www.nephrologyrounds.org OCTOBER 2006 Volume 4, Issue 8

NEPHROLOGY Rounds®

AS PRESENTED IN THE ROUNDS OF THE NEPHROLOGY DIVISION OF BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MASSACHUSETTS

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***Nephrology Rounds* is approved by the Harvard Medical School Department of Continuing Education to offer continuing education credit**

Molecular Mechanisms Underlying Diabetic Nephropathy

By ALICE M. SHERIDAN, MD

Every year, 40% of the patients who start on dialysis have diabetic nephropathy as the cause of their end-stage renal disease (ESRD).[1] The mortality of dialysis patients with diabetic nephropathy is higher than that for non-diabetic patients.[2] Although clinical trials have firmly established the importance of glycemic control[3,4] and inhibition of the renin-angiotensin system (RAS)[5-7] in slowing, but not preventing, the decline in renal function, the mechanisms that underlie diabetic nephropathy remain incompletely understood. Whether glomerular hemodynamic changes or mesangial structural lesions initiate the pathogenetic signaling is a topic of debate. However, data emerging over the past decade emphasize that there is a close association between these two factors. This issue of *Nephrology Rounds* examines the molecular mechanisms underlying diabetic nephropathy.

Structure and function

Early diabetic nephropathy is characterized by hyperfiltration and low-level excretion of albumin. A progressive decline in the glomerular filtration rate (GFR) is associated with marked increases in albumin excretion.[1] The structural lesions that characterize diabetic nephropathy include the expansion of mesangial volume with an increase in matrix proteins, thickening of the peripheral glomerular basement membrane, and tubulointerstitial fibrosis.[8] The degree of mesangial expansion and interstitial fibrosis, but not glomerular basement membrane thickness, correlates with creatinine clearance.[8] It is hypothesized that the increased mesangial matrix obliterates neighboring capillaries, causing a decrease in filtration surface area.[9]

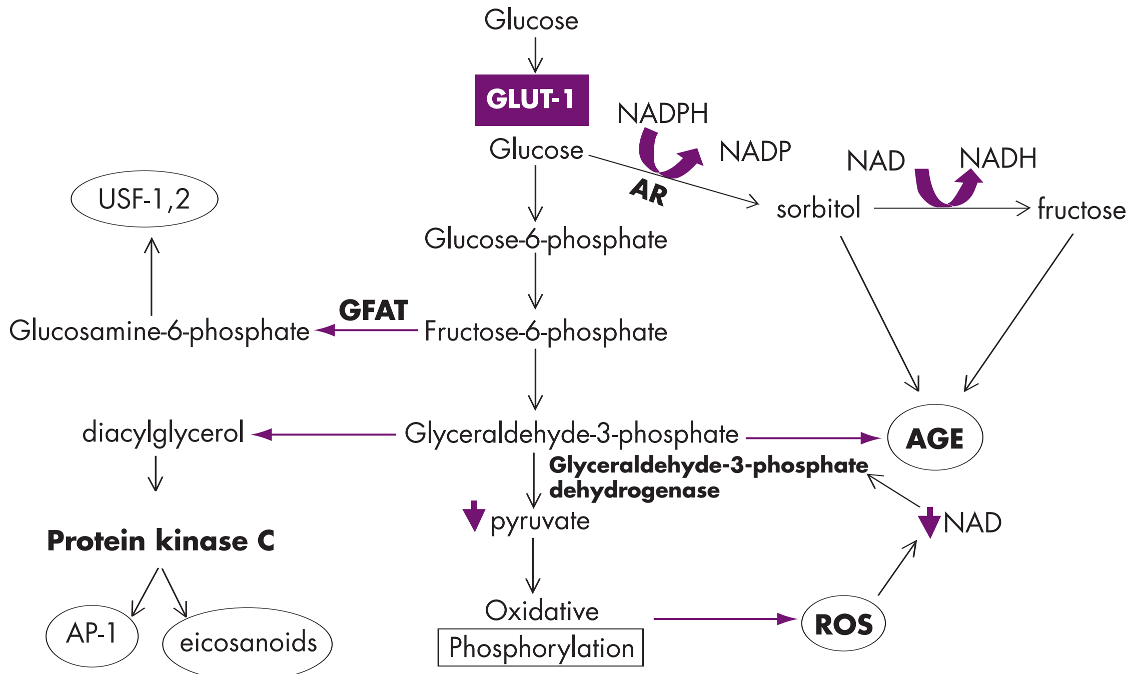
Glucose

The magnitude of hyperglycemia correlates with the functional and structural changes of diabetic nephropathy. Clinically, strict glycemic control inhibits both the functional decline in GFR[3,4] and the formation of characteristic structural lesions.[10] The restoration of euglycemia reverses structural changes.[11] Exposure to high glucose causes an increase in matrix protein generation and cell cycle arrest by cultured cells.[12,13]

