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Chapter III

Role of Hyperfiltration in the Pathogenesis of Diabetic Nephropathy

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Abstract

Diabetes mellitus, Type 1 and Type 2 combined, is the leading cause of end stage renal disease in the United States. While the disease is complex and mutifactorial, one of the major factors leading to diabetic nephropathy is glomerular hyperfiltration. Hyperfiltration and the rise in single nephron glomerular filtration are believed to occur during the first five years of the disease. In the 30-40% of susceptible individuals an increase in both mesangial matrix and microalbuminuria (20 to 200micrograms/min) heralds the onset of diabetic nephropathy. Of the hemodynamic factors involved in the rise in single nephron glomerular filtration rate increases in glomerular pressure as well as glomerular blood flow appear to be of critical importance. The cellular effects resulting from sheer stress (or stretch) may be the major cause for the rise in glomerular pressure although other factors are also involved. Inhibition of the renin-angiotensin-aldosterone axis appears to slow the progression of diabetic nephropathy as does good long term control of hyperglycemia. Many patients with diabetes mellitus have hyporeninemic-hypoaldosteronism. The pathogenesis and management of this interesting subdivision of diabetic nephropathy is also discussed.

Introduction

Diabetes mellitus has been known since antiquity, but it was not until the second half of the 20th century that we began to see the myriad of complications of this disease. In 1921,

Banting and Best [1 for Review] at the University of Toronto showed that an extract of the pancreas lowered blood glucose in dogs with hyperglycemia. One year later, the very first diabetic patient was treated with insulin. In 1923, Banting and Macleod (the prize committee never did acknowledge Best) won the Nobel Prize in Medicine and Physiology for this monumental work.

In 2008, the Center of Communicable Diseases estimated that 24 million people in the United States have diabetes mellitus [2]. It is thought that about six million or 25% of these patients are not yet diagnosed. Approximately 57 million people in the United States are believed to be pre-diabetic because of risk factors such as family history, ethnicity, body habitus, and age. The vast majority of diagnosed patients (i.e., 90%) have Type 2 diabetes (insulin resistance). In the United States 13% of Type 2 diabetics are black, 15% are Amerindian, and 10% are Hispanic [2]. Of the patients with Type 2 diabetes mellitus 30-40% will develop diabetic nephropathy [3, 4].

Only a small percentage of diabetic patients (i.e., 5-10%) have Type 1 diabetes mellitus. This form of the disease is associated with insulin deficiency, typically the result of autoimmune destruction of the β-cells of the pancreas. Type 1 diabetics are usually young and Caucasian. It has long been thought that 30-40% of these patients will develop nephropathy after 10 years of the disease. A recently published prospective report (The Oxford Regional Prospective Study) has cast doubt on these numbers [5]. The Oxford Study showed that 51% of these patients developed microalbuminuria and 14% had macroalbuminiuria by 10 years after diagnosis and that more females than males developed diabetic nephropathy. The patients in this study were recruited within 3 months of developing Type 1 diabetes mellitus and were an average of 8.8 years of age. They were followed prospectively for 10 years and most were on no medications other than insulin. The mean Hemoglobin A_1C of these patients was 9.8% [5]. Good glycemic control is considered to be less than 7.0%. This report of 527 patients documents a much higher cumulative incidence of albuminuria than the 30-40% that has been previously reported. It further suggests that even at the young age of 9, most of these patients should be on renoprotective therapy and have tighter blood glucose control.

Increasingly, we have recognized the importance of gestational diabetes mellitus. This disorder affects 2 to 5% of all pregnancies. It is estimated that 20-50% of these women will develop diabetes mellitus later in life [6, 7]. Most will have the Type 2 form of the disease.

The medical cost for managing diabetes mellitus in the United States now exceeds $132 billion annually [1]. It is the leading cause of chronic renal failure, blindness in adults, and amputation of limbs in the United States. It is one of the major factors contributing to cardiovascular morbidity and mortality. According to the Centers for Medicare and Medicaid, in 2009, there were 18 million people diagnosed as having some degree of chronic kidney disease; more the 410,000 of these patients are now receiving renal replacement therapy (i.e., dialysis or kidney transplant) [7]. As stated above, diabetic nephropathy is the leading cause of renal failure in the United States, accounting for >50% of cases (hypertension is the second most common cause of chronic renal failure).

In 1975, three years after the End Stage Renal Disease program was started, patients with diabetes mellitus comprised less than 5% of dialysis patients. In the intervening time there has been an explosion of Type 2 diabetes mellitus, due in part to obesity, easy access to food, and lack of exercise. Also, we have not restricted dialysis or renal transplant to people with the disease. We both remember the "death boards," weekly meetings deciding who would be able to receive dialysis and who would not (i.e., those who would live and those

who would die). In the 1970's, patients with diabetes mellitus (as well as the aged) were in the latter group.

Diabetic Nephropathy in Humans

Renal biopsies of patients with diabetes mellitus reveal both macrovascular as well as microvascular disease. This has been noted in a large number of cases, even in the absence of abnormal renal function. Much of our understanding of the natural course of diabetic nephropathy comes from the original observations of young adults with Type 1 diabetes. Prior to 1922, virtually all of these patients died from severe ketoacidosis with hyperglycemia and hyperkalemia. Chronic renal failure or uremia by and large was not seen because of the short duration of the disease. In the mid-1930s, by contrast, approximately 15 years after insulin first had been administered clinically, it became clear that the disease affected many organs, including the kidney. Cardiovascular disease, retinopathy, peripheral vascular disease, and autonomic dysfunction (gastroparesis, cystopathy, and peripheral polyneuropathy) are the rule rather than the exception in diabetes mellitus [8]. Upwards of 75% of these patients will die from cardiovascular disease whether they have nephropathy or not.

Renal histology in these early patients showed nodular glomerulosclerosis, first described at autopsy by Kimmelsteil and Wilson [9] at Harvard. It is now apparent that this lesion is but one of several abnormalities which occur in the diabetic kidney. The most common glomerular lesion is diffuse intercapillary glomerulosclerosis, characterized by an eosinophilic deposition of matrix in the mesangium and thickening of the glomerular basement membrane. Nodules are invariably found in diffuse intercapillary glomerulosclerosis due to deposition of mesangial matrix. The only pathognomonic tubular change noted and one we rarely see today is the Armani-Ebstein lesion. This is characterized by pale glycogen-filled cells of the *pars recta* of the proximal tubule. There is also hyaline deposition in the afferent and efferent arterioles. In advanced diabetic nephropathy, the tubules atrophy and one often sees interstitial edema, fibrosis, and cellular inflammation with macrophages and polymorphonuclear leukocytes [8]. The various factors involved in the development of nephropathy as well as those interventions which may alter the progression to renal failure are the subject of this book.

