

# Arginine Metabolism: Enzymology, Nutrition, and Clinical Significance

## L-Arginine and Hypertension<sup>1,2</sup>

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**ABSTRACT** Hypertension is a major healthcare problem afflicting nearly 50 million individuals in the United States. Despite its strong causal association with cardiovascular disease complications including myocardial infarction, heart failure, and stroke, the majority of patients with hypertension do not achieve optimal blood pressure control. The prevalence of hypertension is expected to increase with the aging population, growing obesity epidemic, and rising incidence of metabolic syndrome. Endothelial dysfunction and reduced nitric oxide (NO) bioactivity represent prominent pathophysiological abnormalities associated with hypertensive cardiovascular disease. Individuals with hypertension exhibit blunted epicardial and resistance vascular dilation to endothelium-derived nitric oxide (EDNO) agonists in the peripheral and coronary circulation that likely contributes to mechanisms of altered vascular tone in hypertension. The amino acid L-arginine serves as the principal substrate for vascular NO production. Numerous studies, though not uniformly, demonstrate a beneficial effect of acute and chronic L-arginine supplementation on EDNO production and endothelial function, and L-arginine has been shown to reduce systemic blood pressure in some forms of experimental hypertension. This brief review discusses the potential role of L-arginine in hypertension, and reviews possible mechanisms of L-arginine action including modulation of EDNO production, alteration of asymmetrical dimethylarginine (ADMA):L-arginine balance, and possible improvement of insulin sensitivity. In view of the rising prevalence of hypertension, randomized human clinical studies investigating the potential therapeutic role of L-arginine may be warranted. *J. Nutr.* 134: 2807S–2811S, 2004.

**KEY WORDS:** • L-arginine • hypertension • endothelium • nitric oxide

Approximately 50 million individuals in the United States are hypertensive as defined by an elevated systolic blood pressure  $\geq 140$  mm Hg (18.7 kPa) or diastolic blood pressure  $\geq 90$  mm Hg (12.0 kPa) (1). Hypertension is directly linked to cardiovascular complications including coronary artery disease, left ventricular hypertrophy, and heart failure. It represents the most common risk factor for stroke, and is a major contributor to the development of renal insufficiency and end-stage kidney disease. Data compiled from the National Heart, Lung, and Blood Institute (NHLBI)<sup>4</sup> Framingham Heart Study and the Seventh Report of the Joint National Committee (JNC-7) indicate that most individuals will de-

velop hypertension during their lifetime (2). It is estimated that for every 20/10-mm Hg (2.7/1.3-kPa) rise in blood pressure, there is a doubling in the risk of cardiovascular death. The number of hypertensive patients is expected to grow because of the progressive aging of the population. In younger individuals, the obesity epidemic that currently afflicts 65% of the U.S. population and its link to elevated blood pressure and metabolic syndrome is likely to produce a staggering rise in the prevalence of hypertension (3). Mortality rates from myocardial infarction, stroke, and other vascular diseases decline steadily with effective antihypertensive therapy, and treatment of hypertension represents the second most common reason for physician office visits and prescription medication therapy. However, national survey data show that only 70% of Americans are aware of their elevated blood pressure, and only one-third of individuals with hypertension are adequately treated or achieve optimal blood pressure control. Adjunct therapeutic interventions to existing treatment paradigms have the potential for marked therapeutic effects.

### Endothelial dysfunction in hypertension

With relevance to the nitric oxide/L-arginine pathway, it is recognized that the endothelium modulates vascular tone through the synthesis and elaboration of vasodilator mediators

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<sup>4</sup> Abbreviations used: ADMA, asymmetric dimethylarginine; cGMP, cyclic GMP; EDNO, endothelium-derived nitric oxide; JNC-7, Seventh Report of the Joint National Committee; L-NMMA: N<sup>G</sup>-monomethyl-L-arginine; NHLBI, National Heart, Lung, and Blood Institute; NO, nitric oxide; NOS: nitric oxide synthase.

