Improving Outcomes in Patients with Diabetic Nephropathy

Robert D. Toto, M.D.

Professor, Department of Internal Medicine

The University of Texas Southwestern Medical Center Dallas

Medical Grand Rounds

October 28, 2011

I. Introduction and Background

Kidney disease is a common complication of diabetes mellitus afflicting approximately one-third of all patients. It is now the leading cause of end-stage renal disease in the Western world and in the accounts for nearly 50% of new cases of end-stage kidney disease in North America.[1,2] The disease is incurable and carries with it a significant increase in risk for cardiovascular morbidity and mortality. It usually progresses ultimately to end-stage or leads to death from a cardiovascular event. The median five year survival rate of patients with type 2 diabetes on maintenance hemodialysis of 25% is similar to stage III colon cancer. Moreover, kidney disease disproportionately afflicts certain racial and ethnic groups for Hispanics, Blacks and Native Americans as compared to non-Hispanic whites. For example, the incidence ratio for end-stage renal disease in Hispanics is approximately 5 to 1.[3] Thirty years ago, the reported prevalence of kidney disease in those with type 2 diabetes was approximately 5% and today this group of patients comprises the great majority of patients with diabetes with kidney disease.[4,5] The prevalence of type 2 diabetes is expected to increase over the next 20 years and without improved methods of prevention, detection, diagnosis and treatment will result in an ever increasing number of patients requiring renal replacement therapy. This in turn will increase health care costs and loss of human productivity.[6,7]

The marked increase in both the incidence and prevalence of ESRD in the United States appears to be driven by the increasing prevalence of type 2 diabetes in the general population (**Figure 1**).

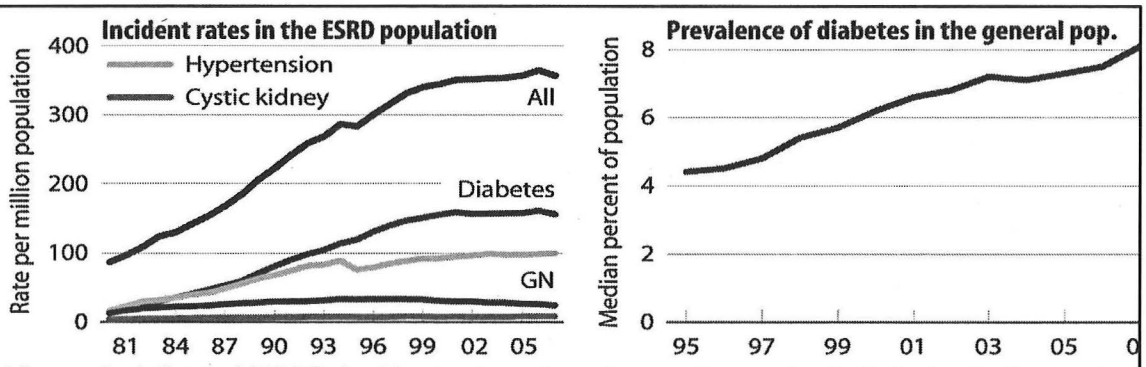


Figure 1 Adjusted ESRD incident rates, by primary diagnosis, & diabetes in the general population (ref 1).

Advances in understanding both the pathogenesis and treatment of diabetic nephropathy have occurred since the publication of trials demonstrating a renal benefit of angiotensin receptor 1 blockade a decade ago.[8-10] Despite overall improvements in glycemia, blood pressure and blockade of the renin-angiotensin aldosterone system, kidney disease continues to progress to ESRD in most patients with type 1 and type 2 diabetes.[11,12] This review focuses on clinical trials aimed at testing interventions on glycemic control, blood pressure, anemia, dyslipidemia and inflammation/oxidative stress contributing to a better understanding of disease prevention and management. This new knowledge is now being translated into better care for our patients and should that will improve outcomes.[12-17] Furthermore, new discoveries have identified a key role for inflammatory and oxidative stress pathways in the pathogenesis of diabetic nephropathy. These discoveries have now been translated into clinical trials in humans with diabetic nephropathy and have the potential to slow, halt, and perhaps prevent this dreaded disease.[18,19]

II. Screening and Detection

All patients with diabetes mellitus should be screened annually for kidney disease by measurement of urine albumin to creatinine ratio (preferably on a first morning voided urine), and a serum creatinine (preferably fasting) to estimate glomerular filtration rate. The presence of persistent microalbuminuria or macroalbuminuria or an estimated glomerular filtration rate $\leq$ 60 ml/min/1.73 m^2 (measured on 2 occasions within a 3 month interval) in a patient with either type 1 or type 2 diabetes should raise the

possibility of diabetic nephropathy.[20-22] Both albuminuria and elevated serum creatinine are useful for detection of kidney disease but neither is specific for the histopathologic diagnosis of diabetic glomerulosclerosis (see below). The combination of albuminuria and an elevated serum creatinine (decreased estimated GFR) portends worse outcomes in patients with diabetes.

Diagnosis: Diabetic Nephropathy is a Clinical Syndrome

The National Kidney Foundation clinical practice guidelines using the following criteria for the diagnosis of chronic kidney disease should be attributed to diabetes: if macroalbuminuria ($\geq$ 300 mg/g albumin/creatinine) is present of if microalbuminuria is present in the presence of diabetic retinopathy or in type 1 diabetes of at least 10years' duration. Note that the diagnosis does not require a kidney biopsy and indeed most patients with diabetic nephropathy do not undergo a kidney biopsy. It is also clear from this definition that the hallmark of diabetic nephropathy is the presence of an increase in urine albumin. In many studies albuminuria strongly correlates with adverse kidney and cardiovascular outcomes and death.[12,23,24 25,26] Typically, these patients have marked albuminuria, arterial hypertension, progressive decline in glomerular filtration and excessive cardiovascular event rates (including myocardial infarction, sudden cardiac death and heart failure).[27]

Diabetic nephropathy in the absence of albuminuria

Whereas albuminuria is an excellent screening tool for detecting kidney disease it is non-specific and its sensitivity is altered by use of drugs that block the renin-angiotensin system, the magnitude is affected by blood pressure level and dietary sodium intake, and it may be transient and in some cases spontaneously reversible. Moreover, an increasing body of evidence indicates that patients with progressive chronic kidney disease in the setting of diabetes may have little or no detectable albuminuria. For example, a substantial fraction of patients with chronic kidney disease in the setting of diabetes have kidney disease other than classical diabetic nephropathy.[28 29] Like those with albuminuria, patients with diabetes and kidney disease without albuminuria share characteristics of hypertension, declining glomerular filtration and severe cardiovascular disease. However, this subgroup of patients does not exhibit a strong correlation between albuminuria and progressive decline in glomerular filtration rate. For instance, in patients with type 1 diabetes, normotension and normoalbuminuria, decline in GFR can occur even in the absence of substantial change albuminuria.[30]

Kidney biopsy

As noted above a renal biopsy is not required for the diagnosis of diabetic nephropathy. Kidney biopsy studies in patients with type 1 and type 2 diabetes have clearly shown the emergence of four different histological patterns as follows: 1) Diabetic Glomerulosclerosis; 2) Diabetic Glomerulosclerosis and a non-diabetic glomerular disease; 3) Non-diabetic glomerular disease without diabetic glomerulosclerosis; and 4) Arterial and arteriolar sclerosis without diabetic glomerulosclerosis.[31-36] Therefore, patients that clinicians label as diabetic nephropathy actually represent a rather heterogeneous group. Still, most clinicians do not perform a kidney biopsy in a patient with diabetes who fulfills the NKF criteria. Instead, biopsy is recommended for patients with diabetes mellitus in whom a primary glomerular disease is suspected on the basis of hematuria, accelerated hypertension and/or accelerated decline in glomerular filtration. Importantly, patients with diabetes without diabetic glomerulosclerosis on biopsy generally have a better prognosis overall.[37]

Since most clinical trials of diabetic nephropathy intervention do not perform at least pre-intervention biopsies they likely include a heterogeneous group as outlined above. This has major implications for trials designed to improve renal outcomes because interventions that may target pathogenetic factors in diabetic glomerulosclerosis may not be as effective for the pathogenetic factors involved in the

other histologic lesions. Thus in a clinical trial in which patients do not undergo kidney biopsy, non-differential misclassification could result in a negative study and a potentially effective therapy may be discarded. A better understanding of the impact, or lack thereof, of interventions, may arise from performing a kidney biopsy at the onset to define the kidney disease particularly in the type 2 diabetes population in which there appears to be more heterogeneity on kidney biopsy. In fact, interventions with proven benefit did not employ biopsy for the diagnosis of diabetic nephropathy and not surprising the response to interventions is heterogeneous and in some individuals provides no benefit at all. This is underscored by the relatively and small effect sizes of 16-20% for angiotensin receptor blockers-a mainstay of therapy for patients with type 2 diabetes and nephropathy.[38] No prospective, long-term intervention trials have attempted to compare outcomes among those with or without albuminuric diabetic nephropathy.

Non-Invasive methods

Both genetic and proteomic methods have been used in attempt to detect early kidney disease and to predict response to treatment with drugs that block the renin-angiotensin system in patients with diabetes.[39-48] In addition a variety of other urinary biomarkers of renal injury have been investigated as a means of detecting early kidney disease and predicting regression of albuminuria.[49 50,51] However, these markers have several limitations including the fact that renal biopsies were not included, albuminuria was used as the marker for kidney disease or no kidney disease and none of the markers associated with early onset nephropathy were validated in a large external populations. Thus, while urine and plasma biomarkers offer great potential for early detection, prognosis and monitoring of kidney disease at the present time urine and plasma biomarkers are important research tools and not available in the clinic. In and effort to predict response to drug therapy in patients with diabetic nephropathy, our laboratory has focused on urinary biomarkers that might predict differential responses to treatment with drugs that block the renin-angiotensin system.[42] This approach may prove beneficial for selecting the right drug for the right patient at the right time. Recent studies using blood-oxygen level magnetic resonance imaging show promise in identifying regional hypoxia in the kidney and tubulointerstitial disease; however, longitudinal studies evaluating the predictive value of these observations is lacking.[52]

In summary, diabetic nephropathy is a clinical syndrome that comprises a heterogeneous a group of patients with varying histopathology and variable degrees of albuminuria. While albuminuria is a harbinger of a worse prognosis, the clinical syndrome of diabetic nephropathy with or without albuminuria includes hypertension, progressive decline in glomerular filtration and heightened cardiovascular morbidity and mortality. Novel laboratory methods using genetic and proteomic methods are under development to improve detection and treatment of diabetic nephropathy.

III. Cardiovascular complications and mortality

It is important to understand that kidney disease is a marker of increased risk for both morbid and fatal cardiovascular events. Cardiovascular disease is more prevalent in patients with diabetic nephropathy as compared to those without nephropathy. Thus, a large body of evidence indicates that the presence of microvascular disease, particularly albuminuria, in diabetic nephropathy increases risk for macrovascular complications such as myocardial infarction, heart failure, sudden cardiac death and cerebral vascular accident.[53] Data from administrative databases, retrospective and prospective cohort studies, Federal registries and clinical trials have consistently found that this is the case.[26,54-56] Moreover, cohort studies and clinical trials also report that patients with diabetic nephropathy are at higher risk for dying of a cardiovascular event than progressing to end-stage

kidney disease.[8,12,57,10 58] An example of the impact of Microvascular disease (albuminuria) on outcomes in patients with type 2 diabetes is illustrated in **Table** 1 below.

