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Renal Function in Diabetic Disease Models: The Tubular System in the Pathophysiology of the Diabetic Kidney

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Abstract

Diabetes mellitus affects the kidney in stages. At the onset of diabetes mellitus, in a subset of diabetic patients the kidneys grow large, and glomerular filtration rate (GFR) becomes supranormal, which are risk factors for developing diabetic nephropathy later in life. This review outlines a pathophysiological concept that focuses on the tubular system to explain these changes. The concept includes the tubular hypothesis of glomerular filtration, which states that early tubular growth and sodium-glucose cotransport enhance proximal tubule reabsorption and make the GFR supranormal through the physiology of tubuloglomerular feedback. The diabetic milieu triggers early tubular cell proliferation, but the induction of TGF- β and cyclin-dependent kinase inhibitors causes a cell cycle arrest and a switch to tubular hypertrophy and a senescence-like phenotype. Although this growth phenotype explains unusual responses like the salt paradox of the early diabetic kidney, the activated molecular pathways may set the stage for tubulointerstitial injury and diabetic nephropathy.

Keywords

tubular transport; glomerular hyperfiltration; tubular growth; sodium-glucose cotransport; diabetic nephropathy

INTRODUCTION

After 10 to 20 years of diabetes mellitus, approximately 20% of patients with either type 1 or type 2 diabetes mellitus (T1DM, T2DM) develop diabetic nephropathy, making diabetes mellitus the leading cause of end-stage renal disease (ESRD). We still do not understand the genetic and environmental factors that determine which patients eventually develop diabetic nephropathy. Thus, there is a need to better understand the pathophysiology and molecular pathways that lead from the onset of hyperglycemia to renal failure. Changes in the vasculature and the glomerulus, including those to mesangial cells, the filtration barrier, and podocytes, play important roles in the pathophysiology of the diabetic kidney (1–3). In addition, the diabetic milieu has primary effects on the tubular system of the kidney, which are the focus of this review.

Diabetes mellitus affects the kidney in stages. At the onset of T1DM or T2DM, a subset of diabetic patients undergo an increase in glomerular filtration rate (GFR) (1, 2). Although there is residual debate on the subject (4), diabetics with early glomerular hyperfiltration appear to be overrepresented among those who develop diabetic nephropathy later in life (5). The early hemodynamic phenotype is imagined to provoke the subsequent demise of a diabetic kidney through glomerular capillary hypertension, although glomerular capillary hypertension is not required for hyperfiltration (6). Investigators have reported many abnormalities that may cause diabetic hyperfiltration through impaired constriction of the afferent arteriole (1). Several years ago, we began encountering situations in diabetic rats in which the concentration and delivery of Na^+ , Cl^- , and K^+ at the luminal macula densa (MD_{NaClK}) and single-nephron GFR (SNGFR) change in opposite directions. We recognized that isolated defects in vasomotion could cause SNGFR and MD_{NaClK} to change only in the same direction but that reciprocal changes in MD_{NaClK} and SNGFR are the expected result when a primary change in proximal tubule reabsorption affects MD_{NaClK} and subsequently SNGFR by negative feedback through the macula densa through a process known as tubuloglomerular feedback. Hence, we proposed the so-called tubular hypothesis of glomerular filtration as an archetype for the kidney in early diabetes (7, 8). Examples in which this hypothesis applies include diabetic hyperfiltration and the salt paradox, a unique phenomenon of the diabetic kidney that refers to the inverse relationship between changes in dietary NaCl intake and GFR.

Although kidney growth shortly after the onset of diabetes in a subset of diabetic patients has been known for many years and kidney size has been linked to the development of diabetic nephropathy (9–13), little attention has been given to this phenomenon. Tubular growth, however, explains early functional changes in the diabetic kidney, including the primary increase in proximal tubule reabsorption, and is thus relevant for the tubular hypothesis of glomerular filtration. Proximal tubule reabsorption is further enhanced in hyperglycemia due to increased glomerular filtration of glucose, which increases proximal tubule reabsorption of glucose and Na^+ through the Na^+ -glucose cotransporters SGLT2 and SGLT1. The interest in SGLT2 has recently been revived due to the current development of SGLT2 inhibitors as new antidiabetic drugs (14, 15).

The molecular signature of proximal tubule growth in the diabetic kidney is unique and includes elements of cell proliferation, hypertrophy, and cellular senescence. This unique growth pattern explains unusual functional responses observed only in the diabetic kidney, like the salt paradox. Through its effects on GFR and the deleterious consequences of hyperfiltration, the salt paradox may account for the unexpected finding of two recent cohort studies in patients with T1DM and T2DM showing that lower NaCl intake is unexpectedly associated with increased rates of ESRD, cardiovascular death, and all-cause mortality (16, 17). Moreover, the molecular pathways involved in the tubular growth of the diabetic kidney are linked to inflammation and fibrosis and may set the stage for renal damage. Therefore, genetic and/or environmental factors that affect the tubular growth response to the diabetic milieu may determine not only the extent of kidney growth, tubular hyperreabsorption, and glomerular hyperfiltration in early diabetes but also the later progression of renal disease.

This review discusses early changes that occur in the tubular system of the diabetic kidney, illustrates their role in the tubular hypothesis of glomerular filtration, and proposes potential links to the later development of diabetic nephropathy. The interested reader is referred to previous reviews on the tubular hypothesis of glomerular filtration and implications of the salt paradox (7, 8, 18, 19) as well as on the link between early tubular changes and the progression of renal disease in diabetes (19–22); we focus this review on the most recent studies.

GLOMERULAR HYPERFILTRATION IN DIABETES MELLITUS AS A PRIMARY TUBULAR EVENT

We begin with a theoretical framework for describing interactions between the glomerulus and tubule. These interactions include a forward effect of SNGFR on the tubule, known as glomerulotubular balance (GTB) or the load dependency of reabsorption (Figure 1). The other component is the tubuloglomerular feedback system, which senses changes in MD_{NaClK} and induces reciprocal changes in SNGFR to stabilize electrolyte delivery to the distal tubule, where fine adjustments of reabsorption and excretion occur. GFR is determined by a balance of forces between primary vascular and primary tubular events. Isolated vascular or tubular events each filtered by the GTB– tubuloglomerular feedback system lead to changes in both MD_{NaClK} and SNGFR. The vascular event causes SNGFR and MD_{NaClK} to change in the same direction, whereas the tubular event causes SNGFR and MD_{NaClK} to change in opposite directions. The tubular component of an outside disturbance dominates the vascular component whenever SNGFR and MD_{NaClK} change in opposite directions (see Supplemental Figure 1; follow the Supplemental Material link from the Annual Reviews home page at <http://www.annualreviews.org>). Identifying that a tubular event is the dominant cause of a change in SNGFR does not preclude the existence of a simultaneous vascular event.