including NO (4). Endothelium-derived nitric oxide (EDNO) regulates arterial tone through a dilator action on vascular smooth muscle cells that depends on soluble guanylyl cyclase activation and consequent increase in intracellular cyclic 3'5'-guanosine monophosphate (cGMP). Studies demonstrating increased blood pressure in animals lacking endothelial nitric oxide synthase (NOS) provide evidence for a role of NO in the regulation of arterial pressure (5). Pharmacological evidence supporting this contention is provided by the observation that infusion of NOS inhibitors such as  $N^G$ -monomethyl-L-arginine (L-NMMA) produces acute blood pressure elevation in animals, and long-term NOS inhibition leads to chronic arterial hypertension (6). Human studies of clinical hypertension that examined vasomotor responses also provide evidence for loss of NO bioaction in this disease state. Coronary vascular dilation to EDNO-agonists is impaired in patients with essential hypertension, and similar findings are reported in most (7,8), but not all (9), clinical studies of forearm circulation. L-NMMA reduces resting blood flow less in patients with hypertension, suggesting a derangement in basal as well as stimulated release of EDNO in hypertension (10). Reduced NO synthesis or increased inactivation may play an important role in alterations of vascular tone contributing to increased arterial resistance. In some studies, the vascular relaxation to nitroglycerin is also blunted (11), indicating associated alterations in vascular smooth muscle response to NO derived from either the endothelium or an exogenous source in advanced hypertension.

Nitric oxide inactivation owing to excess generation of reactive oxygen species, increased production of endogenous vasoconstrictors such as angiotensin-II and endothelin, decreased bioavailability of L-arginine, and defects in intracellular transduction pathways are several proposed mechanisms implicated in the pathophysiology of hypertension (12). In addition to functional abnormalities in the systemic vasculature, animal studies of hypertension also provide information about the mechanisms of impaired EDNO action in the renal regulation of plasma volume and hemodynamics, although findings are highly dependent on the experimental model. For example, in Dahl salt-sensitive rats, sodium chloride loading induces endothelial dysfunction and hypertension, whereas blood pressure and vasodilator responses remain normal when these animals consume a low-salt diet. In this model, L-arginine prevents the development of hypertension, and this effect can be overcome by an inhibitor of NOS (13). It is now understood that renovascular NO production modulates salt and water excretion, and that salt-sensitive hypertension may reflect an impairment of NO action (14). In contrast, in spontaneously hypertensive rats, L-arginine does not prevent development of elevated blood pressure, arguing against an absolute or relative deficiency of NOS substrate in some mechanisms of hypertension.

Whether endothelial dysfunction develops as a consequence of chronically elevated blood pressure or is involved in the pathogenesis of hypertension itself remains unclear. In rats, blockade of NO synthesis by chronic administration of L-NMMA produces severe hypertension (15), and mice deficient in the endothelial NOS gene are hypertensive (5,16). Normotensive offspring of patients with essential hypertension demonstrate blunted acetylcholine-mediated relaxation that may be improved by administration of L-arginine, implying a primary abnormality or genetic basis for a defect in NO activity in some forms of hypertension (17). In contrast, evidence that vasomotor dysfunction may be a secondary phenomenon is suggested by studies demonstrating that acute hypertension

impairs microvascular responses, and antihypertensive treatment has the potential to restore EDNO action (18).

Recent longitudinal studies demonstrate that loss of endothelial homeostasis in hypertension plays a key role in myocardial, cerebral, and renal complications associated with the disease process. In support of this, Perticone et al. (19) examined vascular responses to acetylcholine in patients with untreated hypertension, and after a 31-mo follow-up period, reported a marked increase in adverse cardiovascular events in patients with endothelial dysfunction. Also important, a recent study by Modena et al. (20) that examined brachial artery flow-mediated dilation in 400 consecutive postmenopausal women with mild to moderate hypertension provides evidence that restoring endothelial homeostasis may be a critical determinant of overall cardiovascular outcome in hypertensive patients. In that prospective study, failure to improve endothelial function within a 6-mo period of pharmacological therapy predicted poor cardiovascular outcome independent of treatment method or magnitude of blood pressure lowering. Taken together, these complementary clinical studies suggest that reversing endothelial dysfunction may be an important therapeutic target in hypertensive vascular disease.

