

Arginine Metabolism: Enzymology, Nutrition, and Clinical Significance

Asymmetric Dimethylarginine, an Endogenous Inhibitor of Nitric Oxide Synthase, Explains the “L-Arginine Paradox” and Acts as a Novel Cardiovascular Risk Factor^{1,2}

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ABSTRACT There is abundant evidence that the endothelium plays a crucial role in the maintenance of vascular tone and structure. One of the major endothelium-derived vasoactive mediators is nitric oxide (NO). Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of NO synthase. ADMA inhibits vascular NO production in concentrations found in pathophysiological conditions; ADMA also causes local vasoconstriction when it is infused intraarterially. Thus, elevated ADMA levels may explain the “L-arginine paradox,” i.e., the observation that supplementation with exogenous L-arginine improves NO-mediated vascular functions in vivo, although its baseline plasma concentration is about 25-fold higher than the Michaelis-Menten constant K_m of the isolated, purified endothelial NO synthase in vitro. The biochemical and physiological pathways related to ADMA are well understood: Dimethylarginines are the result of degradation of methylated proteins; the methyl group is derived from S-adenosylmethionine. Both ADMA and its regioisomer, symmetric dimethylarginine, are eliminated from the body by renal excretion, whereas only ADMA is metabolized via hydrolytic degradation to citrulline and dimethylamine by the enzyme dimethylarginine dimethylaminohydrolase (DDAH). DDAH activity and/or expression may therefore contribute to the pathogenesis of endothelial dysfunction in various diseases. Plasma ADMA levels are increased in humans with hypercholesterolemia, atherosclerosis, hypertension, chronic renal failure, and chronic heart failure. Increased ADMA levels are associated with reduced NO synthesis as assessed by impaired endothelium-dependent vasodilation. In several prospective and cross-sectional studies, ADMA evolved as a marker of cardiovascular risk. With increasing knowledge of the role of ADMA in the pathogenesis of cardiovascular disease, ADMA is becoming a goal for pharmacotherapeutic interventions. Among other potential strategies that are currently being tested, administration of L-arginine has been shown to improve endothelium-dependent vascular functions in subjects with high ADMA levels. Finally, ADMA has gained clinical importance recently because several studies have shown that ADMA is an independent cardiovascular risk factor. J. Nutr. 134: 2842S–2847S, 2004.

KEY WORDS: • endothelium • nitric oxide • atherosclerosis • coronary heart disease • prognosis • oxidative stress

The endothelium plays a crucial role in the maintenance of vascular tone and structure. One of the major endothelium-derived vasoactive mediators is nitric oxide (NO), which is formed from the amino acid precursor L-arginine by nitric oxide synthase (NOS).⁴ NO is involved in a wide variety of

regulatory mechanisms of the cardiovascular system, including vascular tone (i.e., it is the major mediator of endothelium-dependent vasodilation) and vascular structure (e.g., inhibition of smooth muscle cell proliferation), and cell-cell interactions in blood vessels (e.g., inhibition of platelet adhesion and aggregation, inhibition of monocyte adhesion). Because of these functions, NO has been summarized as an endogenous antiatherosclerotic molecule.

Dysfunction of the endothelial L-arginine–nitric oxide pathway is a common mechanism by which several cardiovascular risk factors mediate their deleterious effects on the vascular wall. Among them are hypercholesterolemia (1), hypertension (2), smoking (3), diabetes mellitus (4), homocysteinemia (5), and vascular inflammation (6).

Vallance et al. (7) first described asymmetric dimethylarginine (ADMA) as an endogenous inhibitor of NOS in 1992. Since then, the role of this molecule in the regulation of

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⁴ Abbreviations used: ADMA, asymmetric dimethylarginine; DDAH, dimethylarginine dimethylaminohydrolase; IC_{50} , inhibitory concentration of 50%; K_m , Michaelis-Menten constant; NO, nitric oxide; NOS, nitric oxide synthase.

endothelial NO synthesis has increasingly attracted attention. This review focuses on evidence that ADMA is an endogenous regulator of endothelial function in humans, which may explain the so-called L-arginine paradox, and the potential role of this molecule as a novel cardiovascular risk factor.

