Asymmetric Dimethylarginine: A Cardiovascular Risk Factor and a Uremic Toxin Coming of Age?

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● The idea that asymmetric dimethylarginine (ADMA) accumulation may be a cardiovascular risk factor in patients with end-stage renal disease was advanced by Vallance in 1992. During the last decade, the relationship between ADMA and adverse cardiovascular events, including death, in dialysis patients has been investigated thoroughly. Several studies have shown that, independently of other risk factors, ADMA is strongly associated with intima-media thickness of the carotid artery and left ventricular mass, particularly concentric left ventricular hypertrophy. Furthermore, cohort studies in both the general population and the dialysis population showed a strong and independent link between ADMA, all-cause mortality, and cardiovascular events. Circumstantial evidence indicates that norepinephrine and ADMA may be in the same causal pathway leading to cardiovascular complications in patients with end-stage renal disease. Several lines of evidence show that high ADMA levels may exert toxic effects in various cell types. High ADMA levels have been associated with alterations in the regulation of cerebral blood flow and neural function, with insulin resistance, thyroid dysfunction, and alterations in bone homeostasis, fertility, and erectile function. The clinical significance of decreasing plasma ADMA concentrations, if any, is unknown. Well-designed and carefully conducted studies are needed to further clarify the role of ADMA in the pathophysiological states of renal disease and explore possible treatment options to improve the prognosis of patients with elevated ADMA levels. ADMA may enable us to predict risk and follow up the course of renal diseases. *Am J Kidney Dis* 46:186-202.

INDEX WORDS: Asymmetric dimethylarginine (ADMA); nitric oxide; end-stage renal disease (ESRD); uremic toxin; chronic kidney disease (CKD); cardiovascular risk; uremia; hypertension.

ASYMMETRIC dimethylarginine (ADMA) is a naturally occurring amino acid known to biochemists for decades.[1] However, the medical community started to gain interest in this substance as late as 1992 when Vallance et al[2] published their landmark report on the elevation in dimethylarginine levels in patients with end-stage renal disease (ESRD). They speculated that the accumulation of ADMA, leading to impaired nitric oxide (NO) synthesis, might contribute to the hypertension and immune dysfunction associated with chronic renal failure. As envisioned by Vallance et al,[2] chronic kidney disease now has emerged as the clinical situation that best epitomizes the potential importance of this substance in human diseases. The wide range of actions of this compound unraveled by studies performed during the last 10 years suggests that it may be involved in several alterations of the uremic state. It therefore is now formally listed as one of the "uremic toxins."[3]

To date, 350 publications on ADMA have been published. These studies form a fairly coherent framework indicating that ADMA is not only a uremic toxin, but also a strong marker of endothelial dysfunction and atherosclerosis and a solid predictor of mortality in selected patient populations.[4-6] Notably, this substance is considered a common pathway mediating the adverse vascular effects of traditional and nontraditional risk factors.[7]

This review summarizes the biological characteristics of ADMA, elucidates its role in cardiovascular physiological and pathophysiological states, and collates available evidence describing the interference of this compound with various

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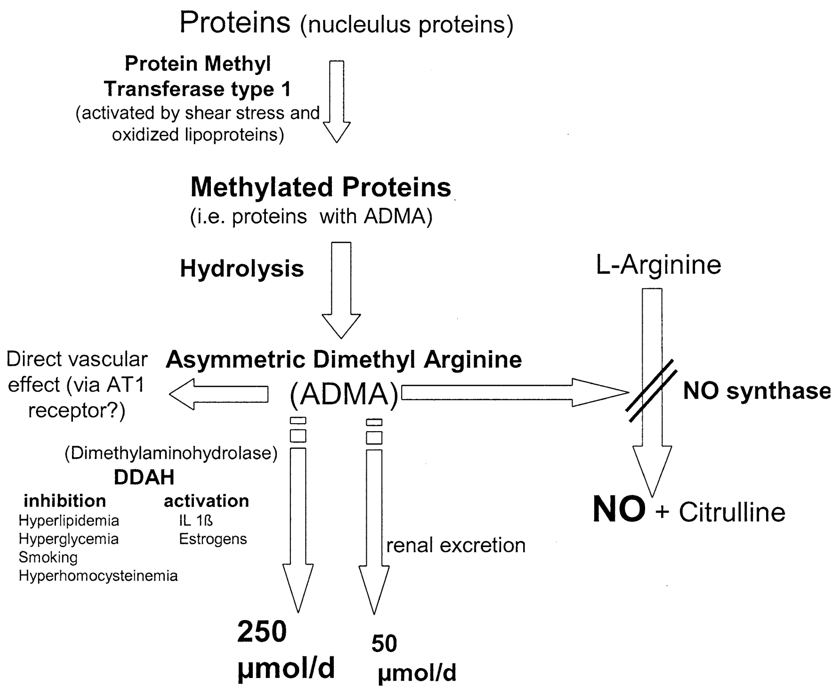

Fig 1. Simplified schema describing ADMA main effects and metabolism (see text).

functional systems, particularly those that are perturbed in patients with chronic kidney disease. We conclude with a discussion on intervention strategies that may test whether ADMA modification may lead to better outcomes in patients with chronic kidney disease and other diseases associated with increased plasma ADMA levels.