The time course for untreated diabetes mellitus in those individuals susceptible to developing diabetic nephropathy may be divided into two stages [8, 10].

1. Early Changes

The early stage (less than 10 years) of diabetic nephropathy is associated with a greater than 30 % increase in glomerular filtration rate (GFR)). There is also a lesser rise in PAH (para-aminohippuric acid) measured effective renal plasma flow such that the filtration fraction (the ratio of GFR to renal plasma flow) increases significantly from a normal of about 18% to 26%. Renal size and the tubular maximum glucose reabsorbtive rate (Tm glucose) are both increased. At this stage hypertension is not often found. Microalbuminuria, perhaps the most important early measure of abnormal renal function, is present and unless

treated, heralds subsequent azotemia and chronic renal failure. Urinary albumin excretion is typically in the range of 30 to 300microgm/24hr (normal is <30microgm/24hr). If renal biopsy is performed at this stage, one sees expansion of the mesangium, an increase in the size of mesangial cells, and thickening of the glomerular basement membrane.

2. Late Changes

The late stage (about 10 to 15 years) of diabetic nephropathy is characterized by proteinuria (>300microgm/24hr), though not necessarily in the nephrotic range. After 30 years, fully one-half of patients with diabetes mellitus and microalbuminuria will develop overt proteinuria. If nephrotic range proteinuria (>3gm/24hrs) occurs, renal replacement therapy typically will be required within 3 years.

In this chapter we shall summarize the role that hemodynamic changes play in early diabetic nephropathy. We also shall discuss the mechanisms responsible for these hemodynamic changes as well as the effect of pharmacologic therapies on these hemodynamic abnormalities. Finally, as we have a long standing interest in the syndrome of hyporeninemic-hypoaldosteronism in diabetes, we shall conclude with a discussion of this disorder.

Determinants of Intrarenal Hemodynamics

In the 1970's the nephrology laboratory at the Peter Bent Brigham in Boston, led by the great renal physiologist, Dr Barry F Brenner, published a series of carefully performed micropuncture experiments [11-20]. The group studied, in superficial cortical nephrons of Munich-Wistar rats, the hemodynamic forces controlling filtration by the glomerulus. Figure 1 is a schematic of a single glomerulus with its afferent and efferent arterioles, the mesangium, the macula densa, the glomerular basement membrane, the urinary space, and the early proximal convoluted tubule [21]. We shall refer to Figure 1 again later in this chapter.

Brenner's group posited that intrarenal physical forces were etiologic in causing chronic renal failure (i.e., glomerulosclerosis) regardless of underlying disease. This hypothesis was based on the well known observation that when renal function decreased in humans to about ½ - ¼ of normal, with time, end stage renal failure inevitably occurred.

Brenner, Deane, Hostetter and colleagues [11-20] measured intrarenal pressures, afferent and efferent blood flow, single nephron glomerular filtration rate (SN_{GFR}), and then calculated the afferent/efferent arteriolar resistances and the filtration coefficient (K_f). After defining these parameters in normal animals, these investigators then characterized what occurred in the remnant kidney where renal function was decreased [16, 18]. These animals, even without an underlying disease such as diabetes mellitus, will follow an inexorable path to end stage renal failure and uremia. Humans behave similarly.

In the 1980's, Brenner's laboratory then concentrated on the intrarenal hemodynamic forces found in experimentally-induced Type 1 diabetes mellitus [22-24]. The effect of therapy on this model was also examined [25]. We shall discuss their findings as it relates to hyperfiltration of diabetes mellitus after a brief review of normal intrarenal hemodynamics.

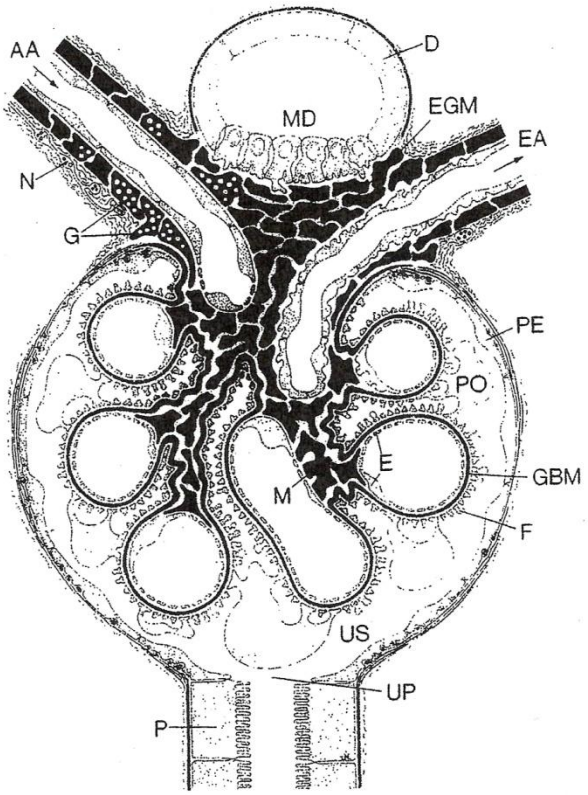


Figure 1.Anatomy of the glomerulus and juxtaglomerular apparatus. Schematic diagram of a section of a glomerulus and its juxtaglomerular apparatus. Structures shown are as follows: afferent arteriole (AA), efferent arteriole (EA), macula densa (MD) of the distal tubule, nerve endings (N), mesangial cell (M), extraglomerular mesangial cell (EGM), endothelial cell (E), epithelial podocyte (PO), with foot process (F), parietal epithelial cell (PE), glomerular basement membrane (GBM), urinary space (US), urinary pole (UP), and proximal tubule (P). G shows the rennin containing granules of the juxtaglomerular apparatus. (Reprinted with permission of the National Kidney Foundation) (Ref 21)

Each normal human kidney contains approximately 1 million nephrons with 75-80% of these nephrons being in the superficial cortex. While it is not possible to study single nephron GFR in humans using micropuncture techniques, it is possible to do so in animals under a variety of conditions, including experimentally-induced diabetes mellitus. The Munich-Wistar rat has been the most extensively studied because it has easily accessible surface nephrons and the preparation is stable for many hours,

There are four main determinants of single nephron glomerular filtration rate (SN_{GFR}): 1) Qa, glomerular plasma flow rate

1. ΔP, mean transmembrane hydraulic pressure difference
2. Πa and Πe, afferent and efferent arteriolar colloid osmotic pressures and
3. K_f, glomerular ultrafiltration coefficient, a parameter which considers the surface area of the glomerulus and its intrinsic permeability.