The L-arginine paradox

Supplementation with excess L-arginine, mostly supplied in drinking water, reduces the impairment of endothelium-dependent vasodilation in rabbits with hypercholesterolemia and atherosclerosis (8–11). Moreover, L-arginine supplementation also enhances endothelium-dependent inhibition of platelet aggregation (12), inhibits monocyte adhesion (13), and reduces vascular smooth muscle proliferation (14). These findings from *in vivo* studies contrast markedly with experimental data showing that the addition of L-arginine to isolated arterial rings *in vitro* does not affect endothelium-dependent vasodilation (15).

Evidence from *in vitro* experiments with cloned, purified endothelial NOS in a cell-free system with optimal concentrations of all cofactors suggests that the Michaelis-Menten constant K_m for this enzyme is $\sim 3 \mu\text{mol/L}$ (16). By contrast, circulating L-arginine concentration in plasma in both healthy humans and patients with vascular disease ranges from 40 to 100 $\mu\text{mol/L}$ (17)—in other words, it is 15- to 30-fold higher than the K_m of endothelial NOS. Even in 1 study that reported reduced plasma L-arginine concentration in hypercholesterolemia, the mean plasma L-arginine concentration was 78 $\mu\text{mol/L}$, compared with 111 $\mu\text{mol/L}$ in normocholesterolemic control subjects (18). These data suggest that at physiological L-arginine concentrations, endothelial NOS should be well saturated with substrate, and the addition of exogenous L-arginine should not affect the enzyme's activity. This discrepancy was termed the "L-arginine paradox." How can it be explained?

Several years ago our group hypothesized that a competitive inhibitor of NOS might be responsible for the L-arginine paradox (19). The presence of a competitive NOS inhibitor in human plasma *in vivo* might explain why L-arginine, although ineffective *in vitro* (15), improves endothelial function *in vivo*: Administration of excess exogenous L-arginine should displace the competitive inhibitor and restore NO production to physiological levels (Fig. 1).

ADMA is an endogenous inhibitor of NO synthesis

In experimental studies with isolated arterial segments *in vitro*, ADMA inhibits vascular NO production at concentrations of 3 to 15 $\mu\text{mol/L}$ (7,20–22). In cultured murine macrophages, ADMA also inhibits NO production (which is probably caused by inducible NOS activity in these cells) in a concentration-dependent manner (23,24). Faraci et al. (21) calculated the inhibitory concentration of 50% (IC_{50}) value for the inhibition of NO production in rat cerebellar homogenate by ADMA as $1.8 \pm 0.1 \mu\text{mol/L}$, and Fickling et al. (24) reported that ADMA concentrations of 2 and 10 $\mu\text{mol/L}$ inhibited nitrite production in LPS-stimulated J774 macrophages by 17 and 33%, respectively.

In experiments with purified macrophage NOS, ADMA inhibited NO production in a concentration-dependent manner as well (7). Our group recently demonstrated that ADMA inhibits endothelial ($IC_{50} = 3.9 \mu\text{mol/L}$) and neuronal NO synthase activities in a concentration-dependent manner (25).

Moreover, there is further indirect evidence that ADMA has a role as an endogenous modulator of NOS activity.