Table 1 Comparison of Outcomes in Subjects with and without Microalbuminuria (HOPE trial)

	Microalbuminuria (N = 1100)	Nomoalbuminuria (N =2398)	Adjusted Risk* (95% CI)
MI, CVA or CV Death	25.0	13.9	1.97 (1.68-2.31)
All-cause mortality	18.6	9.4	2.09 (1.84-2.38)
CHF hospitalization	8.5	2.5	3.70 (2.64-5.17)

Among 3498 patients with type 2 diabetes enrolled in the Heart Outcomes Protection Study, those with microalbuminuria as compared to no albuminuria had 2-5 fold higher rates of major cardiovascular events. The cardiovascular event rates are higher among patients with more advanced kidney disease as comorbidities mount.[59] It should be noted that multiple cardiovascular risk factors including hypertension, hyperglycemia, dyslipidemia, smoking, obesity and family history are highly prevalent among patients with diabetic nephropathy and no doubt contribute to the high rates of cardiovascular complications. These findings have transformed the way nephrologists and cardiologists think about and design clinical trials in patients with diabetic nephropathy. Thus, several large-scale outcomes trials in patients with kidney disease focus on both cardiovascular and renal endpoints in their design (see below).

IV. Predictors of Outcomes

Older age, poor glycemic control, albuminuria, elevated serum creatinine, hypertension, family history, ethnicity, race, hypoalbuminemia and more recently cardiac markers including troponin T and NT-proBNP have been reported to be associated with increased risk for ESRD and cardiovascular death (**Table 2**.).[60] Importantly, many of these factors are amenable to interventions that indeed have been shown to lower the risk for these events including tighter glycemic control, blood pressure lowering, angiotensin II blockade and reducing albuminuria.

Table 2: Predictors of Adverse Renal and Cardiovascular
Age
Hyperglycemia
Hypertension
Albuminuria
Activation of renin-angiotensin system
Family History
Ethnicity (Black, Native American, Asian Pacific Islander)
Race (Hispanic)
Hypoalbuminemia
NT-proBNP and Troponin
C-reactive protein
Smoking

V. Pathogenesis: Focus on inflammation and oxidative stress

The pathogenesis of diabetic nephropathy is complex and incompletely understood. Family history and sibling studies indicate a strong genetic predisposition to nephropathy.[61] While polymorphisms in genes regulating nitric oxide metabolism have been associated with accelerated decline in kidney function in humans, specific gene mutations causing nephropathy in humans with either type 1 and 2 diabetes remain elusive.[62-66] Hyperglycemia and hemodynamic factors are important factors in the pathogenesis and pathophysiology of diabetic nephropathy. Hyperglycemia induces cell hypertrophy, extracellular matrix accumulation, inflammation and oxidative stress in the kidney. For example, hyperglycemia increases intracellular glucose that in turn: 1) activates RhoA and downstream mediators of

inflammation, and 2) increases intracellular glycerol upregulating of protein kinase C isoforms. Selective inhibition of the beta-1 isoform with ruboxistaurin slows kidney disease progression in animal models and reduces albuminuria in patients with type 2 diabetes and nephropathy.[67,68]

Activation of the renin-angiotensin system also plays a key role in the pathogenesis of kidney injury and progression of kidney disease again through multiple mechanisms including activation of inflammation and oxidative stress. In addition to causing renal vasoconstriction, renal hypertrophy vascular injury and hypertension, angiotensin II up regulates a number of inflammatory mediators in part through activation of NADPH oxidase. [69-76] This action is coupled to the formation of oxidative stress and down regulation of nitric oxide-a vasodilator and anti-proliferative molecule. Still, the primary renal injury signal and the molecular pathogenesis have not been clearly elucidated.

Several recent reviews on the subject of pathogenesis emphasize the role of inflammation and oxidative stress particularly in animal models of nephropathy.[77] Over the past 10 years an increasing body of experimental animal models of diabetic nephropathy have led to new insights that clearly indicate an important role for inflammation and oxidative stress as major factors in the development and progression of kidney disease.[78-92] Because an exhaustive review of induction and mechanisms of inflammation and oxidative injury is beyond the scope of this discussion, focus will be placed on key regulatory elements involved in inflammation and oxidative stress in diabetic nephropathy.

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that has emerged as an important modulator of animal models of renal disease including diabetic nephropathy.[93,94] Studies in mouse models of diabetic nephropathy are popular and have provided new insights into the molecular pathogenesis of glomerular injury. For example genetic deletion of the mammalian Target of Rapamycin receptor mTORC) has been shown to cause severe loss of glomerular podocytes, proteinuria and progressive glomerulosclerosis. In these studies loss of mTOR activity was found to be crucial for maintenance of normal glomerular barrier function and predisposed to glomerular injury and disease. And, in mice by genetically reducing mTORC1 copy number in podocytes prevented glomerulosclerosis and significantly ameliorated the progression of glomerular disease in diabetic nephropathy. These results suggested the possibility that mTOR inhibition can protect podocytes and prevent progressive diabetic nephropathy.[95] Several studies in rodent models of diabetic nephropathy have demonstrated that inhibitors of mTOR such as rapamycin may prevent or ameliorate diabetic renal injury and reduce proteinuria.[93,94,96,97]

Nuclear factor erythroid-2-related factor-2 (Nrf2) and diabetic nephropathy

Activation of NK-κB, a master switch in cellular inflammation, leads to upregulation of a number of inflammatory mediators including chemokines, cytokines, reactive oxygen species and enzymes (COX-2, iNOS) (**Figure 2 initiation phase**). Several studies indicate that NF-κB expression is upregulated in diabetic nephropathy in animals and man.[98-105] However, activation of inflammation is accompanied by counter inflammatory signaling that dampens the activation of NF-KB and its potent and broad downstream effects to increase inflammation and oxidative stress. As illustrated in **Figure 2 (resolution phase)** this counter regulatory cascade involves the release of the transcription factor Nrf2. Nrf2 regulates the basal and inducible expression of numerous detoxifying and antioxidant genes and its action is repressed by the cytoplasmic protein The Kelch-like ECH associated protein1 or Keap1 (**Figure 2**). Under quiescent conditions, Nrf2 is anchored in the cytoplasm through binding to Keap1, which, in turn, facilitates the ubiquitination and subsequent proteolysis of Nrf2. Activation of Keap1 by anti-inflammatory mediators leads to inhibition of IKKβ, which in turn blocks activation of NF-κB. Nrf2 is translocated into the nucleus where it upregulates superoxide mutase and a variety of

anti-oxidant and anti-inflammatory mediators that counteract the inflammatory effects of NF-κB activation.

Nrf2 can be activated by cyclopentane prostanoids and triterpeniods such as bardoxolone. Importantly Nrf2 expression is reduced in animal models of chronic kidney disease.[106] Bardoxolone methyl, a derivative of the natural product oleanolic acid interacts with cysteine residues on Keap1, allowing Nrf2 translocation to the nucleus and subsequent up-regulation of a multitude of cytoprotective genes. It also The structure and activity profile of bardoxolone methyl resemble those of the cyclopentenone prostaglandins, endogenous Nrf2 activators that promote the resolution of inflammation. Like cyclopentenone prostaglandins, bardoxolone methyl exerts anti-inflammatory effects by inhibiting the proinflammatory NF-κB pathway and production of reactive oxygen species (**Figure 2.**)[107] Recent studies from the laboratory of Dr. Christopher Lu in nephrology at UT Southwestern demonstrated that bardoxolone could ameliorate renal inflammation in the ischemia reperfusion model of acute renal failure in mice.[108] This agent is now use in clinical trials of patients with diabetic nephropathy and its demonstrated effect to lower serum creatinine in patients with established nephropathy (see below).[18,19]

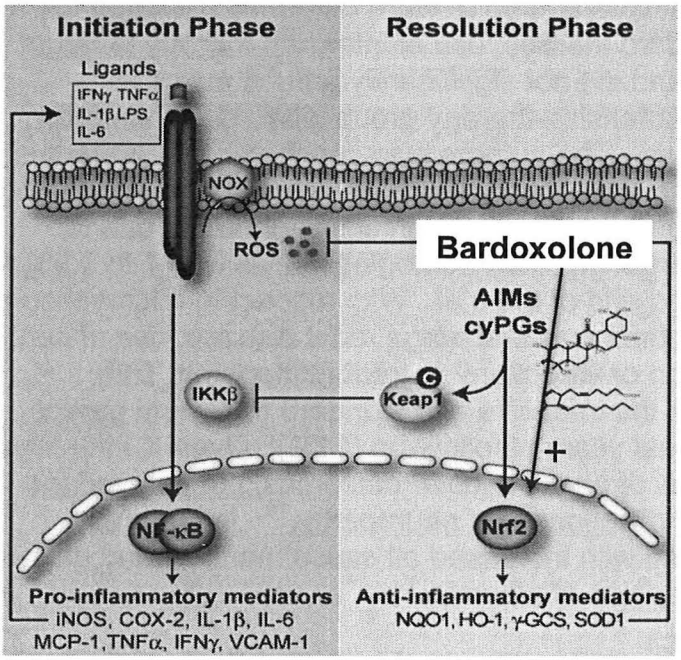


Figure 2 Pro-Inflammatory and Anti-inflammatory pathways in the kidney.

VI. Improving Outcomes: Evidence from Clinical Trials

Several clinical trials have clearly demonstrated the benefit of blockade of the renin-angiotensin system by ACE inhibition or by blockade of the angiotensin type 1 receptor and these drugs have FDA indications for treatment of nephropathy in type 1 and type 2 diabetes respectively. In addition, treatment with an ACE inhibitor alone has been shown to reduce cardiovascular morbidity and mortality in patients with type 2 diabetes.[27] These studies will not be reviewed here. This section will focus on more recent studies that use combinations of ACE inhibition plus either angiotensin receptor blockade or mineralocorticoid receptor blockade. In addition, more recent studies examining the effects of tight glycemic control, tight blood pressure control anemia treatment and treatment of dyslipidemia on renal and cardiovascular outcomes will be reviewed. Importantly, two new studies using anti-inflammatory/antioxidant drug bardoxolone to prevent progression of nephropathy will be reviewed.

