DIABETIC NEPHROPATHY: CAN IT BE PREVENTED?

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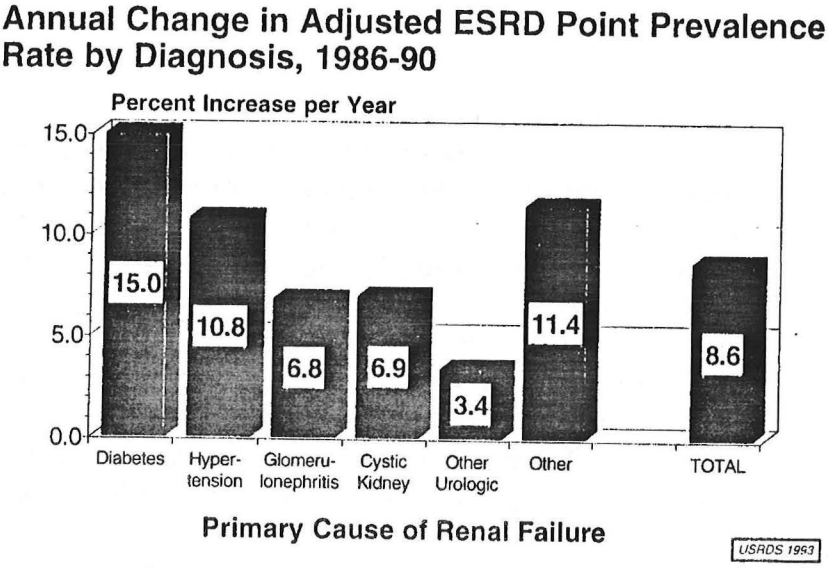
I. INTRODUCTION

End-stage renal disease (ESRD) is a catastrophic illness that afflicts nearly 250,000 Americans. Diabetic nephropathy is the most common cause of both nephrotic syndrome and end-stage renal disease in the United States. Moereover, approximately 10,000 new cases of diabetic ESRD are reported annually and the rate has been increasing steadily over the past decade (1,2). To date there is no cure for renal disease. However, recent experimental and clinical studies have taught us that poor metabolic control and systemic and glomerular hypertension play independent and interdependent roles in the development and progression of diabetic renal disease. The purpose of this Grand Rounds is to review the recent evidence indicating why glucose control and increased blood pressure are not only important pathophysiological mechanisms of diabetic renal disease but also strategic targets for effective prevention and treatment. As I will show it is possible that both "tight" blood glucose control and administration of angiotensin-converting enzyme inhibitors can stall, if not prevent, the development of nephropathy in both type I and type II diabetes. In fact both the National Kidney Foundation and American Diabetes Associations are now developing documents outlining the recommended approach to prevention and therapy of renal disease . However, additional studies will be necessary before these agents can be recommended without reservation as a preventative measures in all diabetics.

II. EPIDEMIOLOGY OF DIABETIC NEPHROPATHY

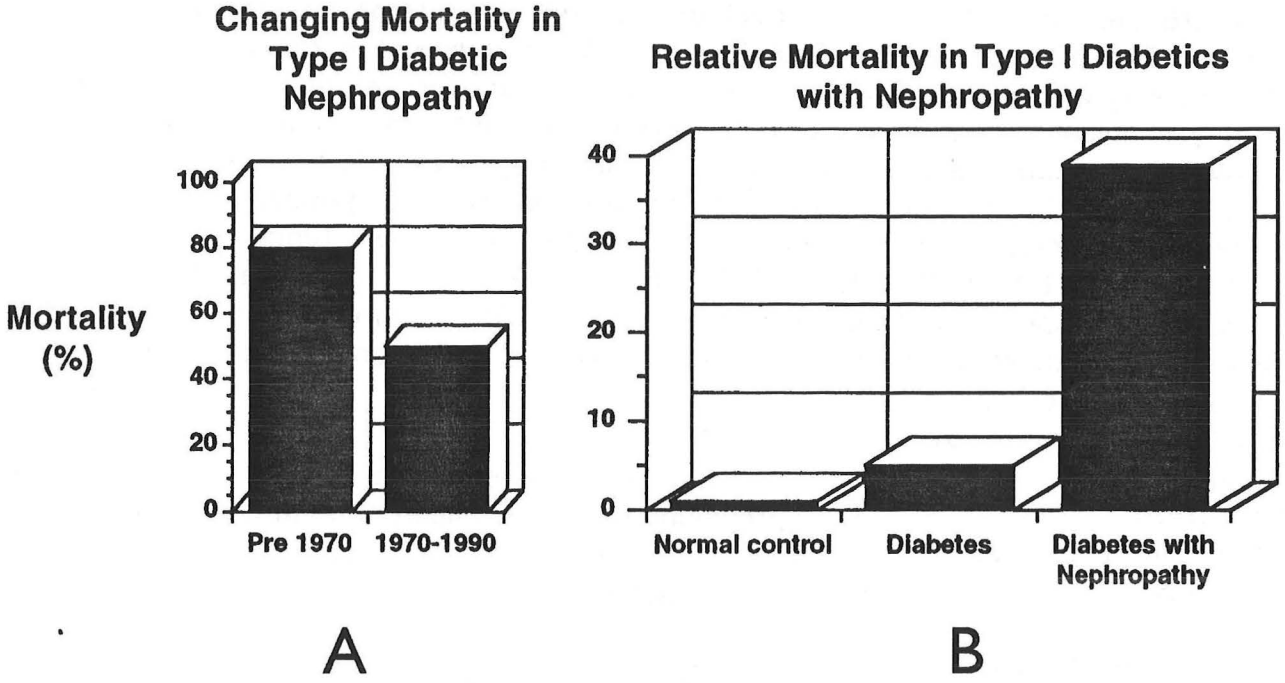
It is estimated that there are approximately 13 million people afflicted with diabetes in the U.S.: 5-10% are type I (insulin-dependent diabetes mellitus) and 90-95% type II (non-insulin-dependent diabetes mellitus). Current data from the United States Renal Data Systems (URDS) indicates that diabetic nephropathy accounts for about 34% of new cases of

Figure 1.Percent increase per year in reported ESRD point prevalence rate per million population between 1986 and 1990, by primary diagnosis. Incidence rates adjusted for age sex and race.



ESRD (1). Type I and type II diabetes each account for about 50% of new cases. There has been a compelling increase in the number of new cases of ESRD apparently caused by diabetes both in the U.S. and in Europe (1-4). However, because of problems with diagnosis coding the distribution among type I and type II diabetes is not known (3). As shown in figure 1, the annual change in adjusted ESRD point

Figure 2. Mortality in Type I Diabetic Nephropathy



prevalence for diabetic nephropathy is about 15%; the greatest increase for all forms of renal disease. It is important to note that these data are derived from registries of patients on treatment for ESRD and therefore do not represent the actual numbers of patients at risk for renal failure. As will be discussed below, the overall incidence of apparent diabetic overt diabetic nephropathy appears to be decreasing in some areas of the world. Nevertheless, in the U.S. the absolute number of patients being offered treatment for ESRD due to diabetes has been increasing. And, the expected remaining lifetime for patients with ESRD of all causes is 7 years at age 49 and 4.3 years at age 59. The corresponding time for the general U.S. population at the same age values are 29.8 years and 21.6 years respectively. The annual total cost for managing diabetic ESRD is approaching $3 billion per year including Medicare and non-Medicare reimbursements.

What's more diabetic nephropathy is a deadly disease. As shown in figure 2 A the mortality rate prior to 1970 was 80% decreasing to 50% in the decade form

1980-1990. Andersen reported that 100% of patients with diabetic nephropathy died within 6 years of the diagnosis (5). In fact, the high mortality in diabetic populations is largely due to nephropathy. The presence of dipstick positive urine test in diabetics is associated with a staggering increase in cardiovascular mortality (figure 2B) (6). There study revealed 9-fold and 40-fold increases in cardiovascular mortality in diabetics with nephropathy as compared to normal controls and diabetics without proteinuria respectively.

III. WHAT IS DIABETIC NEPHROPATHY?

A. DEFINITION

Diabetic nephropathy is defined as the presence of overt proteinuria, which means persistent dipstick positive urine protein in a patient with long-standing diabetes (usually more than 10 years), in association with rising blood pressure, diabetic retinopathy and in the absence of other known causes of renal disease. This is a clinical definition, that is, it does not require a renal biopsy. This definition is applicable to patients with either Type I or Type II disease. It is important to note that the definition requires the presence of at least enough protein in the urine to be detected by routine urine dipstick screening test, which generally indicates a daily excretion rate of at least 300 mg (macroalbuminuria). In contrast to the overt proteinuria, microalbuminuria indicates an abnormal amount of urine albumin that is not detectable by routine screening tests. Microalbuminuria is defined as an albumin excretion rate in the range of > 20 ug/min (28 mg/d, or urine albumin/creatinine ratio of 30 mg/g creatinine on a spot urine sample) and < 200 ug/min (300 mg/d). In the following discussions the term Overt Nephropathy refers to patients with macroalbuminuria (as defined above) and the term Incipient Nephropathy refers to patients with persistent microalbuminuria.

B. NATURAL HISTORY

Type I Diabetes

The natural history of diabetic nephropathy has been best characterized in Type I diabetes mellitus, albeit incompletely. However, it is generally agreed that once overt diabetic nephropathy has been diagnosed, renal disease progresses inexorably to end-stage renal disease. Mogensen has proposed a staging scheme based on clinical studies in humans in which it is conceived that patients progress through five stages (7). In stage I enlargement of the kidneys with Hyperfiltration, transient albuminuria, normal blood pressure and glomerular volume expansion are present and blood pressure is normal. (In animal models increased intraglomerular pressure is present at this stage.) During stage II, patients undergo a period of 2-15

years of silent disease in which urine albumin excretion is normal, but basement membranes are thickening and GFR is either supranormal or normal, blood pressure remains normal. In stage III, also called incipient nephropathy, urine albumin excretion is persistently elevated, glomerular basement membrane and mesangial expansion progress and GFR is supranormal or normal. Blood pressure may be normal but is often elevated compared to healthy controls at this stage. In stage IV, clincial diabetic nephropathy, overt proteinuria is present with mesangial expansion and closure of some glomerular loops, GFR is normal or decreased and overt hypertension is present in the vast majority of cases. As this stage progresses hypertrophy of glomeruli and tubules are noted along with collapsing glomerular capillary loops. As a result GFR progressively declines. In stage V, uremia develops, there is generalized glomerular closure, albuminuria decreases because of severe reduction in GFR and end-stage renl disease occurs.

Table 1. Comparison of key characteristics in Type I and Type II Diabetes with Nephropathy

Characteristic	Type I	Type II
Microalbuminuria reliable predictor of overt proteinuria	++	±
Hypertension precedes overt nephropathy	Rare	Common
Early glomerular Hyperfiltration and Hypertrophy	+	+
Progressive proteinuria	0.33	0.5
Progressive renal failure	0.33	6-15%
Familial predisposition	+	+
Diffuse glomerulosclerosis	+	+
Hyperlipidemia	+	+

In type I diabetes the first clinical sign of renal disease is microalbuminuria. Microalbuminuria increases at a rate of about 10-30% per year and correlates with poor glycemic control. It is generally regarded as a good predictor of future overt proteinuria if it develops before 20 years of disease. Some studies report rates of 80% or more of patients with microalbuminuria progressing to overt nephropathy over 6-15 years (8-10). However, in a recent study microalbuminuria was found to be a less precise predictor of future macroalbuminuria in patients who develop it after > 20 years of disease (11). Approximately 30-40% of type I patients develop overt renal disease.

Type II Diabetes

The natural history of type II diabetes is not as clear. In this condition,

hypertension is often present prior to onset of albuminuria. In addition, the cause for microalbuminuria is not certain, as it may relate to hypertension per se and not diabetic nephropathy (see below). Moreover, although the frequency of proteinuria is higher in patients with Type II diabetes as compared to Type I disease, the rate of decline in creatinine clearance is slower in comparison to Type I patients in some studies (12). It remains to be determined whether microalbuminuria is a reliable predictor of progression to overt nephropathy in patients with type II diabetes. Nevertheless, like type I diabetes, onset of overt diabetic nephropathy is a sign that renal disease is progressive. Some key comparisons between type I and type II diabetes are depicted below in Table 2.

