Diabetic Nephropathy

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Abstract

Diabetes is the most common cause of end-stage kidney disease in the world. Diabetic nephropathy is due to cellular and subcellular mechanisms and involves induction of signaling pathways in the kidney which perpetuate the destruction of glomeruli, the intrarenal vasculature, and the interstitium. Diagnosis and prevention center on the detection of albuminuria, tight plasma glucose control, as well as primary interruption of the renin–angiotensin–aldosterone system, which reduces the transglomerular hydrostatic pressure. Some of the newer glucose control therapeutic agents have shown benefit in diabetic nephropathy, and the future holds promise for specific inhibitors of inflammation, as well as inhibitors of microRNA species. Comorbid conditions such as large vessel disease are also commonly associated and require vigilance on the part of the physician and those supervising the predialysis and dialysis patients.

Keywords

Nephropathy • Renin-angiotensin • Inflammation • microRNA • Genetics • Novel therapy

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The Impact of Diabetic Renal Disease

Diabetes mellitus (DM) remains the most common primary cause of incident and prevalent chronic kidney disease (CKD) requiring renal replacement therapy in the United States [1], the developed [2], and the emerging world [3]. In the United States, more than 44% of the new CKD diagnoses in 2012 were attributable to diabetes: a total of 50,517 patients, at a rate of 155 per million/population. Although the absolute number of

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new CKD patients each year is increasing due to population growth, the rate of prevalent CKD from diabetes has decreased during the period from 1998 to 2012 from 43.1% to 39.2%. CKD attributable to DM remains disproportionately high among blacks, Hispanics, and Native Americans and continues to increase in the elderly and younger (age 30–39) black adults. The economic impact of end-stage kidney disease from diabetes is enormous – total CKD expenditure in 2012 was $28.6 billion (excluding Medicare part D costs), and diabetic patients incurred the highest per-person per-year cost. Patients with diabetes have the highest hospitalization rates and mortality (cardiovascular, infectious, and all-cause) among all dialysis patients. They are also less likely to be listed for or to receive a kidney transplant. Diabetic individuals fare worse than nondiabetic patients after transplantation, with higher mortality and morbidity from infection [4]. Furthermore, new onset diabetes mellitus (NODM) following kidney transplantation and the use of tacrolimus therapy as the immunosuppressive agent is often associated with obesity and accelerated complications [5]. Advanced understanding of vascular biology in DM will likely improve management of cardiovascular disease in the diabetic population. Efforts to attenuate the progression of diabetic nephropathy in the large pre-CKD-5 population [6] represent the greatest opportunity to improve CKD outcomes in DM.

Pathophysiology of Diabetic Nephropathy

While the pathophysiology of diabetic nephropathy is incompletely understood, several cardinal etiologic features have emerged. Persistent hyperglycemia (sustained hemoglobin A1c >7%), glycosylation of circulating proteins as well as renal parenchymal proteins, systemic hypertension (including a family history of hypertension), abnormal alteration of intrarenal hemodynamics, as well as smoking play major roles. Since diabetic nephropathy does not develop in every diabetic patient, genetic factors also play a role. Early physiologic abnormalities include increased transglomerular pressure leading to hyperfiltration, manifesting initially with increased glomerular filtration rate (GFR) especially in type 1 diabetes, and moderately increased albuminuria (formerly called "microalbuminuria"). Detection of moderately increased albuminuria (30–300 mg/day, or random urinary albumin of 30–300 mg/g creatinine) is essential in diagnosis and follow-up of the disease, since the onset of severely increased albuminuria (formerly called "macroalbuminuria") of greater than 300 mg/day heralds the progression to renal failure. Factors contributing to the renal lesions in both type 1 and type 2 diabetic nephropathy are shown in Table 1.

Appearance of urine albumin of glomerular origin is caused by increased intraglomerular pressure, loss of negatively charged glycosaminoglycans in the basement membrane, and eventually, increased basement membrane pore size. Microscopically, there is a thickening of the glomerular basement membrane, an increased mesangial matrix, and an increased population of mesangial cells [7]. Mesangial expansion is associated with a decrease in capillary filtration surface area, which also correlates with (decreased) glomerular filtration rate. Tubulointerstitial disease develops probably as a result of an inflammatory response to albumin accumulation in

Table 1 Factors contributing to development of diabetic nephropathy

Sustained hyperglycemia (HbA 1c > 7.5–8%)	
Familial hypertension (in a parent or sibling)	Abnormalities in red blood cell Na/Li countertransport
	Genetic polymorphism for the DD genotype of the angiotensin-converting enzyme in type I diabetes
Familial diabetic nephropathy	Twins
Ethnic diversity	Native Americans
	African-Americans
	Mexicans
	Hispanic Americans
	Japanese
Metabolic syndrome	

proximal convoluted tubule cells [8]; this results in thickening of the tubular basement membrane, tubular atrophy, interstitial fibrosis, and arteriosclerosis. The podocyte also has a role in the progression of diabetic nephropathy. Podocyte foot processes interdigitate upon and support the glomerular basement membrane, preventing protein escape. Normally negatively charged, the podocytes repel negatively charged molecules such as albumin. The loss of charge demonstrated in diabetic nephropathy (and other glomerular diseases) explains the passage of proteins into the urinary space. One of the mechanisms by which this occurs is the loss of nephrin and other podocyte proteins (podocin). Eventually the podocytes fuse (or efface) and their slit diaphragms disappear. These changes result in proteinuria and loss of podocyte-controlled pressure-sensitive maintenance of intraglomerular pressure.

