Mechanisms of Diabetic Nephropathy
Role of Hypertension

Sara Giunti, David Barit, Mark E. Cooper

Diabetic nephropathy is a major microvascular complication of diabetes, representing the leading cause of end-stage renal disease in the Western world, and a major cause of morbidity and mortality in both type 1 and type 2 diabetic subjects. Clinical hallmarks of diabetic nephropathy include a progressive increase in urinary albumin excretion and a decline in glomerular filtration rate (GFR), which occur in association with an increase in blood pressure, ultimately leading to end-stage renal failure. These renal functional changes develop as a consequence of structural abnormalities, including glomerular basement membrane thickening, mesangial expansion with extracellular matrix accumulation, changes in glomerular epithelial cells (podocytes), including a decrease in number and/or density, podocyte foot process broadening and effacement, glomerulosclerosis, and tubulointerstitial fibrosis.

Diabetic nephropathy occurs only in a minority of subjects with either type 1 or type 2 diabetes and seems to result from the interaction between genetic susceptibility and environmental insults, primarily metabolic and hemodynamic in origin. Over the last decade, the cellular and molecular mechanisms by which these insults translate to structural and functional abnormalities leading to diabetic nephropathy have been increasingly delineated. In particular, it has been determined that both metabolic and hemodynamic stimuli lead to the activation of key intracellular signaling pathways and transcription factors, thus triggering the production/release of cytokines, chemokines, and growth factors, which mediate and/or amplify renal damage.

In the present review, we summarize molecular and cellular mechanisms that seem to be responsible for hypertension-induced renal injury in diabetes, with particular focus on the role of increased intracapillary glomerular pressure, more recently discovered components of the renin–angiotensin system (RAS), such as angiotensin-converting enzyme (ACE) 2, and the increasing knowledge that has been gained emphasizing cross-talk between metabolic and hemodynamic pathways in amplifying diabetes-related renal injury.

Impact of Hypertension on Diabetic Nephropathy

The relationship between hypertension and poor vascular outcomes, including progression of renal disease, is unequivocal and independent of other confounding factors. The impact of hypertension on outcomes is exponential rather than linear.

A sustained reduction in blood pressure seems to be currently the most important single intervention to slow progressive nephropathy in type 1 and type 2 diabetes. Long-term follow-up studies of initially normotensive diabetic subjects without renal disease demonstrate a blood pressure–dependent decline in GFR with blood pressure levels within the reference range. Patients with a blood pressure corresponding to <130/80 mm Hg rarely develop microalbuminuria and show an annual decline in GFR close to the age-matched normal population. Diabetic patients with a blood pressure between 130/80 and 140/90 mm Hg have a greater decline in GFR, with 30% of patients developing associated microalbuminuria or proteinuria over the subsequent 12 to 15 years.

In microalbuminuric and proteinuric type 1 and 2 diabetic patients, numerous studies have demonstrated that treatment of hypertension, irrespective of the agent used, produces a beneficial effect on albuminuria. Aggressive targets for blood pressure control in diabetic patients have been shown to result in reduced development and retardation in the progression of incipient and overt nephropathy, as well as decreasing macrovascular events.

Genetic Susceptibility, Hypertension, and Diabetic Nephropathy

The finding that diabetic nephropathy occurs only in a subset of subjects with either type 1 or type 2 diabetes and tends to cluster in families is consistent with the hypothesis of a role of genetic susceptibility in the development of this complication. The observation that subjects with diabetic nephropathy and their first-degree relatives are characterized by higher frequency of hypertension has led to the postulate that susceptibility to develop nephropathy in the setting of diabetes might be influenced by an inherited predisposition to essential hypertension.

