The traditional risk factors of hypercholesterolemia, hypertension, diabetes mellitus, and tobacco exposure identify a subset of patients at greater cardiovascular risk. A variety of clinical phenotypes, biochemical markers, and genetic polymorphisms have been proposed to explain the variance in risk not explained by the traditional factors. Notably, all of the traditional risk factors, as well as the great majority of new risk markers, are associated with endothelial vasodilator dysfunction.

Because the end points (endothelial dysfunction leading to plaque formation, progression, and rupture) are the same, it follows that diverse risk factors ultimately share common pathways(s) of pathobiology. We and others have provided evidence for a ubiquitous mechanism of endothelial pathobiology shared by all risk factors and markers examined to date. This mechanism of endothelial derangement is mediated by an endogenous inhibitor of nitric oxide synthase (NOS), a molecule known as asymmetrical dimethylarginine (ADMA). Risk factors impair endothelial vasodilator function by causing the accumulation of ADMA. Furthermore, by blocking NO generation, ADMA initiates and promotes processes involved in atherogenesis, plaque progression, and plaque rupture. This review examines the burgeoning body of literature that supports ADMA as an "Über marker," a biochemical factor mediating the adverse vascular effects of many other risk factors and markers.

ADMA: A Major Cause of Endothelial Dysfunction

Endothelial NOS converts the amino acid L-arginine into L-citrulline and NO. The importance of NO in vascular homeostasis has been discussed elsewhere.1 In addition to its vasodilator activity, NO inhibits key processes involved in vascular disease, including leukocyte adhesion, platelet aggregation, and vascular smooth muscle cell proliferation. In animal models, alterations in vascular NO synthesis profoundly influence the progression of atherosclerosis and restenosis.2–6 These experimental observations have gained greater significance with recent reports that impairment of the NOS pathway independently predicts cardiovascular events.7–11

Major causes of impairment of the NOS pathway are the endogenous NOS inhibitors ADMA and N-monomethylarginine (MMA). Plasma levels of ADMA are 10-fold greater than those of MMA.12 Because it is the predominant species in plasma, most studies to date have focused on ADMA. The importance of ADMA as an endogenous inhibitor of NOS was first recognized by Vallance and colleagues12 in patients with end-stage renal disease. In these patients, ADMA accumulates as a result of reduced renal clearance. Dialysis reduces plasma ADMA levels and normalizes endothelial function. Associations between increased levels of ADMA and many cardiovascular risk factors such as age, hypertension, diabetes, insulin resistance, hypercholesterolemia, hypertriglyceridemia, and hyperhomocysteinemia have been documented.13–20 Furthermore, evidence for a causal relationship between increased ADMA levels and endothelial vasodilator dysfunction has been demonstrated in many of these conditions. In hypercholesterolemic adults (but not children), elevated ADMA levels are inversely correlated with endothelium-dependent vasodilation in the forearm.18,21 Consistent with the notion that ADMA is a competitive inhibitor, in hypercholesterolemic adults an intravenous infusion of L-arginine restores endothelial function and increases urinary nitrate excretion (a surrogate parameter of NO production).

Plasma ADMA levels can change quite rapidly in humans, temporally associated with alterations in endothelial vasodilator function. A single high-fat meal doubled ADMA levels in diabetic patients and was temporally associated with a significant impairment of flow-mediated endothelium-dependent vasodilation in the forearm.22 A single oral dose of methionine increases plasma homocysteine levels, paralleled by an increase in plasma ADMA and a decline in endothelium-dependent vasodilation.20 In humans with salt-sensitive hypertension, administration of a high-salt diet increases plasma ADMA and blood pressure and reduces urinary nitrogen oxides.15 A low-salt diet reverses these abnormalities.

