

Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION



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Arterioscler Thromb Vasc Biol 2004;24;1023-1030; originally published online Apr 22, 2004;

DOI: 10.1161/01.ATV.0000128897.54893.26

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association,
7272 Greenville Avenue, Dallas, TX 75214

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ISSN: 1524-4636

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Cardiovascular Biology of the Asymmetric Dimethylarginine:Dimethylarginine Dimethylaminohydrolase Pathway

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Abstract—An increasing number of reports indicate that endogenously produced inhibitors of nitric oxide synthase, particularly asymmetric dimethylarginine (ADMA), regulate nitric oxide generation in disease states. This article describes the biology of ADMA and the implications for cardiovascular physiology and pathophysiology. (*Arterioscler Thromb Vasc Biol.* 2004;24:1023-1030.)

Key Words: asymmetric dimethylarginine ■ dimethylarginine dimethylaminohydrolase
■ nitric oxide synthase ■ endothelial dysfunction

Asymmetric dimethylarginine (ADMA) is a naturally occurring amino acid that circulates in plasma, is excreted in urine, and is found in tissues and cells.¹⁻³ It has aroused interest because it inhibits nitric oxide synthases (NOSs)¹ and therefore has the potential to produce considerable biological effects, particularly in the cardiovascular system. Recently, several studies have suggested that the plasma concentrations of ADMA provide a marker of risk for endothelial dysfunction and cardiovascular disease.^{1,4-6} This article describes the biology of ADMA and the implications for cardiovascular physiology and pathophysiology.

How Is ADMA Made?

ADMA is synthesized when arginine residues in proteins are methylated by the action of protein arginine methyltransferases (PRMTs).^{7,8} Protein arginine methylation is a post-translational modification that adds either 1 or 2 methyl groups to the guanidine nitrogens of arginine incorporated into proteins. There are 2 broad types of PRMTs: type 1 catalyze the formation of ADMA, whereas type 2 methylate both of the guanidino nitrogens and so result in the formation of symmetric dimethylarginine (SDMA; Figure 1). Both types of PRMT, of which there are several isoforms, can also monomethylate, leading to the formation of *N*^G-monomethyl-L-arginine (L-NMMA).^{7,8} Once the proteins are hydrolyzed, free methylarginines appear in the cytosol. The asymmetrically methylated arginines (ADMA and L-NMMA) are inhibitors of NOS, whereas SDMA is not.

There is potentially a very broad range of substrate proteins for type 1 PRMTs,⁹ and the enzymes and their substrates are widely distributed throughout the body.¹⁰ The role of protein arginine methylation is unclear, but this process has been

implicated in regulation of RNA binding, transcriptional regulation, DNA repair, protein localization, protein-protein interaction, signal transduction, and recycling or desensitization of receptors. However, it is only after the proteins are degraded that free methylarginines appear in the cytosol; to date, no direct route of synthesizing ADMA from free arginine has been identified. Thus, the amount of ADMA generated within a cell is dependent on the extent of arginine methylation in proteins and the rates of protein turnover.

Because of the complex process leading to generation of free ADMA, it is unclear whether ADMA generation is fairly constant, it alters with PRMT activity, or if rates of protein turnover are the most important influence. Recently, studies with relatively nonspecific and low-potency PRMT inhibitors have suggested that PRMT activity over 24 to 48 hours contributes to rates of generation of free ADMA and that there is a relationship between PRMT expression levels and free ADMA production.¹¹ Further studies are required to identify more clearly the likely variations in ADMA production rates.

In the cardiovascular system, type 1 PRMTs are expressed in the heart, smooth muscle cells, and endothelial cells. The expression pattern has not been documented in detail, but PRMT -1, 3, 4, and 6 (all type 1 PRMTs) are all expressed in vascular cells. Interestingly, the expression of PRMT-1 in endothelial cells is increased in response to shear stress and this effect can be blocked either by suppression of I κ B kinase or by the peroxisome proliferator-activated receptor (PPAR) γ activator troglitazone.¹² This altered expression of PRMT-1 was associated with corresponding changes in ADMA release, suggesting that rates of ADMA generation in the vessel wall may be regulated in part through alteration in PRMT

Received February 17, 2004; revision accepted April 8, 2004.