A number of candidate genes for diabetic nephropathy involved in blood pressure regulation have been studied with controversial results mainly ascribed to the small size of the studies and the heterogeneity of the populations examined. Because of the central role of the RAS in the initiation and progression of diabetic nephropathy and the use of agents that interrupt the RAS in this condition, particular interest has
been addressed to genes encoding for various components of this system. A number of studies have focused on the role of the ACE gene insertion/deletion (I/D) polymorphism, with a recent meta-analysis, including 14,727 subjects from 47 studies, supporting the association between the ACE gene I/D polymorphism and diabetic nephropathy.7 Specifically, the risk of diabetic nephropathy seems to be reduced in both type 1 and type 2 diabetic white subjects and, most markedly, in type 2 diabetic Asian subjects with the II genotype.7 Furthermore, current literature suggests that the ACE D/D genotype predicts poor renal response to ACE inhibitors and to agents that do not block the MAS in subjects with diabetic nephropathy.8

Other genes that have been implicated in the development of diabetic nephropathy include the D3S1308 marker located in the angiotensin II type 1 (AT1) receptor region9,10 and genes linked to sodium homeostasis, such as the SLC12A3 (solute carrier family 12 member [sodium/chloride]) gene.11–13 Furthermore, other genes linked to the MAS, such as the AT1 receptor,9,10 as well as other vasoactive hormones, such as endothelin,9 urotensin II,9 and atrial natriuretic peptides,9 have been examined, although results in this area have generally been negative.

As outlined previously, it is currently suggested that diabetic nephropathy occurs as a result of the interaction between genetic and environmental factors. This concept does not diminish the importance of the study of specific genetic polymorphisms, which might allow for identification of groups with high risk of developing diabetic nephropathy, thus providing novel therapeutic targets or individualized treatment strategies8 for both the prevention and treatment of this complication.14

The Role of Glomerular Capillary Hypertension

Classical renal micropuncture studies performed >20 years ago suggested that glomerular hypertension, as seen in experimental diabetes even in the setting of a normal systemic blood pressure, was central to the initiation and progression of diabetic nephropathy.15 Diabetes amplifies the deleterious effects of blood pressure within the glomerulus by inducing impairment in the autoregulation of the glomerular microcirculation. This consists of vasodilatation of both the afferent and the efferent arteriole, with a more pronounced effect on the afferent arteriole, thus resulting in an increase in intraglomerular capillary pressure.16 A number of observations suggest a role for glomerular capillary hypertension in the pathogenesis of diabetes-associated kidney disease. Glomerular structural abnormalities reminiscent of diabetic nephropathy have been observed in various forms of progressive renal disease characterized by increases in intraglomerular capillary pressure.16–18 Furthermore, strategies that increase glomerular capillary pressure, including contralateral renal arterial clipping19 and ablation of the contralateral kidney,20 enhance the severity of glomerular lesions in diabetic rats.17 In addition, prevention of glomerular capillary hypertension via either ACE inhibition or low protein diets has been shown to be renoprotective in experimental diabetes.17,21 Indeed, the efficacy of ACE inhibitors in retarding the progression of diabetic nephropathy has been considered to partly occur as a result of their hemodynamic effect, specifically decreasing glomerular capillary pressure through a vasodilatory effect on the glomerular efferent arteriole.22

Because of the elastic properties of the glomeruli, changes in glomerular capillary pressure are paralleled by changes in overall glomerular volume.23,24 These changes in volume are mild in physiological states and become significantly amplified in conditions characterized by impaired autoregulation, including diabetes.24 Cyclic changes in glomerular volume lead to recurrent episodes of stretch and relaxation of all of the glomerular structural components,23,24 including mesangial cells25 and podocytes.26 The cellular and molecular mechanisms by which glomerular capillary hypertension leads to changes that promote glomerulosclerosis in these cell types have been explored and partly delineated only in recent years using the in vitro model of mechanical stretch.

Mesangial cells, when exposed to continuous cycles of stretch/stretch, alter their morphology. Specifically, these cells change from their normal stellate to a straplike appearance, aligning with their long axis perpendicular to the direction of stress.26 This leads to enhanced proliferation26 and increased production of extracellular matrix components. This prosclerotic effect of stretch occurs partly as a result of increases in gene and/or protein expression of extracellular matrix components, such as collagen I,26–28 III,26,27 and IV,27,28 fibronectin,27,28 and laminin.27,28 This effect is proportional to the degree of stretch, with the greatest increase at the periphery of the culture dish at the point of greatest deformation.27,28 Furthermore, this accumulation of extracellular matrix, which occurs as a result of mechanical stretch, is markedly enhanced in a milieu with a high glucose concentration.29

