

## ADMA

# Asymmetric Dimethylarginine: A Modifiable Risk Factor for Cardiovascular Disease

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## Abstract

The endothelium-derived relaxation factor, nitric oxide, provides a unifying mechanism for the action of many cardiovascular disease risk factors. Nitric oxide cannot easily be directly measured, but the inhibitor of its formation, asymmetric dimethylarginine (ADMA) can be. The function of ADMA in modulating nitric oxide production has a significant impact on cardiovascular endothelial function. There are inexpensive and safe ways to improve endothelial nitric oxide responses that can be monitored by following ADMA responses. These interventions promise to significantly reduce the mortality and morbidity statistics for heart disease. Plasma ADMA is now available as a routine laboratory evaluation.

## The role of nitric oxide

Cardiovascular disease remains the number one cause of death in the United States. This fact indicates that there is more to learn regarding risk factors and how these contribute to cardiovascular disease (CVD). Because of its cell regulatory functions, nitric oxide (NO) is involved in the pathogenesis of many chronic degenerative disorders. Atherosclerotic heart disease, type 2 diabetes, metabolic syndrome (X), Crohn's disease, and arthritis are some of the major diseases where pathogenesis involves NO metabolism. Anytime a molecule with such powerful and wide ranging physiological consequences is produced as a normal cell regulatory mechanism, there must be offsetting ways of controlling its synthesis. The focus of this review is on the control of NO synthesis by the endogenous regulator ADMA and its relationship to cardiovascular disease.

***Nitric oxide protects the heart, stimulates the brain, kills bacteria, etc.***

“NO acts as a signal molecule in the nervous system, as a weapon against infections, as a regulator of blood pressure and as a gatekeeper of blood flow to different organs.”

*From 1998 award announcement for the Nobel Prize in Physiology or Medicine to Robert F. Furchgott, Louis J. Ignarro and Ferid Murad.*

Nitric oxide is a gas with molecular dimensions similar to molecular oxygen (O<sub>2</sub>). In fact, the competitive blocking of mitochondrial oxygen uptake is the mechanism used by fireflies for flashing. In humans, one mechanism of NO action is to signal the formation of the intercellular messenger, cyclic guanosine monophosphate (cGMP), thereby eliciting cellular responses like smooth muscle relaxation. NO is formed from the amino acid L-arginine under the action of a family of enzymes, the nitric oxide synthases, found in endothelial, neuronal, and inducible isoforms. Some of the tissue-specific functions of NO are summarized in Table 1.

**Table 1. Tissue-specific functions of nitric oxide**

<b>SYSTEM</b>	<b>FUNCTION OF NITRIC OXIDE</b>
<b>Cardiovascular system</b>	Exerts tonic vasodilator tone. Prevents cellular adhesion to vessel walls. Inhibits platelet activation. Retards development of atherosclerosis. Stimulates angiogenesis. [1] Induces regression of preexisting intimal lesions. [2]
<b>Brain</b>	Modulates neurotransmission. Excess causes neurodegeneration
<b>Peripheral nervous system</b>	Regulates pain perception. Mediates non-adrenergic, non-cholinergic neurotransmission in the gut, genitourinary system and vasculature.
<b>Immune system</b>	Mediates cytotoxicity and regulates immune cell function. [3]

### ***Nitric Oxide and the cardiovascular system***

It is now widely understood that heart disease is really a problem of the vascular system and vascular health is largely a question of endothelial function. In a single adult human, the approximately 100,000 miles of blood vessels are lined with endothelial cells comprising the surface area of eight tennis courts. It is the response of that tissue that determines cardiovascular health. [4]

Endothelial cells respond to signals for dilation or contraction. The response is mediated first by stimulation of nitric oxide synthase enzymes in the endothelial cells to produce nitric oxide. The gas quickly diffuses into the muscular layer where it triggers the formation of cyclic GMP, thus causing the shifting of ionic calcium and culminating in relaxation of the smooth muscle cells surrounding the arteries. This process must function smoothly and continuously during waking activities and digestion to regulate changes in blood flow.