Biochemical mechanisms involved in the pathogenesis of diabetic nephropathy (Fig. 1) include direct glucose toxicity, glycation of proteins, formation of advanced glycation end products (AGEs), and increased flux through the polyol and hexosamine metabolic pathways, resulting in overproduction of reactive oxygen species (ROS), molecules which stimulate each of the above pathways [9]. Glucose itself stimulates some signaling molecules (see below), as does the raised intraglomerular pressure. Several isoforms of protein kinase C, diacyl glycerol, mitogenic kinases, and transcription factors may also be activated in diabetic nephropathy.

In addition, a large number of growth factors may be implicated [10]. Transforming growth factor β1 and connective tissue growth factor may result in mesangial and interstitial fibrosis. Growth hormone and insulin-like growth factor-1 are associated with glomerular hyperfiltration and hypertrophy. Circulating and intraglomerular vascular endothelial growth factor (VEGF) increases are evident [11], while inhibition of VEGF has been associated with improved diabetic retinopathy [12]. Angiotensin II has several important pathophysiologic roles: by its pressor effect, it

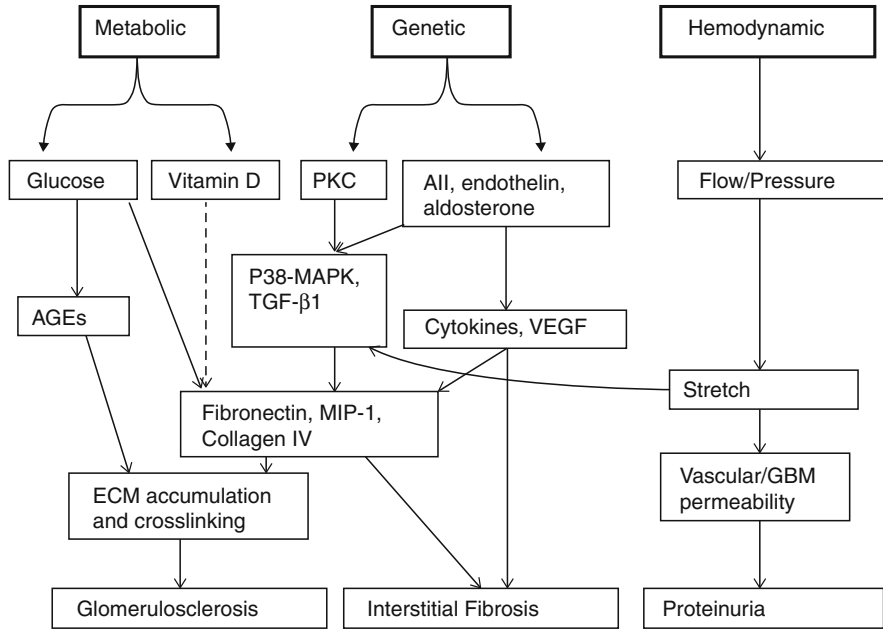

Fig. 1 Schematic of pathogenesis of diabetic nephropathy. Abbreviations: *PKC* phosphokinase C, *AII* angiotensin 2, *P38-MAPK* P38-mitogen-activated protein kinase, *TGF-β1* transforming growth factor β1, *AGEs* advanced glycosylation end products, *VEGF* vascular endothelial growth factor, *MIP-1* macrophage-inhibitory protein – 1, *ECM* extracellular matrix, - - - - - - inhibitory

causes preferential constriction of the efferent glomerular arteriole [13]; it increases glomerular capillary permeability to proteins; and its growth effects stimulate mesangial cell proliferation and accumulation of mesangial matrix. Via stretch receptors stimulated by increased efferent glomerular pressure, the mesangial cell induces transforming growth factor β1 and fibronectin expression [14]. Highlighting the importance of growth factors is the recent demonstration that imatinib (an inhibitor of tyrosine kinase) ameliorates the effect of platelet-derived growth factor (PDGF) in promoting collagen formation, interstitial macrophage infiltrates, and glomerular injury in a mouse model of accelerated diabetic nephropathy [15].

Recent studies have highlighted the role of inflammation in the pathogenesis of diabetic nephropathy: heparanase (which degrades heparan sulfate glycosaminoglycan in extracellular matrix and cell surfaces) is upregulated by hyperglycemia, albumin, and AGEs. Subsequently heparanase is activated postranslationally by tubule-derived cathepsin L to modulate macrophage production of TNF-α, and along with heparan sulfate degradation products, to induce renal injury [16]. In addition, epigenetic phenomena [17] such as DNA methylation and histone modification induced by growth factors, cytokines, AGEs, and oxidized lipids may augment long noncoding RNAs (lncRNA) and TGF-β1-stimulated microRNA (miRNA) formation which may in turn induce fibrosis, podocyte effacement, apoptosis, glomerulosclerosis, and tubulointerstitial fibrosis. The miRNA of great interest in diabetic nephropathy is miR-192, which via a specific target causes mesangial expansion – a hallmark of diabetic nephropathy [18, 19]. Mi-R192 has been shown to arrest G_2/M growth in aristolochic acid nephropathy (Chinese herb nephropathy) [20]. Many other miRNAs have become the focus of interest in chronic kidney disease of varying etiology [21] and renal transplantation [22]. Many single or multiple miRNAs have become targets of directed therapies in a vast array of disease states.

Parathyroid hormone (PTH) is known to have a mitogenic effect in the kidney, and there is upregulation of parathyroid hormone-related protein (PTHrP) in diabetic nephropathy as well as the PTH1 receptor, probably as a result of hyperglycemia, and also through stimulation by angiotensin II [23]. Of more recent interest is the relevance of vitamin D deficiency in the pathogenesis of diabetic nephropathy. Cultured glomerular podocytes have mRNA for 1,25-dihydroxy vitamin D3, vitamin D receptor, and calbindin D28K; in the presence of high glucose, these mRNA concentrations increase [24]. High glucose concentrations also result in the production of fibronectin and collagen IV protein, a process which is blocked by 1,25-dihydroxy vitamin D3. Additionally, 1,25-dihydroxy vitamin D3 blocks the high glucose-induced macrophage-inhibitory protein-1 (MIP-1) [25], the renin–angiotensin system, and TGF-β in mesangial and juxtaglomerular cells [26]. Thus, there seems to be an emerging role for vitamin D in the suppression of diabetic nephropathy; clinical trials are underway in diabetes and other glomerular diseases.