Mechanical stretch may promote extracellular matrix accumulation in cultured mesangial cells, not only by increasing synthesis of extracellular matrix components, but also by decreasing the activity of degradative enzymes.27 Furthermore, mechanical stretch induces both gene27,30 and protein30 expression of transforming growth factor (TGF)-β1, a potent prosclerotic cytokine and a well-known mediator of extracellular matrix accumulation in diabetic nephropathy.31 In addition, stretch enhances both gene and protein expression of the TGF-β receptors I and II.32 Therefore, mechanical stretch influences TGF-β activity by several mechanisms including altering mRNA levels and secretion, activation of the molecule itself, and increasing expression of 2 key TGF-β receptors.32 However, the relative importance of TGF-β1 expression in mediating extracellular matrix deposition in this context remains controversial. Indeed, a previous study suggested that it was unlikely that the increase in gene expression of extracellular matrix proteins by mechanical stretch was secondary to increased production of TGF-β1.33 By contrast, other studies have demonstrated an important role of mechanical stretch-induced TGF-β1 as a mediator of extracellular matrix deposition in mesangial cells.33,34 In particular, it has been suggested that stretch induces fibronectin via a biphasic signaling pathway.34 Specifically, stretch, via the intracellular signaling molecule protein kinase C, causes early activation of p38 mitogen-activated protein kinase, which induces TGF-β1 and fibronectin production.34 In turn, TGF-β1 maintains a late phase of p38 mitogen-activated protein kinase activation, which perpetuates fibronectin production.34 Me-
Mechanical stretch has also been shown to induce gene expression but not protein secretion of connective tissue growth factor, a downstream mediator of TGF-β1 signaling. Therefore, mechanical stretch in mesangial cells results in increased extracellular matrix production directly, via the release of prosclerotic cytokines, and via decreases in extracellular matrix degradation. Interestingly, it seems that only stretch-simulating pathological conditions (10% elongation) are necessary to induce cytokine or matrix production, because a physiological degree of stretch (4% elongation) does not result in these effects. Mechanical stretch may also induce extracellular matrix deposition by interacting with specific metabolic, hemodynamic, and inflammatory pathways. Specifically, stretch amplifies the effects of glucose and angiotensin II (Ang II) and enhances the expression of the chemokine monocyte chemotactic protein-1 (MCP-1) and the adhesion molecule intercellular adhesion molecule-1 (ICAM-1).37

Mechanical stretch in mesangial cells upregulates protein expression of the facilitative glucose transporter (GLUT)-1, as well as basal glucose transport through a TGF-β1-dependent mechanism.38 The importance of GLUT-1 per se is suggested by studies where specific GLUT-1 overexpression in mesangial cells in a normal glucose medium leads to excess extracellular matrix production.38,39 Therefore, mechanical stretch-induced GLUT-1 expression may represent a mechanism whereby mechanical forces interact with glucose-mediated pathways in the pathogenesis of the glomerular injury. This concept is further strengthened by the in vivo observation where an 80% increase in GLUT-1 expression was observed in hypertensive Dahl salt-sensitive rats, a model of systemic hypertension accompanied by glomerular hypertension and injury, but not in similarly hypertensive spontaneously hypertensive rats that do not have a concomitant increase in intraglomerular pressure.38

The powerful vasoconstrictor and trophic hormone Ang II is a central mediator of renal damage in diabetes. In mesangial cells, it produces effects similar to those seen with mechanical stretch, including cell hypertrophy and hyperplasia, accumulation of extracellular matrix, and production of cytokines, chemokines, and growth factors, such as TGF-β1, connective tissue growth factor, interleukin-6, MCP-1, and vascular endothelial growth factor (VEGF). The existence of an interaction between Ang II and mechanical stretch in cultured mesangial cells has been reported by Gruden et al.40 Both Ang II40 and mechanical stretch40,41 can independently induce the production of VEGF. Furthermore, pre-exposure to mechanical stretch results in upregulation of the AT1 receptor (AT1R) subtype, which, in turn, translates to an enhanced cellular response to Ang II. Specifically, Ang II and stretch confer additive effects on VEGF production, a potent promoter of vascular permeability, which specifically in this cell type induces proliferation52 and enhances collagen synthesis.53