The exact percentage of patients with type II disease who develop overt nephropathy progressing to ESRD is not known. However, it is estimated that about 6-15% of patients with type II diabetes will develop ESRD. Although a value of 6% is only 1/5 - 1/6 the value (30-40%) compared to type I diabetes, because there are 6 times as many patients with type II diabetes, approximately 50% of cases of diabetes (at least in the U.S.) arise in type II diabetics.

IV. RISK FACTORS IN THE DEVELOPMENT OF NEPHROPATHY

A. RACE

Several studies have shown that the incidence of new cases of nephropathy are not equally distributed among races. In comparison to Caucasians, African-Americans have a 4-fold (1), and Mexican Americans and Native American a 6-fold higher incidence (13-16) of diabetic nephropathy. In Mexican American women, obesity has been shown to be closely linked with the development of Type II DM. In comparison to Caucasians, African-Americans with ESRD due to diabetes have a 2.6-fold higher incidence even after adjustment for the increased incidence of Type II DM in the African-American population. Moreover, most African-Americans with ESRD are Type II, whereas the majority of Caucasians are Type I diabetics (17).

Table 2. Risk Factors for Development of Diabetic Nephropathy

Race
Hyperglycemia
Hypertension
Microalbuminuria
Early Hyperfiltration
Familial Predisposition
Smoking

B. Hyperglycemia

A multitude of studies indicate that poor glucose control is an important risk factor for development of diabetic nephropathy in both Type I and Type II

patients (13-15,18-22). In addition, several trials indicate that higher average blood glucose concentrations over long periods of time are associated with greater amounts of urinary albumin excretion and that lowering blood glucose can reduce urinary albumin excretion rate indefinitely (23-26).

C. Hypertension

Hypertension is a critically important risk factor for progression to end-stage renal disease in both type I and type II diabetes (13,27-32). In addition, prospective studies have shown that normoalbuminuric patients who progress to microalbuminuria have higher average (although normal) blood pressure (9,33). For instance in a study by Mathieson of 205 type I normoalbuminuric patients followed for 60 months, 7 progressed to microalbuminuria. In these patients albumin excretion rate and glycosylated Hgb levels were higher than in non-progressors (9). During follow-up BP rose progressively and to a greater extent than patients who did not progress. However, baseline BP was similar and albuminuria occurred prior to development of hypertension. In another study involving 137 normoalbuminuric type I patients, 11 patients progressed over a 4 year follow-up period. In comparison to non-progressors patients developing microalbuminuria had higher mean baseline blood pressure and glycosylated hemoglobin (34). Furthermore, in type I patients with renal disease, blood pressure is higher as compared to matched patients without renal disease. Moreover, others have reported that family history of essential hypertension is an important risk factor for development of nephropathy in type I diabetes (35,36), athough this has not been a consistent observation, (37).

D. Microalbuminuria

The normal urinary excretion rate of albumin is about 7 ug/min or 10 mg/d. The presence of persistent abnormal amount of urinary albumin (> 20 ug/min or 30 mg/day) in patients with long-standing diabetes has been shown to be associated with an increased risk for development of overt nephropathy in type I diabetes mellitus (see above). The prevalence of microalbuminuria in Type I with diabetes greater than 10 years is about 30%, and in type II of similar duration about 27% (38). In both type I and type II diabetes there is a close relationship between microalbuminuria and hypertension; however, it seems clear that microalbuminuria precedes onset of hypertension in type I diabetes (39). In type II diabetes this is not clear. Moreover, because hypertension is so prevalent in this population and because hypertension in the absence of diabetes may cause albuminuria, the significance of albuminuria as a predictor of future diabetic renal disease is not clearly established (40).

E. Early Hyperfiltration

Increased glomerular filtration rate (above 130 ml/min/1.73 m^2), or glomerular hyperfiltration is a common feature in patients with type I diabetes mellitus (41-44) and has also been reported in type II patients (45,46). An increase in kidney size, poor or uncontrolled blood glucose and microalbuminuria are associated with hyperfiltration in early stages of diabetes. Microalbuminuria at this stage is readily reversible with improved blood glucose control and is not considered a risk factor if only demonstrated at this stage. Some but not all studies have suggested that patients with early hyperfiltration are at increased risk of development of future nephropathy (39).

F. Familial Predisposition

Many clinicians have observed that diabetes runs in the family. Recently, sibling analysis of type I diabetics who did and did not develop renal disease has revealed that familial clustering of diabetic kidney disease does occur (47). This has been confirmed by additional studies by Borch-Johnssen (48). In addition, DNA sequence differences in ACE gene may contribute to genetic susceptibility to diabetic nephropathy in type I dieabetes (49).

G. Smoking

Two clinical studies have reported that smoking is an independent risk factor for progression from microalbuminuria to overt proteinuria (50,51).

V. STRUCTURAL-FUNCTIONAL RELATIONSHIPS IN DIABETIC NEPHROPATHY

The known structural and functional relationships in diabetes mellitus have been worked out primarily in type I diabetes. Pathologic changes in renal structure in advancing diabetes involve all four compartments of the kidney: 1) Glomerular circulation; 2) Blood vessels (including arterioles and arteries); 3) Renal tubules; and 4) Interstitium. As already noted albuminuria is the key clinical parameter in the progression from incipient to overt renal disease and is closely associated with development of hypertension and subsequent deterioration in renal function. Because persistent albuminuria arises from abnormal glomerular permselectivity, it is indicative of glomerular dysfunction. Consequently, the majority of studies have focused on the interrelationships between proteinuria, blood pressure and glomerular structure (and function). However, tubulointerstitial disease is always present in advanced renal disease and increasing evidence indicates that tubulointerstitial fibrosis correlates well with renal disease progression and glomerular mesangial expansion (52,53). The following discussion will focus on these two areas. However, it should be noted that arteriolar hyalinosis of afferent and efferent arterioles, a common finding in diabetes, is associated with abnormal intrarenal resistance which in turn disrupts renal

autoregulation, glomerular plasma flow and glomerular capillary pressure. The latter two factors are important determinants of altered glomerular function in this condition.

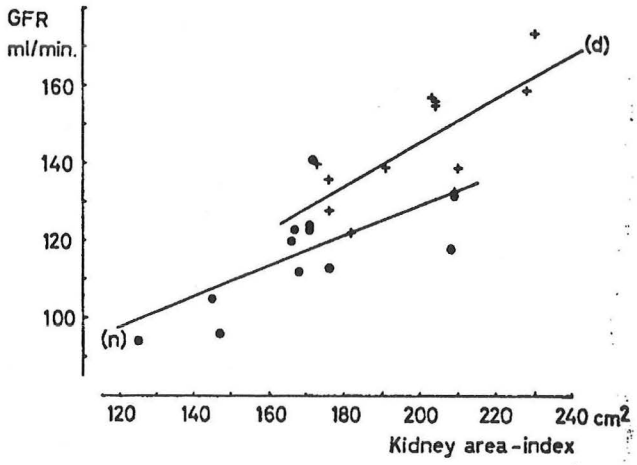
A. GLOMERULAR STRUCTURE and FUNCTION in RELATION TO PROTEINURIA

Early Hypertrophy and Hyperfiltration

The earliest structural changes in type I diabetic nephropathy are glomerular hypertrophy (54). Both glomerular cellular elements and extracellular matrix are increased. Moreover, there is an increase in capillary luminal volume indicating that the filtration surface area is increased. These structural alterations are accompanied by increased renal plasma flow, glomerular hyperfiltration and microalbuminuria in both type I and type II diabetics (41-43,46,55). There is a linear relationship between GFR and kidney size (figure 3). These alterations in structure and function probably occur in most type I, and in some type II, diabetics. However, as shown below in figure 4 these parameters ared partially normalized with improved glucose control. Whether this causes irreversible glomerular injury is still not certain(42,56-58).

The mechanism for hypertrophy and hyperfiltration at this stage are unknown, but hyperglycemia and attendant alterations in hormonal milieu undoubtedly play a role. As discussed below increased glucose concentration may stimulate hypertrophy. In addition, hyperglycemia is associated with reversible increases in renal plasma flow. Hyperfiltration may result from increases in plasma growth hormone, Insulin-like growth factor I, atrial natriuretic peptide, or a combination of these hormones (55,59). I n addition, renal hypertrophy in rats with streptozotocin-induced diabetes mellitus can be partially blocked by administration of converting enzyme inhibitor, suggesting that the renin-angiotensin system may also play a role in hypertrophy (60).

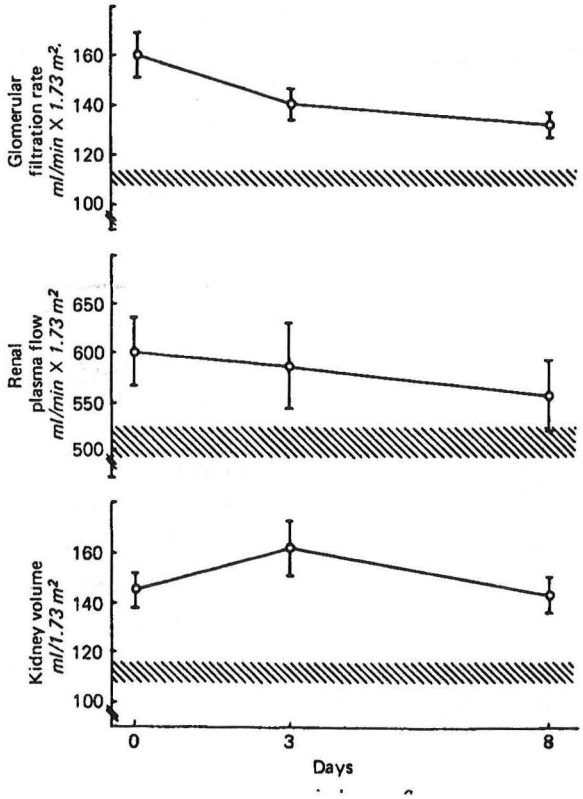
Figure 3. Relationship between GFR and Kidney Size in early type I Diabetes



GFR and kidney area-index in 12 normal (•) and 12 diabetic (+) subjects.
Regression line for normals (n)
—"— diabetics (d)

It has been suggested that early hyperfiltration is a predictor of future diabetic nephropathy (39). In the most recent study, Rudberg reported that of 65 patients with type I diabetes (average duration 11.6 years) only with a GFR > 125 ml/min/1.73 m^2 developed overt nephropathy after an 8 year follow-up period (39) (figure 5). Average baseline GFR, mean blood pressure and albuminuria were normal in these patients. The time course of blood pressure, albuminuria, glucose control and GFR in the five patients who developed overt nephropathy is shown below in figure 5. The positive predictive value of glomerular hyperfiltration was only 53%; however, the negative predictive value of a GFR < 125 ml/min/1.73 m^2 was 95%. Unfortunately this study did examine whether GFR at the onset of diabetic renal disease is associated with progression since the patients had diabetes for an average of nearly 12 years prior to measurement. However, it is noteworthy, that glycemic control did not appear related to progression to nephropathy in patients with hyperfiltration. Still, albuminuria appeared to precede the onset of hypertension in patients who progressed. If diabetic hyperfiltration is associated with development of progressive renal disease then it would be reasonable to assume that GFR would decrease more rapidly in this group. In a recent study of adolescent diabetics with normoalbuminuria, Bognetti (44) showed that patients with hyperfiltration had a greater decrease in GFR than non-hyperfiltering patients followed for 30 months.