From the BHF Laboratories, Division of Medicine, University College London, London, UK.

This work was supported by British Heart Foundation grant no. RG2000007.

Correspondence to Patrick Vallance, University College London, Rayne Institute, 5 University St, London WC1 6JJ, UK. E-mail patrick.vallance@ucl.ac.uk

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Arterioscler Thromb Vasc Biol. is available at <http://www.atvbaha.org>

DOI: 10.1161/01.ATV.0000128897.54893.26

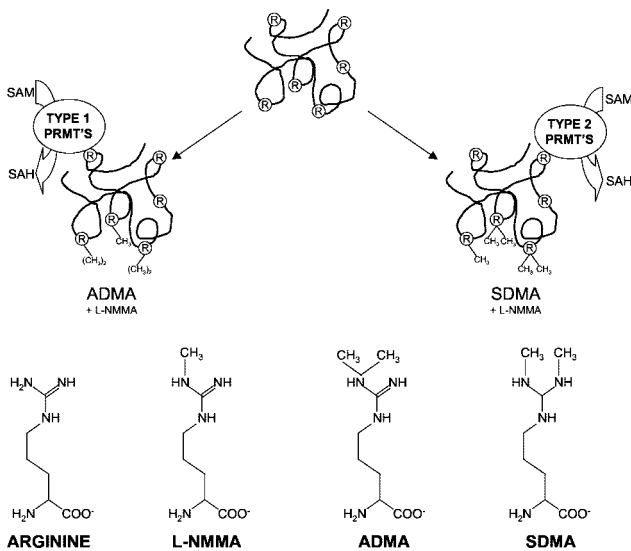


Figure 1. The synthesis of methylated forms of arginine. Arginine residues (circled R) that lie within appropriate consensus sequences in proteins can be post-translationally methylated by the action of PRMTs. S-adenosylmethionine (SAM) is the methyl donor in these reactions, and S-adenosylhomocysteine (SAH) is produced. After proteolysis of arginine-methylated proteins, free L-NMMA, ADMA, and SDMA are released into the cytosol. L-NMMA and ADMA are competitive inhibitors of all 3 isoforms of NOS; SDMA has no inhibitory activity.

expression. PRMT-1 expression is also increased by low-density lipoprotein (LDL) expression, and again the effect seems to correlate with altered ADMA generation.¹¹

Targets for ADMA

ADMA inhibits all 3 isoforms of NOS and is approximately equipotent with L-NMMA.^{1,13} The IC₅₀ is dependent on the prevailing arginine concentration,¹³ and the effects can be reversed by adding excess L-arginine. In addition to blocking NO formation, L-NMMA treatment may uncouple NOS and

lead to the generation of superoxide,^{14,15} and it is likely that ADMA can do the same. Although NOS is the most obvious target for ADMA, it is not clear yet whether it is the only target. At very high concentrations, ADMA and SDMA can compete with arginine for transport through the Y⁺ transporter system,¹⁶ and at millimolar concentrations, guanidine compounds may inhibit Na⁺/K⁺ ATPase.¹⁷ The effects of methylarginines on the Y⁺ system probably only occur at concentrations too high to be physiologically relevant. One potential target for ADMA would be arginine-glycine amidino transferase, an enzyme with a similar structure to the dimethylarginine dimethylaminohydrolases (DDAHs) that metabolizes ADMA (see below).¹⁸ However, our unpublished observations suggest that ADMA is a weak inhibitor of this enzyme. Although it is unnecessary to invoke additional targets for ADMA, it is worth noting that many microbes produce ADMA and express the enzymes necessary to metabolize ADMA¹⁹ yet do not express NOS, perhaps suggesting some additional unrecognized action of ADMA.

