# Nitric Oxide, Oxidative Stress, and Progression of Chronic Renal Failure

By Paul S. Modlinger, Christopher S. Wilcox, and Shakil Aslam

**Cellular injury or organ dysfunction from oxidative stress occurs when reactive oxygen species (ROS) accumulate in excess of the host defense mechanisms. The deleterious effect of ROS occurs from 2 principal actions. First, ROS can inactivate mitochondrial enzymes, damage DNA, or lead to apoptosis or cellular hypertrophy. Second, nitric oxide (NO), which is a principal endothelial-derived relaxing factor, reacts with superoxide anion ($O_2^-$) to yield peroxynitrite ($ONOO^-$), which is a powerful oxidant and nitrosating agent. The inactivation of NO by $O_2^-$ creates NO deficiency. Oxidative stress can promote the production of vasoconstrictor molecules and primary salt retention by the kidney. Several hypertensive animal models showed increased activity of nicotine adenine dinucleotide phosphate (NADPH) oxidase, which is the chief source of $O_2^-$ in the vessel wall and kidneys. NO regulates renal blood flow, tubuloglomerular feedback (TGF), and pressure natriuresis. Animal models of NO deficiency develop hypertension, proteinuria, and glomerulosclerosis. Evidence is presented that chronic renal failure (CRF) is a state of NO deficiency secondary to decreased kidney NO production and/or increased bioinactivation of NO by $O_2^-$. Patients with CRF show decreased endothelium-dependent vasodilatation to acetylcholine, have increased markers of oxidative stress, and diminished antioxidant activity. Therapy for oxidative stress has focused on antioxidants and agents that modify the renin-angiotensin system. The effects of such treatments are more compelling in animal models than in human studies.**



OXIDATIVE STRESS occurs when the formation of reactive oxygen species (ROS) exceeds the body's ability to metabolize them. The most important ROS include superoxide anion ($O_2^-$), hydrogen peroxide ($H_2O_2$), and hydroxyl radical ($OH^-$). Oxidative stress causes cell injury. It has been linked to the development of atherosclerosis, hypertension, neurodegenerative disease, aging, and malignancy. As a consequence of these conditions, the formation of ROS is increased, leading to a potential positive feedback response. Oxygen radicals inactivate mitochondrial enzymes, directly damage DNA and DNA repair enzymes and transcription factors, and lead to cell death.[1,2] ROS-mediated lipid peroxidation is a first step in the development of atherosclerotic disease, by initiating inflammation and foam cell formation.[1,3,4] ROS activate critical transcription factors including nuclear factor $\kappa$B, activator protein-1 (AP-1) and hypoxia inducible factor-1$\alpha$ (HIF-1$\alpha$), which have widespread effects including an increase in vascular smooth muscle cell (VSMC) hypertrophy and hyperplasia.[5]

A second important effect of $O_2^-$ is the inactivation of the endothelium-derived relaxing factor nitric oxide (NO). NO increases renal blood flow, blunts tubuloglomerular feedback (TGF), and promotes pressure natriuresis. $O_2^-$ reacts with NO to form a very powerful oxidant and nitrosating agent, peroxynitrite ($ONOO^-$). $ONOO^-$ oxidizes lipids, DNA, and proteins, and can inactivate proteins by forming 3-nitrotyrosine (3-NT) residues.[1] This reaction of $O_2^-$ generates toxic molecules while diminishing the generally protective functions of NO.

Oxidative stress may contribute to the progression of renal disease indirectly by promoting hypertension and atherosclerosis or directly by inducing glomerular damage and renal ischemia. Much interest centers on the concept that the dramatic increase in mortality in patients with chronic renal failure (CRF) may be secondary to the deleterious cardiovascular effects of oxidative stress.[6] This review focuses on the role of NO as a regulator of kidney function, the ability of oxidative stress to alter kidney function, and the evidence that renal insufficiency is a state of increased oxidative stress and functional NO deficiency that contributes to

*From the Division of Nephrology and Hypertension and the Cardiovascular-Kidney Institute, Georgetown University, Washington, DC.*

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*Address reprint requests to Christopher S. Wilcox, MD, PhD, Division of Nephrology and Hypertension, Georgetown University Medical Center, 3800 Reservoir Rd, NW, PHC F6003, Washington, DC 20007. Email: wilcoxch@georgetown.edu*



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progressive damage of the kidneys and cardiovascular system.