A Primary Increase in Proximal Tubule Reabsorption in Diabetes Mellitus

Lithium clearance is a useful, albeit imperfect, indicator of proximal reabsorption and NaCl delivery to the macula densa. Lithium clearance data published 20 years ago revealed increased proximal reabsorption in patients with early **T1DM** (23) or **T2DM** (24). The authors did not consider the macula densa mechanism but posited excessive proximal reabsorption as a cause of systemic volume expansion that led to hemodynamic consequences in diabetes (23). However, extracellular fluid expansion is not required for diabetic hyperfiltration (25). Additional studies showed that fractional proximal reabsorption was elevated and positively correlated with **GFR** in patients with T1DM (25, 26), and Hannedouche and colleagues (26) speculated that the ensuing decrease in distal Na^+ delivery could deactivate the tubuloglomerular feedback response and contribute to glomerular hyperfiltration in some diabetics. Likewise, investigators recently found a strong correlation between newly discovered T2DM, glomerular hyperfiltration, and decreased lithium clearance in a large trial involving subjects of African descent (27).

The present concern is to distinguish primary changes in tubular reabsorption from secondary changes in reabsorption that begin as primary vascular events and then impact tubular reabsorption via **GTB**. When GTB operates normally, fractional reabsorption declines as GFR increases. Therefore, when diabetic hyperfiltration is accompanied by increased fractional reabsorption of the proximal tubule or the segments upstream of the macula densa (28–30), this scenario is consistent with primary hyperreabsorption. In addition, we artificially activated tubuloglomerular feedback as a tool to manipulate **SNGFR** in order to compare tubular reabsorption at similar SNGFRs. Data were thus obtained for expressing proximal reabsorption as a function of SNGFR in individual nephrons. This approach confirmed a major primary increase in proximal reabsorption in rats with early **streptozotocin (STZ)** diabetes, a model of T1DM (31, 32).

Early Distal $NaClK$ Delivery Is Reduced in Diabetes Mellitus

For this primary increase in proximal reabsorption to be the dominant cause of glomerular hyperfiltration, the diabetic nephron must operate with MD_{NaClK} below normal. In fact, data on the ionic content and $NaClK$ delivery to the early distal nephron in diabetes show values substantially below normal (28–30, 32). Micropuncture in rats with superficial glomeruli

allows the collection of tubular fluid close to the macula densa. This approach revealed ambient early distal tubule concentrations of Na^+ , Cl^- , and K^+ in nondiabetic rats of 21, 20, and 1.2 mM, respectively; these values (together with their absolute deliveries) were reduced by 20–28% in hyperfiltering STZ-diabetic rats (Figure 1) (30).

Resetting of the Tubuloglomerular Feedback Curve in Diabetes Mellitus

SNGFR collected from the proximal tubule also increases in diabetes. Because tubuloglomerular feedback is inoperative during proximal tubule fluid collections, the tubuloglomerular feedback curve must shift upward in diabetes. The curve can be influenced by events outside of the juxtaglomerular apparatus, and the upward shift in the tubuloglomerular feedback curve in diabetes may represent a primary vascular event mediated by any number of the factors affecting the afferent arteriole. Nonetheless, reduced MD_{NaClK} invokes the tubule as the dominant controller of SNGFR, with a primary vascular effect as runner up. Furthermore, the upward shift of the tubuloglomerular feedback curve may be explained by tubuloglomerular feedback resetting from within the juxtaglomerular apparatus. The juxtaglomerular apparatus of each nephron can adjust its own tubuloglomerular feedback response and tends to invoke this capacity to align the steep portion of its tubuloglomerular feedback curve with the ambient tubular flow (33). In accordance and as illustrated in Figure 2, the entire tubuloglomerular feedback curve in diabetes resets leftward and upward, and the greatest tubuloglomerular feedback efficiency resides close to the ambient operating point (29).

Manipulating the Tubuloglomerular Feedback Response Affects Diabetic Hyperfiltration

The tubular hypothesis predicts that diabetic hyperfiltration will be attenuated or blunted in a tubuloglomerular feedback--less mouse. Adenosine mediates the tubuloglomerular feedback response by activation of adenosine A_1 receptors (A_1R), and mice lacking these receptors ($\text{A}_1\text{R}^{-/-}$ mice) have no acute tubuloglomerular feedback response. Two recent studies reported glomerular hyperfiltration in diabetic $\text{A}_1\text{R}^{-/-}$ mice (34, 35). As discussed above, the tubular hypothesis invokes feedback from the tubule as the dominant controller of GFR in early diabetes but does not require tubuloglomerular feedback to be the only controller. The theory allows for additional primary defects in afferent arteriolar vasoconstriction and predicts such defects will be unmasked when feedback from the tubule is eliminated. Under these conditions, some degree of hyperfiltration would persist in the absence of A_1R . Moreover, in one of the two studies the nondiabetic but not the alloxan-diabetic $\text{A}_1\text{R}^{-/-}$ mice were hypotensive compared with wild-type controls during measurements of GFR (34). As a consequence, the higher GFR in normotensive alloxan-diabetic $\text{A}_1\text{R}^{-/-}$ mice may reflect impaired renal autoregulation, a known trait of the tubuloglomerular feedback--less mouse. The other study used severely hyperglycemic Akita-diabetic $\text{A}_1\text{R}^{-/-}$ mice, which have blood glucose levels of 600 to 900 mg dl^{-1} (35). At those levels, which far exceed the glucose transport maximum, glucose becomes a net proximal diuretic (36), and the primary increase in proximal reabsorption, which characterizes diabetic hyperfiltration by the tubular hypothesis with modest hyperglycemia (30, 31), disappears. As a consequence, tubuloglomerular feedback activation may limit glomerular hyperfiltration during severe hyperglycemia. Furthermore, the severity of diabetes may affect the tubuloglomerular feedback--independent influence of adenosine on GFR and thereby the net response to A_1R blockade or knockout. In accordance with this discussion and the tubular hypothesis of GFR in diabetes, glomerular hyperfiltration was blunted when $\text{A}_1\text{R}^{-/-}$ mice were exposed to STZ-induced, more moderate hyperglycemia (37). Tubular control of GFR has also been proposed in dogs, in which acute hyperglycemia increased GFR, but only if tubuloglomerular feedback was intact (38).

Determinants of Primary Proximal Tubule Hyperreabsorption in Diabetes Mellitus

A primary increase in proximal reabsorption in diabetes is the natural consequence of tubular growth because a larger tubule reabsorbs more, and of moderate hyperglycemia, which provides more substrate for proximal tubule Na⁺-glucose cotransport (Figure 1). Conversely, inhibiting tubular growth or Na⁺-glucose cotransport prevents or reduces hyperreabsorption. Moreover, and in accordance with the tubular hypothesis of glomerular filtration, these maneuvers also suppress glomerular hyperfiltration. Proximal tubule glucose transport and tubular growth are important for the pathophysiology of the diabetic kidney, and therefore they are separately and in more detail discussed below, including discussion of their role in primary proximal tubule hyperreabsorption.

GLUCOSE TRANSPORT IN THE DIABETIC PROXIMAL TUBULE

Under normoglycemic conditions, most of the tubular glucose uptake across the apical membrane occurs in the early proximal tubule and is mediated by the high-capacity Na⁺-glucose cotransporter **SGLT2** (SLC5A2) (Figure 3). Most of the remaining luminal glucose is taken up in further distal parts of the proximal tubule by low-capacity SGLT1 (SLC5A1). In accordance, SGLT2 and SGLT1 protein expression has been located in the brush border membrane of the early and later proximal tubule sections, respectively (39, 40). Expression of the human genes in HEK293 cells confirmed that the Na⁺:glucose coupling ratio equals a value of 1 for hSGLT2 and 2 for hSGLT1, indicating a greater concentrative power of the latter, whereas hSGLT2 and hSGLT1 transport glucose with similar affinity (5 mM versus 2 mM) (41).