BIOCHEMISTRY AND CELL PHYSIOLOGY

NO is the most potent endogenous vasodilator. It has a critical role in inhibiting such key processes of atherosclerosis as monocyte adhesion, platelet aggregation, and vascular smooth muscle cell proliferation. Hence, endothelial dysfunction as a result of reduced NO activity is an early step in the course of atherosclerotic vascular disease, and evidence has accumulated that inhibition of NO synthesis by endogenous substances may be causally involved in this process.[7] A family of NO synthases (NOSs) with endothelial, neuronal, and macrophage isoforms converts L-arginine to NO and citrulline by means of stereospecific oxidation of the terminal guanidino nitrogen of the amino acid L-arginine.[8] This process can be inhibited selectively by competitive blockade of the NOS active site with such naturally occurring guanidino-substituted analogues of L-arginine as N^G-monomethyl-L-arginine (L-NMMA) and ADMA (Fig 1).[9] Because the blood concentration of the latter is approximately 10-fold greater than that of L-NMMA, it is considered the predominant endogenous NOS inhibitor, which most studies have focused on.

Generation of ADMA

Humans generate approximately 300 μmol (~60 mg) of ADMA per day.[10] Although an analogue of L-arginine, to date, no direct route of synthesizing ADMA from the free amino acid has been identified. Instead, a rather complex process leads to the generation of ADMA. It is released from proteins that have been posttranslationally methylated and subsequently hydrolyzed. These proteins are found largely in the nucleolus and appear to be involved in RNA processing and transcriptional control.[11] Protein-arginine methyltransferases (PRMTs) catalyze the formation of methylarginine residues. Of the 7 types of PRMTs described, only type I PRMT forms ADMA and L-NMMA residues; type II

PRMT and type VII PRMT form symmetrical dimethylarginine (SDMA), the stereoisomer of ADMA, which has no (direct) effect on NOS activity.[12,13] A number of cell types, including human endothelial cells, elaborate ADMA.[14] To date, it is unclear whether ADMA generation is constant or increased PRMT activity and/or increased protein turnover might contribute to elevated ADMA levels. The latter explanation seems to be possible given the increased protein catabolic rate of patients with ESRD, who have very high ADMA levels. To date, we know that PRMT activity is influenced by oxidized lipoproteins in vitro,[15] and PRMT I expression in endothelial cells and thereby ADMA level increases in response to shear stress.[16] Therefore, (local) ADMA generation may be regulated in part through alterations in PRMT I.

Elimination of ADMA: The Double Function of the Kidney

Because ADMA levels are elevated in patients with ESRD, renal excretion of ADMA was considered to be the main route of elimination.[2] However, an early study from McDermott[17] in rabbits showed that renal excretion of ADMA could not be the major route of elimination. From these studies, it was deducted that a catabolic pathway had to be present. This major metabolic pathway was found to be degradation by the enzyme dimethylarginine dimethylaminohydrolase (DDAH), first isolated from the rat kidney, which hydrolyzes ADMA to dimethylamine and L-citrulline (Fig 1).[18] To date, 2 isoforms of DDAH have been characterized and cloned: DDAH I is found in tissues that express neuronal NOS, whereas DDAH II is found in tissues that express endothelial NOS.[19] DDAH I is encoded by genes on chromosome 1, and DDAH 2, by genes on chromosome 6.[20] Colocalization of DDAH and NOS in various cell types, including renal tubular cells, supports the hypothesis that the intracellular concentration of ADMA is actively and cell-specifically regulated in NO-generating cells.[21] DDAH I and II have emerged as key enzymes regulating cellular and tissue ADMA levels because only 50 μmol/d of ADMA is excreted in urine, but approximately 250 μmol/d of ADMA is eliminated through degradation of DDAH.[10] This is in line with clinical data showing that patients with incipient renal disease and normal renal excretory function, documented by measurement of true glomerular filtration rate with inulin clearance, already have markedly elevated plasma ADMA levels.[22] This possibly could be a result of decreased activity of DDAH I, which is highly expressed in brain, kidney, pancreas, and liver.[19] A study of humans showed greater renal extraction of ADMA compared with SDMA.[23] Thus, the kidney has a predominant role in ADMA elimination by combining 2 mechanisms; urinary excretion and metabolization of ADMA.

DDAH: The Key Mechanism in Regulating Plasma ADMA Levels

The central role of DDAH in regulating plasma ADMA levels was shown by using a DDAH inhibitor. Pharmacological inhibition of DDAH activity with *S*-2-amino-4(3-methylguanidino) butanoic acid causes ADMA to accumulate and thereby induces dose-dependent vasoconstriction of isolated vascular rings in vitro that could be reversed by the addition of L-arginine.[14] Degradation of ADMA by DDAH probably involves a nucleophilic attack on the guanidino portion of the molecule by a cysteine held in an activated state in the tertiary structure of the enzyme.[24] Many factors, such as oxidized low-density lipoprotein cholesterol, inflammatory cytokines, hyperhomocysteinemia, hyperglycemia, infectious agents, and high doses of erythropoietin, have been shown to attenuate DDAH activity, allowing ADMA to accumulate and block NO synthesis.[25-30]

Recently, it was shown that recombinant, as well as mammalian, DDAH is reversibly inhibited after incubation with NO donors in vitro. Hence, under certain conditions, when NO generation increases, DDAH activity decreases, leading to ADMA accumulation and NOS inhibition.[31] This may serve as an important feedback mechanism to inhibit overwhelming NO synthesis. Compelling evidence for the critical role of DDAH activity in regulating NO synthesis in vivo was shown in a transgenic DDAH mouse. The increased DDAH activity in these animals led to a decrease in ADMA levels by more than 50%, which, in turn, increased NOS activity and thereby decreased blood pressure.[32] Leiper et al[31] found that DDAH activity decreased in rats treated with endotoxin to induce inducible NO synthase, suggesting that the phenomenon also occurs in vivo. However, the hypothesis that ADMA may serve as an endogenous suppressor of NO synthesis in the situation of massive NO production is not in line with the observation that ADMA levels decrease in a rat model of endotoxinemia.[33] Intravenous administration of *Escherichia coli* endotoxin in humans did not increase ADMA levels[34]; however, septic patients, who frequently experience acute renal failure, have not been studied longitudinally concerning their ADMA levels. Teleological reasoning would suggest that in the specific situation of sepsis, the defensive properties (eg, bacterial killing) of NO outweigh the risk posed by excessive nitro-oxidative stress.