Figure 4. Insulin treatment reduces hyperfiltration and kidney Size in Type I Diabetes

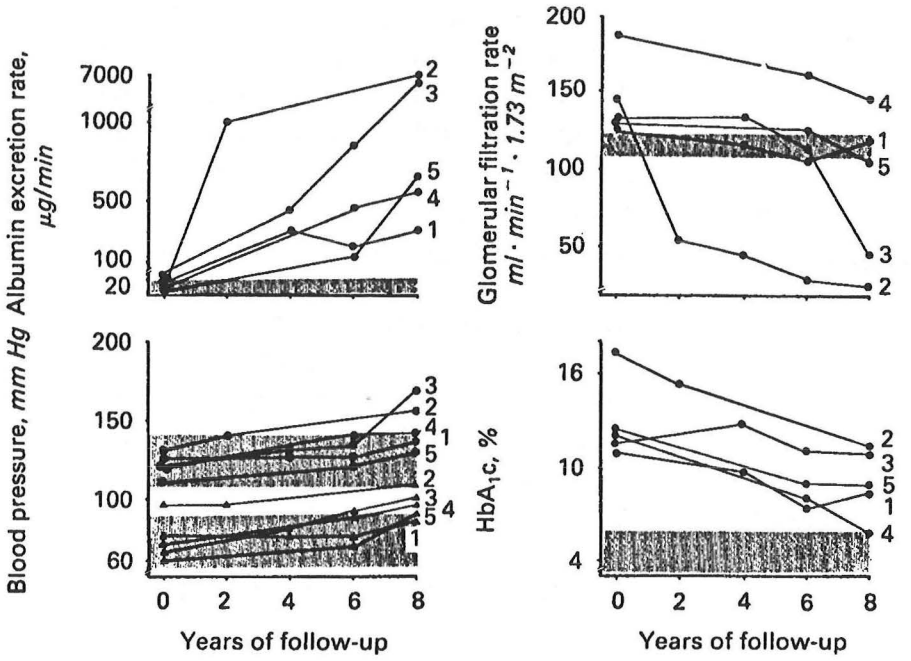


Later Glomerular Changes

Glomerular Basement Membrane

The first irreversible change in glomerular structure in diabetes is an increase in glomerular basement membrane thickness which is detectable within 2 years after onset of the disease. Recent studies in identical twins discordant for type I diabetes confirm this observation (54). However, the increase in GBM thickness is not related to and does not correlate with the development of proteinuria or hypertension in patients who develop overt renal disease. Nevertheless, alterations in the charge density of the GBM can be demonstrated early on even in the absence of detectable albuminuria. These early changes are not associated with mesangial expansion or changes in its chemical composition.

Figure 5. Progressive nephropathy in Type I diabetes with hyperfiltration and poor glucose control



Glomerular Mesangium

The structural hallmark of progressive diabetic nephropathy is an increase in mesangial matrix accumulation. Normally, the mesangium accounts for about 15% of the glomerular volume. Increased matrix begins to develop within 2-3 years of diagnosis and progresses substantially after 10-15 years of disease. The mesangium expands disproportionately relative to other glomerular components such that the fraction of glomerular volume occupied by matrix (Vvmes) becomes quite marked as disease progresses. About 2/3 of the increase in mesangium is extracellular components and 1/3 is cellular expansion. Cell hypertrophy is the main reason for increase; however, there may also be an increase in cell number.

Mesangial Expansion and GFR

There is a close association between the degree of mesangial expansion and GFR in diabetes. As shown below in figure 6A there is a strong inverse linear relation between the fractional mesangial volume and GFR in patients with advancing diabetic nephropathy. It is believed that expansion of the mesangium reduces the glomerular capillary surface area available for filtration ultimately leading to a progressive decline

Figure 6. Glomerular Filtration Rate decreases with increasing Mesangial Expansion in Type I Diabetes

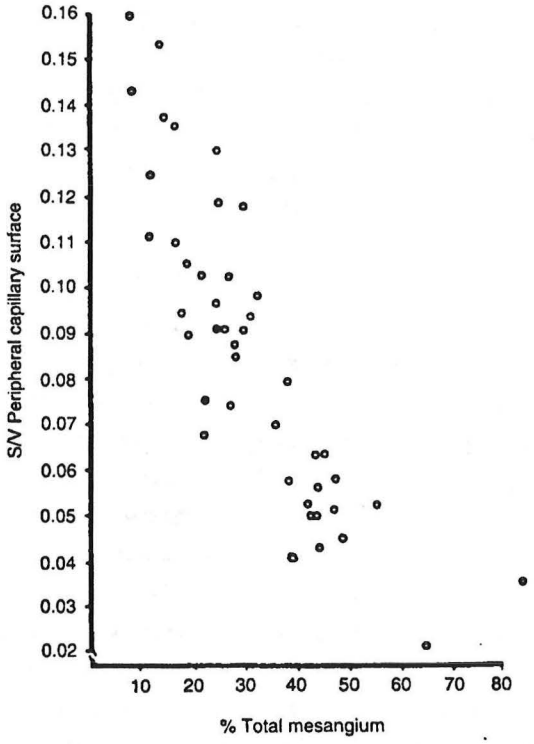


Fig. 4. *Relationship between the surface density (Sv) of the peripheral capillary filtration surface and the total % mesangium or mesangial volume fraction (Vv).*

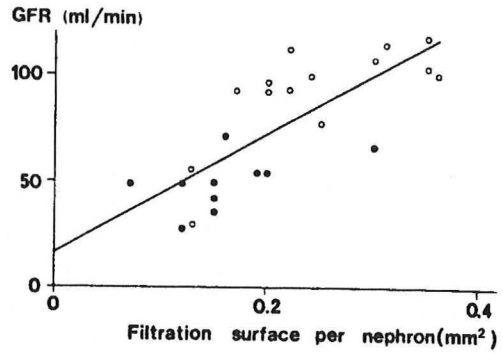


Fig. 4. Relationship between glomerular filtration rate (GFR) (uncorrected) and the estimates of filtration surface per nephron. The regression line is shown ($r=0.77$, $2p<10^{-4}$). Open circles (○) represent cases 1-14 (no antihypertensive treatment), closed circles (●) cases 15-24 (antihypertensive treatment)

in GFR. This view is supported by the strong inverse correlation between totalmesangium and peripheral capillary loop surface area as shown below in figure 6B.

Mesangial Expansion, Hypertension and Proteinuria As previously noted patients with diabetes who develop microalbuminuria tend to have increased blood pressure or are overtly hypertensive. In type I diabetes, all patients with advanced mesangial expansion (> 37% of glomerular volume) develop overt proteinuria and at least 75% of these patients are hypertensive. Thus hypertension in a type I diabetic is usually associated with microalbuminuria, although essential hypertension can occur in diabetics without albuminuria. Chavers performed renal biopsy on three groups of type I diabetic patients: Group I had normal GFR, normal urine albumin

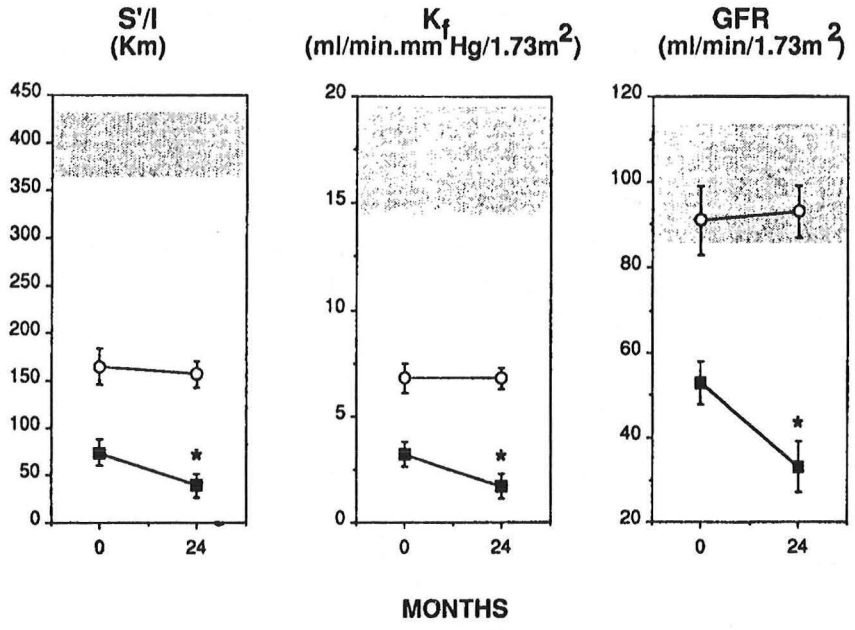
excretion rate and normal blood pressure; Group II had microalbuminuria (> 20 ug/min) and normal GFR and blood pressure; and Group had microalbuminuria, and either a decreased GFR or hypertension or both (54). They measured the mesangial expansion as assessed by the fractional glomerular volume occupied by the mesangium (Vvmes). They found that patients with normoalbuminuria had either normal or increased mesangium and that the patients with microalbuminuria with normal GFR and blood pressure completely overlapped with this group. In contrast, patients with microalbuminuria and hypertension with or without reduced GFR had significantly greater degree of mesangial expansion compared to normotensive microalbuminuric patients. They concluded that normoalbuminuria does not preclude abnormal mesangial expansion; however microalbuminuria associated with hypertension or reduced GFR suggests more advanced lesions are present. These data seem to suggest that additional factors besides an increase in mesangial matrix are responsible for the development of albuminuria in patients with diabetes. Such factors might include hyperfiltration, glomerular hypertension and alterations in the composition of the glomerular basement membrane structure and function.

Glomerular Function

At the glomerular level the single nephron GFR is mathematically expressed as the product of the ultrafiltration coefficient Kf and times the net pressure driving forces acting across the capillary wall as shown: $SNGFR = Kf(\Delta P - \Delta\pi)$ where ΔP represents the difference between hydrostatic pressure and proximal tubular pressure and $\Delta\pi$ the difference between plasma oncotic pressure and Bowman's space oncotic pressure. Recent studies by Austin et al (61) have delineated the changes in functional parameters of the glomerular basement membrane in patients with early vs late stages of overt nephropathy followed for a period of 24 months. As shown below in figure 7, patients with early nephropathy (open circles) have reduced filtration pore density (S'/l), decreased ultrafiltration coefficient (K_f), but a normal GFR, which is maintained during 2 years of follow-up. Since GFR is in the normal range but Kf is reduced, the single nephron net transcapillary pressure gradient ($\Delta P - \Delta\pi$) must be increased. This is likely to be caused by an increase in glomerular capillary pressure. Patients with more advanced disease have a more sever reduction in filtration pore density and K_f and as a result have a decreased GFR to begin with. Furthermore, GFR declines over time in these patients indicating they have progressive renal disease. In these patients glomerular pressure is also high in compensation for the decrease in membrane surface area. These data suggest that glomerular hypertension is likely to be present in patients with early diabetic nephropathy and that it persists during the progression of disease.

Implications for treatment

Figure 7. Reduced Ultrafiltration capacity is reduced in early and late Type I Diabetes.



The structural and functional studies suggest that the mechanism of defective glomerular barrier function in diabetes which causes albuminuria probably results from at least two mechanisms. First, there is an increase in diffusion of protein because of abnormal structural component which leads to leakage of proteins through a damaged basement membrane and altered mesangial matrix. Second, an increase in glomerular pressure leads to convective transport of protein through these glomerular pores. It is therefore likely that in diabetes, these two factors act in concert to cause the proteinuria and may indeed act synergistically to cause the massive, nephrotic range proteinuria often seen with advanced diabetic nephropathy.

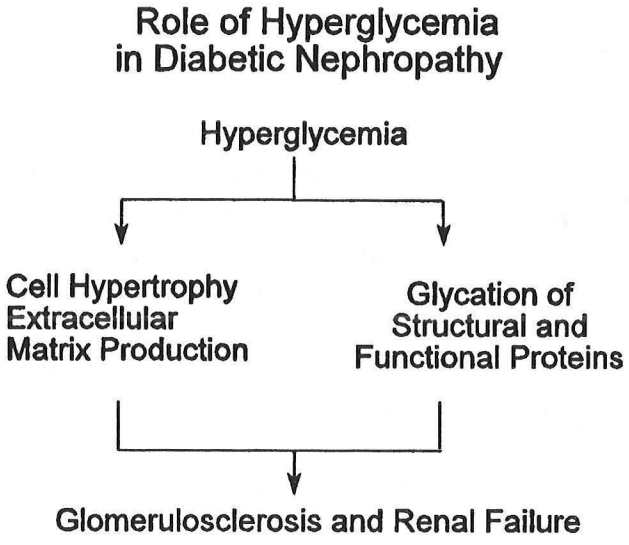
Tubulointerstitial changes

This topic has been recently reviewed in detail (52,53). Renal tubular epithelium and the interstitium are hypertrophic, and in fact account for the majority of the increase in renal mass associated with renal hypertrophy in diabetes mellitus. Interstitial fibrosis is a major pathologic finding in advanced renal disease. As renal disease progresses the interstitium undergoes fibrosis (52). It has been suggested that altered blood flow through the fibrosed interstitium or altered vascular supply to the interstitium (arteriolar hyalinosis, not discussed) may play a role. Long-standing glycation of various membrane and extracellular proteins may also play a role in the process in addition to the effects of various local growth factors (e.g. angiotensin II, platelet derived growth factor, endothelin, etc.) which may contribute to the process (see below).

