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Arginine, arginine analogs and nitric oxide production in chronic kidney disease

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Summary

Nitric oxide (NO) production is reduced in renal disease, partially due to decreased endothelial NO production. Evidence indicates that NO deficiency contributes to cardiovascular events and progression of kidney damage. Two possible causes of NO deficiency are substrate (L-arginine) limitation and increased levels of circulating endogenous inhibitors of NO synthase (particularly asymmetric dimethylarginine [ADMA]). Decreased L-arginine availability in chronic kidney disease (CKD) is due to perturbed renal biosynthesis of this amino acid. In addition, inhibition of transport of L-arginine into endothelial cells and shunting of L-arginine into other metabolic pathways (e.g. those involving arginase) might also decrease availability. Elevated plasma and tissue levels of ADMA in CKD are functions of both reduced renal excretion and reduced catabolism by dimethylarginine dimethylaminohydrolase (DDAH). The latter might be associated with loss-of-function polymorphisms of a *DDAH* gene, functional inhibition of the enzyme by oxidative stress in CKD and end-stage renal disease, or both. These findings provide the rationale for novel therapies, including supplementation of dietary L-arginine or its precursor L-citrulline, inhibition of non-NO-producing pathways of L-arginine utilization, or both. Because an increase in ADMA has emerged as a major independent risk factor in end-stage renal disease (and probably also in CKD), lowering ADMA concentration is a major therapeutic goal; interventions that enhance the activity of the ADMA-hydrolyzing enzyme DDAH are under investigation.

Keywords

arginase; asymmetric dimethylarginine; cardiovascular events; dimethylarginine dimethylaminohydrolase; L-arginine transporters

Introduction

Net nitric oxide (NO) deficiency develops as a result of chronic kidney disease (CKD). Total NO production can be determined by quantifying the stable oxidation products of NO ($\text{NO}_2 + \text{NO}_3 = \text{NO}_x$; only valid under conditions of dietary NO_x control).¹ By this measure, the total amount of NO_x 'excreted' in a 24-h period is decreased in patients with end-stage renal disease (ESRD) receiving peritoneal dialysis or hemodialysis, as well as in CKD patients with approximately 25% residual renal function compared with controls (Figure 1).^{2–4} Similar conclusions have been reached by Blum *et al.*⁵ and Wever and colleagues⁶ (the latter group measured conversion of $^{15}\text{N}_2$ -labeled arginine to citrulline in people with CKD). Although

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Lau and co-workers reported increased rates of arginine conversion to citrulline in ESRD patients⁷—perhaps reflecting activation of inducible nitric oxide synthase [NOS] by hemodialysis—most evidence indicates that total NO production is decreased in humans with CKD or ESRD compared with healthy individuals. In the absence of acute inflammatory events, decreased total NO production (expressed as the qualitative index UNO_XV [urinary excretion of NO_X]) has also been reported in animal models of CKD.^{8–13}

NO is produced in many cell types and organs. Total UNO_XV reflects the sum of all NO produced; it does not yield information on regional NO production and does not discriminate between total NO formed and ‘bioactive’ NO.¹ Because the vascular endothelium is the largest ‘organ’ in the body, a drop in endothelial NO production is likely to contribute to decreased UNO_XV in CKD and ESRD. Indeed, endothelial dysfunction does occur in CKD (characterized by blunted release of endothelial NO) and in ESRD, even during early stages of disease.^{14–18} Although no clinical evidence is available, animal studies implicate intrarenal NO deficiency in CKD, and this is another factor that would contribute to falls in the UNO_XV index.^{7–13} Given the persistent oxidative stress induced in early-stage CKD,¹⁹ reduced NO synthesis is likely to be widespread in both CKD and ESRD (see below).

Chronic inhibition of NOS in otherwise normal animals produces hypertension and focal segmental glomerulosclerosis, the hallmark of progressive CKD.²⁰ It therefore seems likely that the NO deficiency associated with CKD contributes to progression of kidney damage and eventual development of ESRD.

