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Pathophysiology of the Diabetic Kidney

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Abstract

Diabetes mellitus contributes greatly to morbidity, mortality, and overall health care costs. In major part, these outcomes derive from the high incidence of progressive kidney dysfunction in patients with diabetes making diabetic nephropathy a leading cause of end-stage renal disease. A better understanding of the molecular mechanism involved and of the early dysfunctions observed in the diabetic kidney may permit the development of new strategies to prevent diabetic nephropathy. Here we review the pathophysiological changes that occur in the kidney in response to hyperglycemia, including the cellular responses to high glucose and the responses in vascular, glomerular, podocyte, and tubular function. The molecular basis, characteristics, and consequences of the unique growth phenotypes observed in the diabetic kidney, including glomerular structures and tubular segments, are outlined. We delineate mechanisms of early diabetic glomerular hyperfiltration including primary vascular events as well as the primary role of tubular growth, hyperreabsorption, and tubuloglomerular communication as part of a “tubulocentric” concept of early diabetic kidney function. The latter also explains the “salt paradox” of the diabetic kidney, that is, a unique and inverse relationship between glomerular filtration rate and dietary salt intake. The mechanisms and consequences of the intrarenal activation of the renin-angiotensin system and of diabetes-induced tubular glycogen accumulation are discussed. Moreover, we aim to link the changes that occur early in the diabetic kidney including the growth phenotype, oxidative stress, hypoxia, and formation of advanced glycation end products to mechanisms involved in progressive kidney disease.

Introduction

Diabetes is the major cause of end-stage renal disease in the United States and elsewhere, and its incidence has increased by about 50% in the past 10 years (644). Notably, only about 20% of individuals with either type 1 or type 2 diabetes (T1DM; T2DM) actually develop nephropathy, indicating that specific genetic and/or environmental factors contribute to its initiation and progression. In fact, family-based studies including genome-wide scans suggest that a significant genetic component confers risk for diabetic nephropathy (73, 265, 266, 336, 553, 567). Moreover, diabetes-induced end-stage renal disease appears to be more

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likely to be present in African Americans (adjusted odds ratio 1.9), Hispanics (1.4), Asians (1.8), and Native Americans (1.9) than Caucasians (733). The pathogenesis of diabetic nephropathy is still incompletely understood and we do not know which genes are critically involved and also can not predict which individual diabetic patient will eventually develop end-stage renal disease. It is urgent to better understand the genes and events that lead from the onset of diabetes to impairment of renal function with the goal of identifying the patients at risk and achieving effective prevention. To prevent diabetic nephropathy, it may prove more reasonable and effective to identify and understand more completely the very early molecular events that initiate the progressive disease.

High-glucose concentrations induce specific cellular effects, which in the kidney affect many types of cells including endothelial cells, smooth muscle cells, mesangial cells, podocytes, cells of the tubular and collecting duct system and inflammatory cells and myofibroblasts. A better understanding of the molecular mechanisms underlying these effects is expected to be crucial to better understand the disease. In this regard, it will be important to identify effects that are not only observed *in vitro* in cultured cells but that are observed in the intact organism and are functionally relevant.

The hemodynamic phenotype in early diabetes is characterized by glomerular hyperfiltration which has been associated with progressive diabetic nephropathy (415), although this is still a matter of debate (see below for further discussion). Glomerular hyperfiltration has been attributed to abnormalities of the glomerulus and preglomerular vessels (447), which are related to changes in the metabolic milieu, vasoactive factors, alterations in signal transduction, as well as intrinsic defects in glomerular arterioles including electromechanical coupling. Albuminuria and proteinuria indicate relevant tissue damage in the diabetic kidney and, besides changes in renal hemodynamics, have been linked to specific alterations in podocyte function. Another notable phenotype of the early diabetic kidney is that it grows. This “growth” phenotype is characterized by enlargement of the kidney through both hyperplasia and hypertrophy which begin at the very onset of diabetes (512). The proximal tubule accounts for most of the cortical mass to begin with, and the proximal tubule also accounts for the greatest share of growth in diabetes (147, 571). As the tubule grows, more of the glomerular filtrate is reabsorbed and less reaches the macula densa (MD) at the end of Henle’s loop. This causes the glomerular filtration rate (GFR) to increase through the normal physiologic action of the tubuloglomerular feedback (TGF) system (648). As a consequence of hyperfiltration and the diabetic milieu, the glomeruli filter increased amounts of proteins, growth factors, and advanced glycation end products (AGEs). The diabetic milieu and the prolonged interaction of these proteins and factors with the tubular system trigger renal oxidative stress and cortical interstitial inflammation (2, 3), with the resulting hypoxia and tubulointerstitial fibrosis determining to a great extent the progression of renal disease (33, 65, 126, 154, 198, 386, 396, 581, 606). Moreover, the unique molecular mechanisms involved in the early growth phenotype of the diabetic kidney may contribute to set the stage for long-term kidney failure.

This review addresses these aspects in detail and aims to link changes that occur early in the diabetic kidney to progressive kidney disease. Most of the evidence currently available on these issues has been derived from patients and experimental models with T1DM. Fewer

data have been acquired on the early renal pathophysiology in T2DM. While for many aspects similar principles may apply, differences in circulating insulin levels (and C-peptide) and comorbidities are expected to modify the phenotypes in T1DM versus T2DM.

General Mechanisms of Glucose-Induced Cell Injury

The diabetic milieu affects most renal cell types and compartments, although the effects and consequences of high glucose and other components of the diabetic milieu may be specific in individual renal cells. Some cells may be more susceptible to high glucose-induced injury than others. As suggested by Brownlee (86), the cell susceptibility to glucose-induced toxicity is determined by its expression of glucose transporters that mediate cellular uptake of glucose from the extracellular compartment, allowing deleterious increases in intracellular glucose concentrations. Thus, the cell populations most susceptible to the changing milieu of diabetes are those that are unable to sufficiently downregulate glucose uptake and prevent high intracellular glucose levels in the setting of hyperglycemia. In this regard, both mesangial cells (228) and proximal tubular cells (418) are unable to decrease glucose transport rates adequately to prevent excessive changes in intracellular glucose when exposed to high glucose concentrations.

The following sections describe general mechanisms of glucose-induced cell injury that have been implicated in functional and structural changes observed in the diabetic kidney. These mechanisms often act upstream from a variety of processes discussed in the later sections dedicated to specific renal cells, compartments, and functions.

Generation and effects of advanced glycation end products

Prolonged hyperglycemia, but also dyslipidemia and oxidative stress in diabetes result in the production and accumulation of AGEs in the kidney, and at other sites of diabetic complications (86, 92, 591, 612). AGEs are formed by nonenzymatic Maillard or “browning” reaction between carbonyl groups of reducing sugars, like glucose, and amino groups on proteins, lipids, or nucleic acids at a rate determined by a number of factors including intracellular glucose concentrations, pH, and time. The first stable adduct between glucose and protein is fructose-lysine or the Amadori product (see Fig. 1). In addition, the glycation of intracellular proteins is initiated by the elevation of intracellular glucose degradation products (such as glyoxal resulting from glycolysis and the tricarboxylic acid cycle), which occurs more rapidly than with glucose itself. These AGEs can be generated from intra-cellular auto-oxidation of glucose to glyoxal, decomposition of early glycation (Amadori) products to 3-deoxyglucosone, and fragmentation of metabolites of the pentose phosphate pathway such as glyceraldehyde-3-phosphate and dihydroxyacetone phosphate to the reactive carbonyl methylglyoxal (13). Subsequent rearrangement and fragmentation reactions lead to the formation of AGEs. Under physiological conditions, these reactions are slow. However, in diabetes, persistent hyperglycemia, dyslipidemia, and oxidative stress all act to hasten the formation of AGEs (86, 87) and modification of both long-lived as well as short-lived proteins (86).

AGEs are a chemically heterogeneous group of compounds. The biochemistry of AGEs is remarkably complex and beyond the scope of this article [see (13, 614) for further details].

In brief, Ne-(carboxymethyl)lysine is the simplest and best characterized AGE, derived predominantly from the carbonyl modification of lysine. Tissue Ne-(carboxymethyl)lysine concentrations are increased in diabetes, and elevated levels are associated with the presence of vascular complications in patients with diabetes (47). Other more complex AGEs form “cross-links” both between and within modified proteins, such as pentosidine, MOLD (methylglyoxal lysine dimer) and GOLD (glyoxal lysine dimer). Once AGEs are formed, they are nearly irreversible, although enzymes, such as glyoxalase-1, have the ability to detoxify AGE precursors and inhibit AGE production (579). AGEs induce cell injury and contribute to diabetic complications by changing protein structure and function, such as the formation of cross-links between key molecules of basement membranes and the extracellular matrix (ECM) proteins (see Fig. 2). Importantly, AGEs also interact with a receptor (RAGE) on the cell surface thereby altering cellular function (see below).

Formation of AGEs in the ECM occurs on proteins with a slow turnover rate. Accumulation of AGEs on large matrix proteins such as collagens, fibronectin, and laminin through AGE-AGE intermolecular covalent bonds or cross linking increases collagen stiffness, reduces thermal stability, and causes resistance to digestion by metalloproteinases (MMPs) [reviewed in (614)]. In the kidney, this phenomenon is manifested by expansion of mesangial ECM (580), one of the hallmarks of diabetic nephropathy. In the vascular system, the process results in increased stiffness of the vasculature (300). The physiological relevance of these mechanisms has been validated in experimental studies showing nephroprotective effects of inhibitors of AGE formation (155, 306, 592) or by applying “cross-link breakers,” compounds that dissolve the above-mentioned cross-links between the ECM molecules in experimental diabetic nephropathy (178).

Glycation of extracellular lipids, as evidenced by the increased lipid-linked AGEs in low-density lipoproteins (LDL) samples from persons with diabetes, may also play a role in pathophysiology. For example, glycated LDL can contribute to endothelial dysfunction by reducing nitric oxide (NO) production and suppressing uptake and clearance of LDL through its receptor on endothelial cells (502).

Receptor-mediated effects of advanced glycation end products and their consequences—Several different receptors for AGEs have been discovered. The best studied AGE receptor, termed RAGE, initiates the intracellular signaling mechanisms that impair cellular function following the binding of AGEs (see Fig. 2). RAGE is a member of the immunoglobulin superfamily of receptors involved in inflammatory response (556). Upregulation of RAGE in diabetes occurs in the kidney, vasculature, and mononuclear phagocytes (557, 591). RAGE ligands include AGEs of at least two varieties: Ne-(carboxymethyl)lysine adducts (313), and hydroimidazolones derived from methylglyoxal and 3-deoxyglucosone. AGE-RAGE interaction stimulates multiple signals and activates important players in the pathophysiology of nephropathy, such as NAD(P)H oxidase, p21 ras, the mitogen-activated protein kinases (MAPKs), extracellular signal-regulated kinase ERK 1/2 and p38, Src, and the GTPases Cdc42 and Rac, resulting in activation and translocation of nuclear transcription factors, including NF- κ B (57,58, 515). Target genes of NF- κ B include genes for endothelin-1 (ET-1), vascular cell adhesion molecule-1 (VCAM-1), inter-cellular adhesion molecule-1 (ICAM-1), tissue factor, thrombomodulin,

vascular endothelial growth factor (VEGF), and likely, proinflammatory cytokines, including interleukins 1 and 6 (IL-1, IL-6), and tumor necrosis factor- α (TNF- α), as well as RAGE itself (57, 313, 515). Moreover, RAGE has been implicated in diabetes-induced mitochondrial superoxide formation in renal cells (123). Blockade of RAGE with anti-RAGE IgG, soluble RAGE (sRAGE), the extracellular ligand that mediates the clearance of AGEs and RAGE knockout all inhibit NF- κ B activation, the production of mitochondrial superoxide, and protect the kidney against deleterious effects of the diabetic milieu in experimental settings (57,58, 123, 515). More recent studies in mice indicated that blockade of RAGE may exert its renoprotective effects in the diabetic kidney in part via induction of the angiotensin (Ang) II type 2 (AT2) receptor (593).

Activation of the hexosamine pathway and consequences

Under normoglycemic conditions, only 3% of total glucose is utilized via the hexosamine pathway. However, when the intracellular concentrations of glucose are high, fructose-6-phosphate, an intermediate in the glycolytic pathway, is partially diverted into the hexosamine pathway (see Fig. 3). Entry into the hexosamine pathway is catalyzed by the first and rate-limiting enzyme glutamine:fructose 6-phosphate (F-6-*P*) amidotransferase (GFAT), which converts F-6-*P* and glutamine to glucosamine 6-phosphate (GlcN-6-*P*) and glutamate. Subsequent steps metabolize GlcN-6-*P* to UDP-*N*-acetylglucosamine (UDP-GlcNAc). UDP-GlcNAc is a molecule of particular interest with respect to the development of diabetic complications. It serves as a substrate of *O*-GlcNAc transferase (OGT). This is a cytosolic and nuclear enzyme that catalyzes a reversible posttranslational protein modification, whereby GlcNAc is transferred in *O*-linkage to specific serine/threonine residues of numerous proteins (86, 93, 555). The sites of *O*-GlcNAc modification (*O*-GlcNAcylation) are often identical or adjacent to known phosphorylation sites, suggesting a regulatory function. Functional significance of *O*-GlcNAcylation has been reported for several proteins, including the transcription factors Sp1 and c-myc, as well as cytosolic and nuclear enzymes, such as glycogen synthase (GS) and RNA polymerase II [reviewed in (93)]. Sp1 was the first transcription factor identified as an *O*-GlcNAc-modified protein; it has multiple *O*-GlcNAc modification sites, and its phosphorylation on serine and threonine residues is inversely proportional to its *O*-GlcNAc modification (93).

The hexosamine pathway has been causally linked to up-regulation of several molecules implicated in the pathophysiology of nephropathy. Glucosamine reproduced the effect of glucose on induction of transforming growth factor β 1 (TGF- β 1), and blocking GFAT activity with antisense GFAT oligonucleotide or with a chemical inhibitor ameliorated high glucose/GlcN stimulation of TGF- β 1 synthesis; vice versa overexpressing GFAT-induced TGF- β 1 and fibronectin expression in mesangial cells incubated in 5 mM glucose (696). A more recent study (695) has suggested an ability of the hexosamine pathway to increase TGF- β 1 promoter activity. Thus TGF- β 1 expression seems to be dependent, at least in part, on the activity of the hexosamine pathway (see Fig. 3). The hexosamine pathway has been also implicated in diabetes-induced expression of plasminogen activator inhibitor 1 (PAI-1) in mesangial cells (271) and in high glucose-induced upregulation of angiotensinogen in renal tubular cells (247). Another line of evidence has shown the capability of hexosamines to activate Rho, a small GTPase protein, and its immediate downstream target, Rho kinase

(ROCK), in vascular smooth muscle cells (303), a pathway recently implicated in the pathophysiology of nephropathy (316, 323, 484).

The O-linked post-translational modification has been described for endothelial NO synthase (eNOS), an enzyme responsible for endothelial NO production and essential for normal endothelial function. Du et al. (150) and Federici et al. (165) reported that hexosamine pathway-induced modification of eNOS protein interferes with its activation by phosphorylation thereby resulting in a reduced ability of eNOS to produce NO. More recent studies have also implicated the pathway in mesangial proliferation mediated by rapamycin-sensitive mTOR complex 1 (mTORC1) as well as in mesangial apoptosis (272). Further studies are necessary to define the relevance of the hexosamine pathway in the diabetic kidney *in vivo*.

The polyol pathway

The key enzyme in this pathway, aldose reductase, catalyses reduction of toxic aldehydes in the cell to inactive alcohols. When the glucose concentration in the cell becomes too high, aldose reductase reduces glucose to sorbitol, which is later oxidized to fructose. In the process of reducing high amounts of intracellular glucose to sorbitol, the aldose reductase consumes the cofactor NADPH, which is the essential cofactor for regenerating the intracellular antioxidant, reduced glutathione. By reducing the amount of reduced glutathione, the polyol pathway increases susceptibility to intracellular oxidative stress (86). In addition, since sorbitol does not cross cell membranes, its intracellular accumulation results in osmotic stress. A small number of studies conducted in the 1990s suggested that aldose reductase inhibition could attenuate glucose-induced activation of protein kinase C (PKC) and TGF- β production in mesangial cells *in vitro* (261), as well as inhibit some other markers of experimental nephropathy (42, 394). Moreover, the polyol pathway has been implicated in glucose-mediated alterations in proximal tubular cell matrix generation (62, 418, 749), whereas it does not seem to contribute to tubular hypertrophy (749). Further studies are necessary to define the significance of the polyol pathway in renal pathophysiology and outcome.

Activation of protein kinase C and downstream targets

The activation of the diacylglycerol (DAG)-PKC pathway is associated with many abnormalities in retinal, cardiovascular, and renal tissues in diabetic- and insulin-resistant states (332). PKC is a family of serine/threonine kinases that consist of 12 isoforms. PKC isoforms are classified according to whether they contain domains that bind calcium or DAG, both of which positively regulate the kinase activity. Conventional PKC isoforms (α , β 1/2, and γ) bind both calcium and DAG, novel PKC isoforms (δ , ϵ , η , θ , and μ) bind DAG, but not calcium, and atypical PKC isoforms (ζ , λ) bind neither. The activation of conventional and novel PKC isoforms require the phosphorylation of the isoforms and the presence of cofactors such as calcium and/or DAG. When phosphorylated, increases in calcium and/or DAG induce PKC translocation to the membranous compartments of the cells to elicit biological actions. Rapid and short-term increases of DAG and calcium levels are usually induced via the activation of phospholipase C. Chronic activation of conventional

and novel PKC isoforms require sustained elevations of DAG, which involves the activation of phospholipase D/C or the *de novo* synthesis of DAG (131, 332).

In diabetes, PKC is activated via multiple mechanisms. PKC activation in diabetic tissues is linked to enhanced formation of DAG. The latter can be formed by *de novo* synthesis from glycolytic intermediates, dihydroxyacetone phosphate and glycerol-3-phosphate, a process stimulated by high glucose levels (86, 131). DAG can be also derived from the hydrolysis of phosphatidylinositides, which are derived from the metabolism of phosphatidylcholine by phospholipase C (131). Another mechanism leading to activation of the DAG–PKC pathway involves hyperglycemia-induced increases in oxidants such as H₂O₂ which are known to activate PKC either directly or by increasing DAG production (442). In addition, PKC is activated as part of post-receptor signaling of vasoactive peptides known to be involved in the pathophysiology of diabetic complications, such as angiotensin (Ang) II (695) or ET-1 (141). AGEs, as another important component of the diabetic milieu, have been also shown to activate PKC, via ROS-mediated mechanisms (177). Thus, PKC activation is part of several signaling pathways implicated in the pathophysiology of the diabetic kidney.

Studies have suggested the importance of the activation of specific PKC isoforms, mainly the α , β 1/2, and δ isoforms, in causing cardiac or microvascular pathologies in diabetic animals (295, 332, 404, 489). In the kidney, activation of PKC has multiple consequences such as altered regulation of endothelial permeability, vasoconstriction, tubular transport, ECM synthesis/turnover, cell growth, angiogenesis, cytokine activation and leukocyte adhesion (156, 445). Selective inhibition of PKC β has been shown to be nephroprotective in several models of diabetic kidney disease, which may involve effects on multiple target cells including mesangial cells, tubular cells, and antigen-presenting interstitial cells (260, 305, 308, 330, 489). First, clinical trials with a selective inhibitor of PKC β showed beneficial short-term effects, whereas long-term kidney outcomes, assessed in patients with diabetic eye disease, were similar to placebo (638, 641). Notably, genetic variants of the PKC β 1 gene have been linked to the development of end-stage renal disease in patients with T2DM (378).

While the activation of PKC has been mostly studied as mediators of diabetes-induced cell injury, it should be noted that some isoforms, such as PKC ϵ , may exert protective actions in the diabetic kidney (400).

Oxidative stress

Reactive oxygen species (ROS) include free radicals such as superoxide anion ($\bullet\text{O}_2^-$), hydroxyl anion ($\bullet\text{OH}^-$), and peroxy ($\bullet\text{RO}_2$) and nonradical species such as hydrogen peroxide (H₂O₂) and hydrochlorous acid (HOCl). It is important to note that there are also reactive nitrogen species produced from similar pathways, which include the radicals NO ($\bullet\text{NO}$) and nitrogen dioxide ($\bullet\text{NO}_2$), as well as the nonradical peroxynitrite (ONOO⁻), nitrous oxide (HNO₂), and alkyl peroxynitrates (RONOO). Of these, $\bullet\text{O}_2^-$, $\bullet\text{NO}$, H₂O₂, and ONOO⁻ have been the most widely investigated in the diabetic kidney (177). Formation of ROS has been intensively studied as one of the principal mechanisms of glucose-induced cell toxicity, resulting in oxidation of important macromolecules including proteins, lipids,

carbohydrates, and DNA. ROS can also act as signaling molecules for growth and vasoactive factors, such as Ang II (209).

The sources of ROS in cells exposed to the diabetic milieu can be divided into mitochondrial and cytosolic, as discussed in the following and outlined in Figure 4.

Mitochondrial source of reactive oxygen species in diabetes—Mitochondria provide energy to the cell as ATP through oxidative phosphorylation. Oxidative phosphorylation is the process by which ATP is formed as electrons are transferred from NADH or FADH₂ (generated through the Krebs cycle or indirectly via glycolysis) to molecular oxygen (O₂). The generation of ROS, specifically •O₂[−], by damaged or dysfunctional mitochondria, has been postulated as the primary initiating event in the development of diabetes complications (86, 177, 442). In diabetes, where there is an excess of fuels supplied as a result of chronic hyperglycemia feeding into the respiratory chain, it has been hypothesized that excess production of •O₂[−] occurs via the premature collapse of the mitochondrial membrane potential, which, rather than driving ATP production, leaks electrons to oxygen to form •O₂[−]. A number of functional enzymes within the mitochondria are particularly susceptible to ROS-mediated damage, leading to altered ATP synthesis, cellular calcium dysregulation, and induction of mitochondrial permeability transition, all of which predispose the cell to necrosis or apoptosis (86, 177). However, it should be noted that these predominantly tissue culture studies (312) remain to be fully substantiated *in vivo*, particularly with respect to their role in nephropathy. More recent studies showed that RAGE induce cytosolic ROS and promote mitochondrial •O₂[−] generation in diabetes. Notably, no increase in the production of mitochondrial •O₂[−] was seen in renal cortices from gene-targeted diabetic mice lacking RAGE (123).

Cytosolic sources of reactive oxygen species in diabetes—The system of NAD(P)H oxidases seems to be, at this stage, the most important source of cytosolic ROS in the diabetic kidney. The phagocyte NADPH oxidase catalyzes the NADPH-dependent reduction of molecular oxygen to generate superoxide anion (•O₂[−]) which is dismutated to form H₂O₂ (177). The enzyme consists of two plasma membrane-associated proteins, gp91*phox* (the catalytic subunit) and p22*phox* which comprise flavocytochrome b558, and cytosolic factors, p47*phox*, p67*phox*, and the small GTPase Rac (177). Studies over the last decade have documented significant NAD(P)H-dependent ROS-producing activities, not only in phagocytes, but also in nonphagocytic cells, including kidney cells (mesangial cells, podocytes, tubular cells) (177, 604).