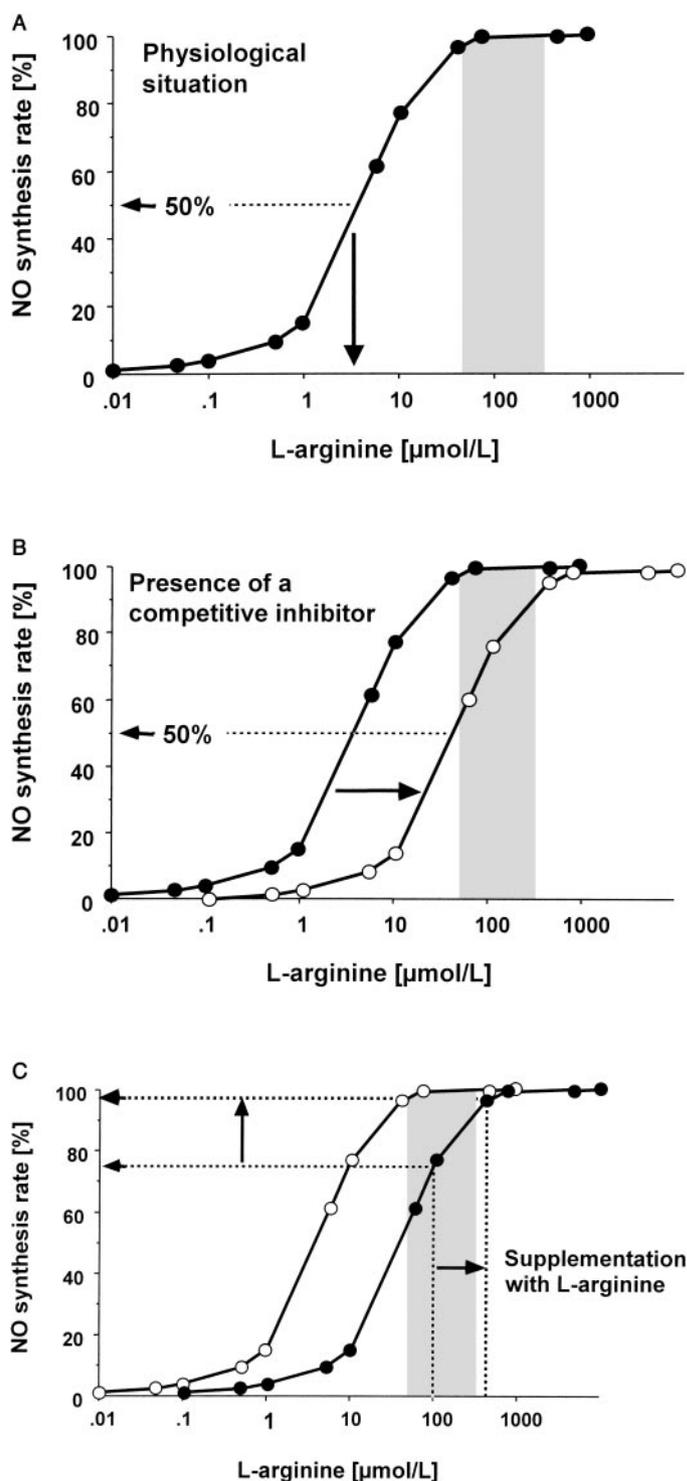


FIGURE 1 The L-arginine paradox from a pharmacological perspective. L-Arginine is the substrate of NOS. The enzyme kinetics of endothelial NOS have been determined biochemically *in vitro*. Data show that physiological plasma L-arginine concentrations (shaded area) are in a range that far exceeds the Michaelis-Menten constant K_m of the enzyme, which indicates 50% of the maximal enzyme activity (panel A). However, in the presence of a competitive inhibitor of NOS, the concentration-response curve for L-arginine is shifted to the right, such that the steep part of the curve may come to lie in the range of physiological L-arginine concentrations (panel B). Under such circumstances, even small changes in L-arginine concentration may cause marked changes in NOS activity. Supplementation with exogenous L-arginine will then enhance NOS activity (panel C).

Inhibition of the enzyme that inactivates ADMA, dimethylarginine dimethylaminohydrolase (DDAH), elevates ADMA levels and causes vasoconstriction of isolated arterial rings in vitro (26). We have also demonstrated that inhibition of DDAH activity causes enhanced endothelial superoxide radical formation (27).

Most recently, it was shown that mice with DDAH gene overexpression (DDAH transgenic mice) have circulating ADMA levels that are about half of those in wildtype animals (0.5 vs. 1.0 $\mu\text{mol/L}$; $P < 0.05$). DDAH transgenic mice have higher rates of systemic NO production, lower systemic blood pressure, and reduced peripheral vascular resistance (28).

In humans, local intraarterial infusion of ADMA into the brachial artery significantly reduces forearm blood flow (29). However, concentrations reached in the local circulation by far exceed those of ADMA that can be measured in patients. Therefore, 2 studies in which ADMA was administered intravenously contributed valuable data on the dose-response relationship of ADMA in humans. In 1 study, ADMA was infused to reach a systemic plasma concentration of $\sim 2.6 \mu\text{mol/L}$ (30). ADMA markedly elevated arterial blood pressure and systemic vascular resistance, reduced cardiac output and heart rate, and decreased the vascular response to exercise. In the other study, ADMA decreased plasma cyclic GMP concentrations at pathophysiologically relevant concentrations, whereas renal plasma flow and glomerular filtration rate were reduced only at very high concentrations (31).