Genetic influences also play a role as evidenced by twin and family studies in type 1 and type 2 diabetes. There is an excess of hypertension, dyslipidemia, insulin resistance, and premature cardiovascular disease in relatives of individuals with proteinuric diabetic nephropathy compared with diabetic individuals with normal albumin excretion [27]. Familial clustering of patients with nephropathy has been observed and may result from environmental influences (poor glycemic or blood pressure control) or from independent genetic influences [28]. Diabetic siblings of patients with combined diabetes and renal disease are five times more likely to develop nephropathy than are diabetic siblings of diabetic patients without renal disease. There is a strong concordance of both nephropathy and renal histopathology in twins with type 1 diabetes [29]. In Brazilian families with two or more diabetic members, the presence of diabetic nephropathy in the propositi is associated with a 3.75-fold increased risk of diabetic nephropathy in the diabetic siblings [30].

In some studies, gene polymorphisms have been reported in the renin–angiotensin pathway, peroxisome proliferator-activated receptor gamma (PPARγ), endothelial nitric oxide, glucose

transporter 1, aldose reductase, and apolipoprotein E [31]. Diabetic nephropathy has been linked to cardiovascular disease and hypertension with inherited abnormalities of sodium-lithium countertransport [32]. In a study of 89 patients with type 1 diabetes, the presence of increased maximal velocity of sodium–lithium countertransport and a parent with hypertension significantly increased the risk of nephropathy [33]. Additionally, parents of patients with type 1 diabetes complicated by nephropathy have decreased survival due to a fourfold increased risk of stroke [34]. Familial clustering and the benefits of angiotensin-converting enzyme (ACE) inhibition in diabetic nephropathy have stimulated investigation into the genetics of the renin–angiotensin system. Increased levels of ACE have been found in patients with type 1 diabetes and nephropathy, particularly in carriers of certain abnormal alleles of the ACE gene [35]. In a study of type 1 patients with CKD compared with type 1 patients with diabetes for at least 15 years without moderately increased albuminuria, the presence of the double deletion (DD) genotype at the ACE locus increased twofold the risk of severe renal failure (CKD-5) [36]. There are also nongenomic and environmental influences on gene polymorphism and physiology which may explain divergent findings of gene polymorphism in diabetic nephropathy [37]. No single gene defect is likely to identify those at risk of nephropathy.

Since CKD is known to be more prevalent in certain ethnic groups – Native Americans, Mexican-Americans, and African-Americans – than in Caucasian-Americans, there should be an increased awareness and increased vigilance of these high-risk populations.

Kidney biopsy is not typically performed to diagnose diabetic glomerulosclerosis, particularly if diabetic retinopathy is present, although hematuria or clinical suspicion for other glomerular pathology may prompt biopsy. The histological picture is diffuse sclerosis of the mesangium and thickening of the basement membrane. Nodular glomerulosclerosis (Kimmelstiel–Wilson kidney) is common and often coexists with global glomerular sclerosis on the same biopsy or autopsy specimen. Classification of severity of pathology by a scoring system of glomerular and interstitial findings has been introduced [38] – no prospective correlations with clinical outcomes have yet emerged.

Clinical Picture and Spectrum of Diabetic Nephropathy

Diabetic nephropathy tends to be a progressive disease that often leads to end-stage renal failure (CKD-5). A succession of stages of nephropathy is well described (Table 2). The clinical problem is that once the disease has become overt, a great deal of renal damage has already occurred, and the opportunity for intervention is limited. When eGFR is >60 ml/min, it may be more accurate to assess kidney function using the CKD-EPI formula [39]. The earliest clinically demonstrable effect of diabetes on the kidney is an increase in glomerular filtration rate, reported in both type 1 [40] and type 2 [41] diabetes. Such hyperfiltration is a harbinger of subsequent deterioration of renal function. It is felt that the increase in glomerular pressure, coupled with hypertrophy, is a stimulus to the processes that ultimately cause glomerular sclerosis. This hypothesis provides a rationale for treatment modalities that lower glomerular capillary pressure (see below). Following the onset of hyperfiltration, there is usually a latency period of 5–20 years during which the basement membranes gradually become damaged, setting off the sequence of events that leads to end-stage renal failure.

Table 2 Classification of chronic kidney disease (CKD) based on glomerular filtration rate (GFR)[a]

Stage 1	GFR > 90 ml/min/1.73 m^2
Stage 2	GFR 60–90 ml/min/1.73 m^2
Stage 3	GFR 30–59 ml/min/1.73 m^2
Stage 4	GFR 15–29 ml/min/1.73 m^2
Stage 5	GFR < 15 ml/min/1.73 m^2

Note: S_{cr} stands for serum creatinine

[a]Modification of diet in renal disease (MDRD) equation for calculation of GFR (calculators found on many internet sites) GFR (mL/min/1.73 m^2) = $175 \times (S_{cr})^{-1.154} \times (Age)^{-0.203} \times (0.742$ if female) $\times$ (1.210 if African-American) (conventional units)

Injury to basement membranes ultimately leads to an increase in glomerular permeability to albumin (vide supra). Normal urinary albumin loss is <10 mg/day. Patients with early diabetic nephropathy develop urinary albumin excretion rates of 30–300 mg/day, moderately increased albuminuria, which may be detected on a 24-h urine specimen or by a "spot" urine albumin:creatinine ratio >0.3 on a random urine specimen. At this stage, a regular urinalysis will be negative for protein. Testing for moderately increased albuminuria should be performed when the patient is feeling well and is at rest, as exercise, fever, acute illness, congestive heart failure, and severe hyperglycemia or hypertension transiently may elevate urinary albumin. Screening for moderately increased albuminuria should be done annually in all patients with type 2 or type 1 diabetes after 5 years or at puberty since urinary albumin excretion increases in all individuals with diabetes at about 20% per year.