Degradation of ADMA: The DDAHs

Methylarginines are eliminated in part by renal excretion. However, although SDMA (the methylarginine that does not inhibit NOS) is eliminated almost entirely by renal excretion, ADMA and L-NMMA are extensively metabolized.^{2,20} Indeed, in some species, >90% of ADMA generated is metabolized rather than excreted.^{2,20–22} The major metabolic route is to citrulline and dimethylamine, a reaction catalyzed by DDAH.^{23,24} The reaction probably involves a nucleophilic attack on the guanidino portion of the ADMA molecule by a cysteine held in an activated state in the tertiary structure of the enzyme.¹⁸ That this cysteine is involved is not in doubt because replacing it with a serine residue renders the enzyme inactive.^{18,25} Furthermore, this cysteine is susceptible to oxidation and regulation by nitric oxide.²⁵ It is not yet clear whether oxidative stress produces irreversible inhibition of

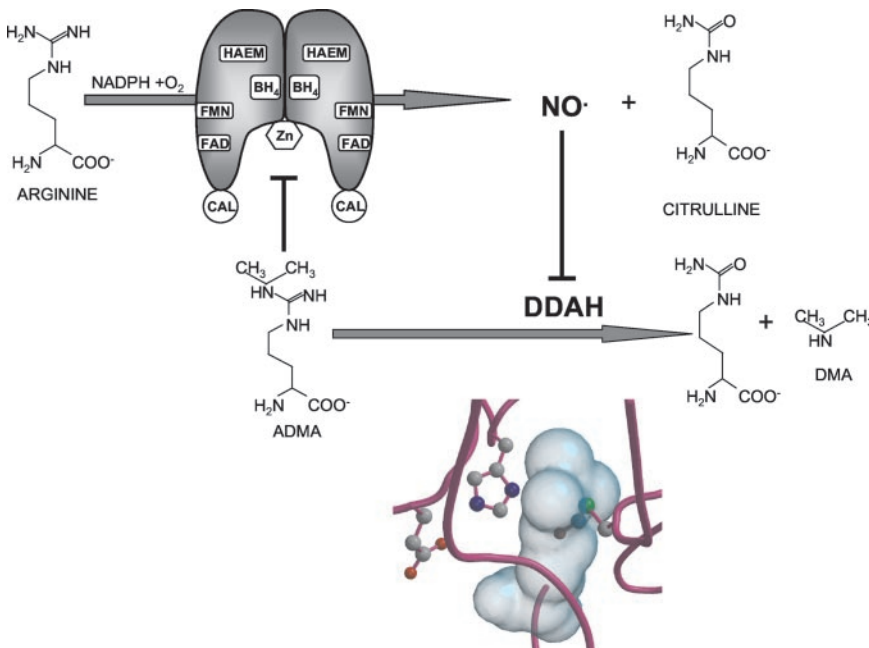


Figure 2. Metabolism of methylated arginine can be directly regulated by NO. NOS catalyzes the conversion of L-arginine and molecular oxygen to citrulline and NO. NOS enzymes are catalytically active as homodimers and require the binding of cofactors (flavin adenine dinucleotide [FAD], flavin mononucleotide [FMN], HAEM, and tetrahydrobiopterin [BH₄]) and calmodulin for optimal activity. Each NOS dimer coordinates a single atom of zinc. NO directly inhibits DDAH by S-nitrosation of the active site cysteine residue. Inhibition of DDAH results in accumulation of ADMA and inhibition of NOS. Inset, A structural model of the active site of DDAH containing ADMA. The catalytic triad of glutamine, histidine, and cysteine residues is shown. S-nitrosation of the sulfur atom (green) of the active site cysteine deactivates this residue and might also sterically hinder the binding of ADMA.

DDAH activity, but certainly nitrosation is reversible. Thus, high-output NO production (eg, from iNOS expression) nitrosates DDAH and inhibits activity. This provides a potentially important homeostatic mechanism whereby increases in NO or changes in the redox environment in which the NO is generated can switch off further NO generation (Figure 2). DDAHs seem to be predominantly cytosolic enzymes with no obvious subcellular localization. No clear cofactor requirement has been identified, although activity is inhibited by certain divalent cations.²³ A requirement for zinc has been identified for a bovine DDAH, but it is not clear whether this is a general requirement for DDAHs.^{26,27}

