From rats to man: a perspective on dietary L-arginine supplementation in human renal disease

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Introduction

Over the last few years enthusiasm has grown for dietary L-arginine supplementation as a novel therapy to slow the progressive course of fibrotic renal disease [1,2]. Although human trials have started [3,4], the emerging picture of L-arginine in renal disease is still puzzling. In disease models characterized by increased renal blood pressure, dietary L-arginine supplementation improves renal haemodynamics and reduces the histological signs of kidney fibrosis [5–17]. In contrast, other data suggests that restriction of dietary L-arginine intake is beneficial and plays a key role in the therapeutic actions of dietary protein restriction [18,19]. The amino acid L-arginine is semi-essential and provides molecular substrate for generation of nitric oxide (NO), polyamines, L-proline and agmatine [1,2,20,21]. All of these L-arginine metabolites have been reported to be involved in renal pathology. NO released in small quantities from endothelial sites is a potent vasodilator. NO generated in cytotoxic quantities has been implicated in immune renal injury. Polyamines and L-proline are important for cell proliferation and fibrotic matrix build-up, both major processes in renal tissue repair.

In a rat model of anti-thymocyte-serum (ATS)-induced glomerulonephritis, we have recently completed a series of four studies comparing both L-arginine supplementation and restriction in the same model under the same conditions [18,19,22,23]. This model is characterized by a sequence of ‘injury’ and ‘repair’, which allows dissection of L-arginine effects on tissue injury and those on the fibrotic response which follows. In this review, we will, (i) give an overview of L-arginine metabolism and function, (ii) summarize our current understanding of the role of L-arginine intake in renal injury and repair and (iii) speculate on the potential use of L-arginine in the management of human renal fibrosis.

L-Arginine metabolizing pathways

Although L-arginine was discovered as a naturally occurring molecule more than 100 years ago, L-arginine research is still an active field of on-going discoveries and surprising results. In the beginning of our century, its occurrence in mammalian protein was demonstrated by Hedin [24]. In the early thirties, Krebs and Henseleit showed that L-arginine is essential for the generation of urea via the urea cycle [25]. In mammals, this is the only pathway to remove continuously generated toxic ammonia from the body. In 1939, Foster et al. showed that L-arginine is also required for the synthesis of creatine [26], which in its phosphorylated form is a crucial energy source for muscle contraction. Because it is cleared by the kidney, the spontaneous degradation product of creatine, creatinine, has become an important marker for renal function. In the eighties, it was discovered that endothelium derived relaxing factor (EDRF) is constitutively generated from L-arginine and that EDRF is the gas NO [1,2,20,21], a discovery awarded the 1998 Nobel Prize in Medicine. Just 5 years ago, a new L-arginine pathway producing agmatine was demonstrated in mammalian tissues [27]. Agmatine’s role in renal physiology and pathophysiology is just starting to emerge. We will give a brief overview of the L-arginine metabolizing pathways with special attention to their role in renal injury and repair. More detailed reviews on L-arginine and NO can be found elsewhere [1,2,21,28,29].

The L-arginine–NO pathway

L-Arginine is the main endogenous source for the generation of NO [1,2,21]. The non-protein amino acid L-citrulline is the co-product. In humans approximately 1% of the daily L-arginine intake is metabolized...
through this pathway [30,31]. The reaction is catalysed by a family of at least three structurally different dimeric enzymes called nitric oxide synthases (NOS), all of which are expressed in the kidney [1,20,21]. Generation of NO depends on the availability of L-arginine, molecular oxygen and reduced nicotinamide adenine dinucleotide phosphate (NADPH) as substrates. Cofactors are a cytochrome P-450 type heme moiety, calmodulin, flavin dinucleotide (FAD), flavin mononucleotide (FMA) and tetrahydrobiopterin (BH4). The generation of NO by all NOS isoforms can be inhibited by L-arginine analogs such as L-nitro-arginine-methyl-ester (L-NAME) or L-nitro-monomethyl-arginine (L-NMMA). Although some NOS isoform specific pharmacological blockade can be achieved with these compounds, truly isoform specific pharmacological NOS blockers are not yet available.

The NO generated from L-arginine is a highly reactive radical gas and is an important molecular messenger [1,20,21]. NO plays a critical role in numerous biological processes ranging from neurotransmission to vasodilatation, and inflammation to cell phenotype regulation (Figure 1). At low physiologic concentrations in vivo, NO acts locally as an important signalling molecule. NO has a greater diffusion coefficient than oxygen and rapidly diffuses through most cells without being utilized or reacting with intracellular molecules. Signalling of NO is mainly mediated by reactions with transition heme groups or critical thiol groups in various molecules and with free radical intermediates.