The first three determinants are measured experimentally and the 4th determinant (i.e., K_f) is calculated.

From the experimentally measured values one can then calculate the resistances of the afferent and efferent arterioles. The formula for SN_{GFR} is:

$$SN_{GFR} \alpha \frac{Q \bullet HP \bullet K_f}{\pi}$$

where,

Q = glomerular plasma flow rate
HP = transmembrane glomerular pressure
K_f = glomerular ultrafiltration coeffient
= intrinsic permeability of the glomerulus • surface area
π = colloid osmotic pressure difference

The one hemodynamic variable which can change most dramatically is glomerular effective renal plasma flow. Compared to other capillary beds, flow to the glomerulus is continuous, not intermittent. Both the afferent and efferent arterioles can dilate or constrict. They do not necessarily do so in tandem, thus glomerular pressure and hence filtration rate may be decreased or increased markedly. In states of volume expansion as is seen in normal pregnancy, flow increases markedly. Such a rise subsequently increases SN_{GFR}, all other things being equal.

A small variation in the mean transmembrane hydrostatic pressure gradient (ΔP) alone is rarely a major factor altering SN_{GFR}. Filtration cannot begin until ΔP exceeds the colloid osmotic pressure at the afferent end of the glomerular capillary. Only then does SN_{GFR} rise as ΔP increases; it does not rise linearly, however. Vasoconstriction of the efferent arteriole will result in an increase in pressure which is transmitted back to the glomerulus. Solely changing colloid osmotic pressure, as by an increase in multiple myeloma proteins will affect the afferent arteriole so much that SN_{GFR} falls.

The ultrafiltration coefficient (K_f) is not a fixed value. Decreases have been reported in experimental glomerulonephritis, acute renal failure, and long standing protein calorie malnutrition. In general, at very low flow rates, K_f has little effect on SN_{GFR}. Angiotensin II (AngII) decreases K_f by decreasing the surface area available for filtration [4, 26]. The contractile elements in the mesangial cell and the afferent arteriole are the primary sites for AngII action. This effect of AngII is independent of its effect as a growth factor or any of its other actions [for in depth review of the renal circulation and glomerular hemodynamics, see also Refs # 8, 27-29].

Pathophsiology of Hyperfiltration (Increased Sn_{gfr}) in Experimental Diabetes Mellitus

About one half century ago it was suggested that as nephrons failed, the remaining ones had to function at a higher level. Bricker and coworkers [30, 31] expanded and clarified this

concept and coined the term "the intact nephron hypothesis". It was not until the 1970s, however, when Brenner's laboratory at the Peter Bent Brigham Hospital began their micropuncture studies in the rat following first unilateral nephrectomy and then more extensive renal ablation that we fully understood the changes in all of the hemodynamic parameters occurring in a single nephron with reduced nephron mass. Based on their results we can now describe the features of these remaining "super nephrons" and theorize on the consequences of single nephron hyperfiltration.

Following uninephrectomy, SNGFR as well as whole kidney GFR increases by about 40% two weeks after surgery although significant increases in both parameters are detectable at 15 hours [16]. Glomerular capillary flow (Qa) rises because both afferent and efferent arteriolar resistances are decreased. Since the fall in the afferent arteriole is much greater, the hydraulic transmembrane pressure (ΔP) rises and SNGFR increases. So the combination of glomerular hypertension and glomerular hyperperfusion explains the "hyperfiltration" which occurs in the remaining nephrons. These changes occur in the absence of uremia. When more extensive renal ablation is performed (i.e., uninephrectomy followed by infarction of the upper two-thirds of the remaining kidney) and uremia developed, SNGFR increased even further, by about 75% [18]. Under these conditions, Qa was about the same as seen with uninephrectomy alone, but the hydraulic transmembrane pressure (ΔP) was more markedly increased. With time, both proteinuria and focal segmental glomerulosclerosis were seen. Eventually the animals will die with uremia.

In the 1980's , the Brenners group (22) published the first paper describing intrarenal hemodynamics in rats with drug-induced diabetes mellitus. The authors sought to answer the question why SNGFR rises in early diabetes mellitus. In a subsequent studies, they examined whether any drug intervention could ameliorate the progression of the renal disease [25].

A single injection of streptozotocin (STZ) destroys the β-cells of the pancreas and the animals develop Type 1 diabetes mellitus. The drug was isolated from a strain of streptomyces in the 1960s and is an alkylating agent. It is transported in to the β-cells of the pancreas by the GLUT2 protein and has been used in the treatment of insulinoma. STZ-treated animals do not develop all the classical findings of diabetic nephropathy and they do not become uremic. The animals do, however, have an increase in kidney size early in the course of the disease; both proteinuria and mesangial cell proliferation also occur [32, 33].

Hostetter et all [22] studied three groups of male Munich-Wistar rats 74 days after a single intravenous injection of 60mg/kg STZ, STZ plus 2 units NPH insulin sc each pm, or diluent-treated controls. On the morning of the micropuncture study, the animals were anesthetized, given an infusion of isoncotic plasma equal to 1% of body weight appropriate for each group; micropipettes were placed in the proximal tubules of surface nephrons for determination of flow rate and inulin concentration. Efferent arteriolar blood samples were obtained to measure total protein concentration. Hydraulic transmembrane pressure was measured in cortical tubules and vessels using the servo-null micropipette technique [27]. Thus, the 4 determinants of SNGFR were measured and calculated. Afferent and efferent arteriolar resistances were also determined. Additionally, catheters were inserted in the femoral artery, the jugular vein, and the urinary bladder for measurements of whole kidney GFR, mean arterial pressure and hematocrit.

Note in Table 1 that at 18 weeks mean arterial pressure was the same in all groups, but whole kidney GFR in the STZ + insulin diabetic group was 50% higher than control. By contrast, in the STZ group where blood glucose was 5 times control and about two-fold

higher than the diabetic animals receiving insulin, GFR was not elevated; it was, in fact, lower than diluent injected controls (discussed subsequently).