Renal micropuncture experiments in knockout mice demonstrated that SGLT2 is responsible for all glucose reabsorption in the early proximal tubule and overall is the major pathway of renal glucose reabsorption (40). The lack of SGLT2 suppresses renal mRNA and protein expression of SGLT1 by approximately 40%, which may prevent excessive glucose uptake in late proximal tubule segments. Whereas mean fractional renal glucose reabsorption at euglycemia was ~40% in *Sglt2* knockout mice, studies in mice lacking *Sglt1* showed normal renal SGLT2 protein expression and a significant but minor reduction in fractional renal glucose reabsorption from 99.8% to 96.9% (42). Similarly, human subjects with *SGLT1* mutations show intestinal glucose malabsorption with little or no glucosuria, whereas individuals with *SGLT2* gene mutations have persistent and prominent renal glucosuria (43). The glucose reabsorption in *Sglt2* knockout mice is thought to reflect the significant capacity of SGLT1 to increase glucose reabsorption when glucose delivery increases.

Expression of SGLT2 and SGLT1 in the Diabetic Kidney

The capacity of glucose transport through SGLT2 and SGLT1 is determined by expression levels, which can increase in diabetes. For example, induction of **STZ** diabetes in rats increased mRNA expression for *Sglt1* and *Sglt2* in the renal cortex (44) and increased renal SGLT1 protein expression (45). Enhanced renal mRNA expression of *Sglt1* and *Sglt2* was also found in diabetic obese Zucker rats compared with age-matched leans (46). Studies in primary cultures of human exfoliated proximal tubule epithelial cells harvested from fresh urine of patients with **T2DM** revealed increased glucose uptake, which was associated with increased mRNA and protein expression of SGLT2 (47). Upregulation of SGLT2 expression in diabetes is linked to the activation of angiotensin II (Ang II) AT₁ receptors (48) and the transcription factor hepatocyte nuclear factor HNF-1 (49), whereas upregulation of SGLT1 is linked to serum- and glucocorticoid-inducible kinase 1 (SGK1) (Figure 3). *SGK1* is upregulated in proximal tubules in STZ-diabetic rats and in patients with diabetic nephropathy (50). Studies in knockout mice implicated SGK1 in the stimulation of SGLT1 activity in proximal renal tubules in diabetes (51). SGK1 may also facilitate proximal tubule

glucose transport by the stimulation of luminal K^+ channels (e.g., KCNE1/KCNQ1) (52), which counteract the depolarization induced by electrogenic Na^+ -glucose cotransport, thereby maintaining the electrical driving force (Figure 3) (53, 54). Moreover, SGK1 may upregulate mRNA levels of the Na-H exchanger *Nhe3* in STZ-diabetic rats (55), an important determinant of proximal tubule Na^+ reabsorption. SGK1 effects on intestinal and proximal and distal tubule transport as well as in fibrosis make SGK1 an interesting target in diabetes (50).

Diabetes may not uniformly increase renal expression of **SGLTs** because some studies found unchanged or even reduced renal SGLT expression and/or activity in diabetic rodent models (19, 56, 57). Studies in primary cultures of renal proximal tubule cells indicated that high-glucose-induced oxidative stress can reduce SGLT expression and activity (Figure 3) (58). This may relate to the downregulation of SGLT1 found in mice lacking *Sglt2* (40) and may limit renal glucose uptake and toxicity. Earlier studies reported that hyperglycemia causes the induction and membrane incorporation of a low-affinity Na^+ -dependent D-glucose transporter in the proximal tubule of 4-day-old STZ-diabetic rats, an effect that was retained for at least 4 weeks, but only when the animals maintained or increased their body weight (59). This effect was lost in severely ill ketoacidotic and cachectic animals (59). These results indicate the importance of metabolic conditions, which may contribute to the different findings on SGLT expression in diabetes. Even with unchanged expression of SGLT2 and SGLT1, the increase in the tubular glucose load associated with hyperglycemia is expected to increase absolute glucose uptake through SGLT2 and SGLT1 because their capacity is not saturated under normoglycemic conditions.

Sodium-Glucose Cotransport Contributes to Primary Proximal Tubule Hyperreabsorption in Diabetes Mellitus

Modeling the effects of Na^+ -linked glucose transport on the active and passive components of proximal reabsorption predicts that modest hyperglycemia enhances Na^+ reabsorption in the proximal tubule (36). Bank & Aynedjian (60) performed microperfusion studies in STZ-diabetic rats and proposed that high glucose in the proximal tubule fluid stimulates Na^+ absorption through Na^+ -glucose cotransport. Increasing luminal glucose (from 100 to 500 mg dl^{-1}) induced significantly greater increases in Na^+ versus glucose absorption on a molar basis, which may reflect the Na^+ :glucose coupling ratio of 2:1 for SGLT1 (41). Increased SGLT-mediated Na^+ transport was confirmed by micropuncture in moderately hyperglycemic STZ-diabetic rats and by the direct application of the nonselective SGLT inhibitor phlorizin into the free-flowing early proximal tubules of superficial glomeruli. In diabetic rats, phlorizin elicited a greater decline in absolute and fractional reabsorption up to the early distal tubule and abolished hyperreabsorption (30). Moreover, this maneuver increased Na^+ , Cl^- , and K^+ concentrations in early distal tubule fluid and reduced diabetic glomerular hyperfiltration, consistent with the tubular hypothesis (30). Studies using the SGLT2 inhibitor dapagliflozin confirmed the acute effects observed with phlorizin on proximal reabsorption and **GFR**. In addition, studies with continuous SGLT2 blockade for 2 weeks from the onset of STZ diabetes suggested such blockade as a means to normalize NaCl delivery to the macula densa and to thereby attenuate hyperfiltration, consistent with a role of SGLT2 in diabetes-induced hyperreabsorption and hyperfiltration (Figures 1 and 3) (61). Finally, the glucose reabsorptive rate in early STZ-diabetic rats increases with kidney weight (62). Tubular growth, the other major cause of proximal hyperreabsorption (see below), may cause proximal hyperreabsorption, in part by enhancing Na^+ -glucose cotransport capacity.