Moderately increased albuminuria has been shown to be a good predictor of progressive diabetic nephropathy [42]. About 75–80% of type 1 and 34–42% of type 2 diabetes patients with moderately increased albuminuria will go on to develop renal dysfunction. The next stage is overt proteinuria (severely increased albuminuria), which is detectable on standard urinalysis. Overt proteinuria generally presages a decline in GFR in 75% of type 1 and 20% of type 2 diabetes patients. The rate of decline is variable from patient to patient (up to 20 ml/min of GFR/year), but the development and severity of hypertension are major influences [43]. Since both diabetes and hypertension can cause endothelial injury, there may be a synergistic effect of these processes on glomerular capillaries [44]. In a large cohort of diabetic patients, it has been shown that low eGFR and albuminuria are both independent risk factors of mortality and progression to ESRD; albuminuria was a stronger predictor of mortality, while low eGFR was a stronger predictor of progression to ESRD [45]. Other risk factors for the progression of the renal dysfunction are listed in Table 1.

Up to this point, the renal dysfunction is usually asymptomatic. However, in the next stage the proteinuria increases to nephrotic levels (above 3 g/day or a urine protein:creatinine ratio >3:1). The full-blown nephrotic syndrome usually ensues, with clinical edema and laboratory evidence of hypoalbuminemia and hyperlipidemia. The latter may, of course, worsen the systemic vascular disease. The nephrotic patient is also at risk for hypercoagulability, which can lead to coronary or cerebral arterial occlusion, peripheral ischemia, or renal vein thrombosis with its risk of pulmonary embolism. By this time, diabetic retinopathy is also usually manifested.

Normal kidneys remove around 1/3 of circulating insulin from the blood [46]. Once GFR falls to around 30 ml/min or less (late stage 3–stage 4 CKD), the half-life of insulin is increased by as much as 2.5-fold [47], so small doses of insulin can have a profound and prolonged hypoglycemic effect. In type 2 diabetes, the temporal rhythms of insulin secretion often become abnormal [40].

Patients with diabetic renal disease whose GFR is <60 ml/min/1.73 m^2 (i.e., stages 3–4) are at risk to develop hyporeninemic hypoaldosteronism. This complication is caused by impaired renin release due to atrophy of the juxtaglomerular apparatus, with low aldosterone levels. The atrophy of renin-secreting cells has been variously attributed to concomitant autonomic neuropathy [48], β-adrenergic stimulation-induced renin secretion, volume expansion inhibiting renin production [49], and suppression of renin by retained potassium [50]. The response to endogenous and exogenous mineralocorticoid is impaired by the tubulointerstitial nephritis that usually accompanies chronic diabetic glomerulosclerosis. Clinically, both hyperkalemia and hyperchloremic metabolic acidosis are seen, due to the failure of mineralocorticoid stimulation of K^+ and H^+ secretion in the distal nephron. Drugs that block the renin–angiotensin–aldosterone axis, which are commonly used in the treatment of diabetic nephropathy, may exacerbate these electrolyte disorders, especially the high K^+. Treatment usually involves a low-potassium diet coupled with a diuretic, pharmacologic doses of mineralocorticoid [51], or sodium bicarbonate.

Risk of Other Complications

Patients with types 1 and 2 diabetes mellitus are at risk for vascular complications, and investigators have typically separated macroangiopathy (coronary syndromes, stroke, and peripheral vascular disease) from microangiopathy (retinopathy and nephropathy). The distinction is largely anatomic, as vascular disease involves a common pathophysiology of endothelial injury, activation of the renin–angiotensin–aldosterone (RAA) system, oxidative stress, inflammation and cytokine dysregulation, and disordered repair/remodeling. While there is evidence of simultaneous damage to the microcirculation of the retina and glomerulus, the clinical presentation may be variably represented in the triopathy of diabetes – retinopathy, nephropathy, and neuropathy. Recently, a link between insulin and cardiovascular disease has been described in type 2 diabetes [52], while a reduced cardiovascular risk was associated with pioglitazone [53] with equivalent glucose control. On the other hand, rosiglitazone has been reported to increase cardiovascular risk [54], although subsequent studies failed to confirm this observation.

Treatment of Diabetic Nephropathy

Diabetes mellitus remains the most common cause of incident ESRD in the United States, and the largest contributor to the alarming cardiovascular morbidity and mortality evident in patients with CKD [1]. Treatment involves interventions to prevent the development or forestall progression of CKD attributable to DM, or diabetic kidney disease (DKD). Interventional clinical trials have demonstrated proteinuria to be a surrogate endpoint for both renal and cardiovascular disease in diabetic individuals. Detection of moderately increased albuminuria indicates incipient nephropathy. Serial quantification of proteinuria allows surveillance and identifies progression, with clinical albuminuria suggesting established nephropathy. This section will review and provide treatment recommendations based upon major clinical trials involving diabetic patients and DKD patients and reporting kidney and/or cardiovascular endpoints. Rather than discrete kidney therapies, many interventions may be inseparable from cardiovascular risk reduction in this population, as evident from trials enrolling patients with DKD and reporting combined cardiovascular endpoints. It is notable that some recent trials suggest a divergence between reduced microvascular risk reduction (i.e., decreased proteinuria, doubling of serum creatinine, or development of ESRD) and CV risk reduction (i.e., events/mortality), observations that underlie most of the current therapeutic controversies in diabetes mellitus.