Table 1.Experimental Type 1 Diabetes Mellitus in Rats

Group	N	Mean arterial P (mmHg)	pGlucose (mg/dL)	aWhole Kidney GFR(mL/min)
Control	8	103	115	1.10
Streptozotocin	7	102	565*	0.76**
Streptozotocin plus NPH insulin sc	6	114	375*	1.47**

aInulin clearance; total blood volume (Cr51 RBC) between the 3 groups was NS
*P<0.001 from control and from each other
**P<0.02 from control and from each other
(Data from Ref # 22)

A rise in GFR has been consistently noted in humans with early diabetes, be it Type 1 or Type 2 and it may last for 5 to 10 years during which time microalbuminuria begins in susceptible individuals.

When micropipettes were placed in the kidney to measure SNGFR and the various parameters which regulate it, the following values were noted (Table 2): SNGFR of the superficial cortical nephrons in the STZ + insulin group was increased by approximately 40%. The increase in SNGFR was due directly to a doubling of glomerular capillary flow rate (Qa) and to a 25% increase in the transmembrane hydraulic pressure difference (ΔP). Because this group of animals was in filtration pressure disequilibrium (with the efferent arteriolar colloid osmotic pressure being less than ΔP), one could calculate Kf and compare it to the control group. When this was done, there was no difference noted between the two groups, a change in Kf could not explain the rise in SNGFR.

While the afferent arteriolar protein concentration and therefore, its colloid osmotic pressure, was higher than control, neither of these parameters could contribute to the rise in SNGFR. If anything, these alterations would make SNGFR fall, as may be seen in some patients with severe multiple myeloma.

The afferent arteriole was markedly vasodilated (i.e., its calculated resistance fell significantly by 36%) and while the efferent arteriolar resistance tended to fall, the value was not significantly different from control at the $P<0.05$ level. These results are qualitatively similar to those of the uremic renal ablation model described at the beginning of this section [18].

Table 2. Glomerular Hemodynamics in Rats with Early Type 1 Diabetes Mellitus in Rats

Group	N	SNGFR	QA	ΔP	Colloid Osmotic	Rt	Kf
		(nL/min)	(nL/min)	(mmHg)	Pressure		
					afferent/efferent		
Control	8	48.9	142	35	18/34	5.0	>0.095
Streptozotocin	7	28.8*	86**	36	b15/32	8.4*	>0.048
Streptozotocin plus NPH insulin sc	6	69.0*	240**	a44	b20/35	3.5*	–

SN_{GFR} = Single nephron glomerular filtration rate
Q_A = Flow
$_{\Delta}P$ = Transmembrane hydraulic pressure difference
R_t = Total renal vascular resistance
K_f = Ultrafiltration coefficient

***** $P<0.05$ from control and from each other

****** $P<0.01$ from control and from each other

a $P<0.005$ from control and from each other

b $P<0.02$ from control and from each other

(Data from Ref # 22)

The significance of the changes noted in the afferent and efferent arterioles will become clear when we later examine the effect of therapy on the rate of progression of diabetic nephropathy.

In the severely hyperglycemic group (STZ) where blood glucose approached 600mg/dl, the story is quite different. These animals must have had severe ketoacidosis and been volume contracted as they lost weight and had an increase in urine output. This group also had a fall in whole kidney GFR and SNGFR. Glomerular capillary pressure and ΔP were not different from control. By contrast, glomerular plasma flow rate (Qa) fell by 40% and this was the cause of the fall in SNGFR. Both afferent and efferent arteriolar resistances both rose substantially, by 57% and 67%, respectively, suggesting there was marked intrarenal vasoconstriction. This vasoconstriction was likely the result of the release of AngII, norepinephrine, or some other vasoactive agent [26].

Critics have said that the STZ model is not a pure one as the animals do not develop all the features of human diabetic nephropathy and the alkylating agent may have direct toxic

effects which substantially alter the results obtained [34,35]. The tremendous amount of data compiled using this model, however, has given us an important basic understanding of the sequence of events occurring in Type 1 diabetes mellitus. With some limitations we believe the findings noted in animals to be compatible with the disease in humans. In order to circumvent the limitations, however, an extensive search has been done over the past decade to develop better animal models. The mouse has proved particularly useful.

Breyer and colleagues [36] have reviewed in depth the mouse models of diabetic nephropathy. We summarize some of their data here. For Type 1 diabetes mellitus we now have several including low dose STZ (40-50mg/kg/d intraperitoneally for 5 days). A milder form of the disease is noted with low grade β-cell damage to the pancreatic islets. After 5 weeks, glomerular hypertrophy as well as albuminuria occurs; at 15-30 weeks some mice have arteriolar hyalinosis and nodular glomerulosclerosis. It is thought that low dose STZ mitigates the toxic effects of the drug, although this is not completely certain. The Insulin-2 Akita mouse also has β-cell failure of the pancreas with the heterozygotes developing hyperglycemia, polyuria, and polydypsia at approximately 3 months of age (the homozygotes die at 2 months and, therefore, cannot be studied). The Non-Obese Diabetic mouse (NOD) is a model in which there is spontaneous autoimmune destruction of the pancreas at 5 months of age. There is a 4:1 female predominance of the disease in this strain. The major problem with the NOD mouse is that there is no good control group to study in parallel.

Since Type 2 diabetes mellitus, that form of the disease associated with insulin resistance and obesity, accounts for 90% of the cases of human diabetes, finding a good animal model is particularly important. Now we have several strains of mice, both mutants and transgenics which should prove useful experimentally. It should be noted that all mouse models, be they Type 1 or Type 2, have the disadvantage of being far more fragile than the Munich-Wistar rat studied by Brenner's group. Mice also respond very poorly to long term anesthesia. These difficulties have been overcome in at least one of the mutant strain which we shall discuss shortly.

Some of the mice with Type 2 diabetes include the Agouti yellow obese mouse, the New Zealand mouse, and several transgenics. There are now mouse strains transgenic for GLUT-1, one for ApoE, and another for advanced glycosylation end products. There is also a transgenic strain having endothelial nitric oxide synthase polymorphism. As stated, Breyer et al (36) have reviewed these and other mouse strains in detail. They specifically point out those strains that develop diabetic nephropathy similar to that seen clinically in humans. The reader is referred to this very important review containing over 200 references.

The most widely studied of the animals with Type 2 diabetes mellitus and the only one thus far which can be prepared for micropuncture, is the db/db mouse. The nomenclature for the db/db mouse has been changed recently but for purposes of clarity we shall use the term db/db for the homozygote and db/m for the heterozygote [36]. The diabetic gene in these animals is transmitted as a recessive trait resulting from a G-to-T point mutation of the leptin receptor.