A prototype of the interaction of NO with heme groups is the binding and activation of the soluble form of guanylate cyclase, which increases cGMP-levels and mediates many of the biological effects of NO including vasodilatation, inhibition of platelet self aggregation and platelet adhesion to endothelium. The action of NO is locally restricted, since once in the vascular space, NO has only a half-life of a few seconds due to its rapid reaction with heme groups of haemoglobin. Some prolongation of its biological intravascular half-time is achieved by binding to specific sites of albumin and haemoglobin [2].

At high concentration in vivo, NO becomes a relatively non-specific effector molecule of the immune system with a key role in mediating host defense and, in the case of autoimmunity, self-destruction (Figure 1) [1,20,21]. Most of NO’s cytostatic and cytotoxic potential is ascribed to the irreversible reaction with superoxide anion (O2-) to produce the potent oxidant peroxynitrite (ONOO-). During inflammation, high output NO generation is often paralleled by increased production of superanions, thus promoting the formation of peroxynitrite. Peroxynitrite itself is a highly cytotoxic radical mediating massive oxidative injury. Peroxynitrite impairs the function of proteins by nitration of their tyrosine residues [32]. The formation of these nitrotyrosines can be detected by immunohistochemistry and has been used as an indicator for the existence and activity of the tissue damaging NOS pathway. In addition through peroxynitrite, NO directly inhibits key enzymes of the respiratory chain and of DNA synthesis by nitrosylation of their iron-containing catalytic elements and can also directly cause fragmentation in DNA. Inducible NO production is also involved in the induction of apoptosis, a constitutively present cascade leading to programmed cell death [2,33].

**NOS isoforms**

The NOS isoforms were originally called neuronal, macrophage and endothelial NOS (nNOS, mNOS and eNOS) according to the cell type from which the NOS enzymes were first cloned and in which they were believed to be principally expressed [21,34]. We know now that there is considerable overlap in the distribution of the NOS isoforms. A new nomenclature has been proposed where nNOS is NOS I, mNOS is NOS II and eNOS is NOS III, according to the order in which they were discovered [34].

Both the neuronal and endothelial NOS are principally constitutively expressed [20,21]. Both isoforms produce a rapid but short-lived production of NO in small amounts for signalling purposes. Their activity is increased by increasing intracellular Ca2+ concentrations involving binding of calmodulin. Although eNOS and nNOS are categorized as constitutive enzymes, recent studies have shown that the mRNA and protein production of both enzymes is somewhat regulated [20,21]. For instance, NOS is transcriptionally altered by variations in salt intake and after chronic hypoxia. eNOS gene transcription changes in response to laminar shear stress, transforming growth factor-β (TGF-β), oestrogen and hypoxia.

**Endothelial NOS (eNOS, NOS III)**

NOS continuously released by endothelial cells, represents the most potent endogenous vasodilator known. Mice with a disrupted eNOS gene are hypertensive and more susceptible to cerebral stroke [35]. In the
kidney, endothelial NO synthesis is important in maintenance of regional vascular tone, renal blood flow and glomerular filtration rate (Figure 1) [1,2]. There is some evidence that NO via a pressure-independent mechanism limits mesangial cell proliferation and matrix production, including a down regulation of the central profibrotic cytokine TGF-β (Figure 2) [36–38]. Within the kidney, endothelial NO production is thought to be a critical homeostatic factor which counterbalances the vasoconstrictive actions of angiotensin II and diminishes angiotensin II driven hypertrophy and matrix production.

The L-arginine–eNOS paradox

In patients with essential or secondary hypertension and in certain animal models of renal disease, L-arginine administration has been shown to reduce blood pressure in parallel with increased NO and L-citrulline production [21,29]. However, the mechanism by which L-arginine supplementation increases endothelial NO synthesis is still unclear [29]. The half-saturating L-arginine concentration (K_m) for eNOS is in the rage of 1–10 μM. Plasma L-arginine concentrations in humans and rats are approximately 100 μM. The intracellular L-arginine concentrations of endothelial cells even when cultured in L-arginine-free medium is well above the K_m of eNOS. Thus, eNOS should always be saturated and not dependent on extracellular L-arginine supply.

Neuronal NOS (nNOS, NOS I)

NO produced by nNOS is an important neurotransmitter involved in memory, learning, sexual behaviour, fluid homeostasis and peripheral vascular resistance [20,21]. nNOS null mice are normotensive and less susceptible to cerebral ischaemic injury [35]. In the kidney, nNOS is found primarily in the macula densa and is involved in the control of glomerular haemodynamics via tubulo-glomerular feedback and the release of renin [1,2,33].