Pathophysiological Importance of ADMA

Data from several experimental studies suggest that ADMA concentrations in a pathophysiologically high range (ie, between 2 and 10 μmol/L) significantly inhibit vascular NO production.[35-38] Furthermore, ADMA competes (to a lesser degree than SDMA) for L-arginine transport mediated by human cationic amino acid transporter-2B into cells, resulting in L-arginine depletion.[39] However, pharmacological ADMA concentrations are necessary to exert this effect in vitro.[40] The broad range of plasma ADMA levels in pathophysiological situations (ie, between 2 and 10 μmol/L) is one reason why the possible role of ADMA in cardiovascular disease is still discussed controversially.[41,42] Until now, we relied on systemic ADMA concentrations, which represent a "spillover" from cells into the blood. ADMA concentrations in the cell and intracellular microdomains in physiological and pathophysiological conditions remain unknown. Thus, we have no solid ground to estimate the biological implications of plasma ADMA levels in various pathophysiological conditions in relationship to levels actually required to inhibit NO synthesis in vitro. The matter is complicated further because ADMA levels regarded as normal or of pathophysiological relevance differ up to an order of magnitude between laboratories. This is attributable in part to different analytical methods.[2,43-54] The main reason to doubt the pathophysiological role of ADMA was the limited evidence from in vivo experiments.

Plasma concentration of the NO precursor L-arginine should be taken into account when interpreting effects of high plasma ADMA levels because, at similar plasma ADMA concentrations, NO synthesis is expected to be less inhibited in the presence of relatively greater L-arginine concentrations. Thus, the L-arginine/ADMA ratio represents a useful index for interpretation of effects of ADMA.

In Vivo Evidence for the Pathophysiological Role of ADMA

To date, only 6 animal studies on the effect of ADMA in vivo exist. Vallance et al[2] described the effect of an ADMA infusion (3 mg/kg/h) in guinea pigs that led to a 15% increase in systolic blood pressure. A bolus of ADMA (3 to 30 mg/kg) caused a dose-dependent increase in mean arterial blood pressure up to 53 mm Hg.[2] These dose-dependent pressor and bradycardic effects of ADMA (1 to 100 mg/kg) were confirmed later in rats.[55] Topical application of 10 and 100 μmol of ADMA through cranial windows in anesthetized rats constricted the basilar artery by 9% and 19%, respectively. In contrast to intravenous injection of ADMA, which caused dose-dependent increases in mean arterial blood pressure, intracerebroventricular injection of ADMA decreased mean arterial blood pressure and heart rate (–39 mm Hg and –50 beats/min, respectively).[56] Investigating the diameter of rat mesenteric arterioles by means of intravital microscopy, ADMA (100 μmol) caused a maximal constriction to 68% of baseline diameter and a 44% decrease in blood flow.[57] A recent study on the effect of long-term (ie, 4 weeks) ADMA infusion in mice showed that it causes significant coronary microvascular lesions.[58] As repeatedly shown for the synthetic NOS inhibitor N^G-nitro L-arginine methyl ester, these long-term vascular effects of ADMA were not mediated solely by inhibition of endothelial NO synthesis, but presumably also by direct upregulation of angiotensin-converting enzyme (ACE), and increased oxidative stress through angiotensin 1 receptor also appears to be involved in this process.

ADMA in Cardiovascular and Renal Physiology in Humans: ADMA and Salt Sensitivity

Part of the pioneering report by Vallance et al[2] was a small uncontrolled observation on the effect of intra-arterial ADMA infusion on the forearm arteriolar bed of healthy volunteers. Intra-arterial application of 8 μmol of ADMA into the brachial artery caused a decrease in forearm blood flow by 28%.[2] Controlled trials examining effects of ADMA on different vascular beds at pathophysiological levels in humans have not been reported for a long time. The first study on the effect of systemic ADMA infusion on renal perfusion with low doses of ADMA (ie, 0.5 and 1.0 mg/kg) that were based on animal data did not show an effect,[59] although the postglomerular renal (micro) vasculature is particularly sensitive to NOS inhibition and can be assessed easily with accurate invasive clearance techniques.[60-62] Conversely, a dose-response study with greater doses of ADMA showed significant effects of ADMA on renal circulation.[63] Acute increases in plasma ADMA levels within the pathophysiologically relevant range, ie, between 2 and 10 μmol/L, were achieved with an ADMA dose of 0.5 and 1.0 mg/kg. With these ADMA doses, a significant decrease in plasma cyclic guanosine monophosphate concentrations was observed. However, these changes may not be related directly to NO production. A significant effect on effective renal plasma flow was documented with infusion of an ADMA dose of 3 mg/kg and greater, whereas glomerular filtration rate remained unaffected.[63] Infusion of ADMA to 7 healthy subjects caused a significant and sustained (lasting 2 hours after the end of infusion) 14% decrease in cardiac output, a significant 11% increase in systemic vascular resistance, and a decrease in heart rate from 58 $\pm$ 7 to 54 $\pm$ 6 beats/min.[63] Mean plasma ADMA concentration increased from 0.95 (baseline) to 22.95 μmol/L at the end of the infusion and was 5.31 μmol/L 2 hours after the end of the infusion, ie, within a pathophysiologically relevant range.[63]

These data are in accordance with data obtained with noninvasive techniques, ie, bioimpedance cardiography.[10] In this study, a bolus of ADMA (3 mg/kg) decreased heart rate by 9% and cardiac output by 15%. ADMA also increased mean blood pressure by 6% and systemic vascular resistance by 24%. Interestingly, in this study, the effect of ADMA on heart rate and cardiac output was not detectable 1 hour after the bolus, in contrast to the study mentioned previously.[10] In a second part of that study, it was shown that handgrip exercise increased cardiac output in control subjects by 96%, but in subjects administered ADMA, cardiac output increased by only 35%. ADMA level 30 minutes after the infusion was 2.6 μmol/L; however, the baseline value was not provided.