#### *L-Arginine, vasomotor function, and hypertension*

Because loss of NO bioactivity is a central feature of endothelial dysfunction in hypertension, providing supplemental substrate to bolster NO production has been suggested as a rational treatment approach. Administration of L-arginine improved endothelium-dependent vasodilation in a number of human clinical studies of hypercholesterolemia and atherosclerosis (21,22). However, only a few studies have examined the effect of L-arginine on vasomotor function specifically in hypertension, and results have been mixed. For example, in 14 subjects with elevated blood pressure, L-arginine infusion did not augment acetylcholine-mediated forearm blood flow, arguing against an absolute or relative deficiency of NOS substrate (23). In contrast, 6 g of oral L-arginine acutely improved brachial artery flow-mediated dilation in patients with essential hypertension, but improved dilator responses were not associated with decreased arterial pressure (24). There is also a paucity of information with regard to the role of L-arginine in modulating hemodynamics specifically in hypertensive patients, although several lines of evidence demonstrate a modest blood pressure-lowering effect with treatment. In a report on patients with newly diagnosed mild to moderate hypertension, oral L-arginine (2 g t.i.d.) reduced arterial pressure and improved endothelial function following 1 wk of treatment (25). In patients with mild hypertension, L-arginine infusion (500 mg/kg for 30 min) lowered mean blood pressure by 8% and reduced renovascular resistance (26). L-Arginine reduces serum endothelin-1 and angiotensin II, which may play a role in its hypotensive effect (27,28). In contrast, L-arginine did not induce hypotension in adults with accelerated malignant hypertension, suggesting that disease duration, severity, and underlying degree of endothelial dysfunction may be important factors in the response to treatment (29). Hemodynamic effects of L-arginine are also evident in normal subjects, who exhibit a dose-dependent reduction in arterial pressure with increasing doses of up to 30 g L-arginine (30). In normotensive adults, a 30-min infusion of L-arginine (500 mg/kg) reduced mean arterial pressure by ~9%, and this hypotensive effect was associated with increased expired NO and plasma L-citrulline, providing evidence for enhanced endogenous NO production (31). These findings extend to normotensive children, who demonstrate a brisk fall in mean systemic pressure in

response to L-arginine (500 mg/kg i.v. over 30 min) that correlates closely with changes in plasma L-citrulline (32).

In animal experiments on systemic hypertension, oral L-arginine treatment appears to regulate hemodynamics and restore renovascular homeostasis, although this effect appears to be specific to salt-sensitive models, as previously mentioned. In Dahl salt-sensitive animals, L-arginine prevents the development of hypertension and corrects blood pressure elevation in rats exposed to a high-salt diet (13). These physiological effects are paralleled by an increase in urinary excretion of cGMP and nitrate, lending support to the hypothesis that L-arginine plays a role in modulating renovascular NO production. In contrast, L-arginine does not affect arterial pressure in spontaneously hypertensive rats, but attenuates pressure-induced cardiac hypertrophy in these rats (33). In humans, the fall in pressure with L-arginine treatment is more pronounced in salt-sensitive subjects (34). From a clinical perspective, these findings raise the question of whether L-arginine would have a greater therapeutic effect in patients susceptible to salt-sensitive mechanisms of hypertension.

### Asymmetric dimethylarginine

Although a convincing number of studies demonstrate a beneficial effect of L-arginine on vascular function and EDNO bioavailability, the precise mechanisms by which L-arginine modulates vasomotor tone remain incompletely understood. (Several proposed mechanisms are listed in Table 1.) Under normal physiological conditions, the availability of L-arginine as a substrate for endothelial NO synthase (eNOS) and NO production does not appear to be rate limiting, because ambient intracellular arginine concentrations are in the millimolar range, whereas  $K_m$  of eNOS for substrate is in the micromolar range (35). In an observational study of middle-aged Finnish men, quintiles of dietary L-arginine intake up to 6 g/d did not correlate with blood pressure or cardiovascular risk (36). Also, a patient with a genetic metabolic deficiency associated with markedly reduced plasma L-arginine concentration did not present hypertension (37). It is difficult to explain a relative deficiency of bioavailable substrate as a dominant mechanism, and alternative actions of L-arginine action deserve consideration. It is possible that under disease conditions, *in vitro* kinetic constants do not apply to compartmentalized *in vivo* conditions. Other factors such as impaired intracellular L-arginine uptake and altered or uncoupled endothelial NO synthase activity may be important. In addition, a growing body of evidence indicates that *in vivo* accumulation of endogenous competitive NOS inhibitors may reach sufficiently high concentrations under pathological disease conditions to shift the enzymatic milieu that renders L-arginine a physiologic factor. One such inhibitor, asymmetric dimethylarginine (ADMA), has received considerable attention. The physiological significance of ADMA was initially described by Valance et al. (38), who reported elevated plasma levels of