ADMA Regulates Vascular Resistance

There is substantial evidence that ADMA regulates vascular resistance in humans. Intra-arterial infusion of ADMA reduces endothelium-dependent vasodilation in the human forearm.12 Moreover, intravenous infusion of ADMA (to increase plasma ADMA levels approximately 3-fold) increases sys-
ADMA and Vascular Structure

By reducing the activity of endothelial NOS, ADMA may affect vascular structure as well as vascular reactivity. Endothelial cells resurfacing an injured vessel manifest higher intracellular levels of ADMA and impaired endothelium-dependent vasodilation.³² The severity of the endothelial dysfunction and the intracellular levels of ADMA are directly related to intimal thickness of the injured vessel.³³ A recent clinical trial is consistent with a role of ADMA in restenosis. Patients undergoing coronary angioplasty and stent placement received a single intramural delivery of L-arginine or vehicle. Intravascular ultrasound at 6 months revealed a 36% reduction of neointimal volume in those patients receiving L-arginine.³²

Exposure of endothelial cells in culture to pathophysiologically relevant concentrations of ADMA reduces NO synthesis, increases superoxide generation, and increases the adhesiveness of endothelial cells for monocytes.²⁹ Evidence also exists in humans that ADMA inhibits endothelium-dependent vasodilation and/or NO synthesis.¹²⁹ In mice genetically engineered to express low plasma ADMA levels, NO synthesis is increased, and vascular resistance is reduced.³⁰

Mechanisms by Which ADMA Becomes Elevated

Generation of ADMA

ADMA is not derived from the methylation of free L-arginine. Rather, ADMA is generated from posttranslational modification of arginine residues within a variety of specific proteins that are predominantly found in the cell nucleus (for review, see Tran et al⁴⁸). Methylation of arginine residues on proteins is catalyzed by a group of enzymes termed protein arginine N-methyltransferases (PRMTs) (Figure). When the proteins undergo proteolysis, free methylarginines are released.

Two distinct PRMT activities (PRMT types I and II) have been classified. Both subtypes can monomethylate arginine to form MMA but differ in that type I asymmetrically dimethylates arginine to form ADMA, whereas type II catalyzes a symmetrical dimethylation of arginine to form SDMA.⁴⁸ Whereas ADMA and MMA each inhibit NOS, SDMA is not capable of doing so.

To date, there is scant evidence that elevated plasma levels of ADMA are due to increased methylation of arginine residues. The methylation of arginine residues on proteins is a highly regulated process, and methylated proteins have a wide range of functions. Although PRMT activity is influenced by oxidized lipoprotein in vitro,⁴⁶ it is unlikely that...
Biochemical pathway for generation, elimination, and degradation of ADMA. ADMA derives from methylation of arginine residues in proteins. The reaction is catalyzed by PRMTs that transfer a methyl group from S-adenosyl-L-methionine (SAM) to each guanidino nitrogen of an arginine residue. This reaction results in a methylated arginine derivative (protein containing ADMA) and S-adenosyl-L-homocysteine (SAH). Hydrolysis of the methylated proteins releases ADMA. ADMA is a competitive inhibitor of endothelial NOS. All methylarginines are excreted into the urine and are in part metabolized to α-keto acids by the enzyme activity of dimethylarginine pyruvate aminotransferase (DPT). The major metabolism of ADMA occurs via degradation through the enzyme DDAH. The enzyme DDAH hydrolyzes ADMA to form dimethylamine and L-citrulline. DM indicates diabetes mellitus; HTN, hypertension, LDL-C, LDL cholesterol; HCY, hyperhomocysteinemia; and CMV, cytomegalovirus.

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primary regulation of ADMA occurs so proximal in the pathway that it would secondarily alter an array of diverse nuclear proteins.

Elimination of ADMA
Humans generate approximately 300 μmol/d (approximately 60 mg) of ADMA. Of this amount, approximately 50 μmol/d is excreted in the urine. Thus, ADMA accumulates in patients with renal failure. Kidney transplantation normalizes SDMA levels, whereas ADMA levels remain elevated. This may be due to persistent impairment in the degradation of ADMA. Degradation of ADMA (but not SDMA) is mediated largely by dimethylarginine dimethylaminohydrase (DDAH). Two isoforms exist: DDAH I predominates in tissues containing neuronal NOS, whereas DDAH II is more prevalent in tissues expressing endothelial NOS. We have proposed that the elevation in plasma ADMA that occurs with vascular disease and risk factors is largely due to impaired activity of DDAH.