Increased *intracellular* glucose initiates changes in the mesangial cell phenotype. This is made possible by the unrestricted entry of glucose in the presence of increased extracellular glucose. The inability to restrict glucose entry is a characteristic of mesangial cells and other tissues that are especially susceptible to the microvascular complications of diabetes, including the capillary endothelial cells of the retina and peripheral neurons.[14] Whereas glucose entry into many tissues, like skeletal muscle, is tightly regulated and does not increase in the presence of increased extracellular glucose,[15] high glucose causes an *increase* in glucose uptake by mesangial cells. Regulation of the expression of facilitative glucose transporters (GLUTs) underlies this distinguishing characteristic of mesangial cells and other susceptible cell types. Glucose transport into all mammalian cells occurs via glucose transporters, including the sodium-coupled and facilitative transporters.[16] The facilitative transporters include 14 members (GLUT 1-14) and all are characterized by 12 homologous transmembrane spanning domains.[16] All members of the facilitative transporter family allow the diffusion of glucose down its concentration gradient.[16] Although GLUT-1, –2, -4, –5, and, most recently, GLUT-12[17] have been identified in the kidney, data have most strongly implicated

Figure 1: Intracellular glucose metabolism is altered by ROS generation.

Glucose enters the cell via the facilitative transporter GLUT-1. ROS are generated as a result of increased electron transfer caused by increased intracellular glucose.[14] ROS deplete cellular NAD, which inhibits the conversion of glyceraldehyde-3-phosphate to pyruvate. This results in the accumulation of pyruvate precursors including glucose, fructose-6-phosphate, and glyceraldehyde-3-phosphate. Glucose is converted by aldose reductase (AR) to sorbitol, which is converted to fructose via the polyol pathway. Fructose-6-phosphate forms hexosamines via the activity of glutamine:fructose-6-phosphate-amidotransferase (GFAT). Glyceraldehyde-3-phosphate forms diacylglycerol which activates protein kinase C isoforms.



USF-1,2 = Upstream stimulatory factor 1 and 2; AP-1 = Activator protein-1; ROS = reactive oxygen species

GLUT-1 in the hyperglycemia-induced increase in glucose uptake by mesangial cells and in the consequent structural lesions.[15]

- First, cultured mesangial cells exposed to high glucose demonstrate a commensurate increase in glucose transport and in GLUT-1 mRNA and protein.[18] Streptozotocin-induced diabetic rats demonstrate higher levels of GLUT-1 protein in the kidney cortex.[19] This upregulation is necessary to allow an increase in glucose uptake, since GLUT-1 is a high-affinity, low-capacity transporter that is saturated at normal glucose levels.[15]
- Second, mesangial cells that express exogenous GLUT-1 and are exposed to *normal* concentrations of glucose, express greater amounts of collagen and fibronectin compared to control cells. This increase is comparable to that observed in non-GLUT-1-transduced cells exposed to high glucose.[9]
- Finally, antisense inhibition of GLUT-1 expression inhibits fibronectin generation by mesangial cells.[20]

Transforming growth factor beta (TGF-β) increases glucose uptake and expression levels of GLUT-1 protein and mRNA by cultured mesangial cells. A neutralizing antibody to TGF-β inhibits the effect of glucose on GLUT-1 expression, suggesting that the effect of glucose on GLUT-1 expression is mediated by TGF-β[21] GLUT-1 expression may also be modulated by transglomerular pressure gradients, suggesting a mechanism by which the increase in transglomerular capillary pressure is transduced to mesangial cells.[22] Thus, GLUT-1 protein is increased in Dahl salt-sensitive rats (characterized by glomerular hypertension), but not in spontaneous hypertensive rats (SHR), nor in normotensive Wistar-Kyoto (WK) rats; both SHR and WK rat strains develop systemic, but not glomerular hypertension. Similarly, mechanical stretch causes the induction of GLUT-1 in cultured mesangial cells.[22] Similar to glucose, the effect of glomerular hypertension and mechanical stretch on GLUT-1 is abrogated by neutralizing anti-TGF-β antibody, suggesting that this effect is mediated by TGF-β.[22]