**TABLE 1**

#### Potential mechanisms of L-arginine action in hypertension

Improved endothelial vasomotor function
Enhanced vascular NO synthesis
Reduced endothelin-1 and angiotensin II activity
Favorable alteration of ADMA:L-arginine ratio
Modulation of renal hemodynamics
Reduced oxidative stress
Improved insulin sensitivity

**TABLE 2**

#### Clinical definition of metabolic syndrome<sup>1</sup>

Risk factor	Defining level
Abdominal obesity (waist circumference)	
Men	≥102 cm
Women	≥88 cm
Triglycerides	≥150 mg/dl
HDL cholesterol	
Men	<40 mg/dl
Women	<50 mg/dl
Blood pressure	≥130/≥85 mm Hg
Fasting glucose	≥110 mg/dl

<sup>1</sup> Definition requires at least 3 of 5 risk factors.

ADMA in patients with end-stage renal disease. In hypercholesterolemic patients, increased ADMA is associated with endothelial dysfunction that is reversed by L-arginine treatment. Increased ADMA levels correlate with disease severity in patients with peripheral arterial disease, and are linked to increased cardiovascular risk (39,40).

The role of ADMA in the pathogenesis of clinical hypertension has not been fully examined, though there are suggestions that elevated physiological concentrations may be linked to systemic pressor actions. In animal models, acute ADMA administration increases peripheral resistance and raises systemic blood pressure that is reversed by L-arginine. In salt-sensitive animal and human studies of hypertension, ADMA status correlates closely with elevation in arterial pressure (41). ADMA may also circulate in sufficient concentrations to directly induce vasoconstriction and contribute to vascular resistance, and the dynamic balance of the L-arginine:ADMA ratio may be an endogenous determinant of arterial tone in some forms of hypertension. More direct evidence that ADMA has a role in modulating cardiovascular hemodynamics was recently provided by Kielstein et al. (42), who conducted a series of controlled experiments with graded intravenous infusions of ADMA in healthy individuals. Acute increases in plasma ADMA within a pathophysiologically relevant range (2–10  $\mu\text{mol/L}$ ) produced marked increases in vascular resistance and mean arterial pressure, and sustained decreases in cardiac output and plasma cGMP concentration. These findings raise the questions of whether chronic elevation of plasma ADMA modulates vascular physiology under certain disease conditions and whether L-arginine therapy has a potential role in abrogating these effects.

### L-Arginine and insulin action

Experimental data demonstrate that insulin mediates vascular dilation and modulates vascular tone through NO-dependent mechanisms (43,44). Insulin resistance is a common feature of hypertension, and insulin-mediated vasodilation is impaired in such patients (45). Abnormalities in glucose, insulin, and lipoprotein metabolism are common in hypertension, and insulin resistance may represent a unifying pathophysiological mechanism (46). This relation has become most evident with the rising obesity epidemic that currently afflicts 65% of the U.S. population (47). Obesity is closely linked to a cluster of physiological abnormalities termed *metabolic syndrome* (see Table 2), defined by glucose intolerance, hypertension, atherogenic dyslipidemia, and central adiposity. The prevalence of metabolic syndrome in adults > 60 y of age is >40%, and >80% in diabetic patients. The rising obesity rates



and the failure of standard treatment regimens to address this healthcare problem may potentially lead to staggering levels of hypertension in the general population.

With regard to L-arginine treatment, experimental studies suggest that the hemodynamic actions of L-arginine may be mediated through an effect on insulin. Small controlled clinical studies in human subjects provide evidence for a role for L-arginine therapy in modulating insulin release or sensitivity. In a study of 10 healthy subjects, intravenous L-arginine (1 g/min for 30 min) increased leg blood flow, raised plasma insulin, and reduced systolic blood pressure by 11 mm Hg (1.5 kPa). These hemodynamic effects were mostly blunted by insulin suppression with octreotide, and were restored by repeat insulin challenge (48). The same group also reports that L-arginine infusion (1 g/min for 30 min) reverses blood pressure elevation produced by acute hyperglycemia (49). In 6 healthy individuals, oral L-arginine (10 g/d for 1 wk), supplied through a natural food diet or pharmacological preparation, reduced blood pressure and fasting plasma glucose without affecting insulin, supporting evidence for a sensitizing effect of treatment (50). Similarly, in subjects with type 2 diabetes, 1 mo of oral L-arginine treatment (3 g t.i.d.) reduced systolic blood pressure, improved insulin sensitivity without affecting glucose, and increased plasma cGMP (51). In diabetic patients with poor glycemic control, acute L-arginine (0.52 mg · kg<sup>-1</sup> · min<sup>-1</sup> infusion) lowered steady-state plasma glucose without changing insulin, and simultaneously enhanced forearm blood flow, assessed by venous plethysmography (52). Taken together, these complementary clinical studies suggest that L-arginine may have endocrine actions that govern insulin sensitivity and vasodilator function.