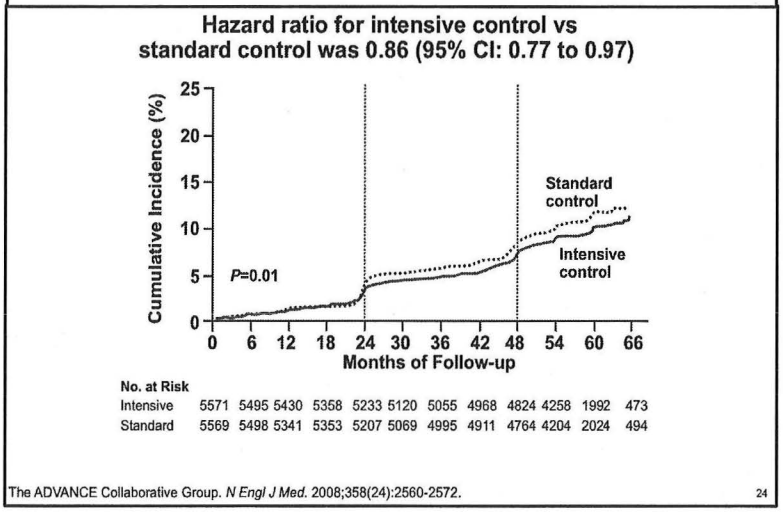
Glycemia

Three recent trials have investigated the impact of tight versus less tight glycemic control on cardiovascular and renal outcomes in patients with type 2 diabetes with our without signs of nephropathy.

1. The Action to Control Cardiovascular Risk in Diabetes (ACCORD) Trial

The objective of the ACCORD study was to determine whether a therapeutic strategy targeting an A1C of <6.0% compared with a strategy targeting an A1C of 7.0%-7.9% in patients with type 2 diabetes mellitus would reduce the rate of cardiovascular events.[109] The study enrolled10, 251 patients randomized to intensive therapy or standard therapy as follows: a) intensive therapy targeted an A1C level of <6.0%; and b) standard therapy targeted an A1C level of 7.0%-7.9%. In addition, 4733 subjects were assigned to lower their blood pressure and 5518 were randomly assigned to receive fenofibrate or placebo. Compared with standard therapy, use of intensive therapy to target normal A1C levels for 3.5 years increased mortality and did not significantly reduce major cardiovascular events. A higher mortality rate in the intensive-therapy group led to discontinuation of intensive therapy after a mean of 3.5 years of follow-up. The authors also concluded that if there is any benefit associated with intensive glucose lowering, it might take several years to emerge, during which time there is an increased risk of death. After termination of the intensive therapy, due to higher mortality in the intensive-therapy group, the target glycated hemoglobin level was 7 to 7.9% for all study subjects, who were followed until the planned end of the trial. With respect to Microvascular outcomes the principal composite microvascular outcome was end-stage renal disease, rise of serum creatinine to >3.3 mg/dL, or need for photocoagulation or vitrectomy to treat retinopathy. This outcome occurred in similar proportions of patients in the intensive and standard treatment groups, both during the study itself (9%) and after 1.5 additional years of follow-up (11%). Overall, intensive therapy did not reduce the risk of advanced measures of microvascular outcomes, but delayed the onset of albuminuria and some measures of eye complications and neuropathy.[14] In summary, intensive glycemic control in ACCORD was associated with increased all cause mortality and did not prevent end stage kidney disease but did slow progression of albuminuria.

Figure 3 Major Microvascular Events in ADVANCE



The ADVANCE Collaborative Group. *N Engl J Med.* 2008;358(24):2560-2572. 24

2. The Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) Trial was a global study that enrolled and randomized 11,140 patients with type 2 diabetes to undergo either standard glucose control or intensive glucose control, defined as the use of gliclazide (modified release) plus other drugs as required to achieve a glycated hemoglobin value of 6.5% or less. All subjects were also randomized to the combination of the ACE inhibitor perindopril and the diuretic indapemide or placebo regardless of baseline blood pressure. The primary end points were composites of major macrovascular and Microvascular events. The study successfully achieved a mean glycated hemoglobin level of 6.5% in the intensive-control group (6.5%) as compared to 7.3% in controls. Intensive control was associated with reduced the incidence of combined major macrovascular and microvascular events (18.1%, vs. 20.0% with standard control; hazard ratio, 0.90; 95% confidence interval [CI], 0.82 to 0.98; P = 0.01), and for major microvascular events (9.4% vs. 10.9%; hazard ratio, 0.86; 95% CI, 0.77 to 0.97; P = 0.01) (**Figure 3**). The latter was largely accounted for by a reduction in the incidence of nephropathy defined as development of macroalbuminuria. There was no significant effect on development of retinopathy. Type of glucose control did not effect major macrovascular events, death from cardiovascular causes, or death from any cause. However, severe hypoglycemia, was more

common in the intensive-control group. The authors concluded that "a strategy of intensive glucose control, involving gliclazide (modified release) and other drugs as required, that lowered the glycated hemoglobin value to 6.5% yielded a 10% relative reduction in the combined outcome of major macrovascular and microvascular events, primarily as a consequence of a 21% relative reduction in nephropathy." In a secondary analysis of the ADVANCE study,

3. The Veteran's Affairs Diabetes Trial (VADT)

Table 3. Nephropathy Outcomes in the Veterans Affairs Diabetes Trial (VADT)

Nephropathy, n/N (%)	Standard (n=899)	Intensive (n=892)	*P*
Serum creatinine >3 mg/dL	16/884 (1.8)	18/882 (2.0)	0.72
GFR <15 mL/min	11/884 (1.2)	7/882 (0.8)	0.35
Any increase in albuminuria	48/731 (6.6)	30/728 (4.1)	0.05

The VADT randomly assigned 1791 veterans to therapy for type 2 diabetes to receive either intensive or standard glucose control. The goal in the intensive-therapy group was an absolute reduction of 1.5 percentage points in the glycated hemoglobin level, as compared with the standard-therapy group. The primary outcome was the time from randomization to the first occurrence of a major cardiovascular event, death from cardiovascular causes, congestive heart failure, surgery for vascular disease, inoperable coronary disease, and amputation for ischemic gangrene. The authors found that after a median follow-up of 5.6 years median glycated hemoglobin was 8.4% in the standard-therapy group and 6.9% in the intensive-therapy group. There was no significant difference between the two groups in any component of the primary outcome or in the rate of death from any cause (hazard ratio, 1.07; 95% CI, 0.81 to 1.42; P=0.62). Overall there were no differences between the two groups for microvascular complications. However, in subgroup analysis the intensive group had less progression of albuminuria (P = 0.01) (**Table 3**).

Blood Pressure Control

The ACCORD trial evaluated blood pressure control in a cohort of those with hypertension. Similar antihypertensive regimens were employed in all subjects. Among 4733 subjects with type 2 diabetes randomly assigned to intensive lowering of systolic BP to < 120 mmHg as compared to < 140 mmHg therapy, the primary composite outcome of nonfatal myocardial infarction, nonfatal stroke, or death from cardiovascular causes did not differ. However, the annual rates of stroke were significantly lower in the intensive versus less intensive group (0.32% and 0.53%, P = 0.01). The mean follow-up was 4.7 years. However, serious adverse events attributed to antihypertensive treatment were more common in the intensive (3.3%) as compared to the standard-therapy group (1.3%) (P<0.001). The authors concluded that overall there was no

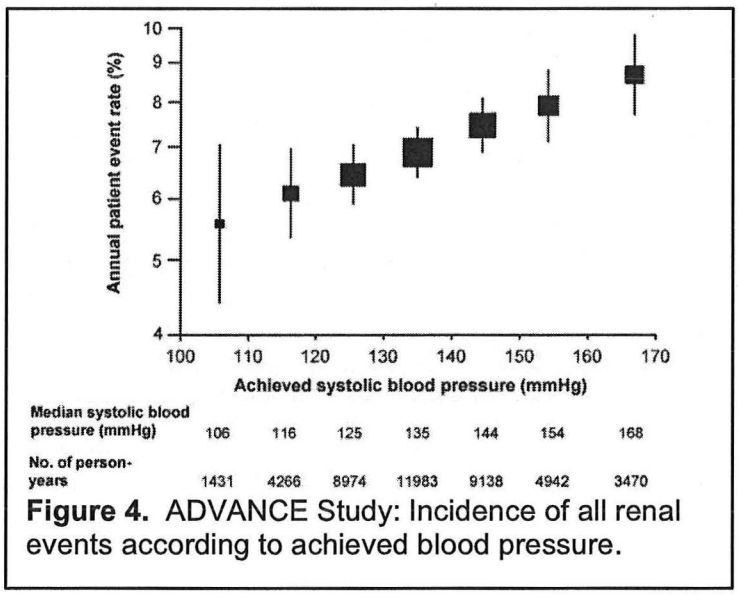


Figure 4. ADVANCE Study: Incidence of all renal events according to achieved blood pressure.

benefit to more aggressive blood pressure lowering in patients with type 2 diabetes and hypertension. Still there was a stroke benefit in the subgroup analysis.

1. The ADVANCE Trial

As noted above study subjects in ADVANCE were randomly assigned to fixed combination perindopril-indapamide or placebo, regardless of their BP at entry. During a mean follow-up of about 4 years, the risk for renal events was decreased by 21% (P=0.0001), which was driven by reduced risk for both microalbuminuria and macroalbuminuria (both P =0.003). The effects of active treatment were consistent across subgroups defined by baseline systolic or diastolic BP. As illustrated in **Figure 4,** lower systolic BP levels even to <110 mmHg, were associated with progressively lower rates of renal events. The authors concluded that BP-lowering treatment with the combination of perindopril plus indapamide provides important renoprotection, even among those with initial BP of120/70 mmHg. And, they could not identify a BP threshold below which renal benefit is lost.

Combined Blockade with an ACEI and an ARB:

1. THE Ongoing Telmisartan Alone and in Combination with Ramipril Global End- point Trial (ONTARGET)

The ONTARGET randomly assigned 25,620 patients with vascular disease or high-risk type 2 diabetes with and without chronic kidney disease to either ramipril 10 mg daily, telmisartan 80 mg daily or the combination. The primary outcome of the trial was primary composite outcome was death from cardiovascular causes, myocardial infarction, stroke, or hospitalization for heart failure. There were no differences in any of the treatment arms with regard to the primary outcome.[110] Subsequent analysis demonstrated that those randomized to the combination had more renal events in particular doubling of serum creatinine and need for dialysis for episodes of acute renal failure. [111] There was no difference in rate of ESRD requiring dialysis; however the rate of decline in estimated GFR was greater in those assigned to the combination as compared to the ACE inhibitor (**Figure 5**).

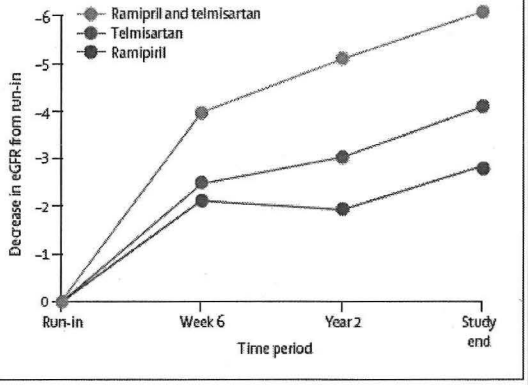


Figure 5. Rate of change in estimated GFR in the ONTARGET STUDY

In contrast, progression of albuminuria was slower in those randomized to the combination. There were no differences in the responses in those with or without diabetes. The authors concluded that overall there was no benefit of combining an ARB with an ACEi in the management of people at high vascular risk. It should be noted that the primary purpose of this study was not to evaluate the effect of combinations on renal failure outcomes; instead it was designed for cardiovascular outcomes. Additional studies using combinations in patients with diabetic nephropathy are now underway (see below). The seeming contradiction that albuminuria increase was attenuated but overall decline in kidney function was accelerated is not explained.