Macrophage-type NOS (mNOS, NOS II)

In contrast to nNOS and eNOS, mNOS is not normally present in most tissues and must be specifically induced leading to the term inducible NOS (iNOS) [20,21]. This isoform is not calmodulin-dependent and can be induced by pro-inflammatory stimuli such as bacterial lipopolysaccharides, tumour necrosis factor-α, interleukin-1 and γ-interferon. Induction of iNOS involves activation of the transcription factor NF-KB. Once activated, the iNOS enzyme produces NO in large quantities for prolonged time periods and its output depends on the extracellular L-arginine supply [23,29]. Since iNOS has to be transcriptionally activated, there is a delay of several hours between induction and subsequent NO synthesis of this isoform. Mice with disrupted iNOS genes are more susceptible to intracellular pathogens and show a blunted hypotensive response to LPS [21,35]. In the diseased kidney, iNOS expression is elevated in both infiltrating macrophages and renal mesangial and tubular cells [39]. Low level constitutive expression of iNOS is found in several renal tubule segments suggesting an uncharacterized homeostatic function of iNOS in these tissues [21].

The L-arginine–ornithine pathway

L-Arginine is also a substrate for a group of enzymes called arginases (Figure 2) [2,40]. While arginase I is mainly expressed in the liver as part of the urea cycle, arginase II is expressed in macrophages and in kidney tissues [40]. Arginases metabolize L-arginine to the non-protein amino acid L-ornithine and urea (Figure 2) [28,41]. Generation of L-ornithine is the first step in the synthesis of polyamines and L-proline.

The L-ornithine–polyamine pathway

The polyamines, putrescine, spermine and spermidine, are aliphatic molecules required for cell replication and differentiation and thus are necessary for the proliferative element of tissue repair. In mammals, polyamines are generated from L-ornithine via the enzyme ornithine decarboxylase (ODC), which is the major regulatory enzyme in polyamine synthesis (Figure 2) [2,28,41]. ODC activity is low in quiescent cells and high in rapidly dividing cells. A rapid and substantial increase of ODC activity is one of the earliest events following the growth-stimulation of resting cells. ODC activity is highly regulated by a number of factors, but is also dependent on the availability its precursors L-arginine and L-ornithine. In a mouse model of carcinogenesis, it was shown that dietary L-arginine restriction lowered tissue L-arginine, L-ornithine and polyamine

Fig. 2. L-Arginine metabolism in renal tissue repair.
levels and was associated with significantly reduced tumour growth [42].

The L-ornithine–L-proline pathway

L-Ornithine is also metabolized by ornithine-amino transferase (OAT) and P5C reductase to form L-proline (Figure 2) [2,28,41]. L-Proline accounts for approximately 22% of the amino acid residues in mammalian collagens. It is, however, unclear whether collagen synthesis and build-up can be directly altered by dietary manipulations in L-arginine. Consistent with a role for dietary L-arginine in L-proline synthesis are data showing that liver OAT activity is regulated by dietary L-arginine intake [43]. In cultured cartilage a minimum of 20% of the L-proline incorporated into collagen is generated from L-ornithine [44]. In hormonally stimulated rat mammary glands, L-arginine is the major precursor for L-proline used in synthesis of breast milk proteins and the enzyme cascade arginase, OAT and P5C reductase is activated [45].

Competition between NOS and arginase pathway

Because both NOS and arginase utilize L-arginine as substrate, competition between these pathways may play a role in the course of renal diseases [2,46,47]. Persistent expression of iNOS may favour continuous injury, while stimulation of arginase activity may support tissue repair. Exemplary studies in dermal wounding have shown that the initial injury is characterized by a marked induction of iNOS and concomitant high NO production [48,49]. This is followed by an increase in arginase activity which progressively depletes the local environment of L-arginine and shifts L-arginine metabolism toward polyamine and proline synthesis, thus favouring tissue repair. In ATS-induced glomerulonephritis, we have shown that L-arginine metabolism is sequentially activated in a similar manner [50]. Early mesangial cell lysis was associated with iNOS induction while the later repair phase was associated with arginase activation and increased expression of the ODC and OAT genes.

The L-arginine–agmatine pathway

Recently, using bovine brain tissues, a new L-arginine metabolizing pathway producing agmatine was discovered in mammalian tissues [1,27]. The existence of this pathway in bacteria and plants has been known for a long time. Agmatine is decarboxylated L-arginine and the catalyzing enzyme is called L-arginine decarboxylase (ADC). Agmatine has clonidine-displacing activity and like clonidine, can bind to and activate both alpha-2 adrenoceptors and imidazol-guanidine receptors [27]. ADC activity is high in normal rat kidney [51]. Using cortical kidney homogenates incubated with radiolabelled L-arginine, agmatine generation was less than ornithine/urea synthesis but much greater than NO/citrulline synthesis [52]. Local micro-infusion of agmatine into renal interstitium increased glomerular filtration and tubular reabsorption [53]. In vitro, agmatine antagonized L-arginine metabolism through iNOS and ODC, while stimulating endothelial NOS synthesis [54–56]. In order to know the importance of this pathway in normal and diseased kidneys and the role of L-arginine intake on this pathway a number of questions must be answered. These include: (i) does the activity of ADC depend on endogenous or extracellular L-arginine concentration, (ii) what factors increase or decrease ADC activity, (iii) do ADC activity and agmatine concentrations change during renal injury and repair and (iv) does ADC compete with NOS and arginase for substrate? Until these questions are answered, it remains possible that some of the observed effects of dietary L-arginine restriction or supplementation are mediated through its metabolite agmatine.