There are many ways in which net NO deficiency could develop (Figure 2). Reduced availability of substrate (L-arginine) might result from decreased dietary intake or endogenous arginine synthesis, which occurs mainly in the kidney, from diversion of L-arginine through other metabolic pathways (e.g. those involving arginase), and from impaired delivery of L-arginine to NOS. NO deficiency could also be a function of increased levels or activity of endogenous NOS inhibitors such as the potent asymmetric dimethylarginine (ADMA), or both. Decreased activity of NOS—due to reduced protein content, changes in phosphorylation, lack of essential cofactors, or inhibitory or stimulatory protein–protein interactions—might also reduce the total NO generated.

Processes independent of NO production could also reduce the levels of bioactive NO. These include enhancement of NO inactivation by oxygen radicals, oxidative stress-induced functional ‘switching’ of NOS to superoxide generators, limiting access of NO to target tissues (e.g. by deposition of advanced glycosylated end products), altering NO receptors and their transduction mechanisms, or both. Oxidative stress is an early feature of CKD that persists throughout the course of disease.¹⁹ Formation of advanced glycosylated end products occurs in patients with renal disease.²¹

This review will focus on some of the proximal causes of NO deficiency in CKD that can be directly assessed in humans, namely substrate availability and ADMA levels.

Regulation of Substrate Availability

Synthesis of arginine

L-Arginine is a ‘semi-essential’ amino acid. During periods of high demand, such as maturational growth or following injury, dietary ingestion of L-arginine is necessary. By contrast, endogenous arginine production satisfies the metabolic requirements of healthy, unstressed adults.²² Endogenous arginine synthesis is concentrated in the liver (where arginine is rapidly hydrolyzed in the urea cycle) and kidney cortex. Most L-arginine produced in the kidney is released into the blood and distributed throughout the body.²³

The kidneys synthesize about 2 g of L-arginine per day; normal dietary intake is approximately 4–5 g per day.²³ In patients with severe CKD or ESRD, who have little functional renal mass, production of L-arginine could be compromised. Indeed, renal L-arginine synthesis is significantly reduced (to approximately 40% of normal levels) in CKD patients;²⁴ however, plasma levels of L-arginine in patients with renal disease are at the low end of the normal range and are always well in excess of the K_m of NOS enzymes.^{2–4} Further, net endogenous L-arginine synthesis is preserved in ESRD patients,⁷ presumably because of compensatory increases in production by nonrenal tissues.

Transport of arginine

Despite maintenance of normal plasma L-arginine levels in CKD, net L-arginine deficiency is indicated by an increase in orotic acid.²⁵ One explanation that reconciles these contradictory findings is that transport of L-arginine into endothelial cells is impaired in CKD. In this scenario, the reduced rate at which L-arginine is eliminated from plasma might maintain plasma L-arginine levels and mask a reduction in intracellular L-arginine availability.

We previously reported that uremic plasma from patients undergoing peritoneal dialysis and hemodialysis inhibits transport of L-arginine into a variety of cultured endothelial cells (Figure 3). A proportion of this inhibitory activity seems to be due to elevated levels of urea.²⁶ This effect of urea is not osmotically mediated or acutely reversible with excess arginine. The increased concentration of urea in the medium induces an ‘all or nothing’ response, which manifests when the concentration of urea exceeds approximately 15 mmol/l²⁶ and requires entry of urea into endothelial cells, since inhibition of urea transport abolishes the inhibitory effect of urea on L-arginine transport.²⁷ So, inhibition of endothelial L-arginine transport by uremic levels of urea might be a functionally significant means of inhibiting endothelial NOS and contributing to endothelial dysfunction. Indeed, in cultured human dermal endothelial cells, 7 days of urea-induced inhibition of L-arginine transport was associated with decreased activity of endothelial NOS in the absence of changes in the enzyme's abundance.²⁶

Our data indicate the presence of other factors in uremia, besides urea, that inhibit L-arginine transport. L-Arginine transport is substantially inhibited *in vitro* by a 1 in 5 dilution of human ESRD plasma, which contains approximately 5 mmol/l urea; a synthetic solution of 5 mmol/l urea has no impact on L-arginine transport.^{26,27} The constitutive endothelial cell transporter that facilitates uptake of L-arginine is cationic amino acid transporter 1 (CAT1). CAT1 transports several cationic amino acids,²⁸ which might competitively inhibit L-arginine uptake. For example, lysine and ornithine have high affinity for CAT1, but their concentrations are either low or normal in uremia.^{24,29,30} Concentrations of the endogenous methylated arginines ADMA and symmetric dimethylarginine (SDMA) are increased in the plasma of ESRD and CKD patients,^{2–4,15,31–34} and these arginines are transported by the y+ CAT family of membrane transporters.^{35,36} Both ADMA and SDMA have a relatively high affinity for CAT1 and inducible CAT2,^{35,36} but *in vitro*, maximal uremic levels of ADMA (10 μ mol/l) are too low to compete with L-arginine for membrane transporters.²⁶ There is currently no information on whether plasma levels of SDMA in CKD or ESRD (1–10 μ mol/l) inhibit L-arginine transport.

