

# Asymmetric dimethylarginine: a new player in the pathogenesis of renal disease?

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## Purpose of review

This review summarizes current knowledge on asymmetric dimethylarginine, renal function in health and disease, and renal disease progression and examines interventions that may modify the plasma concentration of this methylarginine.

## Recent findings

Nitric oxide deficiency may occur in patients with chronic kidney disease and may contribute to accelerate progression of chronic kidney disease, hypertension and cardiovascular complications. An increase of endogenous nitric oxide inhibitors like asymmetric dimethylarginine seems to play a major role in this process. The kidneys are crucial in both, in reabsorbing and generating L-arginine as well as in eliminating asymmetric dimethylarginine primarily by the enzyme dimethylarginine dimethylaminohydrolase and to a minor degree by urinary excretion. Asymmetric dimethylarginine accumulation predicts both accelerated renal function loss and death in patients with chronic kidney disease and incident cardiovascular complications in patients with end stage renal disease.

## Summary

Asymmetric dimethylarginine is a new risk factor potentially implicated in the progression of renal insufficiency and in the high rate of cardiovascular complications of patients with chronic kidney disease.

## Keywords

asymmetric dimethylarginine, cardiovascular risk, chronic kidney disease, hypertension, nitric oxide, renal disease progression

## Abbreviations

<b>ACE</b>	angiotensin-converting enzyme
<b>ADMA</b>	asymmetric dimethylarginine
<b>CKD</b>	chronic kidney disease
<b>DDAH</b>	dimethylarginine dimethylaminohydrolase
<b>EPC</b>	endothelial progenitor cell
<b>GFR</b>	glomerular filtration rate
<b>NADPH</b>	nicotinamide adenine dinucleotide phosphate

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## Introduction

The mounting epidemics of chronic kidney disease (CKD) and the strong association between minor and moderate degrees of renal insufficiency and cardiovascular risk are the major problem that contemporary nephrology faces. The high risk associated with moderate renal insufficiency is well demonstrated in various populations [1,2] and in disparate clinical situations, from hypertension [3] and cardiac ischemia to heart failure [4], stroke [5], and cognitive impairment [6]. Undoubtedly demographic factors such as age and sex are of primary importance for renal and cardiovascular disease as well. Observations made within the framework of the NHANES III (National Health and Nutrition Examination Survey) have shown that CKD is linked to diabetes and to the metabolic syndrome [7], and recent analyses of the Atherosclerosis Risk in Communities study have also documented that this syndrome predicts the incidence rate of CKD [8\*\*]. Furthermore, smoking has now fully emerged as a relevant environmental risk factor for renal disease [9]. Hypertension and proteinuria are the strongest modifiable risk factors for renal disease progression [10], whereas old age [11], male sex, ethnicity, and genetic factors represent the main nonmodifiable risk factors for CKD. The 'hyperfiltration theory' has marked a paradigm shift in the interpretation of renal diseases [12] and the potential for prevention or interventions based on the inhibition of the renin-angiotensin system is now a cornerstone of modern nephrology [13]. Established risk factors, however, completely account for neither the epidemics of CKD nor renal disease progression. Even considering the metabolic syndrome and smoking and the full set of known risk factors, we are still unable to explain more than 50% of the variance in renal disease progression in patients who do not have diabetes [14]. The incomplete knowledge on risk factors is also epitomized by data showing that altogether sociodemographic factors, lifestyle, and Framingham risk factors

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explain only 44% of the excess risk for CKD experienced by African Americans [15]. Thus the challenge of modern nephrology is twofold: first, to identify and overcome barriers that still hinder large-scale application of effective, well established treatments [16], and second, to identify new modifiable risk factors for renal disease progression.