2. Combined blockade with an ARB and a direct renin inhibitor: The Aliskiren in the Evaluation of Proteinuria in Diabetes (AVOID) Trial

In contrast to the ONTARGET, the AVOID study was designed to test the hypothesis that adding a

direct renin inhibitor to standard of care in patients with type 2 diabetes, hypertension and urine albumin/creatinine ratio ≥ 300 mg/g macroalbuminuria would retard progression of albuminuria. Approximately 600 patients receiving 100 mg of losartan daily were randomly assigned to receive 6 months of treatment with aliskiren 150 (first 3 months) then titrated to 300 mg (final 3 months) or placebo, in addition to losartan. The primary outcome was a reduction in the ratio of albumin to creatinine, as measured in an early-morning urine sample, at 6 months. Treatment with 300 mg of aliskiren daily, as compared with placebo, reduced the mean urinary albumin-to-creatinine ratio by 20% (P<0.001), with a reduction of 50% or more in 24.7% of the patients who received aliskiren as compared with 12.5% of those who received placebo (P<0.001). There was no significant difference in blood pressure. The authors concluded that aliskiren may have renoprotective effects independent of its blood-pressure-lowering effect in patients with hypertension, type 2 diabetes, and nephropathy who are receiving the recommended renoprotective treatment.[112] There was no reported increase in acute renal failure or significant hyperkalemia in this study. Based on the results of this trial a large scale combined cardiovascular and renal outcome study in patients with type 2 diabetes is now underway (see below).

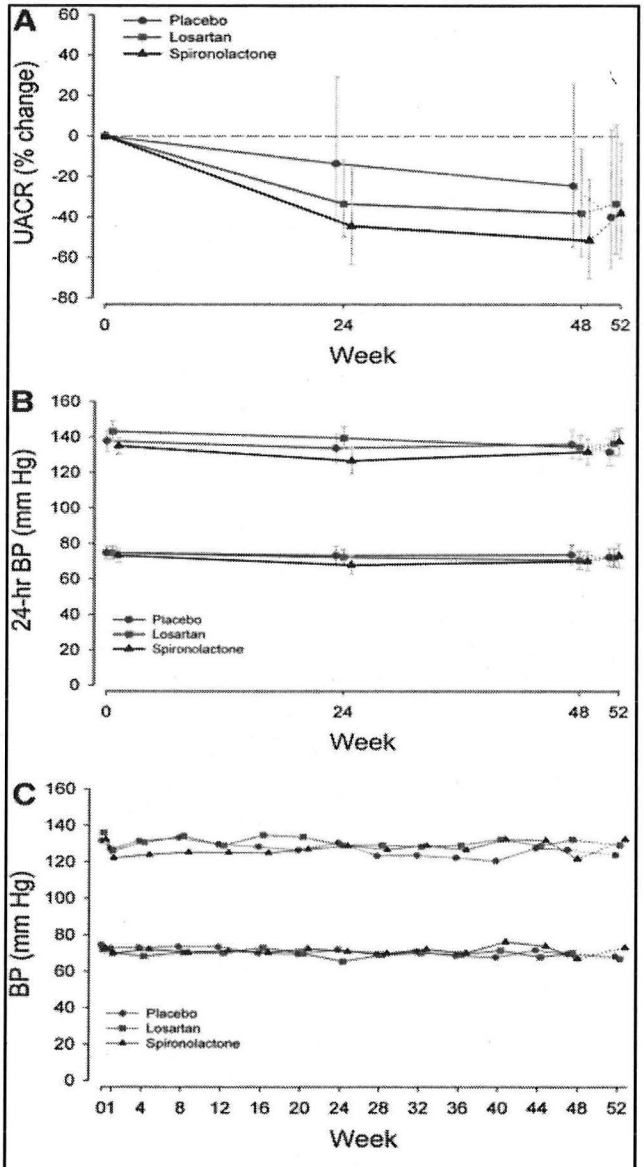


Figure 6. Effect of adding spironolactone or losartan to high dose lisinopril (80 mg daily) on urine albumin creatinine ratio (UACR) 24 hour BP and clinic BP in patients with diabetic nephropathy.

3. Combined Blockade with an ACE inhibitor and a mineralocorticoid antagonist

Aldosterone promotes glomerular and tubular sclerosis independent of angiotensin II in animal models of diabetic nephropathy. Most human studies testing the renoprotective benefit of adding an angiotensin receptor blocker or a mineralocorticoid receptor antagonist to a regimen based on inhibition of angiotensin-converting enzyme (ACE) used relatively low doses of ACE inhibitors. Furthermore, these studies did not determine whether antiproteinuric effects were independent of BP lowering. We conducted a double blind, placebo-controlled trial in 81 patients with diabetes, hypertension, and albuminuria (urine albumin-to-creatinine ratio > or =300 mg/g) who all received lisinopril (80 mg once daily). We randomly assigned the patients to placebo, losartan (100 mg daily), or spironolactone (25 mg daily) for 48 week at UT Southwestern.[113] We obtained blood and urine albumin, urea, creatinine, electrolytes, A1c, and ambulatory BP at baseline, 24, and 48 wk. Compared with placebo, the urine albumin-to-creatinine ratio decreased by 34.0% (95% CI, -51.0%, -11.2%, P = 0.007) in the group assigned to spironolactone and by 16.8% (95% CI, -37.3%, +10.5%, P = 0.20) in the group assigned to losartan (**Figure 6**). Clinic and ambulatory BP, creatinine clearance, sodium and protein intake, and glycemic control did not differ between groups. Serum potassium level was significantly higher with the addition of either spironolactone or losartan. In conclusion, the addition of spironolactone, but not losartan, to a regimen

including maximal ACE inhibition affords greater renoprotection in diabetic nephropathy despite a similar effect on BP. There are no studies evaluating the effect of adding a mineralocorticoid antagonist to standard of care in patients with type 1 or type 2 diabetes and nephropathy. Based on the survival benefit of addition of this class of agents to standard care in patients with heart failure, it would seem reasonable to propose a similar trial for patients with kidney disease. The major drawback is the potential for serious hyperkalemia, an expected adverse event in patients with diabetes and kidney disease. Still, the results of our study support the need to conduct a long-term, large-scale, renal failure outcomes trial.

Anemia

Anemia is a common complication of nephropathy in patients with diabetes and is associated with progression of renal failure and death.[114] Three trials have evaluated the impact of partial or full correction of anemia using erythropoietin stimulating agents.[12,115,116] The **Trial to Reduce cardiovascular Events with Aranesp Trial (TREAT)** was the only randomized, double-blind and placebo-controlled trial.[12] The TREAT study was a multinational study designed by an executive committee that included investigators from UT Southwestern. The study randomized 4,038 subjects with type 2 diabetes, chronic kidney disease (estimate GFR 20-60 ml/min/1.73 m2) and anemia (hemoglobin < 11 g/dl) to placebo or darbepoetin therapy to achieve a goal hemoglobin of ≤13 g/dl. The primary outcome of the trial was a composite of cardiovascular morbidity and death and development of end-stage renal disease and death. Although darbepoetin administration increased hemoglobin significantly and reduced the need for blood transfusion, as illustrated in **Figure 7**, darbepoetin therapy there was no benefit on either the cardiovascular or end-stage renal disease outcomes. And although the effect on overall cardiovascular outcomes was

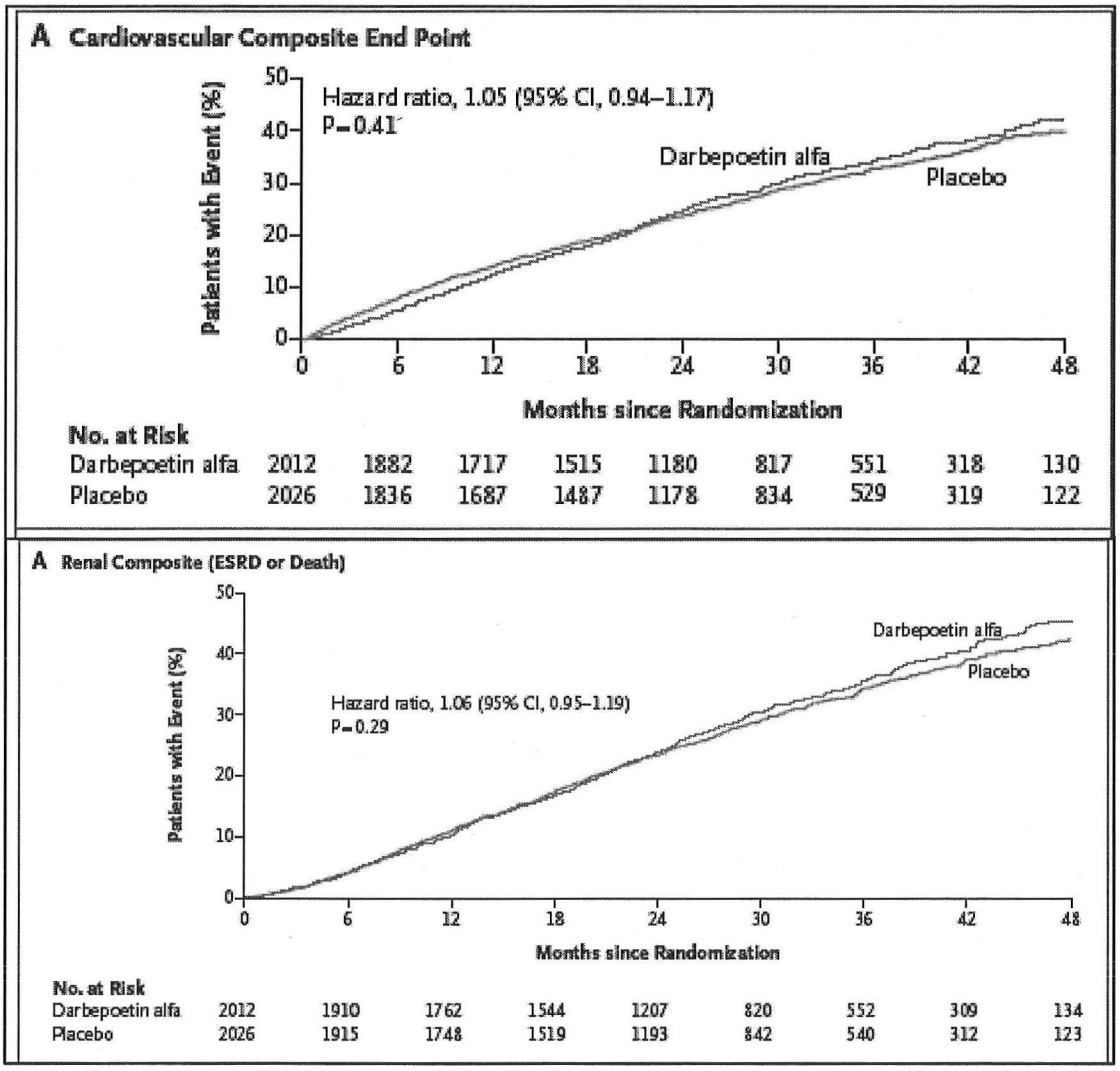


Figure 7. Effect of darbepoetin alpha on cardiovascular and renal composite outcomes in the TREAT.

neutral, there was a doubling in risk for stroke in those treated with darbepoetin. While the main metric for patient reported outcomes, the FACT fatigue index, was significantly improved with darbepoetin the effect was modest. We concluded that
Further analysis of the TREAT study clearly demonstrated that the risk for adverse events is much higher in those individuals who are resistant to the hematopoietic effect of darbepoetin.[117] For this reason, when using an ESA to treat anemia in patients with chronic kidney disease, clinicians should first ensure that iron stores are normal. Oral or intravenous iron can be used to treat iron deficiency in this situation and should be continued during ESA therapy. Importantly, the lowest dose of an ESA needed to prevent blood transfusion should be used and if the patient does not respond (e.g. an increase in hemoglobin of 0.5 g/dl within 4-8 weeks) increasing the dose may be unwarranted.