Studies by Schwartz and colleagues indicate that L-arginine transport is attenuated in the aortae and glomeruli of rats with CKD, concomitant with decreased abundance of CAT1.³⁷ By contrast, L-arginine transport into erythrocytes and platelets is enhanced in ESRD patients compared with controls.^{38,39} The presence of inhibitors of endothelial L-arginine transport, low levels of endothelial transporters in ESRD patients, or both, could explain the poor response to supplementation with excess L-arginine in the patients with renal failure. Studies have shown that neither acute nor chronic administration of L-arginine improve arterial endothelial function in adults or children with chronic renal failure,^{40,41} although acute L-arginine supplementation improves acetylcholine-induced venodilation in ESRD.⁴² Chronic L-arginine therapy (6

months) has no impact on progression of CKD in patients with mild renal impairment⁴³ or in young renal transplant recipients with graft dysfunction.⁴⁴ The results from these studies are disappointing, particularly given the positive outcomes of studies of rat CKD models,²⁵ although as pointed out by Peters *et al.*,⁴⁵ L-arginine has an adverse effect—even in animal models—during acute, ongoing inflammatory responses in which inducible NOS is activated.

Relative importance of substrate availability to endothelial NOS activity

Intracellular levels of L-arginine are very high, in the mmol/l range, while the concentration of extracellular L-arginine is approximately 100 μ mol/l. Because the K_m s for NOS enzymes are approximately 1–20 μ mol/l, NO synthesis should never be substrate limited. Nevertheless, L-arginine supplementation stimulates NO production, at least *in vivo*, creating the 'arginine paradox'. This paradox might be explained by a coupling between caveolar endothelial NOS (eNOS) and the L-arginine transporter.⁴⁶ This has led to the suggestion, discussed above, that eNOS activity depends on an extracellular source of L-arginine. This is a controversial area; some workers believe that NO stimulation *in vivo* with high doses of L-arginine is secondary to other actions such as insulin release.⁴⁷ It is true that cultured endothelial cells can make NO in the absence of L-arginine for up to 24 h.⁴⁸ In a recent study, a stimulatory protein–protein interaction between caveolar CAT1 and eNOS that promotes NO formation was confirmed.⁴⁹ Stimulation of NO production persisted, however, when L-arginine transport was inhibited, indicating that the protein–protein interaction is not dependent on increased rates of L-arginine delivery. It is clear that there are many unanswered questions, even with regard to the issue of something as fundamental as the source of L-arginine for eNOS activity. Some of the differences in the literature might relate to the tremendous degree of endothelial cell heterogeneity within the kidney and throughout the circulation.⁵⁰ Some differences might reflect the use of static endothelial-stimulated versus shear stress-stimulated NO release, and others probably reflect variations between *in vivo* and *in vitro* studies. Of note is that inhibition of *in vivo* L-arginine transport (with lysine) produced substantial and rapid falls in vascular NO production.⁵¹

Utilization of arginine by non-NO-producing pathways

In addition to NO synthesis, L-arginine is utilized during synthesis of creatine (by L-arginine: glycine amidinotransferase), agmatine (by arginine decarboxylase), and urea and ornithine (by arginase) (Figure 4). Synthesis of NO accounts for only a small proportion of total L-arginine turnover; the majority of L-arginine is utilized during creatine production and by arginases.^{22, 23} The relative activity of these other pathways will determine the availability of L-arginine as a substrate for NOS.