Glycemic Control

Glycemic control is effective in the prevention and treatment of established nephropathy, although practitioners should consider the CV risk and benefit of intensive glycemic control for an individual patient. In type 1 diabetes, intensive insulin therapy (decreasing Hgb A1c to 7.1–7.3% for 6.5–7.5 years) reduces the risk of development of moderately increased albuminuria, progression to severely increased albuminuria, and the rate of urinary albumin excretion (UAE) [55, 56]. Tight glycemic control with an intensive insulin regimen also appears to provide sustained benefit (for more than a decade) in incident moderately increased albuminuria, severely increased albuminuria, and CV events and death, even with later recidivism in the degree of glycemic control [57].

Improved glycemic control also reduces microvascular disease in type 2 diabetes. In the UKPDS, intensive blood glucose control (reducing Hgb A1c to 7.0%) with sulfonylureas, metformin, or insulin over 10 years reduced the risk of microvascular disease (albeit mostly retinopathy requiring photocoagulation) by 25% in older, obese patients, when compared with dietary control [58]. The UKPDS investigators demonstrated a strong association between treatment of hyperglycemia and reduction in diabetic complications, with a 37% microvascular risk reduction for every 1% decrease in mean

hemoglobin A1c [59]. Significantly, the UKPDS patients on intensive insulin therapy gained more weight and had more hypoglycemia; there was no macrovascular benefit or improvement in any of the CV outcomes with the intensive glycemic control. This observed dichotomy between microvascular and macrovascular endpoints with intensive glycemic control in T2DM is also evident in several large recently published clinical trials. In the ADVANCE trial, intensive glycemic control (to A1c of 6.5%) versus standard control (A1c 7.3%) over 5 years in patients with T2DM reduced moderate albuminuria, severe albuminuria, and progression to ESRD [60]. ADVANCE (an international multicenter trial) showed no CV benefit or harm with intensive glycemic control [61]. The debate over optimal glycemic control was amplified with the results of the ACCORD trial (North America only), terminated due to significantly increased all-cause and cardiovascular mortality with intensive (targeting HbA1c <6%) versus standard (HbA1c 7–7.9%) glycemic control. Disproportionate weight gain and the increased use of thiazolidinediones (TZDs) in the intensive therapy group of ACCORD have been suggested as causes for this increased CV mortality.

Nevertheless, glycemic control remains a mainstay of DKD prevention and treatment, with target HgbA1c likely <7% for most adult diabetic patients. Treating physicians should be aware of the risks of weight gain and hypoglycemia that may accompany the insulin therapy required for intensive glycemic control. Targeting HgbA1c <6.5% may be acceptable in individual patients without established coronary artery disease, high CV risk, and who do not demonstrate subsequent episodes of hypoglycemia [62].

Blood Pressure Reduction

Blood pressure (BP) management is another well-established intervention for diabetic nephropathy. The approach to optimal management in diabetic patients is informed by prospective observational data. UKPDS-36 reported that systolic BP less than 120 mmHg confers the lowest risk of microvascular complications, with a more than 13% risk reduction observed for each 10 mmHg decrease in systolic BP [63]. Optimal BP lowering cannot, however, be determined from trial data, as there are insufficient randomized trials enrolling hypertensive diabetic patients (measuring attenuation of DKD or CV events) with such BP lowering. Unfortunately, recent changes in guidelines have added to confusion and uncertainty regarding BP treatment thresholds and targets for many diabetic individuals. The current evidence-based (JNC8) threshold for initiating pharmacologic therapy is 140/90 mmHg and the target for lowering of BP is <140/90 mmHg in patients with DM as well as CKD, recommendations based on expert opinion [64]. In fact, the authors point to scant high-quality evidence in diabetic individuals for CV or CKD benefit to BP lowering below 150 mmHg. More intensive blood pressure reduction may decrease microvascular complications of diabetes, and may have either benefit or harm with regard to macrovascular endpoints, and clinicians should be aware of the lack of evidence in this area. Some basis for concern is the diabetic group of the INVEST trial, in which patients with hypertension had similar all-cause mortality, nonfatal MI or nonfatal stroke with tight control (<130 mmHg), and usual control (at or above 130 to <140 mmHg) of systolic blood pressure, but increased all-cause mortality in the tight control group [65].

Finally, a threshold for initiating therapy below 140 mmHg in patients with diabetes (blood pressure lowering in diabetic patients without hypertension) as primary prophylaxis against proteinuria/nephropathy is also uncertain [66].

In summary, diabetic patients probably have proteinuria reduction and attenuated DKD with BP lowering to <140/90 mmHg, a level – based on available data – that also likely confers benefit in mortality and CV risk reduction.