Once inside the cell, glucose is metabolized via multiple pathways that increase the generation of reactive oxygen species (ROS), enhance cell susceptibility to oxidative injury by reducing glutathione, and generate metabolites that activate protein kinase C (PKC) or directly induce the expression of TGF-β1 (Figure 1). The majority of intracellular glucose is metabolized in the cytosol via glycolysis, which generates pyruvate. Pyruvate is oxidized in the mitochondria via the tricarboxylic acid (TCA) cycle. Both glycolysis and the TCA cycle generate nicotinamide adenine dinucleotide (NADH) that acts as an electron donor for mitochondrial oxidative phosphorylation. Excessive electron transfer caused by increased intracellular glucose generates a high electrochemical potential difference that prolongs the half-life of superoxide-gener-

ating electron intermediates in the mitochondria.[23] Some argue that the generation of superoxide and other ROS underlie most, if not all, the pathogenetic effects of intracellular glucose.[2,24] Clearly, ROS generation plays a significant role in altering the phenotype of mesangial cells exposed to high glucose.[25,26] ROS increase TGF-β-mediated matrix synthesis and the expression of plasminogen activator inhibitor,[27] possibly via the activation of NF-κB, and the transcription factor, activator protein (AP-1),[28] since both have been shown to activate the TGF-β1 promoter.[29] Perhaps, most importantly, ROS formation alters glucose metabolism such that alternative pathways to glycolysis are increased, resulting in the accumulation of metabolites that initiate pathogenetic signaling. First, ROS accumulation depletes cells of NAD because of the massive ROS-triggered activation of the NAD-consuming DNA repair enzyme, poly-adenosine diphosphate (ADP)-ribose ploymerase-1 (PARP-1).[24] This alters the NADH/NAD ratio of the cell that shuts down the NAD-dependent enzyme, glyceraldehyde-3-phosphate dehydrogenase, and thus inhibits the formation of pyruvate. As a result, upstream pyruvate precursors accumulate, forcing their metabolism via alternative pathways. For instance, glyceraldehyde-3-phosphate is metabolized to diacylglycerol (DAG) and phosphatidic acid[24] and both of these metabolites activate PKC isoforms.[24] PKC induces TGF-β via activation of the transcription factor, AP-1, and increases eicosanoid generation. Eicosanoids may contribute to the afferent vasodilatation that underlies the increase in transcapillary pressure[30,31] and may also have direct effects on mesangial cell cycle regulation.[32]

The pyruvate precursor, fructose-6-phosphate, is converted to the hexosamine, glucosamine-6-phosphate, via action of the rate-limiting enzyme, glutamine: fructose-6-phosphate-amidotransferase, or GFAT. Hexosamines activate TGF-β1 transcription by increasing the transcription factors, upstream stimulatory factors (USFs), which bind to a glucose response element present in the TGF-β1 promoter. Elevated glucose and GFAT overexpression have both been shown to increase USF expression.[33] The administration of the hexosamine, D-glucosamine, increases matrix synthesis and the expression of TGF-β, as does the overexpression of GFAT in mesangial cells grown in physiologic concentrations of glucose.[34] Furthermore, the inhibition of GFAT prevents the effect of high glucose on TGF-β.[35,36]

The pyruvate precursor, glucose, is also shunted from the glycolytic to the polyol pathway.[24] Aldose reductase is the first and rate-limiting enzyme of the polyol pathway and generates sorbitol that is oxidized to fructose.[24] Excessive activity of aldose reductase depletes NAD-phosphate (NADPH), which is essential for the regeneration of glutathione, thus enhancing cell susceptibility to oxidative injury.[2] Furthermore, the oxidation of sorbitol to fructose worsens the NADH:NAD ratio, exacerbating the inhibition of pyruvate formation. The increased expression of aldose reductase has been demonstrated in cells derived from diabetic patients.[37] Mesangial cells derived from transgenic mice overexpressing aldose reductase demonstrate increased collagen synthesis and increased expression of TGF-β1 that is abrogated by the inhibition of aldose reductase.[38]

Finally, increases in glucose and in metabolites of the polyol and glycolytic pathways favor the formation of advanced glycation endproducts (AGEs). AGEs are proteins and lipids that have been modified by the nonenzymatic covalent addition of a sugar residue via a series of biochemical reactions collectively termed the "maillard reaction."[39] Levels of AGEs, while detectable under physiologic conditions, are markedly increased in diabetic patients and studies in experimental diabetic animals have suggested that AGEs contribute to lesions in mesangial cells and podocytes. The inhibition of AGE formation prevents mesangial expansion and decreases albuminuria,[40] and the intraperitoneal injection of AGE-modified albumin increases the expression of glomerular collagen and TGF-β1 in the streptozotocin-induced diabetic rat.[41]