In a very recent study, it was documented that acute systemic administration of a suppressor dose of the (endogenous) NOS inhibitor ADMA to healthy subjects decreased NO generation, renal perfusion, and sodium excretion without affecting the renin-angiotensin system and sympathetic activity.[64] The absence of changes in blood pressure and cardiovascular hormone levels points to a direct effect of ADMA on renal function. Renal effects of ADMA may be of clinical importance because in salt-sensitive hypertensive patients, salt loading increases plasma concentrations of ADMA, an effect strictly associated with salt-induced suppression of NO plasma concentration.[65] These results are coherent with findings in Dahl rats, ie, an animal model of salt-sensitive hypertension, in which high salt intake increases the ADMA production, blunts NO synthesis, and increases blood pressure.[66] Salt sensitivity is an almost universal phenomenon in chronic renal insufficiency. Thus, ADMA accumulation in this disease may be a factor limiting the salt-excretory potential of the failing kidney.

Collectively, these results document that ADMA has well-defined effects on cardiovascular and renal function in healthy subjects and hypertensive patients. It therefore is conceivable that ADMA causes sustained changes in vascular function through an intracellular action in endothelial cells at blood concentrations found in patients with cardiovascular pathological states.

ADMA as a Cardiovascular Risk Factor in Patients With ESRD

Since the seminal study by Lindner et al,[67] who first noted the excessive cardiovascular morbidity and mortality caused by complications of premature atherosclerosis in patients with ESRD, this problem remains unresolved. Depending on age, patients on renal replacement therapy encounter a 5- to 500-fold risk for dying of cardiovascular events. It was shown that NO production in this patient population was significantly decreased,[68,69] and it appears most likely that this phenomenon depends on accumulation of ADMA, which had been described repeatedly. Hence, the well-documented endothelial dysfunction in patients with ESRD[70-74] could be the consequence of an increased plasma ADMA concentration, a hypothesis fully supported by a cohort study showing a strong and independent link between ADMA, mortality, and cardiovascular events in patients with ESRD.[6]

As noted, under normal conditions, the endothelium continuously generates NO, thus maintaining the circulatory system in a state of active vasodilatation. NO has a protective role for the cardiovascular system because it not only modulates arterial compliance and peripheral vascular resistances, but also inhibits vascular muscle cell proliferation, platelet aggregability, and adhesion of monocytes to the endothelium, all processes that trigger atherosclerosis. When NO production is decreased, atherosclerosis ensues.[7]

One of the major factors that regulate NO synthesis is the parasympathetic mediator acetylcholine.[8] There is a delicate balance between the parasympathetic and sympathetic systems in which sympathetic overdrive suppresses parasympathetic tone, and vice versa. Thus, sympathetic overactivity also may induce endothelial damage by tilting the autonomic balance in such a way that acetylcholine production is inhibited and NO synthesis is decreased.[75] NO is generated in all cellular species of the cardiovascular system, including myocardiocytes, in which it modulates cell growth. For this reason, lack of NO may induce left ventricular hypertrophy by direct (cell hypertrophy) and indirect mechanisms (left ventricular adaptation to arterial rigidity and hypertension).

Observations by Kielstein et al[76] in 1999 refreshed the interest in the potential role of ADMA in accelerated atherosclerosis in patients with ESRD. This cross-sectional study was the first to document that ADMA levels are greater in dialysis patients with than without cardiovascular complications. These observations were followed by a series of studies that thoroughly reexamined the problem by analyzing the relationship between ADMA and several solid outcome measures; namely, carotid intima-media thickness, left ventricular mass and geometry, and mortality and incident cardiovascular events. ADMA appeared to be associated strongly with intima-media thickness in patients with ESRD,[77] and this link was independent of other cardiovascular risk factors because the association was unmodified after appropriate adjustment for other predictors of intima-media thickness, such as plasma homocysteine level and age ($\beta = 0.24$; $P = 0.01$). Furthermore, a prospective cohort study showed that ADMA level also predicted progression of intimal lesions, an association again independent of other risk factors.[77] Likewise ADMA level is associated strongly with intima-media thickness in patients with mild to moderate chronic renal insufficiency (Prabath W.B. Nanayakkara; personal communication; November 2004), implying a role of ADMA in atherosclerosis well beyond the end-stage phase in patients with renal diseases. These observations are in line with data showing a similarly strong independent link between ADMA level and intima-media thickness in apparently healthy individuals in the general population.[78]

A convincing link between endothelial function and vascular hypertrophy is shown in hypertensive humans because in these patients, left ventricular mass is related inversely to forearm blood flow response to the endothelium-dependent vasodilating agent acetylcholine; the endothelial dysfunction is particularly pronounced in hypertensive patients with concentric hypertrophy.[79] The heart and arterial system form an integrated unit that coherently responds to hemodynamic stimuli, and the endothelium has a pivotal role in regulating cardiovascular remodeling. Accordingly, in physiological and disease states, such as in uremic patients, cardiac and arterial remodeling proceed in parallel.[80,81]

The relationship between endothelial dysfunction and cardiovascular remodeling in patients with ESRD has been tested in only 1 study.[82] In this study, the reactive hyperemic response of forearm blood flow (an outcome measure strongly influenced by endothelial function) was related to common carotid intima-media thickness and left ventricular mass, suggesting that endothelial dysfunction may promote structural changes in the cardiovascular system in dialysis patients. ADMA is perhaps the most likely culprit of these alterations because there is an independent strong association between this substance and left ventricular mass.[83] Of note, ADMA levels are much