## NO: REGULATOR OF KIDNEY FUNCTION

NO is produced from L-arginine by nitric oxide synthase (NOS). This enzyme is expressed as 3 isoforms, all of which have been isolated from the kidney: endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). iNOS has low basal expression until activated by an immune response, whereas eNOS and nNOS are expressed constitutively, although both are regulated by specific factors. The kidney is unusual in having significant basal expression of iNOS, which may be a consequence of the production of cytokines by the tubules of normal kidneys. Moreover, the kidney produces 2 distinct but homologous transcripts for iNOS.[7] eNOS has been localized to the endothelial cells of the renal vasculature, including the glomerulus, arcuate vessels, and arterioles, and to a lesser extent to tubular cells.[8] nNOS is heavily expressed in the macula densa cells of the juxtaglomerular apparatus but also is expressed in Bowman's capsule, efferent arterioles, thick ascending limbs, and collecting ducts.[9-11] iNOS is expressed in vascular smooth muscle cells (VSMCs), mesangial cells, interstitial cells, tubular cells of the thick ascending limbs, inner medullary collecting ducts, cortical collecting ducts, proximal and distal tubules, and in intrarenal nerves.[10,12,13] For further details see Wilcox.[14]

Infusion of the NOS inhibitor, $N^G$-nitro-L-arginine methyl ester (L-NAME) into a normal rat causes systemic hypertension, an increase in afferent and efferent arteriolar resistance, and a decrease in filtration coefficient and single-nephron glomerular filtration rate.[15-17] Over time these animals develop proteinuria and glomerulosclerosis. L-NAME enhances the contraction of the afferent arteriole to angiotensin II (Ang II).[15] Moreover, micropuncture studies show that NO produced by nNOS in the macula densa antagonizes the vasoconstriction generated by the action of TGF on the afferent arteriole.[9] TGF is activated by chloride delivery and reabsorption at the macula densa, which leads to the release of mediators that include adenosine and adenoside triphosphate, which constrict the afferent arteriole and decrease the single-nephron glomerular filtration rate.[18] Wilcox et al[9] first showed that the release of NO by the macula densa occurs only during high NaCl delivery and reabsorption. Braam and Koomans[19] showed that NO opposes Ang II–induced vasoconstriction of the afferent arteriole.[19] Further studies have shown that NO blunts TGF only during normal or high salt intake.[20] Thus, NO can cause vasodilatation of the afferent arteriole, buffer its responses to vasoconstrictors, and defend against salt sensitivity during salt loading. These studies distinguish NO as a significant regulator, and in many cases protector, of renal blood flow, glomerular filtration rate, and salt and fluid homeostasis. NO becomes especially important under conditions of enhanced afferent arteriole vasoconstriction (congestive heart failure, cirrhosis, nonsteroidal inflammatory use) and during salt loading.

The ability of NO to protect kidney function is highlighted by recent studies by Gschwend et al[21] who used a 5/6 nephrectomy rat model of CRF. The renal insufficiency in this model is accompanied by hyperfiltration injury of the remaining nephrons and increased intraglomerular pressure. The investigators studied the endothelium-dependent vasodilatation of the interlobar artery at the time of nephrectomy. They found that the degree of this vasodilatation was correlated inversely to the animal's subsequent development of proteinuria and renal insufficiency. In contrast, endothelium-independent vasodilatation with sodium nitroprusside was not correlated to renal injury. They concluded that the endothelium, and its release of NO, protected rats from glomerular injury.

NO is critical for the kidney's ability to maintain salt and water homeostasis. NOS inhibitors, when infused locally into the kidney, reduce sodium excretion and urine flow, which can be reversed by an NO donor.[13] NO inhibits tubular NaCl transport in the thick ascending limbs,[22] collecting ducts, and proximal tubules.[13,14] NO also can influence salt and water handling in the kidney indirectly by regulating medullary blood flow. Infusion of L-NAME,[15] nNOS antisense, or local iNOS inhibition in the renal medulla[23] decreases medullary blood flow and causes salt-dependent hypertension. NO also increases renal interstitial hydrostatic pressure independent of medullary blood flow,[24] which diminishes tubular NaCl transport. The pressure natriuresis response itself is linked to kidney NO production.[13]

On the contrary some studies suggest that NO has antinatriuretic properties. Knockout mice lacking eNOS and iNOS have defective tubular

$NaHCO_3$ absorption and develop metabolic acidosis.[25] Normal rats infused systemically with NOS inhibitors have an induced diuresis and natriuresis, but this may be secondary to an increase in blood pressure. These conflicting studies exemplify the many sites at which NO can interact to alter salt and water excretion.[14]