#### **Beneficial effects of NO on blood vessels:**

- Stimulates dilation and sustained relaxation of arteries
- Reduces platelet and macrophage adhesiveness, thus lowering the inflammatory cascade
- Keeps blood vessels pliant and elastic
- Regulates oxidative enzymes preventing free radical pathology
- Slows plaque growth
- May reverse plaque formation to clear atheroma

#### **Clinical benefits of increased NO production:**

- Less discomfort from angina and other symptoms of heart disease
- Increased pain-free exercise time
- Improvement in erectile dysfunction

### ***Nitric oxide formation from L-arginine***

The normal precursor of NO is the amino acid L-arginine. Thus, the first modifiable factor is to increase plasma arginine by supplementation with 3gm L-arginine or more. [5] Arginine is not considered an essential nutrient because it is formed in human tissues as an intermediate in the urea cycle. Thus, the ubiquitous amino acid glutamate is transformed to citrulline that combines with carbamoyl phosphate (from CO<sub>2</sub> and NH<sub>3</sub>) to form arginine. So, it might seem that one would never run out of arginine because it can easily be made from virtually any amino acid source. The problem is that arginine is also required for synthesis of almost every protein in the body and supply of glutamate is limited. Demands for arginine in the urea cycle and in protein synthesis can cause circulating free arginine concentration in plasma to fall below optimal for NO synthesis.

L-Arginine infusion has been shown to enhance nitric oxide generation and inhibit lesion formation after balloon angioplasty. [6] The communication of vascular dilation signals occurs via NO diffusion from the endothelial layer into the muscular layer. However, the supply of arginine as substrate to the endothelial nitric oxide synthase (NOS) may depend on arginine status in vascular smooth muscle cells, which have unique affinity for uptake of arginine, even in polymeric forms. [7]

Other modifications act on the enzyme activity of NOS. Adding 10 mg of folic acid per day to healthy subjects prevents nitric oxide synthase dysfunction induced by nitroglycerin and nitrate tolerance in the arterial circulation. [8] The results point to oxidative stress as the origin of NOS activity decrease.

Interventions to increase NO	
• Increase circulating arginine concentrations	• Restore folate status
• Increase exercise	• Increase phytoestrogens
• Lower ADMA (see below)	

### ***Nitric oxide regulation by ADMA***

Because it elicits a wide array of powerful cellular responses, NO production must be tightly regulated. The small NO molecule permeates all surrounding cellular compartments and rapidly travels into adjacent cells. NO is converted to nitrite in 10 seconds or less, so cellular concentrations are dependent on rates of synthesis by the enzyme nitric oxide synthase (NOS). Therefore, cellular concentrations of NO depend on NOS regulation. The primary factors controlling NOS activity are the concentrations of the substrate, L-arginine, and the NOS inhibitor, ADMA. Enzyme concentrations are also variable; genes controlling the inducible form of NOS (iNOS) are activated by inflammatory cytokines.

When hyperhomocysteinemia is induced in humans by oral methionine loading, the resulting endothelial vasodilator dysfunction is temporally related to, and quantitatively correlated with, elevations in plasma ADMA. [9] These results support and extend the findings that patients with ischemic stroke have significantly higher concentrations of plasma ADMA than controls [10], and that homocysteine impairs the NO synthesis pathway. [11]

ADMA is produced by methylation of specific arginine residues of certain cellular proteins. Most of the proteins that have been found to undergo significant arginine methylation are found in the nucleus. [12] The methylation produces monomethylarginine and two forms of dimethylarginine, depending on whether the methyl groups are on the same nitrogen atom (ADMA) or two different nitrogen atoms in the side chain. In the latter case, the product is symmetrical dimethylarginine (SDMA), which has insignificant inhibitory effects on NOS. When the proteins are degraded, ADMA and the other isomers are released. Tables 2 and 3 illustrate the range of studies that have demonstrated clinically significant associations with ADMA levels.

#### **ADMA significance**

- Better predictor of endothelial impairment than cholesterol
- Mediates adverse responses to homocysteine
- Mediates cardiovascular effects of cigarette smoking
- Highly correlated with serum triglyceride levels
- Better indicator of insulin resistance than any other single marker

### ***Mechanisms of ADMA action and effects on cardiovascular function***

An early observation that we now know is related to ADMA was that atherosclerosis is marked by impaired vasodilation in response to normal physiological stimuli, and the impairment can be relieved by intravenous L-arginine. [13]. A number of standard heart disease risk factors (smoking, hypertension, hyperlipidemias) are related to vasodilatory impairment, but the strongest correlation may be the ratio of arginine to ADMA in plasma [14]. This ratio has been used to assess the balance of stimulatory and inhibitory effects on NO synthesis [15]. Elaboration of endothelium-derived nitric oxide affects the behavior of circulating T lymphocytes and monocytes. Mononuclear cell adhesiveness is inversely correlated with the plasma L-arginine/ADMA ratio, and the effect is reversed by restoration of the ratio to control levels with oral administration of 14–21 grams of arginine. [16] These changes lead to lower rates of atherosclerotic plaque formation and lower risk of heart disease.