Selective inhibitors of SGLT2 are currently in clinical trials to inhibit renal glucose reabsorption and to increase renal excretion, thereby lowering hyperglycemia (14, 15). This

approach can reduce plasma glucose without inducing increased insulin secretion, hypoglycemia, or weight gain. This approach would constitute a major advance and appears to have a good safety profile. Under hyperglycemic conditions, such inhibitors are expected to lower proximal tubule Na⁺ reabsorption and thereby diabetic glomerular hyperfiltration. Shifting glucose reabsorption from SGLT2 to SGLT1, which has a greater Na⁺:glucose coupling ratio, is expected to attenuate the renal Na⁺ loss in response to SGLT2 inhibition. Preventing the early proximal tubule from sensing episodes of hyperglycemia through SGLT2 may attenuate the negative effects of glucose on tubular growth and function. However, blocking apical glucose entry via SGLT2 may simply increase basolateral glucose entry when blood glucose is rising and may thus inhibit Na⁺ reabsorption and glomerular hyperfiltration, but not the other nefarious effects of high intracellular glucose. Moreover, basolateral glucose uptake may be particularly important for the induction of transforming growth factor β 1 (TGF- β 1) (Figure 3) (21). Studies in mice with a loss of SGLT2 protein function revealed no evidence for tubular injury (63). Induction of STZ diabetes in these mice showed a higher risk for infection and an increased mortality rate, although the influence of the greater total STZ doses required to achieve similar hyperglycemia compared with wild-type mice remained unclear. In patients treated with SGLT2 inhibitors, the increased glucosuria appears to increase the risk of genital infections, but not the risk of ascending urinary tract infections (14). Moreover, patients with familial renal glycosuria due to mutations in *SGLT2* do not show signs of general renal tubular dysfunction or other pathological changes and seem to have normal life expectancies (43). Future studies will help to better define and understand the consequences that occur along the nephron and the urinary system when SGLT2 is inhibited under diabetic conditions, including the role of SGLT-mediated increases in intracellular Na⁺ concentration as a trigger of proximal tubule growth in diabetes (Figure 3) (64).

Luminal Translocation of GLUT2 in the Diabetic Proximal Tubule

Proximal tubule cells do not use glucose for energy production, and glucose that is reabsorbed across the luminal membrane leaves the cell across the basolateral membrane by the low-affinity glucose transporter, GLUT2, in the S1 segment and by high-affinity GLUT1 in the S3 segment. Upregulation of GLUT2 expression occurs in renal proximal tubule cells in diabetic patients (47) and in diabetic rats (65) and has been linked to transcriptional activity of both HNF-1 α and HNF-3 β (Figure 3) (66). The gene for HNF-1 α is mutated in maturity-onset diabetes of the young type 3 (MODY3), an autosomal dominant form of non-insulin-dependent diabetes, and mice lacking HNF-1 α have a renal glucose reabsorption defect (43). In contrast, GLUT1 is downregulated in cortical tubules in diabetes (65) or is unchanged (57).

Diabetes mellitus also enhances glucose absorption in the small intestine, where high luminal glucose concentrations lead to the rapid insertion of GLUT2 into the brush border membrane to operate in parallel with SGLT1-mediated glucose uptake. This luminal insertion of GLUT2 involves a Ca²⁺- and protein kinase C (PKC) α -dependent mechanism (67). Similarly, an increase in the facilitative glucose absorption associated with the translocation of GLUT2 and GLUT5 (but not of GLUT1) to the luminal brush border of the proximal tubule was observed in hyperglycemic STZ-diabetic rats, whereas normalization of blood glucose levels by overnight fasting reversed the translocation of GLUT2, but not that of GLUT5 (57). Similar to the mechanism in enterocytes, the luminal targeting of GLUT2 in the proximal tubule was linked to a Ca²⁺- and PKC α -dependent mechanism but involved the PKC δ isoform and not PKC β (Figure 3) (68). PKC δ is expressed in the brush border of the proximal tubule, where its expression and activity increase in STZ-diabetic rats (68–70).

Because GLUT5 is thought to transport fructose in vivo and has a low affinity for glucose, the relevance of its translocation to the luminal brush border in the diabetic kidney is less clear. With a predicted K_m of 20 to 40 mM for glucose, the luminal translocation of GLUT2 implicates a role for GLUT2 in glucose reabsorption in diabetes to the extent that luminal glucose concentrations exceed peritubular concentrations as a consequence of tubular fluid reabsorption and luminal glucose concentrations saturating the **SGLTs**. Inducing acute hyperglycemia (20 to 35 mM) increased glucose concentrations in late proximal tubule fluid to plasma levels (71), which may reflect equilibration through luminal and basolateral GLUT2. In the small intestine, SGLT1 senses the high glucose concentrations and is required for the insertion of GLUT2 into the brush border membrane (42, 67). If SGLT2 has a similar role in the early proximal tubule (Figure 3), then SGLT2 inhibitors may also lower renal glucose reabsorption during hyperglycemia by inhibiting luminal GLUT2 translocation.

EARLY TUBULAR GROWTH IN DIABETES MELLITUS

The kidney in general and the proximal tubules in particular grow large from the onset of diabetes mellitus (19). Proximal tubule growth involves an early period of hyperplasia followed by a shift to hypertrophy (72), as discussed in this section and illustrated in

Early Hyperplastic Phase

Numerous growth factors contribute to the early proliferation phase of the diabetic tubular system, including insulin-like growth factor 1 (IGF-1), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and epidermal growth factor (EGF) (73, 74). Recent studies proposed that mouse proximal tubule cells express an endogenous renin-angiotensin system (RAS) that is activated by high glucose (see also Figure 3) to stimulate VEGF synthesis through activation of the **Ang II** AT₁ receptor and the extracellular signal--regulated kinase (ERK) pathway (75). Glucose and Ang II activate intracellular signaling processes, including the polyol pathway and generation of reactive oxygen species (**ROS**) (including H₂O₂ and O₂⁻), which activate the Janus kinase (JAK)/signal transducers and activators of transcription (STAT) signaling cascades (76). Ang II activates the JAK/STAT pathway via AT₁ receptors (77) and increases cell proliferation, which is enhanced by high glucose (78). Diabetes and high glucose concentrations also induce suppressors of cytokine signaling (SOCS1, SOCS3) in tubular cells. SOCS1 and SOCS3 are intracellular negative regulators of JAK/STAT activation that inhibit the expression of STAT-dependent genes and high-glucose-induced cell proliferation (79). The JAK/STAT pathway is linked to the induction of immediate early genes like *c-jun* and *c-fos* (76), which can activate ornithine decarboxylase (ODC) (80), the rate-limiting enzyme in polyamine synthesis. In the early diabetic kidney, ODC is required for hyperplasia and most likely also for hypertrophy of the proximal tubule (Figure 4) (31, 80, 81). The rapid yet transient renal induction of IGF-1 correlates with the upregulation of renal ODC expression and activity, the induction of intracellular polyamines in the kidney cortex, and the early proliferative phase (80). Deng et al. (82) proposed that the increase in ODC expression in early diabetes occurs mainly in the distal nephron and that polyamines may pass from the distal tubule to the proximal tubule in a paracrine fashion to trigger proximal tubule growth. Further studies are needed to confirm these findings and to determine the mechanisms that induce ODC expression in the distal tubule.