To replace the 1.5 g of creatine broken down and lost as creatinine each day, approximately 2.5 g of L-arginine are required.²³ Only about 15% of this L-arginine is produced in the kidney, which utilizes approximately 0.5 g of L-arginine per day. The remainder is probably synthesized in the liver, which facilitates recycling of ornithine into L-arginine, thus conserving the body's L-arginine supply.²³ Agmatine is found in many mammalian tissues, including the kidney and liver.²² An α -2 adrenergic receptor agonist, agmatine interacts with other arginine biosynthetic pathways. This agonist is a weak inhibitor of NOS, a feedback inhibitor of arginine decarboxylase, and also inhibits ornithine decarboxylase, thus reducing proline formation and inhibiting cell growth.⁵² Agmatine also acts as a renal vasodilator via an NO-dependent mechanism⁵³ and is a potent stimulator of endothelial NOS.⁵⁴ Morrissey and Klahr suggested that agmatine functions in endothelium as an autocrine regulator (inhibitor) of mitochondrial NO production, while also serving as a paracrine NO stimulatory agent leading to vasodilation.⁵⁴ The overall importance of agmatine to physiological regulation of renal function and vascular tone, and details of its synthesis, remain unknown. There is no information on the impact of CKD on agmatine levels.

Production of urea and polyamines via arginase is a major consumer of L-arginine. In the liver, a considerable amount of L-arginine is synthesized but also metabolized by arginase I and thus never leaves the organ.²³ The kidney contains arginase II, and endothelial cells contain both isoforms.^{23,55,56} Arginases compete with NOS for L-arginine and can thereby limit NO production; indeed, NO production is attenuated in endothelial cells overexpressing arginase I and arginase II.⁵⁵ Vascular arginase activity is increased in experimental hypertension and inhibition of arginase restores endothelial NO synthesis.^{56–59} Arginase I and arginase II are upregulated in the vasculature of Dahl salt-sensitive rats maintained on a high-salt diet;⁵⁸ similar findings have been reported for spontaneously hypertensive rats.⁵⁹ Particularly relevant is that inhibition of arginase protects the kidney from structural damage in the 5/6 renal mass ablation/infarction model of CKD,⁵⁶ indicating that arginase inhibition might be a novel therapeutic approach to slowing progressive renal damage.

Renal reabsorption of arginine

At plasma concentrations of 80–100 $\mu\text{mol/L}$, approximately 3 g of L-arginine are filtered by the kidneys of healthy individuals each day. Almost all L-arginine is reabsorbed in the proximal tubule by a cationic amino acid antiporter that requires sodium, b^{0+} .²³ By contrast, ESRD patients lose L-arginine during dialysis.⁶⁰ Given the importance of the kidney to synthesis and salvage of approximately 5 g of L-arginine per day, it seems inevitable that L-arginine deficiency will occur in CKD/ESRD. These dialysis-mediated losses, together with increased levels of competitive inhibitors of NOS in renal disease (see following section), are likely to promote a state of profound net NO deficiency.

Endogenous NOS Inhibitors

Several endogenous NOS inhibitors have been identified, including methylguanidines and methylated arginines such as N-monomethyl-L-arginine (L-NMMA) and ADMA.^{61,62} ADMA is a potent competitive inhibitor of NOS and has been the focus of much recent investigation. Acute infusion of ADMA in healthy humans causes renal and systemic vasoconstriction, and causes heart rate and cardiac output to drop.^{63,64} Chronically elevated levels of endogenous ADMA have been implicated in endothelial dysfunction, and increased cardiovascular morbidity and mortality, in many diseases including atherosclerosis, heart failure, hypertension, diabetes and ESRD.^{15,65} Plasma ADMA, which has spilled out of the cells in which it was produced, is the measured variable. In vascular endothelium, ADMA levels are approximately 10-fold those of plasma,⁶⁶ and concentrations are extremely high in the kidney and spleen.⁶⁷ It is the local intracellular level of ADMA that regulates NOS activity⁶⁵ and this probably varies greatly between organs.

Regulation of ADMA levels

Synthesis of ADMA—Regulation of ADMA levels is complex. During the production phase, intact proteins are methylated by protein methyl transferases (PRMTs) and methylated amino acids are released during protein catabolism.^{68,69} The type 1 PRMTs catalyze methylation of arginine residues of HISTONES^{68,69} and are responsible for ADMA production. Arginine methylation determines the activity of RNA-binding proteins.⁷⁰ There is a relationship between LDL-induced increases in expression of PRMT1 and ADMA levels,⁷¹ but there is little evidence that regulation of methylation of proteins, such as arginine, is directly related to cardiovascular control mechanisms. Endothelial shear and high LDL concentrations do, however, upregulate PRMT1, thereby increasing ADMA release.^{71,72} Oxidative stress, as occurs in CKD,¹⁹ is associated with enhanced ADMA synthesis via stimulation of PRMT1.⁷¹ At present, however, regulation of ADMA levels in relation to cardiovascular control is thought to be mainly a function of ADMA clearance.