VI. PATHOGENESIS and PATHOPHYSIOLOGY

The pathogenesis and pathophysiology of diabetic renal disease are not completely understood. However, there is general agreement that both metabolic and hemodynamic abnormalities are involved. It is also clear that these factors are interdependent at least during certain stages of the disease process (e.g. early hyperfiltration is related to poor glycemic control, see above discussion). Although it is still a matter of debate as to whether a metabolic (hyperglycemia) versus a hemodynamic (glomerular hypertension) factor is the primary cause for development of nephropathy, both factors play a role during the evolution of renal disease. The purpose of the following discussion is not to debate or evaluate the merits of these two processes as competing hypotheses. Instead the purpose is to outline the rationale for the theory that renal disease is caused by derangements in both metabolism and hemodynamics in diabetes mellitus.

Figure 8



Role of hyperglycemia

There are two basic ways in which hyperglycemia may cause renal damage in diabetes (figure 8). First, hyperglycemia directly induces renal cell hypertrophy both in glomeruli and tubules and stimulates production of excess and abnormal extracellular matrix proteins. Second, glycation of membrane proteins (and perhaps albumin) may lead to alterations in structural integrity which affect critical transport functions of the kidney including glomerular filtration of proteins and ion transport in tubules. Numerous clinical studies in both type I and type II have shown that poor diabetes control is associated with more advanced renal pathologic findings, albuminuria and hypertension (20,21,23,55,62-65). For example, in a recent study Walker et al have shown that there is a close correlation between GBM thickness, mesangial matrix increase, albuminuria and poor glycemic control (55). Hyperglycemia can induce both structural and functional changes in the kidney of diabetics (Table 3.)

Hypertrophy and increased extracellular matrix production

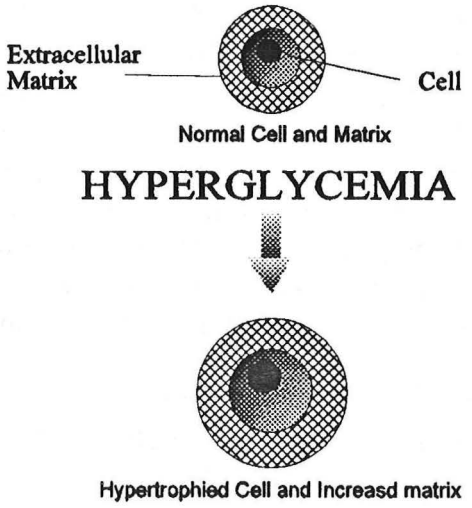
Table 3. Effect of Hyperglycemia on Renal Structure and Function

Structure
Cell hypertrophy: Mesangial, endothelial, tubular
Increased extracellular matrix mass
Altered extracellular matrix composition
Formation of Advanced Glycosylation end products
Function
Hyperfiltration
Increased glomerular permeability to proteins

Glomerular and tubular hypertrophy is common in diabetes, particularly in poorly controlled diabetics, and reducing blood glucose can reverse hypertrophy in humans. The mesangial cell is believed to play a key role in the development of diabetic renal disease. It synthesizes and secretes extracellular matrix, regulates glomerular filtration rate and participates in normal glomerular barrier

Figure 9

Hyperglycemia causes Mesangial Cell Hypertrophy and Expansion of Extacellular Matrix in Diabetes



function which governs filtration of plasma proteins. High ambient glucose concentration has been shown to alter both structure and function of mesangial cells (figure 9). First, it causes mesangial cell hypertrophy which is partially retarded by TGF-β, a potent antiproliferative cytokine (45,66). In addition, high glucose concentration increases in production of extracellular matrix components including type IV collagen, laminin, and fibronectin (13,21,50,67). Moreover, it can inhibit cytosolic calcium signaling which may in turn alter the contractile state of the mesangial cell and thereby enhance glomerular filtration (68). The mechanism of these effects is not completely known; however, several

studies indicate that high glucose concentrations activate cell growth factors, particularly protein kinase C (PKC). PKC activation in mesangial cells is associated with mesangial and endothelial cell hypertrophy. In mesangial cells the activation of PKC has been linked to increased matrix production, altered mesangial cell contractile function, eicosanoid synthesis (especially thromboxane A_2) cell hypertrophy, alterations in cell contraction, eicosanoid synthesis and matrix protein production. It is also associated with endothelial cell hypertrophy which may be accompanied by altered permeability to plasma proteins. This may in part explain altered capillary permeability in diabetes (66). Furthermore, high glucose induces proximal tubule cell hypertrophy, TGF-β gene expression and increased collagen synthesis (21,69,70).

Advanced glycation end products

Chronic hyperglycemia is known to cause glycation of both structural and functional proteins. The glycation of hemoglobin is a good example since it is used as an index of glycemic control in diabetes. More germane to diabetic nephropathy is the effect of glycation on tissue proteins. Glycation of tissue protein, results from interaction between the ketone group in glucose and amino groups (usually on histidine) in protein molecules forming a Schiff base and subsequently the more stable Amadori product (a ketoamine) (Figure 10). Cross-linkages between Amadori products and adjacent tissue protein molecules result in formation of advanced glycosylation end products (AGEs). Formation of AGEs in tissue may profoundly affect the normal structure and function of extracellular matrix (71). AGEs interact with specific receptors of macrophages and activate these cells promoting growth factor release from endothelial cells and collagenase release from mesenchymal cells. Recent studies in mesangial cells in culture have shown that AGEs increase transcription, translation and production of type IV collagen by these cells. This effect also appears to be receptor specific and is mediated by release of platelet-derived growth factor (72). In addition, AGEs have been shown to reduce proteoglycan charge of extracellular matrix (73). Since the

Figure 10. Advanced Glycoslyation Products-Prevention of Cross-Linking by Aminoguanidine

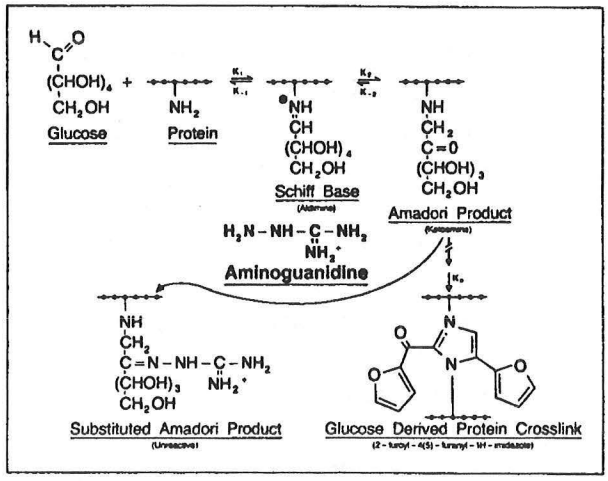


Figure 3. Prevention of Advanced Glycosylation End-Product–Protein Crosslinking by Aminoguanidine.

Aminoguanidine binds preferentially to reactive precursors of advanced glycosylation end products (represented by an Amadori product), and forms unreactive substituted products that can no longer form cross-links.

glomerular barrier to negatively charged proteins such as albumin is critically dependent upon the net negative charge in endothelial cells, glomerular basement membrane and other mesangial matrix, loss of negative charge contribute to increased glomerular permeability to plasma proteins thereby enhancing proteinuria. It has been speculated that disruption of tissue proteins by AGEs in the kidney could result in abnormal assembly of GBM protein leading to loss of normal size and charge selectivity thereby increasing proteinuria (71). Although loss of heparan sulfate proteoglycan from the glomerular basement membrane has been observed in patients in type I diabetics with advanced nephropathy, there is no evidence that this mechanism contributes to the development of microalbuminuria (74). Nevertheless, attempts to block formation of AGEs in renal tissue in experimental animals using Aminoguanidine have been performed. As shown in figure 10 above, Aminoguanidine has an affinity for the Amadori product leading such that reaction with it prevents the formation of a stable Glucose derived protein crosslink. In diabetic rats treated with Aminoguanidine chronically, prevention of glomerular basement membrane matrix accumulation and basement membrane thickening has been documented as shown below in Table 4. In addition to the effects of AGEs on tissue proteins, Ziyeda and Cohen have recently reported that glycated albumin increase collagen type IV production by mesangial cells (4).

Table 4. Aminoguandine protects against Diabetic Renal Damage in Rats (from reference)

	Untreated Group		Aminoguanidine Treatment	
Measurement	Normal	Diabetic	Normal	Diabetic
Glomerular Matrix Cross-linked IgG	0.50 ± 0.2	2.4 ± 0.2	0.40 ± 0.2	0.43 ± 0.1
GBM thickness	Normal	Increased	Normal	Normal

In summary clinical and experimental data support the view that hyperglycemia plays an important role in the pathogenesis and progression of diabetic nephropathy. Hyperglycemia not only induces cell hypertrophy and increased mesangial matrix deposition, the hallmark of diabetic nephropathy, but also induces glycation of tissue proteins which may alter both structure and function of renal cells and basement membranes as well as contribute to increased extracellular matrix production.

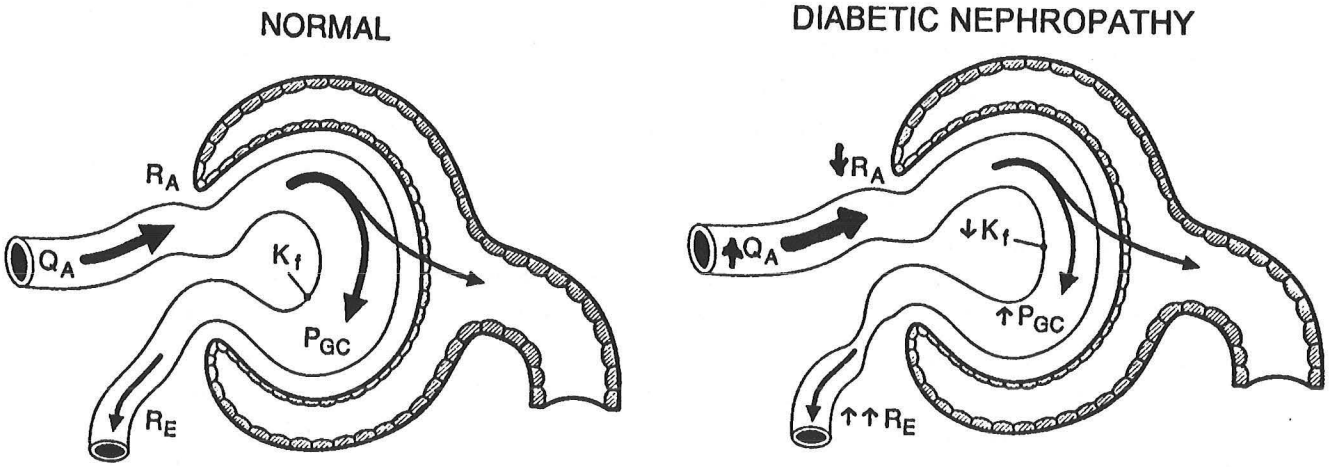
Hemodynamic Factors

Renal hemodynamics in diabetes mellitus are abnormal even during early phases of the disease. In patients with diabetes there are increases in renal plasma flow and glomerular filtration rate and a decrease in renal vascular resistance. In experimental

rat models of both type I and type II diabetes increases in RPF and GFR as well as decreased renal vascular resistance is also found (75). In order to better understand the pathogenetic role of abnormal renal hemodynamics in diabetic nephropathy it is useful to review the normal components of glomerular filtration dynamics. As shown in figure 11 the key hemodynamic variables that govern glomerular filtration are the glomerular plasma flow rate (Q_A), the afferent resistance (R_A), efferent resistance (R_E), the glomerular ultrafiltration coefficient (K_f), glomerular capillary oncotic pressure(π_a) the glomerular hydrostatic pressure (P_{GC}) and the proximal tubular pressure (P_T) (not shown). Single nephron GFR (SNGFR) can be calculated as SNGFR = $K_f(P_{GC}-P_T - \pi_A)$ Glomerular plasma flow rate can be directly measured in humans and K_f can be estimated and is related to total glomerular capillary surface area;which is known to be elevated in early diabetes.