Taken together, the aforementioned studies provide plenty of evidence that ADMA modulates endothelial NOS activity within the concentration range found in patients with vascular disease. Moreover, this evidence also suggests that even small modifications in ADMA concentration markedly affect vascular NO production, vascular tone, and systemic vascular resistance. This is evidence enough to call ADMA a *novel marker of endothelial dysfunction*.

Mechanisms regulating ADMA concentration

ADMA is released when methylated proteins are degraded into their amino acid components during hydrolytic protein turnover (Fig. 2) (32). Protein arginine methylation is a ubiquitous mechanism of posttranslational protein modification that occurs in almost all cell types, due to the activity of specific methyltransferase enzymes [for review, see Aletta et al. (33)]. There is no evidence that the free amino acid, L-arginine, can be methylated by these enzymes. Once ADMA is released from degraded proteins, its elimination from the body is brought about by renal elimination or by enzymatic inactivation. Although renal clearance was the first mechanism for the elimination of ADMA to be reported, with reports of the clinical consequences of ADMA accumulation in patients with advanced renal failure (7,34,35), enzymatic degradation of ADMA by DDAH has attracted much attention recently. DDAH degrades ADMA to dimethylamine and L-citrulline. DDAH activity is found in almost all tissues, with high activities in kidney and liver (36). There are 2 allelic isoforms of this enzyme, DDAH-1 and DDAH-2, which show different tissue expression patterns. DDAH-1 is present in many tissues that also express neuronal NO synthase, whereas DDAH-2 is mainly present in vascular tissues that coexpress endothelial NOS (37). DDAH activity is reduced by oxidative stress induced by TNF- α or oxidized LDL in vitro (38); moreover, it is modulated by S-nitrosylation (39).

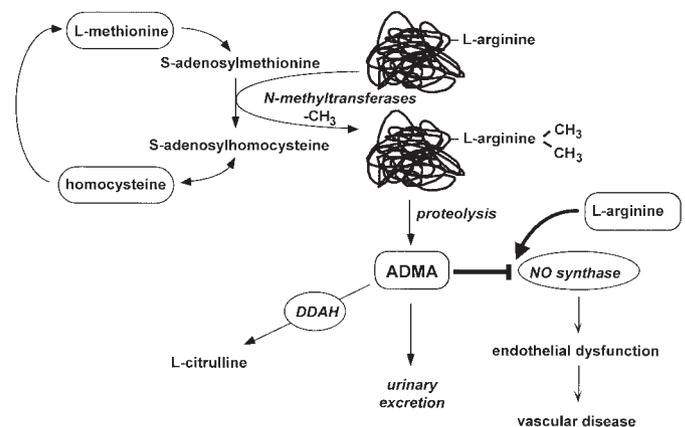


FIGURE 2 Schematic overview of biochemical pathways related to ADMA. Methylation of arginine residues within proteins or polypeptides occurs through *N*-methyltransferases, which utilize *S*-adenosylmethionine as a methyl group donor. After proteolytic breakdown of proteins, free ADMA is present in cytoplasm. It can also be detected circulating in human blood plasma. ADMA acts as an inhibitor of NOS by competing with the substrate of this enzyme, L-arginine, and causes endothelial dysfunction and, subsequently, atherosclerosis. ADMA is eliminated from the body via urinary excretion and, alternatively, via metabolism by the enzyme DDAH to citrulline and dimethylamine. Reproduced from Böger (32) with permission of the publishers.

ADMA and cardiovascular risk and mortality

Case-control studies. There are numerous studies to date that show a relationship between elevated ADMA concentration and cardiovascular disease. Elevated ADMA concentration is highly prevalent in hypercholesterolemia (1), hyperhomocysteinemia (40), diabetes mellitus (41), peripheral arterial occlusive disease (42), hypertension (43,44), chronic heart failure (45), and coronary artery disease (46) (Table 1). All of these studies were designed as case-control studies, and therefore do not allow one to draw any conclusions as to the possible cause-effect relation between ADMA and cardiovascular disease. However, the observation that ADMA levels increase early in the development of atherosclerosis suggests that ADMA has the potential to be not only a marker but a mediator of vascular lesions. Data recently accumulated from a series of clinical studies confirm this hypothesis:

Miyazaki et al. (47), in a study that included 116 healthy humans, were the first to describe the relation between ADMA and carotid intima-media-thickness, a well-accepted surrogate marker for atherosclerosis progression. They found that ADMA concentration was associated with carotid artery intima-media thickness in stepwise regression analysis.