Clearance of ADMA—Humans generate approximately 300 μmol of ADMA per day, which is due to degradation of methylated proteins. About 50 μmol of ADMA per day is excreted via the kidneys while approximately 250 μmol per day are broken down by dimethylarginine dimethylaminohydrolase (DDAH).⁶³ DDAH is widely distributed. In rats, DDAH is particularly abundant in the kidney, and is expressed at high levels in the liver, pancreas, brain and aorta.^{73–75} Two isoforms of DDAH co-segregate with NOS and are present at high concentrations in the kidney and aorta. DDAH2 is found predominantly in endothelium and DDAH1 is located in neuronal and epithelial tissues.^{73,74} DDAH is present in neutrophils and macrophages.⁷⁵

Increased plasma levels of ADMA in CKD indicate that the kidney has a major role in ADMA clearance.^{15,31–34} Although originally assumed to reflect impaired renal excretion due to reduced renal function, it is now apparent that relatively little ADMA appears in the urine even when renal function is normal. In rats, urinary ADMA excretion is negligible despite renal extraction of approximately 30% of circulating ADMA.⁷⁶ Studies of normal humans indicate that ADMA is catabolized in the kidney; a small amount of uncatabolized ADMA is excreted in the urine.⁷⁷ These observations indicate that DDAH in the kidney is an important mediator of catabolic clearance of ADMA, a process that is presumably impaired in CKD (see below).

Maintenance of DDAH activity, and thereby control of ADMA levels, is crucial for optimal NO production; for example, inhibition of DDAH decreases NO synthesis in cultured endothelial cells.⁷⁸ Overexpression of DDAH *in vitro* enhances endothelial NO production and mice that overexpress human DDAH produce NO in excess, in association with decreased levels of plasma ADMA, plus reduced blood pressure and systemic vascular resistance.⁷⁹

Polymorphisms of DDAH genes—Two genes for DDAH have been identified. *DDAH1* predominates in tissues expressing neuronal forms of NOS and maps to chromosome 1p22, whereas *DDAH2* is located on chromosome 6p21.3 and is found in the heart, kidney and other vascularized tissues that express endothelial forms of NOS.^{73,80} Six polymorphisms of the *DDAH2* gene have been identified, one of which is associated with increased expression of *DDAH2*.⁸¹ Although *DDAH2* was originally considered to be the endothelial isoform, the *DDAH1* gene is present in some endothelial cells, including human umbilical vein endothelial cells (Y Chen, personal communication). A preliminary report indicates that a polymorphism in the *DDAH1* gene is associated with increased levels of plasma ADMA, and increased risk of cardiovascular disease and hypertension, among Finnish men (VP Valkonen, personal communication). In a Finnish case-control study, three out of eight *DDAH1* polymorphisms were associated with pre-eclampsia and plasma ADMA concentrations were increased in this condition.^{82,83} Mutations of either DDAH isoform, therefore, probably affect cardiovascular function by causing ADMA levels in endothelial cells and other tissues to increase. Subsequent elevation of plasma ADMA levels would in turn enhance ADMA transport into endothelial cells via CAT.

Activity of DDAH—In addition to regulation at the gene level, the activity of DDAH enzymes is controlled post-translationally. Of particular relevance to CKD is that oxidation of a sulfhydryl group in the active site of the enzyme inhibits DDAH activity, causing ADMA levels to increase.⁸⁴ DDAH activity is also inhibited by S-nitrosylation.⁸⁵ Specific inhibitory signals include hypoxia,⁸⁶ high glucose levels,⁸⁷ high concentrations of homocysteine,⁸⁸ presence of oxidized LDL, and TNF- α .⁸⁴ As mentioned above, oxidative stress is also associated with enhancement of ADMA synthesis via stimulation of PRMT1.⁷¹ The oxidative state is probably an important biological regulator of DDAH activity. Indeed, antioxidants lower ADMA levels *in vitro*⁸⁹ and in a preliminary study, the antioxidant vitamin E decreased plasma ADMA concentrations in six of eight CKD patients.⁹⁰ Other therapeutic interventions that might lower

levels of ADMA by stimulating DDAH activity include vitamin A,⁹¹ aspirin⁹² and estrogen.⁹³