Central Role of DDAH
The first evidence that DDAH is a critical regulator of the NOS pathway came from observations regarding the DDAH inhibitor, 4124W. Addition of 4124W to an isolated vascular segment induces a gradual vasoconstriction, which is reversed by addition of L-arginine to the medium. This finding is most consistent with the view that ADMA is constantly being produced in the course of normal protein turnover. The production of ADMA is balanced by its metabolism by DDAH. Accordingly, pharmacological inhibition of DDAH activity causes ADMA to accumulate, to disrupt NO synthesis, and to thereby induce vasoconstriction.

We have shown that impaired DDAH activity is a central mechanism by which cardiovascular risk factors disrupt the NOS pathway. The activity of DDAH is impaired by oxidative stress, permitting ADMA to accumulate. A wide range of pathological stimuli induces endothelial oxidative stress such as oxidized LDL cholesterol, inflammatory cytokines, hyperhomocysteinemia, hyperglycemia, and infectious agents. Each of these insults attenuates DDAH activity in vitro and in vivo. The attenuation of DDAH allows ADMA to accumulate and to block NO synthesis (Figure). The adverse effect of these stimuli can be reversed in vitro by antioxidants, which preserve the activity of DDAH.

The sensitivity of DDAH to oxidative stress is conferred by a critical sulfhydryl in the active site of the enzyme that is required for the metabolism of ADMA. This sulfhydryl can also be reversibly inhibited by NO in an elegant form of negative feedback. We have shown that homocysteine mounts an oxidative attack on DDAH to form a mixed disulfide, inactivating the enzyme. By oxidizing a sulfhydryl moiety critical for DDAH activity, homocysteine and other risk factors cause ADMA to accumulate and to suppress NO activity.

In apolipoprotein E-deficient mice, hypercholesterolemia is associated with increased levels of plasma ADMA and attenuated angiogenesis. The effect of ADMA on angiogenesis can be reversed by administration of supplemental L-arginine. These data are consistent with previous observations disclosing a critical role of endothelium-derived NO in angiogenesis.61 The role of ADMA in modulating angiogenesis was strengthened by the finding that C6 glioma cells genetically engineered to constitutively overexpress the enzyme DDAH resulted in tumors that were more vascular and grew faster than wild type. Expression of DDAH can also be increased by exposing endothelial cells to retinoic acid. This effect is associated with reduced accumulation of ADMA and increased endothelial cGMP levels.

In experimental models of pulmonary hypertension, a reduction in pulmonary DDAH activity or expression is associated with an increase in plasma ADMA levels and reduced pulmonary NO synthesis. A reduction in DDAH activity could explain elevated plasma ADMA levels and L-arginine responsiveness in patients with pulmonary hypertension. The critical role of DDAH activity in regulating NO synthesis in vivo was convincingly demonstrated by our group with the use of a transgenic DDAH mouse. In this animal, the activity of DDAH is increased, and plasma ADMA levels are reduced by 50%. The reduction in plasma ADMA is associated with a significant increase in NOS activity, as plasma and urinary nitrate levels are increased 2-fold. The increase in NOS activity translates into a 15-mm Hg reduction in systolic blood pressure in the transgenic mouse. This study provides compelling evidence for the importance of DDAH activity and plasma ADMA levels in the regulation of NO synthesis.
ADMA and the ‘l-Arginine Paradox’

The l-arginine paradox relates to observations first made by our group that the administration of l-arginine can reverse endothelial vasodilator dysfunction under some conditions.12 Later, biochemists who had purified NOS enzyme found that its $K_m$ for l-arginine was in the range of approximately 5 $\mu$mol/L. Because l-arginine plasma levels are in the range of 50 $\mu$mol/L, it was paradoxical that l-arginine could be rate limiting. Possible explanations for this phenomenon include nonenzymatic generation of NO from l-arginine, release of growth hormone or insulin, or effects at the level of the y$^+$-transporter responsible for cellular uptake of l-arginine. However, reversal of the effect of ADMA represents a more likely mechanism.