Diabetes mellitus activates **PKC**, which can produce a myriad of consequences, including a mitogen-induced early proliferation phase (83). In particular, diabetes can enhance proximal tubule activity of the PKC α isoform--(68, 70) and PKC δ has been implicated in Akt activation in the renal cortex of diabetic rats (84). In accordance with a role of PKC δ in kidney growth, the early diabetes-induced increase in kidney weight was blunted in mice

lacking this PKC isoform (85). ACE inhibition inhibits diabetes-induced activation of renal PKC α (70), consistent with PKC α being downstream of Ang II. Downstream signaling events of IGF-1 and VEGF include activation of the phosphoinositide 3-kinase (PI3K)/Akt pathways and thus merge with PKC α -activated pathways; both pathways are linked to ODC activation (80). Diabetic renal growth is also associated with reduced phosphorylation of AMP-activated protein kinase (AMPK) (86). Phosphorylated AMPK inhibits the activity of mammalian target of rapamycin complex 1 (mTORC1) by phosphorylating and activating tuberous sclerosis complex (TSC2) (87). As a consequence, mTOR activity is enhanced in the diabetic kidney, and increasing AMPK phosphorylation reverses mTOR activation and inhibits renal growth without affecting hyperglycemia (86). Together these studies propose that the early tubular proliferation phase in diabetes is the consequence of high-glucose-induced oxidative stress and activation of the tubular RAS, enhanced glomerular filtration and tubular expression of growth factors, activation of PKC α and the JAK/STAT pathway, AMPK inhibition, and activation of both mTORC1 and ODC (Figure 4).

Transition from the Hyperplastic Phase to the Hypertrophic Phase

The transition of the diabetic kidney from hyperplastic to hypertrophic growth occurs early (at approximately day 4 in the model of STZ diabetes) (72, 82) and is mediated by TGF- β 1 (88). In accordance, primary tubule cells from TGF- β 1 knockout mice respond to high glucose concentrations with an increased rate of proliferation compared with cells from wild-type littermates but show no hypertrophy (89). The JAK/STAT signaling pathway (90) and PKC δ (91) can induce TGF- β 1 expression in the diabetic kidney (Figure 4). In addition, ERK and p38 have been implicated in high-glucose-induced TGF- β 1 expression and cellular hypertrophy in renal tubular cells of STZ-diabetic rats (92).

TGF- β 1 can induce a G₁ phase cell cycle arrest by induction of the cyclin-dependent kinase (CDK) inhibitor p27^{KIP1} (p27) (93), which can also be induced in diabetes by PKC δ (94). Diabetes also increases the renal expression of the CDK inhibitor waf1/cip1 (p21) (95, 96), and loss of p21 increases tubular cell proliferation (96). Consistent with a role of ROS, the antioxidants N-acetylcysteine and taurine attenuated high-glucose-induced activation of the JAK/STAT signaling pathways, p21 and p27 expression, and hypertrophic growth in renal tubular epithelial cells (97). Antioxidants also attenuated the enhanced p21 expression and cellular hypertrophy induced by advanced glycation end products (AGE) and its receptor (RAGE) in human renal proximal tubule cells (98). Thus, signaling pathways that initially induce proliferation subsequently provoke a switch to hypertrophy through the induction of TGF- β 1 and CDK inhibitors in the diabetic tubule (Figure 4). Sustaining kidney hypertrophy and size in the long-term diabetic state involves additional mechanisms, including decreased proteolysis (99).

Tubular Growth as a Determinant of Proximal Tubule Hyperreabsorption in Diabetes Mellitus

It is easy to imagine that an increase in proximal tubule length and diameter enhances proximal tubule reabsorptive capacity. Difluoromethylornithine (DFMO), an ODC inhibitor, attenuates kidney growth in early STZ-diabetic rats (81) and was used to test whether tubular growth contributes to the primary increase in proximal reabsorption in early diabetes mellitus. DFMO had no significant effect on kidney weight or GFR in nondiabetic rats (31). In comparison, DFMO attenuated kidney growth and glomerular hyperfiltration in similar proportions in diabetic rats. Moreover, DFMO eliminated the primary increase in proximal reabsorption in STZ-diabetic rats; i.e., at the same level of single-nephron GFR, proximal reabsorption was lower in DFMO-treated than in untreated STZ-diabetic rats (31).

Diabetes stimulates the basolateral Na-K-ATPase activity of the proximal tubule that has been associated with and implicated in renal hypertrophy (64, 100), although the involved mechanisms still remain unclear. PKC may be part of this link because the kinase is involved in tubular growth in diabetes (85) and has been implicated in the activation of Na-K-ATPase and Na⁺ transport in the proximal tubule (Figure 3) (101). Because PKC inhibition can also lower diabetic hyperfiltration (91), we speculate that this effect involves inhibitory effects on proximal tubule growth and reabsorption (70).

Tubular Senescence in the Early Diabetic Kidney

Senescence is a tumor suppressor mechanism that involves CDK inhibition to halt cells from replicating and passing on a damaged genome (102). Transient induction of p21, p16^{INK4A} (p16), and/or p27 is involved in prototypical senescent arrest or senescence-like growth arrest. Satriano et al. (95) showed an early transient induction of growth-phase components in the kidney followed by their suppression at day 10 after the onset of STZ diabetes. These events were concurrent with the induction of CDK inhibitors p16, p21, and p27 and the expression of senescence-associated α -galactosidase activity in cortical tubules (Figure 4) (95). Moreover, they showed that proximal tubule cells in culture transition to senescence in response to oxidative stress. An accelerated senescent phenotype was also found in tubule cells of patients with **T2DM** and nephropathy (103). Senescent cells are relatively well differentiated but skewed in several aspects, including a striking increase in the secretion of proinflammatory cytokines and the production of growth factors and extracellular matrix (ECM), and are resistant to apoptotic remodeling (102). Whereas the senescent arrest of tubular cells may be triggered by glucotoxic signals to prevent excessive proliferation, we speculate that such arrest contributes to inflammation and fibrosis in the diabetic kidney (Figure 4) and alters early proximal tubule function. One example for the latter effect may be the so-called salt paradox of the diabetic kidney, which is discussed in the following section.

THE SALT PARADOX OF THE DIABETIC KIDNEY

In normal subjects, GFR is insensitive to dietary NaCl or to changes in the same direction as the change in NaCl intake (32, 104). In 1995, we reported that a low-NaCl diet reduced renal vascular resistance and increased renal blood flow, GFR, and kidney weight in male STZ-diabetic rats (105). In contrast, female rats with early (1 week) or established (4–5 weeks) STZ diabetes responded to a high-NaCl diet with renal vasoconstriction (106). To describe the inverse relationship between dietary NaCl and GFR that is counterintuitive with regard to NaCl homeostasis, we coined the term salt paradox of the diabetic kidney. The salt paradox was confirmed in STZ-diabetic mice (37); in Long-Evans rats (107); and, most importantly, in diabetic patients, including young patients with uncomplicated **T1DM**, in whom restriction of dietary Na⁺ to 20 mmol day⁻¹ decreased renal vascular resistance and increased effective renal plasma flow and GFR (108).

How Can Dietary NaCl Suppress Glomerular Hyperfiltration, and Why Is This Unique to the Diabetic Kidney?