In summary, erythropoietin stimulating agent treatment of anemia in patients with diabetic nephropathy does not improve cardiovascular or renal outcomes and may increase risk for cardiovascular events when hemoglobin levels above 12 are targeted.

Dyslipidemia
Three large-scale trials have evaluated the effect of pharmacologic lowering of cholesterol on cardiovascular outcomes in patients with chronic kidney diseases including diabetes.

1. The Die Deutsche Dialysis in Diabetes (4D) trial
The 4D study enrolled 1255 subjects all of whom had type 2 diabetes on maintenance hemodialysis and randomized them in a double-blind fashion to receive atorvastatin 10 mg daily or placebo.[15] The primary outcome of the trial was a composite of a composite of death from cardiac causes, nonfatal myocardial infarction, and stroke. Atorvastatin lowered LDL cholesterol 42% as compared to 1.3% in placebo. Atorvastatin reduced the rate of all cardiac events combined but not combined cerebrovascular events or total mortality. The authors concluded that atorvastatin had no statistically significant effect on the composite primary end point of cardiovascular death, nonfatal myocardial infarction, and stroke in patients with diabetes receiving hemodialysis.

2. A Study to Evaluate the Use of Rosuvastatin in Subjects on Regular Hemodialysis: An Assessment of Survival and Cardiovascular Events (AURORA)
The AURORA trial was an international, multicenter, randomized, double-blind, prospective trial involving 2776 subjects undergoing maintenance hemodialysis. Patients with diabetes comprised approximately 20% of the study population. Subjects were randomly assigned patients to receive rosuvastatin, 10 mg daily, or placebo. The primary end point was a composite of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke. Rosuvastatin lowered LDL-cholesterol levels 43% from a mean baseline level of 100 mg/dl. During follow-up period 396 patients in the rosuvastatin group and 408 patients in the placebo group reached the primary end point (P=0.59). Also, rosuvastatin had no effect on

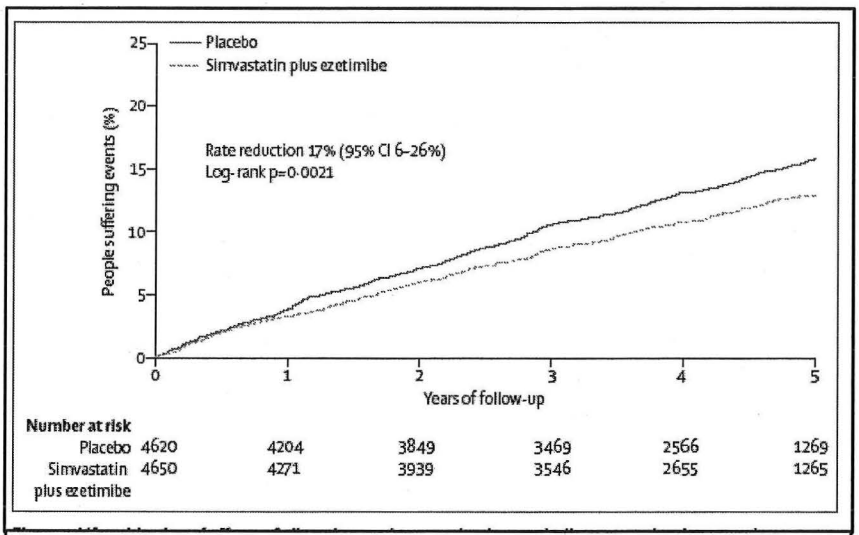


Figure 8. Effects of allocation to simvastatin plus ezetimibe versus placebo on major atherosclerotic events.

individual components of the primary end point and no significant effect on all-cause mortality. The authors concluded that in patients undergoing hemodialysis, the initiation of treatment with rosuvastatin lowered the LDL cholesterol level but had no significant effect on the composite primary end point of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke.

3. The Study of Heart and Renal Protection (SHARP)

The SHARP study was a randomized double-blind trial that included 9270 patients with chronic kidney disease of whom 3023 were on dialysis and 6247 were not. Patients aged 40 years and older were eligible to participate if they had chronic kidney disease with more than one previous measurement of serum or plasma creatinine of at least 1.7 mg/dL in men or 1.5 mg/dL in women, whether receiving dialysis or not. Patients with type 2 diabetes comprised 23% of the study population. Study subjects were randomly assigned to simvastatin 20 mg plus ezetimibe 10 mg daily versus matching placebo. The primary outcome was first major atherosclerotic event (non-fatal myocardial infarction or coronary death, non-hemorrhagic stroke, or any arterial revascularization procedure). Active therapy lowered LDL cholesterol as expected. Overall, during follow-up (median 4·9 years) active therapy produced a 17% proportional reduction in major atherosclerotic events (p=0·0021) (**Figure 8**). Fewer subjects randomized to simvastatin plus ezetimibe had a non-fatal myocardial infarction or died from coronary heart disease but this was not statistically significant. The benefit of this intervention was similar in those on dialysis. There was a small excess risk of myopathy (9 [0·2%] *vs.* 5 [0·1%]). The authors concluded that reduction of LDL cholesterol with simvastatin 20 mg plus ezetimibe 10 mg daily safely reduced the incidence of major atherosclerotic events in a wide range of patients with advanced chronic kidney disease.

In summary, cholesterol lowering with statin alone is not efficacious in the hemodialysis population. Combination therapy with simvastatin and ezetimibe is effective in reducing the risk for major cardiovascular effects.

Potential Novel Therapies: Anti-inflammatory/Antioxidant Interventions

Hyperglycemia and hypertension induce increases in reactive oxygen species and common inflammatory pathways (NF-κB). These in turn lead to glomerular endothelial dysfunction, mesangial proliferation, expansion and inflammation, basement membrane thickening and reduction in GFR. The reduction in GFR is mediated in part by decreasing surface area for filtration and by promoting glomerular and interstitial fibrosis and scarring. In patients with diabetic nephropathy chronic inflammation and oxidative stress are common.[75,76,118-120] As noted above, bardoxolone methyl is an antioxidant inflammation modulator that activates the Keap1–Nrf2 pathway-an important pathway for maintaining kidney function and structure.[107,121,122] Two recent clinical trials in patients with type 2 diabetes and nephropathy have investigated the effects of bardoxolone methyl on kidney function. Positive results from these trials suggest that bardoxolone has great potential as a novel therapeutic agent for diabetic nephropathy.

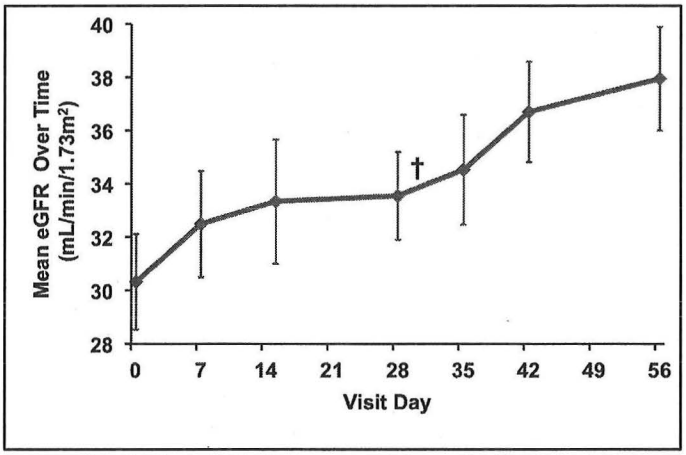


Figure 9. Dose-response curve for bardoxolone methyl on estimated GFR in patients with type 2 diabetes (n=20).

In a phase 2a study of 20 patients with diabetic nephropathy on standard of care with ACEi or ARB, bardoxolone methyl was administered for 56 days. In this study, increased the estimated glomerular

filtration rate (GFR) and 24-hour creatinine clearance (**Figure 9**).[19] The effect on eGFR persisted 30 days after discontinuation of bardoxolone. There was no change in the 24-hour creatinine excretion rate. Markers of vascular injury and inflammation were improved by treatment with bardoxolone and no life-threatening adverse events or drug-related serious adverse events were reported.

The 52-Week Bardoxolone Methyl Treatment: Renal Function in CKD/Type 2 Diabetes (BEAM) trial was a phase 2b study, in which we investigated the effect of bardoxolone methyl on kidney function in a randomized, placebo controlled trial. In this dose ranging study the effects of bardoxolone were examined at 24 and 52 weeks of study drug and at 56 weeks, four weeks after discontinuation of study drug. The primary outcome was the change in eGFR at 24 weeks and secondary outcomes included the change in eGFR at 52 weeks and 4 weeks after drug withdrawal. Two-hundred twenty seven study subjects with nephropathy (defined as an estimated glomerular filtration rate of 20 to 45 ml per minute per 1.73 m2) were randomly assigned in a 1:1:1:1 ratio to receive placebo or bardoxolone methyl at a target dose of 25, 75, or 150 mg once daily. Subjects receiving bardoxolone methyl had significant increases in the mean (±SD) estimated GFR, as compared with placebo, at 24 weeks (with between-group differences per minute per 1.73 m2 of 8.2±1.5 ml in the 25-mg group, 11.4±1.5 ml in the 75-mg group, and 10.4±1.5 ml in the 150-mg group; P<0.001) (**Figure 10**). The increases were maintained through week 52, with significant differences per minute per 1.73 m2 of 5.8±1.8 ml, 10.5±1.8 ml, and 9.3±1.9 ml, respectively. Serum creatinine, blood urea nitrogen, serum phosphorus and serum uric acid all decreased in the bardoxolone treated groups. Side effects included hypomagnesemia, muscle cramps and elevation of ALT. We concluded that bardoxolone methyl was associated with improvement in the estimated GFR in patients with advanced CKD and type 2 diabetes at 24 weeks. The improvement persisted at 52 weeks, suggesting that bardoxolone methyl may have promise for the treatment of CKD.