There is growing evidence that the well-known connection of elevated homocysteine with cardiovascular disease is mediated by a mechanism involving ADMA [12]. Homocysteine has become recognized as an important risk factor for heart disease. Multiple data sets on homocysteine elevation have been compiled in a meta analysis giving estimates that

lowering average homocysteine concentrations by 3 micromol/L would reduce the risk of ischaemic heart disease by 16%, deep vein thrombosis by 25%, and stroke by 24%. [17] Recent research indicates that the effects of homocysteine on cardiovascular health are mediated by ADMA. [8, 10]

In addition, the association of elevated LDL cholesterol with increased risk of heart disease may be as a covariable in the oxidative activation of ADMA synthesis. Oxidized LDL particles stimulate the expression of enzymes that generate ADMA [18]. These enzymes are discussed further below.

In patients with type two diabetes mellitus, plasma ADMA increases from 1.0 to 2.5 mmol/L five hours after ingestion of a high-fat meal. ADMA may contribute to abnormal blood flow responses and to atherogenesis in type 2 diabetics. [19] In hypertensives, the urinary excretion rate of nitrite plus nitrate (NO<sub>x</sub>), an index of endogenous NO production, was lowered from 77 to 56 micromol/mmol creatinine, while plasma levels of ADMA were increased from 1.1 to 2.4, indicating ADMA inhibition of NO production. [20] ADMA plays an important role in the pathogenesis of hypertension associated with the experimental focal and segmental glomerulosclerosis. [21] Other findings relevant to the role of ADMA in renal disorders are presented in Table 3.

**Table 2. Associations of ADMA with cardiovascular health**

<b>Elevated ADMA is associated with</b>
• Increase in <b>blood pressure</b> during high salt intake. [40]
• Pathophysiology of <b>coronary vasospasm</b> . [41]
• <b>Carotid intima-media thickness</b> in end-stage renal disease. [42]
• <b>Borderline hypertension</b> in young subjects – independent of hypercholesterolemia. [43]
• <b>Endothelium dysfunction</b> as a mediator of homocysteine degenerative effects. [44]
• <b>Hyperhomocysteinemia</b> in patients with <b>ischemic stroke</b> . ADMA levels above the 90 <sup>th</sup> percentile increased risk for stroke by six-fold. [10]
• Increases in endothelin-1 and reductions in insulin-induced increments in plasma NO <sub>x</sub> and cGMP in patients with <b>cardiac syndrome X</b> (angina pectoris and normal coronary arteriograms). All of the effects are reversed by intravenous L-arginine. [45]
• <b>Acute coronary events</b> . For men who do not smoke, those in the highest quartile for ADMA have 4-fold greater risk. [46]
• <b>Essential hypertension</b> , but not post-transplant hypertension. [47]
• <b>Congestive heart failure</b> , via NO-mediated vasodilatation inhibition by endothelin-1. [48]

### **Generation and clearance of ADMA**

The relationship of methylation to the formation of methylated arginines is important for understanding the rates of formation of ADMA. The conversion can be stimulated by methionine as a direct donor of methyl groups via enzymes called protein arginine N-methyltransferases (PRMTs). Many, if not most proteins that contain ADMA are found in the nucleus and they interact with RNA, exerting transcriptional control. [22] PRMT activity is increased by methionine loading. In fact, acute methionine loading in human subjects induces endothelial dysfunction because of increased methylation of L-arginine residues in endothelial proteins, forming ADMA residues. Upon proteolysis, free ADMA is released for down-regulation of NOS. [12]

Once ADMA is released from methylated proteins by proteolysis, the two principal factors controlling levels are renal clearance and hepatic metabolism. Normally, slightly more ADMA is cleared by hepatic metabolism than by renal excretion. The hepatic enzyme dimethylarginine dimethylaminohydrolase (DDAH) is important for clearing ADMA by conversion to citrulline. Over 4 mmol (>1gm)/day of ADMA is extracted by the liver (700 times the amount circulating in blood). [23] Several lines of evidence show that renal clearance is the other primary mechanism for control of ADMA concentrations. ADMA is not excreted in patients with chronic renal failure, and concentrations in plasma are two to six times higher in uremic patients than in healthy control individuals. [24-26] All-trans-retinoic acid increases the expression of DDAH II, the predominant DDAH isoform in endothelial cells, resulting in increased synthesis of NO. [27] On the other hand, nerve damage causes marked increase in the expression of DDAH as part of the mechanism to protect neuronal cells from the effects of excess NO. [28]