This mutant strain exhibits many histologic features of diabetic nephropathy. At 10 weeks of age the mice begin to develop hyperglycemia. At 2 to 4 months of age features of diabetic nephropathy appears with a three-fold increase in mesangial matrix. Albuminuria of 70 to 600 micrograms/24 hrs also has been documented. Arterial hyalinosis has been described, but there is not yet evidence of the tubulointerstitial fibrosis seen in humans with longstanding disease.

Recently, David Levine's laboratory at the University of Ottawa has developed a stable preparation in the db/db Type 2 diabetic mouse [37, 38]. They have performed very careful micropuncture studies looking at intrarenal glomerular hemodynamics in the db/db animals before [37] and after uninephrectomy [38]. They have compared the results to heterozygous littermates (db/m) as well as to the wild type (WT) control strain from which they were developed.

Levine et al [37] studied the animals at 10-11 weeks of age performing whole animal clearance studies, microalbumin excretion, and micropuncture of the superficial cortical nephrons at two sites for proximal and distal SNGFR. Histopathology was performed on a separate group of identically treated animals when they were 30-40 wks of age. At the time of the micropuncture study, the animals varied in weight from 34gm to 51gm, with the highest weight noted in the db/db mutant. Results of the whole animal data are shown in Table 3. Note that blood glucose was > 400mg/dl in the db/db Type 2 diabetic mouse at the time of micropuncture. Urine flow was 3-fold higher in the homozygotes than the other two groups, being 10.5µl/min. Whole kidney GFR was almost two-fold higher in the db/db group than in either the heterozygotes or the WT while systolic pressure was identical in all three groups. Urine albumin excretion rate was highest in the db/db mouse measuring 303µg/24hr ($P<0.02$ from the other two groups). It was 83µgm/24 hr in the db/m group and 62µg/24hr in the WT controls. The micropuncture data are shown in Figure 2. Note first that the SNGFR in the db/db group is significantly higher than either WT controls or the heterozygotes. In the db/db mouse SNGFR was 11.6nl/min measured proximally and 9.3nl/min measured distally.

It is possible to test tubuloglomerular (TG) feedback by subtracting the proximal SNGFR minus distal SNGFR in the same nephron [28, 37]. Proximal SNGFR eliminates the TG feedback signal. Distal SNGFR measured in the same nephron bypasses both the macula densa and the afferent arteriole and is a true steady state value. In WT control mice, proximal SNGFR exceeds distal by approximately 16%. In the db/db mouse proximal minus distal SNGFR was increased to about 25% ($P<0.05$ from the other two groups). The heterozygotes had a proximal minus distal SNGFR value of about 11%, a value not different from their WT littermates. These results suggest that there was a "resetting" of feedback control in the diabetic mice. The mechanism involved in this resetting is not completely known.

Since alterations in nitric oxide release or action have been suggested to be involved in the pathogenesis of diabetic nephropathy, the group examined one aspect of the system. They gave an intravenous infusion of the specific neuronal nitric oxide synthase inhibitor (S-methylthiocitrulline) at a dose that had no effect on blood pressure (i.e., acute nitric oxide inhibition). There was a dramatic fall in proximal SNGFR in the db/db mouse but distal SNGFR in these animals did not change. In the heterozygous db/m and the WT control mice, both proximal and distal SNGFR were decreased significantly. One would expect such results if nitric oxide were of major importance in the hyperfiltration of early diabetes mellitus. However other studies including some of Levine et al have underscored the importance of nitric oxide in the hyperfiltration of early diabetic nephropathy (A detailed discussion of nitric oxide dysfunction in diabetic nephropathy is provided in another chapter in this book).

Histopathology showed that mean glomerular diameter was significantly higher in the db/db group as compared the WT animals (95.4 microns vs. 75.6 microns, respectively, $P<0.001$). Glomerular diameter of the heterozygotes measured an intermediate value. Mesangial expansion was noted only in the db/db group, being two-fold higher than the heterozygotes ($P<0.001$); arteriolar hyalinosis and luminal narrowing were also noted.

Table 3.Type 2 Diabetes Mellitus in the db/db Mouse

Group	Blood glucose (mg/dL)	Body wt (g)	+GFR (µl/min)	Systolic BP (mmHg)	Urine flow (µl/min)
Homozygous (db/db)	266*	51*	422*	107	10.5*
Heterozygous (db/m)	100	25a	179a	95a	3.6
Wild Type **(WT)**	113	34	262	100	3.4

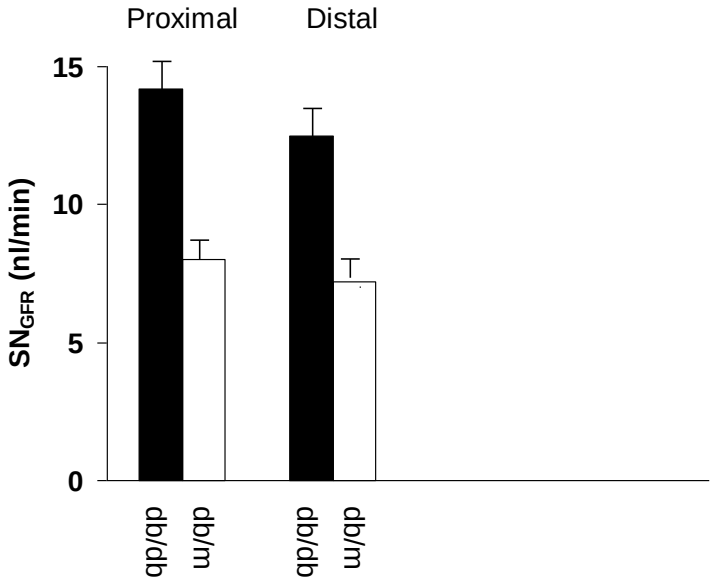
+Insulin clearance; N = >6 animals/group

*****P<0.02 from heterozygous db/m or WT control

a P<0.02 from the other 2 groups

Microalbumin excretion, 303 µg/24 in db/db vs. 62 µg/h in db/m mice (P<0.02) @ 10-11 weeks old

(Data from Ref # 37)



(Data from Ref # 37).