ADMA in end-stage renal disease

There is no doubt that plasma levels of ADMA are elevated in ESRD^{2,3,15,31–34} but is this functionally significant? When cultured endothelial cells from several species and locations in the circulation were exposed to a 1 in 5 dilution of plasma from an ESRD patient, the rate of NO production decreased as a result of the presence of a competitive inhibitor of NOS.⁶⁰ The plasma of all ESRD patients ($n = 11$) studied exerted this effect while responses were more variable when plasma from CKD patients was used. In a small group of patients ($n = 11$) with various primary diseases and approximately 30% residual renal function, an inhibitor of endothelial NOS was detected in the plasma of only 5 patients.⁹⁴ As shown in Figure 5, plasma ADMA levels in the CKD patients were predictive of endothelial NOS activity. ADMA concentrations were not correlated with primary disease or medication.⁹⁴ There was no relationship between plasma creatinine or blood urea nitrogen and ADMA (Figure 5), indicating that reduced catabolism of ADMA rather than impaired renal clearance was responsible for elevated plasma levels in the affected subgroup of CKD patients. This hypothesis was reinforced by the finding that plasma levels of SDMA (which is not a substrate for DDAH) were uniformly elevated, as was plasma creatinine (Figure 5).

One problem with interpreting the clinical literature on ADMA is that different laboratories report widely disparate baseline values as a result of using different analytical methods.^{2,3,25,31–34,64,95} Nonetheless, most groups find plasma ADMA levels in ESRD patients to be in excess of 2 $\mu\text{mol/l}$, a concentration sufficient to inhibit endothelial cell NO synthesis *in vitro*.⁶⁰ In the cell culture studies described above, however, in which a 1 in 5 dilution of ESRD plasma inhibited the activity of endothelial NOS, the concentration of ADMA would have been 1 $\mu\text{mol/l}$ or less (the concentration of undiluted plasma ADMA from ESRD patients on hemodialysis is $4.1 \pm 0.8 \mu\text{mol/l}$). An ADMA concentration of 1 $\mu\text{mol/l}$ or less is too low to inhibit endothelial NOS activity in this preparation;⁶⁰ therefore, there must be unidentified NOS inhibitors accumulating in the plasma of patients with ESRD. In other words, the elevated plasma level of ADMA in these patients is likely to be both a mediator of endothelial dysfunction and a marker of other uremic toxins that inhibit the activity of endothelial NOS.

Cardiovascular morbidity and mortality are increased in ESRD,¹⁵ disproportionately so in younger patients.¹⁶ Although plasma levels of ADMA are elevated in all patients with ESRD, the magnitude of this increase is highly variable, which has an impact on cardiovascular risk. As shown in Figure 6, ESRD patients with plasma ADMA concentrations above the mean of 2.52 $\mu\text{mol/l}$ have a much greater rate of cardiovascular events than those in the lower 50th percentile.^{15,96} In patients with ESRD, plasma ADMA predicts carotid artery intima-media thickness and the rate of worsening of carotid atherosclerosis,⁹⁷ as well as the extent of left ventricular hypertrophy and dysfunction.⁹⁸ Increased ADMA levels have been reported in a variety of conditions associated with cardiovascular risk—including essential hypertension⁹⁹ and peripheral arterial occlusive disease^{15,33}—even when renal function is normal. In healthy, male nonsmokers, elevated plasma ADMA concentration is associated with a fourfold increased risk of acute coronary events.¹⁰⁰