Endocrine functions of L-arginine

L-Arginine has also been shown to enhance the release of a number of hormones, including corticotrophin releasing hormone, prolactin, growth hormone, somatostatin, insulin, glucagon, cortisone and aldosterone, some of which have been implicated in tissue fibrosis [1,2]. Parenteral administration of large quantities of L-arginine stimulates release of pituitary growth hormone and has been used for decades as a test for growth hormone deficiency in humans. With regard to the L-arginine–eNOS paradox discussed above, a recent study has shown that the blood pressure lowering effect of L-arginine administration is at least in part mediated through insulin release rather than through increased substrate availability for endothelial NO production [57].

Importance of dietary L-arginine intake

A special feature of L-arginine is that its intake is semi-essential. Under normal physiological conditions endogenous L-arginine synthesis is sufficient to maintain whole body L-arginine metabolic homeostasis and dietary L-arginine intake is dispensable [1,2,58,59]. Endogenous net L-arginine synthesis occurs primarily in the proximal tubule of the kidney using the non-protein amino acid L-citrulline as precursor [60]. Minor endogenous L-arginine synthesis has been observed in endothelial cells and macrophages [1,2]. However, the rate of endogenous L-arginine production is constant and in conditions of increased demand such as growth, tissue inflammation or wound healing, L-arginine intake may become critical [48,59]. Studies in dermal wound healing and renal disease have shown that local L-arginine levels markedly decrease [8,23,48], consistent with the concept that exogenous dietary L-arginine supply may become rate-limiting to the L-arginine pathways.
Dietary L-arginine in renal injury and repair

Impact of injury and repair on renal fibrosis

A hallmark of both experimental and human chronic renal disease is the progressive expansion of extracellular matrix, which impairs renal function and leads to organ failure [61]. It is our current hypothesis that the extent of renal fibrosis results from two major variables: the extent of tissue injury and the activity of the tissue repair process which follows [33,62]. Well-known injurious stimuli to the kidney are increased blood pressure, hyperglycaemia or autoimmune tissue destruction. The degree of the resulting tissue injury depends on the frequency, duration and severity of these injurious stimuli.

Injury to the kidney leads to a predictable repair reaction, characterized by matrix expansion [61]. Over-expression of the profibrotic cytokine TGF-β has been identified as a key factor mediating, orchestrating and promoting the activity of the molecular cascades leading to expansion of extracellular matrix in various organs (Figure 3). TGF-β strongly induces the synthesis of most matrix proteins, inhibits matrix degradation and stimulates local expression of matrix binding receptors. Blocking the actions of TGF-β with neutralizing antibodies has been shown to prevent excessive matrix accumulation after injury in a number of tissues including the kidney [61,63]. A number of factors known to be injurious to the kidney at the cellular level have now been shown to directly induce TGF-β over-production, including angiotensin II, mesangial cell stretch, fluid shear stress, high glucose levels, hypoxia, immune-complexes, protein trafficking, platelet derived growth factor and TGF-β itself (Figure 3) [62]. The degree of TGF-β over-expression generally correlates with the rate of matrix accumulation. Mesangial cell lysis in ATS-induced glomerulonephritis is a strong and rapid inducer of glomerular TGF-β overproduction and subsequent matrix production. Other stimuli such as increased blood sugar moderately increase renal TGF-β production resulting in a slow rate of matrix accumulation. The same appears to be true in human disease. Rapidly progressing diseases, such as HIV-nephropathy, show marked over-expression of TGF-β, while slowly progressing diabetic nephropathy shows more slowly increasing TGF-β over-expression [64].

In addition to the severity and the nature of tissue injury, the degree of TGF-β over-expression and the subsequent matrix deposition are influenced by factors directly interacting with the repair process [33,62,65]. Over the last years, the octapeptide angiotensin II has been identified as key factor determining the activity of TGF-β and the repair process [65]. In addition to its impact on hypertensive tissue injury, there is substantial evidence that angiotensin II directly induces and promotes TGF-β over-expression, which in turn promotes further matrix expansion [66,67]. Very recent data have shown that angiotensin II prevents the normal action of TGF-β to down-regulate its own receptors [68] which suggests that angiotensin II may prevent TGF-β from self-limiting the repair response and could be a key event in shifting acute wound repair to progressive tissue fibrosis. In addition, the direct action of angiotensin II on TGF-β over-expression may well explain the experimental and human experience that pharmacological angiotensin II blockade prevents renal fibrosis more effectively than other anti-hypertensive drugs. Another important determinant of ongoing matrix accumulation seems to be insufficient endogenous production of the proteoglycan decorin, a naturally occurring TGF-β antagonist [69]. Experimental strategies supplying decorin have been shown to lead to a direct and injury-independent decrease in TGF-β over-expression [70,71].