Early on in experimental diabetes in rats there is a decrease in R_A, an increase in Q_A and an increase in P_{GC}. As a result there is an increase in the net transmembrane hydrostatic pressure gradient, or ΔP ($\Delta P = P_{GC} - P_T$,) (76). Thus, there is not only glomerular hyperfiltration but also glomerular capillary hypertension. These abnormalities along with hyperglycemia persist. As disease progresses, proteinuria, hypertension and progressive focal glomerulosclerosis develop. It is

Figure 11. Glomerular hemodynamics in Normal and Diabetes



important to note that this model is similar to human disease in many respects, except one important one. Namely, the glomerular pathology in rats is not typical of human

disease. Instead of diffuse (and nodular) glomerulosclerosis, a pattern of focal segmental sclerosis is observed. This has led some to question the utility of this animal model. Nevertheless, these animal models have contributed in a major way to a better understanding of human disease and have provided a rationale for specific therapy, namely angiotensin converting enzyme inhibitors.

Based on these observations, therapeutic interventions designed to reduce glomerular hyperfiltration and hypertension have been developed. It appears that both hyperfiltration and hypertension contribute to the development of proteinuria and renal damage in this model (59,77-81). Both reduced dietary protein intake and antihypertensive therapy are known to lower glomerular pressure and reduce hyperfiltration. As shown below in figure 12, reducing dietary protein intake in streptozotocin-induced diabetes markedly attenuates glomerular hyperfiltration,

Figure 12. Low Protein diet protects the Kidney in Diabetic Rats

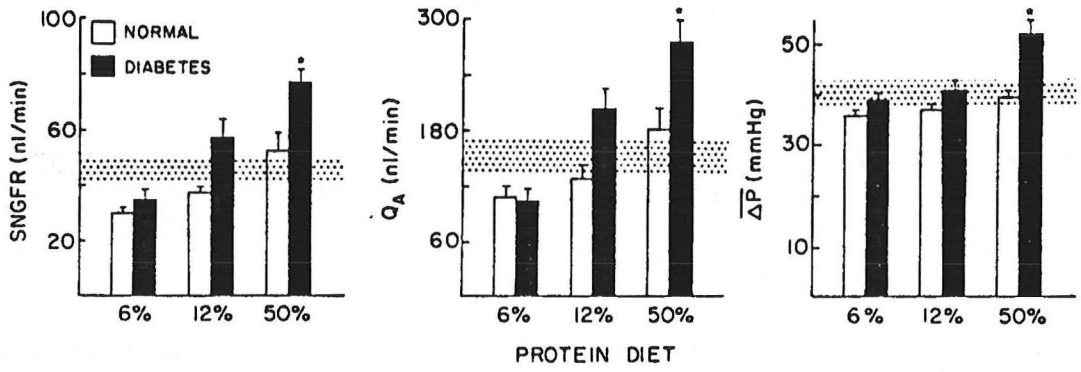


FIG. 1. Mean values for SNGFR, Q_A, and $\overline{\Delta P}$ 2–10 weeks after induction of diabetes or sham treatment. Open bars represent normal rats and solid bars represent insulin-treated diabetic rats. The stippled horizontal bands indicate normal values (±1 SEM) observed in rats fed 24% protein chow (31). Asterisks denote significant differences from normal. Note that only D50 rats had significant increases in all three hemodynamic parameters.

glomerular capillary hypertension, systemic hypertension, proteinuria (and progressive glomerulosclerosis, not shown) (79). These findings obtain even in the presence of persistent hyperglycemia. This led to the suggestion that hemodynamic factors predominate in the progression of experimental renal disease.

To explore the mechanism further studies were performed to determine whether lowering the transcapillary hydrostatic pressure gradient ΔP might also

protect the kidney. It is known that AII enhances renal efferent arteriolar tone and that efferent tone is increased in relation to afferent tone in diabetic animals. Therefore, enalapril, an angiotensin-converting enzyme inhibitor, was used to lower glomerular capillary pressure. In this experiment, systemic blood pressure was normal in control animals and animals with streptozotocin-induced diabetes mellitus. As shown below in figure 13, diabetic animals with normal blood pressure have increased glomerular capillary pressure and flow in comparison to controls. Enalapril treatment attenuated the increase in SNGFR and glomerular plasma flow rate. The increase in transcapillary hydrostatic pressure gradient (ΔP) was completely abrogated by enalapril treatment. Of note is the fact that systemic blood pressure was also lowered by enalapril even though systemic pressure was in the normal range. In addition, proteinuria was completely prevented by enalapril. Finally, glomerulosclerosis was prevented in the enalapril-treated animals. These studies strongly suggest that elevated glomerular capillary pressure and in turn transcapillary hydrostatic pressure. studies also support a role for elevated pressure in the pathogenesis and progression of diabetic nephropathy. Several studies have documented the fact that deterioration in renal function is accelerated by systemic hypertension (28,30,82-84). Moreover, the decline in renal function appears to be continuous even at levels of diastolic blood pressure in the range of 70-90 mmHg as shown in figure 14. Although glomerular pressures cannot be measured in humans, it is reasonable to assume that lowering of blood pressure in hypertensive diabetics also lowers glomerular pressure. Furthermore, several prospective studies of treatment of hypertension have established that lowering blood pressure preserves renal function in patients with overt, advanced nephropathy. In type I diabetics reduction in systemic blood pressure is associated with sharp reductions in albuminuria and the rate of decline in glomerular filtration rate(31,32,83,85-87).

Figure 13. Enalapril prevents diabetic nephropathy in Rats

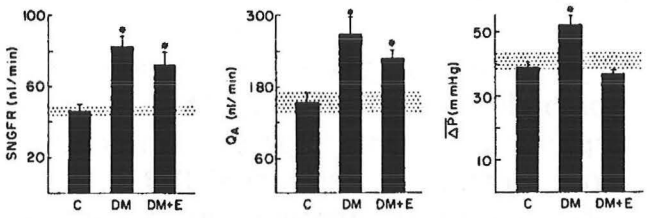


Figure 2. Glomerular hemodynamics 4–6 wk after induction of diabetes. SNGFR and Q_A were augmented in diabetic rats (group DM) and not significantly affected by enalapril treatment (group DM+E), compared to non-diabetic control rats (group C). $\overline{\Delta P}$ was also elevated in DM rats but was normalized by enalapril treatment. The stippled horizontal bands denote normal values (±1SEM) observed in this laboratory.

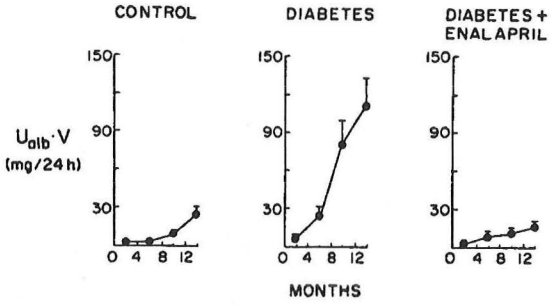


Figure 3. Sequential average daily albumin excretion rates ($U_{alb} \cdot V$) in control (C), untreated (DM), and enalapril-treated (DM+E) diabetic rats. In contrast to the marked rise in $U_{alb} \cdot V$ in the DM group, values in the DM+E group were indistinguishable from control.

Anecdotal reports in humans also favor this view. In diabetics with unilateral renal artery stenosis, the stenosis prevents increases in glomerular plasma flow and pressure in the stenotic kidney. Nodular glomerulosclerosis occurs in the contralateral kidney but not in the stenotic kidney (59). Taken together the experimental and clincal data have taught us that renal damage and dysfunction in diabetic humans results from hemodynamic forces as well as hyperglycemia.

Figure 14. Relationship between diastolic BP level and rate of progression of renal failure in Diabetes

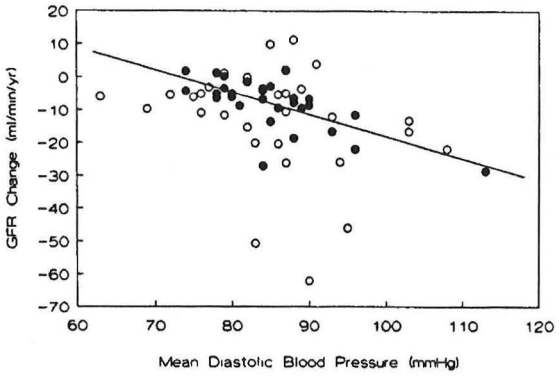


Fig 1. Rate of GFR change versus mean diastolic blood pressure. The observations are divided between those receiving more (●) or less (○) statistical weight ($r = 0.70$; $P < 0.0001$).

Hyperlipidemia

Hypercholesterolemia and hypertriglyceridemia occur commonly in type I and type II diabetics with advancing nephropathy. There is evidence that patients with hyperlipidemic patients progress toward end-stage renal disease at faster rates than normolipidemic patients (20,88,89). In a recent study by Shoji, pravastatin treatment of hyperlipidemia in albuminuric diabetics with normal serum creatinine reduced albuminuria after 3 months follow-up. However, at present there are no trials which have shown that treatment of dyslipidemia slows the rate of progression or development of renal disease.

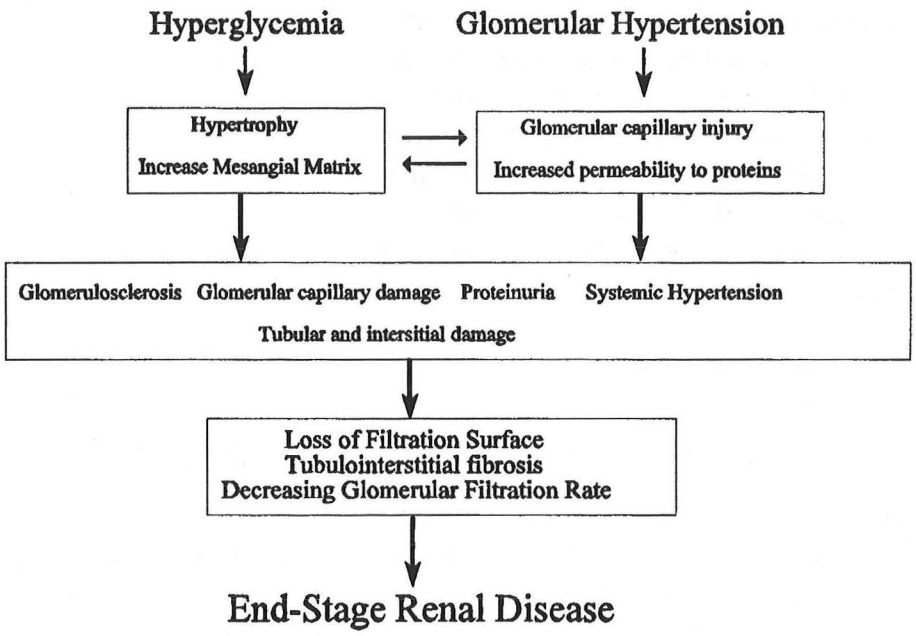
In summary, the pathogenesis of diabetic neprhopathy is complex. Both metabolic and hemodynamic factors play a role in the pathogenesis and progression of diabetic nephropathy. Hyperglcyemia together with glomerular hypertension act in concert to cause structural damage leading to proteinuria, systemic hypertension and progressive, irreversible glomerular and tubular damage culminating in terminal renal failure. Figure 15 illustrates a possible scheme in which these facgtors may interact to cause renal disease.

VII. CAN DIABETIC NEPHROPATHY BE PREVENTED?

Theoretically it is possible to prevent diabetic nephropathy. Although a clear picture of the pathogenesis has not yet emerged, we have a better understanding of factors that contribute importantly to development and progression of the disease. Over the past 10 years interventions designed to optimize blood glucose control and lower glomerular pressure have been tested in humans. These studies have provided

the following important conclusions: 1) "tight" blood glucose control reduces the risk of development of nephropathy; 2) antihypertensive treatment slows the rate of progression of established overt diabetic nephropathy; 3) treatment of normotensive and hypertensive diabetics with overt nephropathy using angiotensin-converting enzyme inhibitors (ACEIs) slows the rate of progression of nephropathy to a greater extent than conventional antihypertensive therapy; 4) ACEIs may prevent the development of overt nephropathy in normotensive microalbuminuric patients. These studies have come from various centers in Europe, Australia and the United States., Thus, the conclusions have come from a global effort to better understand and treat the disease. Further studies at very early stages as well as life-long follow-up data will be needed to prove unequivocally that the disease can be prevented. Although there is no evidence that overt diabetic nephropathy can be cured or stopped once it develops, progress in the past 10 years gives us reason to be optimistic about the future of preventing the disease. These new studies strongly suggest that nephropathy is already being prevented at least in some patients. The following discussion is focused on the results of these important studies with the aim at arriving at a rationale new approach to prevention and clinical management of diabetic nephropathy.