There is also evidence to suggest that L-arginine supplementation may offer synergy to existing treatment modalities in patients with insulin resistance. In a study of 10 newly diagnosed non-insulin-dependent diabetic patients with mild hyperglycemia, L-arginine infusion (1g/min for 30 min) raised plasma insulin and caused a 6% decrease in systemic blood pressure (53). Metformin treatment for 8 wk amplified the hypotensive effect of L-arginine but did not affect fasting or stimulated insulin concentrations, supporting the proposal that metformin increases sensitivity to L-arginine-induced hemodynamic changes. Metformin treatment is also associated with decreases in ADMA concentration and favorable increases in the L-arginine:ADMA ratio (54). Other therapeutic interventions such as weight loss and lifestyle modifications also appear to augment the vascular effects of L-arginine. In a study of premenopausal women, L-arginine (3 g i.v.) decreased blood pressure in obese subjects by one-third of the dilator response exhibited by matched lean control subjects. After a 12-mo multidisciplinary program to reduce body weight by 10%, the vasodilator response to L-arginine improved markedly and did not differ from that of the control group (55).

### Future directions

Both experimental and human clinical data suggest that L-arginine treatment produces a modest decrease in blood pressure in normotensive individuals and individuals with some forms of hypertension, but the mechanisms are not clearly defined. Many of the data are accrued from small clinical studies involving 10 to 20 patients, designed to be predominantly exploratory and mechanistic in nature, with variable dosing regimens. In addition, treatment durations were short, ranging from acute infusions to several weeks of therapy. There are no large, randomized controlled trials of L-arginine therapy for hypertension, and the effects of long-

term treatment remain unknown. Given the efficacy of currently available pharmacological agents for the treatment of hypertension, any additive effect of L-arginine therapy remains an open question. Further, the relations among duration of clinical hypertension, extent of endothelial vasomotor dysfunction, and response to treatment should be explored, because L-arginine may be less effective in advanced hypertensive disease. Given the critical importance of NO metabolism in vascular homeostasis, future studies addressing these issues should be considered to examine any potential role of therapy as an adjunct to existing treatment paradigms. Further exploration of the possible endocrine effects of L-arginine in modulating insulin bioaction may have widespread implications with regard to the current obesity epidemic and associated metabolic abnormalities.