Thy 1 glomerulonephritis

While most experimental renal diseases involve continual and overlapping cycles of tissue injury and repair, the model of ATS-induced glomerulonephritis involves a sequence of distinct phases of ‘injury’ and ‘repair’, making it amenable to dissection of effects on both processes. Injection of an antibody to a Thy 1-like epitope causes dose-dependent complement-mediated lysis of a portion of glomerular mesangial cells [33]. This ‘injury’ is followed by a marked upregulation of TGF-β and a fibrotic ‘repair’ phase with glomerular matrix expansion. Although the injury, namely antibody-mediated mesangial cell lysis, is not shared by all renal diseases, the histological and molecular pattern of the acute repair reaction is very similar to that found in both chronic experimental and human renal disease [62]. Acute and chronic, experimental and human kidney disease, all share the feature that, where there is tissue fibrosis there is over-expression of TGF-β. Thus, we believe that studies on matrix expansion in ATS-nephritis are relevant for human renal fibrosis. This view is supported by recent studies showing that angiotensin II blockade and dietary protein restriction, both of which are anti-fibrotic in chronic experimental renal disease and slow the

Fig. 3. Central position of TGF-β in tissue injury (top) and tissue repair (bottom) [62]. Abbreviations used are: AGE’s, advanced glycosylation end-products; MCP-1, macrophage chemoattractant protein-1; PDGF, platelet derived growth factor and TGF-β, transforming growth factor-β.
progression of chronic human renal insufficiency, also markedly limit TGF-β over-expression and matrix accumulation in acute ATS-induced glomerulonephritis [33,72].

Using the ATS-induced model of glomerulonephritis, we have recently completed a series of four studies, comparing and contrasting the effects of both L-arginine restriction and L-arginine supplementation in the same model and under very similar conditions [18,19,22,23]. With major emphasis on therapeutic potential, we related both restriction and supplementation of dietary L-arginine intake to the two major determinants of renal fibrosis: (i) ‘injury’ (here ATS-induced mesangial cell lysis) and (ii) ‘repair’ (TGF-β over-expression and matrix accumulation).

In the case of L-arginine restriction, L-arginine intake was reduced to the level found in low protein diet (6% casein). In the case of L-arginine supplementation, 1% L-arginine was given in the drinking water. This supplementation protocol has been used frequently in previous studies and increases L-arginine intake in rats on a normal protein diet approximately 4-fold. To investigate the effects of L-arginine on renal injury, rats were pretreated with L-arginine restriction or supplementation for 7 days before ATS injection. The severity of ATS-induced mesangial cell lysis was examined 4–6 h after disease induction, where previous studies had shown that mesangial cell lysis was almost complete. To investigate the effects of L-arginine on renal repair, L-arginine restriction or supplementation were started after disease induction and continued for 6–7 days, a time point when TGF-β over-expression and rate of matrix expansion is maximally induced.

### Dietary L-arginine in renal injury

In renal tissue injury, the role of dietary L-arginine intake is primarily defined through its metabolite NO and both detrimental and protective actions of modifying L-arginine intake have been shown (Figure 1) [5–18,23]. While enhanced endothelial NO production appears to mediate protective effects, L-arginine metabolized through the high output iNOS isoform seems to be a critical effector molecule in immune mediated tissue damage.

### Dietary L-arginine restriction in immunologic renal injury

A central feature of immune-mediated tissue injury is a marked local de novo expression of iNOS and a high NO production, which has been demonstrated in models of immune-complex glomerulonephritis, ATS-induced glomerulonephritis, lupus nephritis and transplant rejection [18,23,46,73–76]. In ATS-induced glomerulonephritis, our laboratory has shown that the initial mesangial cell lysis is mediated through cytotoxic amounts of NO produced by iNOS (Figure 4A) [18]. In rats with short-term pretreatment of the NOS inhibitor L-NMMA before ATS administration, mesangial cell lysis was inhibited by approximately 90%. The number of infiltrating macrophages was not affected by L-NMMA treatment. A similar critical role of inducible NO production in renal tissue injury has been shown in models of acute ischaemic renal failure and lupus nephritis [74,77] and has been suggested in experimental kidney transplant rejection [73].