### Nitric oxide and the kidney: from renal hemodynamics to renal sodium handling

Nitric oxide is a major player in the regulation of renal function and a primary factor in the long-term regulation of blood pressure [17], a concept well supported by the fact that intrarenal suppression of nitric oxide synthesis eventuates in systemic hypertension in experimental models [18]. Nitric oxide produces both afferent and efferent arteriolar vasodilation, increases the glomerular filtration rate (GFR), and interferes with sodium reabsorption in the thick ascending limb, in the distal tubule, and in the collecting duct [19]. The renal effects of nitric oxide are integrated with those of the renin–angiotensin system because nitric oxide affects renin secretion. Nitric oxide synthase inhibition by *N*-nitro-L-arginine methyl ester (L-NMA), is an experimental manoeuvre to unravel the physiological role of nitric oxide synthesis both at systemic [20] and at organ [21] level. Observations made with this nitric oxide synthase inhibitor are of relevance because the system is endowed with endogenous nitric oxide synthase inhibitors such as asymmetric dimethylarginine (ADMA). Acute elevation of plasma ADMA levels by infusion of exogenous ADMA in healthy humans leads to a dose-dependent decrease in renal plasma flow and to an increase in renal vascular resistance [22], whereas the GFR remains unchanged.

As anticipated, nitric oxide inhibits sodium reabsorption along various tubule segments [23]. Although some of the effects of this substance at tubular level remain unclear, the large majority of experimental data *in vitro* and *in vivo* are in line with the natriuretic and diuretic actions of nitric oxide *in vivo* and with the antinatriuretic effect of nitric oxide synthase inhibition. The three nitric oxide synthase isoforms are expressed with different intensity in the proximal and in the distal nephron, suggesting specific roles for each isoform, an issue that remains to be investigated [24<sup>••</sup>]. The activity of nitric oxide synthase 1 and 2 is directly related to intracellular pH [25] so that acidosis inhibits nitric oxide formation. This depends on a direct effect of protons on these enzymes and on availability of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. During acidosis, because it is saturated by the high H<sup>+</sup> concentration, NADPH is unable to take up electrons from nitric oxide synthase during production of nitric oxide. This nitric oxide synthase–NADPH uncoupling switches nitric oxide synthase activity from nitric oxide to superoxide production, a reactive species that scavenges any nitric oxide

produced. Thus superoxide is a major control factor of nitric oxide bioavailability [26,27]. This might be aggravated by the fact that the optimal pH for the enzyme that degrades the endogenous nitric oxide synthase inhibitor ADMA, dimethylarginine dimethylaminohydrolase (DDAH), is approximately 7.4 [28]. Hence acidosis is likely to increase the concentration of ADMA *in vivo*, thereby inhibiting nitric oxide production.

Acidosis, superoxide production, and nitric oxide synthase inhibition are common features of salt-sensitive hypertension both in experimental models [29] and in human hypertension [30]. In salt-sensitive rats, sodium loading augments NADPH oxidase activity, leading to superoxide production, a phenomenon that eventuates in reduced nitric oxide synthesis. Oral L-arginine supplementation normalizes NADPH activity in the kidney cortex [31]. This effect suggests that L-arginine may recouple the nitric oxide synthase–NADPH oxidase activities by specifically reestablishing the production of nitric oxide by nitric oxide synthase. It is important to note that high salt in Dahl rats triggers a marked increase in the urinary excretion of ADMA, reflecting an increased renal synthesis of this nitric oxide synthase inhibitor [32]. Altered nitric oxide synthesis in this model is of relevance because Dahl rats display the typical lesions of nephrosclerosis [33]. Overall nitric oxide is a fundamental counterregulatory factor buffering vasoconstrictor hormone–induced reduction of medullary blood flow [34]. As previously suggested, when nitric oxide synthase activity is reduced within the renal medulla, experimental animals become exceedingly sensitive to vasoconstrictors and develop hypertension. Reduced cellular uptake of L-arginine in the medullary tissue may play a role in the hypertensive renal-dependent mechanisms [35]. ADMA is perhaps the most important endogenous regulator (inhibitor) of renal nitric oxide synthase. Although less potent than the synthetic nitric oxide synthase inhibitor L-NAME (*N*-nitro-L-arginine methyl ester), ADMA exerts sustained effects on renal circulation that persist at least 2 hours after the end of an exogenous infusion. High plasma ADMA concentrations have been found in salt-sensitive individuals [36,37]. Subpressor doses of ADMA cause a significant decrease in urinary sodium excretion that leads to sodium retention [38], supporting the notion that increased ADMA blood levels may cause persistent renal vasoconstriction and sodium retention.