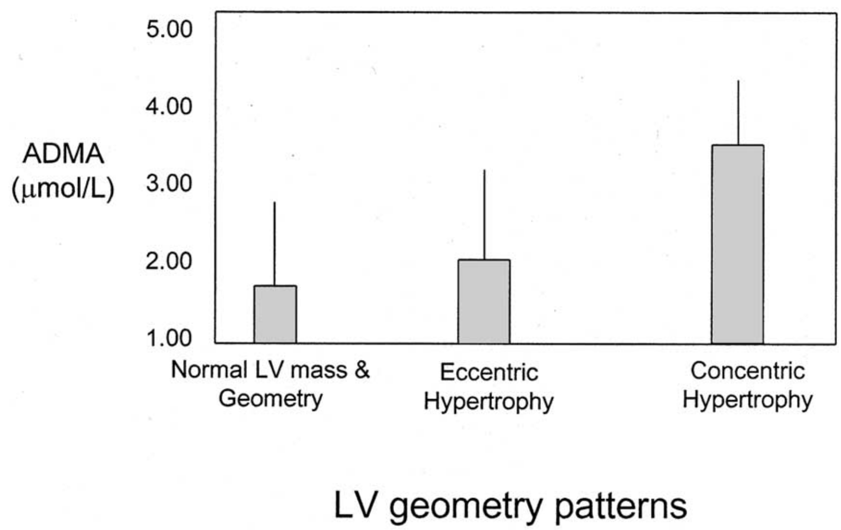

Fig 2. Plasma ADMA levels according to left ventricular (LV) geometry patterns in patients with ESRD. Redrawn from data reported in Zoccali et al.[83]

greater in patients with concentric left ventricular hypertrophy than in those with eccentric left ventricular hypertrophy or normal left ventricular mass (Fig 2). The strong link between ADMA level and concentric left ventricular hypertrophy may underlie a causal relationship because concentric left ventricular hypertrophy occurs in experimental models of chronic NO inhibition[84] and severe concentric hypertrophy and vascular damage is a hallmark in the knockout mice lacking the NOS and ApoE genes.[85] As discussed, these observations are also in accordance with the strong association between endothelial dysfunction and concentric left ventricular hypertrophy in patients with essential hypertension.[79] Thus, ADMA may be an important factor in a pathophysiological process that may result in severe clinical sequels ranging from heart failure to coronary and cerebrovascular complications.

As anticipated, this hypothesis was supported by a cohort study of 225 patients with ESRD. In this study, median plasma ADMA concentration was 3 times greater in dialysis patients than in healthy subjects,[6] and survival analysis by means of the Cox regression model adjusting for a series of potential confounders showed that mortality and cardiovascular events rates were much greater in patients in ADMA level quartile 4 than in those in other quartiles and a dose-response relationship exists between ADMA plasma concentration and these outcomes (Fig 3).

As mentioned, acetylcholine is a potent stimulator of NO synthesis. By inhibiting acetylcholine release, high sympathetic activity may reduce NO synthesis and thereby lead to endothelial dysfunction. The NO–sympathetic system link appears clinically relevant because in patients with ESRD, not only ADMA, but also norepinephrine, is associated strongly with incident cardiovascular events.[86] ADMA level is associated strongly with plasma norepinephrine level in patients with ESRD, and this association is largely independent of arterial

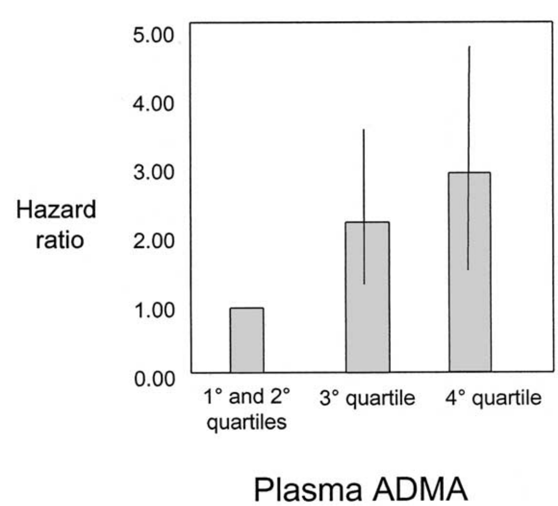

Fig 3. Hazard ratio for fatal and nonfatal cardiovascular events by plasma ADMA concentration. Drawn from data reported in Zoccali et al.[6]