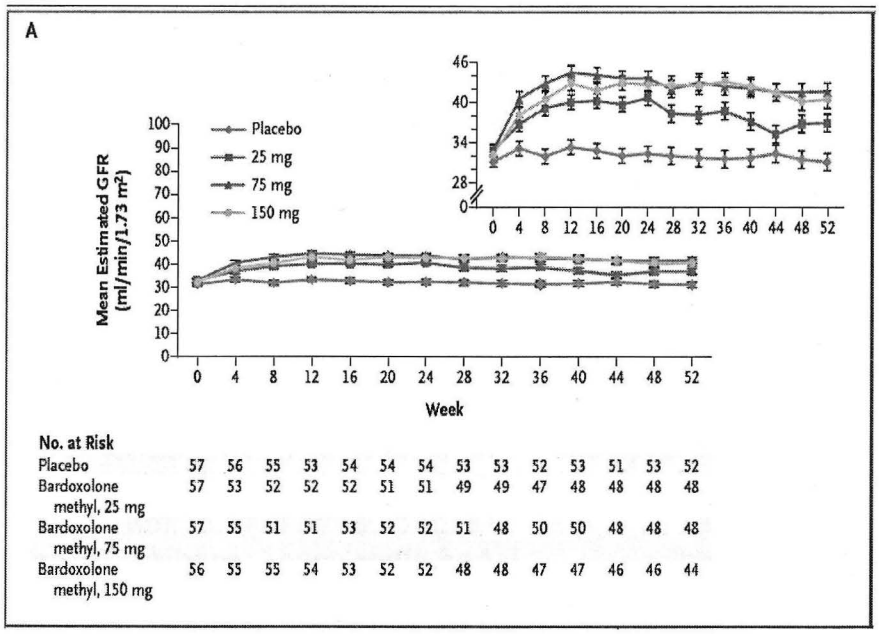


Figure 10. The Effect of Bardoxolone Methyl dosing on estimated glomerular filtration rate in patients with type 2 diabetes and nephropathy (n=227).

Ongoing Trials

1. BEACON Based on the data from the BEAM study, the study sponsor, REATA pharmaceuticals-a pharmaceutical company originated by investigators at UT Southwestern and based in Irving Texas has initiated a phase 3, large-scale outcomes trial to determine whether bardoxolone methyl at a dose of 75 mg added onto conventional therapy can reduce end-stage renal disease events and death in patients with type 2 diabetes and advanced nephropathy (Bardoxolone Methyl Evaluation in Patients with Chronic Kidney Disease and Type 2 Diabetes: The Occurrence of Renal Events, The BEACON study).

2. VA Nephron-D

The VA NEPHRON-D is a randomized, double-blind, multicenter clinical trial to assess the effect of combination losartan and lisinopril, compared with losartan alone, on the progression of kidney

disease in 1850 patients with diabetes and overt proteinuria. The primary endpoints are time to (*1*) reduction in estimated GFR (eGFR) of > 50% (if baseline < 60 ml/min/1.73 m2); (*2*) reduction in eGFR of 30 ml/min/1.73 m2 (if baseline > 60 ml/min/1.73 m2); (*3*) progression to ESRD (need for dialysis, renal transplant, or eGFR < 15 ml/min/1.73 m2); or (*4*) death. The secondary endpoint is time to change in eGFR or ESRD. This is the first large-scale outcomes trial to assess the effects of combination drug therapy on renal outcomes in a population of patients with established diabetic nephropathy.

3. The ALiskiren Trial In Type 2 diabetes Using cardiorenal Disease Endpoints (ALTITUDE)

The ALTITUDE study is a multinational, double-blind randomized placebo-controlled trial designed to determine whether addition of the direct renin inhibitor aliskiren to a standard of care regimen in patients with type 2 diabetes and renal or cardiovascular disease and microalbuminuria can reduce risk for cardiovascular morbidity and mortality and progression to end-stage renal disease. The trial is fully recruited and currently has 8,671 study subjects enrolled. It is anticipated to be completed in 2012. This is the first large-scale trial utilizing the orally active direct renin inhibitor in the treatment of diabetic nephropathy and follows from the observations of the above described AVOID trial.

VII. Current Management Recommendations based on the evidence

Based on the available clinical trial evidence the following are recommendations for the clinician to guide prevention and management of patients with diabetes at risk for or with established nephropathy (**Table 4**) (see www.kidney.org for most up to date clinical practice guidelines). Below is a brief discussion of the parameters in the table.

Table 4. Recommendations for Detection and Management of Diabetic Nephropathy

Parameter	Goal	How to get to Goal
Estimate GFR	Stable or improve	MDRD equation (lab report)
Measure urine albumin/cr ratio	< 300 mg/g (for macro) < 30 mg/g (for micro)	BP control, RAAS blockade
Glycemia (A1C)	< 7%	Oral agents ± injectables
Blood Pressure	< 130/80 mmHg	RAAS blockade + other agents[123]
RAAS blockade	Reduce BP and albuminuria	Maximum tolerated ACEi or ARB
Anemia	Improve symptoms Goal ~Hb 10-12 g/dl	Diagnose and correct Iron And other cause, consider ESAs
Dyslipidemia	LDL < 100 mg/dl (< 70 mg/dl)	Statin, possibly ezetimibe (not FDA approved for kidney disease)
Smoking	Cessation	Cessation programs
Overweight/Obese	Ideal BMI	Diet and exercise

See www.kidney.org for calculator if not reported by lab

Glycemia

It seems clear from the clinical trial data on glycemic control that targeting an A1c in the range of 6-7% reduces risk for onset and progression of albuminuria. The optimal regimens for accomplishing this are not entirely clear and more research in this area is needed. It is also important to remember that the risk for hypoglycemia is higher regardless of the regimen used to more aggressively lower blood glucose.

Blood Pressure Control

The recommended target for blood pressure control in patients with diabetes and chronic kidney disease of < 130/80 mmHg has not been proven in a clinical trial. However, compelling evidence from the ADVANCE study (see above) suggests that lower blood pressure with a combination regimen including an ACE inhibitor and indapemide may slow progression of albuminuria. However, it should be noted that outcomes studies including doubling of serum creatinine or end-stage renal disease have not been conducted with this combination. At the present time it seems reasonable to consider targeting the recommended level of 130/80 mmHg until we have data from substantial large outcomes trials on more aggressive blood pressure control. For a comprehensive review on pathophysiology and treatment of hypertension in diabetic nephropathy see article by Dr. Peter Van Buren[123]

Blockade of the Renin-Angiotensin System

ACE inhibition or angiotensin II receptor blockade should be prescribed for patients with diabetic nephropathy and evidence from the HOPE trial strongly suggests that an ACE inhibitor can reduce the risk for cardiovascular events in patients with nephropathy.

Anemia

Available evidence indicates that treatment of anemia with ESA therapy does not improve cardiovascular or renal outcomes. These drugs should not be prescribed for this purpose. However, ESA treatment is appropriate for patients with symptomatic anemia and a blood hemoglobin level below about 10 g/dl. The safe upper limit of hemoglobin for ESA has not been clearly established. However, a level of 12 g/dl is a reasonable target to aim for. Importantly, the use of ESAs should be limited to this purpose. If no demonstrable improvement in patient symptoms or functionality is observed or if the patient is resistant the drug should be discontinued.

Dyslipidemia

The American Heart Association recommends use of statins to lower LDL-cholesterol in patients with type 2 diabetes due to the high risk for myocardial infarction. The National Kidney Foundation recommends lowering LDL-cholesterol below 100 mg/dl in all patients with chronic kidney disease using statin and other therapy as needed. For some high risk patients lowering the LDL to < 70 mg/dl may be indicated. From the SHARP trial we have learned that administration of a simvastatin in combination with ezetimibe in patients with nephropathy (including diabetes) may also provide benefit for reducing risk of major cardiovascular events. Unfortunately, it is not clear whether a statin alone can accomplish the same outcome in patients not on dialysis.

Beyond the evidence

Although there are no controlled trials, it seems prudent to recommend smoking cessation for cigarette smokers and weight loss and exercise. In addition to its adverse effects on cardiovascular and pulmonary systems, smoking has been shown to accelerate decline in kidney function in patients with diabetic nephropathy. Further, although not proven in clinical trials, weight loss for overweight/obese patients with diabetes may reduce their risk for developing hypertension or kidney disease.

VIII. Conclusions

Significant progress has been made in better understanding the mechanisms and spectrum of kidney disease in patients with diabetes mellitus. Results from clinical trials have taught us how we can better manage patients to prevent onset and progression of nephropathy and reduce risk for major cardiovascular events. Use of existing interventions are effective for slowing progression of kidney disease and in some cases lowering cardiovascular risk (e.g. ACE inhibition and statin+ezetimibe). Ongoing research utilizing drugs that act as anti-inflammatory and antioxidants show promise for preserving kidney function beyond current medical management. Taken together, we have reason to be cautiously optimistic that we will soon discover better detection, prevention and treatment of diabetic nephropathy and thereby improve the public health.

References

1. Reutens AT, Atkins RC. Epidemiology of diabetic nephropathy. Contributions to nephrology 2011;170:1-7.
2. information CIfH. Treatment of End Stage Organ Failure in Canada, 1996 to 2005. Canadian Institute for Health information 2007;2007 Annual Report – ttawa, ON: CIHI: 2008.
3. Lora CM, Daviglus ML, Kusek JW, et al. Chronic kidney disease in United States Hispanics: a growing public health problem. Ethnicity & disease 2009;19:466-72.
4. Fabre J, Balant LP, Dayer PG, Fox HM, Vernet AT. The kidney in maturity onset diabetes mellitus: A clinical study of 510 patients. Kidney international 1982;21:730-8.
5. Report USRDSAD. 2011 Atlas of CKD & ESRD. 2011.
6. Parving HH, Lewis JB, Ravid M, Remuzzi G, Hunsicker LG. Prevalence and risk factors for microalbuminuria in a referred cohort of type II diabetic patients: a global perspective. Kidney international 2006;69:2057-63.
7. WHO. Diabetes Programme. 2010.
8. Brenner BM, Cooper ME, de ZD, et al. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. The New England journal of medicine 2001;345:861-9.
9. Parving HH, Lehnert H, Brochner-Mortensen J, Gomis R, Andersen S, Arner P. The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes. The New England journal of medicine 2001;345:870-8.
10. Lewis EJ, Hunsicker LG, Clarke WR, et al. Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. The New England journal of medicine 2001;345:851-60.
11. Rosolowsky ET, Skupien J, Smiles AM, et al. Risk for ESRD in type 1 diabetes remains high despite renoprotection. Journal of the American Society of Nephrology : JASN 2011;22:545-53.
12. Pfeffer MA, Burdmann EA, Chen CY, et al. A trial of darbepoetin alfa in type 2 diabetes and chronic kidney disease. The New England journal of medicine 2009;361:2019-32.