RAAS Blockade

Renin–angiotensin–aldosterone system blockers are the preferred first-line agents for diabetic

patients with hypertension or nephropathy in many guideline statements [67]. This preference is sensible in view of the pathophysiologic activation of the RAAS system among diabetic subjects and the advantageous effects of some antagonists on systemic hypertension, intraglomerular hypertension, and proteinuria. Decreased risk of doubling of serum creatinine, death, dialysis, and transplant as well as progression to clinical proteinuria has been demonstrated with angiotensin-converting enzyme inhibitors (ACEIs) in the Collaborative Study Group [68] and Micro-Hope [69] trials, respectively. Angiotensin receptor blockers (ARBs) also decreased progression to clinical albuminuria in IRMA-II [70] and to doubling of serum creatinine, progression to CKD, and death in both the RENAAL [71] and IDNT [72] trials. Claims of specific renoprotective benefit in many of the trials may be confounded by insufficient BP data and unequal blood pressure reduction as compared with a placebo. Furthermore, in trials where equivalent blood pressure reduction was achieved, ACEIs were not superior to a β-blocker [73] nor a dihydropyridine calcium-channel blocker [74] in reducing proteinuria. A meta-analysis [75] has also concluded that when compared with other active intervention providing equal BP reduction, ACEIs and ARBs provide no specific renoprotection in diabetic patients with regard to creatinine, GFR, or progression to CKD, although they improved proteinuria. The preponderance of evidence suggests that achievement of sufficient blood pressure reduction appears to be more beneficial than use of any particular class of antihypertensive agent. Nevertheless, it is apparent that patients with DKD will need multiple medications to achieve BP control, and intervention with RAAS antagonists is likely to have a role in BP lowering, proteinuria reduction, and CV risk reduction in this high-risk population.

Angiotensin II (AII) and aldosterone (more below) likely contribute to glomerulosclerosis and proteinuria in experimental nephropathy [76], and aldosterone breakthrough in diabetic patients on ACEI monotherapy is associated with refractory proteinuria [77] and declining GFR [78]. Aldosterone breakthrough is likely the result of AII breakthrough due to either inadequate ACE inhibition [79] or non-ACE-dependent generation of AII [80]. Therapeutic methods to antagonize breakthrough have been explored, including high-dose ARB therapy [81], combination ACEI and ARB [82], and use of ARB or ACEI with aldosterone antagonists (MRAs) [83]. While these measures have all been demonstrated to further reduce proteinuria (and in most studies, provide additional BP reduction) in diabetic nephropathy, there is concern for increased adverse outcomes and hyperkalemia, and such measures are not advised in advanced CKD without potassium monitoring. Furthermore, combination ARB and ACEI cannot be recommended in DKD, following the publication of trial data showing lack of benefit, increased AKI, and intolerable hyperkalemia [84].

Aldosterone is a steroid hormone that activates mineralocorticoid receptors, regulating sodium and potassium excretion, and exerting profibrotic and proinflammatory effects [85]. Mineralocorticoid receptor antagonists (MRAs) prevent renal fibrosis, mesangial expansion, and glomerulosclerosis, via their action on TGF-β1, PAI-1, local oxidative stress, and endothelial function [86, 87]. Aldosterone is associated with insulin resistance and gluconeogenesis, and as insulin sensitivity decreases, nighttime hypertension and drug-resistant hypertension is more likely to occur. Mineralocorticoid receptor antagonists lower renin levels and blood pressure, and the effect is more prominent in patients with a low renin state than a high renin state [88]. In diabetic patients with uncontrolled hypertension and on ACEI/ARBs, adding eplerenone in a dose of 37.5 mg/day can reduce daytime and nighttime blood pressure [89, 90]. Multiple studies have investigated the effects of MRAs on proteinuria. In patients with diabetic nephropathy, an MRA can be added to ACEI/ARBs to improve blood pressure, insulin resistance, and DKD progression [91], with a pronounced effect on proteinuria [92]. Patients taking ACEIs or ARBs with spironolactone may have a greater degree of proteinuria reduction than with other methods of combined RAAS blockade. A well-powered

study involving eplerenone (a more selective MRA) in combination with ACEI, suggests that eplerenone is well tolerated in diabetic patients, and provides proteinuria reduction at 50 mg, independent of blood pressure reduction [93]. Unfortunately, there are no adequately powered studies reporting clinical outcomes from combination aldosterone antagonists and ACEIs or ARBs in patients with DKD. It should be noted that the addition of mineralocorticoid receptor antagonists (spironolactone and eplerenone) to therapy including ACE inhibitors or ARBs have shown mortality benefit in patients with congestive heart failure and left ventricular dysfunction post-myocardial infarction [94, 95]. It is the practice of the authors, in our dedicated diabetic nephropathy clinic, to begin ARB monotherapy and add mineralocorticoid receptor antagonists in any patients observed or suspected to have aldosterone breakthrough, with careful surveillance for hyperkalemia or AKI.

Aliskiren, an alternative agent for blockade of the renin–angiotensin–aldosterone axis through direct renin inhibition, has been approved in the treatment of hypertension and has been examined for renoprotective effects. The AVOID trial (Aliskiren in the Evaluation of Proteinuria in Diabetes), was a multicenter, randomized, double-blind, placebo-controlled trial that examined the effect of aliskiren in 599 type 2 diabetes patients already on maximal dose of losartan (ARB). After 24 weeks of treatment with aliskiren, there was a significant 20% reduction of urinary albumin-to-creatinine ratio (UACR) [96]. However, the ALTITUDE trial (Aliskiren Trial in Type 2 Diabetes Using Cardio-Renal Endpoints) was terminated early as the aliskiren arm showed no benefit in the primary outcome and increased rates of stroke and other adverse events, namely, hyperkalemia and hypotension [97]. Combination therapy including a renin antagonist and an ACEI or ARB cannot be recommended as an intervention to attenuate DKD.

In summary, glycemic control, systemic blood pressure reduction, and the use of ACE inhibitors or ARBs as monotherapy to antagonize the RAAS system are the established therapies for DKD intervention. Intensive glycemic control (A1c $< 6.5\%$) may, in some patients, increase CV risk. Despite additive reduction in blood pressure and proteinuria, combination of ARB with either ACE inhibitor or direct renin inhibitor cannot be recommended due to increased adverse events in multiple studies. The combination of ARB or ACE inhibitor with aldosterone antagonists on clinical outcomes in DKD has not been adequately studied, but may be an effective therapeutic strategy.