Figure 15



Blood Glucose Control

In attempt to determine whether there is a cause-effect relationship between blood glucose control and diabetic microvascular complications, Reichard et al (69) randomized 102 type I diabetics with nonproliferative retinopathy and normal serum creatinine but poor glycemic control to standard or intensive glucose control and followed them for 7.5 years. Nephropathy (manifest by overt proteinuria) developed i n 1/48 patients in the intensive treatment group and 9/54 patients assigned to the intensive treatment group. The odds ratio for developing nephropathy in the intensively treated group was 0.10, a highly significant outcome. As shown in the Table accompanying Figure 15, none of the microalbuminuric patients in the intensely treated group progressed to overt proteinuria and only one normoalbuminuric patient progressed. These findings strongly support the possibility that renal disease may be prevented or at least forestalled for prolonged periods of time.

In striking contrast to the increasing incidence of ESRD due to diabetes (see above), Bojestig et al

Figure 15. Intensive Blood Glucose Control Reduces the Risk of Diabetic Nephropathy

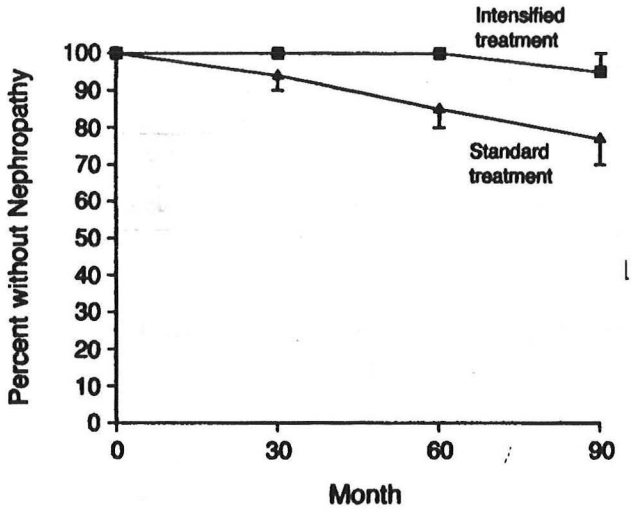


Figure 2. Mean (±SE) Proportions of Patients in the Intensified-Treatment and Standard-Treatment Groups without Nephropathy, According to Life-Table Analysis.

Table 3. Urinary Albumin Excretion at Base Line and after 7.5 Years in the Intensified-Treatment and Standard-Treatment Groups.

Albuminuria	Intensified Treatment	Standard Treatment	P Value
	no. of patients		
At base line			
Normoalbuminuria	34	33	
Microalbuminuria	8	13	
Nephropathy	2	3	0.56
After 7.5 yr			
Normoalbuminuria	33	26	
Microalbuminuria	8	11	
Nephropathy	3	12	0.04

Figure 16. Declining Incidence of Nephropathy in Type I Diabetes

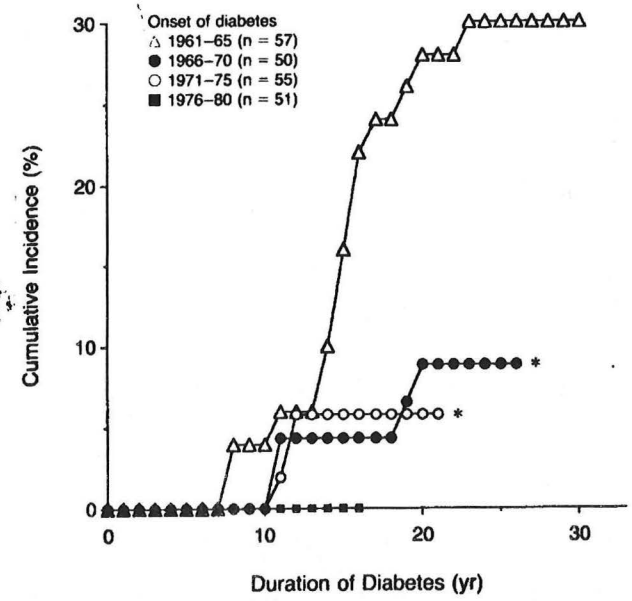


Figure 1. Cumulative Incidence of Persistent Albuminuria among Patients in Whom Insulin-Dependent Diabetes Began before the Age of 15 Years, According to the Year of Onset.

Each asterisk denotes a significant difference in incidence (P = 0.01) between the group indicated and the group with onset of diabetes from 1961 to 1965.

reported declining incidence of nephropathy in type I diabetes (90). In this study they reported glycosylated hemoglobin and urinary albumin excretion rates in 213 type I diabetics diagnosed before the age of 15 between 1961 and 1980. Glycosylated hemoglobin was measured in all patients beginning in 1980 and 92% of patients were followed up to 1991. As shown below in figure 16, they found that the cumulative incidence of persistent microalbuminuria after 25 years of diabetes decreased from 30% among patients who developed the disease between 1961 and 1965 to 8.9% among patients who developed disease from 1966 to 1970. And after 20 years of diabetes the cumulative incidence of microalbuminuria decreased form 28% in patients diagnosed for n 19611965 to 5.8% in patients diagnosed from 1971-1975. Most important, no patient diagnosed between 1980-1985 had developed microalbuminuria. They also found that glycosylated hemoglobin levels were higher in patients with microalbuminuria as compared with those patients who did not develop albuminuria.

In a long-term study averaging 11.9 (range 9-14) years, Gilbert et al (75) followed 44 patients (both type I and type II), 22 of whom progressed from microalbuminuria to overt nephropathy and 22 age and duration of disease matched patients who did not progress. They found that in progressors the rate of increase in albuminuria correlated with the level of glycosylated hemoglobin. This study provided evidence that progression of subclinical, i.e. microalbuminuria with normal

Figure 17. Strict Blood Glucose Reduces the Risk of Nephropathy in Type I Diabetes (The Diabetes Complications and Control Trial)

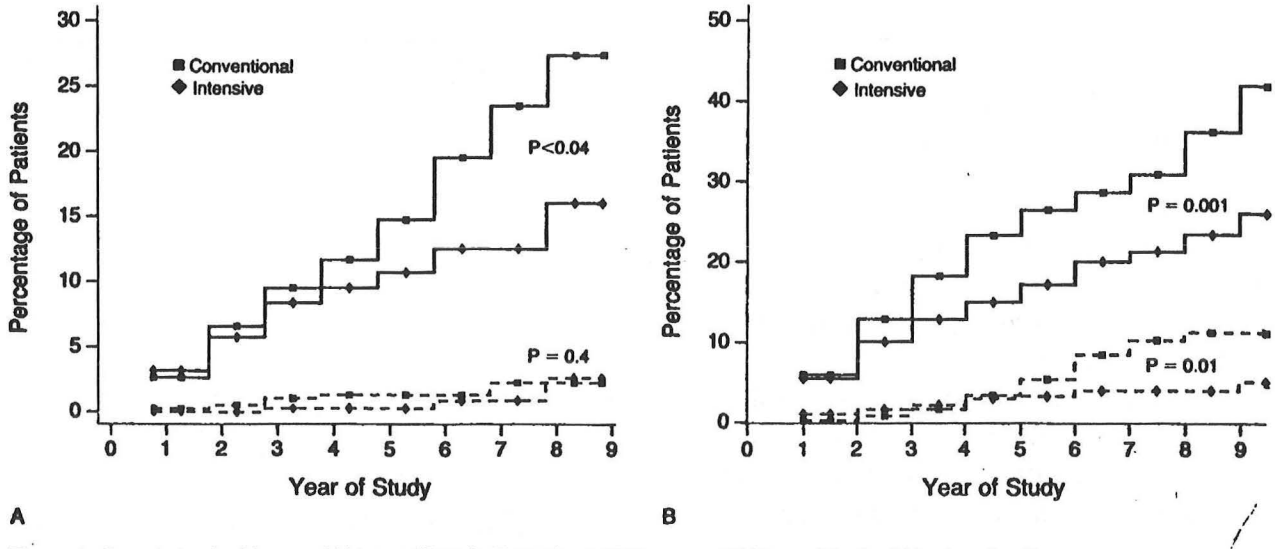


Figure 3. Cumulative Incidence of Urinary Albumin Excretion ≥300 mg per 24 Hours (Dashed Line) and ≥40 mg per 24 Hours (Solid Line) in Patients with IDDM Receiving Intensive or Conventional Therapy.

In the primary-prevention cohort (Panel A), intensive therapy reduced the adjusted mean risk of microalbuminuria by 34 percent (P<0.04). In the secondary-intervention cohort (Panel B), patients with urinary albumin excretion of ≥40 mg per 24 hours at base line were excluded from the analysis of the development of microalbuminuria. Intensive therapy reduced the adjusted mean risk of albuminuria by 56 percent (P = 0.01) and the risk of microalbuminuria by 43 percent (P = 0.001), as compared with conventional therapy.

blood pressure and glomerular filtration rate, nephropathy to overt nephropathy is linked to glycemic control. Importantly they also found that type I patients who developed nephropathy had higher blood pressure at the end of study as compared to the non-progressors.

The most powerful and largest study to indicate that tighter glycemic control can prevent nephropathy is the Diabetes Complications and Control Trial was extensively reviewed by Dr. Raskin in a recent Grand Rounds (91). In this study which enrolled 1441 patients followed for nine years there was a striking risk reduction for development of albuminuria in the primary prevention cohort (N=726) and in development of albuminuria as well as microalbuminuria in the secondary intervention cohort patients (N=715, retinopathy but no microalbuminuria). The risk reduction was 34% in the primary intervention cohort and 56% in the secondary intervention cohort (Figure 17).

Taken together these studies strongly suggest that optimal blood glucose control can forestall if not prevent the development of diabetic nephropathy at least in type I patients. It is possible to overinterpret the results of these trials because there is a small chance in each trial that the biases introduced by study design influenced the outcome of the trial. Moreover, the conclusions are based on probability that an event did not occur as a result of random variation but rather because of the treatment intervention. Finally, the studies are not life-long trials, thus it is possible that the development of persistent proteinuria is only stalled not prevented by the treatment. However, it should be recalled that in patients who develop microalbuminuria after more than 20 years of diabetes, the incidence of progression to overt nephropathy is much lower (11). Still, the optimistic view of these data are that overt diabetic renal disease is a preventable entity.

Antihypertensive therapy, Angiotensin Converting Enzyme Inhibitors and Calcium Channel Blockers.

Less than 20 years ago Mogensen reported (29) that hypertension was associated with progression to overt nephropathy and accelerated renal failure in diabetes and that lowering blood pressure may be beneficial in slowing deterioration in renal function. Since then numerous studies have been conducted in diabetic patients with hypertension and microalbuminuria as well as overt nephropathy. All studies indicate that lowering blood pressure plays a critical role in preserving renal function in diabetes.

Over the past 10 years there has been an emphasis on the use of angiotensin converting enzyme inhibitors (ACEIs) to control blood pressure and prevent nephropathy, because these agents could prevent nephropathy in experimental animals. A number of recent studies have taken these observations to the bedside. The results indicate that indeed these agents are the most effective agents for slowing progression of renal disese in both type I and type II diabetics with overt

nephropathy. Moreover, they are also effective in preventing the development of overt nephropathy in type I and type II patients. The key finding is that blood pressure control is of paramount importance in patients with diabetic nephropathy.

Recall that microalbuminuria does not necessarily indicate that overt nephropathy will develop but it does increase the likelihood that it will. In the following discussion prevention of diabetic nephropathy means that overt nephropathy does not develop over the time interval of the clinical trial.

Do Angiotensin-Converting Enzyme Inhibitors Slow the rate of progression of overt Nephropathy?

Studies in Hypertensive Type I Diabetics

There are several studies in hypertensive type I diabetics with microalbuminuria and overt nephropathy which have shown that ACEIs reduce blood pressure and albuminuria and preserve GFR (92-94). These studies involved small numbers of patients followed for 2.5-3 years. More recently two important long-term, prospective clinical trials have shown an important therapeutic advantage to treatment of type I diabetics with overt nephropathy. Bjorck et al (95) have reported that the rate of decline in renal function was significantly slower with enalapril as compared to metoprolol in a cohort of 40 type I patients despite similar levels of systemic blood pressure (Figure 18). In other words lowering blood pressure with the ACEI conferred additional renal protection.