The family of gp91*phox* homologues termed Nox (for NAD(P)H oxidase) proteins consists of five members (Nox1–5), of which Nox2 is gp91*phox* or the neutrophil isoform (347). These oxidases are proposed to play a role in a variety of signaling events, such as cell growth, cell death or survival, oxygen sensing, and inflammatory processes. The kidney seems to be particularly susceptible to NADPH oxidase-induced oxidative damage. The isoform Nox4/Renox was cloned from the kidney and found to be highly expressed in renal tubular epithelial and mesangial cells (205). Enzyme activity and subunit expression are upregulated in response to high glucose in vascular and renal cells as well as in experimental models of diabetes and mediate diabetes-induced oxidative stress and downstream signaling,

as well as renal cell structural and functional abnormalities characteristic for the diabetic state (177, 205, 235, 547, 604).

Cytosolic ROS are also formed during the formation of intracellular AGEs (177) and as a result of activation of the polyol pathway, processes discussed above in more detail. Other sources of cytosolic ROS include uncoupled eNOS (235) and xanthine oxidase (177). Further information about the individual sources of ROS and their pathophysiological roles in specific kidney compartments and cells is provided in the following sections.

In parallel with enhanced ROS generation in the diabetic environment, the cells exposed to high glucose and other components of the diabetic milieu lose their ability to fight oxidative stress. Several mechanisms of endogenous antioxidant protection have been shown to be altered in diabetic kidney as discussed in the following.

Superoxide dismutase—Superoxide dismutase (SOD) is the major antioxidant enzyme for superoxide removal, which converts superoxide into H_2O_2 and molecular oxygen (184). The hydrogen peroxide is further detoxified to H_2O by other important components of the cellular antioxidant system, catalase or glutathione peroxidase. In mammals, three SOD isoforms exist: cytoplasmic CuZnSOD (SOD1), mitochondrial MnSOD (SOD2), and extracellular CuZnSOD (SOD3, ecSOD) (177). In addition to experimental evidence, lower SOD activity has also been observed in the diabetic patients with nephropathy as compared with those without diabetic complications (177).

Overexpression of cytoplasmic Cu/ZnSOD1 in mice attenuated diabetes-induced albuminuria, glomerular hypertrophy, and ECM accumulation (142). Recent studies in mice with different susceptibility to the development of nephropathy have shown that the differences in strains may be attributable to differences in activities of SOD isoforms (189). AGE modifications could be responsible for altered activity of these enzymes in diabetic nephropathy (177).

Glutathione—The tripeptide glutathione (GSH, glutamylcysteinylglycine) is the abundant intracellular nonprotein thiol. Some 20% of intracellular glutathione is located in the mitochondria where it helps protect against mitochondrial ROS production. There are several mechanisms whereby hyperglycemia may bring about oxidative stress via changes in glutathione metabolism (86, 142, 177). Excessive glucose resulting in mitochondrial or cytoplasmic ROS generation (described above) may exhaust glutathione pool. Furthermore, as mentioned, hyperglycemia results in increased flux through the polyol pathway causing glutathione depletion.

Thioredoxin—The major thioredoxins are thioredoxin-1, a cytosolic and nuclear form, and thioredoxin-2, a mitochondrial form. Thioredoxin-1 efficiently reduces cellular ROS through thioredoxin peroxidases, which belong to a conserved family of antioxidant proteins, the peroxiredoxins. The reduced form of thioredoxin peroxidase scavenges oxidant species such as H_2O_2 and in the process homo- or heterodimerizes with other family members. Thioredoxin interacting protein (Txnip), also known as vitamin D3 upregulated protein-1 or thioredoxin binding protein-2, is the endogenous inhibitor of cellular thioredoxin,

inactivating its antioxidative function. Hyperglycemia promotes oxidative stress, in part, through inhibition of thioredoxin activity by increased expression and activity of Txnip (566), mechanism recently demonstrated to be operating also in the diabetic kidney (12).

Detailed discussion of these exceedingly complex biochemical reactions is beyond the scope of this articles and the readers are referred to excellent reviews addressing this topic (86, 177, 475).

Glomerular Pathophysiology in Diabetes

The diabetic milieu has profound effects on glomerular function and morphology, affecting all types of glomerular cells and structures. In the following section, we will discuss diabetes-induced mechanisms of glomerular cell injury in specific cell types and their consequences for the development of proteinuria and structural changes.

Mesangial cell pathophysiology in the diabetic kidney

Although there are some exceptions to the rule, the hypertrophic phenotype prevails over the hyperplastic phenotype in diabetic glomeruli. Mesangial expansion is considered to be one of the most important structural lesions during the development of nephropathy in T1DM. The importance of mesangial expansion was proposed in seminal human biopsy studies by Mauer et al. (396) and Osterby et al. (464, 466). These studies formulated the so-called structural-functional relationship in diabetic nephropathy based on tight correlations between the degree of mesangial expansion and clinical characteristics of nephropathy, such as proteinuria, blood pressure, and the rate of decline in GFR. Mesangial expansion is determined both by mesangial cell hypertrophy and accumulation of mesangial matrix. These processes are closely related and are discussed together. Many of the following pathways are further discussed and illustrated in the section on tubular growth.

Factors driving mesangial expansion

Factors that are driving mesangial expansion are discussed in the following. We begin with growth factors and cytokines, which are stimulated by hyperglycemia, AGEs, oxidative stress, and physical forces, such as mechanical stress (211), and then discuss the role of cell-cycle regulation.

Transforming growth factor- β system—This system includes TGF- β peptides and receptors as well as Smad signaling molecules. TGF- β signals via TGF- β types I and II receptors. Upon TGF- β binding to the type II receptor on the cell surface, this complex activates the type I receptor, which then phosphorylates and activates members of the Smad family (Smad2 and Smad3). The activated Smads form oligomers with the unique co-Smad, Smad4, and rapidly translocate to the nucleus to regulate expression of target genes. Smad7 is an inhibitory Smad that regulates TGF- β signaling, preventing recruitment and phosphorylation of Smad2 and Smad3 (364). In addition, recruitment of a complex of Smad7 with Smurf1 or Smurf2 to the type I TGF- β receptor results in receptor ubiquitination by the Smurf proteins and targets the receptor for degradation.

TGF- β is a prosclerotic factor considered to be crucial for the development of mesangial cell hypertrophy and production of ECM proteins. This factor not only stimulates genes for the components of ECM (68), but also inhibits the synthesis of MMPs, enzymes responsible for ECM degradation, and stimulates production of inhibitors of metalloproteinases (TIMP). Thus, TGF- β is one of the major determinants of the dysbalance between production and degradation of ECM in the diabetic kidney (68). In addition, TGF- β influences the cell cycle as discussed below (706).

Numerous studies have shown that TGF- β plays a key role in the process of mesangial expansion and the development of glomerulosclerosis. Renal expression of TGF- β and other components of this system are increased in the kidney both in diabetic patients and in a variety of experimental models of diabetes (71, 191, 220, 263, 331, 575, 578, 723, 748). In addition to the components of the diabetic milieu discussed in the introductory part of this article (103), it is important to note that TGF- β is also activated by factors more typical for the metabolic environment in T2DM, such as leptin (220, 709).

TGF- β is also activated by Ang II and acts as one of the mediators of growth and prosclerotic effects of the renin-angiotensin-aldosterone-system (RAS) (69, 119, 286, 707). Indeed, inhibitors of the RAS such as ACE inhibitors and Ang AT1 receptor blockers (ARB), or more recently with renin inhibitors (251) reduce TGF- β expression in the diabetic kidney, which is considered to be important for antifibrotic effects of these agents (70, 191). This phenomenon is discussed in more detail in a section devoted to the intrarenal activation of the RAS in the diabetic kidney. In addition, TGF- β has been shown to operate downstream of other vasoactive and growth factors acting in the diabetic kidney such as ET-1 (231) or vasoconstrictor prostanoids (107, 430, 712).

The role of TGF- β as a mediator of diabetic glomerulosclerosis, as well as interstitial fibrosis (discussed in the following sections) has been documented in a number of studies using a variety of experimental approaches. A seminal study by Ziyadeh et al. (748) utilized long-term treatment with a neutralizing antibody to demonstrate that TGF- β inhibition ameliorated mesangial expansion and glomerulosclerosis in db/db mice, a model of T2DM. Interestingly, these protective effects were not associated with a significant reduction of proteinuria, suggesting that the glomerular pathology is driven by multiple factors acting on various cell types. More recently, the results by Ziyadeh et al. (748) were reproduced in studies with Smad3 knockout (KO) mice (686).

Several proteins from the TGF- β family act as endogenous antagonists of TGF- β and have also been implicated in the pathophysiology of nephropathy. For example, bone morphogenic protein-7 (BMP-7) prevents the development of nephropathy in streptozotocin (STZ)-diabetic rats (314). The small proteoglycan decorin is another endogenous inhibitor of TGF- β , acting at least in part downstream of the protective hepatocyte growth factor (HGF) (315).

Connective tissue growth factor—Connective tissue growth factor (CTGF), a 36 to 38 kDa cysteine-rich secreted protein, has been identified as a prosclerotic cytokine involved in the regulation of ECM accumulation by acting as a downstream mediator of TGF- β (91,

521). However, there is also evidence for TGF- β -independent regulation of CTGF by other components of the diabetic milieu (684). Exposure of mesangial cells to high glucose concentrations, mechanical strain, AGEs, or TGF- β leads to increased levels of CTGF gene expression and protein secretion (91, 525). In their seminal study, Riser et al. (525) examined cultured rat mesangial cells, kidney cortex, and microdissected glomeruli from diabetic db/db mice, a model of T2DM, and their control counterparts. Their results suggested that diabetes or hyperglycemia-induced CTGF upregulation is important for mesangial matrix accumulation and progressive glomerulosclerosis, acting downstream of TGF- β . Similarly, in STZ-diabetic mice, a model of T1DM, Roestenberg et al. (529) reported that CTGF expression coincided with the initiation of mesangial expansion and preceded increases in renal fibronectin and collagen IV expression.

Importantly, several observations have suggested that urinary CTGF may serve as an early biomarker and predictor of progression of nephropathy in diabetic patients. In patients with diabetes and microalbuminuria or overt diabetic nephropathy, urinary CTGF mRNA (10) or protein excretion are increased (197, 436, 522) and correlate with urinary albumin excretion and GFR. Moreover, a prospective study by Nguyen et al. has identified plasma CTGF as an independent predictor of end-stage renal disease and mortality in patients with diabetic nephropathy (437). Similarly to TGF- β , CTGF is present in the renal tubular fluid where it may act directly on tubular epithelial cells and mediate epithelial-mesenchymal transition (91), a process discussed in more detail in the following sections.

Further support for a pathogenetic role of CTGF in nephropathy provided studies with antisense oligodeoxynucleotides. This intervention was successful in reducing proteinuria and expression of genes involved in mesangial matrix expansion in mouse models of diabetes (212). In a first small and open-label phase 1 safety trial in microalbuminuric diabetic patients, the human monoclonal antibody to CTGF, FG-3019, rapidly and significantly lowered urine albumin to creatinine ratios, which warrant further prospective, randomized and blinded clinical trials (11).

Growth hormone and insulin-like growth factor axis—Diabetes is associated with complex alterations of growth hormone (GH) and insulin-like growth factor I (IGF-I) secretion, activity and actions. Although a very detailed analysis is beyond the scope of this article, it is important to note that relatively abundant evidence has been amassed with respect to renal actions of these hormones and their possible contribution to the development of nephropathy. The kidneys are an important source for the synthesis of IGF-I, some of which is released into the circulation (670). GH and IGF-I have profound effects on renal growth, glomerular hemodynamics, and tubular function. Administration of IGF-I to animals promotes renal growth through a process of cellular hypertrophy and hyperplasia, and induces a rapid increase in renal blood flow and GFR together with a reduction in renal vascular resistance (163, 670). Subjects with diabetes and microalbuminuria present several alterations in the GH-IGF-IGF-binding proteins (IGFBP) system, with increased IGF-I renal levels and IGFBP-3 protease activity, increased excretion of bioactive GH, IGF-I, and IGFBP-3, but decreased circulating IGFBP-3 levels. Circulating levels of IGF-I are normal or reduced in patients with diabetes when compared with normal controls, but renal IGF-I

production can be increased and contributes to the pathogenesis of diabetic nephropathy (670).

The most relevant evidence about the roles of the GH-IGF-I pathway in diabetic renal pathophysiology has been provided by studies with inhibitors of the system. Antagonists of the GH-IGF-I pathway, including somatostatin analogues or GH and IGF-I receptors antagonists, have been found to have beneficial effects on the diabetic kidney in animal models (340, 568). Landau et al. (340) investigated the effects of PTR-3173, a novel somatostatin analogue which exerts a prolonged inhibition of the GH-IGF axis without affecting insulin secretion, on markers of diabetic nephropathy in the nonobese diabetic (NOD) mouse. The treatment with PTR-3173 blunted renal and glomerular hypertrophy, albuminuria, and glomerular hyperfiltration in diabetic NOD mice. These effects were associated with the prevention of renal IGF-I accumulation.

Specific inhibitors of GH action [i.e., specific GH receptor antagonists (GHRAs)] represent another group of agents with nephroprotective potential in diabetes. Diabetic mice treated from the onset of diabetes with the GHRA, G120K-PEG, showed normalization of diabetes-induced renal hypertrophy, glomerular enlargement, and slower development of albuminuria (568). In addition, beneficial renal effects were reported even after late intervention with GHRA in NOD mice with manifest renal changes (568).

Platelet-derived growth factor—Platelet-derived growth factor (PDGF) is expressed mainly in glomerular mesangial and epithelial cells and it plays an important role in the production of ECM proteins in renal diseases. Several studies have suggested that PDGF may be involved in the pathogenesis of DN, and increased glomerular expression of this factor has been documented in models of diabetic nephropathy *in vitro* (633) and *in vivo* (306), as well as in renal biopsies from patients with nephropathy (345). However, the pathogenetic role of PDGF in diabetic nephropathy still remains to be tested in studies with selective inhibitors.

The role of cell-cycle regulation—Although diabetes-induced changes in cell-cycle regulation occurs in a variety of cell types, this topic is introduced in this section and further discussed in the section on tubular growth. Following activation, for example by growth factors, quiescent cells in G₀ phase, progress through G₁ phase and initiate DNA synthesis in S phase. During the G₂ phase, cell growth continues and proteins are synthesized in preparation for entry into the M phase, where mitosis occurs. The process requires orchestrated activation of cyclin-dependent kinases (CDK), which, after binding specific proteins (cyclins) form active complexes, and act as positive regulators of the cell cycle. The cyclin-CDK complexes are regulated by multiple phosphorylation and dephosphorylation events and by several proteins called CDK-inhibitors. Two classes of CDK-inhibitors have been identified: the INK4 family and the Cip/Kip family.

The transition from late G₁ into the S phase determines the cell's growth characteristics. G₁ arrest results in hypertrophy, whereas G₁ exit is associated with apoptosis. Transition from G₁ to S phase results in DNA synthesis and proliferation. On entry into G₁, cells normally undergo a physiologic increase in protein synthesis before S phase DNA synthesis. In

hypertrophic cells, as in the kidney cells exposed to the diabetic milieu, this increase in protein content is not matched by a concurrent increase in DNA. Consequently, one mechanism underlying cellular hypertrophy is cell-cycle arrest at the G₁/S checkpoint, leading to increased protein to DNA ratio (388, 573).

Mesangial cells respond to high glucose with an initial proliferation phase, which is followed by G₁ arrest and hypertrophy (732). Hyperglycemia increases the expression of cyclin D1 and the activation of CDK4, evidence of cell-cycle entry (167). Importantly, exposure to high glucose increases the levels of both CDK-inhibitors p21 Cip1 and p27 Kip1 in cultured mesangial cells (709), and antisense oligonucleotides (ASOs) to p21 Cip1 or p27 Kip1 reduce the hypertrophic effects of hyperglycemia (163). Further support for the roles of CDK inhibitors in diabetes-induced mesangial hypertrophy has been provided in studies with p21 or p27 KO mice. High glucose-induced mesangial hypertrophy was not observed in cells harvested from p21 KO or p27 KO mice. Moreover, the reconstitution of p27 Kip1 by transfection in p27 KO mesangial cells restored the hypertrophic phenotype (417, 710). In addition, both p21 Cip1 and p27 Kip1 are required for maximal mesangial cell hypertrophy induced by TGF- β (163) and hypertrophic effects of CTGF are at least in part mediated by increased expression p15 INK4, p21 Cip1, and p27 Kip1 (4).

The *in vitro* evidence discussed above has been reproduced in *in vivo* studies in experimental models of diabetic nephropathy. In STZ-diabetic mice, glomerular hypertrophy was associated with a selective increase in p21 Cip1 (338). Diabetic p21 KO mice are protected from glomerular hypertrophy and development of progressive renal failure (16). Diabetic p27 KO mice exhibit only mild mesangial expansion without glomerular hypertrophy, despite increases in glomerular TGF- β (32, 708). In summary, these cell culture and experimental models show that p21 Cip1 and p27 Kip1 play a critical role in mediating diabetes-associated glomerular hypertrophy and the consequential increase in matrix proteins, associated with the deterioration in renal function. These pathways are further discussed and illustrated in the section on tubular growth.

Notably, an influence of the presence of ECM proteins on the response of mesangial cells to diabetes has been indicated in a recent study in STZ-diabetic mice. Using KO mice for type VIII collagen, Hopfer et al. linked mesangial expansion and cellularity, ECM expansion and albuminuria to the expression of type VIII collagen which is enhanced in this model (243).

Changes in the Glomerular Filtration Barrier in the Diabetic Kidney

The glomerular filtration barrier (GFB) is composed of glomerular endothelial cells (GEC), the glomerular basement membrane, and podocytes (glomerular epithelial cells). All three components of the barrier undergo changes in diabetes and contribute both to the structural glomerular alterations and changes in the permeability to macromolecules. In addition to other functions, the integrity of the GFB determines the extent to which albumin and other proteins will be filtered into the urinary space and, thereby, contributes to the development of albuminuria/proteinuria, a hallmark of diabetic nephropathy. Figure 5 illustrates and summarizes diabetes-induced changes in the GFB.

The GFB contributes to the impermeability to macromolecules by size and charge selectivity. Under physiological conditions, low molecular weight proteins (40 kDa) are essentially freely filtered, whereas, high molecular weight proteins (100 kDa) are almost completely restricted (125, 390). The molecular weight of albumin is 69 kDa, that is, of borderline size. In patients with diabetes and low-grade albumin excretion (microalbuminuria), no changes in size selectivity, determined mainly by the radius of glomerular endothelial pores, have been reported (550), suggesting a possible role for alterations in the charge barrier at the early stages of microalbuminuria. The charge barrier is formed by anionic sites within the GBM, on podocytes and the endothelial glycocalyx. As the disease progresses, anatomical derangements of the GFB result in gradual loss of selectivity.

Changes in the glomerular endothelium in the diabetic kidney

The changes in the glomerular endothelium are similar to the well-described generalized endothelial dysfunction in diabetes, including reduced bioavailability of NO, impaired endothelium-dependent regulation of vascular tone, upregulation of adhesion molecules, development of a pro-thrombotic phenotype and increased permeability (203, 318, 500, 551). These changes are not specific for GEC, but are applicable to the entire renal vascular tree.

Unlike other capillaries, glomerular capillaries have a high permeability to water (hydraulic conductivity), yet, like other capillaries, they are relatively impermeable to macromolecules. GEC are highly specialized cells with regions of attenuated cytoplasm punctuated by numerous fenestrae, circular transcellular pores 60 to 80 nm in diameter (36). The glycocalyx is a layer on the luminal surface of GEC, largely composed of glycoproteins and proteoglycans with adsorbed plasma proteins. Heparan sulphate proteoglycans (HSPGs) may be responsible for the negative charge characteristics of the glycocalyx (36). Relevant for the pathophysiology of diabetic nephropathy, ROS have been shown to disrupt the glycocalyx (230). In diabetes, a specific assessment of structural changes in GEC and the associated glycocalyx has not been performed. However, indirect evidence supports the notion that abnormalities of the glycocalyx exist and may be relevant. For example, total systemic glycocalyx volume is reduced by acute hyperglycemia in humans (440). Furthermore, patients with T1DM have decreased systemic glycocalyx volume, which correlates with the presence of microalbuminuria (439).

Impaired activity of endothelial nitric oxide synthase and of nitric oxide—eNOS
and eNOS-derived NO are crucial molecules for endothelial function and integrity. NO acts as a potent vasodilator and as a molecule with potent anti-thrombotic and anti-growth properties in the vasculature (318). Earlier studies suggested several direct mechanisms whereby the diabetic milieu antagonizes NO bioavailability. These mechanisms include NO quenching by AGEs (89, 673) or ROS (235), and NO capture by glucose (82).

More recent studies revealed a spectrum of mechanisms related to posttranslational alterations of eNOS molecules in diabetes. To function as an endothelial NO-producing enzyme, eNOS requires a battery of co-factors, posttranslational modifications such as

phosphorylation and dimerization, protein-protein interactions, and subcellular targeting (207). Phosphorylation of eNOS on Ser¹¹⁷⁷ by several serine/threonine kinases, such as Akt (protein kinase B) (143, 192) or protein kinase A (PKA) (94), in response to a variety of physiological stimuli, is a critical control step for NO production by the enzyme. This process enhances the rate of electron flux from the reductase to the oxygenase domain of eNOS, and reduces the calcium requirements for the enzyme, thus increasing NO synthesis (398). Also the formation of a homodimer is crucial for NO production by eNOS (229, 350), creating high affinity binding sites for the NOS substrate L-arginine, and enabling electron transfer from the reductase domain of one NOS monomer to the oxygenase domain of the other (541). Finally, activation of eNOS is not only dependent on phosphorylation by upstream kinases and conformational changes, but is also determined by its specific subcellular localization with the activatable enzyme being localized within the plasma membrane.

As mentioned in the “*General mechanisms of glucose-induced cell injury*” section, hexosamine pathway-induced modification of eNOS molecule *in vitro* interferes with its stimulatory phosphorylation resulting in a reduced ability of eNOS to produce NO (150, 165). To address these issues in *in vivo* settings, Komers et al. (326) analyzed renal cortical samples from control and STZ-diabetic rats, and described impaired eNOS dimerization, membrane targeting, and stimulatory phosphorylation at Ser¹¹⁷⁷ in diabetic samples despite of similar whole cell eNOS expression in diabetic and control animals. These findings further suggest that a process called “eNOS uncoupling” operates also in the diabetic kidney. During this process, electron transfer within the active site of eNOS becomes “uncoupled” from L-arginine oxidation resulting in reduction of molecular oxygen to superoxide (669, 720). Thus, eNOS can become a ROS producing enzyme in sharp contrast to its protective role as a machinery for production of protective NO. Indeed, renal production of ROS has been shown to be attenuated by L-arginine-analogues (547) that act as NOS inhibitors. The mechanisms of uncoupling are currently being investigated, but well-described diabetes-induced deficiency in co-factors, such as tetrahydrobiopterin (402, 503), necessary for conformational changes of eNOS allowing NO production, are likely to play a role.

Moreover, as postulated by Nakagawa et al. (423) high glucose-induced posttranslational changes in eNOS may be responsible for uncoupling of VEGF actions from eNOS, allowing unhindered growth and vasoactive signaling of VEGF (discussed in detail below) without parallel activation of protective eNOS-derived NO production. VEGF normally stimulates endothelial NO release and acts in concert with elevated NO levels as a trophic factor for vascular endothelium. The increased NO derived from the endothelial cell acts as an inhibitory factor that prevents excess endothelial cell proliferation, mesangial or vascular smooth muscle cell proliferation, and macrophage infiltration. In the setting of reduced NO bioavailability, high levels of VEGF lead to excessive endothelial cell proliferation, stimulation of macrophage chemotaxis, and mesangial or vascular smooth muscle cell activation.