Endothelial cells exposed for 24 hours to concentrations of ADMA that exist in the plasma of hypercholesterolemic individuals generate less NO and more superoxide anion and are more adhesive for monocytes.29 Similarly, a reduction of plasma ADMA levels from 1.6 $\mu$mol/L (in normal mice) to 0.7 $\mu$mol/L in our transgenic DDAH mice is associated with a 2-fold increase in plasma and urinary nitrogen oxides.80 The striking effect of modest changes in plasma ADMA is surprising given the high intracellular concentrations of l-arginine, which are almost 3 orders of magnitude greater. However, intracellular l-arginine may not have access to NOS (intracellular compartmentalization). Indeed, eNOS co-localizes with plasma membrane regions referred to as caveolae.66 Finally, there is evidence that ADMA may also “uncouple” endothelial NOS, such that molecular oxygen becomes the substrate for electron transfer rather than arginine.70 Under these conditions, endothelial NOS generates superoxide anion to increase oxidative stress, attenuate NO bioactivity, and induce additional endothelial dysfunctions.

However, an elevation of plasma ADMA does not necessarily predict a beneficial response to l-arginine supplementation. In a small study of men with stable angina and elevated plasma ADMA levels, oral l-arginine supplementation did not improve forearm endothelial vasodilator function, treadmill walking time, or time to ST segment depression.71 The lack of benefit of l-arginine in this study likely reflects the multifactorial mechanisms of endothelial vasodilator dysfunction. For example, NOS may become a major source of superoxide anion and endothelial dysfunction when l-arginine availability becomes rate limiting. However, deficiencies or reduced activity of tetrahydrobiopterin, heat shock protein 90, or specific tyrosine kinases would probably not be addressed by supplementation with l-arginine.72-74 L-Arginine may not be useful in later stages of atherosclerosis, in which cytokine- or lipid-induced instability and/or reduced transcription of NOS may decrease its expression.75 Endothelial dysfunction secondary to certain NOS gene polymorphisms10 might be unresponsive to supplemental l-arginine.

Therapeutic Modulation of Plasma ADMA

Notably, plasma ADMA can be reduced by pharmacotherapy. When glycermic control is improved by administration of metformin, plasma ADMA levels are reduced in diabetics.76 In patients with insulin resistance, rosiglitazone improved insulin resistance and lowered plasma ADMA levels.77 Treatment of hypertensive patients with a converting-enzyme inhibitor or angiotensin receptor antagonist reduces plasma ADMA levels.78,79 Estrogen therapy, alone or with progestogens, modestly reduces plasma ADMA (but not SDMA) levels, an effect that may be due to estrogen-induced increases in DDAH activity.80,83 However, hormonal replacement may also reduce plasma l-arginine levels, so that the l-arginine/ADMA ratio is not favorably affected.82 Strategies to reduce oxidative stress would likely enhance DDAH activity and thereby reduce plasma ADMA levels, but the results of 2 small studies of antioxidant vitamins have been negative or inconclusive.21,85 In the future, a likely target for pharmacotherapy is the enzyme DDAH.

Conclusion

To summarize, there is compelling evidence that ADMA plays an important role in the regulation of vascular tone by acting as an endogenous inhibitor of NO synthesis. By inhibiting NO synthesis, plasma ADMA may reduce vascular compliance, increase vascular resistance, and limit blood flow. Furthermore, plasma ADMA may promote atherogenesis as it opposes the vasoprotective effects of NO. Thus, elevations in plasma ADMA may accelerate the progression of atherosclerosis and increase the risk of cardiovascular events. ADMA may mediate the effect of many risk factors and risk markers on the NOS pathway. Plasma ADMA level may be an “Über marker,” reflecting the summative effect of various risk factors on endothelial health.

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References


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