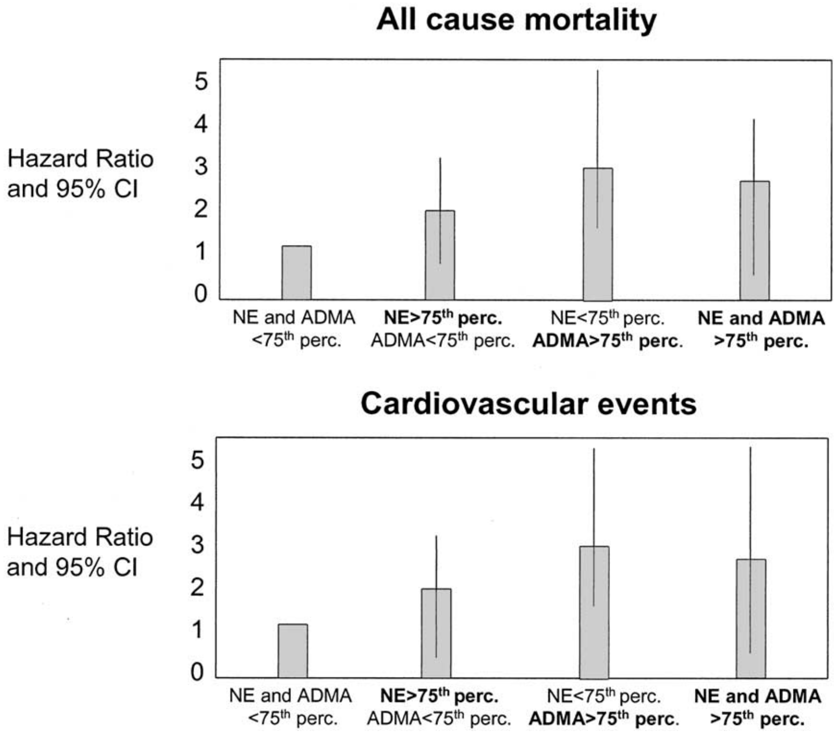

Fig 4. Risk for all-cause and cardiovascular mortality in patients stratified according to plasma norepinephrine (NE; < or > 75th percentile) and plasma ADMA (< or > 75th percentile) levels in patients with ESRD. The risk associated with high plasma ADMA levels is superior to that associated with elevated plasma NE levels, but there is no further risk increase in those who show both high ADMA and high NE levels. This phenomenon suggests that the 2 risk factors are in the same causal pathway leading to adverse outcomes. Redrawn from data reported in Mallamaci et al.[87]

pressure and other risk factors. Of note, detailed statistical analysis of the relationship between ADMA and norepinephrine levels and cardiovascular outcomes suggests that norepinephrine and ADMA are in the same causal pathway leading to the high rate of cardiovascular events in patients with ESRD[87] (Fig 4). However strong, the link between ADMA, cardiovascular damage, and incident cardiovascular complications may not be causal. Causality demands that ADMA is biologically active in humans at plasma concentrations found in patients with ESRD and other pathophysiological situations. Furthermore, ADMA-related cardiovascular complications should be prevented by interventions aimed at reducing ADMA concentrations. However, confusion still abounds regarding issues of association and causation. We still do not have intervention studies based on ADMA modification to support this hypothesis. Nonetheless, previously discussed studies by Achan et al[10] and Kielstein et al[63] shed interesting light on this potentially causal relationship. They convincingly showed in vivo in humans that ADMA is biologically active when administered at doses adequate to bring plasma concentrations up to pathophysiological levels.

Minor Renal Dysfunction

Even minor renal dysfunction was recognized lately as a cardiovascular risk factor.[88] Interestingly, several recent studies found markedly elevated plasma ADMA levels not only in patients with ESRD, but also in patients with progressive chronic kidney disease and even those with incipient renal disease and normal renal function.[22,69,89] Accumulation of ADMA could be a cause for impaired acetylcholine-induced endothelium-dependent vasorelaxation in patients with incipient renal disease (such as adult polycystic kidney disease) and normal renal function.[90] These findings are of particular relevance because the hemodynamic response to acetylcholine is associated strongly with renal function in patients with untreated uncomplicated essential hypertension,[91] and the response to acetylcholine also is related strongly to plasma ADMA levels in these patients.[92]

ADMA and Aging

In humans, as in many species, a consequence of aging includes deterioration in renal function, even without primary renal disease. Past and more recent studies documented that aging changes renal hemodynamics, particularly by an increase in renovascular tone with reduced ability of postglomerular vessels to dilate in response to such stimuli as acetylcholine or amino acids.[93-96] Furthermore, in senescent individuals, reduced availability of NO is thought to be linked to the increase in blood pressure and renovascular resistance, possibly a reflection of arteriosclerosis.[97,98] In comparison to young healthy adults, ADMA concentrations are markedly increased in normotensive elderly people and particularly in hypertensive elderly people.[99] The significant relationship between high ADMA blood levels, reduced renal perfusion, and high blood pressure values is compatible with the notion that accumulation of this NOS inhibitor in senescent individuals is involved in the decrease in renal perfusion and increase in blood pressure. Recent studies indicated that ADMA may have far-reaching effects on the senescence process because it shortens telomere length in vitro by activating telomerase.[100]

ADMA as a Full-Scale Toxin: Potential Implication in Dementia, Endocrine Alterations, Bone Disease, and Sexual Function

The pathophysiological relevance of ADMA goes beyond its interference with cardiovascular function. There is experimental evidence that ADMA may be implicated in dementia, endocrine alterations, osteoporosis, and fertility, ie, in alterations typically found in patients with advanced renal insufficiency.

NO has a prominent role in the regulation of cerebral blood flow and modulation of cell-to-cell communication in the brain.[101] In addition to regulation of cerebral blood flow, NO has an important role in numerous physiological neuronal functions, such as liberation of neurotransmitters in vitro[102] and in vivo.[103] NO can prevent or induce neuronal apoptosis, depending on its concentration and cellular redox state. Generally speaking, NO is considered a neuroprotective compound because it increases the expression of such cytoprotective genes as *HSP70, heme oxygenase*, and *Bcl-2*. Moreover, NO (through the cyclic guanosine monophosphate pathway) activates the antiapoptotic serine/threonine kinase Akt by protein kinase G–dependent activation of phosphatidylinositol 3-kinase.[104] However, a high concentration of NO and peroxynitrite, which results from NO and superoxide anion reaction, can promote apoptotic pathways in neuronal cells through the indirect activation of caspases. As a modulator of NO synthesis, ADMA is a likely player in the regulation of neural function. ADMA levels are much increased and NO levels are decreased in plasma of patients with Alzheimer disease.[105] These data suggest that inhibition of endothelial NO synthesis by ADMA may impair neural function and cerebral blood flow, thus contributing to the development of cerebrovascular disease, including Alzheimer disease. It is well known that patients with advanced renal diseases often experience an accelerated decline in cognitive function.[106] Thus, the potential interference of ADMA with neuroprotective mechanisms and the regulation of cerebral blood flow suggest that accumulation of this substance alters cognitive processes in patients with ESRD. ADMA also may contribute to the increased risk for stroke, which is approximately 6 times greater in patients with ESRD than in the general population.[107] An association between cerebrospinal fluid levels of ADMA and cerebral vasospasm in a primate model of subarachnoid hemorrhage has been shown.[108]