Role of vascular endothelial growth factor in the early and later stages of the diabetic kidney—Several kidney biopsy studies have confirmed increased glomerular vascularity and endothelial cell proliferation at early stages of diabetic nephropathy (465).

VEGF, a pro-angiogenic factor, is prominently produced by podocytes (122). VEGF may affect podocyte function in an autocrine fashion (via VEGFR1) (106). VEGF protein also crosses the GBM and acts on GEC (via VEGFR1 and VEGFR2) to promote endothelial cell survival and to induce the formation of fenestrae, which enhance glomerular endothelial permeability. VEGF expression is increased at early stages of nephropathy, and has been implicated in diabetic renal patho-physiology, and in accord with its actions, as a mediator of albuminuria or even structural changes (122, 134). Indeed, inhibition of VEGF by a variety of means reduces albuminuria in diabetic rodents (134, 337, 603).

However, the role VEGF and angiogenesis in the development of diabetic kidney disease, even albuminuria is more complex. VEGF may be elevated in the early phases of the diabetic kidney, but it may not be maintained as more chronic fibrotic changes occur in the kidney, and VEGF may decrease in the advanced stages of the disease. In a remnant kidney model, VEGF levels are reduced, correlating with the progression of renal damage (294). In human as well as experimental diabetic nephropathy, more advanced stages are also associated with lower VEGF expression (34, 195, 238, 372). These observations suggest that the upregulation of VEGF in early stages of diabetic nephropathy may provide a mechanism for the initial progression of the disease, leading to excessive blood vessel formation and albuminuria. The decline of VEGF in the later phase of diabetic nephropathy may reflect a loss of endogenous VEGF due to the disruption of podocytes and tubular cells in chronic kidney damage. In fact, at later stages of chronic kidney disease, VEGF is currently considered to be a protective factor with beneficial effects on glomerular endothelium, podocyte viability, and, as described in the remnant kidney models and in diabetic patients, on the peritubular network of capillaries (372), preventing the progression of interstitial fibrosis.

Role of inhibitors of angiogenesis and angiopoietins in the diabetic kidney—

The pathophysiological roles of angiogenic factors in the diabetic kidney are further supported by studies focusing on endogenous inhibitors of angiogenesis. These molecules originate as cleavage products of ECM proteins (tumstatin and endostatin) or plasminogen (angiostatin) and specifically target endothelial cells and inhibit their proliferation, survival, migration, and sprouting (218, 459, 460). The levels of these proteins are dramatically decreased in the kidney of STZ-diabetic rats, most likely due to decreased activity of MMPs [see section “*Glomerular pathophysiology in the diabetic kidney*” and (740)].

Administration of tumstatin (a cleavage product of collagen IV), endostatin (a cleavage product of collagen XVIII), or angiostatin has been shown to reduce glomerular hypertrophy, hyperfiltration, albuminuria, mesangial matrix expansion, ECM accumulation, endothelial cell proliferation, and monocyte/macrophage infiltration in STZ-induced diabetic mice (255, 724, 740) in parallel with the suppression of multiple molecular markers of nephropathy. The treatments also increased nephrin expression (255, 724) and enhanced the levels of pigment epithelium-derived factor, another endogenous antiangiogenic factor (740). Similar renoprotective effects have been observed in STZ-diabetic mice treated with vasohibin-1, an endogenous angiogenesis inhibitor that is, unlike the angiostatins discussed above, induced in endothelial cells by proangiogenic factors (428).

The studies with endogenous angiogenesis inhibitors should be also interpreted with caution. Despite impressive effects of these molecules on a variety of functional, structural and molecular markers of nephropathy, these studies were conducted only for several weeks in STZ-diabetic mice known to be relatively resistant to the development of structural changes characteristic of diabetic nephropathy. Although those studies suggest beneficial effects of angiogenesis inhibition at early stages of nephropathy, their long-term nephroprotective effects, and consequently the role of angiogenesis later in the course of the disease, remain to be established. Moreover, observed beneficial effects of angiogenesis inhibitors in mesangial cells and podocytes suggest involvement of additional, less understood, effects unrelated to modulation of angiogenesis.

Angiopoietins are another family of vascular growth factors that have been implicated in the pathophysiology of nephropathy. The best-studied are angiopoietin 1 (Ang-1) and Ang-2. During normal development, they are considered critical for vascular differentiation through angiogenesis, the process of growth and remodeling of existing vessels. In mature organisms, angiopoietins are involved in maintenance and turnover of blood vessels (715). Ang-1 causes enhanced endothelial survival and endothelial cell stabilization, whereas Ang-2 acts as a natural antagonist of Ang-1 (310). The *in vivo* biologic effects of the angiopoietins also depend on ambient levels of VEGF-A (715). Angiopoietins seem to be linked to nephrin expression, since Ang-2 overexpression in non-diabetic mice leads to downregulation of nephrin (132). Experimental models of T1DM are associated with altered renal expression of angiopoietins. Rizkalla et al. (527) reported that in STZ-diabetic rats whole-kidney Ang-1 and Ang-2 mRNA and protein levels rose at 4 weeks, but at 8 weeks, Ang-1 levels were lower than those in nondiabetic controls, whereas Ang-2 remained elevated. Ang-1 was immunolocalized in diabetic kidney tubules, whereas Ang-2 was prominent in glomerular endothelia and podocytes. Yamamoto et al. (724) also reported renal upregulation of Ang-2 in STZ-diabetic mice. In accordance with this *in vivo* evidence in models of diabetes, more recent *in vitro* studies in endothelial cells identified mechanisms of high glucose-induced Ang-2 expression (727). These observations suggest that a decreased ratio of Ang-1/Ang-2 might play a role in the pathobiology of glomerular disease in diabetic nephropathy. However, considering the above discussed effects of angiogenesis inhibitors in the diabetic kidney, the pathophysiological roles of Ang-1/Ang-2 remain rather murky. It is also possible, that the primary target of these molecules is not the GEC but the podocytes.

Other factors influencing glomerular endothelial permeability in diabetes—

Glomerular endothelial permeability can also be increased by proinflammatory cytokines and adipokines, such as TNF- α , IL-6 and leptin, that is, factors more typical for T2DM [reviewed in (546)]. In contrast, adiponectin, unlike other adipokines, appears to have protective effects in the vasculature by reducing endothelial cell activation and inflammation, in addition to protective and antiproteinuric effects on podocytes (577).

The glomerular basement membrane in the diabetic kidney

The normal GBM is a 300- to 400-nm thick structure consisting predominantly of type IV collagen, laminin, nidogen, and heparan sulfate proteoglycans (HSPGs). In T1DM, GBM

thickening occurs early in the course of the disease, long before the development of microalbuminuria. Thickening of the capillary basement membrane has been observed in other vascular beds in patients with T1DM and does not seem to be specific for the kidney (149, 392). The thickening of the GBM is due to the accumulation of ECM, which originates most likely in the podocyte (see Fig. 6).

The GBM may constitute a charge barrier, due to its HSPG content. The evidence for a role of HSPG in the diabetic kidney remains controversial with equally persuasive reports supporting a role of loss of HSPG in diabetes (136, 404, 508, 586, 610) or arguing against it (666, 700, 701).

Paradoxically, diabetes-induced increases in GBM thickness might be expected to reduce protein transit, but it is associated with proteinuria. However, as postulated by Jefferson and coworkers (273), the GBM thickening occurs in an irregular manner with areas of thinned GBM with presumably enhanced permeability for proteins. Moreover, altered GBM may affect the adjacent cellular elements, reducing cell binding and promoting podocyte detachment (see below).

Podocyte pathophysiology in diabetes

Podocytes are terminally differentiated and highly specialized cells. They line the urinary side of the GBM and function as a fine filter contributing to ultimate size-selectivity and permitting permeability to molecules smaller than albumin. They also synthesize components of the GBM and counteract capillary hydrostatic pressure. The podocyte is composed of the cell body and interdigitating foot processes. The narrow gaps (30–40 nm) between neighboring processes are bridged by the glomerular slit diaphragm. This crucial structure is a highly specialized gap junction with small pores, permeable to water and solutes but relatively impermeable to plasma proteins. The shape of podocytes is supported by an actin cytoskeleton, which allows the podocyte to dynamically change its shape. Podocytes are anchored to the GBM by integrins and α - and β -dystroglycans. Similar to GEC and GBM, podocytes are also negatively charged on their apical membrane domain owing to surface anionic proteins such as podocalyxin, podoplanin, and podoendin. The negative charge helps limit passage of negatively charged molecules (like albumin). However, podocytes also serve as a size barrier to proteins, predominantly due to the properties of the slit diaphragm.

When exposed to the diabetic milieu, podocytes undergo a spectrum of changes that ultimately contribute to the development of proteinuria and glomerulosclerosis. These include a reduction in podocyte number (podocytopenia), foot process widening and effacement, and altered secretory functions. These processes are closely linked, but will be, for better clarity, discussed separately and are summarized in Figure 5. Importantly, kidney biopsies from Pima Indians with type II diabetes suggested that podocyte loss contributes to the progression of diabetic nephropathy (354, 355, 468).

Reduction in podocyte number (podocytopenia)—Several mechanisms have been implicated in podocyte loss in the diabetic kidney as outlined in the following.

Podocyte detachment: The appearance of podocytes in the urine (podocyturia), which has been described in 53% of microalbuminuric and 80% of macroalbuminuric patients with T2DM, while being absent in subjects with normal albumin excretion (424, 488), has been considered to be a persuasive *in vivo* indicator of podocyte detachment in the diabetic kidney. It has been proposed that this abnormality occurs due to reduced expression of the $\alpha\beta 1$ integrin, the predominant integrin tethering the podocyte to the GBM (102).

Podocyte apoptosis: There is increasing and relatively abundant evidence implicating apoptosis of podocytes as another cause of podocytopenia in diabetes. Several mechanisms might underlie podocyte apoptosis in diabetes. Studies by Susztak et al. (604) have implicated high glucose-induced oxidative stress and proapoptotic p38 MAPK signaling in this process. Importantly, this study demonstrated that apoptosis preceded podocyte depletion and coincided with the onset of albuminuria. It should be noted, however, that early activation of p38 MAPK has also been associated with maintenance of the actin cytoskeleton in diabetic podocytes, and its loss correlated with progression of proteinuria (130). Other factors operating in the diabetic kidney, such as TGF- β (554, 683) or activation of RAS (151), have also been shown to induce podocyte apoptosis. Other studies have implicated a stimulation of RAGE, which is overexpressed on podocytes in experimental T2DM, in proapoptotic signaling (699).

More recently, Isermann et al. (258) reported interesting findings implicating activated protein C (APC) formation in several alterations in the diabetic kidney. Protein C is an antithrombotic system, regulated by endothelial thrombomodulin. Plasma protein C activity is reduced in diabetic patients (682), but with no clear link to the pathophysiology of diabetic nephropathy. Isermann and colleagues described a lower activity of a new pathway involving thrombomodulin-dependent APC formation in the diabetic kidney, resulting in attenuated cytoprotection in renal cells and enhanced glomerular apoptosis including podocytes. APC prevented the mitochondrial apoptosis pathway via the protease-activated receptor PAR-1 and the endothelial protein C receptor EPCR in glucose-stressed cells. *In vivo*, maintaining high APC levels during long-term diabetes protected against diabetic nephropathy in mice (258).

Activation of the Notch signaling pathway has been the most recent addition to a spectrum of mechanisms underlying podocyte apoptosis in diabetes. Notch proteins and numerous components of their down-stream signaling pathway are known to play a crucial role in mammalian kidney development. However, Notch1 is practically undetectable in the mature kidney. Niranjan et al. (441) have reported the expression of genes in the Notch pathway in mature podocytes in humans with diabetic nephropathy and in mice with STZ-induced diabetes. Moreover, expression of ICN1, a Notch intracellular domain, in podocytes resulted in apoptosis *in vitro* and led to podocyte loss, albuminuria, and glomerulosclerosis *in vivo*. Conversely, genetic or pharmacological inhibition of Notch signaling prevented podocyte apoptosis and albuminuria. Most recently, Lin et al. (370) extended these observations and showed that, in addition to the antiapoptotic effect, inhibition of Notch1 abrogated high glucose-induced VEGF synthesis and nephrin loss in podocytes, with a beneficial effect on proteinuria in diabetic animals.

Inability to proliferate and restore podocyte number: In contrast to mesangial cells, mature podocytes do not actively synthesize DNA nor proliferate under normal conditions (480). In the diabetic kidney, where podocytes undergo detachment or apoptosis, the relative inability of these cells to proliferate may lead to an eventual decline in number and become a contributing factor for the reduced podocyte number. As shown in several studies, high expression of CDK inhibitors may be responsible for this phenomenon (573).

High expression of CDK inhibitors has also been linked to another diabetes-induced podocytic change, namely hypertrophy (708). Under diabetic conditions, podocytes undergo hypertrophic changes like mesangial cells, resulting in increased cell size (517). The high glucose-induced podocyte hypertrophy can be ameliorated by Ang II AT1 receptor blockade, suggesting the involvement of RAS activation (365). Downstream effectors may include parathyroid hormone-related protein, which induces hypertrophy in podocytes via TGF- β 1 and p27 Kip1 (530). In addition to high glucose, cyclic mechanical stretch, as a result of elevated intraglomerular pressure and impaired autoregulation (see below), can induce podocyte hypertrophy (487). The pathophysiological consequences of the increased size of podocytes are not clear. However, as shown by Wolf et al., the protective effect against the development of nephropathy in STZ-diabetic mice by deletion of the CDK inhibitor p27 Kip1 was associated with reduced podocyte hypertrophy and collagen type IV and laminin expression (708). Additional studies indicated that exposure of podocytes in culture to AGE-modified bovine serum albumin (AGE-BSA) induces cell-cycle arrest and cell hypertrophy by a mechanism that involves upregulation of p27 Kip1 (535). More recently, Bondeva et al. exposed differentiated mouse podocytes in culture to AGE-BSA. Using differential display and real-time PCR analyses they observed a downregulation of neuropilin-1 and confirmed a reduction in neuropilin-1 expression in glomeruli of diabetic db/db mice and in renal biopsies from patients with diabetic nephropathy compared to transplant donors (66). Additional *in vitro* studies indicated that AGE-BSA inhibited podocyte migration by downregulating neuropilin-1. The authors proposed that decreased podocyte migration could lead to adherence of uncovered areas of the glomerular basement membrane to Bowman's capsule thereby contributing to focal glomerulosclerosis.

Podocyte foot process widening and effacement—Foot process effacement is a result of retraction, widening, and shortening of the podocyte foot processes (see Fig. 6). The retraction results in large areas of flattened epithelium covering the capillary loop. This leads to a decrease in slit diaphragm pores per unit of length of GBM, causing a reduction in filtration surface. However, as postulated by Jefferson et al. (273), focal epithelial defects may allow increased protein flux across these denuded areas. This may be further facilitated by defects in slit diaphragm protein composition. This phenomenon is not specific for the diabetic kidney, and proteinuria may occur without effacement (730). Conversely, effacement is not always associated with proteinuria (273). Mundel and Shankland (419) proposed that the underlying mechanisms leading to foot process effacement include (1) changes in slit diaphragm-associated proteins, (2) actin cytoskeleton abnormalities, (3) alterations in the negative apical membrane domain of podocytes, and (4) interference with podocyte-GBM interaction.

Slit diaphragm abnormalities: The slit diaphragm, which bridges adjacent foot processes derived from different podocytes, functions as the ultimate molecular size filter. Several podocyte-specific proteins, such as nephrin, NEPH1, P-cadherin, and FAT-1 have been located at this region, whereas CD2AP, podocin, and zonula occludens-1 (ZO-1) are found in the slit diaphragm inserted region of the foot process [reviewed in (365)]. The relevance of these proteins has been documented by detection of foot process effacements, often associated with proteinuria, in patients with mutations of these genes or experimental models with targeted deletion or administration of antibodies to some of these molecules (365).

Proteinuria is a hallmark of diabetic nephropathy. Consequently, studies have focused on the changes in slit diaphragm-associated molecules in diabetic nephropathy. Thus far, nephrin is the best studied slit diaphragm protein in the diabetic context. Bonnet et al. (67) demonstrated a reduction in nephrin mRNA and protein expression in STZ-induced diabetic spontaneously hypertensive rats at 32 weeks after diabetes induction. These experimental findings are in accord with observations in kidney biopsies derived from patients with nephropathy and T1DM or T2DM (148). These changes in nephrin expression have been linked to activation of PKC α (403) and/or the RAS (67, 323, 344). Similar changes in nephrin expression were observed in cultured podocytes exposed to glycated albumin and Ang II (148). In contrast, Aaltonen et al. (1) showed an increase in nephrin mRNA levels in STZ-diabetic rats and in nonobese diabetic mice even before the development of significant albuminuria. In another study, nephrin protein expression was increased in renal cortex of db/db mice (603), whereas others found no changes in renal cortical nephrin expression in diabetes (337). The reasons for the disparate findings may be due to differences in duration of diabetes, the possibility of a biphasic response in nephrin expression and the influence of confounding factors, like hypertension.

Compared to nephrin, there have been relatively fewer investigations on the changes of other slit diaphragm proteins. P-cadherin mRNA and protein expression were reduced in diabetic glomeruli and in podocytes exposed to high glucose, a process mediated by PKC (722). Another study revealed decreases in ZO-1 protein expression in glomeruli of animal models of T1DM and T2DM and in high glucose-stimulated podocytes (520). In comparison, the expression of CD2AP and podocin has been reported to be unchanged under diabetic conditions (51, 329).

Actin cytoskeleton abnormalities: The actin cytoskeleton and several actin-binding proteins such as α -actinin-4 and synaptopodin are important in maintaining podocyte shape. The main cytoskeleton components of the foot process are microfilaments, containing a dense network of F-actin and myosin. Dai et al. (130) studied the podocytic cytoskeletal response to diabetes both *in vitro* and *in vivo*. They reported early high glucose- or diabetes-induced activation of the p38 module of MAPK and its downstream effector small heat-shock protein 25 (HSP25), resulting in a stabilization of fibrillar actin (FA) and prevention of its cleavage to G-actin, and, thus, maintenance of the actin cytoskeleton. This response was gradually lost with increasing duration of diabetes, correlating with slit diaphragm pore density and albuminuria. The authors concluded that p38 MAPK and HSP25 activation comprise an acute adaptation to glycemic stress with beneficial effects on actin cytoskeleton,

which is being lost with increasing duration of the disease with structural and functional consequences.

α -Actinin-4 is an actin cross-linking protein, and its mutation in human and animal leads to proteinuria and foot process fusion. A recent study revealed that glomerular α -actinin-4 mRNA expression is increased in glomeruli from db/db mice with T2DM, which might reflect a repairing process (383).

Small GTPase RhoA and its downstream effectors Rho-associated kinases (ROCK) are the major signaling pathway involved in regulation of actin cytoskeleton (162). Therefore, the Rho-ROCK pathway has attracted considerable interest as a potential mediator of podocytic cytoskeletal changes in proteinuric kidney disease. The pathway mediates the podocyte cytoskeletal response (F-actin reorganization) to mechanical stress (160). More recently, RhoA has been linked to synaptopodin, another important actin-associated protein. Asanuma et al. reported that synaptopodin induced stress fibers in podocytes by competitive blocking of Smurf1-mediated ubiquitination of RhoA and promoted contractile stress fiber formation in the cell body (30). Moreover, gene silencing of synaptopodin in podocytes causes loss of stress fibers, formation of aberrant nonpolarized filopodia, and impairment of cell migration (30). This suggests that synaptopodin is essential for the integrity of the podocyte actin cytoskeleton and for the regulation of podocyte cell migration. Despite the functional role of synaptopodin in maintaining podocyte shape, only one study by Blanco et al. (60) investigated the changes in synaptopodin expression in diabetic nephropathy. They observed a trend toward a decrease in glomerular synaptopodin expression in obese Zucker rats with T2DM.

The above-mentioned evidence suggests that the Rho-ROCK pathway is important for maintaining podocyte cytoskeletal integrity and associated cell functions. However, as indicated by studies with ROCK inhibitors, the pathway plays a role in progressive kidney disease (262, 291, 443, 444), including diabetic nephropathy (202, 316, 323, 484). Interestingly, long-term inhibition of ROCK led to amelioration of foot process effacement in STZ-diabetic rats (484), and to nephrin upregulation in uninephrectomized diabetic rats with accelerated nephropathy (323). These effects were associated with blood pressure-independent modest antiprotein-uric effects (323, 484). Further studies are needed to establish whether the nephroprotection of ROCK inhibition is mediated via its effects on podocytic cytoskeleton.

Alterations in the negatively charged apical membrane domain of podocytes: Similar to GEC or GBM, the apical membrane domain of podocytes is negatively charged due to the surface anionic proteins, such as podocalyxin and podoplanin. As the cytoplasmic domain of podocalyxin is linked to the actin cytoskeleton, podocalyxin is also considered important in maintaining podocyte shape. mRNA and protein expression of podocalyxin have been shown to be suppressed in podocytes cultured in high glucose conditions, and glomerular podocalyxin protein expression was decreased in STZ-induced diabetic rats (152). Podoplanin is another apical membrane protein. In contrast to animals with puromycin aminonucleoside nephrosis (77), podoplanin mRNA expression was increased in human

diabetic glomeruli and was positively correlated with the mean foot process width (329), suggesting a presumable compensatory reaction to the damage inflicted on the podocyte.

Interference with podocyte-GBM interaction: This phenomenon is discussed above as a possible cause of podocyte detachment. In addition to its role in podocyte detachment, decreased expression of $\alpha 3\beta 1$ integrin is associated with foot process effacement, suggesting a role for $\alpha 3\beta 1$ integrin in maintaining cell shape (9). However, the evidence indicating a role for this mechanism specifically in the patho-physiology of diabetic nephropathy is still scarce. In addition to $\alpha 3\beta 1$ integrin, the DG protein complex, another podocyte anchoring molecule, is also important in keeping foot process shape. DG1, a heavily glycosylated peripheral membrane protein located in podocytes, is known to be decreased in various glomerular diseases with foot process effacement (517). However, this has not been observed in experimental diabetic nephropathy. In fact, an increase in DG1 expression was reported in isolated glomeruli from db/db mice (383) suggesting involvement of this protein in a glomerular repair process.

Altered secretory functions of podocytes—In addition to the functions described above, podocytes act as secretory cells. These actions and consequences on the surrounding cells and structures are described in detail in other sections describing alterations of the GFB in diabetes. In brief, podocytes exposed to the diabetic milieu could be an important source of the components of the RAS including Ang II, angiotensinogen and renin as indicated in studies in immortalized cultured podocytes (151). Podocytes also secrete GBM proteins (296) and act as an important source of glomerular VEGF (122) and lipid mediators, such as 12-lipoxygenase (296) and cyclooxygenase (COX) products (390).

The Unique Growth Phenotype and Morphological Changes of the Tubular System in the Diabetic Kidney

Growth of the kidney occurs with the onset of diabetes (116, 413). Understanding the involved mechanisms is crucial because diabetic kidney growth has been associated with the development of nephropathy (44, 64, 349, 738). Zerbini et al. (738) observed patients with T1DM and normal renal function for a mean duration of 9.5 years and found that increased kidney volume enhanced the risk of progression to microalbuminuria along with a faster decline in GFR. Diabetes-induced kidney growth in early diabetes may affect not only mesangial and podocyte function (see above), but also tubular hyperreabsorption, and as a consequence, glomerular hyperfiltration. In addition, the unique molecular mechanisms involved in early tubular growth in diabetes may set the stage for progressive tubulointerstitial damage. In the following we discuss the unique mechanisms involved in the growth of the tubular system and other morphological changes in the diabetic kidney.