In our first study, we found that restriction of L-arginine intake in the form of low protein feeding significantly prevented ATS-induced mesangial cell lysis as well (Figure 4B) [18]. Addition of L-arginine to the drinking water to normalize L-arginine intake increased mesangial cell lysis to the same extent seen in animals on a normal protein diet. Although parameters of NO synthesis were not measured, these findings suggest that dietary L-arginine restriction limits glomerular tissue injury by limiting the substrate for cytotoxic NO generation, a mechanism which may have great importance in the beneficial effects of low protein diets in renal disease [2,18].

### Dietary L-arginine supplementation in immunologic renal injury

In our second study, 1% L-arginine in the drinking water was given rats on a normal protein diet [23]. We were uncertain whether L-arginine supplementation would worsen ATS-induced glomerular injury. Studies by Klahr’s group have shown that L-arginine supplementation may have important anti-inflammatory actions [7]. In models of obstructive nephropathy and puromycin-induced nephrosis, L-arginine feeding markedly reduced macrophage infiltration and increased endogenous corticoid production, both of which may be protective in inflammatory tissue injury.

However, confirming and expanding our initial study, we found that supplementing L-arginine intake before ATS-injection significantly increased mesangial cell lysis measured at 6 h compared to nephritic rats with normal L-arginine intake (Figure 4C) [23]. In additional groups of rats, L-arginine administration was stopped 16 h after antibody injection to let the repair process proceed with normal L-arginine intake. The fibrotic response, as measured by glomerular TGF-β over-expression and histological matrix accumulation, was significantly increased 6 days after disease induction as compared to disease control animals that had never been exposed to high L-arginine. This finding is consistent with the concept that the extent of repair reflects the degree of the initial injury. Interestingly, L-arginine administration did not appear to reduce the inflammatory response following ATS-injection since macrophage infiltration and glomerular iNOS and protein levels did not differ in L-arginine supplemented and non-supplemented rats. However, 6 h after disease induction, plasma levels of L-arginine and nitrate/nitrite, the stable end products of NO, were significantly higher in L-arginine supplemented rats than in those drinking tap water only. In addition, NO production by cultured glomeruli harvested 6 h after ATS-injection was highly dependent on the extracellular
l-arginine concentration with a marked increases in NO production between the l-arginine concentrations seen in nephritic rats without and with l-arginine supplementation. These results strongly suggest that l-arginine supplementation can worsen tissue injury and subsequent fibrosis in immune-mediated renal disease through a substrate-dependent mechanism. Similar detrimental effects of l-arginine administration have been demonstrated in experimental acute tubular necrosis, immune-mediated dermal vasculitis, hypoxic lung injury, toxic colitis and brain reperfusion [78–82]. These models appear to share the feature that NO synthesis is greatly increased through induction of iNOS.

In conclusion, our studies on manipulations of dietary l-arginine intake are consistent and reveal that l-arginine restriction limits and l-arginine supplementation augments mesangial cell injury (Figure 4B and C). The data show that this occurs through a substrate-dependent effect on NO production by iNOS.

**Immune-complex glomerulonephritis**

While studies of ATS-induced glomerulonephritis, lupus nephritis, transplant rejection and acute tubular necrosis point to a critical role for iNOS in renal tissue damage, experimental immune-complex glomerulonephritis appears to be an exception. While there is substantial evidence that iNOS is expressed and active in this model of kidney disease [46,75,76,83,84], the high NO production does not seem to contribute to tissue damage. Experiments designed to decrease NO production via iNOS, including the use of systemic arginase administration to deplete l-arginine levels and the use of non-specific NOS inhibition showed increased signs of renal damage [85,86]. Consistent with these findings are recent data demonstrating that genetic disruption of the iNOS gene in mice does not ameliorate anti-glomerular basement membrane glomerulonephritis [87].

**Dietary l-arginine in hypertensive renal injury**

Beneficial effects of dietary l-arginine supplementation have been reported in models of renal disease characterized by increased renal blood pressure where there is likely to be a component of hypertensive tissue injury. l-Arginine supplementation has been reported to improve impaired renal haemodynamics in 5/6 nephrectomy, ureteral obstruction, puromycin-induced nephrosis and nephropathy secondary to diabetes, salt-sensitive hypertension, cyclosporin, radiocontrast drugs or ageing [5–17]. This reduction in hypertensive injury is reflected in a reduction in the fibrotic repair response in 5/6 nephrectomy, ureteral obstruction and nephropathy secondary to salt-sensitive hypertension, cyclosporin or ageing [5,9,11,15,16]. Similar to the vascular dysfunction seen in essential and secondary hypertension, these models seem to share the feature of chronically deficient renal NO synthesis [2].