Investigational Therapeutic Strategies

Despite established therapy – tight glycemic control, blood pressure reduction, and renin–angiotensin–aldosterone system blockade – to delay the progression of diabetic nephropathy, current strategies remain unsatisfactory, and a significant proportion of diabetic patients will ultimately develop progressive CKD and ESRD. There is an ongoing search for novel therapeutic targets and clinical investigation of promising therapies for diabetic nephropathy.

Hyperglycemia triggers intracellular events in glomerular and tubular cells including generation of reactive oxygen species, protein kinase C, mitogen-activated protein kinase activation, and transcription factor inductions [98–100]. With these mechanisms, high glucose enhances inflammation and fibrosis [101]. Findings also suggest that high glucose levels activate the Janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling cascade [102, 103]. A phase II trial is currently investigating the effect of an oral JAK1 and JAK2 inhibitor, baricitinib – initially developed for rheumatoid arthritis rather than renal protection. Baricitinib will be evaluated as an adjuvant to RAAS blockade in diabetic subjects with kidney disease and severely elevated proteinuria. The primary outcome measure is a change from baseline urinary albumin-to-creatinine ratio (UACR) at 24 weeks of treatment [104].

Certain hypoglycemic agents have been speculated to have renoprotective effects. Thiazolidinedione (TZD) studies have shown mixed results. The PROactive (Prospective Pioglitazione Clinical Trial in Macrovascular Events) post hoc

analysis revealed that CKD patients who received pioglitazone were less likely to have cardiovascular and cerebrovascular events than placebo. Moreover, the study showed a greater improvement in estimated GFR in the pioglitazone group compared to placebo [105]. However, in a meta-analysis of TZD trials involving both pioglitazone and rosiglitazone, the 2860 patients involved did not show significant reduction in albuminuria [106]. Dipeptidyl peptidase-4 (DPP-4) inhibitors are another class of glucose-lowering agents found to be renoprotective in experimental animal models: Alter et al. showed that combined treatment with linagliptin and the ARB telmisartan in mice models had a greater reduction in albuminuria than either telmisartan or linagliptin alone [107]. In a Japanese patient cohort, 12 weeks of alogliptin showed a significant reduction in albuminuria in type 2 diabetic patients [108]. In a pooled analysis of four similarly designed randomized, double-blind, placebo-controlled trials, the addition of linagliptin to RAAS blockade in type 2 diabetes with chronic kidney disease led to a significant reduction in albuminuria [109]. Mori et al. conducted an open-label, prospective randomized study in 85 patients with type 2 diabetes and stable RAAS blockade regimens comparing the effect of sitagliptin on moderately increased albuminuria compared with other oral hypoglycemic agents. The study revealed that sitagliptin significantly lowered urinary albumin excretion at 6 months [110]. Ongoing clinical trials of DPP-4 inhibitors in patients with DKD will provide evidence involving clinical rather than surrogate renal endpoints.

Pirfenidone (PFD), an antifibrotic agent that inhibits production of both TGF-α and TGF-β, has shown potential in diabetic nephropathy treatment. In animal models, PFD decreased serum levels of TGF-α and TGF-β, disrupting signaling pathways and gene transcription responsible for extracellular matrix deposition and production of reactive oxygen species. In mice models, Rao et al. showed that PFD administration resulted in significant reduction in mesangial matrix expansion and expression of renal matrix genes, although treatment did not affect albuminuria [111]. A small randomized, double-blind, placebo-controlled study of 77 subjects with diabetic nephropathy was conducted by Sharma et al. Although the dropout rate was higher in the high-dose PFD group, results demonstrated an increase in GFR in the lower dose PFD group compared to placebo [112].

Glycosaminoglycans (GAGs) are essential in the composition of the glomerular basement membrane and extracellular matrix. GAGs also play a major role in providing the anionic charge through the presence of heparan sulfate. The anionic charge renders the glomerular basement membrane less permeable to albumin. A study in rat models demonstrated that exogenous GAG administration had a favorable effect on GBM morphology and albumin excretion rates [113]. Smaller studies also showed promise in mitigating moderately and severely elevated albuminuria in both type 1 and type 2 diabetes [114–116]. Sulodexide is a purified mixture of sulfated glycosaminoglycans that contains low-molecular-weight heparin, high-molecular heparin, and dermatan sulfate. The Di. N.A.-S. study – a randomized, double-blind, placebo-controlled, multicenter trial – demonstrated that high doses of sulodexide significantly improved albuminuria, an action that persisted for 4 months after discontinuation [117]. In 2012, Packham et al. conducted the Sun-MACRO trial – another randomized, double-blind, placebo-controlled study – that evaluated the renoprotective effects of sulodexide in patients with type 2 diabetes, renal insufficiency, and significant proteinuria, on maximal doses of ARBs. The trial was terminated after enrolling 1248 patients as the sulodexide group failed to demonstrate substantial benefit compared to the placebo [118].

Protein kinase C-β plays a major role in the signal pathway responsible for cellular growth, fibrosis, and tissue injury seen in diabetic nephropathy. Ruboxistaurin, a selective protein kinase C-β inhibitor, showed early promise in diabetic rat models. A randomized, double-blind, placebo-controlled, multicenter pilot study was performed to evaluate the effect of ruboxistaurin in patients with type 2 diabetes with persistent albuminuria despite treatment with ACE inhibitors or ARBs. After 1 year, the ruboxistaurin

group had a significant decrease in UACR compared to the placebo group [119]. In contrast, a retrospective analysis of data of 1157 patients from 3 trials originally designed to assess the effect of ruboxistaurin on diabetic retinopathy (the PKC-Diabetic Retinopathy Study, PKC-Diabetic Macular Edema Study, and the PKC-DRS2), showed no difference in kidney outcomes between treatment and placebo groups [120].