13. Patel A, MacMahon S, Chalmers J, et al. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. The New England journal of medicine 2008;358:2560-72.
14. Ismail-Beigi F, Craven T, Banerji MA, et al. Effect of intensive treatment of hyperglycaemia on microvascular outcomes in type 2 diabetes: an analysis of the ACCORD randomised trial. Lancet 2010;376:419-30.
15. Wanner C, Krane V, Marz W, et al. Atorvastatin in patients with type 2 diabetes mellitus undergoing hemodialysis. The New England journal of medicine 2005;353:238-48.
16. Fellstrom BC, Jardine AG, Schmieder RE, et al. Rosuvastatin and cardiovascular events in patients undergoing hemodialysis. The New England journal of medicine 2009;360:1395-407.
17. Baigent C, Landray MJ, Reith C, et al. The effects of lowering LDL cholesterol with simvastatin plus ezetimibe in patients with chronic kidney disease (Study of Heart and Renal Protection): a randomised placebo-controlled trial. Lancet 2011;377:2181-92.
18. Pergola PE, Raskin P, Toto RD, et al. Bardoxolone methyl and kidney function in CKD with type 2 diabetes. The New England journal of medicine 2011;365:327-36.
19. Pergola PE, Krauth M, Huff JW, et al. Effect of bardoxolone methyl on kidney function in patients with T2D and Stage 3b-4 CKD. American journal of nephrology 2011;33:469-76.
20. KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease. AmJKidney Dis 2007;49:S12-154.
21. Buse JB, Ginsberg HN, Bakris GL, et al. Primary prevention of cardiovascular diseases in people with diabetes mellitus: a scientific statement from the American Heart Association and the American Diabetes Association. Circulation 2007;115:114-26.
22. Gross JL, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T. Diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes care 2005;28:164-76.
23. Keane WF, Brenner BM, de ZD, et al. The risk of developing end-stage renal disease in patients with type 2 diabetes and nephropathy: the RENAAL study. Kidney Int 2003;63:1499-507.
24. Schwartz MM, Lewis EJ, Leonard-Martin T, Lewis JB, Batlle D. Renal pathology patterns in type II diabetes mellitus: relationship with retinopathy. The Collaborative Study Group. Nephrology DialTransplant 1998;13:2547-52.
25. Toto RD. Microalbuminuria: definition, detection, and clinical significance. JClinHypertens(Greenwich) 2004;6:2-7.
26. Toto RD. Heart disease in diabetic patients. SeminNephrol 2005;25:372-8.
27. Mann JFE, Gerstein HC, Pogue J, Bosch J, Yusuf S. Renal insufficiency as a predictor of cardiovascular outcomes and the impact of ramipril: the HOPE Randomized Trial. Annals of internal medicine 2001;134:629-36.
28. Macisaac RJ, Jerums G. Diabetic kidney disease with and without albuminuria. Current opinion in nephrology and hypertension 2011;20:246-57.
29. Kramer HJ, Nguyen QD, Curhan G, Hsu CY. Renal insufficiency in the absence of albuminuria and retinopathy among adults with type 2 diabetes mellitus. JAMA : the journal of the American Medical Association 2003;289:3273-7.
30. Mauer M, Zinman B, Gardiner R, et al. Renal and retinal effects of enalapril and losartan in type 1 diabetes. The New England journal of medicine 2009;361:40-51.
31. Biesenbach G, Bodlaj G, Pieringer H, Sedlak M. Clinical versus histological diagnosis of diabetic nephropathy--is renal biopsy required in type 2 diabetic patients with renal disease? QJM : monthly journal of the Association of Physicians 2011;104:771-4.
32. Helal I, Goucha R, Karoui C, et al. Nodular glomerulosclerosis in patients' without history of diabetes mellitus: a case report. Cases journal 2009;2:6792.
33. Prakash J, Sen D, Usha, Kumar NS. Non-diabetic renal disease in patients with type 2 diabetes mellitus. The Journal of the Association of Physicians of India 2001;49:415-20.

34. Wirta O, Helin H, Mustonen J, Kuittinen E, Savela T, Pasternack A. Renal findings and glomerular pathology in diabetic subjects. Nephron 2000;84:236-42.
35. Richards NT, Greaves I, Lee SJ, Howie AJ, Adu D, Michael J. Increased prevalence of renal biopsy findings other than diabetic glomerulopathy in type II diabetes mellitus. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 1992;7:397-9.
36. Parving HH, Gall MA, Skott P, et al. Prevalence and causes of albuminuria in non-insulin-dependent diabetic patients. Kidney international 1992;41:758-62.
37. Chang TI, Park JT, Kim JK, et al. Renal outcomes in patients with type 2 diabetes with or without coexisting non-diabetic renal disease. Diabetes research and clinical practice 2011;92:198-204.
38. Eijkelkamp WB, Zhang Z, Remuzzi G, et al. Albuminuria is a target for renoprotective therapy independent from blood pressure in patients with type 2 diabetic nephropathy: post hoc analysis from the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) trial. JAmSocNephrol 2007;18:1540-6.
39. Papale M, Di Paolo S, Magistroni R, et al. Urine proteome analysis may allow noninvasive differential diagnosis of diabetic nephropathy. Diabetes care 2010;33:2409-15.
40. Merchant ML, Perkins BA, Boratyn GM, et al. Urinary peptidome may predict renal function decline in type 1 diabetes and microalbuminuria. Journal of the American Society of Nephrology : JASN 2009;20:2065-74.
41. Thongboonkerd V. Study of diabetic nephropathy in the proteomic era. Contributions to nephrology 2011;170:172-83.
42. Fisher WG, Lucas JE, Mehdi UF, et al. A Method for Isolation and Identification of Urinary Biomarkers in Patients with Diabetic Nephropathy. Proteomics Clinical applications 2011.
43. Parving HH, de ZD, Cooper ME, et al. ACE Gene Polymorphism and Losartan Treatment in Type 2 Diabetic Patients With Nephropathy. JAmSocNephrol 2008.
44. Egido J, Ortiz A. ACE gene polymorphism and the prognosis and treatment of overt diabetic nephropathy. Nature clinical practice Nephrology 2008;4:472-3.
45. Kure M, Pezzolesi MG, Poznik GD, et al. Genetic variation in the matrix metalloproteinase genes and diabetic nephropathy in type 1 diabetes. Molecular genetics and metabolism 2011;103:60-5.
46. Pezzolesi MG, Skupien J, Mychaleckyj JC, Warram JH, Krolewski AS. Insights to the genetics of diabetic nephropathy through a genome-wide association study of the GoKinD collection. Seminars in nephrology 2010;30:126-40.
47. Pezzolesi MG, Poznik GD, Mychaleckyj JC, et al. Genome-wide association scan for diabetic nephropathy susceptibility genes in type 1 diabetes. Diabetes 2009;58:1403-10.
48. Rogus JJ, Poznik GD, Pezzolesi MG, et al. High-density single nucleotide polymorphism genome-wide linkage scan for susceptibility genes for diabetic nephropathy in type 1 diabetes: discordant sibpair approach. Diabetes 2008;57:2519-26.
49. De Carvalho JA, Piva SJ, Hausen BS, et al. Assessment of urinary gamma-glutamyltransferase and alkaline phosphatase for diagnosis of diabetic nephropathy. Clinica chimica acta; international journal of clinical chemistry 2011;412:1407-11.
50. Vaidya VS, Niewczas MA, Ficociello LH, et al. Regression of microalbuminuria in type 1 diabetes is associated with lower levels of urinary tubular injury biomarkers, kidney injury molecule-1, and N-acetyl-beta-D-glucosaminidase. Kidney international 2011;79:464-70.
51. Wolkow PP, Niewczas MA, Perkins B, et al. Association of urinary inflammatory markers and renal decline in microalbuminuric type 1 diabetics. Journal of the American Society of Nephrology : JASN 2008;19:789-97.

52. Inoue T, Kozawa E, Okada H, et al. Noninvasive evaluation of kidney hypoxia and fibrosis using magnetic resonance imaging. Journal of the American Society of Nephrology : JASN 2011;22:1429-34.
53. Rosenson RS, Fioretto P, Dodson PM. Does microvascular disease predict macrovascular events in type 2 diabetes? Atherosclerosis 2011;218:13-8.
54. Mogensen C, Christensen C. Mortality of diabetes in type II DM with proteinuria. The New England journal of medicine 1984;311:89-93.
55. Mogensen CE. Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. NEnglMed 1984;310:356-60.
56. Mann JF, Yi QL, Gerstein HC. Albuminuria as a predictor of cardiovascular and renal outcomes in people with known atherosclerotic cardiovascular disease. Kidney international Supplement 2004:S59-62.
57. Keith DS, Nichols GA, Gullion CM, Brown JB, Smith DH. Longitudinal follow-up and outcomes among a population with chronic kidney disease in a large managed care organization. ArchInternMed 2004;164:659-63.
58. National Kidney F, Supplement. Clinical Practice Guidlines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disase. In: Tuttle K, ed. American Journal of Kidney Diseases; 2007:S1-S123.
59. Gerstein HC, Mann JF, Yi Q, et al. Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. Journal of American Medicine Association 2001;286:421-6.
60. McMurray JJ, Uno H, Jarolim P, et al. Predictors of fatal and nonfatal cardiovascular events in patients with type 2 diabetes mellitus, chronic kidney disease, and anemia: An analysis of the Trial to Reduce cardiovascular Events with Aranesp (darbepoetin-alfa) Therapy (TREAT). American heart journal 2011;162:748-55 e3.
61. Igo RP, Jr., Iyengar SK, Nicholas SB, et al. Genomewide linkage scan for diabetic renal failure and albuminuria: the FIND study. American journal of nephrology 2011;33:381-9.
62. Breyer MD, Qi Z, Tchekneva EE, Harris RC. Insight into the genetics of diabetic nephropathy through the study of mice. Current opinion in nephrology and hypertension 2008;17:82-6.
63. Zanchi A, Moczulski DK, Hanna LS, Wantman M, Warram JH, Krolewski AS. Risk of advanced diabetic nephropathy in type 1 diabetes is associated with endothelial nitric oxide synthase gene polymorphism. Kidney international 2000;57:405-13.
64. Shin Shin Y, Baek SH, Chang KY, et al. Relations between eNOS Glu298Asp polymorphism and progression of diabetic nephropathy. Diabetes research and clinical practice 2004;65:257-65.
65. Zhao HJ, Wang S, Cheng H, et al. Endothelial nitric oxide synthase deficiency produces accelerated nephropathy in diabetic mice. Journal of the American Society of Nephrology : JASN 2006;17:2664-9.
66. Mueller PW, Rogus JJ, Cleary PA, et al. Genetics of Kidneys in Diabetes (GoKinD) study: a genetics collection available for identifying genetic susceptibility factors for diabetic nephropathy in type 1 diabetes. Journal of the American Society of Nephrology : JASN 2006;17:1782-90.
67. Tuttle KR, Anderson PW. A novel potential therapy for diabetic nephropathy and vascular complications: protein kinase C beta inhibition. American Journal of Kidney Diseases [Online] 2003;42:456-65.
68. Tuttle KR, Bakris GL, Toto RD, McGill JB, Hu K, Anderson PW. The effect of ruboxistaurin on nephropathy in type 2 diabetes. Diabetes care 2005;28:2686-90.
69. Kanwar YS, Sun L, Xie P, Liu FY, Chen S. A glimpse of various pathogenetic mechanisms of diabetic nephropathy. Annual review of pathology 2011;6:395-423.

