Sci 2001;100:161–7.

34. Fujiwara N, Osanai T, Kamada T, Katoh T, Takahashi K, Okumura K. Study on the relationship between plasma nitrite and nitrate level and salt sensitivity in human hypertension: modulation of nitric oxide synthesis by salt intake. Circulation 2000;101:856–61.

35. Osanai T, Saitoh M, Sasaki S, Tomita H, Matsunaga T, Okumura K. Effect of shear stress on asymmetric dimethylarginine release from vascular endothelial cells. Hypertension 2003;42:985–90.

36. Moss MB, Brunini TM, Soares De Moura R, et al. Diminished L-arginine bioavailability in hypertension. Clin Sci 2004;107:391–7.

37. Arnal JF, Munzel T, Venema RC, et al. Interactions between L-arginine and L-glutamine change endothelial NO production. An effect independent of NO synthase substrate availability. J Clin Invest 1995;95:2565–72.

38. Bogle RG, Baydoun AR, Pearson JD, Mann GE. Regulation of L-arginine transport and nitric oxide release in superfused porcine aortic endothelial cells. J Physiol 1996;490:229–41.

39. Morris CR, Poljakovic M, Lavrisha L, Machado L, Kuypers FA, Morris SM Jr. Decreased arginine bioavailability and increased serum arginase activity in asthma. Am J Respir Crit Care Med 2004;170: 148–53.

40. Iwata S, Tsujino T, Ikeda Y, et al. Decreased expression of arginase II in the kidneys of Dahl salt-sensitive rats. Hypertens Res 2002;25: 411–t8.

41. Rodriguez S, Richert L, Berthelot A. Increased arginase activity in aorta of mineralocorticoid-salt hypertensive rats. Clin Exp Hypertens 2000;22:75–85.