### LITERATURE CITED

1. Chobanian, A. V., Bakris, G. L., Black, H. R., Cushman, W. C., Green, L. A., Izzo, J. L., Jr., Jones, D. W., Materson, B. J., Oparil, S. et al. (2003) The seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *J. Am. Med. Assoc.* 289: 2560–2572.
2. Vasan, R. S., Beiser, A., Seshadri, S., Larson, M. G., Kannel, W. B., D'Agostino, R. B. & Levy, D. (2002) Residual lifetime risk for developing hypertension in middle-aged women and men: the Framingham Heart Study. *J. Am. Med. Assoc.* 287: 1003–1010.
3. Haffner, S. & Taegtmeier, H. (2003) Epidemic obesity and the metabolic syndrome. *Circulation* 108: 1541–1545.
4. Dominiczak, A. F. & Bohr, D. F. (1995) Nitric oxide and its putative role in hypertension. *Hypertension* 25: 1202–1211.
5. Huang, P. L., Huang, Z., Mashimo, H., Bloch, K. D., Moskowitz, M. A. & Bevan, J. A. (1995) Hypertension in mice lacking the gene for endothelial nitric oxide synthase. *Nature* 377: 239–242.
6. Baylis, C., Mitruka, B. & Deng, A. (1992) Chronic blockade of nitric oxide synthesis in the rat produces systemic hypertension and glomerular damage. *J. Clin. Invest.* 90: 278–281.
7. Brush, J. E., Faxon, D. P., Salmon, S., Jacobs, A. K. & Ryan, T. J. (1992) Abnormal endothelium-dependent coronary vasomotion in hypertensive patients. *J. Am. Coll. Cardiol.* 19: 809–815.
8. Panza, J. A., Quyyumi, A. A., Brush, J. E. & Epstein, S. E. (1990) Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N. Engl. J. Med.* 323: 22–27.
9. Cockcroft, J. R., Chowienczyk, P. J., Benjamin, N. & Ritter, J. M. (1994) Preserved endothelium-dependent vasodilation in patients with essential hypertension. *N. Engl. J. Med.* 330: 1036–1040.
10. Calver, A., Collier, J., Moncada, S. & Vallance, P. (1992) Effect of local intra-arterial N<sup>G</sup>-monomethyl-L-arginine in patients with hypertension: the nitric oxide dilator mechanism appears normal. *J. Hypertens.* 10: 1025–1031.
11. Gokce, N., Holbrook, M., Duffy, S. J., Demissie, S., Cupples, L. A., Biegelsen, E., Keaney, J. F., Jr., Loscalzo, J. & Vita, J. A. (2001) Effects of race and hypertension on flow-mediated and nitroglycerin-mediated dilation of the brachial artery. *Hypertension* 38: 1349–1354.
12. Gokce, N., Keaney, J. F., Jr. & Vita, J. A. (1998) Endotheliopathies: clinical manifestations of endothelial dysfunction. In: *Thrombosis and Hemorrhage* (Loscalzo, J. & Shafer, A. I., eds.), pp. 901–924. Williams and Wilkins, Baltimore, MD.
13. Chen, P. Y. & Sanders, P. W. (1991) L-Arginine abrogates salt-sensitive hypertension in Dahl/Rapp Rats. *J. Clin. Invest.* 88: 1559–1567.
14. Shultz, P. J. & Tolins, J. P. (1993) Adaptation to increased dietary salt intake in the rat: role of endogenous nitric oxide. *J. Clin. Invest.* 91: 642–650.
15. Arnal, J. F., Warin, L. & Michel, J. B. (1992) Determinants of aortic cyclic guanosine monophosphate in hypertension induced by chronic inhibition of nitric oxide synthase. *J. Clin. Invest.* 90: 647–652.
16. Shesely, E. G., Maeda, N., Kim, H. S., Desai, K. M., Krege, J. H., Laubach, V. E., Sherman, P. A., Sessa, W. C. & Smithies, O. (1996) Elevated blood pressure in mice lacking endothelial nitric oxide synthase. *Proc. Natl. Acad. Sci. U.S.A.* 93: 13176–13181.
17. Taddei, S., Virdis, A., Mattei, P., Chiadoni, L., Sudano, I. & Salvetti, A. (1996) Defective L-arginine-nitric oxide pathway in offspring of essential hypertensive patients. *Circulation* 94: 1298–1303.
18. Luscher, T. F., Vanhoutte, P. M. & Raji, L. (1987) Antihypertensive treatment normalizes decreased endothelium-dependent relaxations in rats with salt-induced hypertension. *Hypertension* 9: III193–III197.
19. Perticone, F., Ceravolo, R., Pujia, A., Ventura, G., Iacopino, S., Scozzafava, A., Ferraro, A., Chello, M., Mastroberoberto, P. et al. (2001) Prognostic significance of endothelial dysfunction in hypertensive patients. *Circulation* 104: 191–196.
20. Modena, M. G., Bonetti, L., Coppi, F., Bursi, F. & Rossi, R. (2002) Prognostic role of reversible endothelial dysfunction in hypertensive postmenopausal women. *J. Am. Coll. Cardiol.* 40: 505–510.