### Nitric oxide, nitric oxide synthase inhibition, and renal senescence

Old age brings a very high risk for CKD. In the NHANES III study [39], the risk of moderate CKD was 12.5 times higher in individuals older than 60 years than in those in the 40–59 years range. Renal histology in senescent kidneys typically shows arteriolar thickening and

hyalinosis [40], segmental glomerulosclerosis, glomerular obsolescence, tubular atrophy and interstitial infiltration by lymphomonocytes, and fibrosis [41]. Senescence of renal cells is characterized by progressive telomere shortening, a phenomenon that contributes to regressive changes [42]. Elderly people are unable to rapidly adjust the renal excretion of salt in situations of salt excess or salt deficiency and are therefore predisposed to a salt-sensitive form of hypertension [43]. Likewise, failure by aged renal tubules to properly concentrate urine makes polyuria and nocturia common complaints in old age [44].

Reduced nitric oxide bioavailability is considered a major factor in the multiple functional and structural alterations of the aging kidney. Endothelial function, measured either on the basis of the hemodynamic response to acetylcholine [45] or by biomarkers [46], is progressively reduced with aging, and this unrelenting process does not spare the renal endothelium. The maintenance of vascular tone in kidney vessels is indeed critically dependent on nitric oxide because nitric oxide synthase inhibition by L-NAME triggers a more pronounced vasoconstriction in renal vessels of old kidneys than in those of young kidneys [47,48]. ADMA may be a causative factor in the functional and structural alterations of the kidney in the elderly (Fig. 1) because in old rats high plasma concentration of this compound is accompanied not only by reduced whole body nitric oxide generation [49,50] but also by declining renal plasma flow [51] and sustained proteinuria [52]. Data in elderly subjects [53] are concordant with observations in the rat [54], suggesting that ADMA accumulation may have a prominent role in the decline of renal perfusion in the elderly. Finally, recent data from Scalera *et al.* [55] show that ADMA accelerates cell senescence by increasing the activity of telomerase,

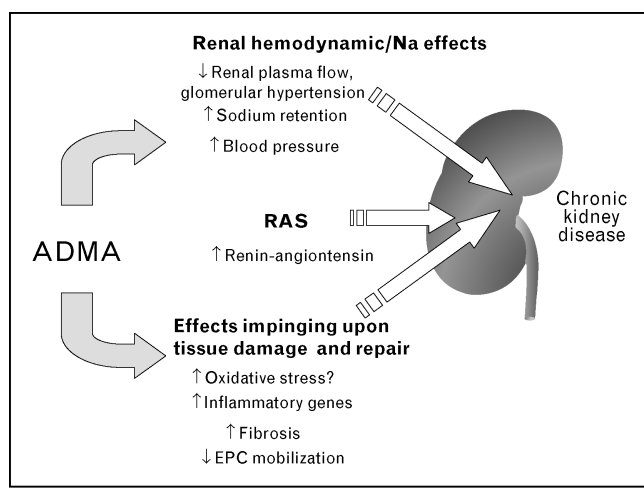
i.e. that it triggers a biologic chain of events that accelerates senescence. These intriguing results indicate that this substance may participate by multiple mechanisms in the disturbances of renal function in old age. The close relationship of nitric oxide, ADMA, DDAH, and the kidney was nicely documented by histologic studies [56]. The kidney is the organ in which DDAH, the key enzyme for regulating ADMA levels, was first discovered [57] and in which it is highly expressed [58]. Furthermore 20% of the 300  $\mu\text{mol}$  of ADMA produced per day is excreted by the kidney [59], making it one of the key organs for regulating the plasma ADMA levels.