DDAHs are highly conserved throughout evolution²⁸ and have been identified in primitive organisms including bacteria. In higher organisms, including humans, 2 isoforms of DDAH have been identified that are encoded by genes located on chromosomes 1 (DDAH-1) and 6 (DDAH-2).^{28,29} The 2 isoforms have distinct tissue distributions but apparently similar activity.²⁹ There is some overlap between the expression of DDAH-1 and neural NOS and DDAH-2 and endothelial NOS (eNOS), but it is clear that both DDAHs are widely expressed and not confined to NOS-expressing cells or tissues.²⁹ Both isoforms have been identified within the cardiovascular system, although it appears as though expression of DDAH-2 is probably more abundant, at least at the mRNA level. Analysis of the promoter region of DDAH-2 suggests that this gene displays many characteristic of a so-called “housekeeping” gene.³⁰ However, although it is highly expressed under basal conditions in many cells, it is also transcriptionally regulated, and expression levels may vary considerably. For example, treatment of endothelial cells with retinoic acid increases DDAH-2 expression by ≈ 2 -fold.³¹ Dissection of the promoter of DDAH-2 has identified several control elements,³⁰ and a region has been identified that seems to promote basal gene expression, at least in certain types of endothelial cells. Overall, available data suggest that the DDAHs are widely expressed genes that nonetheless are regulated at the level of transcription.

Cellular ADMA

ADMA is generated within cells^{32–34} and there is evidence that the cellular levels alter with pathophysiology. For example, endothelial cells re-populating blood vessels after balloon denudation have higher concentrations of ADMA than do control cells.³⁵ Although the precise concentration of ADMA within cells is unclear, endothelial cells concentrate methylarginines so that if methylarginines are added to culture medium, the concentrations in the cell rise to approximately 5-fold higher than in surrounding medium.¹⁶ This concentration of methylarginines is probably attributable to transport by the arginine transport system referred to as the Y⁺ transporter.¹⁶ It is not yet clear whether there is any compartmentalization of ADMA that might lead to pockets of very high concentrations, but the observation that the *K_m* of DDAH for ADMA is rather high (above 100 $\mu\text{mol/L}$) might suggest that ADMA levels reach very high local concentrations under certain conditions. Endothelial cells express both PRMTs and DDAHs,³⁶ and inhibition of DDAH leads to significant accumulation of ADMA.^{36–38} Furthermore, func-

tional studies in vascular rings suggest that inhibition of DDAH produces changes in endothelial function consistent with substantial concentrations of ADMA in the vicinity of eNOS.³⁶ The overall output of ADMA from endothelial cells presumably is a balance between rates of arginine methylation, rates of degradation of proteins containing methylated arginine, rates of metabolism of ADMA by DDAHs, and the rates of active extrusion from the cell. The relative importance of each component is not yet known, but the metabolic capacity of DDAH is high and it seems likely that DDAH activity will usually be the major determinant of overall ADMA levels within a cell.

The system of generation of ADMA is intriguing and seems rather cumbersome as a route to generate a regulatory mediator. Indeed, rates of free methylarginine production might be expected to remain relatively constant. It remains to be determined how rates of protein turnover are linked to ADMA generation and whether the ADMA is simply an “unfortunate” byproduct of the breakdown of proteins that has to be eliminated by DDAH before it causes problems or there is some important biological link between the degradation of certain types of proteins and the NO pathway.

ADMA is relatively stable and can diffuse between cells. Indeed, the ADMA made in one cell is capable of inhibiting NOS in a second cell type.³⁹ This interaction has been clearly demonstrated for macrophages and endothelial cells³⁹ and presumably may also occur for smooth muscle cells and endothelial cells. This raises the possibility that ADMA provides a mechanism by which smooth muscle cells may signal to the endothelium.

Circulating ADMA

The plasma concentration of ADMA has been assessed in a wide variety of cardiovascular and other conditions.^{40,41} Because the kidneys provide one route for clearance of methylarginines, it is perhaps not surprising that ADMA and SDMA concentrations increase in patients with renal failure.^{1,42,43} The SDMA increases in line with creatinine and reaches higher levels than the ADMA.⁴² This is not surprising because ADMA is also metabolized by DDAH, whereas