Micropuncture studies established that the salt paradox occurs because diabetes causes proximal tubule reabsorption to become markedly sensitive to changes in dietary NaCl such that eating more NaCl leads to greater suppression of proximal tubule reabsorption and greater **MD_{NaCl}** and vice versa for a low-NaCl diet, with secondary consequences on GFR via tubuloglomerular feedback (Figure 1) (32). In accordance, the salt paradox is absent in the STZ-diabetic, tubuloglomerular feedback--less A₁R--/– mouse (37). In comparison, nondiabetic rats on various NaCl intakes manage NaCl balance primarily in the distal nephron downstream of the macula densa, and thus a tubuloglomerular feedback--mediated

inverse effect of dietary NaCl on GFR does not occur (32). Considering the need to maintain effective circulating volume, if dietary NaCl restriction progresses to actual NaCl depletion, the salt paradox will become imperceptible (7).

The Salt Paradox Is Linked to Tubular Growth

Ang II and renal nerves are prominent effectors that link proximal reabsorption to total body NaCl, but neither chronic renal denervation (109) nor chronic Ang II AT₁ receptor blockade (105) prevented the salt paradox in **STZ**-diabetic rats. Supporting a role of diabetic kidney growth, pharmacological inhibition of ODC, which inhibited tubular growth and hyperfiltration (see above), also prevented the salt paradox (110). As discussed above and as illustrated in Figure 4, hypertrophic proximal tubule cells in early diabetes are continuously stimulated by mitogens, at the same time being prevented from entering the cell cycle, and have a senescent phenotype. These tubular cells may have lost the programming of a differentiated proximal tubule cell. For example, normal proximal tubule cells do not respond to moderate changes in dietary NaCl, and nephron segments downstream of the macula dense normally attend to this balance. The diabetic proximal tubule may have lost this characteristic of a differentiated nephron segment and responds strongly to dietary NaCl, forming the basis for the salt paradox. Thus, in addition to tubular hyperreabsorption and glomerular hyperfiltration, the tubular growth phenotype also contributes to this phenomenon in the diabetic kidney.

An Anomalous Role for Dietary NaCl in Diabetes Mellitus Beyond the Salt Paradox?

Many guidelines recommend low NaCl consumption for patients with **T1DM** or **T2DM** (111), even though the impact or relationship of dietary NaCl to overall mortality or **ESRD** has not been firmly established. Two recent prospective studies provided unexpected findings in patients with T1DM and T2DM. Both studies estimated NaCl intake on the basis of 24-h urine collections and followed up on the patients over a median of 10 years. The first study reported that survival tracked monotonically with urinary Na⁺ in patients with T2DM such that a daily 100-mmol increase in Na⁺ intake predicted 30% lower cardiovascular and all-cause mortality (16). The second study reported that in patients with T1DM the relationship of all-cause mortality to Na⁺ intake was biphasic, with a minimum at 100–200 mmol day⁻¹, a steep rise at lower Na⁺ intake, and a gradual rise at higher intake. However, the cumulative incidence of ESRD was monotonically associated with Na⁺ intake such that reducing Na⁺ intake from the ninetieth percentile to the tenth percentile mapped to a tenfold increase in the likelihood of developing ESRD (17). These studies question the notion that a lower NaCl intake is always better for patients with T1DM and T2DM and indicate the need for intervention studies.

The studies also pose the question as to whether there is something unique about diabetes with regard to the response to dietary NaCl. The kidney and cardiovascular system in diabetes may be predisposed to damage by counterregulatory and NaCl-conserving sympathetic nerves and hormones (like Ang II) that are progressively activated by a decline in extracellular volume and thus by a low NaCl intake. Another mechanism that may make the diabetic kidney vulnerable to chronic damage on a low-NaCl diet is the above-described salt paradox, in which a low-NaCl diet can increase **GFR** and can also augment diabetic kidney growth, which can predispose diabetic patients to renal failure later in life (see above). Further studies are needed to better understand the link between (a) dietary NaCl and (b) ESRD and mortality in diabetes, including the influence of NaCl intake on tubular growth and the involved molecular pathways.

LINKING THE MOLECULAR SIGNATURE OF TUBULAR GROWTH TO TUBULOINTERSTITIAL INJURY AND PROGRESSION OF DIABETIC KIDNEY DISEASE

The diabetic milieu and the prolonged interaction of albuminuria, AGE, and other factors in the glomerular filtrate with the tubular system trigger renal oxidative stress and cortical interstitial inflammation; the resulting hypoxia and tubulointerstitial fibrosis determine to a great extent the progression of diabetic renal disease (1, 19–22, 112). In addition, the molecular mechanisms involved in the early growth phenotype of the diabetic kidney may set the stage for long-term progression of diabetic kidney disease. This scenario is consistent with the observation that kidney size is linked to diabetic nephropathy (9, 11–13). This principle was recently confirmed in a cross section of older patients with longstanding diabetes mellitus and an estimated GFR of $<60 \text{ ml min}^{-1}$. Among these patients, those with larger kidneys were more likely to develop ESRD after 5 years than were those who entered the study with smaller kidneys (10).

TGF- may have a special role in linking the early tubular growth phenotype of the diabetic kidney to inflammation as well as to fibrotic changes, scarring, and impairment of renal function (21, 113), as illustrated in Figure 4 and as further discussed below. We have outlined various factors that are upstream of **TGF-**, including **ROS**, **Ang II**, the **JAK/STAK** pathway, and **PKC**. New insights into C-peptide have recently been gained. C-peptide is coreleased with insulin, and therefore plasma levels of C-peptide are low in **T1DM**. Substitution of C-peptide reduces diabetic kidney growth (114), tubular hyperreabsorption, and glomerular hyperfiltration (115) and lowers albuminuria/proteinuria in patients and animal models of **T1DM** (114, 116). Moreover, in vitro studies in human kidney proximal tubule cells showed that C-peptide can enhance the expression of hepatocyte growth factor, thereby reversing the effects of **TGF-1** (117). Here we summarize recent studies that link elements of the molecular pathways involved in diabetic tubular growth (depicted in Figure 4) to tubular injury.

Links to Inflammation

The release of chemokines and macrophage infiltration are important for the initiation of pathological changes in **STZ**-diabetic rats and human diabetic nephropathy (118–120). Growth factors like **IGF-1** and **TGF-** are important for diabetic tubular growth, as discussed above, but their interaction with their respective receptors on proximal and distal tubules and on collecting ducts also enhances the levels of cytokines like monocyte chemotactic protein-1 (MCP-1 or CCL2), RANTES (CCL5), and **PDGF-**, which activate the proliferation of fibroblasts as well as of macrophages (Figure 4) (74, 120, 121). Recent studies implicated **TGF-1** in (a) the high-glucose induction of macrophage inflammatory protein-3 in human proximal tubule cells (122) and in (b) IL-18 overexpression in human tubular epithelial cells in diabetic nephropathy (123). The synergism of high glucose concentrations with cytokines such as **PDGF** or the proinflammatory macrophage-derived cytokine IL-1 can further stimulate **TGF-1** synthesis in proximal tubule cells (21), indicating local positive feedback loops. **ROS** stimulate many proinflammatory mediators relevant to chronic kidney disease, including MCP-1 and RANTES (124, 125). Furthermore, the gene expression of osteopontin, which promotes inflammation and cell recruitment, is increased by high glucose in diabetic rat proximal tubules and in rat immortalized renal proximal tubule cells via **ROS** generation, intrarenal **RAS** activation, **TGF-1** expression, and **PKC-1** signaling (126), all of which are involved in diabetic tubular growth. In contrast, the small leucine-rich proteoglycan decorin suppressed **TGF-1**, connective tissue growth factor (CTGF), and p27 in tubular epithelial cells of **STZ**-diabetic mice and reduced

upregulation of the proinflammatory proteoglycan biglycan, the infiltration of mononuclear cells, and ECM accumulation (127). These effects reflect the critical role of TGF- β 1 in the promotion of both inflammation and fibrosis in the diabetic kidney.