21. Quyyumi, A. A., Dakak, N., Diodati, J. G., Gilligan, D. M., Panza, J. A. & Cannon, R. O. (1997) Effect of L-arginine on human coronary endothelium-dependent and physiologic vasodilation. *J. Am. Coll. Cardiol.* 30: 1220-1227.
22. Lerman, A., Burnett, J.C.J., Higano, S. T., McKinley, L. J. & Holmes, D. R. J. (1998) Long-term L-arginine supplementation improves small-vessel coronary endothelial function in humans. *Circulation* 97: 2123-2128.
23. Panza, J. A., Casino, P. R., Badar, D. M. & Quyyumi, A. A. (1993) Effect of increased availability of endothelium-derived nitric oxide precursor on endothelium-dependent vascular relaxation in normal subjects and in patients with essential hypertension. *Circulation* 87: 1475-1481.
24. Lekakis, J. P., Papathanassiou, S., Papaioannou, T. G., Papamichael, C. M., Zakopoulos, N., Kotsis, V., Dagher, A. G., Stamatelopoulos, K., Protogerou, A. & Stamatelopoulos, S. F. (2002) Oral L-arginine improves endothelial dysfunction in patients with essential hypertension. *Int. J. Cardiol.* 86: 317-323.
25. Pagnotta, P., Germano, G., Grutter, G., Leonardo, F., Rosano, G. & Chierchia, S. (1997) Oral L-arginine supplementation improves essential arterial hypertension. *Circulation* 96 (suppl. 8S): 538-1.
26. Higashi, Y., Oshima, T., Ozono, R., Watanabe, M., Matsuura, H. & Kajiyama, G. (1995) Effects of L-arginine infusion on renal hemodynamics in patients with mild essential hypertension. *Hypertension* 25: 898-902.
27. Piatti, P., Fragasso, G., Monti, L. D., Setola, E., Lucotti, P., Fermo, I., Paroni, R., Galluccio, E., Pozza, G. et al. (2003) Acute intravenous L-arginine infusion decreases endothelin-1 levels and improves endothelial function in patients with angina pectoris and normal coronary arteriograms: correlation with asymmetric dimethylarginine levels. *Circulation* 107: 429-436.
28. Higashi, Y., Oshima, T., Ono, N., Hiraga, H., Yoshimura, M., Watanabe, M., Matsuura, H., Kambe, M. & Kajiyama, G. (1995) Intravenous administration of L-arginine inhibits angiotensin-converting enzyme in humans. *J. Clin. Endocrinol. Metab.* 80: 2198-2202.
29. Sato, K., Kinoshita, M., Kojima, M., Miyagawa, K., Takase, H., Suzuki, S. & Dohi, Y. (2000) Failure of L-arginine to induce hypotension in patients with a history of accelerated-malignant hypertension. *J. Hum. Hypertens.* 14: 485-488.
30. Bode-Boger, S. M., Boger, R. H., Galland, A., Tsikas, D. & Frolich, J. C. (1998) L-Arginine-induced vasodilation in healthy humans: pharmacokinetic-pharmacodynamic relationship. *Br. J. Clin. Pharmacol.* 46: 489-497.
31. Mehta, S., Stewart, D. J., & Levy, R. D. (1996) The hypotensive effect of L-arginine is associated with increased expired nitric oxide in humans. *Chest* 109: 1550-1555.
32. Nelin, L. D. & Hoffman, G. M. (2001) L-Arginine infusion lowers blood pressure in children. *J. Pediatr.* 139: 747-749.
33. Matsuoka, H., Nakata, M., Kohno, K., Koga, Y., Nomura, G., Toshima, H. & Imaizumi, T. (1996) Chronic L-arginine administration attenuates cardiac hypertrophy in spontaneously hypertensive rats. *Hypertension* 27: 14-18.
34. Campese, V. M., Amar, M., Anjali, C., Medhat, T. & Wurgaft, A. (1997) Effect of L-arginine on systemic and renal haemodynamics in salt-sensitive patients with essential hypertension. *J. Hum. Hyper.* 11: 527-532.
35. Loscalzo, J. (2000) What we know and don't know about L-arginine and NO. *Circulation* 101: 2126-2129.
36. Venho, B., Voutilainen, S., Valkonen, V. P., Virtanen, J., Lakka, T. A., Rissanen, T. H., Ovaskainen, M. L., Laitinen, M. & Salonen, J. T. (2002) Arginine intake, blood pressure, and the incidence of acute coronary events in men: the Kuopio Ischaemic Heart Disease Risk Factor Study. *Am. J. Clin. Nutr.* 76: 359-364.
37. Kamada, Y., Nagaretani, H., Tamura, S., Ohama, T., Maruyama, T., Hiraoka, H., Yamashita, S., Yamada, A., Kiso, S. et al. (2001) Vascular endothelial dysfunction resulting from L-arginine deficiency in a patient with lysinuric protein intolerance. *J. Clin. Invest.* 108: 717-724.