Mechanical stretch may also trigger an inflammatory response in mesangial cells. Indeed, stretch induces both gene expression and protein secretion of MCP-1 and reduces both gene and protein expression of CC chemokine receptor 2,44 the cognate MCP-1 receptor. MCP-1 secretion paralleled by CC chemokine receptor 2 downregulation is a common pattern of response to known inducers of MCP-1, as reported previously in mesangial cells exposed to high glucose54 and in monocytes exposed to tumor necrosis factor-α, lipopolysaccharides, and interferon-γ. It has been postulated that at sites of inflammation, this response, by enhancing local MCP-1 availability, favors recruitment of monocytes. Accordingly, stretch-induced MCP-1 significantly enhances the ability of mesangial cells to attract monocytes. Furthermore, both stretch37,44 and MCP-1-44 independently induce ICAM-1 expression, which, in turn, significantly enhances phagocyte leukocyte adhesion to mesangial cells. This physical interaction is a key event in the amplification of the inflammatory cascade and may contribute to glomerular injury by inducing the release of cytotoxic reactive oxygen species.51

These proinflammatory effects of mechanical stretch provide a further mechanism by which glomerular capillary hypertension may exert deleterious effects in diabetic glomerulopathy. Indeed, glomerular infiltration of macrophages has been histologically demonstrated in renal biopsies from patients with diabetic nephropathy,52 with both MCP-1 and ICAM-1 having been implicated in the pathogenesis of diabetic nephropathy. Specifically, both proteins are overexpressed in the glomeruli from diabetic animals.53,54 Furthermore, there is amelioration of both proteinuria and glomerular histological damage in both ICAM-152 and MCP-1-156 knockout mice in the setting of concomitant diabetes. The mechanisms described above by which mechanical stretch may contribute to mesangial cells extracellular matrix deposition are summarized in Figure 1.

As mentioned previously, diabetic nephropathy is characterized by additional structural abnormalities and, in particular, a decrease in the number and/or density of podocytes. These epithelial cells play a major role in the synthesis of matrix proteins of the glomerular basement membrane, in the maintenance of glomerular barrier function, and in counteracting capillary wall distension. Therefore, podocytes are constantly exposed to mechanical stress. Furthermore, these cells are very sensitive to mechanical force, which, specifically, reduces the size of the cell bodies of the podocyte and induces a reversible reorganization of the actin cytoskeleton consisting of a disappearance in transverse stress fibers and formation of radial stress fibers connected to an actin-rich center. It has been suggested that podocytes assume a state of intermediate adhesion as a strategy to avoid mechanical damage. However, this state of intermediate adhesion may ultimately be maladaptive, because reduced adhesion may lead to progressive podocyte loss, with these cells detaching more readily from the glomerulus.

Mechanical stretch may favor a decrease in podocyte number through several mechanisms, including effects on proliferation, apoptosis, and cell adhesion to the basement membrane (Figure 2). Stretching podocytes in vitro reduces proliferation by decreasing the expression of positive cell cycle regulatory proteins and by increasing the expression of cyclin-dependent kinase inhibitors. In addition, mechanical stretch has been shown to induce Secreted Protein Acidic and Rich in Cysteine, a matrix-cellular protein mediating cell–matrix interactions, which diminishes proliferative capacity in various tissue systems. It has been postulated that this induction of secreted protein acidic and rich in cysteine may lead to a progressive reduction in podocyte
number, potentially via a TGF-β1–dependent mechanism.60 Furthermore, podocyte exposure to mechanical stretch results in the activation of a local tissue angiotensin system, consisting of increased production of Ang II and increased expression of the AT1R, which ultimately leads to an increase in podocyte apoptosis.62 Interestingly, stretch-induced apoptosis is not completely abrogated by Ang II blockade, suggesting that mechanical stretch may also induce apoptosis via non-Ang II–dependent pathways.62 A potential mediator could be TGF-β1, of which the gene expression is induced by mechanical stretch in podocytes.62 Indeed, this growth factor has been reported to induce podocyte apoptosis,60 although the relative importance of TGF-β1 in explaining podocyte depletion in diabetes remains to be fully clarified. Other than the proapoptotic effect, Ang II in podocytes results in effects that may be relevant for extracellular matrix production and alteration of the permselectivity of the glomerular filtration barrier. Specifically, Ang II enhances production of collagen IV and VEGF signaling63 and downregulates nephrin expression64 in podocytes. Nephrin is a protein specifically localized to the slit pore diaphragm of the podocyte and is implicated in the pathogenesis of proteinuria. Indeed, nephrin downregulation may be closely linked to the pathogenesis of diabetic nephropathy. A decrease in expression of nephrin occurs in vitro in podocytes exposed to glucose-modified proteins known as advanced glycation end products (AGEs)64 and in vivo in streptozotocin-induced diabetic rats65,66 and human diabetic nephropathy.64,67 Furthermore, in both experimental65,66 and human67 diabetes, this depletion in renal nephrin expression is prevented by RAS blockade. Although Ang II has been reported to promote extracellular matrix accumulation and nephrin downregulation in podocytes, it remains to be determined whether stretch via an Ang II–dependent pathway has similar effects in these glomerular epithelial cells.