AGEs have both receptor-independent and receptor-dependent effects. The receptor-dependent effects are mediated via their interaction with RAGE (Receptor for AGE), a 35 kDa transmembrane receptor that belongs to the immunoglobulin superfamily.[39] Originally described as a scavenger receptor that removes AGEs from cells, RAGE is now known to be a multiligand receptor that may induce the vascular endothelial growth factor (VEGF).[42,43] RAGE is upregulated in podocytes from patients with diabetic nephropathy and in db/db diabetic mice, and it colocalizes with VEGF. Streptozotocin-treated, RAGE-null mice, unlike wild-type streptozotocin-treated mice, fail to show upregulation of VEGF.[42] The inhibition of RAGE activation by the intraperitoneal administration of soluble RAGE (sRAGE), which comprises the ligand-binding domain of RAGE, decreases TGF-β and prevents both the mesangial expansion and glomerular basement membrane thickening.[42]

In summary, mutiple metabolic derangements result from increased intracellular glucose and culminate in TGF-β-induced increase in matrix protein synthesis, growth arrest of mesangial cells, and changes in growth factor expression. How do these events relate to the hemodynamic changes that characterize diabetic nephropathy? Critical studies have delineated the contribution of hemodynamic changes to diabetic nephropathy; the interruption of the renin-angiotensin system has become a cornerstone in the treatment of diabetic nephropathy. However, whether the glomerular hemodynamic changes are causal or secondary to mesangial lesions remains controversial.

Hemodynamic changes/ renin-angiotensin system

Hyperfiltration is characteristic of early diabetic glomerulopathy.[44] Hyperfiltration is due to increased

glomerular plasma flow rate and transcapillary hydraulic pressure differences.[45] The increased plasma flow rate is due to the decreased resistance of the afferent arteriole.[45] The mechanism of afferent arteriole vasodilatation is not known, although multiple putative mediators have been suggested, including alterations in eicosanoids, nitric oxide, and atrial natriuretic peptide, which results in a decreased response to angiotensin II.[45,46] Recent data suggest that TGF-β may contribute to glomerular afferent dilatation that underlies glomerular hyperfiltration by inhibiting angiotensin II-induced calcium mobilization from vascular smooth muscle cells derived from afferent arterioles.[47] Studies of animal models have proven that these hemodynamic changes contribute to the diabetic structural changes and functional deterioration. Increased glomerular sclerosis is observed in streptozotocin-treated rats after unilateral nephrectomy,[48] or unilateral clipping,[49] or consumption of a high-protein diet.[50] Angiotensin-converting enzyme (ACE) inhibition prevents the increase in the transcapillary hydraulic pressure gradient and has been demonstrated to decrease both the structural lesions and the proteinuria observed in streptozotocin-treated rats.[51]

The primary mechanism by which inhibition of the renin-aldosterone-angiotensin system alters extracellular matrix formation has yet to be completely clarified. On one hand, studies have suggested that intraglomerular capillary pressure changes are directly transmitted to mesangial cells, increasing TGF-β-mediated extracellular matrix formation.[52-54] Thus, mesangial cells that are subjected to mechanical strain have increased synthesis of matrix protein.[52] TGF-β1 mRNA and protein,[53] and TGF-β receptors[55] are increased in mesangial cells subjected to mechanical stretch. These data suggest that the observed effect of ACE inhibitors on glomerulosclerosis may occur via a decrease in transglomerular pressure. On the other hand, angiotensin II increases the expression of TGF-β and the synthesis of matrix proteins by cultured mesangial cells, under conditions where the effect of glomerular hypertension is obviated.[56] The effect of angiotensin II on matrix protein synthesis is blocked by neutralizing the antibody to TGF-β, suggesting that TGF-β is causal in the fibrogenic effect of angiotensin II.[56]