Tubular growth in diabetes

The kidney in general and the proximal and distal tubules in particular, grow large from the onset of diabetes with the proximal tubule accounting for the greatest share of growth (116, 510, 511, 571). Rasch described in 50-day STZ-diabetic rats that the length of the proximal and distal tubules increased by 22% and 20%, respectively. Whereas no structural

abnormalities were found in the proximal tubules, the total increase of the distal tubule was due to abnormal tubules confined to the cortex and the outer stripe of the outer medulla, which were filled with glycogen deposits, had strikingly few organelles and the basal infoldings were greatly reduced (see below for further details). In comparison, the total length of the collecting ducts was not affected in diabetes and most cells appeared normal (510). As described above for mesangial cells, both hyperplasia and hypertrophy are involved in diabetes-induced tubular growth (512), as illustrated in Figure 7.

The role of ornithine decarboxylase and polyamine synthesis in the growing diabetic tubule

—The growth of the early diabetic kidney depends, at least in part, on overexpression of the enzyme, ornithine decarboxylase (ODC) (482). ODC converts ornithine to putrescine. This is the first and rate-limiting step in polyamine synthesis and appears to be a necessary early step for all cell division and for some forms of hypertrophy. In the early diabetic kidney, polyamines are required components for hyperplasia and most likely also for hypertrophy of the proximal tubule (140, 481, 482, 617). Renal ODC mRNA and protein expression are increased as a very early response to the onset of diabetes (358, 481, 617). Increased ODC activity can persist in the STZ-diabetic kidney for several weeks (482, 617). The specific mechanisms leading to sustained ODC expression in diabetic kidneys are not known, but the finding is characteristic of this form of kidney growth. The earliest change in the local environment of the proximal tubular cell in diabetes is the increase in filtered glucose leading to increased apical entry of sodium and glucose via sodium glucose co-transport (SGLT). One might imagine glucose and/or sodium in the cell providing some stimulus for the cell to grow (339, 595). However, based on immunostaining, Deng et al. proposed that the increase in ODC expression in early diabetes mainly occurs in the distal nephron and suggested that polyamines may pass from the distal to proximal tubule in a paracrine fashion to trigger proximal tubular growth (140). More studies are necessary to clarify this issue and what may trigger ODC expression in the distal tubule.

Growth of the proximal tubule in diabetes — early hyperplasia—For the early growth of the proximal tubule in the diabetic kidney, a period of hyperplasia precedes hypertrophy (250). DNA synthesis is increased in the proximal tubules of STZ-induced diabetes, peaking at day 2 (512). Numerous growth factors are associated with the early onset of diabetes and diabetic kidney growth, including IGF-I, HGF, PDGF, fibroblast growth factor (FGF), VEGF, epidermal growth factor (EGF), and DAG (134, 176, 199, 538, 712). All of these factors can induce ODC activity (549). IGF-I may be the most studied mediator of the very early growth phase in diabetes, and its effects are attributed principally to activation of the phosphoinositide 3-kinase (PI3K)/Akt pathways (see Fig. 7). At the onset of STZ-diabetes, the rapid yet transient renal induction of IGF-I (171, 506) temporally correlates with the upregulation of renal ODC expression and activity (140, 481, 617), induction of intracellular polyamines in the kidney cortex (140), and the proliferative phase. Also VEGF, which is induced in both the STZ-model (T1DM) and the db/db mouse model (T2DM), increases protein synthesis in renal epithelial cells utilizing the PI3K/Akt pathway (569). Diabetes also enhances proximal tubular activity of PKC β 1 (489). PKC activation can produce a myriad of consequences in diabetes including a mitogen-induced early

proliferation phase (85). PKC β has been implicated in Akt activation in the renal cortex of diabetic rats (719). Activation of PI3K/Akt (574) and PKC pathways (245, 277) can both increase ODC activity. PKC activation of ODC is likely by induction of immediate early genes c-myc, c-jun, and c-fos (232, 698). In accordance with a role of PKC β in kidney growth, the early diabetes-induced increase in kidney weight was blunted in mice lacking this PKC isoform (401).

In rats with T1DM, renal growth is associated with reduced phosphorylation of AMP-activated protein kinase (AMPK) (351). Phosphorylated AMPK inhibits the mammalian target of rapamycin (mTOR) activity by phosphorylating and activating tuberous sclerosis complex (TSC). TSC negatively regulates mTOR complex 1 (mTORC1) by inhibiting Rheb, a small cytoplasmic GTPase and activator of mTORC1 [for review see (369)]. In accordance, mTOR activity is enhanced in the diabetic kidney and increasing AMPK phosphorylation by pharmacological means reversed mTOR activation and inhibited renal growth without affecting hyperglycemia (351). Together these studies propose a link between diabetes-induced growth factor filtration and expression, activation of PKC, inhibition of AMPK, and activation of both mTORC1 and ODC in the early tubular proliferation in diabetes (see Fig. 7).

Growth of the proximal tubule in diabetes — switch from hyperplasia to hypertrophy—The diabetic kidney switches from hyperplastic to hypertrophic growth very early in the course of hyperglycemia, for example, at around day 4 in the model of STZ-diabetes (250), which matches the time frame of hyperplasia observed using 5-bromodeoxyuridine (BrdU) incorporation (140), an indicator of cell-cycle progression. TGF- β 1 is a critical mediator of this growth switch in diabetes and critical to diabetic kidney growth in general (219) (see Fig. 7). PKC β has been implicated in the induction of TGF- β in the diabetic kidney (260, 308, 330, 331). PKC β 1 has been localized to the proximal tubule of the rat (490) and its membrane-associated expression (activation) was further upregulated in response to STZ-diabetes (489). Other studies indicated that ERK and p38, which are activated in renal tubular cells of STZ-diabetic rats, may mediate high glucose-induced TGF- β expression and cellular hypertrophy (190). High glucose administered to primary tubule cells from TGF- β KO mice induced an increased rate of proliferation relative to isolated cells from wild type littermates, and no hypertrophy (105). Expression of the CDK inhibitor p27^{KIP1} (p27) increases in response to hyperglycemia or diabetes, which, based on studies in non-tubular cells, can be attributed to induction by PKC (711) and TGF- β (288). TGF- β can arrest cells in the G₁ phase of the cell cycle by induction of p27 (288). In mesangial cells, it is this G₁ arrest that likely switches the hyperplastic growth to the hypertrophic phenotype observed in diabetes (32, 417, 708, 710). In accordance, targeted disruption of the p27 gene does not affect hyperglycemia in the STZ diabetic model, but decreases glomerular hypertrophy (32, 708) (also see sections on glomerular pathophysiology above). Experimental diabetes also increases the renal expression of p21 (16, 548). Moreover, the loss of p21 increases tubular cell proliferation, consistent with a role of p21 in the switch from hyperplasia to hypertrophy in the diabetic kidney (16) (see Fig. 7).

By 7 days after STZ administration, renal ODC activity is still elevated (482, 617), and may drop to below control by 3 or 4 weeks (358, 587). A temporal activation of ODC activity may be attributed to temporal changes in the local growth factor/mitogen content. For example, IGF-I induction is transient, with levels peaking at day 3 and normalizing by day 7 (171). Therefore, the sustained hypertrophy observed later in diabetes may be more a consequence of decreased proteolysis than cell-cycle arrest (179), and may also not be dependent upon growth factor/PI3K/Akt induction (179).

Tubular senescence in the early diabetic kidney

Satriano et al. recently demonstrated that tubular hypertrophy in the STZ-diabetic kidney is associated with a phenotypic transition to senescence (548) (see Fig. 7). Senescence is a tumor suppressor mechanism to halt cells from replicating and passing on a potentially damaged genome. Like early diabetic hypertrophy, senescent arrest requires upregulation of CDK inhibitor (518). Although the prototypical senescent arrest involves the temporal induction of p21^{WAF1} (p21) and p16^{INK4A} (p16), studies demonstrate that induction of p27 can impose a senescent-like growth arrest (17, 729). Satriano et al. further described that proximal tubule cells in culture transition to senescence in response to oxidative stress. Moreover, STZ-diabetic kidneys exhibited an early temporal induction of growth phase components followed by their suppression at day 10 after STZ application concurrent with the induction of CDK inhibitors p16, p21, and p27 and markers of senescence including expression of senescence associated beta-galactosidase activity in cortical tubules (548). Notably, kidneys of patients with T2DM and nephropathy display an accelerated senescent phenotype in tubule cells and, to a lesser extent, in podocytes (675). Senescent cells exhibit several aspects of a fairly well differentiated phenotype; however, this phenotype is skewed in several parameters (518), including the release of inflammatory cytokines, production of growth factors and ECM, and a resistance to apoptotic remodeling (18, 583). Thus, one may speculate that the senescent arrest of tubular cells may not only alter the early transport phenotype [e.g., the “salt paradox” of the diabetic kidney (651)] but also contribute to the development of diabetic nephropathy (see Fig. 7).

Evidence for epithelial-mesenchymal transition in the diabetic kidney

Tubulointerstitial fibrosis represents the final common pathway of many forms of kidney disease including diabetic nephropathy (426). The accumulation of fibrotic material in the tubular interstitium tends to accompany disease progression, correlating with a gradual decline in renal function in patients with T1DM (302). Much of the accumulated interstitial matrix in fibrosis is synthesized by interstitial myofibroblasts. In addition to interstitial fibroblasts and circulating precursor cells, a proportion of interstitial myofibroblasts in fibrotic kidneys can originate from tubuloe epithelial cells through epithelial to mesenchymal transition (EMT) (264, 374, 433, 599, 600, 725). In the model of unilateral ureter occlusion, very elegant studies using bone marrow chimeras and transgenic reporter mice indicated that EMT may account for at least a third of the fibroblasts (264). EMT is associated with an early decrease in the expression of adhesion proteins that keep cell polarity and intercellular junctions, including E-cadherin and zonula occludens protein ZO-1 (234, 287). Upregulated EMT markers include metalloproteinases MMP-2 (108, 109, 601) and MMP-9 (601, 725) involved in the degradation of the tubular basement membrane, as well as alpha-SMA (236,

433) and integrin linked kinase (ILK) (367, 368), which increase cell contractility/motility and movement, respectively. EMT leads to enhanced formation of ECM proteins (e.g., fibronectin) (287, 599).

The extent to which EMT contributes to renal fibrosis *in vivo*, especially in human, remains a matter of intense debate (371, 498, 679) and may depend on the experimental and clinical context [for review see (375)]. Features of EMT have been observed in human renal biopsies from diseased kidney, in which the number of tubulointerstitial cells with EMT features were associated with serum creatinine and the degree of interstitial damage (513). Markers of EMT have also been observed in kidneys of STZ-diabetic uninephrectomized mice (368), STZ-diabetic Wistar Kyoto (457), Sprague Dawley (91, 457), uninephrectomized Sprague Dawley (323), and Ren-2 rats (240), and in a renal biopsy from a patient with T1DM (457). Recent studies using cell lineage-tracing techniques indicated that a substantial number of interstitial fibroblasts, including in the diabetic kidney, also come from capillary endothelia by endothelial-to-mesenchymal transition (363, 736). Endothelial cells are a specialized type of epithelia and, thus, the latter process represents another form of EMT occurring in the injured kidney.

TGF- β 1 is upregulated in the kidney in experimental diabetes and in patients, plays a critical role for tubulointerstitial fibrosis (493), and is the most potent inducer that is capable of initiating and completing the entire EMT course (90, 234, 374) (see Fig. 7). Other endogenous factors of EMT include EGF, FGF-2, IL-1, Ang II, AGEs, Kruppel-like factor 6 (KLF6), CTGF, and MMP-2, part of which act through TGF- β 1 [KLF6 (240), IL-1, MMP-2] or exhibit a dramatic additive or synergistic effects with TGF- β 1 to promote EMT (EGF, FGF-2, Ang II) [for review see (374)]. Signaling events in EMT downstream of TGF- β receptor activation include activation of MAPK, Smad/ILK, and CTGF (90, 91, 234, 287, 374). Recent results suggested that parathyroid hormone (PTH)-related protein (PTHrP), TGF- β 1, EGF, and VEGF might cooperate through activation of ERK1/2 to induce EMT in renal tubulointerstitial cells (27). In this regard, PTHrP and its receptor are upregulated in tubules and glomeruli of STZ-induced diabetic mice (267) and a preliminary report has shown that PTHrP upregulation also occurs in both tubules and glomeruli in patients with diabetic nephropathy (72). In primary cultured renal proximal tubular epithelial cells, hyperglycemia induces EMT through ROS, PI3K/Akt, GSK-3 β , snail, and beta-catenin (352) (see Fig. 7).

Conversely, application of BMP-7 (737) and HGF (129) has been shown to inhibit fibrosis and markers of EMT in the kidney *in vivo*, the latter in the diabetic kidney of mice. HGF appears to specifically induce Smad transcriptional co-repressor SnoN expression, which in turn physically interacts with activated Smad-2 by forming a transcriptionally inactive complex and blocks the trans-activation of Smad-mediated genes, including EMT mediator ILK (368). Thus, there may be intrinsic renoprotective factors that can safeguard tubular epithelial phenotypes by preventing EMT, which may have therapeutic potential.

A partial EMT phenotype has been recently described (356) that is not necessarily associated with E-cadherin down-regulation, and there is evidence that this process might operate in the diabetic kidney of mice (687). It has been postulated that the entire classical

EMT program observed primarily *in vitro*, with the associated decrease in E-cadherin, may not be critical for the development and progression of tubulointerstitial fibrosis (687). Both TGF- β and CTGF increase ECM expression independently of E-cadherin. While TGF- β lowers E-cadherin, CTGF (a driver of partial EMT) increases E-cadherin expression. In the nondiabetic context, an increase in E-cadherin has been recently reported in rats with ureteral obstruction, a classical model of EMT-mediated kidney fibrosis (144). Further studies are needed to better understand the relevance, the molecular signature, and the therapeutic potential of EMT in the diabetic kidney, especially in human.

Hyperglycemia Induces Glycogen Accumulation in Thick Ascending Limb and Further Distal Cortical Nephron Segments

In hyperglycemic conditions, cells of the thick ascending limb (TAL) and, to a lesser extent, of the distal convoluted tubule and the collecting duct show glycogen deposits, also known as glycogen nephrosis or Armanni-Ebstein lesions (54, 239, 510). Holck and Rasch reported that on a continuous basis, glycogen is present only in the cortical TAL, whereas scattered dot-shaped and diffuse glycogen accumulations are discretely distributed in the MD, the distal convoluted tubule and the cortical collecting duct system (239). The cells appear either empty (“clear cells”) or full of a PAS-positive material, digestible with α -amylase, and at the electron microscope level the cytoplasm contains glycogen-like granules, strikingly few organelles and the basal infoldings are greatly reduced (510). Notably, these abnormalities are confined to the cortex and the outer stripe of the outer medulla (510). The fate of glycogen-accumulating “clear cells” and the role in diabetic kidney disease is still unclear. Under severe hyperglycemic conditions and ketoacidosis, apoptosis of these cells may occur (39).

Holck and Rasch proposed that the glycogen accumulation in the distal nephron of diabetic rats correlates with the delivery of glucose via the tubular fluid (239) (see Fig. 8). Alternatively, glycogen accumulation in the TAL in diabetes may reflect activation of GS. Studies by Cammisotto et al. in normal rats indicated that globular adiponectin (derived from white adipocyte secretion and being filtered by the glomeruli) through binding to its receptor, ADIPOR1, in the luminal membrane of the TAL activates AMPK leading to inhibition of GS. Moreover, they showed that this regulation is impaired in STZ-diabetic rats, which might contribute to glycogen nephrosis (95) and is similar to adiponectin resistance in other tissues (88). This may be due to high glucose-induced dephosphorylation of AMPK (351), inasmuch as high glucose-activation of the phosphatase PP2A can dephosphorylate AMPK and prevent the association of AMPK subunits (127, 201, 278). Moreover, TALs from diabetic animals have higher concentrations of glucose-6-phosphate (95), an allosteric activator of GS. Further studies are necessary to better understand the tubular formation of glycogen deposits and their relevance in the diabetic kidney.

Mechanisms of Tubulointerstitial Injury in the Diabetic Kidney

Glomerular mesangial expansion and podocyte loss are important early features of diabetic nephropathy, whereas tubulointerstitial injury and fibrosis are critical for progression of diabetic nephropathy to kidney failure. Increased proximal tubular basement membrane

width is an integral component of early nephropathology in patients with T1DM (80). Tubulointerstitial injury is a final common pathway to end-stage renal failure in many forms of kidney disease (425,426) and the focus of this section. Important primary factors contributing to tubulointerstitial injury include hyperglycemia, proteinuria, AGEs and chronic hypoxia. For recent reviews see (112, 297, 382, 581, 751). Figure 9 aims to integrate and illustrate some of the aspects that are discussed in the following.

Enhanced glomerular filtration of proteins, growth factors, and other molecules in diabetes

The mechanisms by which proteinuria results in tubular and interstitial damage may include lysosomal injury, energy depletion and direct tubular injury by various components in the proteinuric urine, such as AGEs, transferrin, albumin, albumin-bound fatty acids, and complement components. Urinary albumin induces pro-inflammatory chemokines in proximal tubular epithelial cells such as IL-8 (382, 611) and monocyte chemoattractant protein 1 (MCP-1), the latter is mediated by NF κ B (694) (see Fig. 9). In addition, ultrafiltered growth factors, such as IGF-I, HGF, and TGF- β have been linked to proteinuria-associated interstitial fibrosis in the diabetic kidney (688, 690, 691). The glomerular filtration of fatty acids bound to albumin has also been implicated in tubular damage. Fatty acids can activate peroxisome proliferator-activated receptor-gamma (PPAR- γ) in human proximal tubular cells and induce apoptotic cell death (28). This, however, is in contrast with the tubuloprotective effects of PPAR- γ agonists in animal models of diabetes against oxidative injury (588), indicating the need for further clarification. Proteinuric urine contains components of the complement system and, in addition, both transferrin and IL-2 can stimulate C3 synthesis (83, 237). In this regard, the degree of nonselective proteinuria correlates with both the level of intratubular C3 activation as well as tubulointerstitial injury (425).

Linking high glucose to tubulointerstitial fibrosis

It has been proposed that it is the tubular injury which initiates the deleterious cascade of events in chronic kidney disease (173). High glucose concentrations induce collagen gene transcription and secretion in a murine cortical tubular cell line (750). Similarly, exposure of primary cultures of human renal proximal tubular cells to high glucose increased the amount of type IV collagen and fibronectin (494). This was associated with a net decrease in gelatinolytic activity which accounted for the accumulation of fibronectin and type IV collagen (492). Exposure of human proximal tubular cells and cortical fibroblasts to high extracellular glucose concentrations can directly stimulate cell growth and collagen synthesis, independent of hemodynamic, glomerular or vascular pathology (283). The polyol pathway has been implicated in high glucose induced alterations in proximal tubular cell matrix generation (62, 418, 749).

In addition to alterations in the turnover of “normal” matrix constituents, the diabetic state may also lead to the *de novo* induction of “abnormal” structural elements, such as hyaluronic acid. Both elevated glucose and IL-1 β can increase hyaluronic acid synthase 2 and HA generation by proximal tubular cells via activation of NF- κ B, whereas the profibrotic cytokines TGF- β 1, PDGF, and FGF2 did not increase hyaluronic acid synthesis

(282). The functional role of hyaluronic acid in the diabetic kidney remains to be established.

A role for transforming growth factor- β —Several growth factors contribute to the pathogenesis of diabetic nephropathy, including TGF- β , CTGF, VEGF, GH, and IGF-I with some evidence being available also for EGF and PDGF (see Fig. 9). They are generally expressed in the normal kidney but their levels further increase in diabetes, where they have been implicated in the control of cell hypertrophy, proliferation and survival, as well as renal matrix composition [for review see (112)]. The expression of TGF- β is elevated in human and experimental diabetic nephropathy (723) and its upregulation is one of the key factors responsible for the fibrotic changes and scarring observed in the diabetic kidney (493, 747). Importantly, TGF- β is relevant to progression of renal disease as indicated by studies in db/db mice, a model of T2DM, in which treatment with a monoclonal anti-TGF- β antibody prevented renal insufficiency (748). In the diabetic kidney, the synergism of high glucose concentrations with cytokines such as PDGF or the pro-inflammatory macrophage-derived cytokine, IL-1 β , can stimulate TGF-1 synthesis by proximal tubular cells (495, 496) (see Fig. 9). The latter studies with IL-1 β further indicated that glucose stimulated leukocyte recruitment contributes to tubular TGF- β 1 protein synthesis indicating a close link between inflammation and the development of fibrosis in the diabetic kidney.

Reciprocal paracrine activation of proximal tubular cells and fibroblasts—

Notably, in chronic kidney disease, the regions of active interstitial fibrosis predominantly exhibit a peritubular rather than perivascular distribution (21), indicating that injured proximal tubular cells may release fibrogenic signals to cortical fibroblasts. In fact, TGF- β 1 can stimulate the release of pre-formed basic fibroblast growth factor (bFGF) from renal proximal tubular cells (284). Glucose can also stimulate the interstitial fibroblasts to increase their synthesis of ECM and fibrillar collagens via an autocrine TGF- β 1 system (221) (see Fig. 9). Importantly, there is evidence for reciprocal paracrine activation of proximal tubular cells and fibroblasts. Proximal tubular cells in the human kidney modulate the biological behavior of neighboring cortical fibroblasts through paracrine mechanisms, which include the production and release of the AB heterodimer of PDGF and TGF- β 1 (280). Vice versa, studies in human renal fibroblasts indicated that they can modulate proximal tubule cell growth and transport via the secretion of IGF-I and IGF binding protein-3 (281) (see Fig. 9). These interactions in the tubulointerstitium appear to be modified by the tubular basement membrane components laminin and collagen type IV: conditioned medium from rat proximal tubular cells cultured on collagen-type IV stimulated proliferation of cortical fibroblast. Moreover, co-culture of proximal tubular cells on laminin with cortical fibroblasts suppressed the activity of MMPs collagenase and gelatinase in both cell types (361). Thus, a complex cross-talk between proximal tubular cells, ECM proteins and fibroblasts appears to contribute to the regulation of ECM and one may speculate that tubular injury in diabetes alters these interactions and contributes to tubulointerstitial fibrosis.

A role for connective tissue growth factor—CTGF is a prosclerotic cytokine that is mainly induced by TGF- β and involved in the regulation of matrix accumulation (542). Vice versa, CTGF may enhance the profibrotic effects of TGF- β by enhancing TGF- β expression

(212) as well as the ability of TGF- β to bind to its receptor at low TGF- β concentrations (6). Urinary CTGF is detectable in diabetic patients (see Fig. 9) and the levels correlate with the degree of microalbuminuria (197, 522). Wang et al. found that CTGF mRNA levels were increased in the renal cortex of rats with STZ-induced diabetes and immunohistology localized the CTGF protein expression particularly to dilated-appearing proximal tubules, where it appeared to colocalize with IGF-I (688). These studies also provided evidence that glomerular ultrafiltrate from diabetic rats, which contained bioactive TGF- β and HGF, can induce CTGF expression in proximal tubular cells (see Fig. 9). CTGF has an IGF-binding domain and binds to IGF-I, and studies in NRK-49F renal fibroblast cells showed that IGF-I increased the activity of CTGF toward the expression of collagen α 1III (688). Guha et al. evaluated the specific role of CTGF in the diabetic kidney by using a CTGF ASO (212). In STZ-diabetic mice, CTGF-ASO reduced renal CTGF expression as well as proteinuria, albuminuria, and kidney growth. In db/db mice, CTGF-ASO reduced serum creatinine and attenuated urinary albuminuria and proteinuria. CTGF-ASO also reduced renocortical expression of fibronectin, collagen (I and IV) and PAI-1, an inhibitor of matrix degradation. Further analyses implicated a role for CTGF in diabetes-induced phosphorylation of p38 MAPK and its downstream target CREB that have been implicated in regulating matrix proteins (212).