Figure 2. SN_{GFR} ± SE in db/db ■ homozygous and db/m □ heterozygous mice. The db/db mice were infused with 0.9% NaCl @ 4.0 mL/100g body weight/hr; the db/m mice were infused with 0.9% NaCl @ 3.3 mL/100g body weight/hr (see also Tables 2 and 3).

True nodules and interstitial fibrosis as seen in long standing human diabetic nephropathy, was not seen in these experiments.

In a second study Levine et al [38] examined the effect of reduced renal mass in the db/db Type 2 diabetic mouse. The authors reasoned that a reduction in nephron mass would hasten the progression of diabetic renal disease, possibly by raising SNGFR even further early on. The additional increase in SNGFR, provoked by the loss of renal mass, would not be sustainable, however, and the glomerular hyperperfusion and hypertension would lead to an inexorable progression to renal failure. To decrease renal mass, right uninephrectomy (Nx) was performed in the db/db mouse at about 10 weeks of age. The animals were allowed to recover and were prepared for micropuncture 3 weeks later. Sham Nx was performed in a group of db/db littermates by only touching the right kidney intraoperatively. The results were compared to WT controls treated similarly. Detailed histopathology was performed in separate animals at 30 to 40 weeks of age.

The db/db Nx mice had an even higher whole kidney GFR and SNGFR than did their db/db sham Nx littermates (Table 4). Histopathology showed a near linear increase in mean glomerular diameter at 15 to 30 weeks and an even higher value at 40 weeks. Glomerular diameter was greater in the db/db Nx group as compared to the WT Nx animals. While albumin excretion was not measured in this study, another early marker of diabetic nephropathy was — the mesangial matrix ratio. Mesangial matrix ratio was much higher in the db/db Nx than in the db/db sham group. These results suggest that a reduction of renal mass (i.e., uninephrectomy) exacerbated the progression of diabetic nephropathy.

Table 4. Uninephrectomy in the Type 2 Diabetes Mellitus in the db/db Mouse

Group	Blood Glucose (mg/dL)	Left Kidney GFR (μl/min)	SNGFR (nl/min)	Glomerular Diameter (μm)	Mesangial Ratio
db/dbNx (homozygous)	239	295**	17.9	95.8c	0.46c
db/db Sham	284	220	13.7b	NS	0.27*
Wild Type Nx	136a	269**	14.5	76.3	0.12
Wild Type Sham WT	151	177	12.3b	?	0.18

Nx = unilateral nephrectomy; inulin clearance for SNGFR (N = >6/group)
* P< 0.05 from WT sham
**P<0.02 from appropriate control
aP<0.05 from db/db Nx
bP<0.05 from appropriate control
cP<0.01 WT Nx
(Data from Ref # 38)

When neuronal nitric oxide synthase was inhibited chronically (by oral administration of S-methylthiocitrulline 24 µg/day for 2-3 weeks after Nx) there was no effect on SNGFR or TG feedback. Thus, neither acute nor chronic inhibition of neuronal nitric oxide synthase altered TG feedback in the db/db Type 2 diabetic mouse. Schnermann [39] has shown recently that a knockout mouse for neuronal nitric oxide synthase in the macula densa had normal TG feedback activity. Similar findings have been reported in a thromboxane receptor knockout mouse. This is in marked contrast to knockouts for AT1 and ACE receptor where there is no TG feedback regulation [4, 39]. It may be that TG feedback is reset at a higher value in the hyperfiltration of early diabetes mellitus and that nitric oxide and Ang II are in some manner involved in the mechanism.

Possible Factors Leading to Hyperfiltration in Diabetic Nephropathy (Excluding Raa System)

A major question is whether the rise in SN_{GFR} in early diabetic nephropathy is the initiating factor leading the end stage renal disease or is it the final common pathway to chronic renal failure. If an elevated SN_{GFR} is the initiating factor, then what are the subsequent steps occurring at the cellular level which leads to end stage renal failure? If it is the final common pathway, what is initially turned on (or off) in the cell to cause the hyperfiltration?

When the "Hyperfiltration Theory" was first proposed by the Brenner laboratory, they felt it was the increase in glomerular pressure, that caused the rise in SN_{GFR} (Figure 3). This changes, if left unabated, would overwhelm the remaining nephrons and lead to glomerulosclerosis by damaging the glomerular endothelial cells. An injury such as this would then cause an increase in flux of macromolecules especially to the mesangial cells (Figure 1). The enhanced flux, would in some manner, increase mesangial matrix and size. With time, nephrons would "drop out", the negative charge on the foot processes would be lost, and macromolecules (i.e., protein) would be lost in the urine. The combination of increased pressure and glomerular flow would only exacerbate this cascade.

The "Hyperfiltration Hypothesis" has been characterized as one broadly relating to physical or hemodynamic factors which will increase SN_{GFR}[40-44]. This is opposed to the many metabolic factors (i.e., uncontrolled hyperglycemia, advanced glycosylation end products, GLUT-1 activity, AngII, cytokines, growth factors) which may be involved in the rise in SN_{GFR}.

In this book there are separate chapters devoted to the role of various factors in the pathogenesis of diabetic nephropathy. These include the role of hyperglycemia and advanced glycosylation end products (Chapters 3 and 4) as well as the role of translational dysfunction (Chapter 5). In our view the role of the renin-angiotensin-aldosterone system (Chapter 6) is extremely important, very complex, and as yet not fully understood. Aspects relating to the role of growth factor activation (Chapter 8) could easily explain the rise in SN_{GFR}. Dysfunction of the nitric oxide system (Chapter 11) and oxidative stress (Chapter 10) are doubtless both crucial in the genesis of inflammation; an association with chronic inflammation and nephropathy has been recently advanced.