Our group measured intima-media thickness by high-resolution ultrasound analysis in 90 patients with end-stage renal disease undergoing chronic hemodialysis (48). Confirming the data of Miyazaki et al. (47), we found that ADMA was highly correlated with intima-media thickness in this population as well. Furthermore, during 1 y of follow-up, the progression of intimal thickening was best predicted by initial ADMA and C-reactive protein levels.

Valkonen et al. (46), in a nested case-control study including 150 middle-aged nonsmoking men from eastern Finland, found that subjects whose ADMA concentration fell into the highest quartile of the distribution in this population (i.e., $>0.62 \mu\text{mol/L}$) had a 3.9-fold elevation of risk for acute coronary events, compared with the other quartiles.

In a multicenter case-control study including 816 patients with objectively proven coronary artery disease and matched

TABLE 1

*Diseases in which ADMA concentration is elevated*¹

Disease	Fold increase in ADMA
Childhood hypertension	2–3
Chronic heart failure	2–3
Chronic renal failure	2–7
Coronary artery disease	~2
Diabetes mellitus type II	2–3
Hepatic failure	2–3
Hypercholesterolemia	~2
Hyperhomocysteinemia	~2
Hypertension	~2
Hyperthyroidism	~2
Peripheral arterial disease	2–3
Preeclampsia	2–3
Pregnancy-induced hypertension	~2
Pulmonary hypertension	~2.5
Stroke	~2

¹ Values are fold increases in ADMA level in the presence of the indicated disease. Only diseases for which data from clinical studies in humans subjects are available are listed.

controls, we found that ADMA was the marker with the best discriminative power to differentiate between cases and controls (49). In adjusted multivariate regression analysis, the odds ratio associated with an increase of ADMA by 1 $\mu\text{mol/L}$ was 2.34.

Prospective clinical studies. In the first prospective trial studying ADMA as a potential new cardiovascular risk factor, we measured ADMA concentration as well as a variety of conventional and new cardiovascular risk factors in 225 hemodialysis patients. After a mean 33.4 mo of follow-up, 120 major cardiovascular events and a total of 83 deaths (53 vascular deaths) had occurred. Using Cox's proportional-hazards model, ADMA and age were the strongest predictors of cardiovascular events and total mortality (35). Although the median plasma ADMA concentration in this population (2.52 $\mu\text{mol/L}$) was higher than in healthy normal adults (usually <1 $\mu\text{mol/L}$), the risk of death by any cause for patients with an ADMA concentration above the 75th percentile within this group was 3-fold that of patients with an ADMA level below the median.

In another prospective study, Nijveldt et al. (50) found that ADMA was the strongest predictor of death among patients on an intensive care unit, with a 17-fold excess in mortality for patients in the highest ADMA quartile, compared to those in the lowest quartile. ADMA was associated with hepatic failure and lactic acid and bilirubin concentrations, suggesting that hepatic function is an important determinant of circulating ADMA concentration.

In a third prospective study, Lu et al. (51) investigated whether ADMA may allow the prediction of outcome after percutaneous coronary intervention in patients with stable angina pectoris. The investigators followed 153 consecutive patients for a median of 16 mo. A total of 51 major cardiovascular events occurred during follow-up. There was a clear increase in risk with increasing ADMA, independent of potential confounding factors in a multifactorial Cox's regression analysis (including age, smoking, hypercholesterolemia, use of stent, and other factors). The remarkable point in this study is that ADMA levels were within a range that many would consider normal; patients in the lowest tertile had a median circulating concentration of 0.42 $\mu\text{mol/L}$, compared with 0.75 $\mu\text{mol/L}$ in the highest tertile.

Taken together, these 3 prospective clinical studies unanimously show that ADMA does act as a novel cardiovascular risk factor.