As anticipated, ADMA interferes with the action of several hormones and is influenced in turn by perturbations in endocrine gland function. In rats with streptozotocin-induced diabetes, ADMA is closely related to metabolic control of the disease.[109] In nondiabetic normotensive subjects, plasma ADMA concentration correlated positively with impairment of insulin-mediated glucose disposal.[28] Interestingly, this substance also is associated strongly with some risk factors that compound the cluster identifying metabolic syndrome, such as fasting triglyceride levels, but not with other risk factors (such as low-density lipoprotein cholesterol levels). Pharmacological treatment with rosiglitazone (a peroxisome proliferator-activiated receptor gamma agonist that ameliorates insulin sensitivity) reduces plasma ADMA concentrations,[28] an effect suggesting that the relationship between insulin sensitivity and ADMA underlies a causal mechanism. This view also is supported because in morbid obesity, a paradigmatic situation of insulin resistance, weight loss after gastroplastic surgery induces a substantial decrease in plasma ADMA levels.[110]

Thyroid function is regulated by the NO system, and ADMA is produced within thyrocytes.[111] ADMA and L-arginine levels are deranged in patients with thyroid disease.[112] Patients with chronic kidney disease often present the typical findings of the "euthyroid sick syndrome." Thyroid function therefore appears to be another interesting area in which ADMA deserves attention, particularly in patients with ESRD.

Osteoporosis and cardiovascular disease generally are considered disparate entities and unrelenting consequences of the aging process. However, endothelial dysfunction and impaired NO expression may represent a shared mechanism leading to these apparently disparate diseases. Endothelial NOS is the most expressed isoform in bone cells, and NO is a fundamental regulator of bone formation because it transduces mechanosensitive signals within the principal cellular species composing the bone system, including osteoblasts, osteocytes, and bone-lining cells.[113] Bone mineral density is decreased in aged animals and elderly humans, and treatment with NO donors prevents bone loss.[114] In rat plasma, ADMA and bone mineral density are inversely associated.[115] ADMA inhibits osteoblastic differentiation in mouse bone marrow–derived mesenchymal stem cells, documented by a dose-dependent decrease in nitrite formation causing a parallel decrease in alkaline phosphatase activity, calcium deposition, and osteoblast-related gene expression.[116] Importantly, concurrent treatment with L-arginine reverses the ADMA-mediated decrease in NO production and restores the differentiation potential of bone stem cells.[116] These data further highlight the role of NO in the local regulation of bone metabolism and suggest that ADMA may act as a uremic toxin on bone through its effect to inhibit NO actions in osteoblasts. To date, there is no study in uremic humans supporting such a hypothesis, but the possibility that ADMA is involved in renal osteodystrophy can be tested in both experimental and clinical studies.

Estrogens stimulate NO production by vascular endothelial cells. This effect depends on increased expression of both the constitutive and inducible isoforms of NOS. A recent study reported that ADMA concentrations in plasma of 15 postmenopausal women decreased after 2-week subcutaneous administration of estradiol.[117] Conversely, human and murine endothelial cell lines exposed to 17β-estradiol showed a dose-dependent decrease in ADMA release, associated with an increase in DDAH levels.[117] Thus, a decrease in ADMA levels could contribute to the positive effect of estrogen on NO synthesis. Conversely, NO appears to be a key mediator in the vasodilation leading to penile erection,[118] suggesting that elevated ADMA levels (a common finding in patients at high cardiovascular risk) may have a role in the pathogenesis of erectile dysfunction often observed in these patients, particularly in patients with ESRD, for whom impotence is a pervasive problem. However, it is well known that NO generated by neuronal NOS is necessary for normal reproductive function. In the male mouse, NO is required for normal mating behavior, follicle-stimulating hormone secretion, and testicular development.[119] Because NOS also is expressed in the gonads, ADMA may cause local NOS inhibition and secondary infertility. ADMA and L-arginine levels were significantly greater and NO levels were lower in a group of patients with newly diagnosed hypogonadotropic hypogonadism than in a group of well-matched males, and these alterations were reversed fully 10 days after the administration of a single dose of testosterone.[120]

Collectively, there is ample evidence that high ADMA levels perturb a wide range of endocrine functions. However, it should be emphasized that studies performed to date can only be considered hypothesis generating, rather than solid proof of the involvement of ADMA in endocrine diseases or endocrine perturbations of ESRD. Intervention studies based on well-defined clinically relevant outcome measures are needed to establish whether high ADMA levels have far-reaching consequences in human endocrinopathies.

ADMA Neoangiogenesis and Vascular Repair

A recent study by Jacobi et al[121] in DDAH-transgenic mice showed enhanced angiogenesis that was attributed to the reduction in ADMA levels and subsequent increase in NOS activity. Therapeutic manipulation of DDAH expression or activity therefore may represent a novel approach to restore tissue perfusion. Endothelial progenitor cells (EPCs) recently have come into focus of cardiovascular research because they are thought to be responsible for endothelial and hence vascular repair.[122] EPCs circulate in the vasculature, where they home and incorporate into sites of active neovascularization.[123] EPCs orchestrate re-endothelialization of damaged vessel walls, also by secreting a large number of important cytokines that attract and govern cells that are indispensable in the process of endothelial repair.[124] Is there an interaction between ADMA and EPCs? A recent study suggested that increased endothelial NO availability is required for improvement of EPC mobilization and myocardial and neovascularization after myocardial infarction.[125] Other investigators found that NO is crucial for vascular endothelial growth factor–[126] or physical exercise–induced[127] mobilization of EPCs. Therefore, it is possible that increased ADMA levels and thereby decreased NO production lead to a deficiency of EPCs.