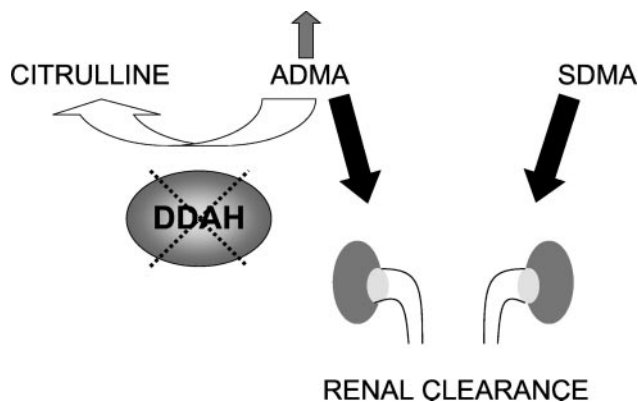


Figure 3. DDAH dysfunction selectively increases circulating ADMA. Both ADMA and SDMA are cleared by the kidney and both increase in renal failure. ADMA is also removed by metabolism catalyzed by DDAH. Impairment of DDAH activity would selectively increase circulating ADMA, whereas SDMA would remain unchanged.

TABLE 1. ADMA: What Is Known and Not Known

Known	Not Known
ADMA is a competitive inhibitor of NOS	Whether endogenous ADMA concentrations increase sufficiently to inhibit NO production in vivo
ADMA is a product of protein turnover	Whether DDAH activity is the major determinant of ADMA levels in vivo
ADMA is metabolized by DDAH	Whether ADMA has a causal role in pathophysiology
ADMA is cleared by the kidney	Other than for renal failure, the data on changes with disease states are not consistent and the reason is not clear
Exogenous ADMA inhibits NO generation in tissue culture and organ bath experiments	
Infused ADMA reduces forearm blood flow in humans	
Infused ADMA reduces cardiac output and increases systemic vascular resistance in humans	
Plasma ADMA concentration is increased in some disease states associated with endothelial dysfunction and/or reduced NO production	

SDMA is not. In other conditions, ADMA seems to be selectively increased with no change in SDMA.^{40,41,44} This pattern of increase is highly suggestive of DDAH dysfunction (Figure 3). It is not known whether it is the circulating ADMA that is biologically active or whether the plasma concentration is simply a marker of high intracellular levels. Concern has been expressed that the typical levels found in health (500 nmol/L–1.2 μ mol/L) or many disease states (up to \approx 3 μ mol/L) are too low to be biologically active.

The concern about ADMA relates to the relative concentrations of ADMA and arginine. Plasma arginine concentrations are in the order of 30 to 100 μ mol/L, and the intracellular concentrations of arginine may be 1 to 2 mmol/L.⁴⁵ With this vast excess of arginine, ADMA should be inert and would not be expected to inhibit NOS. However, experimental evidence suggests that despite the theoretical concerns, even very low concentrations of methylarginines exert profound effects. Infusions of ADMA into healthy volunteers show that increases of blood pressure and vascular resistance and a fall in cardiac output and heart rate occur when plasma concentrations of ADMA are within the pathophysiological range.^{46,47} Interestingly, the fall in heart rate seen was large and occurred very rapidly, even before changes in vascular resistance were evident.^{46,47} This suggests that the effects on heart rate may be a direct effect on NOS systems in the heart rather than reflex effects secondary to hemodynamic effects.

The proportion of circulating ADMA eliminated by renal excretion and by DDAH activity seems to differ between species. In the rat, it has been estimated that the majority (in the order of 90%) of ADMA is metabolized with only a small fraction appearing unchanged in the urine.^{20,21} In humans, infusions of ADMA lead to an increase in dimethylamine production, suggesting that there is substantial DDAH activity.⁴⁶ Indeed, it has been calculated that \approx 260 μ mol (\approx 50 mg) of ADMA is metabolized each day and \approx 60 μ mol is excreted. The amount present in urine is enough to produce concentrations as high as 20 to 30 μ mol/L, and this may be significant in terms of renal tubular NOS activity. Complete failure of elimination of ADMA would be expected to increase plasma ADMA concentrations by as much as 5 μ mol/L each day.⁴⁶ An implication of this finding is that it is unlikely that plasma ADMA levels vary significantly over

short time periods. Indeed, some of the rapid changes seen in certain studies are difficult to understand unless there is significant proteolysis leading to a sudden increase in release of ADMA or sudden flux of ADMA from tissue into plasma.