A potential role for serum- and glucocorticoid-inducible kinase 1—The serum- and glucocorticoid-inducible kinase 1 (SGK1) is a serine-threonine kinase that has been implicated in many forms of fibrosis and is stimulated by multiple diabetes-associated factors and conditions including high glucose, hyperosmolality, TGF- β , FGF, PDGF, AGEs, and insulin (341). In primary culture of human proximal tubular cells, high glucose increased SGK-1 mRNA and protein expression (538). Moreover, high glucose and overexpression of SGK-1 increased Na/H-exchanger NHE3 mRNA and EGFR phosphorylation. SGK-1 overexpression increased proximal tubular cell growth, progression through the cell cycle, and inhibited apoptosis. Upregulation of tubular SGK-1 mRNA was confirmed in STZ-diabetic animals *in vivo*. These *in vitro* and *in vivo* effects of high glucose were sensitive to the EGFR blocker PKI166, indicating that the effects of high glucose on proximal tubular cell proliferation, reduced apoptosis and increased NHE3 mRNA levels are mediated by EGFR-dependent upregulation of SGK-1 (538). Importantly, SGK transcription was markedly enhanced in patients with diabetic nephropathy, with particularly high expression in mesangial cells, interstitial cells, and cells in TAL of Henle's loop and distal tubules (343). SGK1 message and protein expression is also upregulated in the kidney of STZ-diabetic mice, where it has been implicated in enhanced proximal tubular glucose reabsorption (7) and fibronectin formation (168). In fibroblasts, SGK1 can induce CTGF promoter activity and CTGF expression through activation of NF κ B (665). Thus, SGK-1 has been linked to TGF- β and CTGF and the outlined studies implicate potential roles of the kinase in the early and late stages of the diabetic kidney including potential effects on kidney growth, transport, and fibrosis. Further studies are necessary to better define the role of SGK1 in the diabetic kidney *in vivo* and determine the relevance of SGK-1 for the long-term renal outcome of the diabetic kidney.

Interrelation between hypoxia and oxidative stress in the diabetic kidney

Abnormalities in oxygen metabolism have been implicated in the development and progression of diabetic nephropathy and include hypoxia, oxidative stress, nitrosative stress, and advanced glycation and/or carbonyl stress [for review see (183, 187, 297, 409)]. The primary cause for local oxidative stress in the diabetic kidney remains a matter of debate (see earlier section on ROS). Most of the ROS are generated during mitochondrial oxidative phosphorylation and smaller amounts via the NADPH-oxidase system (200, 293, 366) (see Fig. 9). Hypoxia has also been implicated as a cause of oxidative stress in the diabetic kidney and in the pathophysiology of diabetic nephropathy (581).

Renal hypoxia has been demonstrated in animal models of T1DM and T2DM (200, 268, 469, 519, 531), particularly in the outer medullary region including the medullary TAL (519, 531). A role of hypoxia in chronic renal disease was proposed by (174) and has been confirmed in human and animal models including the diabetic kidney (426, 581). Hypoxia can be due to enhanced oxygen consumption, as shown *ex vivo* in cortical and medullary tubular cells of STZ diabetic rats (469). Changes in vasoactive factors such as Ang II and/or NO can lower postglomerular blood flow in diabetes thereby contributing to hypoxia (471–473, 582). In later stages of the disease, glomerular lesions and interstitial vascular rarefaction, which is associated with reduced VEGF-A expression in human diabetic nephropathy (372), further impair peritubular blood flow and oxygen delivery to the tubules (426). Hypoxia can be further enhanced by concurrent anemia (35) and enhanced sodium transport load and oxygen consumption in remnant nephrons (429) (see Fig. 9).

In general, the cells in hypoxia depend on anaerobic glycolysis to generate ATP. However, due to the residual low oxygen supply, some level of oxidative ATP production remains via the Krebs cycle and electron transport chain. Electron leakage from the mitochondrial electron transport chain under hypoxic conditions results in excessive ROS generation. Studies in diabetic rats indicated increased mitochondrial expression of uncoupling protein 2 (UCP-2) in renal proximal tubular cells, which mediates mitochondrial uncoupling and increased O₂ consumption (186). Whereas this may protect against diabetes-induced oxidative stress, it may aggravate hypoxia (see Fig. 9).

Hypoxia can enhance oxidative stress as outlined. Vice versa, oxidative stress can enhance hypoxia. Overproduction of ROS, in part due to activation of NADPH oxidase with translocation of p47phox to the membrane, limits NO generation in the diabetic kidney (29), which enhances hypoxia by affecting the use and supply of oxygen (411, 471, 473). The latter is enhanced through NO quenching by AGEs (89, 673) or ROS (235), and NO capture by glucose (82). Superoxide can induce renal vascular constriction (752) and enhance tubular salt reabsorption (285), which can enhance renal hypoxia., treatment of STZ-diabetic rats with the antioxidant alpha-tocopherol prevented diabetes-induced disturbances in oxidative stress, oxygen tension, and oxygen consumption. Notably, diabetic hypertrophy and glomerular hyperfiltration were unaffected by alpha-tocopherol (469).

Hypoxia and oxidative stress also affect the function of the endoplasmatic reticulum (ER) which is critical for the processing and function of newly synthesized proteins (721). Impaired ER function—ER stress—has been documented in the diabetic kidney (373). ER

stress but also oxidative stress and nitrosative stress (50, 458), carbonyl stress and AGE formation (410, 427) are sensitive to Ang II AT1 receptor blockade (or ACE inhibition). In addition, these maneuvers can improve tissue hypoxia by increasing postglomerular peritubular blood flow (384) and/or through preferred activation of Ang II AT2 receptor activation and NO production (470) as shown in other models of kidney injury. A protective role of these effects has been implicated in experimental models of T2DM (268), which support a prominent role of the RAS in the diabetic kidney beyond blood pressure regulation.

Stimulation and inhibition of hypoxia inducible factor in the diabetic kidney—

Defense against hypoxia involves the hypoxia-inducible factor (HIF) (561). Its activation induces a wide variety of genes [e.g., erythropoietin, VEGF, hemoxygenase 1 (HO-1)], which can help to protect hypoxic tissues. The cell-protective effect of HO-1 relies, at least in part, on its ROS scavenging ability (5). The stability of HIF is reduced through hydroxylation by the oxygen sensor prolyl hydroxylase (PHD). Rosenberger et al. showed that STZ-diabetic and Cohen diabetes-sensitive rats transiently upregulate the hypoxia marker pimonidazole (PIM) and HIF-1 α , HIF-2 α , as well as HO-1 in the renal outer medulla, with increases at 14 and 30 days and normalization after 90 days (531). PIM was detected in TALs and CDs, increasing with distance from vascular bundles. Whereas HIF-1 α appeared in the same cells as PIM, HIF-2 α was localized to capillary endothelial cells and in the interstitial cells of the inter-bundle zone. The HIF target gene HO-1 was also detected in interstitial cells (531). Studies by Katavetin et al. showed that the activation of the HIF hypoxia-responsible element pathway is blunted in STZ-diabetic rats (301). Superoxide has been proposed to reduce HIF activity (726). In accordance treatment of diabetic rats with the SOD mimetic tempol increased HIF-1 α , HIF-2 α , and HO-1 in the renal outer medulla (531) indicating that oxidative stress in the diabetic kidney may suppress HIF activation (see Fig. 9). Similarly, high glucose levels inhibit the hypoxia-induced activation of HIF and VEGF expression in rat proximal tubular cells *in vitro*; this effect was reversed by the antioxidant α -tocopherol (301).

The role of HIF was further tested in spontaneous hypertensive rats (SHR)/NDmcr-cp rats, a hypertensive model of T2DM, by application of cobalt (for 20 weeks), which inhibits HIF degradation by PHD (454). Cobalt did not affect hypertension and metabolic abnormalities but reduced proteinuria and histological kidney damage. This was associated with increased expressions of HIF-regulated genes, including erythropoietin, VEGF, and HO-1. Moreover, cobalt reduced the renal expressions of TGF- β and AGE formation (454). The toxicity of cobalt prohibits its use in humans. Small molecular activators of HIF that are currently developed include inhibitors of PHD1, which can induce hypoxia tolerance by reprogramming basal oxygen metabolism (25, 559), without impairing the regulation of angiogenesis and erythropoiesis mediated by PHD2 (608, 609).

Linking hypoxia and oxidative stress to the accumulation of extracellular matrix—*in vitro* studies linked renal hypoxia to enhanced ECM: studies in human proximal tubular epithelial cells showed that hypoxia (1% O₂, 24 h) increased total collagen production, which was associated with decreased MMP-2 activity and increased tissue

inhibitor of metalloproteinase-1 (TIMP-1) protein (461). Collagen IV mRNA levels decreased while collagen I mRNA increased, suggesting induction of interstitial collagen. Although hypoxia stimulated TGF- β production, this did not appear to mediate the pro-fibrogenic stimulus of hypoxia (461). Similarly, hypoxia (1% O₂) applied to human renal tubular endothelia, interstitial fibroblasts and microvascular endothelial cells *in vitro* increases ECM production, which was associated with decreased turnover via effects on matrix-degrading enzymes and their inhibitors (446). These effects were independent of HIF but involved activation of both PKC- and tyrosine kinase-mediated signal transduction pathways (446). Superoxide activated ERK-dependent fibrosis-stimulatory factor and ECM gene transcription have been implicated in STZ-diabetic rats. These findings link both hypoxia and oxidative stress to the accumulation of ECM and fibrosis.

Inflammatory response in the diabetic kidney

Renal inflammation is an important pathway that is common to diverse chronic kidney diseases, precluding renal fibrosis and driving progression to end-stage renal failure (153). Studies in STZ-diabetic rats have demonstrated prominent macrophage infiltration (545, 732). Moreover, studies of renal biopsies taken from patients with T2DM have suggested a role for macrophages and their products in the initiation of the pathological changes of human diabetic nephropathy (193). Chemokines are important for the recruitment of specific inflammatory cells into the kidney including MCP-1, which has a key role in the recruitment of macrophages as indicated in diabetic animal models and in renal biopsies from patients with T1DM and T2DM [for review see (537)]. As mentioned above, urinary albumin can induce pro-inflammatory chemokines in proximal tubular epithelial cells including IL-8 (611) and MCP-1 (694) (see Fig. 9).

Interaction between growth factors, such as IGF-I, HGF, and TGF- β , and their respective receptors in the apical membranes in proximal and distal tubules and collecting ducts enhances the levels of MCP-1, RANTES, and PDGF- β , which activate the proliferation of fibroblasts but also of macrophages (112, 237, 689). Many proinflammatory mediators, including chemokines MCP-1 and RANTES, are under the tight control of transcription factor NF κ B (213). ROS are thought to be critical inducers of renal inflammation and destruction (455, 572). In accordance, recent studies in renal epithelial cells and in animal studies showed that candesartan, by a direct antioxidant effect, independent of Ang II AT1 receptor blockade, inhibits NF κ B activation and chemokine production, including MCP-1 and RANTES, thereby ameliorating renal inflammation and injury (104) (see Fig. 9).

Stimulation of proximal tubular TGF- β 1 synthesis by IL-1 β derived from pro-inflammatory macrophages has been described in the diabetic kidney (496). High glucose has also been shown to induce macrophage inflammatory protein-3 alpha (MIP-3 α) in human proximal tubular cells in a TGF- β 1-dependent way (507) (see Fig. 9). Moreover, MIP-3 α was upregulated in the dilated tubules of diabetic rats, which were surrounded by CD3-positive cells, a marker of T lymphocytes. Notably, this upregulation was attenuated in the presence of an ACE inhibitor (507). Blockade of the renin-angiotensin-aldosterone system is currently the only clinical used anti-inflammatory strategy to treat diabetic nephropathy. Studies in STZ-diabetic rats revealed that ACE inhibition by lisinopril reduced the renocortical

accumulation of interstitial cells that have activated PKC β 2 and express markers of antigen-presenting cells and of macrophages that are infiltrating affected tissue (489). PKC β 2 has been implicated in glucose-dependent monocyte transformation to macrophages (101). This would indicate that pharmacological inhibition of ACE or PKC β 2 could have nephroprotective effects in diabetes in part by inhibiting this transformation and renal inflammation. The clinical relevance of new experimental approaches like chemokine receptor antagonists or even immunosuppressive therapy to prevent/treat diabetic nephropathy remains to be established (537).

Nuclear hormone receptors in the diabetic kidney

Many nuclear hormone receptors have been suggested to provide protection against metabolic, inflammatory and cardiovascular diseases. A number of studies indicated a potential role for PPAR α (476, 477), PPAR γ (532), PPAR δ (532), estrogen receptors (ER) (100, 387), hepatic nuclear factor 4 alpha (HNF4 α) (438) and, especially, the vitamin D receptor (VDR) (135, 741, 743, 744) and the farnesoid X receptor (FXR) (279, 693) in the pathogenesis of diabetic nephropathy. Notably, activation of all of these nuclear hormone receptors has been linked to lowering proteinuria, while some have, in addition, been implicated in attenuating mesangial expansion (PPARs, ER, VDR, FXR), renal fibrosis (PPARs, ER, FXR), and macrophage infiltration (PPARs, VDR, FXR) [for review see (692)]. Although there is first evidence that at least some of these receptors play a direct role in the diabetic kidney, the interpretation of renal outcomes in the setting of diabetes has been confounded by extrarenal effects on metabolism and other functions with potential secondary effects on the kidney. Studies using animal models with cell-specific knockdown of these genes in the kidney will greatly facilitate to separate the systemic versus kidney-specific effects of these nuclear hormone receptors in the diabetic kidney.

Glomerular Function in Diabetes

Early glomerular hyperfiltration in T1DM, which is often associated with nephromegaly, has been recognized for many years (413). Similar studies have more recently been performed in the much larger patient population with T2DM. These studies revealed a significant variation in renal hemo-dynamics, but provided clear evidence for elevations of GFR and renal plasma flow (RPF) in significant proportions of patients with T2DM of Caucasian, Native and African American origin (420, 474, 680). Compelling evidence for the presence of renal hemodynamic abnormalities in T2DM has also been reported in Pima Indians (432), in which transition from impaired glucose tolerance to T2DM was accompanied by a 30% increase in GFR. An increase in GFR has been also reported in obesity (215), a condition which often accompanies T2DM.

Alterations in renal hemodynamics in diabetes can also be associated with loss of renal functional reserve, that is, the ability to increase GFR in response to amino acid infusion or to ingestion of a meal rich in protein (540). These maneuvers may identify altered renal hemodynamics in a subset of patients with GFR within the normal range. Notably, another study found in fasted diabetic patients who have normal renal function that the renal

hemodynamic responses to increased plasma amino acid concentrations were augmented (640).

It has been proposed that early glomerular hyperfiltration may predispose the subsequent development of diabetic glomerulopathy (22, 128, 412, 414, 533, 596). Early support for this hypothesis emanated from the finding that diabetic glomerulopathy only occurred in the nonstenosed kidney in the setting of unilateral renal artery stenosis (52). However, the clinical relevance of early hyperfiltration as a risk and/or pathogenic factor for the later development of overt nephropathy is debated since other studies have failed to document such a relationship (357, 728), including a recent analysis by Ficociello et al. (172). The ongoing debate is well documented by two recent publications: a meta-analysis of 10 studies in T1DM conducted by Magee et al. (380) concluded that the presence of hyperfiltration at baseline more than doubled the risk of developing micro- or macroalbuminuria at follow-up. In contrast, a thorough analysis of available data led Jerums et al. (276) to the notion that the available data in support of a pathogenetic role for hyperfiltration in diabetic nephropathy are at present too weak to allow reliable conclusions. The available studies suffer from one or more of the following defects: they are too small, far too short and have failed to control for confounders. In addition, the putative link between hyperfiltration and onset of nephropathy has focused entirely on albuminuria and the definition of progression of albuminuria has been variable. By contrast, no study has shown prospectively that hyperfiltration leads to subnormal GFR independently of other variables.

Renal hemodynamics in experimental diabetes mellitus

Despite the controversy in human diabetes concerning the significance of hyperfiltration in the subsequent development of overt nephropathy, extensive experimental data discussed in this section provides considerable insight into the importance of hemodynamic factors in the initiation and progression of diabetic glomerulopathy. As in diabetic patients, diabetic rats tend to exhibit reduced values for whole kidney GFR during periods of severe uncontrolled hyperglycemia; single nephron GFR (SNGFR) and plasma flow rates are also normal or reduced in animals in such catabolic states (244). In the more clinically applicable model of T1DM with moderate hyperglycemia, whole kidney GFR and SNGFR increase as compared to normal rats (244, 274, 734, 735). Reductions in intrarenal vascular resistances result in elevation of glomerular plasma flow rate, Q_A . Despite normal blood pressure levels, transmission of systemic pressures to the glomerular capillaries can be facilitated by proportionally greater reductions in afferent compared to efferent arteriolar resistances (see Fig. 10). This phenomenon was documented not only by micropuncture studies, but also by direct measurements of afferent and efferent arteriolar diameters using videomicroscopic techniques (99). Consequently, the glomerular capillary hydraulic pressure (P_{GC}) can rise, although this is not a prerequisite for diabetic hyperfiltration (274, 735). Thus, the observed single nephron hyperfiltration can result from both glomerular capillary hyperperfusion and hypertension (244, 734, 735). The potential minor contribution of a lower hydrostatic pressure in Bowman space (274, 654, 657, 734) as a cause of a greater effective filtration pressure in diabetes is further outlined in the tubular section below.

In long-term studies, diabetic rats develop morphologic changes reminiscent of those in the diabetic human, including glomerular basement membrane thickening, renal and glomerular hypertrophy, mesangial matrix thickening and hyaline deposition, and ultimately glomerular sclerosis (734, 735). In comparison, mice in general are more resistant to the development of diabetic nephropathy (79, 84) (see animal models below for further discussion). Evidence that glomerular hemodynamic maladaptations contribute to the development and progression of diabetic glomerulopathy has been shown by studies of maneuvers which aggravate or ameliorate glomerular hyperperfusion and hyperfiltration, without affecting metabolic control. Uninephrectomy, which increases SNGFR, Q_A and P_{GC} in normal rats, accelerates the development of albuminuria and glomerular sclerosis in diabetic rats (448). Intensification of glomerular lesions is observed in the unclipped kidney of diabetic rats with two-kidney Goldblatt hypertension, while the clipped kidney is substantially protected from glomerular injury (395). Diabetic renal injury is similarly amplified by augmentation of dietary protein content, which increases glomerular perfusion and filtration (735): diabetic rats fed a high protein diet exhibited glomerular capillary hyperfiltration, hyperperfusion, and hypertension, and marked increases in albuminuria and glomerular morphologic injury. By contrast, dietary protein restriction, which reduces SNGFR, Q_A and P_{GC} in other models, limited SNGFR in long-term diabetes by reducing the elevated P_{GC} and Q_A , and virtually prevented albuminuria and glomerular injury (735). As there were no differences in metabolic control between the various groups, this study provided strong evidence that amelioration of the maladaptive glomerular hemodynamic pattern can dramatically lower the risk of diabetic glomerulopathy and renal disease.

Mechanisms of hyperfiltration in diabetes

The pathogenesis of diabetic hyperfiltration is multifactorial. Numerous mechanisms and mediators for this effect have been proposed, and are briefly reviewed here. For better clarity, these mechanisms are divided in five areas separately discussed below. However, the reader should be aware of overlap between these processes, and the fact that they act in concert to promote renal hemodynamic alterations in diabetes.

Metabolic milieu—Considering the simple fact that without hyperglycemia and other factors characteristic for the diabetic milieu there would be no hemodynamic changes or nephropathy, the diabetic metabolic milieu must contribute. Hyperglycemia and/or insulinopenia *per se* (118), together with augmented GH and glucagon levels (117, 478) have been invoked in this process. Reduction of plasma glucose with initial institution of therapy reduces GFR in patients with both T1DM and T2DM (118, 680). In moderately hyperglycemic diabetic rats, normalization of blood glucose levels reverses hyperfiltration (594), and insulin infusion reduces P_{GC} (562). By contrast, insulin infusion sufficient to produce hyperinsulinemia, with euglycemia, increases P_{GC} and hyperfiltration in normal rats (635). Further, infusion of blood containing early glycosylation products reproduces glomerular hyperfiltration in normal rats (539). However, it should be appreciated that the most pronounced changes in renal hemodynamics as compared to nondiabetic animals occur in moderately hyperglycemic rats treated with suboptimal doses of exogenous insulin. Hyperfiltration may not always develop in rats without exogenous insulin treatment, despite moderate hyperglycemia. This finding suggests that some insulin levels, in concert with

hyperglycemia, may facilitate the development of renal hemodynamic changes in diabetes. Whether these effects are linked to vasodilator actions of insulin remains to be established. Furthermore, C-peptide (685), which is a part of the endogenous insulin molecule but that is not contained in exogenous insulin preparations, has its own separate physiological and pathophysiological effects. In addition to alterations in plasma insulin levels and actions, it has been suggested that the lack of C-peptide also contributes to diabetic hyperfiltration (233, 249, 514).

Vasoactive factors

Apart from the above-mentioned mechanisms, which are closely related to the diabetic metabolic milieu, there is substantial evidence suggesting that renal hemodynamic alterations are a consequence of an imbalance between the vasoactive humoral systems controlling the glomerular circulation. There is evidence that the balance between factors influencing the afferent arteriolar tone is shifted toward vasodilators, whereas opposite changes may occur on the efferent arteriole.

Atrial natriuretic peptide—Atrial natriuretic peptide (ANP) which induces afferent dilation and efferent constriction represents one such promising candidate for mediating diabetic hyperfiltration. Plasma ANP levels are elevated in diabetes (462), and blockade of the ANP action with an antibody (462) or a specific receptor antagonist (739) blunts hyperfiltration in diabetic rats. It is likely that altered levels of ANP in diabetes are a consequence of an increase in total exchangeable body sodium and the hypervolemic state (166, 450), although resistance to ANP may be also involved (175). Although these observations may suggest that sodium homeostasis is an important factor in the pathogenesis of hyperfiltration, it is clear that the latter does not absolutely depend on accumulation of NaCl in the body, since GFR can increase relentlessly in early diabetes, notwithstanding a decline in extracellular volume (283).

Nitric oxide—NO is a potent vasodilator acting on both afferent and efferent arterioles, presumably with predominant afferent actions *in vivo* (139). In mammalian tissues, NO is synthesized by a family of isoenzymes known as NO synthases (NOS). Considering its renal actions, an enhanced NO activity appears to be a good candidate for mediating diabetic hyperfiltration. Paradoxically, diabetes is considered to be a state with reduced NO bioavailability (203, 499). However, the situation in the diabetic kidney, in particular at the early stages of diabetes, is more complex (318).