THERAPEUTIC STRATEGIES

Because ADMA has a low molecular weight (~202 d), similar to that of urea (60 d), renal replacement therapy seems to be the option of choice for removing ADMA in patients with ESRD, resulting in improvement in organ dysfunction and clinical symptoms. However, clinical studies concerning the impact of hemodialysis (HD) on ADMA blood levels indicate that this view is oversimplified. In studies showing a significant decrease in ADMA plasma levels by HD, the reduction ranged between 23%[41] and 65%.[128] However, several studies did not see a significant decrease in ADMA levels comparing predialysis and postdialysis ADMA levels,[76,129,130] in particular, not in patients prone to hypotension.[131] Interestingly, some studies showed that SDMA, although of similar molecular weight, was removed much more easily by dialysis than ADMA.[41,130,131] Recent data suggest that dialysance and thus removal of ADMA during regular HD is less than could be expected with regard to its molecular weight, in part because of significant protein binding of ADMA.[129] Only approximately 37 μmol, ie, 12% of the ADMA produced per day, were found in dialysate after a regular HD treatment.[129] Of note, these results are in line with a recent study by Achan et al,[10] who found that in healthy men, only 50 μmol/d are excreted in urinary ways. It therefore is reasonable to assume that dialysis will not be more effective in respect to ADMA elimination than the normal kidney. Aside from method differences in that study, HD does not sufficiently remove this putative uremic toxin, although high-flux membranes[128] and hemodiafiltration[132] might be more effective in that respect. However, a recent prospective study comparing predilution online hemofiltration and low-flux HD failed to show an advantage of the convective method in decreasing ADMA plasma levels.[133] These studies leave us with several incompletely answered questions and indicate that HD might affect ADMA beyond diffusive clearance. A rather complex scenario affecting DDAH activity because of changes in oxidative stress, inflammatory cytokine levels, and pH seems to be likely. However, this remains to be investigated.

The view on the influence of peritoneal dialysis on plasma ADMA levels also is controversial. Although 2 studies did not find differences in ADMA levels between HD and peritoneal dialysis patients,[41,134] 1 group reported significantly lower ADMA levels in patients treated with peritoneal dialysis on the morning after cycler therapy.[76] This is in line with 2 studies by Schmidt et al[68,128] that also found lower ADMA levels in patients treated by peritoneal dialysis[68] than those treated by HD,[128] although no direct comparison was performed. Interestingly, standard peritoneal dialysis solutions have been shown to reverse the hemodynamic effects of ADMA (100 μm) on rat mesenteric arterioles in vivo within minutes.[57]

L-Arginine, the substrate of NOSs, is found in mammalian organisms at concentrations far exceeding Michaelis constant values of these enzymes. Therefore, additional L-arginine should not enhance NO formation. However, in vivo, supplementing L-arginine and thereby increasing the L-arginine/ADMA ratio (a key determinant of NOS activity[135]) has been shown repeatedly to increase NO production, as recently reviewed.[136,137] This phenomenon has been named the L-arginine paradox.[138] In patients with uremia, this seems to be even more important because urea inhibits the cell L-arginine transporter in vitro at concentrations commonly observed in uremic patients.[40] Doubt remains about whether L-arginine supplementation to patients with ESRD will be beneficial. Although 1 study showed a positive effect on endothelial function, the other did not.[71,139] The increasing use of modern batch dialysis systems throughout Europe might provide an interesting opportunity to supplement L-arginine through dialysate because L-arginine supplementation was shown to exert beneficial effects in many patients with disease states.

There is increasing evidence that plasma ADMA levels can be decreased by pharmacotherapy; however, the clinical significance, if any, is unknown. The ACE inhibitor perindopril decreased ADMA plasma levels in 11 patients with non–insulin-dependent diabetes mellitus.[140] Both the ACE inhibitor enalapril and the angiotensin receptor blocker eprosartan decreased ADMA levels in 20 patients with primary hypertension.[141] This was confirmed for the ACE inhibitors zofenopril and enalapril in a larger study involving 96 patients with essential hypertension.[142] Whether this effect is related to the decrease in blood pressure or a direct effect on ADMA metabolism remains to be elucidated. Conversely, a recent double-blind parallel-group study using olmesartan in 35 patients with non–insulin-dependent diabetes mellitus during 12 weeks did not see an effect on plasma ADMA levels despite a significant decrease in 24-hour ambulatory blood pressure, an increase in effective renal plasma flow, and a decrease in markers of oxidative stress.[143] Improved glycemic control with metformin decreased ADMA plasma levels in patients with diabetes.[144] Rosiglitazone improved insulin resistance and decreased plasma ADMA levels in patients with insulin resistance.[28] Estrogen therapy in postmenopausal woman, either alone or in combination with progestogens, modestly decreased ADMA levels.[145,146] This probably is caused by an estrogen-induced increase in DDAH activity.[117] Based on current knowledge, treatment aimed at reducing oxidative stress should decrease ADMA levels.[147] Two preliminary studies on the effect of folic acid and vitamin E showed a small beneficial effect of this treatment.[148,149] Decreasing homocysteine levels had no effect in plasma ADMA levels in either monkeys or humans.[150,151] Interestingly, neither pravastatin nor simvastatin,[152-154] but rosuvastatin,[155] decreased ADMA plasma levels. New specific therapeutic agents to be designed based on the structure of DDAH[24] preferably should not only decrease plasma ADMA levels, but also influence tissue DDAH activity in target organs.

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