How does elevated plasma ADMA increase cardiovascular risk? One possibility is that reduced endothelial NO production leads to endothelial dysfunction, hypertension and proatherosclerotic changes.^{15,65} Indeed, elevated plasma levels of ADMA were found to be the sole determinant of endothelial dysfunction (assessed as flow-mediated dilation) in patients with hyperhomocysteinemia.¹⁰¹ *In vitro* studies have shown that high ADMA levels accelerate senescence of endothelial cells.¹⁰² In addition to promoting injury, high concentrations of ADMA probably inhibit repair because NO is required for production and homing of

endothelial progenitor cells (EPCs) to damaged sites.¹⁰³ ADMA inhibits both mobilization of EPCs from bone marrow and homing to vascular endothelium *in vitro*.¹⁰⁴ Inhibition of ADMA by overexpression of *DDAH2* increases expression of vascular endothelial growth factor in endothelial cells and enhances tube formation; chemical inhibition of DDAH has the opposite effects.¹⁰⁵ Elevation of endogenous ADMA in human umbilical vein endothelial cells (by silencing the *DDAH1* gene) also impairs cell proliferation and tube formation (Y Chen, personal communication). Increases in the ADMA : arginine ratio interfere with neointimal repair and restoration of endothelial function after balloon injury.¹⁰⁶ There are fewer EPCs in the circulation of patients with ESRD and advanced CKD. Plasma from uremic patients inhibits EPC differentiation and tube-forming capacity.¹⁰⁷

ADMA in chronic kidney disease

The risk of cardiovascular events is increased in CKD patients, as are inflammation, oxidative stress and endothelial dysfunction, even when renal insufficiency is mild.^{14,16–19} The impact of CKD on plasma ADMA levels is not yet clear. In a small series ($n = 11$) we found that plasma ADMA levels are variable in CKD patients and are not correlated with severity of CKD.⁹⁴ Similar findings have been reported by Saran and colleagues.⁹⁰ By contrast, Fleck *et al.* reported mild but consistent elevation of plasma ADMA levels (30–40% higher than normal) in CKD patients, which did not correlate with concentrations of plasma creatinine.³² Kielstein and colleagues found that plasma ADMA levels were uniformly and markedly elevated (300–400%) in CKD. These increases affected patients with CKD of varying severity and were not correlated with creatinine levels.⁹⁵ Even the plasma ADMA concentrations of patients with near-normal renal function (plasma creatinine <1.3 mg/dl [$<114.9 \mu\text{mol/l}$]) were substantially increased compared with controls.⁹⁵

In contrast to the data cited above, two recent studies have reported that plasma ADMA concentration varies inversely with the severity of CKD.^{108,109} The authors of these studies also concluded that the higher the initial concentration of plasma ADMA, the greater the rate of progression of CKD. It should be noted, however, that in these study populations, high baseline ADMA was associated with high plasma creatinine, which is a known predictor of loss of renal function.⁶⁵

The literature indicates that there is considerable variability in the relationship between renal function and plasma (and by inference tissue) ADMA levels in CKD. This variability presumably reflects differences in DDAH activity in the populations studied. Perturbation of renal urinary clearance will cause plasma levels of ADMA to rise, an effect that will be amplified in some patients by concomitant reduction of DDAH activity. The early increase in ADMA concentration might be prevented in patients with active DDAH. It is likely that loss of renal DDAH activity occurs in CKD as functional renal mass is lost and that loss of activity also contributes to elevated plasma and tissue levels of ADMA. To confirm that high concentrations of ADMA accelerate progression of CKD, prospective studies that compare groups with equivalent levels of renal function and normal versus elevated levels of ADMA are needed.

Conclusions

Most evidence shows that total NO production is decreased in renal disease and this is likely to be due, in part, to impaired NO production by several mechanisms; for example, substrate limitation due to decreased renal synthesis of L-arginine and competition from other metabolic pathways such as those including arginase. The accumulation of cationic amino acid transport inhibitors in ESRD might impair L-arginine delivery into cells, thus potentiating intracellular substrate deficiency. Besides dietary L-arginine supplementation—which seems to have only a limited effect, perhaps because of the presence of transport inhibitors—boosting substrate

availability in CKD might be achieved by supplementation with citrulline, the substrate for L-arginine biosynthesis, and by inhibition of arginase.

Increased plasma levels of ADMA in CKD and ESRD are emerging as major cardiovascular risk factors, and are probably primary causes of the endothelial dysfunction associated with renal disease. Reducing ADMA concentrations is an important therapeutic goal. Data conflict as to whether hemodialysis removes ADMA. Most groups report significant removal of ADMA by this modality^{60,110,111} whereas Kielstein and colleagues reported low rates of elimination,¹¹² which they attribute to plasma proteins binding ADMA. Boosting the activity of DDAH enzymes (e.g. using antioxidants) is a promising therapeutic goal. Nevertheless, ADMA probably represents the 'tip of the iceberg' in regulation of NO production and specific targeting of DDAH enzymes might leave many unidentified inhibitory agents active.