Other data suggests that human endothelial cell PRMT activity is up-regulated by LDL cholesterol, due in part to the enhanced gene expression of PRMTs. [18] Thus, we find ADMA intermeshed with both cholesterol and homocysteine cardiovascular risk factors. Hyperglycemia is another factor related to ADMA. Human endothelial cells exposed to high glucose concentrations show impairment of DDAH and accumulation of ADMA that may contribute to endothelial vasodilator dysfunction in diabetes mellitus. [29] Improved glycemic control in patients with type 2 diabetes results in lowering of plasma ADMA levels. Metformin treatment achieved the lowering of ADMA without simultaneous sulfonylurea treatment. [30]

Feedback regulation of NO on DDAH is achieved by nitrosylation of active site residues. This explains why expression of iNOS often leads to inhibition of constitutively expressed NOS isoenzymes. [31] As average nitric oxide concentrations increase, nitrosylation increases and enzyme activities fall. Other lines of evidence indicate that reactive oxygen species are responsible for loss of DDAH activity. For example, the endothelial dysfunction caused by methionine loading can be reversed by administration of ascorbic acid. [32]

Since vascular responses are so strongly dependent on NO production, it is important to assess the interplay of other treatments for hypertension and hypercholesterolemia. Plasma ADMA concentrations are unaffected by statin treatments that lower LDL cholesterol. [33] On the other hand, angiotensin converting enzyme (ACE) inhibitors lower plasma ADMA concentrations from 1.69 to 1.41 umol/L and the effect is independent of blood pressure. [34] Long-term use of ACE inhibitors caused reduced ADMA, increased NO<sub>x</sub>, and increased ratio of L-Arg/ADMA in syndrome X patients, giving improved coronary microvascular function and reduced myocardial ischemia. [35] Estrogen replacement therapy with oral conjugated estrogen lowers plasma ADMA. [36]

Some studies have examined the relative benefits of homocysteine normalization vs NO stimulation. Peripheral arterial occlusive disease patients with hypercholesterolemia and hyperhomocysteinemia showed no improvement in endothelium-dependent vasodilation with combined B vitamin therapy designed to lower homocysteine (folate, 10mg; vitamin B<sub>12</sub>, 200mcg; vitamin B<sub>6</sub>, 20 mg/day). Oral L-arginine (24 g/d) significantly improved endothelial function in these patients. [37] The NO stimulatory affect of arginine supplementation in this study was more beneficial for this group of patients than was homocysteine lowering. Thus, under hyperhomocysteinemic conditions, the controlling factor for endothelial functions is accumulation of ADMA that is relieved by arginine supplementation.

**Interventions to lower ADMA**

- Increase exercise
- Reduce oxidant stress
- Increase antioxidant levels
- Normalize folic acid, vitamin B<sub>12</sub>, and vitamin B<sub>6</sub>

There are multiple dietary and nutrient-dependent steps involved in the control of ADMA synthesis and clearance. L-Arginine stimulates NO synthesis to overcome the inhibitory effects of high ADMA. Low fat meals help to reduce the synthesis of ADMA by PRMT. Vitamin A induces expression of DDAH, causing a lowering of ADMA. Antioxidants prevent the inhibition of DDAH, thus enhancing the removal of ADMA. Normalization of elevated homocysteine with vitamins B<sub>6</sub>, B<sub>12</sub>, and folic acid reduces ADMA by controlling endothelial oxidative effects. Folic acid supplementation alone has been shown to lower both ADMA and arginine in plasma of hyperhomocysteinemic individuals. [38] In addition, individuals with persistent ADMA elevations may be managed with hormonal interventions such as ACE inhibitors and natural estrogen replacement therapies. Exercise has a strong positive effect on the arginine/ADMA ratio. Pain-free walking distance is linearly correlated with lower ADMA levels in atherosclerotic patients. [39]