### Does asymmetric dimethylarginine predict renal outcomes?

As discussed, the nitric oxide system is central to the maintenance of normal renal function in health and in disease [60] states because it appears to protect renal function from factors that may jeopardize renal hemodynamics. Experimental data indicate that nitric oxide synthase inhibition does not modify the GFR in the normal rat but that it critically reduces the GFR in diabetic rats [61]. In pregnancy, augmented nitric oxide production is a major factor sustaining the increased renal blood flow of this condition [62]. The importance of adequate nitric oxide production in preventing pregnancy-related complications is indicated by the fact that high ADMA precedes and accurately predicts preeclampsia [63] and by the fact that L-arginine attenuates the severity of this disease [64]. Molecular genetics studies support the notion that nitric oxide is nephroprotective because nitric oxide synthase 3 polymorphisms associated with reduced nitric oxide synthase activity are more frequent in patients with end-stage renal disease [65]. Overall altered nitric oxide bioavailability is deemed to be a relevant factor in progressive nephropathies [66].

As alluded to previously, there is solid evidence that ADMA interferes with renal function in humans (Fig. 1). At levels attained only in pathophysiologic conditions, ADMA triggers renal vasoconstriction and reduces renal plasma flow but does not modify the GFR. Unmodified GFR in the face of reduced renal plasma flow underlies high glomerular capillary pressure. Interestingly, Kielstein *et al.* [53] pointed out that this effect is not unique to ADMA because angiotensin II also exerts vasoconstrictor effects only at supraphysiologic concentrations. High tissue concentration of angiotensin II has strong atherogenic effects in the cardiovascular system [67] and alters renal function and structure [68]. By analogy, high ADMA at tissue level may engender arterial and renal damage as well. Of note, ADMA has been associated with oxidative stress and endothelial dysfunction in a large series of patients with CKD of various causes [69]. The possibility that ADMA is involved in the evolution of progressive nephropathies

**Figure 1 Potential mechanisms whereby asymmetric dimethylarginine (ADMA) may engender or aggravate renal insufficiency**



is suggested by two recent studies. The first study [70\*\*] was performed in an incident cohort of patients enrolled in an Italian centre and the second [71\*\*] in a prevalent cohort of patients being followed up in several centres in Austria, Italy, and Germany. Although patients in the first study were more than 20 years older and had more advanced renal failure than those in the second study, in both studies high ADMA consistently predicted a faster rate of renal function loss. These consistent, prospective observations, which seem to be independent of age and degree of renal impairment, lend support to the hypothesis that ADMA may engender renal damage both because it triggers glomerular hypertension and because it sets in motion several effects ultimately leading to endothelial damage, salt accumulation, and cell senescence. It is of interest that even patients with incipient renal disease due to autosomal dominant polycystic kidney disease or IgA nephropathy have been shown to have elevated ADMA plasma levels [72]. This might help to explain the fact that patients with autosomal dominant polycystic kidney disease have defective endothelium-dependent relaxation even when they are normotensive and have normal renal function [73]. Similar observations were made in type 1 diabetes, in which ADMA was inversely associated with the GFR and with atherosclerosis [74]. In these patients, however, ADMA failed to predict renal disease progression [75].

#### **The second hit: asymmetric dimethylarginine inhibits vascular repair**

Besides leading to renal damage, ADMA may also be involved in inhibiting repair of renal lesions by endothelial progenitor cells (EPCs). EPCs have recently come into focus in cardiovascular research because they are thought to be responsible for endothelial repair [76]. EPCs circulate in the cardiovascular system, where they home and incorporate into sites of active neovascularization [77]. EPCs orchestrate reendothelialization 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 [78]. A recent study suggests that increased endothelial nitric oxide availability is required for improvement of EPC mobilization, myocardial repair, and neovascularization after myocardial infarction [79\*\*]. Increased ADMA level and thereby a decreased nitric oxide production may lead to a deficiency of EPCs. Indeed, a recent study by Thum *et al.* [79\*\*] has shown an inverse correlation between ADMA plasma concentration and circulating EPC levels in patients with coronary artery disease [80].