Finally, mechanical stretch may interfere with podocyte adhesion to the glomerular basement membrane. The integrin α3β1 is an adhesion molecule, which is primarily involved in podocyte adhesion to the glomerular basement membrane.68 The expression of α3β1 integrin is reduced in vitro in podocytes exposed to high glucose and in vivo in podocytes not only from streptozotocin-induced diabetic rats but also in patients with diabetic nephropathy.68 Preliminary evidence suggests that mechanical stretch may reduce both the expression of the integrin α3β1 and podocyte adhesion to an extracellular matrix substrate,69 providing a mechanism whereby glomerular

Figure 1. Schematic representation of mechanisms whereby stretch may induce extracellular matrix (ECM) deposition in mesangial cells.

Figure 2. Schematic representation of mechanisms whereby stretch may lead to a decrease in podocyte number. SPARC indicates secreted protein acidic and rich in cysteine; CDK, cyclin-dependent kinases; GBM, glomerular basement membrane.
capillary hypertension may favor the detachment of podocytes from the glomerular basement membrane. Overall, these mechanisms of stretch-induced podocyte abnormalities may provide a key explanation for progressive podocyte loss and glomerulosclerosis in diseases associated with glomerular capillary hypertension, including diabetic nephropathy.62

**Role of ACE2**

A major role for the local RAS in the development and progression of diabetic nephropathy has been clearly demonstrated and reviewed elsewhere70 and is beyond the scope of this review. Here, we would like to focus on a new component of the RAS, ACE2, a homologue of ACE, which may be relevant in various pathophysiological states, including hypertension,71–74 cardiovascular disease,74,75 and diabetic nephropathy,75–77 thus representing a novel treatment target for these conditions.78,79

ACE2 is part of the enzymatic cascade of the RAS. Specifically, it seems to act as a negative regulator of the RAS, counterbalancing the function of ACE,78 thus promoting vasodilation. Indeed, it is implicated in the conversion of Ang I to Ang (1-9) and in the degradation of Ang II to Ang (1-7), a peptide that has been postulated to counteract the potentially detrimental actions of Ang II via the AT1R, resulting in vasodilator, nitricre, and antiproliferative effects.75,80 Furthermore, ACE2 is involved in cleavage of other vasoactive peptides, such as apelin, neurotensin, kinetensin, and des-Arg bradykinin.75

ACE2 is highly expressed in a variety of tissues, although its distribution is much less widespread than ACE. Such sites of ACE2 expression include the heart and kidney. In the kidney, ACE2 has a distribution similar to ACE with major localization in renal tubules.76,77

Both ACE2 gene and protein expression are downregulated in the kidneys in experimental models of hypertension74 and in streptozotocin-induced diabetic Sprague–Dawley rats after 24 weeks of diabetes.76 It is not yet known whether this reduction in ACE2 is of pathophysiological significance, but it has been postulated that renal ACE2 deficiency as occurs in long-term diabetes leads to a local increase in tubular Ang II, which, in turn, may promote tubulointerstitial fibrosis.76 Interestingly, ACE inhibition seems to prevent the diabetes-associated decreases in renal ACE2 expression, suggesting that ACE inhibition may confer some of its renoprotective effects via modulation of ACE2 expression.76