Links to Fibrosis

High glucose induces cell growth but also increases the amount of type IV collagen and fibronectin in primary cultures of human renal proximal tubule cells (21, 128) as a consequence of decreased degradation reflecting reduced gelatinolytic activity (21, 129). The regions of active interstitial fibrosis in chronic kidney disease exhibit predominantly a peritubular distribution (130), indicating that proximal tubule cells may release fibrogenic signals to cortical fibroblasts (Figure 4). In fact, TGF- β 1 can stimulate the release of preformed basic fibroblast growth factor from renal proximal tubule cells (131). In contrast, studies in human renal fibroblasts indicated that they can modulate proximal tubule cell growth and transport via the secretion of IGF-1 and IGF-binding protein-3 (132). Although TGF- β can induce epithelial-mesenchymal transition, the extent to which this process contributes to renal fibrosis in vivo, especially in humans, remains a matter of intense debate and may depend on the experimental and clinical context (for review, see References 1 and 133). CTGF is another prosclerotic cytokine that is induced mainly by TGF- β and IGF-1, particularly in dilated-appearing proximal tubules. CTGF is involved in the regulation of matrix accumulation and determines the outcome of diabetic renal injury in human and animal models (120, 134, 135).

Clinical trials and experimental studies implicated the importance of epigenetic processes in the development of diabetic complications. EGF contributes to diabetic kidney growth, and EGF signaling is altered by the acetylation status of histone proteins. Recent studies revealed that pharmacological inhibition of histone deacetylase (HDAC) reduced early tubular epithelial cell proliferation in diabetic rats and blunted renal growth, which may be mediated in part through downregulation of the EGF receptor (136). Other studies implicated HDAC-2 in the development of ECM accumulation in the diabetic kidney and showed that ROS mediate TGF- β 1-induced activation of HDAC-2 (137). These findings indicate that epigenetic processes affect renal growth and fibrosis of the diabetic kidney.

Links to Hypoxia and Apoptosis

Hypoxia can be due to enhanced tubular oxygen consumption, as shown ex vivo in cortical and medullary tubule cells of STZ-diabetic rats (138). Hypoxia has been implicated as a cause of oxidative stress in the diabetic kidney and in the pathophysiology of diabetic nephropathy (112). Superoxide enhances Na-K-2Cl cotransporter activity in the thick ascending limb (TAL), which can further aggravate renal hypoxia (139). Defense against hypoxia involves hypoxia-inducible factor (HIF). STZ-diabetic rats and Cohen diabetes--sensitive rats, a nonobese normolipidemic genetic model of diet-induced T2DM, transiently upregulated the hypoxia marker pimonidazole and HIF-1 α , primarily in the TAL of the renal outer medulla (140). Whether there is any link to the formation of glycogen deposits (Armanni-Ebstein lesions), which are found particularly in the cells of the TAL, is not known (1). Importantly, diabetes or high glucose levels appear to blunt the hypoxia-induced HIF pathway by inducing oxidative stress, as observed in the kidneys of STZ-diabetic rats (141) and in rat proximal tubule cells in vitro (Figure 4) (140, 141). In accordance, dietary eicosapentaenoic acid has beneficial effects on STZ-diabetic kidney injury by suppressing ROS generation and mitochondrial apoptosis, partly through augmentation of the HIF-1 response (142). Overexpression of catalase in renal proximal tubule cells not only attenuated apoptosis in STZ diabetes (143) and in *db/db* transgenic mice (144) but also reduced interstitial fibrosis in the latter model (144). Studies in *db/db* mice implicated Nox4-based NADPH oxidase in glucose-induced oxidative stress in proximal tubules and fibrosis (145).

Inhibition of JAK2 protected renal endothelial and epithelial cells from oxidative stress (146), and a direct relationship between tubulointerstitial JAK/STAT expression and progression of kidney failure was found in patients with **T2DM** (147). In accordance, SOCS proteins inhibit the expression of STAT-dependent genes (involved in cell proliferation, inflammation, and fibrosis) and improve renal function in diabetes (79). Besides **ROS** (148), both **PKC** (149) and the TSC2/mTOR pathway (150) have been implicated in promoting apoptosis of proximal tubule cells in diabetes.

PERSPECTIVES

Inhibition of proximal tubule glucose reabsorption via **SGLT2** is a promising new therapeutic approach that can lower blood glucose levels and attenuate glomerular hyperfiltration in diabetes, and ongoing studies are assessing the long-term safety of this approach. Tubular glucose uptake contributes to tubular injury, but further studies are needed to better define the role of **GLUT2** translocation and the role of SGLTs and GLUT2 in tubular injury and growth in the diabetic kidney. Further studies are required to elucidate the proposed deleterious effects of dietary NaCl restriction on kidney function and mortality in diabetic patients. The unique early growth phenotype of the proximal tubule is important for early tubular hyperreabsorption, glomerular hyperfiltration, and the salt paradox. However, we speculate that the molecular signature of tubular growth sets the stage for the development of inflammation, fibrosis, tubulointerstitial injury, hypoxia, and apoptosis, which would explain the strong correlation between kidney size and prognosis of kidney outcome in diabetic patients. Genetic and environmental factors may modulate the tubular response to hyperglycemia, thereby contributing to the fact that only some diabetic patients develop large kidneys, tubular hyperreabsorption, glomerular hyperfiltration, and diabetic nephropathy. Finally, we need to better understand why it takes 10 to 20 years for the diabetic milieu to cause renal failure when many of the proposed deleterious molecular pathways can be activated within hours, days, or weeks of hyperglycemia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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KEY TERMS AND DEFINITIONS

GFR	glomerular filtration rate
SNGFR	single-nephron glomerular filtration rate
TGF-	transforming growth factor
T1DM	type 1 diabetes mellitus
T2DM	type 2 diabetes mellitus
MD_{NaClK}	concentration and delivery of Na ⁺ , Cl ⁻ , and K ⁺ at the luminal macula densa
SGLT	Na ⁺ -glucose cotransporter

GTB	glomerulotubular balance
Ang II	angiotensin II
Streptozotocin (STZ)	injected to induce a model of type 1 diabetes mellitus
GLUT	glucose transporter
RAS	reninangiotensin system
ROS	reactive oxygen species
ODC	ornithine decarboxylase
PKC	protein kinase C
CDK	cyclin-dependent kinase
ESRD	end-stage renal disease
IGF-1	insulin-like growth factor 1
VEGF	vascular endothelial growth factor
JAK/STAT	Janus kinase/signal transducers and activators of transcription
HIF	hypoxia-inducible factor