Glomerular hypertension contributes to tissue injury in many chronic renal disorders. Such injury is not generally characterized by increased expression of inducible NOS. Dietary l-arginine supplementation is believed to overcome chronic NO deficiency by enhancing endothelial NO production, increasing vasodilation and thereby limiting hypertensive injury to the kidney (Figure 1). This view is supported by data indicating increased NO synthesis following l-arginine administration in models of subtotal nephrectomy, ureteral obstruction and Dahl/Rapp nephrosclerosis. In ureteral obstruction and in salt-sensitive Dahl/Rapp rats, l-arginine administration also reduced arterial pressure [7,88]. In rats with 5/6 nephrectomy, l-arginine supplementation normalized glomerular pressure [6]. Thus, the main therapeutic effect of l-arginine
supplementation in these models appears to be a reduction in hypertensive tissue injury which in turn leads to less subsequent renal fibrosis.

**Dietary L-arginine in renal tissue repair**

As mentioned above, the ATS model is characterized by a distinct 'repair' phase. Although glomerular haemodynamic changes have been reported following ATS-injection, the degree of the fibrotic response is mainly attributed to the degree of immunologic injury. In our third and fourth study, both L-arginine restriction or supplementation were started after diseases induction [19,22], when mesangial cell lysis had occurred and the same degree of tissue injury had been achieved. After 6–7 days, a time where previous studies had demonstrated that TGF-β over-expression and the rate of matrix expansion is maximal, therapeutic effects were determined.

**Dietary L-arginine restriction in renal tissue repair**

In the third study, we found that restriction of L-arginine intake in rats on isocaloric normal protein diet limited glomerular TGF-β and matrix accumulation comparable to that seen in nephritic rats fed a low protein diet [19]. In contrast, when low protein was combined with L-arginine in the drinking water to normalize L-arginine intake, the beneficial effect of protein restriction was abolished. Though it has yet to be proven, we speculated that this effect of L-arginine restriction on repair may be due to local limitation of substrate for polyamine and L-proline production which may become rate-limiting for the proliferative and fibrotic repair responses (Figure 2). Together with the beneficial action of L-arginine restriction on immune tissue injury, these data strongly suggest that L-arginine is a key amino acid in the therapeutic actions of low protein diets.

**Dietary L-arginine supplementation in renal tissue repair**

In the fourth study, we determined the effect of supplementing 1% L-arginine in the drinking water on the repair of ATS glomerulonephritis [22]. Because we had shown anti-fibrotic effects of dietary L-arginine restriction [19] and others had reported anti-fibrotic effects of dietary L-arginine supplementation in other models of renal disease [5,9,11,15,16], we were uncertain whether increasing dietary L-arginine intake after ATS-injection would worsen or ameliorate the glomerular fibrotic response.

We found that L-arginine supplementation started after mesangial cell lysis had occurred, significantly reduced glomerular TGF-β over-expression and matrix in animals fed a normal protein diet [22]. The anti-fibrotic effect was similar to that seen in nephritic rats fed a low protein diet. Combined therapy of low protein diet and 1% L-arginine produced additional reductions in pathological glomerular TGF-β over-expression and matrix expansion. Given the anti-proliferative, anti-fibrotic and TGF-β-reducing potential NO has shown in vitro (Figure 2) [36–38], this therapeutic action may be mediated through increased endothelial NO production. However, indices for NO synthesis (glomerular NO production, plasma and urinary NO levels) did not differ between diseased animals on the same diet with and without L-arginine, although L-arginine supplementation significantly increased arterial L-arginine levels. It may be that in a normotensive model such as ATS nephritis, the impact of L-arginine supplementation on endothelial NO synthesis is below the detection limit of our methods, but it may also be that other pathways are involved.

Taken together, in rats following induction of mesangioproliferative glomerulonephritis both dietary L-arginine restriction and L-arginine supplementation significantly limit glomerular TGF-β over-expression and subsequent matrix accumulation. Because normal L-arginine intake produces more severe disease than either restriction or supplementation, these data suggest a bell-shaped effect of L-arginine intake on the renal repair process.

**From rats to man**

L-Arginine research in rat models has improved our knowledge and understanding of renal physiology, pathophysiology and pathology. Although animal models give us critical information on the potential of new therapeutic approaches, it remains hazardous to extrapolate experimental results to human renal disease. With this precaution in mind, we will speculate on the therapeutic potential of modifying dietary L-arginine intake for human renal disease.