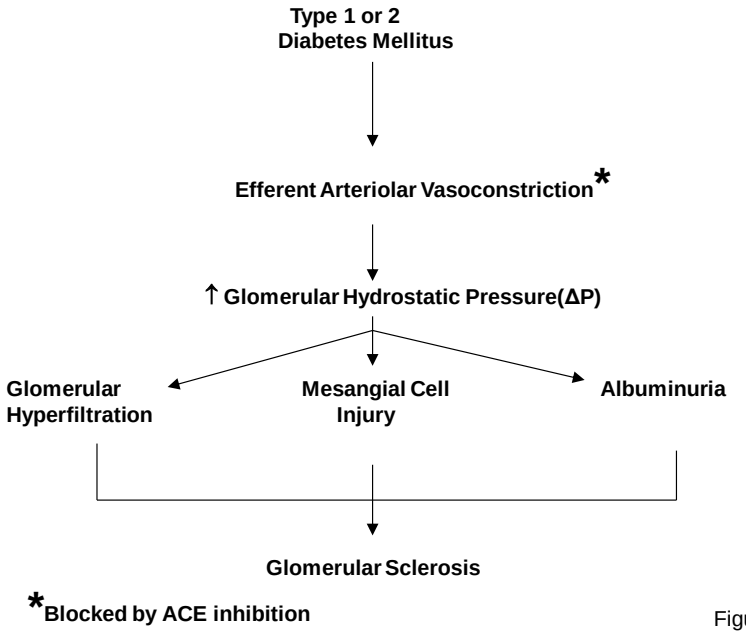


Figure 3

Figure 3. Schema whereby glomerular sclerosis could occur in diabetes mellitus. Once a certain degree occurs further renal injury would progress. Poor glycemic control would only aggravate intrarenal glomerular hemodynamics.

Any one or several of these factors in a genetically susceptible individual may be enough to tip the scale towards fulminate renal disease in diabetes mellitus.

We will concentrate on a physical process that increases flow and pressure on vessel walls and that is stretch or sheer stress. We believe this to affect growth factors and endothelial cell function and to be part of the pathogenesis of hyperfiltration.

Sheer Stress and Stretch

One of the major perturbations in blood vessels when there is an increase in pressure is a rise in wall tension and stretch. Such a rise may directly cause a cascade of events in each of the individual glomeruli and mesangial cells and this, in turn, may increase SN_{GFR} and affect matrix biosynthesis adversely. Narin's laboratory [45, 46] was one of the first groups to examine how pressure and stretch may be involved in the rise in SN_{GFR}. In rat mesangial cells in culture these investigators examined the effect of prolonged cyclic *in vitro* stretch on mesangial extracellular matrix (ECM) protein deposition Also examined was the effect of increasing perfusion pressure (from 0 to 200mmHg) on volume of isolated perfused rat cortical glomeruli. These authors found that, if one increased perfusion pressure *in vitro* from 60 to 100mmHg, values comparable to *in vivo* glomerular transcapillary pressures of 32 to 52mmHg, glomerular volume increased markedly from 8.3% to 17%, respectively($P<0.05$). Quantitatively similar effects were seen regardless of baseline glomerular volume.

When a stretch of 7% to 12% was applied to a cultured monolayer of human mesangial cells for 20 hours to 100 hours, a linear increase in total collagen synthesis occurred, indicating that the extracellular matrix was increased. When the specific components of the

mesangial matrix were analyzed, enhanced production of collagen I, collagen IV, fibronectin and laminin was noted. While their studies did not examine the cellular mechanisms which may be involved, some of the possibilities include the generation of cyclic AMP or cyclic GMP, changes in cytosolic calcium, protein kinase C inhibition, and the enhanced release of specific growth factors.

Homma et al [47] showed that *in vitro* stretch of 12% for 24 hours to cultured mesangial cells not only increased collagen production, but it enhanced non-collagen protein synthesis by 25%. Thus, not only did "stretch" increase synthesis of various components of the collagen family, it also caused the synthesis of new proteins. Addition of the growth factor AngII stimulated the synthesis of both protein fractions and did so even if the cells were not proliferating. Addition of epidermal growth factor was different as it increased only collagen production. Both growth factors stimulate phosphorylation of S6 kinase in mesangial cells when they are subjected to repeated mechanical stress. S6 kinase is the term used for a family of cyclic nucleotide serine/threonine kinases which act via protein kinase C mechanisms.

Both AngII and epidermal growth factor are important within the kidney [for reviews see Refs 4 and 48]. AngII stimulates EGF-dependent mitogenesis in the proximal tubule. In fact, the kidney is one of the major sites of epidermal growth factor biosynthesis. Receptors for this growth factor are found in mesangial cells, medullary interstitial cells, the basolateral membrane of the proximal tubule and the collecting duct. In early diabetes mellitus increases in epidermal growth factor may play a role in renal enlargement. Urinary excretion of this growth factor is increased 3-fold while its mRNA rises 4 to 8 times early in experimentally-induced disease [48]. The time course for changes in epidermal growth factor level may not be rapid enough to explain the hyperfiltration of early diabetic nephropathy.

Another growth factor thought etiologic in stimulating matrix protein synthesis in mesangial cells in response to repeated mechanical stretch is the cytokine transforming growth factor-beta (TGF-β) [4, 8, 42, 48]. There are several proteins in the TGF-β superfamily, including bone morphogenetic proteins, inhibins, and activins. These regulatory growth factors are unique in their broad effects on the extracellular matrix. They stimulate fibronectin, osteopontin, and thrombospondin production as well as inhibiting tissue collagenases. TGF-β actively modulates tissue repair and when present in excess leads to fibrosis. In addition to stretch there are a number of other stimulators of TGF-β which may be relevant to diabetes mellitus. They include AngII, uncontrolled hyperglycemia, advanced glycosylation end products, oxidative stress, and aging.

TGF-β 1 appears to be the isoform important in the kidney. All three of the isoforms and their specific receptors are present in the kidney[4]. If mesangial cells are subjected to *in vitro* stretch, first TGF-β 1 mRNA increases, then cellular TGF-β 1 rises, and finally mesangial matrix protein synthesis is stimulated [45]. When neutralizing TGF-β 1 antibody is added to the media the increase in matrix proteins does not occur [49]. While Yasuda et al [49] also found an important role for enhanced TGF-β1 production in the proteinuria of diabetic nephropathy, they do not believe it to be the initiating factor primarily because the time course seems too long.

It seems clear that stretch (i.e., in afferent arteriolar stretch), secondary to an increase in glomerular pressure results in one of the early findings of diabetic renal disease, that of mesangial expansion. It is likely that stretch, by increasing the size of the glomerulus results in damage to the foot processes (by increasing the space between the podocytes and

decreasing the effectiveness of the negatively charged barrier) subsequently resulting in proteinuria.