Selective inhibitors of sodium-glucose co-transporter 2 (SGLT-2) block the reabsorption of glucose in the proximal tubule. By increasing urinary glucose excretion, the use of SLGT-2 inhibitors has proved to be another effective strategy in achieving optimal glucose control. While experimental animal models have shown that selective inhibition of SLGT-2 does lead to improvement of diabetic nephropathy, there are few human clinical trials [121, 122]. A multicenter, phase III, randomized, double blind, noninferiority trial – CATATA-SU (Canagliflozin Treatment and Trial Analysis versus Sulfonylurea) – consisting of 1450 subjects compared the efficacy of canagliflozin with glimepiride in patients with type 2 diabetes inadequately controlled with metformin. The SGLT-2 inhibitor groups showed greater reductions in HbA1c, initial improvement followed by stabilization of eGFR as compared to eGFR decline with the sulfonylurea, but more adverse events such as genital mycotic infections, urinary tract infections, and osmotic-related diuresis events [123].

Breaking Clinical Trials

Dietary advanced glycation end products (AGEs) increase oxidative stress and inflammation and contribute to the development of diabetes and diabetic complications. Restriction and elimination of dietary AGEs is an emerging therapy in the treatment of diabetic patients [124]. Sevelamer carbonate prevents the absorption of dietary AGEs, and in a 6-month trial in patients with stage 2–4 DKD, HbA1c >6.5%, and albuminuria (>200 mg/g of creatinine), sevelamer reduced AGEs and oxidative stress but did not reduce HbA1c or proteinuria [125].

Bardoxolone methyl is a synthetic antioxidant and anti-inflammatory molecule that activates nuclear erythroid 2-related factor (Nrf2) transcription pathway and inhibits nuclear factor kB (NF-kB) [126]. Bardoxylone was noted in early clinical investigation to improve eGFR, and in a phase 2 study (BEAM) over 52 weeks, Bardoxylone combined with RAAS blockade increased eGFR in patients with T2DM and stage 3b-4 CKD [127]. A subsequent phase 3 study (BEACON) of bardoxolone methyl with background therapy including RAAS blockade was terminated early due to safety concerns [128]. Although therapy increased eGFR compared to placebo (5.5 ml/min/1.73 m^2 versus −0.9 ml/min/1.73 m^2), there was an increased risk of heart failure, nonfatal myocardial infarction, and nonfatal stroke, as well as increased systolic and diastolic blood pressure, and brain-type natriuretic peptide (BNP).

Vitamin D receptor (VDR) activators have been used to decrease proteinuria. Observational studies have shown that vitamin D deficiency is associated with increased all-cause mortality, hypertension, inflammation, immune dysfunction, endothelial dysfunction, and cardiovascular disease [129, 130]. In animal models of diabetes, vitamin D deficiency increased albuminuria, whereas treatment with the VDR activators calcitriol or paricalcitol had antiproteinuric and anti-inflammatory effects [131]. The VITAL and PROCEED trials investigated the effect of VDR activators in diabetic subjects with chronic kidney disease. VITAL randomized diabetic patients with albuminuria receiving ACEIs or ARBs to either placebo or paricalcitol (1 or 2 mcg/day) for 24 weeks, with a primary endpoint of change in mean UACR. The 2 mcg/day paricalcitol dose decreased proteinuria and lowered systolic blood pressure and eGFR, with a renoprotective effect postulated from suppression of renin, and/or antiproliferative and antifibrotic effects of VDR activation [132]. The PROCEED trial investigated the effect of paricalcitol (2 mcg/day) in diabetic patients on stable RAAS blockade

without advanced CKD (Cr < 2 mg/dl) and urinary albumin-to-creatinine ratio >300 mg/24 h [133]. Paricalcitol decreased eGFR reversibly only by 5%. Some side effects of high-dose paricalcitol included acute myocardial infarction, coronary artery disease, chest pain, fluid overload, cerebrovascular accident, and hypercalcemia.

Endothelins are small vasoactive peptides with pleiotropic actions that contribute to hypertension, albuminuria, insulin resistance, inflammation, fibrosis, and endothelial dysfunction [134]. Endothelin 1 via activation of the endothelin type A receptor may have a central role in the pathogenesis of proteinuria, and endothelin-receptor antagonists have been evaluated for the prevention of progression of diabetic nephropathy. The ASCEND study investigated the use of avosentan on overt diabetic nephropathy [135]. Avosentan was compared at 2 dosage regimens of 25 mg/day or 50 mg/day against placebo with the primary outcome of doubling of serum creatinine, ESRD or death; secondary outcomes were changes in UAE and eGFR as well as cardiovascular outcomes. The trial was terminated early due to unusually high number (74%) of deaths due to cardiovascular causes in the treatment groups compared to the placebo group. Although Avosentan reduced albuminuria by 40–50%, there was also a higher incidence of pulmonary edema, CHF and decrease in hemoglobin, hypoglycemia, and hypotension. Due to concern for increased mortality and known adverse events of similar antagonists, an ongoing study has excluded patients with peripheral edema, elevated BNP, and history of CHF or pulmonary disease. The SONAR phase III trial is currently assessing the effect of atrasentan versus placebo as an adjuvant to RAS blockage in patients with type 2 DM, DKD with eGFR of 25–75 ml/min/1.73 m^2, and UACR 300–5000 mg/g. The study completion date is July 2018 [136].

Despite some success with proteinuria reduction or other surrogate endpoints, few novel therapies have been demonstrably safe and effective in the prevention of DKD. Clinicians eagerly await the results of ongoing and future clinical trials.

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