## PRODUCTION OF NO IN CRF

L-arginine is a semi-essential amino acid. It is formed from citrulline, which is a product of amino acid metabolism in the gut wall.[26] L-arginine is converted from L-citrulline via argininosuccinate synthetase and argininosuccinate lysase predominantly in the proximal tubules of the kidney.[27,28] Although increased levels of citrulline have been documented in patients with CRF,[27] normal L-arginine levels are found in mild renal insufficiency, likely secondary to hypertrophy of the remnant proximal tubular cells.[8,27] Arginine levels are decreased in end-stage renal disease (ESRD).[29,30] ESRD places a limitation on total body L-arginine production and reduces L-arginine concentration.[30] Normally, plasma arginine levels are determined primarily by arginine degradation. Metabolism of arginine by arginase-1 in hepatic cells to ornithine and urea is limited by the expression of the cellular transporter cationic amino acid transferase (CAT)-2A.[31] The activity of NOS in the macula densa and kidney is limited during salt restriction not by nNOS or eNOS expression, which actually are increased,[32] but rather by arginine delivery and uptake via CAT-1.[20] Thus, studies in salt-restricted rats have shown that microperfusion of L-arginine, and its tubular uptake, enhances macula densa NO generation and blunts TGF.[20] Likewise, intrarenal L-arginine infusion and uptake via CAT increases renal blood flow selectively in salt-restricted rats.[33] Therefore, the generation, delivery, metabolism, plasma levels, and uptake of L-arginine into specific cells are regulated heavily[26] and determine the activity of NOS, which is a key enzyme for blood pressure and salt homeostasis. These facts led Kitiyakara et al[34] to propose that L-arginine be considered a hormone.

The kidney's central role in the production of the NO precursor, together with the anorexia and protein catabolism of advanced chronic renal insufficiency and the development of oxidative stress, place these patients at high risk for NO deficiency. However, the available data for total NO production in CRF are conflicting and suggest that NO deficiency is a regional rather than a global defect. On the one hand, studies of the platelet dysfunction of uremia in animal models of CRF have concluded that excess NO production inhibits platelet aggregation.[35] A study by Aiello et al[36] in a rat model of CRF found that, although there was decreased renal production of NO, there was an increase in total body NO production secondary to increased systemic vascular NOS activity. These investigators suggested that this upregulation of vascular NOS activity might be caused by guanidinosuccinate, which is a toxin that accumulates in CRF and can increase NO production in cultured endothelial cells. Such a finding also could account for the platelet dysfunction. Clearly, any increased NO production would be a protective mechanism against the development of hypertension in CRF. However, Vaziri et al[28,37] argued that it was the severe hypertension rather than renal failure in the animal model by Aiello et al[36] that increased vascular NOS. These investigators created a rat model of CRF with only mild hypertension and found significant reductions in both the renal and vascular production of NO. However, established hypertension is accompanied by endothelial dysfunction[38] and NO deficiency that can be ascribed to the effects of Ang II acting on $AT_1$ receptors[39] and to the development of oxidative stress.[40]

Studies in patients with CRF or ESRD show a substantial decrease in NO generation. Blum et al[41] studied 3 groups of patients with different kidney function and concluded that renal $NO_X$ excretion (metabolites of NO) correlated with creatinine clearance. However, plasma $NO_X$ levels were higher than in healthy controls. The investigators concluded that CRF is a state of NO deficiency, but that $NO_2$ and $NO_3$ accumulate in the plasma because of decreased renal excretion. Schmidt and Baylis et al[42,43] placed hemodialysis (HD) patients on a fixed $NO_X$ intake and accounted for all $NO_X$ elimination by measuring $NO_X$ in the urine and dialysate.[42] They concluded that NO generation was reduced substantially in ESRD.[43] These investigators also showed that patients receiving peritoneal dialysis have a 60% reduction in NO generation, a 25% reduction in plasma L-arginine levels, and a 5-fold increase in asymmetric dimethylarginine (ADMA), an endogenous NOS inhibitor.[30]

These investigators later studied factors in the plasma of dialysis patients that affected endothelial cells in culture. They reported that the plasma contained factors, including urea, that impaired L-arginine uptake into these cells, and factors, including ADMA, that impaired L-arginine metabolism by NOS.[44,45]

Probably the most accurate method to quantitate NO generation in humans is from the rate of conversion of administered [$^{15}$N]-L-arginine to [$^{15}$N]-L-citrulline or [$^{15}$N]-$NO_X$. Such studies have shown a 50% reduction in NO generation in patients with CRF[46] and a strictly similar reduction in patients with essential hypertension.[47] These findings collectively document a profound reduction in L-arginine availability and metabolism to NO in CRF, and raises the hypothesis that this may be mediated in a large part by the effects of prolonged hypertension. Indeed, in a recent study, Wang et al[48] contrasted endothelial function and NOS activity of small vessels dissected from fat biopsy specimens from humans with essential hypertension or autosomal-dominant polycystic kidney disease (ADPKD). Endothelial dysfunction and reduced NOS activity were found in patients with ADPKD even before development of hypertension or renal insufficiency. However, with the development of hypertension, these defects became more severe. The group with hypertension and azotemia were no more impaired than those with hypertension alone, and were similar to those with essential hypertension. These studies showed that ADPKD causes endothelial dysfunction and reduced NOS activity and that it is the development of hypertension, rather than azotemia, that leads to a sharp further decrease in function.