#### **Interventions that may modify asymmetric dimethylarginine levels**

L-Arginine, the substrate of nitric oxide synthase, is actively transported through the cationic amino acid y+ transporters into cells yielding intracellular concentration

of L-arginine as high as 2 mM [81]. Because these concentrations by far exceed the K(M) values of these enzymes [82,83], additional L-arginine should not enhance nitric oxide formation. *In vivo*, however, increasing L-arginine concentration in the plasma has been shown to increase nitric oxide generation [84]. This phenomenon has been named the L-arginine paradox [85]. Doubt remains as to whether L-arginine supplementation to patients with ESRD will be beneficial because studies showed positive [86] as well as no effects [87] on endothelial function. Prospective long-term and large-scale clinical studies still need to be performed, however.

There is sparse evidence that plasma ADMA levels can be reduced by pharmacotherapy. The angiotensin-converting enzyme (ACE) inhibitor perindopril lowered the ADMA plasma level in 11 patients with non-insulin dependent diabetes mellitus [88]. Both the ACE inhibitor enalapril as well as the ARB eprosartan lowered the ADMA level in 20 patients with primary hypertension [89]. This was confirmed for the ACE inhibitors zofenopril and enalapril in a larger study involving 96 patients with essential hypertension [90]. A recent randomized controlled study by Fliser *et al.* [90], however, failed to show an effect of a 4-week treatment with the ARB olmesartan (40 mg/day) on plasma ADMA levels in 35 patients with non-insulin dependent diabetes mellitus [91]. Whether ACE inhibitors/ARBs have a direct effect on the ADMA metabolism or whether the change in ADMA levels is a result of changes in blood pressure is still unclear.

In contrast to the conflicting data on the effect of ACE inhibitors and ARBs, oral antidiabetic drugs appear consistently effective in reducing ADMA levels. Improved glycemic control with metformin reduced ADMA plasma levels in patients with diabetes [92]. Rosiglitazone improved insulin resistance and lowered plasma ADMA levels in seven insulin-resistant subjects with hypertension [92]. This effect likely results from the upregulation of DDAH. Indeed, in the rat the peroxisome proliferator activated receptor gamma (PPARgamma) ligand, pioglitazone, increases nitric oxide production partly by upregulating tissue DDAH II expression and by decreasing systemic ADMA levels [93\*]. That this is indeed a PPARgamma ligand-specific action on DDAH rather than an effect of better glycemic control is also suggested by a recent study in humans using acarbose. Despite blunting the response to postchallenge hyperglycemia in subjects with impaired glucose tolerance, acarbose did not influence plasma ADMA levels [94].

Estrogen therapy in postmenopausal women, either alone or in combination with progestogens, modestly reduced ADMA levels [95,96], probably due to an estrogen-induced increase in DDAH activity [97].

Based on our current knowledge, treatment aimed at reducing oxidative stress should lower ADMA levels [98]. Two preliminary studies on the effect of folic acid and vitamin E showed a small beneficial effect of this treatment [99,100], but lowering homocysteine had no effect on plasma ADMA levels, either in monkeys or in humans [101,102].

Interestingly, neither pravastatin nor simvastatin [103–105] lowers ADMA plasma levels, but rosuvastatin does [106]. Treating 45 patients with erectile dysfunction with sildenafil for 70 days did not influence plasma ADMA levels [107].

New, specific therapeutic agents to be designed based on the structure of DDAH [108] should preferably not only lower plasma ADMA levels but also influence tissue DDAH activity in target organs.

## Conclusion

The recent advent of gene-manipulated mice either overexpressing [109] or deleting DDAH [110] will help to further elucidate the pathophysiologic role of ADMA in renal disease and will hopefully help to generate new opportunities for intervention in renal disease progression.

## References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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