**L-Arginine metabolism in humans**

All of the L-arginine metabolizing pathways expressed in rodents, are expressed in humans, including eNOS, bNOS, iNOS, arginase, ODC, OAT and ADC. Thus, L-arginine effects seen in rodent models of renal disease should be very similar in humans. However, there is some debate whether the iNOS enzyme is actually functioning in humans. The discussion results from early data showing that rodent cells in vitro produce large amounts of NO in response to simple immunologic stimuli, while human macrophages in vitro show little or no response [89]. However recently, evidence has accumulated demonstrating that human iNOS is active in vivo, but requires more specific immunologic stimuli which are difficult to mimic in vitro. An active iNOS enzyme has been demonstrated in several inflammatory human diseases including tuberculosis, urinary tract infection, inflammatory arthritis, leishmaniosis and immunodeficiency virus (HIV) type 1 encephalitis and renal transplant rejection [89–95]. Also, evidence for an active tissue damaging NO pathway in humans has been provided by immunohistochemical detection of nitrotyrosine [32], with demonstration of increased staining in a number of human diseases, including atherosclerosis, myocardial...
ischaemia, inflammatory bowel disease, septic lung disease, renal manifestations of Wegener’s granulomatosis and kidney transplant rejection [32,95,96]. Furthermore, induction of the iNOS enzyme has been demonstrated in a number of immunologic human renal diseases (Table 1) [95–99]. Interestingly, in the studies by Kashem et al. and Furusu et al. the degree of iNOS expression correlated with the severity of histological signs of renal damage supporting the concept that a destructive iNOS pathway is operating in human disorders [97,98].

Are we ready for human trials?

Although angiotensin II blockade and dietary protein restriction slow the rate of deterioration, halting the progressive course of chronic human renal disease remains one of the greatest challenges in nephrology. Thus, it may not be surprising that the positive effects of l-arginine administration in experimental renal diseases has resulted in some enthusiasm for human trials. However, as outlined in this review, dietary l-arginine supplementation has the potential both to protect and to harm the kidney. Animal data has provided substantial evidence that in renal diseases where iNOS is increased, l-arginine supplementation can worsen renal injury and the subsequent fibrotic response through enhancing generation of cytotoxic NO suggesting a dark side of l-arginine supplementation. This is particularly relevant to immune-mediated inflammatory disorders, where the use of l-arginine supplementation as a therapy may be potentially harmful and l-arginine restriction may be warranted.

On the other hand, l-arginine supplementation may be useful in some human renal diseases, especially in later stages when inflammation is over and the progressive loss of renal function is mainly driven by glomerular hypertension. However, we believe that there are some critical questions which should be addressed before human trials begin. (i) In what renal disorders will l-arginine supplementation be safe? How is iNOS expressed in hypertensive and diabetic nephropathy? (ii) How effective can l-arginine supplementation be? The data in hypertensive models of renal disease suggests that l-arginine simply acts as a vasodilator via endothelial NO synthesis. Thus, it is relevant to ask how the beneficial effect of l-arginine supplementation compares to other blood pressure reducing drugs? How does the l-arginine effect compare to angiotensin II blockade in a side-by-side comparison? Will l-arginine supplementation result in an additional anti-fibrotic effect in the presence of angiotensin II blockade? (iii) Is l-arginine supplementation really feasible? To supplement dietary l-arginine in humans would require l-arginine in the range of 15–30 g/day which equals an additional daily protein intake of 15–30 g. Will this be practical in patients with chronic renal insufficiency who are supposed to restrict their daily protein intake to approximately 60 g? (iv) Is the beneficial effect of l-arginine supplementation indeed mediated through NO and can we mimic its therapeutic potential with NO donating drugs in doses designed to overcome deficient endothelial NO production, but far below NO levels involved in tissue injury? This strategy would overcome the detrimental effects of l-arginine supplementation in situations where iNOS is activated and would be easier to add to a low protein diet. If so, would we be advised to start a human trial testing these drugs rather than l-arginine supplementation?

Summary

Experimental studies have shown both therapeutic and detrimental consequences of modifying dietary l-arginine intake in renal diseases which likely reflect the complexity of l-arginine metabolism. l-Arginine intake is semi-essential and provides substrate for a number of l-arginine metabolites involved in renal pathology. Dietary l-arginine restriction has been identified as a key mediator of the beneficial effects of low protein diets on human renal fibrosis. Supplementing dietary l-arginine in renal diseases with increased iNOS expression appears to be detrimental and thus, may be harmful in immune-mediated human kidney disorders. Increasing l-arginine intake is beneficial in experimental models of hypertensive renal disease. Based upon available data, we believe additional questions must be answered experimentally, not only to prevent an adverse outcome in humans, but to enhance our chances of human trials which will result in substantially better amelioration of disease than currently available.

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References


<p>| Table 1. Human renal disease with increased iNOS expression |</p>
<table>
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<tr>
<th>Disease</th>
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<tbody>
<tr>
<td>IgA nephropathy</td>
<td>[97,98,99]</td>
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<tr>
<td>non-IgA mesangiproliferative glomerulonephritis</td>
<td>[97]</td>
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<tr>
<td>Wegener’s granulomatosis</td>
<td>[96]</td>
</tr>
<tr>
<td>Transplant rejection</td>
<td>[99]</td>
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<tr>
<td>Lupus nephritis</td>
<td>[97,98]</td>
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