The largest trial performed so far has involved 409 patients with type I diabetes, a urinary protein excretion rate > 500 mg/day and baseline serum creatinine ≤2.5 mg/dl (96). The patients were randomly assigned to receive either captopril 25 mg TID or placebo (+ conventional antihypertensives if necessary) in a double-masked fashion. 75% of the patients were hypertensive and 25% normotensive at the time of randomization. Goal diastolic blood pressure was < 140/90 mmHg. The primary study endpoint was a doubling of serum creatinine to at least 2.0 mg/dl. Secondary endpoints include development of ESRD and death. As shown in figure 19 below, there was a significant reduction in the number of patients with primary and secondary endpoints in the study in patients treated with captopril. Moreover, renal protection was most apparent in patients with the most advanced disease, i.e. those with higher baseline serum creatinine levels. The risk reduction was 48% in the captopril group as a whole, 76% in the patients with baseline Scr of 2.0, 55% for a creatinine of 1.5 mg/dl and 17% for patients with creatinine of 1.0 mg/dl at baseline. The impact of blood pressure lowering per se was not addressed in this paper. However, blood pressure was comparable and within normal range in both groups. This important study established that captopril protects against deterioration in renal function in type I diabetics and is significantly more effective than blood

pressure control alone. It would seem that this effect may in part be due to the capacity for the ACEI to reduce glomerular capillary pressure in the diabetic subjects. Further analysis of data on glomerular filtration rate, proteinuria and long-term blood pressure control will be forthcoming from this trial and should prove interesting. It is possible that this analysis will provide more information on the optimal blood pressure control level with an ACEI for slowing the rate of progression of renal disease in diabetics.

Figure 18. Enalapril is superior to Metoprolol in Preserving GFR in Diabetic Nephropathy

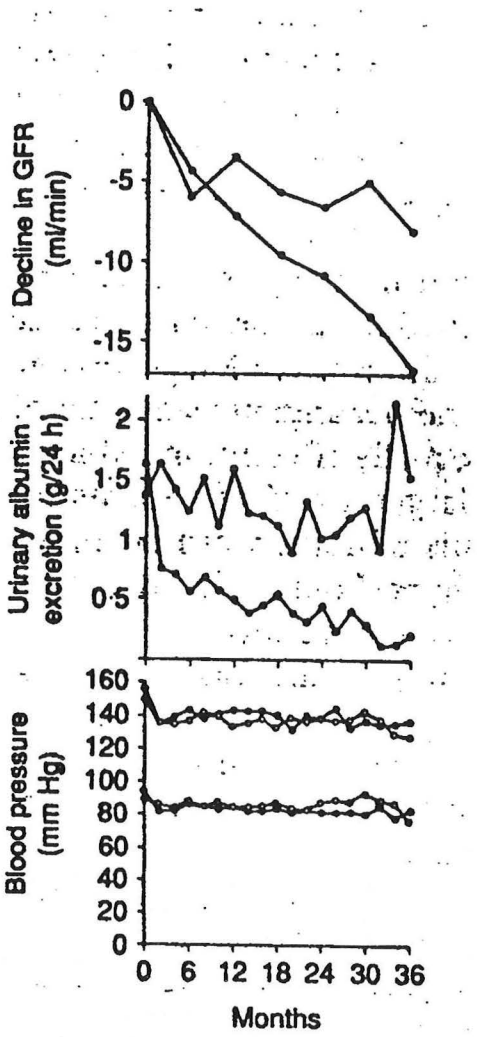


FIG 1—*The decline in glomerular filtration rate (GFR), urinary albumin excretion, and blood pressure before and during treatment with enalapril (●) or metoprolol (○) in 40 patients with diabetic nephropathy*

Figure 19. Captopril Reduces the Rate of Progression of Renal Disease in Type I Diabetics with Overt Nephropathy

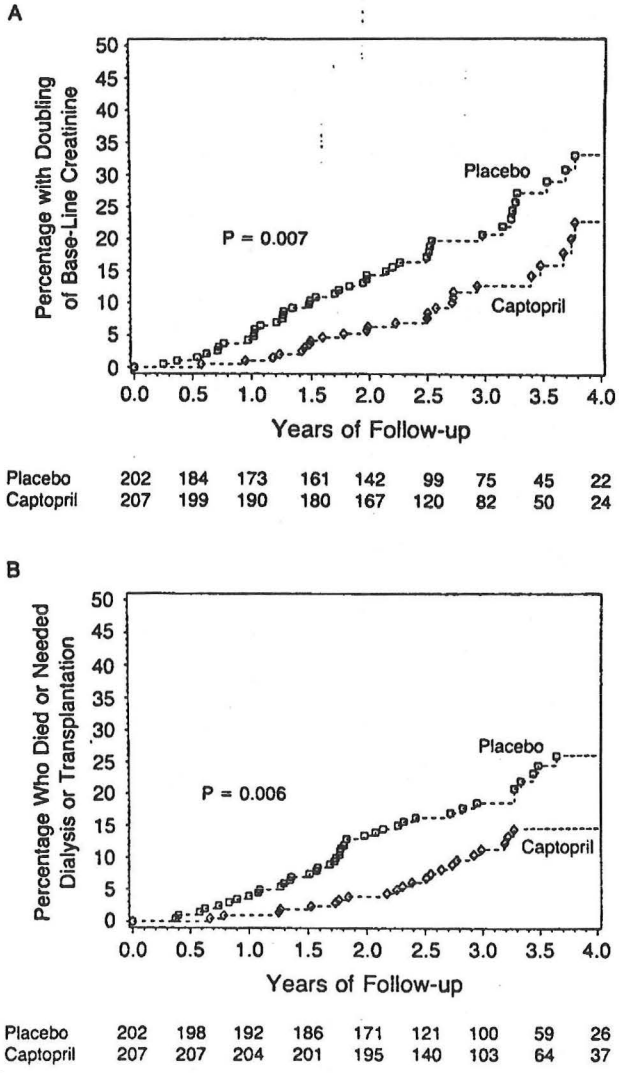


Figure 1. Cumulative Incidence of Events in Patients with Diabetic Nephropathy in the Captopril and Placebo Groups.

Panel A shows the cumulative percentage of patients with the primary end point: a doubling of the base-line serum creatinine concentration to at least 2.0 mg per deciliter. Panel B shows the cumulative percentage of patients who died or required dialysis or renal transplantation. The numbers at the bottom of each panel are the numbers of patients in each group at risk for the event at base line and after each six-month period.

Table 3. Percent Reduction in the Overall Risk of Progression of Diabetic Nephropathy with Captopril Treatment and According to the Base-Line Serum Creatinine Concentration.*

Event	Percent Reduction in Risk (95 Percent Confidence Interval)	
	Unadjusted for mean arterial pressure	Adjusted for mean arterial pressure
Doubling of base-line serum creatinine		
All patients	48 (16 to 69)	43 (6 to 65)
Base-line serum creatinine†		
1.0 mg/dl	17 (−97 to 65)	4 (−121 to 58)
1.5 mg/dl	55 (25 to 73)	50 (16 to 70)
2.0 mg/dl	76 (55 to 87)	74 (52 to 86)
Death, dialysis, or transplantation		
All patients	50 (18 to 70)	46 (10 to 68)
Base-line serum creatinine†		
1.0 mg/dl	7 (−127 to 62)	4 (−129 to 16)
1.5 mg/dl	54 (22 to 73)	51 (16 to 71)
2.0 mg/dl	78 (57 to 89)	75 (51 to 87)

*Proportional-hazards regression analysis was used to estimate the 95 percent confidence interval of the percent reduction in risk with mean arterial pressure as a time-dependent covariate and without it; a negative number indicates an increase in risk. The chi-square statistic for the interaction of the base-line serum creatinine concentration with the effect of captopril on the risk of doubling the concentration was 5.09 (P = 0.02) without adjustment for mean arterial pressure and 6.10 (P = 0.014) after adjustment for mean arterial pressure; that for the interaction with the effect of captopril on the combined end points of death, dialysis, and renal transplantation was 5.97 (P = 0.02) without adjustment for mean arterial pressure and 5.34 (P = 0.021) after adjustment for mean arterial pressure.

†The results given are for patients with the exact serum creatinine concentrations shown, as representative of the continuum of base-line serum creatinine concentrations.

Studies in Hypertensive Type II diabetics

Six recent clinical trials have focused attention on the effect of ACEIs on control of blood pressure progression of renal disease in Type II diabetics (97-102). In general these studies have all shown that ACEIs lower blood pressure, albuminuria. In patients with normal renal function at onset of the study no effect on GFR or serum creatinine was discernable. However, in studies involving patients with overt nephropathy with reduced glomerular filtration rate at baseline, the data indicate that the ACEI provides greater renal protection. In two comparison trials in which Lisinopril was compared with either guanfacine or atenolol, the rate of deterioration in renal function was significantly less in the lisinopril treated group (97,101). More recently, Lebovitz et al (102) conducted a prospective, randomized, double-masked, placebo-controlled trial. As shown in Table 5, patients treated with Enalapril had a significantly slower rate of decline in GFR as compared to conventional-treated subjects. However, mean arterial perssure was significantly lower in the enalapril-treated group. Therefore, although the effect of the ACEI was favored in this trial it may have been due to better blood pressure control and not a selective effect of the drug per se on renal glomerular hemodynamics. This again raises the question of optimal blood pressure control in the hypertensive diabetic with renal disese. At present there is no study which has compared different levels of blood pressure control with or without an ACEI to specifically address this issue.

Table 5. Enalapril is superior to conventional therapy in Type II Diabetics with overt nephropathy

Baseline and treatment data for patients with sub-clinical albuminuria[a] at baseline

	Control (N = 40)	Enalapril (N = 35)	Significance
Baseline GFR *ml/min/1.73 m²*	76.4 (3.12)	82.9 (2.57)	0.12
Baseline serum creatinine *mg/dl*	1.31 (0.048)	1.25 (0.034)	NS
Final serum creatinine *mg/dl*	1.57 (0.096)	1.39 (0.056)	NS
Rate of change of GFR during maintenance period *ml/min/1.73 m²/month*	−0.33 (0.124)	0.20 (0.129)	0.0044
MAP during maintenance period *mm Hg*	100.7 (0.920)	95.8 (0.820)	0.0001
Correlation MAP with rate of change in GFR	r = 0.08	r = 0.15	0.026

Data are mean. Numbers in parentheses are SEM. Abbreviation is: MAP, mean arterial pressure.

[a] Subclinical albuminuria = urinary albumin excretion ≤ 300 mg/24 hr or urinary protein excretion ≤ 500 mg/24 hr

Do Angiotensin-Converting Enzyme Inhibitors Prevent Diabetic Nephropathy?