Most of the renal hemodynamic studies conducted in hyperfiltering diabetic rats demonstrated increased renal hemo-dynamic responses and near-normalization of renal hemodynamics in response to inhibition of NO synthesis with nonspecific NOS inhibitors (i.e., affecting all NOS isoforms) (317, 391, 626). Subsequent studies attempted to identify the contribution of individual NOS isoforms in the process and focused on renal hemodynamic roles of neuronal NOS (nNOS, NOS1). Under physiological conditions, NOS1-derived NO from the MD counteracts afferent vasoconstrictor signals mediated by the TGF mechanism, thus inducing a rightward shift and attenuation of the slope of the TGF function and contributing to the control of Q_A and P_{GC} (662, 663, 702). The renal vascular

tree is more sensitive to systemic NOS1 inhibition in diabetic rats as compared to controls (320, 324). Furthermore, we observed complete amelioration of hyperfiltration in response to acute intrarenal selective NOS1 inhibition in conjunction with increased number of nNOS-positive cells in MD regions of diabetic kidneys (320). Thomson et al. confirmed the GFR-lowering effect of acute selective NOS1 inhibition in STZ-diabetic rats, when the drug was given intravenously, and reported an ongoing overactivity of MD NOS1 that is not regulated by MD salt (585). These observations implicated NOS1-derived NO as an important player in the pathogenesis of diabetic hyperfiltration, and are in accordance with previous reports showing a modest attenuation of the slope of the TGF function in diabetic rats (61, 647), as further discussed below. Moreover, unlike the chronic treatment with nonspecific NOS inhibitors, which aggravates the course of experimental nephropathy, selective NOS1 inhibition slowed down the development of nephropathy in uninephrectomized diabetic rats most likely via a hemodynamic mechanism (324).

There is also evidence suggesting that the activity of the endothelial NOS isoform (eNOS, NOS3) may be increased, and responsible for enhanced renal NO production in diabetes (602, 671). However, the relevance of such findings could not be further validated due to the lack of specific eNOS inhibitor. The contribution of eNOS to the development of renal hemo-dynamic changes in diabetes is in our opinion less likely, due to the complex pathobiochemistry of this enzyme as discussed in more detail in the sections devoted to endothelial dysfunction. Notably, genetic knockout of *Nos3* has been used to induce nephropathic changes in mouse models of both T1DM and T2DM that mimic many aspects of human disease (278, 392, 398, 707) (see discussion of animal models below). A possible role for NO-ROS interactions in the regulation of efferent arteriolar tone is outlined below.

In addition to possible direct effects of diabetes on NOS activities, the NO-mediated alterations in renal hemodynamics may be related to increased activity of factors that act as NO-dependent vasodilators or activate the NO-cGMP pathway as a part of their signal transduction. De Vriese et al. (134) reported that neutralization of VEGF with an antibody ameliorated diabetic hyperfiltration. VEGF has been implicated in nonhemodynamic pathways in the pathogenesis of diabetic complications, but also possesses vasomotor effects mediated by NO. However, as discussed above and below, the more recently described phenomenon of uncoupling of VEGF with the eNOS pathway as a mechanism for diabetic vasculopathy (423) does not support the role of this mechanism in the pathogenesis of hyperfiltration.

Cyclooxygenase—A role for COX metabolites of arachidonic acid in the pathogenesis of diabetic nephropathy has been suggested in a number of clinical and experimental studies. Schambelan et al. (552) demonstrated an increase in conversion of exogenous arachidonate to prostaglandin E2 (PGE2), prostaglandin F2, prostaglandin D2, and thromboxane B2 (TxB2) in glomeruli from diabetic rats. In the early stages of nephropathy, vasodilator prostaglandins, such as PGE2 and prostacyclin, have been implicated in mediating alterations in renal hemodynamics in humans with T1DM (161, 194, 242, 677), as well as in experimental models of diabetes (124, 275, 299, 486). As demonstrated by Jensen et al. (275), inhibition of PG synthesis results in significant reductions in SNGFR, Q_A and P_{GC} in diabetic rats.

The above-mentioned evidence about the role of prostaglandins in the renal hemodynamics in diabetes relied on measurements of renal function in response to nonspecific inhibitors that inhibit COX-1 and COX-2 isoenzymes. Follow-up studies demonstrated an increased expression of the COX-2 isoform in the diabetic kidney and a modest GFR lowering effect of selective COX-2 inhibition in these diabetic rats; in comparison, COX-1 expression was unchanged in diabetic rats and acute COX-1 inhibition ineffective with regard to renal hemodynamics (321). These experimental observations, indicating a role for COX-2 in the regulation of renal hemodynamics in the diabetic kidney, were more recently reproduced in the clinical setting (111).

Other mediators—Diabetes-related abnormalities of other vasodilator mechanisms have also been suggested, including activation of the kallikrein-kinin system (96, 269). However, studies with kinin receptor antagonists have been inconsistent (270, 319, 681).

Efferent arteriolar vasoconstrictors—Reduced afferent arteriolar or mesangial constriction may occur in the diabetic kidney due to reduced glomerular receptor sites for the vasoconstrictors Ang II and thromboxane (38, 703, 704) and altered vascular responsiveness (452). Notably, inhibition of vasoconstrictor systems such as the RAS (734), ET (49), or thromboxane A₂ (328, 643) has beneficial effects on the development of nephropathy including amelioration of diabetic hyperfiltration. The most plausible explanation is, at least in part, inhibition of their efferent arteriolar vasoconstrictor actions. This suggests that diabetes is associated with normal or increased efferent actions of vasoconstrictors. Supporting this view are our whole kidney data showing enhanced renal vasodilation to Ang II inhibition in diabetic rats (309, 324) in conjunction with reduced filtration fraction. Also Hollenberg's group has documented enhanced activity of the renal RAS in both types of diabetes by demonstrating enhanced hemodynamic responses in these patients as compared to nondiabetic subjects (346, 505).

Small GTPase RhoA and its down-stream effectors ROCK mediate vasoconstrictor signals and recent studies showed altered renal hemodynamic responses in the diabetic kidney. Unlike the control rats, diabetic animals responded to acute inhibition of ROCK (with the selective inhibitors Y27632 or fasudil) with renal vasodilation and lower filtration fraction (322). Considering the hemodynamic patterns induced by inhibitors of these systems, which include reductions in filtration fraction and thus potentially in P_{GC} , it is possible that these interventions have important efferent actions and target diabetes-induced dysbalances in afferent and efferent vasoconstrictor signals.

It should be noted that similar to the glomerular micro-circulatory pattern, renal hemodynamic responses to various mediators in diabetes are largely dependent upon the state of metabolic control and insulin treatment. These differences may explain some disparate findings of studies exploring the activities of vasoactive systems in diabetes.

Reactive oxygen species—Enhanced production of ROS has been also implicated in the renal hemodynamic changes of the diabetic kidney. ROS can induce renal vasoconstriction by decreasing the bioavailability of NO or limiting the buffering capacity of NO against vasoconstrictors (451, 558, 565). Therefore, one would expect that enhanced ROS may

induce renal vasoconstriction in diabetes. Importantly, there is no evidence demonstrating the effect of ROS scavenging on basal renal arteriolar tone in diabetic rats (558). Furthermore, considering the described NO-ROS interaction and a dominant role of NO on the afferent arteriole, one would expect enhanced afferent vasodilator responses to antioxidant treatment in diabetes. In contrast, *in vivo* studies demonstrated that treatment with the antioxidant α -tocopherol normalizes glomerular hyperfiltration in diabetic rats (333). This reduction in GFR was associated with a decrease in filtration fraction, which may implicate an effect of the antioxidant treatment on the efferent arteriole, which may include an NO effect. The observed renal microvascular effects of antioxidant treatment may also include other mechanisms, such as attenuation of PKC activity (333) or Ang II signaling via ROS (208).

Alterations in signal transduction—In addition to a myriad of other effects in diabetic cells and tissues, PKC transduces vasoactive signals. Importantly, some PKC isoenzymes are not only activated by hyperglycemia via *de novo* synthesis of DAG, but also operate in signaling cascades of some vasoactive peptides, such as Ang II (564). The role for PKC β in altered hemodynamics in the diabetic kidney is best documented by amelioration of hyperfiltration, associated with an overall renoprotective effect in the diabetic kidney, in diabetic rats and, as shown more recently, in patients with T1DM treated with the PKC β inhibitor ruboxistaurin (110, 260). PKC β is also expressed in the brush border of the proximal tubule (489, 490) and thus may affect GFR in diabetes indirectly via its tubular effects as discussed below. In addition to PKC, other signaling pathways have been studied as potential mediators of diabetic hyperfiltration, in particular those that are involved in the control of vascular tone. For example, selective inhibition of p38 MAPK near-normalized hyperfiltration in diabetic rats, however, this effect was not specific for the diabetic state and similar effects of this intervention were observed in control animals (325).

Renal sodium handling and tubuloglomerular feedback—In the normal kidney, cells of the MD sense early distal intratubular concentrations of Na⁺ and Cl[−] and in response to increasing ion concentrations, they send vasoconstrictor signals to the afferent arteriole. This autoregulatory mechanism, known as TGF, prevents excessive NaCl losses from the organism and contributes to renal autoregulation. The contribution of TGF to the pathogenesis of glomerular hyperfiltration is discussed below in detail as part of the “tubulocentric concept” of renal hemodynamics in the early diabetic kidney.

Intrinsic defects in glomerular arterioles, electromechanical coupling—Data originating mainly from Carmines' laboratory have indicated primary changes in preglomerular microvascular smooth muscle function as an underlying mechanism of afferent arteriolar dilation during the hyperfiltration state of diabetes. Using videomicroscopy in isolated blood-perfused juxtamedullary nephrons, they described several diabetes-induced alterations in afferent arteriolar ion channels that can result in increased baseline diameter and impaired responses to vasoconstrictors. This impaired electromechanical coupling in afferent arteriolar smooth muscle could impair vasoactive responses to multiple humoral agents as well as the myogenic response, TGF, and, ultimately, autoregulation of renal blood flow and GFR.

They showed that STZ-diabetic rats have suppressed vasoconstrictor-induced increases in intracellular Ca^{2+} concentrations due to functional defects in afferent arteriolar L-type calcium channels (99). Brosius and colleagues had earlier proposed that diabetes attenuates glucose uptake via GLUT4 in afferent microvessels and renal glomeruli, and they proposed that this may induce vasodilation by lowering intracellular glucose-induced ATP formation and thus the inhibition of ATP-sensitive K^+ channels (K-ATP) (376, 385). Subsequent studies (256) implicated increased expression and function of K-ATP channels in the renal afferent arteriolar dilation in experimental diabetes. Pharmacological blockade of K-ATP channels contracts afferent arterioles from rats with STZ-induced diabetes mellitus while having minimal impact on afferent arterioles from normal rats (256). More recent studies evaluated the contributions of other K^+ channels to the regulation of afferent arteriolar tone in normal and diabetic rat kidney using pharmacological inhibitors of voltage-gated K^+ channels (KV), inward rectifier K^+ channels (KIR), Kir1.1 and Kir3.x subfamilies of KIR channels, large-conductance Ca^{2+} -activated K^+ channels (BKCa), and small-conductance Ca^{2+} -activated K^+ channels (SKCa) (631). While Blockers of SKCa and BKCa had no discernible impact on afferent arteriolar tone in normal rat kidney, STZ-diabetic rats displayed altered relative contributions of specific K^+ channels to afferent arteriolar tone, such that there was an increased tonic dilator impact of KIR channels (including Kir1.1 and/or Kir3.x). Thus, exposure to blockers of K-ATP or KIR channels reversed the afferent arteriolar dilation in diabetic rats. This observation suggests that K^+ channel activation is important for sustaining tonic afferent arteriolar dilation during the hyperfiltration stage of diabetes mellitus in the rat. These K^+ channels can contribute to afferent arteriolar dilation in diabetes mellitus by promoting vascular smooth muscle membrane hyperpolarization, thereby reducing Ca^{2+} influx through voltage-gated channels and, ultimately, decreasing cytosolic Ca^{2+} concentration. This situation can be exacerbated by the mentioned suppression of afferent arteriolar Ca^{2+} influx responsiveness to membrane potential in kidneys from diabetic rats (99). In the more complex *in vivo* setting, systemic application of the K-ATP channel blocker, U37883A, did not alter renal vascular resistance, renal blood flow, or GFR in both control and STZ-diabetic rats (645).

Role of glomerular capillary hypertension in renal injury—Of the glomerular hemodynamic determinants of hyperfiltration, the available evidence suggests that glomerular capillary hypertension plays the key role in progression of renal injury. Studies in a variety of experimental models, including diabetes, have consistently shown that interventions which control glomerular capillary hypertension are associated with marked slowing of the development of structural injury (23).

Glomerular capillary hypertension can modify the growth and activity of glomerular cells, inducing the elaboration or altered expression of growth factors and cytokines, enzymes and a variety of signaling molecules (37, 334, 453, 467, 485, 523, 742). As outlined above, podocytes may be particularly sensitive to mechanical stress and capillary stretch associated with increases in P_{GC} . The enhanced expression and/or release of these mediators then stimulate mesangial matrix production and promote structural injury as discussed in various contexts in this article.

Tubular Function in Diabetes

Hyperglycemia induces specific primary effects on the tubular system of the kidney. This includes changes in tubular growth (discussed above) and transport with secondary consequences on renal hemodynamics via the physiology of tubuloglomerular communication. In addition to primary defects in the GFB (see above), an inhibition of proximal tubular reabsorption of albumin has been proposed to contribute to the manifestation of albuminuria in early diabetic nephropathy (534, 637). An increased sensitivity of proximal tubular NaCl reabsorption to changes in dietary NaCl intake explains the unique phenomenon of the “salt paradox” of the early diabetic kidney. Moreover, kidney growth via its role in the development of tubulointerstitial injury is thought to play a crucial role in the long-term progression to kidney failure in diabetes. This article summarizes the effects of hyperglycemia on tubular transport of glucose, NaCl, and fluid [for previous reviews see (382, 493, 613, 646, 648)]. We also include a brief discussion on hyperinsulinemia-induced renal sodium retention in T2DM.

Evidence for a primary increase in reabsorption by the proximal tubule

Hyperfiltering adult patients with T1DM (81, 223) or T2DM (397) have increased absolute and fractional proximal reabsorption as determined by lithium clearances. Enhanced fractional proximal reabsorption was confirmed in children with T1DM (449). Similarly, hyperfiltering rats with STZ-diabetes (40, 501, 647, 657) have increased absolute and fractional reabsorption in the nephron segments upstream from the MD associated with lower early distal delivery of Na^+ , Cl^- , and K^+ (501, 647, 657). Operating solely under the influence of glomerulo-tubular balance (GTB), that is, load-dependence of tubular reabsorption, an increase in GFR should lead to an increase in absolute reabsorption. Due to the imperfect nature of GTB, however, an increase in GFR should also decrease fractional reabsorption and increase the distal delivery. Therefore, if fractional reabsorption and GFR happen to change in the same direction, this cannot be explained by GTB alone. There must be a change in the number of transporters or in their avidity, that is, a primary change in tubular reabsorption. To follow up on this issue, we performed micropuncture experiments in STZ-diabetic rats. In these experiments, SNGFR was manipulated by perfusing Henle's loop to activate TGF. This enabled proximal reabsorption to be established as a function of SNGFR. Using this method to control for the effects of SNGFR, a major primary increase in proximal reabsorption was documented for rats with early STZ-diabetes (617, 651) (see Fig. 11). The following paragraphs discuss mechanisms that contribute to this primary increase in proximal tubular reabsorption in the early diabetic kidney.

Tubular growth as a cause of primary tubular hyperreabsorption in diabetes

The kidney in general and the proximal tubule in particular, grow large from the onset of diabetes (116, 511, 571) (see above). On the basis of the principle of mass action, an increase in the size of the tubule results in increased proximal reabsorption (648). To test whether tubular growth per se contributes to the primary increase in proximal reabsorption in early diabetes mellitus, we used difluoromethylornithine, an inhibitor of ODC, which had been shown previously to attenuate kidney growth in early STZ-diabetic rats (482). We

observed that difluoromethylornithine not only attenuated kidney growth but also eliminated the primary increase in proximal reabsorption in STZ-diabetic rats (617).

Tubular growth in diabetes involves PKC β activation (see above). Moreover, the PKC β 1 isoenzyme is expressed in the brush border of proximal tubule (490) and stimulation of sodium transport in proximal tubule by Ang II is mediated by PKC β activation (157). STZ-diabetes activates PKC β 1 in the proximal tubule, which was inhibited by ACE inhibition (489). Further studies are needed to more directly test the role of PKC β 1 for proximal tubular hyperreabsorption, which could contribute to the beneficial effects of specific inhibition of PKC β isoenzymes on the diabetic kidney (260). Notably, genetic variants of the PKC β 1 gene have been linked to the development of end-stage renal disease in patients with T2DM (378).

Sodium-glucose co-transport as a cause of primary tubular hyperreabsorption in diabetes

The kidneys reabsorb large amounts of glucose, thereby contributing to the overall metabolic balance of the body. In healthy humans, about 180 g/day of glucose are filtered through the renal glomerulus, more than 99% of which is reabsorbed along the tubular system. Glucose entry into proximal tubular cells is insulin-independent, which makes proximal tubular cells particularly sensitive to the deleterious effects of chronic hyperglycemia in diabetic conditions. Transport of glucose across cell membranes is accomplished by two gene families: the facilitative glucose transporters—the GLUTs—and by an active sodium-dependent transport process mediated by the SGLT. At least two sodium-coupled glucose transporters, SGLT1 and SGLT2, play an important role in the apical membrane of proximal tubular cells in the kidney (718). The high-capacity, low-affinity SGLT2 is found primarily in the apical membrane of the S1 segment of the proximal tubule, whereas the low-capacity, high-affinity SGLT1 is located more distally in the S3 segment of the proximal tubule (290, 716, 718, 731). Two basolateral membrane glucose transporters facilitate transcellular glucose transport—the low-affinity GLUT2, which works in concert with SGLT2 in the S1 segment, and the high-affinity GLUT1, which works with SGLT1 in the S3 segment (114, 713).

The phenotype of humans carrying gene mutations in the genes for SGLT1 (*SLC5A1*) and SGLT2 (*SLC5A2*) provided much of the evidence for the quantitative contribution of these proteins to renal glucose reabsorption. Gene mutation of SGLT1 has serious intestinal consequences for afflicted individuals, who experience glucose-galactose malabsorption, frequent watery diarrhea, and dehydration, demonstrating the role of SGLT1 in intestinal glucose uptake (717). However, these individuals have little or no glucosuria. In contrast, those who have SGLT2 gene mutations have persistent renal glucosuria, with glucose excretion of up to 160 g/day in severe cases (180, 381, 543, 563, 667). Recently we directly localized the expression of SGLT2 protein to the apical brush border of the early proximal tubule and demonstrated by free-flow micropuncture that SGLT2 KO mice lack glucose reabsorption in the early proximal tubule and that fractional renal reabsorption of glucose is reduced compared with wild-type mice (36 ± 8 vs. $99.7 \pm 0.1\%$) and varied inversely with the amount of filtered glucose in the KO mice (between 60% and 10%) (656). Thus, early proximal SGLT2 reabsorbs the bulk of glucose filtered by the kidneys.

The capacity of glucose transport through SGLTs depends on the level of SGLT protein expression. Moreover, modeling the effects of sodium-linked glucose transport on the active and passive components of proximal reabsorption predicts a positive effect of filtered glucose on net proximal sodium reabsorption up to the point where the filtered load approximately doubles the transport maximum for glucose (697). Glucosuria starts to develop when blood glucose levels reach the renal threshold for reabsorption, which is about 8 to 10 mmol/l (180 mg/dl). Thus, modest hyperglycemia enhances sodium reabsorption in the proximal tubule, and the extent of sodium reabsorption depends on the expression of SGLT. In this regard, STZ-diabetic rat renal cortex was found to contain increased mRNA expression for SGLT 1 and SGLT 2 (676) and greater renal SGLT1 protein expression (678). Likewise, renal SGLT1 and SGLT2 mRNA levels in diabetic obese Zucker rats were higher than in age-matched leans (605). Primary cultures of human exfoliated proximal tubular epithelial cells from fresh urine of patients with T2DM indicated an increased renal glucose uptake associated with increased mRNA and protein expression of SGLT2 and GLUT2 compared with healthy control subjects (509). Upregulation of SGLT2 expression in diabetes may involve Ang II AT1 receptors (463). In accordance, treatment of rats with either ramipril or losartan showed significant reduction in the intensity of immunostaining and levels of SGLT2 protein and mRNA (45). Upregulation of SGLT2 expression in diabetes may also involve the transcription factor, hepatocyte nuclear factor HNF-1 α (181). There is also evidence for upregulation of GLUT2 mRNA and protein expression in renal proximal tubules in diabetic rat models (113, 145, 289), which may be due to transcriptional activity of both HNF-1 α and HNF-3 β (182). Whereas multiple studies found evidence for an upregulation of SGLT2 expression in the diabetic kidney, other studies showed unchanged (8) or reduced (15) renal expression in diabetic rodent models, and *in vitro* studies indicated that high glucose-induced oxidative stress can inhibit Na⁺/glucose co-transporter activity in renal proximal tubule cells (222). Downregulation of SGLT could be an approach to limit glucose uptake and glucose toxicity. Notably and possibly relevant in this regard, gene-knockout of SGLT2 lowers renal SGLT1 mRNA and protein expression by 40% (656).

The serum and glucocorticoid-inducible kinase SGK1 is upregulated in proximal tubules in STZ-diabetic rats (538) and in patients with diabetic nephropathy (343). Excessive SGK1 expression in the diabetic kidney contributes to stimulation of SGLT1 activity in proximal renal tubules, which attenuates glucosuria (7). SGK1 could also increase proximal renal tubular glucose transport by stimulation of the K⁺ channel KCNQ1/KCNE1 (159), which establishes the electrical driving force for electrogenic glucose transport in the proximal tubule (649, 650). Moreover, SGK1 has been proposed to mediate in STZ-diabetic rats the upregulation of mRNA levels of the Na-H-exchanger NHE3 (538), which is of critical importance for proximal tubular sodium reabsorption (660). SGK1 effects on proximal and distal tubular transport (see below) as well as fibrosis (see above) make this kinase an interesting targeting in diabetes (342). NHE3 is thus also a candidate to be involved in proximal tubular hyperreabsorption in the diabetic kidney. Whereas renal NHE3 expression was found to be increased, unchanged or decreased in STZ-diabetes (431, 590, 678), the expression was found to be reduced in Zucker diabetic fatty rats (55, 63).

Based on microperfusion studies in normal and STZ-diabetic rats, Bank and Aynedjian proposed that high glucose in the proximal tubular fluid stimulates sodium absorption

through SGLT (40). They found that increasing luminal glucose (from 100 to 500 mg/dl) induced significantly greater increases in sodium versus glucose absorption on a molar basis, which may reflect, in part, the well-documented sodium:glucose coupling ratio for SGLT1 of 2:1 [in comparison, the ratio is 1:1 for SGLT2 (718)]. The contribution of SGLT1 to renal glucose absorption is expected to increase in diabetes when the luminal glucose delivery overwhelms the capacity of SGLT2. Confirmation of increased SGLT-mediated sodium transport was demonstrated with micropuncture in STZ-diabetic rats with moderate hyperglycemia, by delivering the SGLT inhibitor, phlorizin, directly into the free-flowing early proximal tubule of nephrons with superficial glomeruli: in diabetic rats, phlorizin elicited a greater decline in absolute and fractional sodium reabsorption up to the early distal tubule and abolished the hyperreabsorption (657). These studies support the concept that moderate hyperglycemia enhances SGLT-mediated glucose and sodium reabsorption in the proximal tubule.