Although ACE2 expression seems to be downregulated in diabetes-associated kidney disease, there is evidence suggesting that ACE2 expression is increased in early phases of diabetes in the absence of renal injury. Indeed, protein expression of ACE2 is increased in renal cortical tubules of diabetic db/db mice, a model of type 2 diabetes, after 3 to 4 weeks of diabetes, when renal complications have not as yet developed.77 Furthermore, in the same model, the increase in ACE2 expression was paralleled by a decrease in ACE protein expression.77 and it has been postulated that this combination may provide renoprotection by attenuating Ang II accumulation and increasing Ang 1-7 formation.77 The finding of increased ACE2 protein expression in renal cortical tubules from young mice prone to type 2 diabetes (db/db mice) does not exclude the possibility of a later reduction in ACE2 during the course of the disease as nephropathy develops.77 Indeed, older ACE2 knockout mice develop glomerulosclerosis that resembles to a certain extent diabetic nephropathy.73 Thus, it would now be of interest to specifically examine various models of ACE2 deficiency and excess in the context of diabetes.

**Interaction Between the Hemodynamic and Metabolic Pathways**

There is increasing evidence that the hemodynamic and metabolic pathways, rather than acting independently, are functionally linked in diabetic nephropathy. As outlined earlier, glucose, per se, amplifies the effects of blood pressure within the glomerulus by inducing impairment in the autoregulation of the glomerular microcirculation. On the other hand, glomerular capillary hypertension enhances GLUT-1 expression with a concomitant increase in intracellular glucose accumulation, amplifying the deleterious effects of glucose and its metabolites within the kidney.58 Therefore, in diabetes, both metabolic and hemodynamic factors interact to modify the glomerular microcirculation, creating an environment conducive to progressive glomerular injury.

Patients with poor glycemic control show a greater reduction in intrarenal vascular resistance after blockade of the RAS than that seen in subjects with good metabolic control,81,82 suggesting that the activity of the intrarenal RAS may be influenced by metabolic factors.52 How glucose, per se, induces injury in the kidney remains an area of active investigation, with various pathways having been identified, including promotion of mitochondrial superoxide production83 and enhanced generation of AGEs. These AGEs occur as a result of a series of biochemical reactions involving glucose attaching to proteins, lipids, and nucleic acids. In vitro and in vivo studies have characterized functional interactions between these AGEs and the RAS. In vitro, in mesangial cells, AGE-mediated reactive oxygen species generation via an AGE receptor known as Receptor for Advanced Glycation End products (RAGE) induces autocrine generation via an AGE receptor known as Receptor for Advanced Glycation End products (RAGE) induces autocrine production of Ang II, which results in activation of TGF-β Smad signaling, cell hypertrophy, and fibronectin synthesis.84 In vivo, in streptozotocin-induced diabetic Sprague–Dawley rats, renal AGE accumulation is attenuated to a similar degree by both an ACE inhibitor and an AGE formation inhibitor.85 Furthermore, in Sprague–Dawley rats, infusion of AGE-modified rat serum albumin results in a significant increase in renal expression of various components of the vasoconstrictor arm of the RAS.86 This is associated with tubular and glomerular hypertrophy and AGE accumulation, which can be antagonized by the AT1R blocker valsartan. In the same model, an infusion of Ang II increases both serum and renal accumulation of AGEs and advanced oxidation products and induces renal hypertrophy that can be antagonized by an inhibitor of AGE formation, pyridoxamine.82

Overall, these in vitro and in vivo observations demonstrate the existence of a functional interaction between AGEs and the RAS. Furthermore, the in vivo findings demonstrate that both pathways and their structural effects may be partly blocked by inhibitors of the other pathway, reinforcing the concept of the potential use of combination therapy in a
diabetic context. Indeed, in studies performed in diabetic spontaneously hypertensive rats, monotherapy with either the ACE inhibitor perindopril or the inhibitor of AGE formation aminoguanidine attenuated development of albuminuria in this model, with the combination even more effective than the individual agents. It remains to be determined as to the clinical translatability of these experimental findings, although the situation with respect to the RAS has generally indicated that positive findings in rodents with agents that interrupt the RAS would translate to humans. For example, the exciting initial findings observed in diabetic rats in response to ACE inhibitors and, subsequently, AT1R antagonists were ultimately translated to the clinic in both type 1 and type 2 diabetes. Therefore, with the widespread use of agents that interrupt the RAS as first-line treatment in diabetic patients at risk or with nephropathy, further studies of new renoprotective therapies will now need to be performed in the context of concomitant ACE inhibitor or AT1R antagonist treatment.

Disclosures

None.

References