**Table 3. Other clinical associations of ADMA**

Elevated ADMA is associated with:
• <b>Multiple organ failure</b> in intensive care units. [49]
• Regulation of <b>maternal and fetal circulation</b> . [50]
• Risk factor of <b>ICU mortality</b> . [51]
• <b>Hyperthyroidism</b> (ADMA increases with TSH). [52]
• <b>Incipient primary chronic renal disease</b> . [53]
• Insulin resistance and <b>metabolic syndrome</b> . [54]
• Low serum folic acid and <b>high serum homocysteine</b> . [55]
• The pathophysiology of <b>erectile dysfunction</b> . [56]
• <b>Overall mortality</b> and cardiovascular outcome in hemodialysis patients. Thus, ADMA is an important risk factor for cardiovascular disease in chronic renal failure. [57]
• <b>Shorter survival</b> and higher rate of incident cardiovascular complications in end-stage renal disease. [58]
• A 3.9-fold higher risk of <b>acute coronary events</b> compared with lower quartiles. [46]
• <b>Type I diabetes</b> and hypercholesterolemia. Pravastatin had no effect on the levels of ADMA in hypercholesterolemic men. [59]
• The pathophysiology of insulin resistance syndrome and <b>non-insulin-dependent diabetes mellitus</b> . [60]
• Normoglycemic women with previous <b>gestational diabetes</b> . The ADMA increase is independent of other risk factors or surrogate markers for diabetes or cardiovascular events. [61]
• <b>Renal insufficiency</b> that causes reduction of ADMA clearance to less than 10% of controls. [62]
• <b>Nerve injury</b> , where ADMA in neurons profoundly modulates NOS function and suppress NO-mediated injury. [28]

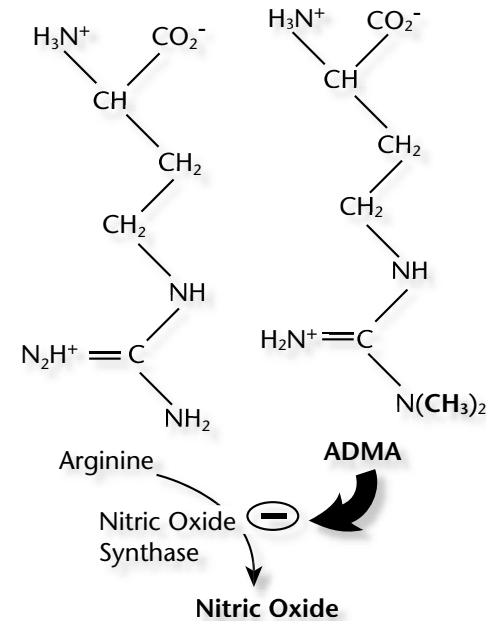
### **Laboratory methodology:**

ADMA has been assayed using high pressure liquid chromatography. [61] This method is time consuming and not suitable for clinical laboratory throughput. A simple, sensitive and fast HPLC – tandem mass spectrometric method was developed with limits of detection of 1 ng/ml. [62] Refinements in this method are making measurement of ADMA more routine.

### Summary and conclusions:

The origins of cardiovascular disease are clarified by knowledge of the mechanism of formation and action of ADMA. Endothelial function depends on finely tuned regulation of NO production. That regulation revolves around two processes. The first is the rate of formation of NO by isoforms of NOS. The therapeutic use of nitroglycerin and the use of supplemental L-arginine are ways of direct stimulation of increased NO production. Increased intake of folic acid protects NOS activity. Finally, exercise stimulates NOS formation, providing the link to another long-recognized lifestyle modification known to improve cardiovascular health.

The second controlling process is the rate of formation of the NOS inhibitor, ADMA. The enzyme that forms ADMA is stimulated by high dietary fat and by high plasma methionine concentrations. Thus, PRMT1 activity is modifiable by dietary factors. DDAH, the enzyme that degrades ADMA is susceptible to oxidative damage. Consequently, antioxidant protection conserves DDAH function and lowers ADMA. Oxidant challenge encompasses factors such as cigarette smoking, exercise-induced myocardial infarction, and oxidized cholesterol (the main link associating total cholesterol). Antioxidant intervention studies include those using purified vitamin E, vitamin C, and dietary antioxidant-rich foods.



*Arginine and ADMA in nitric oxide synthesis*

ADMA levels may be reduced by bringing inflammatory processes under control, by increasing antioxidant protection, and by assuring adequate functional status of nutrients that are required for the control of inducing factors such as homocysteine and metabolic syndrome. Macronutrient intake also affects ADMA, especially high fat meals that acutely elevate ADMA levels. Exercise has strong positive effects on ADMA, lowering the plasma arginine to ADMA ratio. Plasma or serum ADMA measurements can be used to monitor the net effect of these multiple health-promoting factors. Significant reduction of morbidity and mortality may be achieved by reducing ADMA in patients with high levels of this inhibitor of cellular nitric oxide production.

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