Cardiovascular Effects of ADMA

ADMA inhibits NOS and produces the effects expected of an isoform-nonspecific NOS inhibitor (Table 1).^{46–49} It elevates blood pressure, causes vasoconstriction, impairs endothelium-dependent relaxation, and increases endothelial cell adhesiveness.^{1,46,50} Extrapolation from other NOS inhibitors suggests that long-term exposure to ADMA would be expected to enhance atherogenesis and produce sustained hypertensive damage to end organs.^{51,52} In addition, in animal models, other NOS inhibitors can reproduce some of the vascular and renal effects of pre-eclampsia.^{53,54} NOS knockouts suggest that prolonged inhibition of NOS predisposes to aneurysm formation,⁵⁵ but it is unclear whether the same would be true for prolonged “pharmacological” inhibition of the enzyme by high-circulating levels of ADMA.

In the heart, ADMA reduces heart rate and cardiac output, and other NOS inhibitors have similar effects.^{46,47} Left ventricular hypertrophy is also a feature of prolonged NOS inhibition. Renal effects of NOS inhibition include reduced sodium excretion,^{56,57} and this may also contribute to the hypertension. High-salt diet may be associated with increased pressor responses to NOS inhibitors, particularly in individuals who are most salt sensitive.⁴⁹

ADMA also inhibits angiogenesis in animal models.⁵⁸ Furthermore, DDAH overexpression promotes angiogenic processes in cells in culture⁵⁹ and in experimental tumors in vivo.^{60,61} DDAH overexpression is also associated with an increase in vascular endothelial growth factor expression, and this seems to be important in promoting the angiogenesis.⁵⁹

Pathophysiology

ADMA accumulation has been reported in a wide range of cardiovascular disorders. In this section, we discuss some of these and the potential biological effects of ADMA.

Renal Failure

As the kidneys fail, ADMA accumulates.¹ Plasma ADMA concentrations increase to between 1 and 3 μ mol/L but are

lower than SDMA levels. There is a variable increase in ADMA,^{1,42,43} probably because of variable DDAH activity and possibly because renal DDAH itself contributes to overall elimination of ADMA, and the effects of different renal pathologies may have different effects on renal DDAH expression. ADMA is removed by dialysis with hemodialysis, producing large swings in ADMA levels that rapidly return toward high pathological values after dialysis.⁶² Interestingly, ADMA fulfills many of the criteria for a uremic toxin: it accumulates as the kidney fails, is a guanidino compound, is a product of protein metabolism, and through effects on NOSs, has the potential to affect multiple biological functions that are deranged in patients with chronic renal failure, including effects on cardiovascular system, bone, and defense against infections.¹

There is a relationship between the plasma levels of ADMA and the degree of endothelial dysfunction,⁶² and in a cohort of patients undergoing hemodialysis, ADMA levels were predictive of future cardiovascular events and overall mortality.^{6,63} These findings are consistent with ADMA, providing a marker of cardiovascular risk in these patients, and there is a plausible biological mechanism for it having a causal role through inhibition of NOS. Although ADMA effects should be reversed by adding L-arginine, data on the effects of arginine in renal disease are inconsistent. Some studies have shown that arginine improves endothelial function in patients with renal failure but others have not.^{64,65} It remains to be determined whether the apparent lack of effect of arginine in some studies is because prolonged exposure to high ADMA levels produces endothelial dysfunction that becomes irreversible if ADMA is a marker rather than a causal agent, if ADMA produces some other effect independent of NOS inhibition, or it eventually leads to an arginine-irreversible inhibition of NOS.

Cardiovascular Risk

Extrapolation from the situation in renal disease led investigators to speculate that an increase in ADMA may also contribute to cardiovascular risk. Increased ADMA has been detected in a variety of cardiovascular risk states, including hypercholesterolaemia,⁶⁶ hypertension,⁶⁷ diabetes,⁶⁸ hyperhomocystinaemia,⁶⁹ and in individuals with overt atherosclerotic disease.⁵ The circulating levels are not increased to the levels seen in renal failure, and the results have not been consistent for all of the risk factors. Indeed, it is probably only for hypercholesterolaemia that any degree of consistency has been seen in the published literature. Most articles that have explored the relationship have found increased ADMA or an increased ADMA/L-arginine ratio in patients with raised cholesterol⁷⁰. However, many of the studies are rather small, a problem caused in part by the laborious methods for measuring ADMA.