NO production may be decreased in patients with CRF secondary to increased plasma levels of ADMA. ADMA is a natural product formed by methylation of arginine. It is a competitive inhibitor of L-arginine uptake into cells and of L-arginine metabolism by NOS. Although some ADMA is excreted in the urine, the majority is metabolized by an enzyme dimethylarginine dimethylaminohydrolase that is coexpressed with NOS in endothelial cells of blood vessels and in renal tubular cells.[32] Infusion of ADMA into the brachial artery of healthy volunteers decreases forearm blood flow, most likely secondary to a decrease in local NO production.[29] In patients with CRF an increase in ADMA combined with a decrease in L-arginine may inhibit the production of NO.[29] If this hypothesis were true, endothelial dysfunction in CRF should be reversed by infusion of L-arginine whereas studies generally have shown little difference in the response to L-arginine in such patients.[49] This suggests that other factors, notably the effects of prolonged hypertension, dyslipidemia in those with the nephrotic syndrome, or the disease process itself in those with ADPKD, and especially the effects of oxidative stress, are the major contributors to the NO deficiency of CRF.

## THE IMPACT OF OXIDATIVE STRESS ON NO AND ENDOTHELIAL FUNCTION

The ability of NO to preserve endothelium-dependent vasodilatation depends not only on its production but also on its rate of bioinactivation. In a reduced cell state, NO can maintain endothelial function, scavenge the low concentrations of ROS, and terminate the lipid peroxidation radical cascade.[1] However, under conditions of oxidative stress, NO is depleted and $ONOO^-$ accumulates and can lead to a cascade that results in vasoconstriction, inflammation, and impaired vascular and renal function.

ROS are produced as a normal byproduct of mitochondrial enzymes and by nicotine adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase, various arachidonic acid monoxygenases including lipoxygenase and epoxygenase, and even NOS itself. NOS fails to complete the 2 electron transfers, and thereby generates $O_2^-$ rather than NO, when depleted of its substrate, L-arginine, or cofactor, tetrahydrobiopterin ($BH_4$).[50] Oxidative stress increases the conversion of $BH_4$ to dihydrobiopterin ($BH_2$), thus potentially perpetuating the production of ROS in endothelial cells. NADPH oxidase is the chief producer of $O_2^-$ in VSMCs[51,52] and kidney cortex and medulla.[53-56] NADPH oxidase in endothelial and VSMCs differs somewhat from the NADPH oxidase in phagocytes that generates the oxidative burst to destroy bacteria. Endothelial NADPH oxidase consists of 5 components. On activation, the cytoplasmic $p47^{phox}$ assembles with $p40^{phox}$ and $p67^{phox}$, which under the influence of rac, joins with $p22^{phox}$ and $gp91^{phox}$. VSMCs express a homologue of $gp91^{phox}$ called NOX-1. The normal kidney contains all these components as well as NOX-4.[56]

### Oxidative Stress in Animal Models of Hypertension

The inactivation of NO by ROS has been implicated in hypertension. Increased levels of ROS have been documented in humans with essential hypertension.[5] Animal models of hypertension have increased production of $O_2^-$ and increased NADPH oxidase activity. Zalba et al[57] showed that the blood vessels of the spontaneously hypertensive rat (SHR) have increased $O_2^-$ levels and up-regulation of $p22^{phox}$ messenger RNA, whereas in the kidney the primary up-regulation is $p47^{phox}$.[56] Up-regulation of NADPH oxidase and $O_2^-$ production in the kidneys occurs in mice infused with Ang II.[58,59] Interestingly, the hypertension, increased $O_2^-$ production, and increased NADPH oxidase expression in these animals is reversed by administering the $AT_1$ receptor antagonist losartan.[59] This suggests that NADPH oxidase is under hormonal control and implicates oxidative stress as a cause of hypertension in this animal model. Recently, we confirmed the presence of oxidative stress in mice undergoing a slow pressor response to Ang II.[58] Co-infusion of tempol, a cell permeant superoxide dismutase (SOD) mimetic, prevented the oxidative stress as well as the hypertension and the afferent arteriolar vasoconstriction. Recent studies in rats infused for 2 weeks with Ang II at a slow pressor rate show oxidative stress accompanied by up-regulation in the kidneys of the messenger RNA for $p22^{phox}$ and Nox-1, and down-regulation of EC SOD.[60] These effects all are prevented by the $AT_1$-receptor antagonist, candesartan, whereas PD-123,319, which is an $AT_2$-receptor antagonist, enhances oxidative stress and $p22^{phox}$ expression. These data implicate Ang II type I receptors in the kidney as causing oxidative stress and show that type 2 receptors may have an important protective role.