38. 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.
39. Boger, R. H., Bode-Boger, S. M., Szuba, A., Tsao, P. S., Chan, J. R., Tangphao, O., Blaschke, T. F. & Cooke, J. P. (1998) Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. *Circulation* 98: 1842-1847.
40. Cooke, J. P. (2000) Does ADMA cause endothelial dysfunction? *Arterioscler. Thromb. Vasc. Biol.* 20: 2032-2037.
41. Matsuoka, H., Itoh, S., Kimoto, M., Kohno, K., Tamai, O., Wada, Y., Yasukawa, H., Iwami, G., Okuda, S. & Imaizumi, T. (1997) Asymmetrical dimethylarginine, an endogenous nitric oxide synthase inhibitor, in experimental hypertension. *Hypertension* 29: 242-247.
42. Kielstein, J. T., Impraime, B., Simmel, S., Bode-Boger, S. M., Tsikas, D., Frolich, J. C., Hooper, M. M., Haller, H. & Fliser, D. (2004) Cardiovascular effects of systemic nitric oxide synthase inhibition with asymmetrical dimethylarginine in humans. *Circulation* 109: 172-177.
43. Scherrer, U., Randin, D., Vollenweider, P., Vollenweider, L. & Nicod, P. (1994) Nitric oxide release accounts for insulin's vascular effects in humans. *J. Clin. Invest.* 94: 2511-2515.
44. Steinberg, H. O., Brechtel, G., Johnson, A., Fineberg, N. & Baron, A. D. (1994) Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. *J. Clin. Invest.* 94: 1172-1179.
45. Ferrannini, E., Buzzigoli, G., Bonadonna, R., Giorico, M. A., Oleggini, M., Graziadei, L., Pedrinelli, R., Brandi, L. & Bevilacqua, S. (1987) Insulin resistance in essential hypertension. *N. Engl. J. Med.* 317: 350-357.
46. Grundy, S. M., Brewer, H. B., Jr., Cleeman, J. I., Smith, S. C., Jr. & Lenfant, C. (2004) Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation* 109: 433-438.
47. Flegal, K. M., Carroll, M. D., Ogden, C. L. & Johnson, C. L. (2002) Prevalence and trends in obesity among US adults, 1999-2000. *J. Am. Med. Assoc.* 288: 1723-1727.
48. Giugliano, D., Marfella, R., Verrazzo, G., Acampora, R., Coppola, L., Cozzolino, D. & D'Onofrio, F. (1997) The vascular effects of L-arginine in humans: The role of endogenous insulin. *J. Clin. Invest.* 99: 433-438.
49. Giugliano, D., Marfella, R., Coppola, L., Verrazzo, G., Acampora, R., Giunta, R., Nappo, F., Lucarelli, C. & D'Onofrio, F. (1997) Vascular effects of acute hyperglycemia in humans are reversed by L-arginine: evidence for reduced availability of nitric oxide during hyperglycemia. *Circulation* 95: 1783-1790.
50. Siani, A., Pagano, E., Iacone, R., Iacoviello, L., Scopacasa, F. & Strazullo, P. (2000) Blood pressure and metabolic changes during dietary L-arginine supplementation in humans. *Am. J. Hypertens.* 13: 547-551.
51. Piatti, P. M., Monti, L. D., Valsecchi, G., Magni, F., Setola, E., Marchesi, F., Galli-Kienle, M., Pozza, G. & Alberti, K. G. (2001) Long-term oral L-arginine administration improves peripheral and hepatic insulin sensitivity in type 2 diabetic patients. *Diabetes Care* 24: 875-880.
52. Wascher, T. C., Graier, W. F., Dittrich, P., Hussain, M. A., Bahadori, B., Wallner, S. & Toplak, H. (1997) Effects of low-dose L-arginine on insulin-mediated vasodilation and insulin sensitivity. *Eur. J. Clin. Invest.* 27: 690-695.
53. Marfella, R., Acampora, R., Verrazzo, G., Ziccardi, P., De Rosa, N., Giunta, R. & Giugliano, D. (1996) Metformin improves hemodynamic and rheological responses to L-arginine in NIDDM patients. *Diabetes Care* 19: 934-939.
54. Asagami, T., Abbasi, F., Stuelinger, M., Lamendola, C., McLaughlin, T., Cooke, J. P., Reaven, G. M. & Tsao, P. S. (2002) Metformin treatment lowers asymmetric dimethylarginine concentrations in patients with type 2 diabetes. *Metabolism* 51: 843-846.
55. Ziccardi, P., Nappo, F., Giugliano, G., Esposito, K., Marfella, R., Cioffi, M., D'Andrea, F., Molinari, A. M. & Giugliano, D. (2002) Reduction of inflammatory cytokine concentrations and improvement of endothelial functions in obese women after weight loss over one year. *Circulation* 105: 804-809.