Plausible mechanisms for increased ADMA have been shown in cell culture, with effects of LDL on both DDAH activity and PRMT expression.^{11,37} However, it is clear that it will be necessary to develop better methods for accurate high throughput measurement of ADMA levels to be able to characterize better the role of ADMA in cardiovascular risk and its use as a risk marker. The recent identification of

functional polymorphic variants of DDAH genes³⁰ should enable genetic association studies to be undertaken.

The relationship between homocysteine and ADMA is of interest because there are many potential interactions. Homocysteine can inhibit DDAH activity,⁷¹ possibly by an interaction with the critical cysteine residue in the active site of the enzyme.¹⁸ However, homocysteine is a key part of the cycle of methylation. S-adenosylmethionine is the methyl donor that allows arginine methylation and yields S-adenosylhomocysteine, which in turn, can be converted to homocysteine. Methionine challenge has been used to increase homocysteine levels,⁷² and this is associated with endothelial dysfunction. However, methionine challenge also increases ADMA,^{69,73} and therefore this might provide an alternative explanation. Again the data are inconsistent, and some investigators have failed to find an increase in ADMA after methionine.⁷⁴ Nonetheless, these studies raise the possibility that any relationship between homocysteine and cardiovascular risk may simply be a reflection of a relationship between ADMA and cardiovascular risk.

Diabetes

Elevated ADMA levels have been found in animal models of type 1 and type 2 diabetes and in patients with overt type 2 diabetes or insulin resistance.^{68,75} Indeed, there seems to be a strikingly close correlation between indices of insulin resistance and ADMA levels.⁷⁶ Glucose itself may suppress DDAH activity⁷⁷ and increase ADMA, but the mechanisms by which diabetes or insulin resistance may increase ADMA have yet to be elucidated.

Both metformin and the thiazolidinediones reduce ADMA levels.⁷⁶⁻⁷⁹ The mechanisms by which these drugs may reduce ADMA levels are not clear. There is a PPAR/retinoid X receptor site in the promoter region of DDAH-2,³¹ and one possibility is that DDAH-2 expression is increased in response to PPAR agonists as it is to retinoic acid. It is not known how metformin works, but it is intriguing to note the structural similarities between metformin and ADMA (Figure 4).

Heart Failure

Levels of ADMA are increased in heart failure,⁸⁰⁻⁸² and ADMA has the capacity to decrease ventricular contraction and heart rate.⁴⁶ It is not yet clear whether there is any causal role for ADMA in either cardiac function or endothelial function in heart failure.

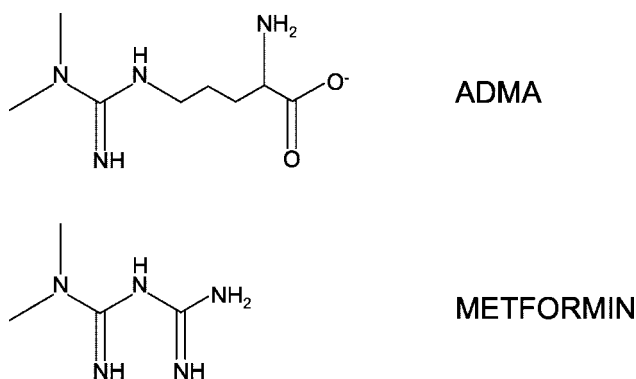


Figure 4. Comparison of the structure of ADMA and metformin. Both ADMA and metformin contain 1 dimethylated and 1 free guanidino nitrogen.