NADPH oxidase activity and $O_2^-$ production is not confined to models of hypertension with excess Ang II. Arterioles from rats on a high-salt diet have increased levels of oxidative stress, impaired endothelium-dependent vasodilatation, and increased activity of NADPH oxidase.[61] Similarly, the deoxycorticosterone acetate–salt rat model of mineralcorticoid excess and low Ang II has increased NADPH oxidase activity and increased $O_2^-$ in aortic rings.[62] Furthermore, inhibition of NADPH oxidase with apocyanin decreases $O_2^-$ production and blood pressure in these animals. Thus, although the cause of increased NADPH oxidase activity in animal models of hypertension still is unclear, accumulating evidence suggests that it may be causative of hypertension. Chabrashvili et al[56] documented an increase in renal $p47^{phox}$ messenger RNA and an increase in lipid peroxidation in young SHR before the development of hypertension.

### ROS-Mediated Vasoconstriction

Decreased NO under conditions of oxidative stress inhibits cytochrome P450 enzymes, which could favor production of vasoconstrictor molecules. The $\omega/\omega$-1-hydroxylase cytochrome P450 enzymes metabolize arachadonic acid into hydroxyeicosatetraenoic acids. This enzyme is inhibited by NO. The SHR, deoxycorticosterone acetate–salt, and Ang II–infused rat models all have increased levels of 20-hydroxyeicosatetraenoic acid, which likely is important for hypertension because inhibition of $\omega/\omega$-1-hydroxylase reduces their blood pressure.[63]

High concentrations of NO normally inhibit cyclooxygenase,[1] whereas a decrease in NO or an increase in $ONOO^-$ enhances its activity.[1,50] Cyclooxygenase metabolizes arachadonic acid into prostaglandin $H_2$ ($PGH_2$), which is metabolized further to thromboxane $A_2$ ($TxA_2$), a potent vasoconstrictor, or $prostacyclin_2$, a vasodilator, or prostaglandin $E_2$. $O_2^-$ and $ONOO^-$ enhance the activity of thromboxane synthase and the production of $TxA_2$ while inhibiting prostacyclin synthase and prostacyclin-2 production.[1,50,64] Arachadonic acid also can be oxidized directly by $O_2^-$ to form 8-isoprostane prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (8-Iso), which is a vasoconstrictor that acts through the thromboxane receptor. 8-Iso is used in animal and human studies as a marker of oxidative stress.

### $O_2^-$ and Salt Balance

$O_2^-$ may also affect blood pressure by regulating the renal handling of salt and water. The SHR has an enhanced TGF response that may contribute to hypertension through renal afferent arteriolar constriction and salt and water retention. The enhanced TGF can be corrected by micro-infusion of the nitroxide SOD mimetic tempol.[50] Indeed, intravenous infusion of tempol normalizes blood pressure in the SHR.[65] Zou et al[53] showed that tempol increased medullary blood flow and urinary

salt excretion in the absence of NO production. These results were corroborated by Majid and Nishiyama[66] in the dog. Makino et al[54] showed that increased medullary oxidative stress decreased medullary blood flow and led to chronic hypertension. Studies of the isolated perfused thick ascending limb have shown that $O_2^-$ enhances NaCl reabsorption.[22]

### Cellular Defense Against Oxidative Stress

The deleterious impact of oxidative stress on NO bioavailability and endothelial function is attenuated by cellular enzymatic and nonenzymatic antioxidants that scavenge ROS. The most important defense derives from SOD, which converts $O_2^-$ to $H_2O_2$. $H_2O_2$ is converted further to $O_2$ and water by catalase and glutathione peroxidase, which uses reduced glutathione (GSH) as its major substrate. Nonenzymatic ROS scavengers include ascorbic acid (vitamin C), and $\alpha$-tocopherol (vitamin E), and glutathione (GSH), all of which have been evaluated as possible therapies against oxidative stress. GSH is an intracellular thiol that can scavenge ROS and regenerate other antioxidants.[67] Vaziri et al[68] showed that normal rats given buthionine sulfoximine to inhibit GSH production became hypertensive and developed oxidative stress,[68] which these investigators attributed to NO inactivation from ROS. Ganafa et al[69] found that rats administered buthionine sulfoximine had increased renal $O_2^-$ production accompanied by decreased plasma prostacyclin-2 and increased $TxA_2$. These studies testify to the importance of the antioxidant system in defense against oxidative stress.