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SUMMARY POINTS

1. Enhanced Na⁺-glucose cotransport and tubular growth cause a primary increase in proximal tubule reabsorption in the diabetic kidney, which through tubuloglomerular feedback induces glomerular hyperfiltration.
2. The kidney in general and the proximal tubules in particular grow large from the onset of diabetes mellitus, and kidney size has been linked to the development of diabetic nephropathy.
3. Hypertrophic proximal tubule cells in early diabetes are continuously stimulated by mitogens, at the same time being prevented from entering the cell cycle, and have a senescent phenotype.
4. The salt paradox, a unique phenomenon of the diabetic kidney that refers to an inverse relationship between changes in dietary NaCl intake and GFR, occurs because diabetes causes proximal tubule reabsorption to become extensively sensitive to changes in dietary NaCl, which is related to the tubular growth phenotype.
5. The molecular signature of tubular growth in the diabetic kidney is linked to tubulointerstitial fibrosis, inflammation, hypoxia, and apoptosis and may set the stage for tubulointerstitial injury and the progression of diabetic kidney disease.
6. Genetic and environmental factors may modulate the tubular response to hyperglycemia, thereby contributing to the fact that only some diabetic patients develop large kidneys, tubular hyperreabsorption, glomerular hyperfiltration, and diabetic nephropathy.

Figure 1.

Tubular hypothesis of glomerular filtration in diabetes mellitus. (a) Glomerular hyperfiltration in diabetes mellitus as a primary tubular event. The single-nephron glomerular filtration rate (SNGFR) is determined by primary vascular events, tubuloglomerular feedback, and tubuloglomerular feedback resetting. Glomerulotubular balance (GTB) and primary tubular events determine the concentration and delivery of Na⁺, Cl⁻, and K⁺ at the luminal macula densa (MD_{NaClK}). A primary vascular event causes SNGFR and MD_{NaClK} to change in the same direction, whereas a primary tubular event causes SNGFR and MD_{NaClK} to change in opposite directions. Hyperglycemia causes a primary increase in proximal tubule reabsorption (the primary tubular event) through enhanced tubular growth and Na⁺-glucose cotransport **1**. This reduces MD_{NaClK} **2** and, via tubuloglomerular feedback **3**, increases SNGFR **4**. Enhanced growth and tubular reabsorption can also reduce the hydrostatic pressure in Bowman space (P_{BOW}) **5**, which by increasing effective filtration pressure can also increase SNGFR **4**. The resulting increase in SNGFR partly restores the fluid and electrolyte load to the distal nephron. (b) The salt paradox. The nondiabetic kidney adjusts NaCl transport to dietary NaCl intake primarily downstream of the macula densa, and thus MD_{NaClK} or SNGFR is not altered. In contrast, diabetes renders reabsorption in the proximal tubule very sensitive to dietary NaCl, with subsequent effects on MD_{NaClK} and SNGFR.

Figure 2.

A proposed role for tubuloglomerular feedback (TGF) resetting in the diabetic kidney. TGF is depicted as the inverse relationship between early distal Na^+ concentration ($[Na^+]_{\text{early distal}}$) and single-nephron GFR (SNGFR). Data from perturbation analysis of late proximal tubule flow rate were combined with data for fractional proximal reabsorption (29), ambient $[Na^+]_{\text{early distal}}$ (30), and the relationship between late proximal flow rate and early distal conductivity (29) in control and STZ-diabetic rats. Similar curves were derived for early distal Cl^- concentration (not shown). Dashed lines represent glomerular-tubular balance (GTB), which refers to the load dependency of reabsorption between the glomerulus and the macula densa. The operating point (*triangles*) of the nephron, where TGF and GTB intersect, is usually located where TGF is steep. A primary diabetes--induced increase in reabsorption (with a parallel shift in the GTB line) lowers $[Na^+]_{\text{early distal}}$, which increases SNGFR but shifts the operating point to the flatter part of the TGF curve. To operate at high TGF efficiency, the TGF curve in diabetes resets upward such that the operating point is restored to the steeper part of the TGF curve.

Figure 3.

Proximal tubule glucose and Na^+ transport in the diabetic kidney. Hyperglycemia increases glomerular filtration of glucose and enhances glucose reabsorption in the proximal tubule via SGLT2 (early segments) and SGLT1 (later segments), thereby enhancing the reabsorption and intracellular concentration of Na^+ ($[\text{Na}^+]_i$). An increase in $[\text{Na}^+]_i$ and activation of basolateral Na-K-ATPase [via diabetes-induced protein kinase C 1 (PKC 1)] have been implicated in diabetic tubular growth. Diabetes increases angiotensin II (Ang II) in the cytosol and in the extracellular space; the latter may include enhanced release of angiotensinogen (AGT) (75). Release of AGT and activation of AT_1 receptors may also occur at the luminal membrane. Ang II and hepatocyte nuclear factor HNF-1 increase SGLT2 and glucose transporter (GLUT)2 expression. Diabetes also induces the serum- and glucocorticoid-inducible kinase SGK1, which increases SGLT1 activity. Induction of oxidative stress (ROS) can inhibit SGLT. Glucose reabsorption via SGLTs is electrogenic, and luminal K^+ channels (e.g., SGK1-activated KCNE1/KCNQ1 in the late proximal tubule) stabilize the membrane potential. Glucose exits via basolateral GLUTs. In diabetes, PKC 1 can shift part of GLUT2 into the apical membrane, which would equilibrate luminal and basolateral glucose concentrations. The induction of transforming growth factor 1 (TGF-1) may be particularly sensitive to basolateral glucose uptake. ROS denotes reactive oxygen species. Modified with permission from Reference 19.

Figure 4.

The early growth phenotype of the diabetic proximal tubule and potential links to renal injury and failure. Diabetes mellitus--induced growth of the proximal tubule includes an initial phase of cell proliferation and an early transition to hypertrophy via G₁ cell cycle arrest and the development of a senescence-like phenotype. The molecular pathways involved in this tubular growth phenotype are linked to tubulointerstitial fibrosis and inflammation and may thus set the stage for renal failure later in life. Abbreviations: AGE, advanced glycation end products; Ang II, angiotensin II; bFGF, basic fibroblast growth factor; CTGF, connective tissue growth factor; Cyc D1, cyclin D1; HGF, hepatocyte growth factor; HIF-1, hypoxia-inducible factor 1; IGF-1, insulin-like growth factor 1; PKC 1, protein kinase C 1; JAK/STAT, Janus kinase/signal transducers and activators of transcription; MCP-1, monocyte chemotactic protein-1; mTORC1, mammalian target of rapamycin complex 1; ODC, ornithine decarboxylase; p16, p16^{INK4A}; p21, the CDK inhibitor waf1/cip1; p27, p27^{KIP1}; pAMPK, phosphorylated AMP-activated protein kinase; PI3K, phosphoinositide 3-kinase; PDGF-, platelet-derived growth factor; RAGE, receptor for AGE; RAS, renin-angiotensin system; ROS, reactive oxygen species; SOCS, suppressor of cytokine signaling; TGF-, transforming growth factor; TSC, tuberous sclerosis complex; VEGF, vascular endothelial growth factor. Modified, with permission, from Reference 1.