TGF-ß

Many, but not all of the effects of hyperglycemia are ultimately mediated by TGF-β. The causal role of TGF-β1 in mesangial matrix expansion was first demonstrated in the antithymocyte model of glomerulonephritis.[57,58] A series of studies on models of diabetic nephropathy, *in vitro* and *in vivo*, confirmed the integral role of TGF-β isoforms in the progression of diabetic nephropathy. Although 3 isoforms of TGF-β have been identified in the mammalian kidney and shown to be fibrogenic in mesangial cells,[59] the majority of evidence implicates TGF-β1 in diabetic nephropathy. TGF-β1 mRNA and protein are elevated in the renal cortices of patients with diabetic nephropathy[60,61] and in experimental animal models,[13,60,61] as well as in cultured, high-glucose-treated proximal tubule[62] and mesangial cells.[63] An elevation of the TGF β-receptor has also been demonstrated in experimental animal models of diabetic nephropathy and this increase is abrogated by insulin.[64] High glucose and exposure to exogenous TGF-β1 both inhibit the proliferation and increase the generation of matrix proteins by cultured cells, and the effect of glucose is abrogated by neutralizing antibody to TGF-β1,[12,63] confirming that TGF-β is an effector of glucose. *In vitro* data are supported by *in vivo* studies. The intraperitoneal injection of a neutralizing antibody to TGF-β1 prevents the glucose-induced increase in TGF-β expression and kidney hypertrophy, as well as matrix protein accumulation in the streptozotocin-treated mouse.[65] Anti-TGF-β1 antibody prevents the decline in creatinine clearance that is observed in the db/db mouse, which expresses a mutant form of the leptin receptor and is a model of type 2 diabetes.[66] Perhaps the most convincing data are from transgenic mice that constitutively express an active form of TGF-β1 under the control of a murine albumin promoter (Alb).[67] Alb/TGF-β1 transgenic mice, selected for high liver and plasma TGF-β1 levels, develop mesangial expansion and thickened glomerular basement membranes by 3-weeks of age, and mice exhibiting the highest levels of TGF-β1 develop proteinuria and progressive renal insufficiency [68] with striking increases in matrix proteins.[69]

However, the role of TGF-β in podocytes has yet to be proven and non-TGF-β1 mediated mechanisms, including VEGF formation, may mediate albuminuria.[42] Although neutralizing antibody to TGF-β1 preserves GFR and prevents structural lesions in the streptozotocin-treated mouse, the antibody does not prevent proteinuria.[66] Cell cycle changes occur in mesangial cells prior to the induction of TGF-β, suggesting that glucose-related pathogenetic mechanisms may occur in parallel to and independent of TGF-β1[63] In addition, the renal effects of TGF-β are incompletely understood. Studies of the TGF-β1 knockout mice suggest that the anti-inflammatory properties of TGF-β may rival its profibrogenic ones. TGF-β knockout mice die of inflammatory lesions by 20 days[70] and experimental models of non-diabetic renal disease have suggested that TGF-β may confer a *protective* effect

against inflammatory renal lesions.[71] As a result, targeting downstream fibrogenic effectors of TGF-β1 (eg, connective tissue growth factor [CTGF], a fibrogenic mediator of TGF-β1), may provide a more viable approach to preserving glomerular structure and function than complete inhibition of gene expression.[72]

Conclusion

Both mesangial structural changes and glomerular hemodynamic alterations contribute to the pathogenesis of diabetic nephropathy. The crosstalk between these structural and functional lesions is extensive and likely, bidirectional. Signals that initiate from mesangial cells in response to hyperglycemia-induced ROS generation may contribute to the afferent dilatation that underlies the increase in transglomerular pressure. Many, but not all of the effects of hyperglycemia and glomerular hemodynamic changes, are mediated by TGF-β.

Dr. Alice Sheridan *is a physician in the Renal Division at Brigham and Women's Hospital.*

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Upcoming Scientific Meetings

14-19 November 2006
ASN Renal Week 2006
San Diego, California
CONTACT: www.asn-online.org

9-12 March 2007
Renal Physicians Association (RPA)
2007 Annual Meeting
Baltimore, MD
CONTACT: Tel. 301-468-3515
Fax: 301-468-3511
Website: www.renalmd.org

10-14 April 2007
National Kidney Foundation (NKF)
2007 Spring Clinical Nephrology Meetings
Walt Disney World Swan and Dolphin Hotel
Orlando, Florida
CONTACT: Tel. 800-622-9010
Fax: 212-689-9261
Website: www.kidney.org

21-25 April 2007
2007 World Congress of Nephrology
Rio de Janeiro, Brazil
CONTACT: Tel. +32 2 743 1546
Fax: +32 2 743 1550
Website: www.WCN2007.org

22-26 June 2007
American Diabetes Association 67th Scientific Session
Chicago, Illinois
CONTACT: http://scientificsessions.diabetes.org

Dr. Sheridan has no conflict of interest regarding the content of this issue. Dr. Sheridan's research is supported by grant number R01 DK054741.

This publication is made possible by an educational grant from

Amgen Inc.

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