## OXIDATIVE STRESS IN CRF

Oxidative stress has been documented in animal models and in patients with renal disease. Some suggest that oxidative stress may play a causative role in the induction or maintenance of renal insufficiency. Recently, the increased oxidative stress in these patients has been associated with their markedly increased cardiovascular mortality.

The 5/6 nephrectomized rat has increased blood pressure, accompanied by increased malondialdehyde (MDA), a marker of lipid peroxidation, increased tissue 3-nitrotyrosine (3-NT),[70] and decreased SOD and up-regulation of $gp91^{phox}$.[71] Hasdan et al[72] showed that 5/6 nephrectomized rats developed early hypertension that was prevented with intraperitoneal tempol.[72] Resistance vessels from these rats with renal failure exhibited a markedly decreased endothelial-dependent relaxation that was improved by SOD. Gaertner et al[73] showed increased $O_2^-$, $H_2O_2$, and $OH^-$ production in the anti-Thy 1.1 glomerulonephritis rat, which is a model of mesangioproliferative glomerulonephritis. The increase in ROS was accompanied by an increase in NADPH oxidase activity and a decrease in activity of SOD, glutathione peroxidase, and catalase.

Studies of forearm blood flow in patients with CRF or ESRD consistently showed significant endothelial dysfunction. Patients with moderate CRF[74] or those with ESRD on peritoneal dialysis[75] have diminished endothelium-dependent vasodilatation of the brachial artery but retain a normal response to the direct vasodilator, sodium nitroprusside. The interpretation of these studies is confounded by co-expression of risk factors for increased endothelial dysfunction such as hypertension, diabetes, and hypercholesterolemia in those with CRF. However, a recent study of normotensive children (mean age, 12 y) with advanced CRF and no risk factors also found defective endothelial-dependent forearm vasodilatation[76] that correlated positively with markers of oxidative stress and cellular oxidative status, and negatively with total antioxidative activity.[77]

There are many studies of patients with CRF or ESRD that show increased markers of oxidative stress including 8-Iso, MDA, and breath ethane, and often and association with altered antioxidant levels.[71,78-81] Mimic-Oka et al[81] found increased levels of $H_2O_2$ and decreased catalase activity in HD patients. Some studies reported normal levels of vitamin A and E in ESRD patients,[78,79] but one study detected a decreased vitamin C level in patients on HD.[79] Another study found decreased plasma GSH levels and glutathione peroxidase activity in patients with CRF.[82] Interestingly, one study of peritoneal dialysis and HD patients did not detect an increase in MDA levels but found increased antioxidant activity as measured by ferric reducing/antioxidant power.[83] This may have protected these patients against oxidative stress. The discrepancy between this study and others may be due to the difference in patient populations, parameters studied, or assaying techniques.

Oxidative stress may contribute to progression of renal disease through the generation of ad-

vanced glycation end products (AGEs). AGEs are formed nonenzymatically by the reaction of carbonyl compounds with a free amino group from proteins, lipids, or amino acids. AGEs are increased in diabetes, aging, and renal insufficiency. They participate in the pathogenesis of atherosclerosis through activation of an AGE receptor, which increases expression of vascular endothelial adhesion molecules that attract inflammatory cells. The components of oxidative stress that increase AGEs by increasing the formation of carbonyl groups are termed *carbonyl stress*.[83] AGEs have been shown to accumulate in the mesangium of rats and humans with diabetic nephropathy.[84,85] Exogenous administration of AGEs to rats decreases glomerular volume and increases proteinuria and glomerulosclerosis.[84,85] Furthermore, interaction of AGEs with AGE receptors increases oxygen radical formation, thus causing a feed-forward cycle.[85]

Oxidative stress has been linked to increases in plasma C-reactive protein and hypoalbuminemia, both of which are independent risk factors for cardiovascular events in the renal failure population. C-reactive protein may be a marker for leukocyte activation, which generates large quantities of $O_2^-$, whereas low albumin levels may diminish plasma antioxidant capacity. In HD patients, C-reactive protein correlates with levels of $F_2$-isoprostane and thiobarbituric acid–reducing substance, which are markers of lipid peroxidation.[85] Malnutrition in patients with CRF increases the plasma levels of markers of oxidative stress.[83,85] Thus, increased oxidative stress in renal failure patients may be at the root of the increased mortality rate, and may provide an explanation for some of the correlates of cardiovascular risk in this special population.