TABLE 2. Therapeutic Strategies to Alter NO Production via Modulation of the DDAH/ADMA/NOS Pathway

To Increase NO Production	To Limit NO Production
Arginine/citrulline supplementation	Small molecule inhibitors of DDAH
Antioxidant supplementation to increase DDAH activity	
Transcriptional upregulation of DDAH	
Small molecule activators of DDAH	
Gene therapy to increase DDAH levels	

Pre-Eclampsia

ADMA levels seem to fall during normal pregnancy⁸³ but are increased in women with pre-eclampsia.⁸⁴ Recently, it has been shown that ADMA levels are increased even before the development of pre-eclampsia,⁸⁵ suggesting that ADMA might provide a novel risk marker for the early detection of women at high risk. It is not known where the ADMA originates, but ADMA is produced by the fetus and is present in large amounts in fetal plasma and urine.⁸⁵ Furthermore, the placenta expresses high levels of DDAH-2,^{28,29} raising the possibility that failure of the placenta to clear the ADMA produced by the fetus is important. In women with high ADMA levels early in pregnancy, there was a clear relationship between the ADMA level and endothelial dysfunction, but this was only seen in the women who subsequently developed pre-eclampsia.⁸⁵ This may suggest that a raised ADMA alone is insufficient, but that some individuals are particularly susceptible to the effects of raised ADMA and they are at most risk of developing complications.

Pulmonary Hypertension

ADMA levels are raised in children with pulmonary hypertension,⁸⁶ and in experimental models of pulmonary hypertension, ADMA is increased and DDAH is defective.^{87,88} DDAH expression is decreased in a porcine and a rodent model of hypoxia-induced pulmonary hypertension, although in one model, the effect seemed to be caused by lack of DDAH-2,⁸⁷ and in the other, it was DDAH-1 that was decreased.⁸⁸ The pulmonary circulation is very sensitive to NOS inhibitors, and L-NMMA causes a rapid and sustained rise in pulmonary artery pressures.⁸⁹ The markedly reduced DDAH expression seen in experimental pulmonary hypertension provides a mechanism for increased NOS inhibition, but it is not known whether hypoxia directly downregulates DDAH expression.

Targets for Treatments

For cardiovascular pathology, the most obvious treatment objective would be to reverse the effects of increased ADMA or reduce the ADMA levels. Theoretically, arginine should be able to displace ADMA and restore NOS activity. Arginine has been reported to improve endothelial function in patients with hypercholesterolaemia and increase walking distance in patients with peripheral vascular disease.⁹⁰ However, studies to date have been relatively small, and the potential therapeutic effects of arginine remain poorly established.

An alternative approach would be to increase DDAH expression or activity (Table 2). The intriguing observations that thiazolidinediones and metformin appear to decrease ADMA levels deserve further study, but it remains to be determined

whether effects on DDAH contribute to this effect or how the actions on ADMA may contribute to their therapeutic efficacy. Modulation of DDAH expression is possible with existing drugs: estrogens increase DDAH expression⁹¹ and reduce ADMA in experimental models, as does retinoic acid.³¹ At this stage, agents that alter DDAH expression are likely to be useful experimental tools to probe the biology of ADMA and DDAH, but it is far too early to know whether increasing DDAH activity is indeed a potentially useful therapeutic goal. Under certain circumstances, short-term inhibition of DDAH may even be useful, for example, to inhibit tumor angiogenesis or decrease excess NO production in acute inflammatory states.⁹² However, increased ADMA production may occur naturally in these situations because of increased protein turnover.

Conclusions

ADMA is a naturally occurring inhibitor of NOS. It is clear that it is generated by many different cell types in the cardiovascular system and can affect vascular and cardiac function. Correlation of ADMA with endothelial dysfunction and cardiovascular risk, together with the associations between cardiac risk factors and ADMA levels, suggest that ADMA is linked to cardiovascular disease, but strong causal relationships have yet to be established. The basic biology of ADMA and DDAH is being revealed, and much is known about the regulation of DDAHs in experimental systems and models. The high degree of conservation of the PRMT-ADMA-DDAH pathway throughout evolution is supportive of an essential biological role. However, to define precisely the clinical significance, it will be necessary first to establish the robustness of ADMA as a marker in the conditions in which it has been implicated. This will require better assay methods and larger studies. Indeed, the wide range of levels between studies remains a matter for concern. Second, it will be important to move beyond association studies into studies of causal relationships. To do this, it will be necessary to use specific inhibitors or activators of DDAH and manipulation of DDAH expression, particularly in animal models. Studies that have taken these approaches have indicated important causal relationships between ADMA and vascular function, and these now hint at therapeutic opportunities.

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