70. Nawroth PP, Isermann B. Mechanisms of diabetic nephropathy--old buddies and newcomers part 1. Experimental and clinical endocrinology & diabetes : official journal, German Society of Endocrinology [and] German Diabetes Association 2010;118:571-6.
71. Yamagishi S, Fukami K, Ueda S, Okuda S. Molecular mechanisms of diabetic nephropathy and its therapeutic intervention. Current drug targets 2007;8:952-9.
72. Schena FP, Gesualdo L. Pathogenetic mechanisms of diabetic nephropathy. Journal of the American Society of Nephrology : JASN 2005;16 Suppl 1:S30-3.
73. Nicholas SB. Advances in pathogenetic mechanisms of diabetic nephropathy. Cell Mol Biol (Noisy-le-grand) 2003;49:1319-25.
74. Hostetter TH. Mechanisms of diabetic nephropathy. American journal of kidney diseases : the official journal of the National Kidney Foundation 1994;23:188-92.
75. Marrero MB, Banes-Berceli AK, Stern DM, Eaton DC. Role of the JAK/STAT signaling pathway in diabetic nephropathy. American journal of physiology Renal physiology 2006;290:F762-8.
76. Marrero MB, Fulton D, Stepp D, Stern DM. Angiotensin II-induced signaling pathways in diabetes. Current diabetes reviews 2005;1:197-202.
77. Mora C, Navarro JF. Inflammation and diabetic nephropathy. Current diabetes reports 2006;6:463-8.
78. Noh H, Ha H. Reactive oxygen species and oxidative stress. Contributions to nephrology 2011;170:102-12.
79. Nitti M, Furfaro AL, Patriarca S, et al. Human mesangial cells resist glycoxidative stress through an antioxidant response. International journal of molecular medicine 2011;27:213-9.
80. Liu CX, Hu Q, Wang Y, et al. Angiotensin-converting enzyme (ACE) 2 overexpression ameliorates glomerular injury in a rat model of diabetic nephropathy: a comparison with ACE inhibition. Mol Med 2011;17:59-69.
81. Kitada M, Kume S, Imaizumi N, Koya D. Resveratrol improves oxidative stress and protects against diabetic nephropathy through normalization of Mn-SOD dysfunction in AMPK/SIRT1-independent pathway. Diabetes 2011;60:634-43.
82. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circulation research 2010;107:1058-70.
83. Fujii H, Kono K, Nakai K, et al. Oxidative and nitrosative stress and progression of diabetic nephropathy in type 2 diabetes. American journal of nephrology 2010;31:342-52.
84. Wu J, Mei C, Vlassara H, Striker GE, Zheng F. Oxidative stress-induced JNK activation contributes to proinflammatory phenotype of aging diabetic mesangial cells. American journal of physiology Renal physiology 2009;297:F1622-31.
85. Whaley-Connell A, Habibi J, Wei Y, et al. Mineralocorticoid receptor antagonism attenuates glomerular filtration barrier remodeling in the transgenic Ren2 rat. American journal of physiology Renal physiology 2009;296:F1013-22.
86. Kakkar R, Mantha SV, Radhi J, Prasad K, Kalra J. Antioxidant defense system in diabetic kidney: a time course study. Life sciences 1997;60:667-79.
87. Ha H, Kim KH. Pathogenesis of diabetic nephropathy: the role of oxidative stress and protein kinase C. Diabetes research and clinical practice 1999;45:147-51.
88. Ha H, Lee HB. Oxidative stress in diabetic nephropathy: basic and clinical information. Current diabetes reports 2001;1:282-7.
89. Rosen P, Nawroth PP, King G, Moller W, Tritschler HJ, Packer L. The role of oxidative stress in the onset and progression of diabetes and its complications: a summary of a Congress Series sponsored by UNESCO-MCBN, the American Diabetes Association and the German Diabetes Society. Diabetes/metabolism research and reviews 2001;17:189-212.
90. Koo JR, Vaziri ND. Effects of diabetes, insulin and antioxidants on NO synthase abundance and NO interaction with reactive oxygen species. Kidney international 2003;63:195-201.

91. Agarwal R, Campbell RC, Warnock DG. Oxidative stress in hypertension and chronic kidney disease: role of angiotensin II. SeminNephrol 2004;24:101-14.
92. Oberg BP, McMenamin E, Lucas FL, et al. Increased prevalence of oxidant stress and inflammation in patients with moderate to severe chronic kidney disease. Kidney international 2004;65:1009-16.
93. Lieberthal W, Levine JS. The role of the mammalian target of rapamycin (mTOR) in renal disease. Journal of the American Society of Nephrology : JASN 2009;20:2493-502.
94. Mori H, Inoki K, Masutani K, et al. The mTOR pathway is highly activated in diabetic nephropathy and rapamycin has a strong therapeutic potential. Biochemical and biophysical research communications 2009;384:471-5.
95. Godel M, Hartleben B, Herbach N, et al. Role of mTOR in podocyte function and diabetic nephropathy in humans and mice. The Journal of clinical investigation 2011;121:2197-209.
96. Cheng L, Chen J, Mao X. Everolimus vs. rapamycin for treating diabetic nephropathy in diabetic mouse model. Journal of Huazhong University of Science and Technology Medical sciences = Hua zhong ke ji da xue xue bao Yi xue Ying De wen ban = Huazhong keji daxue xuebao Yixue Yingdewen ban 2011;31:457-62.
97. Yang Y, Wang J, Qin L, et al. Rapamycin prevents early steps of the development of diabetic nephropathy in rats. American journal of nephrology 2007;27:495-502.
98. Ohga S, Shikata K, Yozai K, et al. Thiazolidinedione ameliorates renal injury in experimental diabetic rats through anti-inflammatory effects mediated by inhibition of NF-kappaB activation. American journal of physiology Renal physiology 2007;292:F1141-50.
99. Romzova M, Hohenadel D, Kolostova K, et al. NFkappaB and its inhibitor IkappaB in relation to type 2 diabetes and its microvascular and atherosclerotic complications. Human immunology 2006;67:706-13.
100. Tang SC, Leung JC, Chan LY, Tsang AW, Lai KN. Activation of tubular epithelial cells in diabetic nephropathy and the role of the peroxisome proliferator-activated receptor-gamma agonist. Journal of the American Society of Nephrology : JASN 2006;17:1633-43.
101. Han SY, Kim CH, Kim HS, et al. Spironolactone prevents diabetic nephropathy through an anti-inflammatory mechanism in type 2 diabetic rats. Journal of the American Society of Nephrology : JASN 2006;17:1362-72.
102. Pillarisetti S, Saxena U. Role of oxidative stress and inflammation in the origin of Type 2 diabetes--a paradigm shift. Expert opinion on therapeutic targets 2004;8:401-8.
103. Mezzano S, Aros C, Droguett A, et al. NF-kappaB activation and overexpression of regulated genes in human diabetic nephropathy. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 2004;19:2505-12.
104. Mezzano S, Droguett A, Burgos ME, et al. Renin-angiotensin system activation and interstitial inflammation in human diabetic nephropathy. Kidney international Supplement 2003:S64-70.
105. Kumar A, Hawkins KS, Hannan MA, Ganz MB. Activation of PKC-beta(I) in glomerular mesangial cells is associated with specific NF-kappaB subunit translocation. American journal of physiology Renal physiology 2001;281:F613-9.
106. Kim HJ, Vaziri ND. Contribution of impaired Nrf2-Keap1 pathway to oxidative stress and inflammation in chronic renal failure. American journal of physiology Renal physiology 2010;298:F662-71.
107. Sporn MB, Liby KT, Yore MM, Fu L, Lopchuk JM, Gribble GW. New synthetic triterpenoids: potent agents for prevention and treatment of tissue injury caused by inflammatory and oxidative stress. Journal of natural products 2011;74:537-45.
108. Wu QQ, Wang Y, Senitko M, et al. Bardoxolone methyl (BARD) ameliorates ischemic AKI and increases expression of protective genes Nrf2, PPARgamma, and HO-1. American journal of physiology Renal physiology 2011;300:F1180-92.

109. Gerstein HC, Miller ME, Byington RP, et al. Effects of intensive glucose lowering in type 2 diabetes. The New England journal of medicine 2008;358:2545-59.
110. Investigators O. Telmisartan, Ramipril, or Both in Patients at High Risk for Vascular Events. New England Journal of Medicine 2008;358:1547-59.
111. Mann JF, Schmieder RE, McQueen M, et al. Renal outcomes with telmisartan, ramipril, or both, in people at high vascular risk (the ONTARGET study): a multicentre, randomised, double-blind, controlled trial. Lancet 2008;372:547-53.
112. Parving HH, Persson F, Lewis JB, Lewis EJ, Hollenberg NK. Aliskiren combined with losartan in type 2 diabetes and nephropathy. The New England journal of medicine 2008;358:2433-46.
113. Mehdi UF, Adams-Huet B, Raskin P, Vega GL, Toto RD. Addition of angiotensin receptor blockade or mineralocorticoid antagonism to maximal angiotensin-converting enzyme inhibition in diabetic nephropathy. Journal of the American Society of Nephrology : JASN 2009;20:2641-50.
114. Mohanram A, Zhang Z, Shahinfar S, Keane WF, Brenner BM, Toto RD. Anemia and end-stage renal disease in patients with type 2 diabetes and nephropathy. Kidney Int 2004;66:1131-8.
115. Drueke TB, Locatelli F, Clyne N, et al. Normalization of hemoglobin level in patients with chronic kidney disease and anemia. The New England journal of medicine 2006;355:2071-84.
116. Singh AK, Szczech L, Tang KL, et al. Correction of anemia with epoetin alfa in chronic kidney disease. The New England journal of medicine 2006;355:2085-98.
117. Solomon SD, Uno H, Lewis EF, et al. Erythropoietic response and outcomes in kidney disease and type 2 diabetes. The New England journal of medicine 2010;363:1146-55.
118. Garrido AM, Griendling KK. NADPH oxidases and angiotensin II receptor signaling. Molecular and cellular endocrinology 2009;302:148-58.
119. Lemley KV, Abdullah I, Myers BD, et al. Evolution of incipient nephropathy in type 2 diabetes mellitus. Kidney international 2000;58:1228-37.
120. Hayashi H, Karasawa R, Inn H, et al. An electron microscopic study of glomeruli in Japanese patients with non-insulin dependent diabetes mellitus. Kidney international 1992;41:749-57.
121. Li J, Stein TD, Johnson JA. Genetic dissection of systemic autoimmune disease in Nrf2-deficient mice. Physiological genomics 2004;18:261-72.
122. Yoh K, Itoh K, Enomoto A, et al. Nrf2-deficient female mice develop lupus-like autoimmune nephritis. Kidney international 2001;60:1343-53.
123. Van Buren PN, Toto R. Hypertension in diabetic nephropathy: epidemiology, mechanisms, and management. Advances in chronic kidney disease 2011;18:28-41.