## TREATMENT OF OXIDATIVE STRESS

Therapy for oxidative stress has focused on antioxidants and renin-angiotensin antagonists. The dramatic effects in animal models have not yet been reproduced so clearly in clinical studies.

Attia et al[86] showed that rats receiving L-NAME infusion for 3 weeks developed severe hypertension, renal failure, proteinuria, endothelial dysfunction, and increased $O_2^-$ levels. Vitamin E therapy prevents renal failure and proteinuria and decreases $O_2^-$ production, but it has no effect on endothelial function or hypertension. Supplements of vitamins C and E in hypercholesterolemic pigs decrease the oxidizability of low-density lipoprotein and improve endothelium-dependent renal blood flow.[87] Vaziri et al[70] showed in 5/6 nephrectomized rats that vitamin E improved blood pressure, increased tissue NO levels, and decreased tissue 3-NT deposition. Buthionine sulfoximine–fed rats given palm oil, which is rich in vitamins A and E, have decreased levels of 8-Iso and $TxA_2$.[88]

Simvastatin, a 3-hydroxy-3-methylglutaryl coenzyme-A inhibitor given to hypercholeterolemic patients reduced 8-Iso levels significantly, but vitamin E produced no further reduction.[89] The investigators suggested that previous trials of vitamin E therapy were hampered by failure to measure oxidative stress, accounting for lipid peroxidation.

L-arginine has been studied as a possible therapy for oxidative stress in renal failure because its depletion with arginase in animal models increases proteinuria and glomerular injury.[90] L-arginine supplementation to 5/6 nephrectomized rats reduced proteinuria and hypertension, and increased urinary $NO_X$. It was as effective as captopril.[91] This renoprotective effect of L-arginine may be similar in mechanism to angiotensin-converting enzyme inhibitors because L-arginine has been shown to decrease glomerular capillary pressure and efferent arteriolar resistance.[92] Diabetic rats that were administered L-arginine had less glomerular hyperfiltration and proteinuria than control rats.[93] Although L-arginine is a promising therapy, one study of oral supplementation[94] and one study of intravenous infusion of L-arginine[49] failed to show any improvement of endothelial function in renal failure patients.

Administration of captopril to 5/6 nephrectomized rats decreased plasma levels of MDA and deposition of 3-NT in the cerebral cortex.[95] Rats with streptozotocin-induced diabetes have increased plasma lipid peroxidation products, increased tissue deposition of 3-NT, and increased renal expression of $p47^{phox}$. All these changes of oxidative stress are prevented by 1-month administration of an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker (ARB) treatment. These effects are independent of blood sugar or blood pressure.[96] The defective endothelial function of SHRs is reversed by the ARB valsartan and/or the angiotensin-converting enzyme inhibitor enalapril. A combination of treatments has the greatest effect.[97] Thus, these 2 meth-

ods to interrupt the renin-angiotensin system may exert their cardiac and renal protective effects through limiting oxidative stress and improving NO bioavailability in the kidney and blood vessels.[39]

An HD treatment can reduce the levels of oxidized protein thiols.[79,82,98] Moreover, HD treatments reverse the increased levels of oxygen radical production by neutrophils in the blood of patients with ESRD.[98] In contrast, 2 studies failed to detect any effect of HD to reduce the high levels of $F_2$-isoprostanes in these patients.[78,80] The investigators suggested that the increased levels may relate primarily to a failure of renal clearance in ESRD patients.[78] There are conflicting data relating to the importance of hemodialysis membrane on oxidative stress. Bioincompatible membranes (cellulose membranes), which have been shown to activate complement and cause inflammation, did not significantly affect $F_2$-isoprostanes compared with biocompatible membranes.[80,82,98] However, as described earlier, these studies are confounded by the finding that $F_2$-isoprostanes themselves are not affected by HD, whereas other indices of oxidative stress appear to be reversed in full.

Another study quantitated oxidative stress by the 8-hydroxy-2′-deoxyguanosine in the DNA of leukocytes as a marker of oxidative damage to DNA.[99] These investigators found that bioincompatible cellulose membranes induced substantially more oxidative stress than biocompatible polysulphone membranes.[100] In a further report, vitamin E–bonded cellulose membranes reduced plasma MDA levels and oxidized low-density lipoprotein levels compared with normal cellulose membranes.[99] Moreover, the vitamin E–bonded membranes slowed the rate of aortic calcification (an index of atherosclerosis) over 2 years.[101] However, it was not possible to dissect whether vitamin E bonding had a specific protective role because the reduction in oxidative stress induced by vitamin E–bonded cellulose membranes was found to be comparable with the reduction with biocompatible membranes.[99] However, more recent studies comparing vitamin E–coated biocompatible dialyzers with uncoated biocompatible dialyzers have shown a reduction in oxidative stress after repeated use as measured by oxidized low-density lipoprotein and MDA[102] and ascorbyl free radical/vitamin C ratio.[103] Thus, vitamin E–coated dialyzers may exert a specific anti-oxidant effect but this will need to be evaluated in larger trials.