Because most patients with type I diabetes and normal blood pressure have microalbuminuria and normal or only slightly reduced glomerular filtration rate, renal protection in such studies is determined by development of overt nephropathy. In essence these studies are focused on preventing diabetic nephropathy

Studies in Normotensive Type I Diabetics

Five studies have examined the effect of ACEIs on normotensive patients. These studies have been carried out for 1-4 years. Four of the five trials includied patients with microalbuminuria only and one trial included only patients with overt nephropathy, but in every trial blood pressure was normal at entry into the trial. In general they all show that ACEIs reduce the average level of albuminuria. Two of the four trials including only microalbuminuric patients demonstrated a substantial and significantly different rate of development of overt nephropathy in the ACEI treated patients (103). Figure 20 shows the results of the trial by Viberti et al in which 94 patients were randomized to either placebo or captopril and followed for 2 years. As can be seen urinary albmin excretion tended to increase in controls and decrease in captopril treated patients. Note that initial GFR was normal and did not change significantly in either group. Blood pressure was reduced significantly by captopril and did not change in the placebo group. Importantly, overt nephropathy developed in 12/46 (24%) placebo-treated patients but in only 4/46 (9%) of captopril-treated patients. It is noteworthy that blood pressure increased independent of treatment in patients who progressed to overt nephropathy. In a similar trial that was open-labeled but substantially longer, Mathiesen et al (104) showed that treatment with captopril for four years resulted in significant reduction in urinary albumin excretion, stabilization of glomerular filtration rate and prevention of overt nephropathy (figure 21). In this trial 0/21 (0%) captopril as compared to 7/23 (30%) placebo patients developed overt nephropathy. This is a very important finding since the reported rates of development of overt proteinuria in patients with microalbuminuria range from 15-70%. Taken together these small trials strongly suggest that diabetic nephropathy may be prevented by long-term treatment with ACEIs. Further follow-up is necessary to confirm that prevention has taken place. Still, it can be strongly argued that this treatment at least significantly delays

Figure 20. Captopril prevents development of overt nephropathy in microalbuminuric Type I

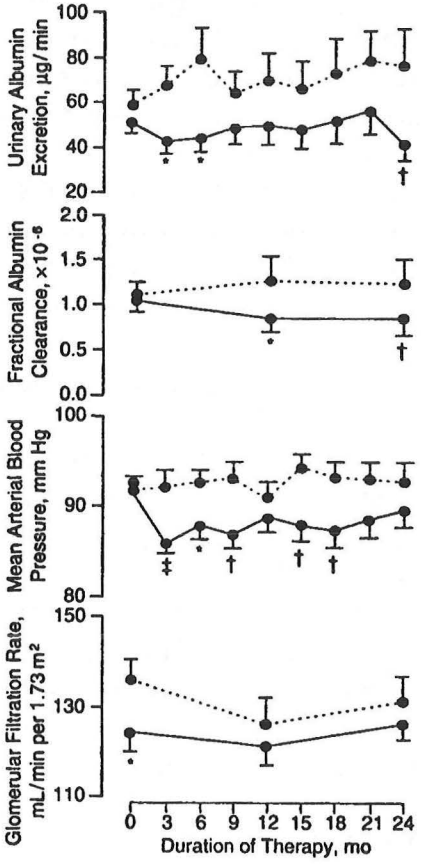


Fig 2.—Albumin excretion rate, fractional albumin clearance, mean arterial pressure, and glomerular filtration rate in patients with insulin-dependent diabetes mellitus who had microalbuminuria and who received either captopril, 50 mg (solid line) or indistinguishable placebo tablets (broken line) twice per day. Values are mean±SEM. Asterisk indicates $P \leq .05$, and dagger, $P \leq .01$, for captopril vs placebo for change from baseline.

the development of overt nephropathy.

Studies in Type II diabetics

Six recent studies have addressed this issue in Type II diabetics (105-110). The studies lasted from 6 months to 5 years. In aggregate, they provide good evidence that ACEIs can also prevent onset of nephropathy in type II diabetics. The most compelling evidence is furnished by Ravid et al (105). This was a randomized, double-masked, placebo controlled trial lasting 5 years in 94 patients. The results of the study are shown below in figure 22. As shown in the figure, placebo-treated patients experienced a progressive rise in albumin excretion and serum creatinine. In contrast, patients treated with enalapril had stabilization of both parameters. Blood pressure did not change significantly in either group. The incidence of overt nephropathy was 12% in the enalapril-treated and 42% in the placebo-treated groups respectively, an overall 30% risk reduction that was statistically significant ($P < 0.001$).

Figure 21. Captopril Prevents Overt Nephropathy in Type I Diabetics

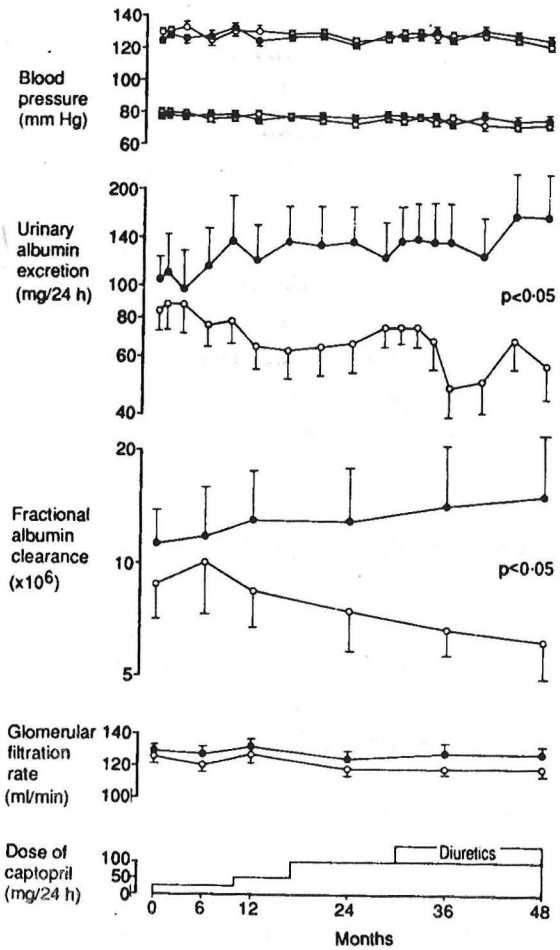


FIG 1—*Time course of mean arterial blood pressure, urinary albumin excretion, fractional albumin clearance, and glomerular filtration rate in normotensive insulin dependent diabetic patients with microalbuminuria. Twenty one patients received captopril (○) and 23 served as untreated controls (●). Ordinates of urinary albumin excretion and fractional albumin clearance are log scales. Bars are SEM*

In summary, in long-term studies involving both type I and type II diabetics treatment with a converting enzyme inhibitor significantly reduces the risk of development of overt diabetic nephropathy. Although these studies generally involved small numbers of patients the effect is quite consistent; therefore, it seems reasonable to expect that similar findings would be likely in a large-scale multicenter study. The ABCD trial is a long-term prospective, randomized controlled, double-blind trial large, multicenteter clinical trials an ongoing trial in type II diabetics including normotensive and hypertensive patients in which the effect on renal function of moderate versus intensive blood pressure control is being investigated. It will also compare the ACEI enalapril with the calcium channel blocker Nisoldipine.

Calcium Channel Blockers vs Angiotensin Converting Enzyme Inhibitors.

Few studies have examined the long-

term effects of calcium channel blockers on diabetic nephropathy. This subject has been recently reviewed (111). In general both calcium channel blockers and converting enzyme inhibitors lower blood pressure to a similar extent in patients with early and late diabetic nephropathy. However, in contrast to the ACEIs the results with Calcium channel blockers with respect to the antiproteinuric effects are more variable. In particular in some studiese, the results with dihydropyridine calcium channel blockers (e.g. nifedipine) are less favorable than verapamil or diltiazem. To date, there are no long-term trials comparing these classes of agents which have shown a clear therapeutic advantage of one class over another. However, in the Melbourne Diabetic Nephropathy Study, which compared nifedipine to perindopril in hypertensive and normotensive type I and type II diabetics with microalbuminuria, not overt proteinuria at entry, both agents lowered blood pressure and albuminuria to similar degrees after one year of treatment (110). Also there was no significant difference between type I and type II patients in response to therapy. In contrast, in a double-blind randomized trial lasting 12 months, Chan et al (97) found that enalapril lowered albuminuria to a greater extent than nifedipine in type II diabetics. However, creatinine clearance decreased to a similar extent in both groups and diuretics were required in a higher percentage of enalapril-treated patients in order to obtain the same level of blood pressure control as compared to nifedipine-treated subjects. Slataper has shown that lisinopril and diltiazem produce similar reductions in blood pressure and albuminuria and slow the progression of renal disease to a similar extent in type II patients with advanced nephropathy studied for 18 months (101). Moreover, either regimen was superior in slowing the rate of progression of renal failure as compared with conventional vasodilator/diuretic therapy. In a short-term trial, DeMarie and Bakris reported that diltiazem but not nifedipine lowers proteinuria in type II diabetics with advanced diabetic nephropathy and heavy proteinuria (112). However, there are no long term head-to-head comparisons of different calcium channel blockers in diabetics.

Combination Therapy with Angiotensin Converting Enzyme Inhibitors and Calcium Channel Blockers

Combination therapy with calcium channel blockers and ACEIs versus either agent alone and with conventional therapy has been studied in a small number of patients by Bakris et al studied for 12 months (98). In this study lisinopril and diltiazem reduced proteinuria and slowed deterioration in GFR to a similar extent and were both renal protective in comparison to the combination of guanfacine and hydrochlorothiazide. The combination of lisinopril and diltiazem reduced proteinuria to a greater extent than either agent alone, but did not improve further on the decline in GFR. However, patients with the greatest reductions in proteinuria tended to have the slowest rate of decline in renal function.

Dietary Protein Restriction

Several studies in patients with type I diabetic nephropathy indicate that lowering dietary protein intake preserves renal function over periods up to 3 years. The most compelling study comes from our institution. Zeller et al (113) showed that lowering dietary protein intake to 0.6 g/kg/d as compared to ≥ 1.0 g/kg/d (plus the urinary loss rate) slowed the average rate of decline in GFR to 0.3 ml/min/1.73 m^2 in the low protein as compared to 0.9 ml/min/1.73 m2 in the normal protein intake group Blood pressure control and dietary phosphate intake were similar between groups. Importantly there was no significant decline in serum albumin or evidence of malnutrition in the patients on the low protein intake. This study stands in contrast to the lack of benefit of dietary protein restriction reported in the Modification of Diet in Renal Disease study. However, type I diabetics were excluded from that trial. This therapy seems to be effective but it requires a highly motivated patient and intensive dietary counseling to maintain the low protein intake.

Future Studies

Two important clinical trials designed to preserve renal function in diabetics are now underway. The ABCD trial has been mentioned. In addition, a multicenter clinical placebo-controlled trial using aminoguanidine, the agent that blocks advanced glycosylation end product formation, to prevent renal disease progression in type I diabetics is in the recruitment process now. This will provide important information of whether altering tissue AGE formation is important in the pathogenesis of progressive renal disease.

VIII. RECOMMENDATIONS for PREVENTION and TREATMENT of DIABETIC NEPHROPATHY

The National Kidney Foundation and the American Diabetes Association are now in the process of developing recommendations for prevention and treatment of diabetic nephropathy; however, there recommendations have not been published as yet. My recommendations are based on this review which includes current experimental and clinical evidence. Until further studies are performed to compare different antihypertensive/antiproteinuric agents and other therapeutic interventions, it seems prudent to recommend the following:

For prevention of future nephropathy

1. Optimize glycemic control to provide glucose control as close to normal as possible. Although complete normalization is ideal any improvement is warranted. Patients should be assisted by all available means in attempt to achieve this goal.

2. Routine screening for albuminuria should be performed on an annual basis

beginning with routine urine dipstick test. If the dipstick is positive, a confirmatory test using a spot morning urine sample to calculate the urinary albumin/creatinine ratio (see Table 6 below for appropriate values). If the ratio is ≥ 30 mg/g the test is positive and can be confirmed further with a 24 hour urine to quantitate proteinuria. Urine albumin excretion rate ≥ 20 ug/min is abnormal and should be treated. If the test is negative the patient should be rescreened annually.

Table 6. Diagnosis of proteinuria in diabetes mellitus

	24-hour collection	Spot urine (adjusted for urine creatinine)	Timed urine (12 or 24-hr) collection
Normal albuminuria	< 30 mg/24 h	30 mg/g cr	< 20 μg/min
Microalbuminuria (incipient nephropathy range)	30-300 mg/24 h	30-300 mg/g cr	20-200 μg/min
Macroalbuminuria (overt nephropathy range)	> 300 mg/24 h	> 300 mg/g cr	> 200 μg/min

3. Treatment with an angiotensin-converting enzyme inhibitor should be instituted in microalbuminuric patients whether blood pressure is increased or not. The dose of drug should be increased to the maximum tolerable amount or until albuminuria is in the normal range.

For treatment of Overt Nephropathy

1. Patients with overt nephropathy should be treated with an angiotensin-converting enzyme inhibitor as noted above unless there is a contraindication.

2. Hypertension control is paramount in diabetes. The optimal level of blood pressure control has not yet been delineated. Recent studies suggest that higher blood pressures even in the normal range may be detrimental to the kidney. The recommended target for now is < 130/85. However, caution should be exerted in patients with autonomic neuropathy because of the risk of orthostatic hypotension.

3. In hypertensive edematous patients dietary sodium restriction to 20 grams per day is advisable. Loop diuretics should be employed to reduced blood pressure and help control edema if ACEI therapy alone is insufficient. Loop diuretics should be added and not substituted for ACEI to control blood pressure.

4. Dietary protein restriction should not exceed 1.0 g/kg/d. Although many patients may not tolerate the level of 0.6 g/kg/d it is possible that many can and will tolerate a level of 0.8 g/kg/d. This should be recommended for most patients and is in keeping with the normal recommended dietary intake in the U.S.

N.B. These recommendations may require revision after release of the NKF and ADA recommendations which are currently under development.

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