Key Points

- Production of nitric oxide (NO; from L-arginine and O₂ via nitric oxide synthase [NOS]) is reduced in renal disease
- As chronic NOS inhibition produces hypertension and renal dysfunction in animals, NO deficiency probably contributes to progression of kidney disease in humans
- Net NO deficiency can develop in response to decreased substrate availability (e.g. perturbed synthesis or transport of arginine) and the action of endogenous NOS inhibitors (e.g. asymmetric dimethylarginine)
- Potential therapies for kidney disease include supplementation of dietary L-arginine, inhibition of non-NO-producing biochemical processes that consume L-arginine, and decreasing the activity of asymmetric dimethylarginine

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Glossary

K_m

Michaelis–Menten constant substrate concentration required for an enzyme reaction to proceed at 50% of maximum

HISTONES

Simple water-soluble proteins containing basic groups that combine with nucleic acids and are complexed with DNA in chromatin

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Figure 1.

The 24-h output of NO₂ + NO₃ (i.e. NO_x, the stable oxidation products of NO) in subjects with normal renal function (control), patients with chronic kidney disease and approximately 25% residual renal function, and patients with end-stage renal disease undergoing peritoneal dialysis or hemodialysis. Data obtained from references 2–4. *, significant difference ($P < 0.05$ vs control).

CKD, chronic kidney disease and approximately 25% residual renal function; ESRD, end-stage renal disease; HD, hemodialysis; PD, peritoneal dialysis.

Figure 2.

Simplified schematic of the biosynthetic pathway for nitric oxide *in vivo*. Reproduced with permission from reference 113 © (2005) Elsevier. ADMA, asymmetric dimethylarginine; NO, nitric oxide; NOS, nitric oxide synthase.

Figure 3.

Effects on L-arginine transport in human dermal microvascular endothelial cells, human glomerular endothelial cells and bovine aortic endothelial cells of 6 h incubation with a 1 in 5 dilution of plasma from healthy subjects (control), or from patients undergoing peritoneal dialysis or hemodialysis. Measurements were made in the baseline state (solid histograms) or in the presence of 5 mmol/l of the endogenous nitric oxide synthase inhibitor *N*-monomethyl-L-arginine (open histograms). Results show the mean $\pm$ SEM of three separate experiments, each performed in triplicate. Reproduced with permission from reference 26 © (2001) American Physiological Society. *, significant difference ($P < 0.01$ vs control); #, significant difference ($P < 0.05$ vs pre-hemodialysis and control).

BAEC, bovine aortic endothelial cells; HD, hemodialysis; HDMEC, human dermal microvascular endothelial cells; HGEC, human glomerular endothelial cells; PD, peritoneal dialysis.

Figure 4. Pathways of L-arginine metabolism. The enzyme at 1 is arginine decarboxylase, at 2 is arginase, and at 3 is L-arginine:glycine amidinotransferase. Reproduced with permission from reference 22 © (1998) Portland Press.

Figure 5.

Relationship between the nitric oxide synthase activity of plasma and plasma concentrations of: the endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine, which is catabolized by dimethylarginine dimethylaminohydrolase; symmetric dimethylarginine, which is not a substrate of dimethylarginine dimethylaminohydrolase; and creatinine and blood urea nitrogen as indices of renal function. Reproduced with permission from reference 94 © (2001) Blackwell Publishing. *, significant difference ($P < 0.05$ vs control); #, significant difference ($P < 0.05$ vs chronic kidney disease I patients with normal nitric oxide synthase activity in plasma).

ADMA, asymmetric dimethylarginine; BUN, blood urea nitrogen; CKD, chronic kidney disease; Cr, creatinine; NOS, nitric oxide synthase; P, plasma; SDMA, symmetric dimethylarginine.

Figure 6.

Kaplan–Meier plot of cardiovascular event rate in patients with end-stage renal disease. Patients were stratified according to percentiles of asymmetric dimethylarginine plasma concentration at baseline, and followed for a mean of 33.4 months. Data obtained from reference 96. Reproduced with permission from reference 15 © (2003) Elsevier. ADMA; asymmetric dimethylarginine.