The use of GSH supplementation was evaluated recently in patients with peripheral vascular disease and claudication.[104] A GSH infusion improved endothelium-dependent vasodilatation to the lower extremities with a concomitant decrease in symptoms, although oxidative stress parameters were not measured.

The SPACE (Secondary Prevention with Antioxidants of Cardiovascular disease in End-stage renal disease) trial randomized 196 HD patients with pre-existing coronary disease to receive vitamin E 800 IU/d or placebo for 519 days.[105] There was a 50% decrease in cardiovascular events in the therapy group, but no significant difference in mortality. Markers of oxidative stress were not measured. These positive results contrasted to 3 earlier studies involving patients without renal failure but at high risk for vascular disease and increased oxidative stress who did not show any benefit from vitamin E supplementation.[6] The available data do not provide consistent guidelines for therapies for oxidative stress. Anti-oxidant trials have been disappointing, but this may indicate that they have not been used in sufficient doses for sufficient time to reverse oxidative stress. The results of the SPACE trial require confirmation but do suggest that vitamin E 800 IU/d may be beneficial for HD patients. More trials are required to evaluate the effects of angiotensin-converting enzyme inhibitors and ARBs on oxidative stress before they can be recommended specifically to reduce oxidative stress in these patients. Clinicians must not forget the importance of diet and exercise because a recent study showed that 45 to 60 minutes of exercise 3 times per week in combination with a low-fat, high-fiber diet, reduced lipids and 8-Iso levels, and increased urinary $NO_X$ levels in obese men.[106]

## CONCLUSIONS

The major pathophysiologic effects of ROS and NO deficiency are summarized in Figure 1. NO regulates the function of the blood vessels and kidneys. Its deficiency in animal models causes hypertension, vascular disease, and kidney damage. Reduced kidney mass that limits L-arginine synthesis, the accumulation of endogenous NOS inhibitors such as ADMA, the accumulation of L-arginine uptake inhibitors such as urea, the anorexia of uremia, and the development of oxidative

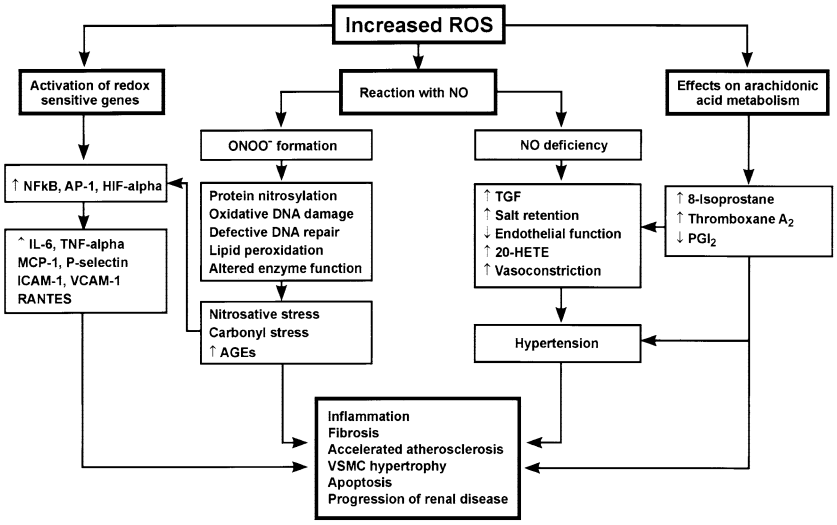


**Fig 1. Diagram of some pathophysiologic processes that follow from increased ROS formation and lead to hypertension and renal and vascular dysfunction.**

stress place CRF patients at high risk for NO deficiency. Oxidative stress plays a significant role in the pathogenesis of hypertension in animal models, in part by inactivation of NO and by generation of 20-hydroxyeicosatetraenoic acids. Oxidative stress is marked not only by the loss of the potent vasodilator/natriuretic pathway based on NO, but also by the generation of vasoconstrictor molecules and primary salt retention by the kidneys. There is evidence that CRF is a state of NO deficiency, increased oxidative stress, and endothelial dysfunction. It is possible that increased oxidative stress and endothelial dysfunction in ESRD patients are at the root of their increased cardiovascular mortality. Antioxidant therapy has been disappointing in clinical trials, but early studies from angiotensin-converting enzyme-I, ARBs, and 3-hydroxy-3-methylglutaryl coenzyme-A trials are more promising.

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