Earlier, Seyer-Hansen had reported that in early STZ-diabetic rats the glucose reabsorptive rate increased to the same extent as kidney weight (570). Hence, the primary increase in proximal reabsorption in early diabetes is the combined result of growth of the tubule and increased SGLT. This could be relevant for the regulation of GFR by TGF in early diabetes (see below).

Inhibition of SGLT2 as a new therapeutic approach in diabetes—Good control of blood glucose levels is important in diabetic patients but difficult to achieve without the potential for hypoglycemia and weight gain. Moreover, existing therapies may not always reduce cardiovascular complications, indicating the need for new therapies with novel mechanisms of action. SGLT inhibition offers the potential to decrease the deleterious effects of hyperglycemia while reducing blood glucose levels by inhibiting renal glucose reabsorption. Promising results have been achieved in diabetic animal with the nonselective SGLT inhibitor, phlorizin (158), its derivative T-1095 and its metabolite T-1095A (26, 642), including beneficial effects on blood glucose levels and HbA_{1c} levels, partial improvement of glucose tolerance and insulin resistance, and suppression of albuminuria and the expansion of the glomerular mesangial area in diabetic rodents (456, 642). Due to their effect on intestinal SGLT1, however, nonselective inhibitors can have unacceptable gastrointestinal side effects and did not proceed to clinical development. Selective SGLT2 inhibitors offer the potential to primarily increase renal excretion of glucose and create a negative energy balance without affecting intestinal function. Therefore, various selective SGLT2 inhibitors are currently in development for the treatment of diabetes mellitus [for review see (421, 661)]. It remains to be determined whether these drugs lower the toxicity of glucose directly on renal cells, independent of hyperglycemia, which may slow or prevent the progressive nature of diabetic nephropathy.

Is there relevant hyperinsulinemia-induced renal NaCl retention in type 2 diabetes mellitus?

Hyperinsulinemia-mediated sodium retention has been hypothesized to contribute to hypertension, particularly in obese subjects with T2DM (137, 170, 516, 526, 544, 624). An acute increase in insulin levels induces an antinatriuretic response, which is at least in part

due to enhanced renal reabsorption, as indicated by studies in human (31, 138, 185, 584, 597) and animals (169, 196, 311, 407). These antinatriuretic effects of insulin have been ascribed to its actions on the proximal tubule (169, 196), loop of Henle (311), and especially on the distal nephron (138, 169, 185, 589, 597). Studies by Tiwari et al. in mice showed that the acute antinatriuretic response to insulin is associated with enhanced expression of total and phosphorylated SGK1 in the membrane fraction as well as trafficking of the epithelial sodium channel, ENaC, to the apical membrane compartment of the distal nephron (623). In accordance, Huang et al. showed that the acute insulin-induced antinatriuresis observed in wild-type mice (during glucose clamp) is significantly blunted in mice lacking SGK1, and these mice lack the salt-sensitizing hypertensive effect of high fructose intake, which is associated with hyperinsulinemia (248). SGK1 may also contribute to the enhanced expression and activation of the thiazide-sensitive Na-Cl co-transporter (NCC or TSC) observed in a rat model of hyperinsulinemia (56, 658).

One study in patients with T1DM reported that the acute sodium-retaining effect of insulin was blunted (483). However, others concluded an intact distal antinatriuretic effect of insulin in T1DM (598), and studies in hyperinsulinemic patients with T2DM and peripheral insulin resistance also showed a preserved acute antinatriuretic effect of insulin (585). Yet, studies in rats showed that insulin receptors are downregulated in the kidneys of insulin resistant animals as well as in STZ-diabetes, possibly mediated by hyperglycemia and Ang II (622). Furthermore, studies with partial knockdown of insulin receptors in renal tubules implicated that activation of renal insulin receptors can actually facilitate renal sodium and water excretion and reduce blood pressure, possibly via an effect on NO production (625). These findings are consistent with the notion that the antinatriuretic and ENaC-mediated effects of insulin are actually mediated by its binding to the IGF-I receptor and downstream activation of SGK1 (204).

Notably, all the currently available data that support a sodium-retaining or blood pressure increasing effect of chronic hyperinsulinemia have been generated in rodent studies (75,76, 399, 589, 628). In comparison, chronic increases in plasma insulin levels in humans and dogs have not been shown to cause hypertension (74, 217), and sodium retention was not apparent during chronic intra-renal insulin infusion in dogs (216). Therefore, further studies are necessary to translate the results from acute insulin infusion studies in humans and chronic rat and mice studies that support a sodium-retaining effect of hyperinsulinemia to the situation of chronic hyperinsulinemia in humans with T2DM.

Role of primary tubular hyperreabsorption for glomerular hyperfiltration in the diabetic kidney

Common sense may suggest that glomerular hyperfiltration must be due to some defect in microvascular function or an imbalance of hormones impinging directly on the glomerulus, as outlined above. However, in early diabetes glomerular filtration is also inordinately influenced by feedback from the proximal tubule and we have proposed a general tubulocentric principle for the control of GFR in early diabetes [reviewed in (648)]. In this principle, primary changes in tubular transport induce *secondary* changes in GFR through TGF (see Fig. 12). Manifestations of this tubulocentric model in the early diabetic kidney

include glomerular hyperfiltration discussed here as well as the salt paradox, which is outlined below.

The TGF system senses changes in the concentration of Na^+ , Cl^- , and K^+ at the luminal MD and induces reciprocal changes in SNGFR (560, 655). TGF, thereby, stabilizes electrolyte delivery to the distal tubule which in these nephron segments allows fine adjustment of reabsorption and excretion according to body needs. As outlined above, there is a primary increase in proximal reabsorption in early diabetes, which reduces delivery of NaCl and K^+ to the MD. Pollock et al. reported that the Na^+ concentration in early distal tubules of hyperfiltering STZ-diabetic Sprague Dawley rats is remarkably low (about 30 vs. 76 mM) (501). We performed similar experiments in diabetic rats of the Hannover-Wistar-Froemter strain with superficial glomeruli from which it is possible to sample tubular fluid closer to the MD. In these studies, respective ambient early distal tubular concentrations of Na^+ , Cl^- , and K^+ in nondiabetic rats were 21, 20, and 1.2 mM, respectively. In hyperfiltering STZ-diabetic rats of this strain, early distal concentrations of Na^+ , Cl^- , and K^+ were reduced by 20% to 28%, consistent with a primary increase in upstream reabsorption (657). The MD senses the decline in salt delivery as an error signal and elicits an increase in GFR, which offsets a portion of the original error signal through negative TGF.

The role of tubular reabsorption and a reduced TGF stimulus as antecedents to diabetic hyperfiltration is further supported by the observation that adding phlorizin to the early proximal tubule of diabetic rats caused a significant increase in early distal electrolyte concentration along with a decisive reduction in SNGFR in diabetic rats (657). Tubular control of GFR has also been demonstrated in dogs where acute hyperglycemia caused GFR to increase, but only if TGF was intact (714). Evidence for a primary hyperreabsorption upstream of the MD and a potential role in glomerular hyperfiltration was also proposed in diabetic patients (81, 223, 674), including studies which showed that fractional proximal reabsorption was elevated and positively correlated with GFR (223). Again, based on the principle that GTB cannot explain a positive correlation between GFR and fractional proximal reabsorption, these findings imply a primary increase in proximal reabsorption leading to a TGF-mediated increase in GFR.

Increasing by pharmacological means the cortical interstitial concentrations of adenosine, which acts as the mediator of TGF (615), normalizes GFR in STZ-diabetic rats (654). Excessive hyperglycemia may inhibit proximal reabsorption (697) and, thus, TGF may limit hyperfiltration under these conditions (164). However, moderate hyperglycemia increases proximal reabsorption and, in accordance, glomerular hyperfiltration is blunted in moderately hyperglycemic STZ-diabetic mice that lack TGF (due to gene-targeting of the adenosine A_1 receptor) (653).

Intervening with difluoromethylornithine, an inhibitor of ODC, to reduce early diabetic tubular hypertrophy and hyperreabsorption also diminished glomerular hyperfiltration in direct proportion to the effect on kidney size (617). Along these lines, each maneuver that succeeds at attenuating kidney growth in diabetes (which may occur with normalization of blood glucose levels by insulin treatment (115, 511, 705), manipulations of GH signaling, etc.) is expected to also reduce GFR by preventing hyperreabsorption. Furthermore, in some

diabetic patients, glomerular hyperfiltration persists even after euglycemia is achieved through aggressive insulin therapy. Since established tubular growth reverses slowly, and complete normalization of kidney size may not be achieved in patients even after intensive treatment with insulin supplementation (115, 639, 640, 705), glomerular hyperfiltration may endure in these patients due to persistent tubular enlargement and hyperreabsorption independent of the average blood glucose level. Moreover, patients may be heterogeneous in their response to hyperglycemia with regard to kidney growth, and therefore in the resulting tubular hyperreabsorption and glomerular hyperfiltration.

The primary increase in tubular reabsorption in diabetes, in addition to reducing the TGF signal, can lower the hydrostatic pressure in Bowman space (P_{BOW}) (274, 654, 657, 734). Enhanced reabsorption is expected to reduce P_{BOW} by lowering the flow rate through distal nephron segments where flow resistance is high (362). Furthermore, in early STZ-diabetic rats, tubular growth increases luminal diameter of high flow resistance distal nephron segments (511). Hence, tubular growth might contribute to the decrease in P_{BOW} in diabetes through its effects on tubular reabsorption and tubular diameter. All else remaining equal, this reduction in P_{BOW} could make a small, but significant, contribution to glomerular hyperfiltration by increasing the effective glomerular filtration pressure in diabetes (657). Figure 12 integrates the outlined concept of a primary role of the proximal tubule and tubuloglomerular communication for glomerular hyperfiltration in early diabetes mellitus.

Reduced efficiency of tubuloglomerular communication and potential consequences in diabetes

The incremental slope of the TGF response to any given positive or negative change in MD salt around the operating point is modestly reduced in STZ-diabetes as determined by free-flow perturbation analysis (647) (see Fig. 13). This is associated with ongoing overactivity of NOS I that is not regulated by MD salt (618). A change in TGF efficiency is irrelevant to an undisturbed nephron in steady state in which SNGFR and P_{GC} would remain constant. However, a consequence is that TGF buffers any given disturbance less efficiently in the diabetic kidney, and a greater variability in glomerular capillary pressure will result, which could contribute to the development of diabetic glomerular sclerosis by exposing the glomerular mesangium and podocytes to greater fluctuating physical stress (14, 335, 524). In addition to TGF, glomerular capillary pressure is stabilized by a myogenic mechanism when blood pressure fluctuates or increases. Whereas micropuncture is restricted to superficial nephrons, diabetes has been found to impair renal autoregulation also at the whole kidney level (225, 226, 259), although improved autoregulation at low perfusion pressures has also been reported (348, 393). Bell et al. measured mean arterial pressure and renal blood flow continuously (18 h/day) in uninephrectomized control and STZ-diabetic rats. Transfer function analysis of blood pressure and renal blood flow power spectra revealed rapid and sustained increases in gain over the TGF and myogenic frequency ranges, suggesting that renal autoregulation is impaired at the very onset of hyperglycemia (48). Using a similar approach in the ZSF1 (fa/fa cp) model of type 2 diabetes, Griffin et al. could not detect a significant difference in renal transmission of blood pressure (210) further indicating that the finding of impaired autoregulation at the whole kidney level is not uniform in diabetes.

The salt paradox in the diabetic kidney

In addition to causing a high baseline GFR, diabetes also alters the renal hemodynamic response to a variety of stimuli. For example, acute insulin application, which increases GFR in the nondiabetic rat, lowers RPF and GFR in STZ-diabetic rats while being glucose clamped at euglycemic levels (636). Changes in dietary salt constitute another such stimulus, the renal response to which is abnormal in diabetes. In 1995, we reported studies in which we placed male STZ-diabetic rats on a low salt diet for 7 to 8 days after 6 weeks of diabetes. Surprisingly, the maneuver increased renal blood flow, GFR and kidney weight in diabetic rats whereas low NaCl diet did not significantly alter these parameters in nondiabetic controls (664). This observation was extended by demonstrating renal vasoconstriction in response to a high NaCl diet among female rats with early (one week) or established (4–5 weeks) STZ-diabetes (652). Since the negative impact of dietary NaCl on GFR in diabetes is counterintuitive with regard to salt balance, we refer to it as the *salt paradox*. We have subsequently confirmed the paradoxical effect of dietary salt by micropuncture on the level of the single nephron in male (651) and female (unpublished observation) rats. More recently, the salt paradox was confirmed in STZ-diabetic mice (659) and STZ-diabetic Long-Evans rats (348).

Most importantly, in 1997 Miller reported the same phenomenon in young patients with T1DM: restriction of dietary sodium to 20 mmol/day was found to induce a fall in renal vascular resistance, accompanied by a rise in effective RPF as well as GFR, in spite of a significant rise in plasma renin activity (406). In 2002 Luik et al. similarly showed that short-term moderate sodium restriction induces relative hyperfiltration in uncomplicated T1DM (377). Almost all evidence currently available on kidney function in early diabetes has been derived from patients and experimental models with T1DM. Fewer data have been acquired on the early renal pathophysiology in T2DM. With regard to the influence of NaCl diet and consistent with the salt paradox, one study reported that T2DM with hypertension is associated with reduced RPF when dietary NaCl intake is high for 5 to 7 days (133). Another study, however, found no significant effect on RPF or GFR by variation in NaCl intake for 9 to 14 days in this kind of patients (97). Future studies are needed to further elucidate this issue and to answer the question whether the salt paradox is present in patients with T2DM in the early phase of the disease and in the absence of confounding complications.

The salt paradox in the diabetic kidney is due to hypersensitivity of proximal reabsorption to dietary salt and linked to tubular growth—In normal subjects, GFR is either insensitive to dietary NaCl or changes in the same direction as dietary NaCl (616, 619, 651). The negative impact of dietary NaCl on GFR in diabetes is counterintuitive, but has been observed in diabetic human and animal subjects, and may reveal something basic about the main controller of renal function in diabetes. The paradoxical effect of dietary NaCl on the diabetic kidney cannot be explained by primary vascular effects of the neurohumoral or pressure natriuresis system (648) (see Fig. 14). We propose that the salt paradox is another manifestation of the tubulocentric nature of glomerular function in diabetes.

Renin Release and the Intrarenal Renin-Angiotensin System in the Diabetic Kidney

The renin-angiotensin system (RAS) is of primary importance for the pathophysiology of the diabetic kidney (214). Human trials show that RAS inhibition significantly slows the progression of renal damage in patients with T1DM and T2DM (78, 359, 360), including early stages of the disease (479). The mechanisms involved in renal protection in diabetes by RAS inhibition are less clear but may include effects on blood pressure and glomerular hemodynamics (214). However, clear evidence for systemic activation of the RAS is difficult to detect in diabetic patients, and plasma renin activity is often normal or even suppressed under regular diet (406, 505, 632, 634). Likewise, supine serum Ang II is suppressed in patients with T1DM (166). It has been proposed that this may be misleading and perhaps reflecting an activated intrarenal RAS system (505). Whereas plasma renin concentration and serum ACE activity were normal in modestly hyperglycemic STZ-diabetic rats, intrarenal renin protein content and renin and angiotensinogen mRNAs were increased, consistent with disproportionate activation of the intrarenal RAS (24). Acute increases in RPF and GFR in response to ACE inhibition in patients with T1DM are consistent with intrarenal activation of the RAS (241). Immunohistochemical studies indicated a glomerular redistribution of ACE activity in the diabetic kidney inasmuch as proximal tubule ACE activity was reduced and ACE immunostaining intensity enhanced in glomeruli and renal vasculature of diabetic rats (24).

Whereas ACE converts Ang I to Ang II, ACE2 is a homologue of ACE that both degrades the vasoconstrictor Ang II and generates the vasodilator peptide Ang 1–7, thereby counterbalancing effects of ACE. ACE2 is highly expressed in the kidney, particularly in the proximal tubules. STZ-diabetes reduced ACE2 protein expression in the kidney, an effect that was prevented by ACE inhibitor therapy (621). Moreover, immunostaining revealed that in the diabetic kidney, ACE2 protein was localized predominantly to renal tubules. Also in mice, induction of STZ-diabetes was associated with a reduction in renal ACE2 expression and Ang 1–7 was decreased (620). The ACE2 inhibitor, MLN-4760, and gene-knockout of ACE2 enhanced diabetes-associated albuminuria and increased blood pressure. However, renal hypertrophy and fibrogenesis were reduced in diabetic mice with ACE2 deficiency, and hyperfiltration was attenuated (620). These data indicate a complex role of ACE2 in the diabetic kidney. In Finnish patients with T1DM, polymorphisms were not associated with diabetic nephropathy or any studied risk factor for this complication (188).

As mentioned before, high glucose can induce podocyte hypertrophy, which can be ameliorated by Ang II AT1 receptor blockade, suggesting the involvement of RAS activation (365). Moreover, podocytes express RAGE and studies in cultured podocytes, which express Ang II AT1 and AT2 receptors, surprisingly, revealed that Ang II induced RAGE mRNA and protein expression through activation of the Ang II AT2 receptor (536). This involves a NF-kappaB binding site on the RAGE promoter region and has functional consequences like AGE-induced increases in the expression of TNF- α , but did not affect podocyte proliferation or apoptosis. Further studies are needed to determine the relevance and consequences for the diabetic kidney *in vivo*.

More recent studies provided strong evidence for a role of the metabolite, succinate, and its receptor in the juxtaglomerular apparatus for an upregulation of intrarenal renin in diabetes and also pointed to a specific role of prorenin in the diabetic kidney, as discussed in the following and illustrated in Figure 15.

A role for succinate and the succinate receptor 1 (SUCNR1) in the regulation of renin in diabetes

Baumbach et al. had shown in 1976 that succinate increases renin release from viable juxtaglomerular cells during superfusion of isolated rat glomeruli (43). More recently, the G protein-coupled metabolic receptor SUCNR1 (previously GPR91) has been identified as a succinate receptor that is highly expressed in the kidney, where it can contribute to renin-dependent activation of the RAS and increases in blood pressure (227). SUCNR1 has been located in the luminal membrane of MD cells of the juxtaglomerular apparatus in close proximity to renin-producing granular cells, as well as in cortical TAL, and cortical and inner medullary collecting duct cells (528). Toma et al. proposed that high levels of glucose enhance the local accumulation of succinate in the juxtaglomerular apparatus and that activation of SUCNR1 in the glomerular endothelium triggers the release of renin (627). In addition, renin may be stimulated in the diabetic kidney by luminal succinate that activates the SUCNR1 in the luminal membrane of MD cells (668).

In patients with T2DM plasma, renin activity can be less suppressible in response to high NaCl intake (504). Further studies are needed to elucidate whether this may be due to tonic activation of the succinate system. Since an increase in systemic renin activity is not a general phenomenon in diabetes, it may be that the succinate system contributes to the activation of a local intraglomerular RAS. Vargas and colleagues showed that STZ-diabetes caused SUCNR1-dependent upregulation of renal renin content (668); the signal transduction pathway activated by succinate included the production of NO and PGE2. Further studies are needed to test whether the succinate-induced NO and PGE2 contribute to the development of diabetic glomerular hyperfiltration. This could constitute an extension of the tubular hypothesis (see above) with luminal succinate delivered from upstream tubular segments affecting glomerular function via SUCNR1 in the MD. Inhibition of SUCNR1 and a better understanding of the determinants of succinate accumulation in tubular fluid and the juxtaglomerular apparatus and its role in the long-term outcome of the diabetic kidney may provide further therapeutic approaches.

Activation of the intraglomerular renin/prorenin system in diabetes

Renin was long suspected to have cellular receptors and in 2002 Nguyen and colleagues reported the cloning of the human renin receptor, a 350-amino acid protein with a single transmembrane domain (435). The receptor specifically binds renin and prorenin. Binding of renin increases the catalytic efficiency of angiotensinogen conversion to Ang I and induces an intracellular signal with phosphorylation of serine and tyrosine residues associated to an activation of MAP kinases ERK1/2. Within the kidney the receptor is localized in the mesangium of glomeruli and in the subendothelium of kidney arteries, associated to smooth muscle cells and co-localized with renin (435). These results are consistent with a prominent intraglomerular RAS and propose renin effects independent of Ang II. In accordance, renin

may exert receptor-mediated, Ang II-independent, tissue-damaging effects by increasing the expression of profibrotic pathways and molecules such as TGF- β 1 (251, 434).

Studies in cultured immortalized mouse podocytes revealed that exposure to high glucose induced a 2-fold increase in Ang II levels mediated through increased renin activity and also increased Ang II AT1 receptor levels (151). The same study proposed a glomerular upregulation of the prorenin receptor in a podocyte distribution early in the course of STZ diabetes in rats. The prorenin-to-active renin ratio significantly increases in the diabetic kidney. Ichihara et al. reported that when a site-specific binding protein interacts with the “handle” region of the prorenin prosegment, the prorenin molecule undergoes a conformational change to its enzymatically active state. Moreover, they provided evidence that the nonproteolytic activation of prorenin may be a significant mechanism in the STZ-diabetic kidney (252). Subsequent studies provided evidence that prorenin binding to its receptor in the diabetic kidney induces kidney injury without its conventional proteolytic activation. Additional studies, using Ang II AT1a receptor gene-deficient mice and (pro)renin receptor blockade with a short peptide of prorenin, indicated that not only Ang II but also intraglomerular activation of MAPK by the receptor-associated prorenin plays a pivotal role in diabetes-induced kidney injury (254). In additional studies, heminephrectomized STZ-diabetic rats with an increased urinary protein excretion and a significant glomerulosclerosis were treated for 12 weeks with a (pro)renin receptor blocker (PRRB), an ACE inhibitor, or vehicle peptide. Whereas treatment with PRRB prevented any further increase in urinary protein excretion or glomerulosclerosis, treatment with an ACE inhibitor only attenuated the further increases (607). The data indicate a prominent role of a local intraglomerular renin system in the diabetic kidney and a potential therapeutic target (253).

The outlined studies in rats and mice were performed in the absence of any insulin treatment and under conditions of severe hyperglycemia (~400–550 mg/dl). Notably, whole kidney Ang II content is lower in STZ-diabetic rats compared with nondiabetic controls when diabetic rats were daily treated with insulin to adjust blood glucose levels around 350 mg/dl (664). In contrast, in the absence of insulin treatment and blood glucose levels of about 500 mg/dl, kidney content of both Ang I and II were reported to be increased in STZ-diabetic rats (252), indicating a potential influence of insulin treatment and/or blood glucose control. Further studies are needed to elucidate the influence of the degree of hyperglycemia and of insulin and other antidiabetic treatments on the activation of the intrarenal prorenin/renin system and, most importantly, its role in long-term diabetic renal failure.

Animal Models of Diabetic Nephropathy

Reliable animal models that mimic the human diabetic kidney disease are very important to better understand the molecular pathways involved and to identify key targets for better prevention and therapy. Diabetic animal models have mainly been developed in the rat and mouse and a brief overview on current models, problems and perspectives is presented. See also (19, 79, 84, 576, 672) for recent reviews on the topic.

Rat models of diabetic nephropathy

Models of nephropathy in type 1 diabetes mellitus—The streptozotocin (STZ)-diabetic rat is the most frequently used model of T1DM. Administration of STZ, a beta cell toxin, leads to beta cell destruction and insulinopenic diabetes within 24 to 36 h. Residual insulin secretion may be detectable in plasma for several weeks. Spontaneous recovery after several weeks of hyperglycemia is also possible. Without insulin treatment, STZ-diabetic rats develop severe hyperglycemia, dehydration, salt wasting, slow weight gain, and corresponding polydipsia and hyperphagia. To improve long-term survival and to create a more clinically relevant model of T1DM and its complications, studies are frequently conducted with STZ-diabetic rats treated with suboptimal doses of insulin to achieve moderate hyperglycemia (~20–25 mmol/l).