Role of ADMA in explaining the beneficial effects of nutritional L-arginine supplementation

Few patients experience pathologically low L-arginine concentrations. However, clinical and experimental evidence suggests that the enzymatic activity of NOS is regulated by the ratio between the concentration of L-arginine (the natural substrate) and that of ADMA (the endogenous inhibitor), such that in the presence of normal L-arginine levels, any elevation of ADMA levels may cause relative L-arginine deficiency with regard to optimal NO synthase activity. Because ADMA is a competitive inhibitor of NO synthase, its inhibitory action can be overcome by increasing the concentration of the enzyme's substrate, L-arginine. The studies cited above show that ADMA levels may increase in situations associated with cardiovascular disease. Elevated ADMA concentration is 1 possible explanation for endothelial dysfunction and decreased NO production in this disease cluster. In this respect, it is interesting to note that we recently observed improved endothelium-dependent vasodilation after L-arginine administration in patients with chronic heart failure (who had elevated ADMA concentrations), whereas L-arginine did not affect endothelium-dependent vasodilation in healthy human subjects (who had low ADMA concentrations) (52). Thus, nutritional supplementation with L-arginine may help to restore the physiological status by normalizing the L-arginine:ADMA ratio, whereas it will do little in humans with an undisturbed L-arginine:ADMA balance.

Azuma et al. (53) showed, in an experimental study, that ADMA accumulates in regenerated endothelial cells after experimental balloon angioplasty. It is well known that regenerated endothelial cells growing at the site of an arterial injury lack full functional capacity, with a major defect in the L-arginine-NO pathway (54). Again, this may explain why local or systemic administration of L-arginine improves local postinjury arterial function (55).

The beneficial vascular effects of cholesterol-lowering drugs (statins) depend in part on their ability to upregulate endothelial NOS gene expression (56). However, the number of clinical research studies that show that statins do not improve endothelium-dependent vasodilation is about equivalent to the number of studies that report that statins cause an improvement. Once more, this discrepancy may be resolved with the help of ADMA: Janatuinen et al. (57) recently found that pravastatin enhances myocardial blood flow (measured by positron electron resonance tomography) only in patients with low ADMA levels, whereas the drug is ineffective in patients with elevated ADMA levels. We investigated the idea that ADMA may block NOS despite its upregulated gene expression after statin treatment, and that this blockade may be overcome by L-arginine supplementation (58). Indeed, we found in a randomized, controlled trial that in patients with elevated ADMA concentration, simvastatin enhances endothelium-dependent vasodilation only when combined with supplemental nutritional L-arginine (59).

Thus, ADMA may explain the discrepant results of clinical trials in which L-arginine sometimes improves endothelial function and sometimes does not—a discrepancy that has so far remained unexplained. The observation of elevated ADMA concentration in a given patient may warrant the administration of nutritional L-arginine supplements, to improve the ability of the endothelium to counteract the insults

to the vascular wall by circulating blood cells, by vasoconstrictors, and by oxygen-derived free radicals.

ADMA as a novel cardiovascular risk factor: implications for daily practice

Research into ADMA was an exotic, purely experimental topic in the 1990s. However, ADMA is now coming of age as a novel cardiovascular risk factor. Efforts are currently under way to define the normal range of ADMA concentration in healthy humans of different age groups and of both sexes. Moreover, an international research initiative is undertaking a comparison of analytical methods, to enable the comparison of data measured in different laboratories around the world. Finally, in addition to elaborate analytical methodologies such as HPLC and MS methods, more widely available and less time consuming (and therefore cheaper) methods of measuring ADMA levels are now available with the presentation of a novel competitive enzyme immunoassay that enables sensitive and specific assessment of ADMA concentration [more information is available at several web sites (60,61)].

As the basis is set for a wider application of ADMA assessment, the question is, who should be tested? Elevated ADMA concentration is associated with almost all traditional cardiovascular risk factors and is seen in patients with established cardiovascular or metabolic disease. However, ADMA concentration varies markedly even within these patient populations. Moreover, multivariate regression analyses that take into consideration all of the traditional and some other novel risk factors show that ADMA remains independently associated with cardiovascular risk or total mortality even after adjustment is performed.

At present, cardiovascular risk is increasingly evaluated using risk factor scores instead of individual risk factors. All of these scores agree that pharmacological treatment should be initiated in patients whose risk exceeds 20% in the next 10 y. However, what about those patients whose risk falls closely short of this line—say, 17% or 18%—or is as low as 11% risk of an acute cardiac event in the next 10 y? Additional risk markers may help to assess risk more accurately for these patients. Thus, ADMA levels will be helpful in estimating the risk of cardiac events for patients who may deserve intensified preventive treatment. L-Arginine supplementation may be 1 means of specifically antagonizing the deleterious effects of ADMA on endothelial function via nutritional supplementation in primary and secondary prevention.

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