STZ-diabetic rats develop early renal and glomerular hypertrophy, initially without any structural changes. Within weeks, kidneys from STZ-diabetic rats display molecular markers of nephropathy including prosclerotic cytokines, growth factors, and components of ECM and a gradual increase in albuminuria as compared to age-matched nondiabetic controls. Although some degree of mesangial expansion or diffuse glomerulosclerosis could be detected earlier, measurable glomerulosclerosis, and in particular tubulointerstitial fibrosis, develop 6 to 12 months after induction of diabetes. Electron microscopy may reveal foot process effacement.

There are differences in rat strains in propensity to diabetes-induced renal pathology. The Sprague-Dawley strain is in our experience more susceptible than Wistar rats. Moreover, male rats are more prone to the development of albuminuria and structural changes than females. Without additional interventions STZ-diabetic rats do not develop decreases in GFR.

The relatively long period of time required for the development of renal structural changes and the lack of progressive decrease in GFR in STZ-diabetic rats motivated additional approaches to accelerate the course of nephropathy. These approaches include induction of STZ-diabetes in rat models of hypertension both spontaneous, such as in spontaneously hypertensive rats (121), and acquired, such as in DOCA-salt hypertensive rats (107). An aggressively progressive course of nephropathy with the development of severe hypertension, albuminuria, progressive decrease in GFR, and glomerular and interstitial lesions is characteristic for STZ-diabetic mRen-2 transgenic rats (307), harboring mouse renin gene. However, this model has been recently increasingly criticized. Massive activation of the RAS and hypertension lead to end-organ damage without concomitant diabetes, and, as demonstrated by Hartner et al. (224) these factors are the major driving force of nephropathy in this model.

The accelerated course of nephropathy can be also achieved by combining STZ-induced diabetes with unilateral nephrectomy, usually performed prior to induction of diabetes. These rats develop modest increases in blood pressure, massive proteinuria, reductions in GFR, and significantly more severe glomerulosclerosis and interstitial fibrosis as compared to uninephrectomized nondiabetic counterparts within 16 to 20 weeks after onset of diabetes (322, 323).

Rat models of nephropathy in type 2 diabetes mellitus—T2DM is part of the “metabolic syndrome”, which is, in addition to T2DM, defined by insulin resistance, truncal obesity, lipid abnormalities, and hypertension. The patients with metabolic syndrome also develop hyperuricemia, hypercoagulation state, and microalbuminuria. Therefore, the models of T2DM and associated vascular complications should be viewed from this broader perspective. Indeed, some of these models display clinical course resembling the evolution of metabolic syndrome and T2DM in humans. Moreover, these animals demonstrate a variety of renal abnormalities prior to the development of hyperglycemia (55, 327, 379), but in parallel with rising plasma insulin, FFA and triglyceride levels.

The best studied model of metabolic syndrome and T2DM with kidney involvement is the Zucker obese (fatty, fa/fa) rat. These rats are metabolically well characterized—an autosomal recessive mutation of the *fagene*, encoding the leptin receptor, results in hyperphagia, obesity, and hyperlipidemia (497). Similar to patients with T2DM, ZDF rats display insulin resistance, hyperinsulinemia, and impaired glucose tolerance, and slowly progressive increases in blood glucose levels (257). During the first 3 to 4 months of age, these rats do not display frank hyperglycemia, and are therefore suitable for investigations of pathophysiological roles of other factors associated with metabolic syndrome insulin resistance and T2DM, for example, increased levels of FFA, hyperinsulinemia, and adipokines. Zucker lean rats without leptin receptor abnormality do not display any abnormalities and serve as controls.

Extensive studies by Kasiske et al. (298) and Coimbra et al. (120) have shown that the first detectable changes in glomerular morphology suggestive of glomerulosclerosis and significant proteinuria occur at about 4 to 5 months of age, whereas tubulointerstitial damage after at least 40 weeks. More subtle changes characterized by glomerular hypertrophy and mesangial matrix expansion and albuminuria occur by 10 to 14 weeks of age. Mild elevations of blood pressure have been also reported (298). Similar to a number of other models of kidney disease, the kidney involvement is more severe in males than in females (146).

Otsuka Long-Evans Tokushima Fatty (OLETF) Strain and Goto Kakizaki (GK) rats are additional models utilized in studies exploring the pathophysiology and management of nephropathy in metabolic syndrome and T2DM. The OLETF Strain, a spontaneously diabetic rat with polyuria, polydipsia, and mild obesity, was discovered in 1984 in an outbred colony of Long-Evans rats. The characteristic features of OLETF rats include late onset of hyperglycemia (after 18 weeks of age), mild obesity, inheritance by males, hyperplastic foci of pancreatic islets; and renal complications including nodular lesions. After 40 weeks of age, the beta cell function is exhausted leading to low plasma insulin levels with gradual development of polyphagia, polyuria, decrease of body weight, and hyperglycemia, that is, symptoms of insulinopenic diabetes. Since this phenomenon occurs in a proportion of patients with T2DM, OLETF rats might be a valuable model for studies focusing on the impact of this transition on progression of microvascular complications. Interestingly, mild glomerular involvement, such as mesangial proliferation and thickening of the basement membranes were observed from week 40, associated with the appearance of glycosuria. The nodular lesions, have been described in 70-week old animals (304).

The GK rat, which was developed by Goto and collaborators (206) is characterized by glucose intolerance, which appears after 2 weeks of age in all animals. The etiology of diabetes in GK rats is thought to be exclusively genetic, because they were obtained by repetitive selective breeding of Wistar rats with abnormal glucose tolerance and became stably diabetic after several generations (206).

Unlike Zucker obese rats, GK rat do not display most of features of metabolic syndrome, such as hyperlipidemia, obesity, and remain normotensive until advanced age. Phillips et al. (491) described the course of renal changes in this model. They showed that GK rats did not develop significant proteinuria, but displayed age-dependent renal structural changes. These changes consisted of thickening of glomerular and tubular basement membranes at week 35. This thickening was accompanied by marked glomerular hypertrophy, although fractional capillary and mesangial volumes remained unchanged. There was evidence of podocyte injury, assessed by *de novo* expression of desmin. Interstitial monocyte/macrophage influx increased significantly in GK rats at 12 weeks compared with Wistar controls. Glomerular macrophage infiltration was elevated significantly in 35-week old GK rats. The authors concluded that structural changes in the GK rats resembled those described in prolonged non-insulin-dependent diabetes mellitus patients without overt renal disease. The model might be suitable to study the effects of hyperglycemia in T2DM on the development of organ complications without other confounding factors such as lipid abnormalities or obesity.

Zucker obese rats can be also crossed with nondiabetic rat models of hypertension to generate models with an accelerated course of nephropathy. For example, hybrid rats derived from the Zucker obese rats and the spontaneous hypertensive heart failure rat (SHHF/Gmifa) have been shown to develop severe renal injury and renal failure (146). A similar approach has been used to create spontaneously hypertensive/NIH-corpulent (SHR/NDmcp (fat/fat)) (405, 427).

Mouse models of diabetic nephropathy and the Animal Models of Diabetic Complications Consortium (AMDCC)

In 2001, the AMDCC (<http://www.amdcc.org>) was initiated by the National Institutes of Health to develop and characterize mouse models that mimic important aspects of human diabetic disease. The availability of >450 inbred strains (46, 389), the ongoing assembly of mice that bear multiple mutations that alter function in each known gene (246, 353, 629), and the application of the available unique strategies for genetic manipulations to such a mouse disease model may facilitate the understanding of the underlying genetic mechanisms that contribute to the development of diabetic nephropathy in human (79). However, no current murine strain of T1DM or T2DM reliably develops all of the features of human diabetic nephropathy (84). The FVB-OVE26 mouse (JAX # 5564) is a transgenic model of early-onset T1DM, generated directly onto the FVB/N background in the Epstein laboratory (746), which has been described to come relatively close. These mice showed progressively enlarged glomeruli and expanded mesangium with diffuse and nodular expansion of mesangial matrix and thickening of the glomerular basement membrane. Moreover, tubulointerstitial fibrosis, and some decrease in GFR in the later stages have been observed

in these mice (746). More extensive studies are necessary to establish the value of this mouse model.

The AMDCC recommends the following criteria for validating a progressive mouse model of diabetic nephropathy: greater than 50% decline in GFR over the lifetime of the animal; greater than 10-fold increase in albuminuria compared with controls for that strain at the same age and gender; kidney pathology including advanced mesangial matrix expansion $\pm$ nodular sclerosis and mesangiolysis, any degree of arteriolar hyalinosis, glomerular basement membrane thickening by $>50\%$ over baseline, and tubulointerstitial fibrosis (84). The explicit focus on renal insufficiency is important, yet no current murine strain of T1DM or T2DM progresses to renal failure. An additional useful source for comparing basal metabolic parameters in the more commonly used inbred strains, including urinary albumin/creatinine ratios, is The Mouse Phenome Database (<http://phenome.jax.org/pub-cgi/phenome/mpdcgi?rtn=docs/home>).

Very helpful are the strategies and insights provided by the AMDCC on phenotyping standards (e.g., analysis of hyperglycemia, HgbA1C, albuminuria, creatinine, and GFR; staining methods for histology), useful T1DM and T2DM mouse models (including use of low-dose STZ protocol), validation criteria, the role of animal age, and the importance of genetic background (79). With regard to the latter, it appears that strains like BKS, 129S6/SvEvTac, KK/HIJ and, in particular, the DBA/2 mouse are more susceptible to diabetic glomerulopathy than the B6 strain, which seems to be the most resistant. However, to which extend these differences are associated with or predictive of renal insufficiency (i.e., a reduction in GFR), is unclear. Considering the pathophysiological importance of podocyte loss and that tubulointerstitial fibrosis determines to a major degree the outcome of the diabetic kidney, these aspects may deserve stronger attention in the model criteria. Furthermore, it seems important to also characterize these experimental models for important early aspects of the diabetic kidney that have been associated with later injury (98) including tubular growth.

The recent AMDCC review (84) concluded that targeting of *Nos3*, the gene encoding eNOS induces nephropathic changes in mouse models of both T1DM and T2DM that mimic many aspects of human disease (292, 416, 422, 745). Notably, this model is one of the very few to develop appreciable degrees of tubulointerstitial fibrosis and available on different backgrounds from The Jackson Laboratory [B6 (JAX# 2684), BKS-db/db (JAX# 8340), and BALB/cBy (JAX# 7073)]. Likewise important are new information on negative diabetic models that unexpectedly did not show disease progression (84). Details on these models including possible explanations are outlined on the AMDCC web site. Examples include diabetes induction in C57B6 mice with *ACE2* gene deficiency, B6-*Sod2* heterozygous mice, and endothelial-specific receptors of advances glycation end-product monotransgenic mice on a mixed B6 and CD-1 background.

Considering the relative resistance of the mouse to diabetic nephropathy, important clues may be derived by comparing gene expression profiles in diabetic human and mice. In this regard, Berthier et al. analyzed the expression of genes in glomeruli and tubulointerstitium in kidney biopsies from diabetic nephropathy patients to identify pathways that may be

activated in humans but not in murine models that are resistant to diabetic nephropathy and kidney failure, using a mRNA microarray approach, which was followed up by real-time RT-PCR and immunohistological staining. db/db C57BLKS and STZ-induced diabetic DBA/2J mice were used as commonly studied murine models of diabetic nephropathy. The study found a direct relationship between tubulointerstitial expression of the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway and progression of kidney failure in patients with T2DM and nephropathy. In contrast, there was little or no increase in expression of these pathways in the nonprogressive murine diabetic mouse models (53).

Conclusion

Much has been learned about the (patho)physiology of high glucose concentrations in the context of a single cell as well as the intact kidney. This includes mechanisms of AGEs formation and oxidative stress and their central role in the diabetic kidney. A better understanding of the molecular determinants and the complex interplay between the diabetic milieu and the structures involved in the GFB, including the podocytes, is emerging. A role for primary vascular as well as tubular events in early diabetic glomerular hyperfiltration has been established, the latter being related to tubular growth and hyperreabsorption and the physiology of tubuloglomerular communication. We are beginning to understand the molecular basis of the complex interactions between the diabetic milieu and the tubulointerstitium as well as of the unique growth phenotype of the diabetic kidney, and their potentially critical role in linking early changes of the diabetic kidney to tubulointerstitial fibrosis, hypoxia, and renal failure. A better understanding of a proposed senescent phenotype of diabetic tubular cells is necessary as well as the molecular signature and the relevance of epithelial mesenchymal transition in the diabetic kidney. More insight in the activation of the intraglomerular renin/prorenin system, including the role of succinate and its receptor, and of their relevance to the long-term kidney outcome is highly desirable. Further insights are also expected from the rapidly expanding field of epigenetics and transcriptional control, which may identify and explain the phenomenon of “metabolic memory” in the pathogenesis and perpetuation of microvascular complications of diabetes including nephropathy. The identification and better understanding of the molecular pathophysiology of key mechanisms may help one day to predict which diabetic patient will eventually develop diabetic nephropathy and renal failure, and to develop new therapeutic strategies that target these mechanisms. Our progress in this regard will also depend on the further development of reliable animal models that mimic the phenotypic and molecular signature of human diabetic kidney disease. To identify and further explore these models, experimental studies are needed that follow up into the later stages of diabetic nephropathy including the decline in GFR and renal failure.

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Figure 1.

Simplified biochemistry of advanced glycation end-product formation. Prolonged hyperglycemia, but also dyslipidemia and oxidative stress in diabetes result in the production and accumulation of advanced glycation end products (AGEs) in the kidney, and at other sites of diabetic complications. AGEs are formed by nonenzymatic Maillard or “browning” reaction between carbonyl groups of reducing sugars, like glucose, and amino groups on proteins, lipids, or nucleic acids. The first stable adduct between glucose and protein is the Amadori product. In addition, the glycation of intracellular proteins is initiated by the elevation of intracellular glucose degradation products or decomposition of early glycation products leading to formation of intermediates such as glyoxal or methylglyoxal. Subsequent rearrangement and fragmentation reactions lead to the formation of AGEs. Under physiological conditions these reactions are slow. However, in diabetes, persistent hyperglycemia, dyslipidemia, and oxidative stress all act to hasten the formation of AGEs.

Figure 2.

Consequences of accelerated formation of advanced glycation end products (AGEs) in the diabetic kidney. The schematic shows major mechanisms whereby AGEs exert their harmful effects in the diabetic kidney. AGEs typically crosslink and alter protein structure and function in extracellular compartment, modify cytosolic molecules and exert receptor-mediated effects that lead to activation of multiple signaling pathways and genes implicated in a variety of pathophysiological mechanisms in the diabetic kidney. RAGE, receptor for AGEs; ROS, reactive oxygen species; MAPKs, mitogen-activated protein kinases; ROCKs, Rho kinases; NO, nitric oxide; BM, basement membrane; ECM, extracellular matrix; NF- κ B, nuclear factor kappa B.

Figure 3.

Activation and downstream targets of the hexosamine pathway in diabetes. When the intracellular concentrations of glucose are high, fructose-6-phosphate, an intermediate in the glycolytic pathway, is partially diverted into the hexosamine pathway. After a series of enzymatic steps glucosamine (GlcNAc) is transferred in *O*-linkage to specific serine/threonine residues of numerous proteins, including transcription factors as well as cytosolic and nuclear enzymes, with patho-physiological consequences in affected cells. Key enzymes in the pathway are highlighted in yellow. More detailed description is provided in the text. G-6-P, fructose-6-phosphate; F-6-P, fructose-6-phosphate; GFAT, glutamine:fructose 6-phosphate amidotransferase; GlcN-6-P, glucosamine 6-phosphate; UDP-GlcNAc, UDP-N-

acetylglucosamine; OGT, O-GlcNAc transferase; eNOS, endothelial NO synthase; mTOR, mammalian target of rapamycin; TGF- β , transforming growth factor- β ; PAI-I, plasminogen activator inhibitor-I.

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Figure 4.

Mechanisms of enhanced formation of reactive oxygen species (ROS) in renal cells in diabetes. High intracellular glucose, stimulation of RAGE, as well as increased activity of vasoactive and pro-growth factors, such as the renin-angiotensin system, lead to formation of cytosolic and mitochondrial ROS via multiple mechanisms. ROS alter protein, lipid and DNA structure, and act as signaling molecules in pathways implicated in the pathophysiology of diabetic complications. See text for further details. Ang II, angiotensin II; AT1R, angiotensin II type 1 receptor; AGEs, advanced glycation end products; RAGE, receptor for AGEs.

Figure 5.

Diabetes-induced alterations of glomerular filtration barrier. The schematic presentation describes major consequences of diabetes-induced changes in glomerular endothelial cells, glomerular basement membrane, and podocytes, as discussed in more detail in the text.

Figure 6.

Podocyte foot process effacement and glomerular basement membrane (GBM) thickening in experimental diabetes. Transmission electron microscopy was used to investigate the glomerular filtration barrier (GFB) in normal (C) and STZ-diabetic DBA/2J mice (V, L, DL, L + DL, and L + DH). Compare the appearance of the GFB in normal (C) and diabetic mice (V), and the effects of nephroprotective treatments [L, DL, L + DL, and L + DH; for details on treatments see (741)]. Stars indicate the areas of podocyte foot process effacement; arrow pairs point to both sides of the glomerular basement membrane (GBM). Adapted with permission from (741).

Figure 7.

Regulation of tubular growth in the diabetic kidney. Illustrated is a conceptional frame work that links hyperglycemia to tubular growth including an early phase of hyperplasia that is followed by G1 cell-cycle arrest and development of hypertrophy and a senescence-like phenotype. Potential links to enhanced formation of extracellular matrix, inflammation, and tubulointerstitial injury are shown. See text for further explanations. ECM, extracellular matrix; EMT, epithelial mesenchymal transition; TSC, tuberous sclerosis complex.

Figure 8.

Hyperglycemia induces glycogen accumulation in thick ascending limb and further distal cortical nephron segments. Glycogen accumulation, also known as glycogen nephrosis or Armani-Ebstein lesions, may relate to increased delivery of glucose via the tubular fluid or peritubular capillaries. Glycogen accumulation may also reflect activation of glycogen synthase, due to accumulation of the allosteric activator, glucose-6-phosphate. Adiponectin is secreted from white adipocytes and signals through its receptor, ADIPOR1, to activate AMP-activated protein kinase (AMPK) thereby inhibiting glycogen synthase. Thus, glycogen synthase can be activated as a consequence of reduced adiponectin levels or adiponectin resistance with impaired activation of AMPK due to activation of phosphatase PP2A. See text for further details.

Figure 9. Mechanisms of tubulointerstitial injury in the diabetic kidney. Illustrated is the interplay of hyperglycemia, luminal factors (derived from glomerular filtration and tubular release), reabsorption, and blood flow in the interaction of tubular cells with fibroblasts and inflammatory cells. TGF- β , chemokines, and the complex interactions between advanced glycation end products (AGE), hypoxia and oxidative stress play key roles in the development of diabetic tubulointerstitial injury. ECM, extracellular matrix. See text for further details.

Figure 10.

Renal hemodynamic changes in diabetes at the whole kidney and single nephron level. Early stages of diabetes are associated with specific renal hemodynamic changes schematically presented in this figure. The schematic shows regulators of afferent and efferent arteriolar tone, which have been implicated in these changes. GFR, glomerular filtration rate; RPF, renal plasma flow; SNGFR, single nephron glomerular filtration rate; Q_A , glomerular plasma flow rate; R_A , afferent arteriolar resistance; R_E , efferent arteriolar resistance; P_{GC} , glomerular capillary pressure; K_f , ultrafiltration coefficient.

Figure 11.

Primary increase in proximal tubular reabsorption and inhibition by high NaCl diet in the early diabetic kidney. Absolute proximal fluid reabsorption (J_{prox}) shown as a function of SNGFR. SNGFR was manipulated by perfusing Henle's loop to activate TGF to characterize proximal reabsorption as a function of SNGFR in control rats (CON) and diabetic rats (STZ) on normal versus high NaCl diet. * $P < 0.05$ for a GFR-independent effect of STZ (left panel) or high NaCl diet (right panel) on proximal tubular reabsorption. Adapted with permission from (651).

Figure 12.

Tubular basis of glomerular hyperfiltration in the early diabetic kidney. Hyperglycemia causes a primary increase in proximal tubular reabsorption through enhanced tubular growth and Na⁺-glucose (Gluc) cotransport (1). The enhanced reabsorption reduces the signal of the tubuloglomerular feedback (TGF) at the macula densa ([Na-Cl-K]_{MD}) (2) and via TGF increases SNGFR (4). Enhanced growth and tubular reabsorption also reduce the hydrostatic pressure in Bowman space (P_{BOW}) (3), which by increasing effective filtration pressure can also increase SNGFR (4). The resulting increase in SNGFR serves to partly restore the fluid and electrolyte load to the distal nephron (5). SNGFR₀ is the input to SNGFR independent of TGF. Adapted with permission from (648).

Figure 13.

Lower efficiency of tubuloglomerular feedback (TGF) in the diabetic kidney. **(A)** *In vivo* free-flow perturbation analysis of tubular flow rate in control and STZ-diabetic rats. The fractional compensation profiles illustrate the ability of the TGF system to stabilize tubular flow in response to a perturbation in late proximal tubular flow (by adding or subtracting tubular fluid). The data show that the efficiency of the TGF system to stabilize tubular flow rate (as an indirect measure of stabilizing SNGFR or P_{GC}) is reduced in diabetic rats with a compensation of small perturbations around the operating point of 40% versus 70% in control rats. **(B)** Data from perturbation analysis of late proximal tubular flow rate were combined with data for fractional proximal reabsorption to synthesize “traditional” TGF functions describing the dependence of SNGFR on late proximal tubular flow rate. The triangles indicate the operating points. The gain of the curve at the operating point is modestly reduced in diabetic rats (red lines). Adapted with permission from (647).

Figure 14.

Tubular basis of the salt paradox in the early diabetic kidney. **(A)** In classical physiology, renal function and total body NaCl are linked by several parallel feedback loops. Paths from dietary NaCl to GFR and renal blood flow (RBF) are highlighted (black arrows). Each highlighted path contains only “+” signs or an even number of “-” signs, indicating a positive influence of dietary NaCl on GFR and RBF. Interfering with these processes can alter the strength of this influence, but cannot make it paradoxical. **(B)** Incorporating tubuloglomerular feedback (TGF) provides a pathway whereby dietary NaCl can inversely impact GFR and RBF via a primary change in proximal (i.e., upstream to macula densa) reabsorption (note the odd number of “-” signs along the dotted path). Thus, GFR and RBF

are subject to competing influences in response to changes in dietary NaCl. The NaCl paradox arises when TGF prevails. Adapted with permission from (648).

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Figure 15.

Diabetes-induced upregulation of the renin-angiotensin-system in glomeruli. Illustrated is a proposed role of succinate acting on its receptor (SUCNR1) in glomerular endothelial cells and macula densa cells in the activation of renin. Renin and prorenin can induce angiotensin II-dependent and independent effects by activation of renin receptors on glomerular structures including mesangial cells and podocytes. See text for further details. ADO, adenosine; Ado A₁, adenosine A1 receptor; Agten, angiotensinogen; RR, renin receptor.