Gruden et al [50] indirectly examined the integrity of the negatively charged barrier by giving an infusion of vascular endothelial growth factor (VEGF) to rats and found that they developed proteinuria. These investigators also noted the presence of this growth factor in the plasma of patients with minimal change nephrotic syndrome. When human mesangial cells are exposed to stretch, VEGF mRNA more than doubled at 6 hours, findings much earlier than any effect of TGF-β 1 [50]. VEGF itself was tripled at 12 hours. Furthermore, when these "stretched" cells were preincubated with H7, an inhibitor of protein kinase C, VEGF levels fell by 75%. Preincubation with herbimycin A, a protein tyrosine-kinase blocker, caused VEGF levels to fall by 80%. These two inhibitors did not alter cell viability nor did they affect basal VEGF secretion. These results suggest that the VEGF system is extremely important in early diabetic renal disease. To further support a key role for this growth factor, if VEGF neutralizing antibodies are given to STZ-treated rats for 6 weeks diabetes associated hyperfiltration is fully abolished [52]. For reviews of VEGF biology, [see Refs 4, 48, 53]

Early in experimental diabetes mellitus, VEGF is up regulated. This growth factor is expressed in the mesangial cell and in the glomerular podocyte, as well as other parts of the kidney (54). It is also one of the most potent mitogenic factors known, and its activity is affected by many variables involved in diabetes mellitus. AngII stimulates its expression and biosynthesis in mesangial cells while preincubation with the A1 receptor blocker, losartan, prevents it. High glucose stimulates VEGF in vascular smooth muscle cells probably via a protein kinase C mechanism.

Genetics

Clearly, genetics affect the propensity to develop diabetic nephropathy. Genetics may predominate in the full expression of the disease or it may be a silent partner, contributing only when things begin to go awry [8, 55-57]. This seems to be true in either Type 1 (autoimmune destruction of the pancreas) or Type 2 (obesity, insulin resistance and aging) diabetes mellitus. Just as there are factors which make some individuals have the propensity to develop the disease and end organ damage, there must be factors which protect people as not all patients with the disease develop renal damage.

For Type 1 diabetes mellitus, there is evidence of genetic susceptibility on chromosome 3. For Type 2, susceptibility has been reported on chromosomes 10, 18, and 20 along with polymorphism to angiotensin converting enzyme. The genetic aspects of the disease and its relevance to nephropathy are discussed later in Chapter 12.

Understanding the genetics of diabetes mellitus is further complicated by the known strong association of the disease with that of "essential" hypertension. Furthermore, the extent of the interrelationships between activation genes and post-translational events are not known. The interactions may be so complicated that they can never be completely dissected. Because research in clinical medicine is so complex and diverse, those trained in one area may not have the expertise to attack a problem from varying and different perspectives. Thus, collaboration in clinical medicine is imperative so that a cohesive picture of the pathophysiology of this important disorder can be developed.

Hyperglycemia and Advanced Glycosylation Products

The role of tight control of blood glucose in the development of diabetic nephropathy continues to be debated. It appears that tight glucose control does not fully protect one against diabetic nephropathy in susceptible individuals. The advanced glycosylation end products resulting from prolonged hyperglycemia, however, may be important in mesangial expansion and loss of fusion of foot processes as these large molecules are continuously recycled and reabsorbed by the proximal tubule 54, 58). This topic is discussed later in this book.

Hypertension

A rise in glomerular pressure, even in the absence of hyperglycemia can lead to glomerulosclerosis and end stage renal disease. This was shown by Brenner's laboratory in the 1970s using the remnant kidney model (i.e., uninephrectomy followed 7 days later by infarction of the upper pole of the remaining kidney) [18]. When these Munich-Wistar rats were prepared for micropuncture 14 days later, clear differences in the glomerular hemodynamics of the remnant kidney model were noted as compared with the STZ-induced diabetic model [24]. Both models, with time, lead to proteinuria, nephrosclerosis, and chronic renal failure. Early, there is hyperfiltration with a significant increase in SN_{GFR} in the remnant kidney. This rise is due primarily to an increase in glomerular capillary pressure. Maneuvers which increase glomerular capillary pressure such as high protein diet or glucocorticoid administration lead to a further significant rise in SN_{GFR} and accelerate the rate of deterioration of renal function.

By contrast, maneuvers which decrease glomerular capillary pressure, such as chronic protein restriction or angiotensin converting enzyme inhibitors, protect the kidney from progressive damage.

Initially it was thought that any maneuver which lowered systemic blood pressure would automatically lower glomerular pressure (ΔP). Brenner's group (23, 59) addressed this issue in diabetic and non-diabetic animals. They gave another group of the remnant kidney animals hydralazine plus reserpine to lower mean arterial pressure to an equivalent degree. The measurements of glomerular hemodynamics were then repeated. The hydralazine/reserpine-treated animals were not protected because glomerular capillary pressure did not fall. Lowering the systemic blood pressure with these two pharmacologic agents vasodilated the afferent arteriole but they did *not* vasodilate the efferent renal arteriole. Consequently, the increased efferent pressure was transmitted back to the glomerulus. These results are very different from those obtained with captopril where systemic pressure falls to an equivalent degree. While captopril decreases afferent arteriolar pressure, it results in a *much larger* fall in the efferent arteriolar pressure, thus decreasing glomerular capillary pressure. It is this large fall in efferent arteriolar pressure which is unique to the angiotensin converting enzyme inhibitors and which may be the mechanism protective to the kidney.

Arguing against glomerular pressure causing the rise in SN_{GFR} and the subsequent cascade of pathologic events are results obtained with some drug models of nephrosclerosis. Fogo's group studied uninephrectomized rats given either puromycin or adriamycin for 2

months and showed that glomerular capillary pressure did not rise despite the development of proteinuria, nephrosclerosis, and renal failure [60]. In fact, with adriamycin administration, glomerular capillary pressure fell. Treating the animals with the angiotensin converting enzyme inhibitor, captopril, markedly decreased the severity of glomerulosclerosis without any change in glomerular capillary pressure. Fogo's group, well known for their precise morphometry, found that glomerular hypertrophy best correlated with the severity of glomerulosclerosis.

It would not be surprising if there were more than one important regulator of the rise in SN_{GFR}, i.e., not just increased glomerular capillary pressure. The more critical an event or factor is which sustains life, the more regulators there are to keep it within the normal physiologic range. Think of the many regulators there are which prevent lethal hyperkalemia, quickly shifting the ion inside cells as well as enhancing its excretion by the kidney.

Effect of Therapy on the Progression of Diabetic Nephropathy

Definitive therapy for reversal of diabetes mellitus and its myriad of complications, including nephropathy, is surgical. Unfortunately, pancreas transplantation is a viable option for only a minute number of patients now and this is not likely to change in the near future. In the United States in 2008, 705 kidney/pancreas transplants and only 307 pancreas transplants were performed [2]. By contrast, there were 13,743 kidney transplants done; a minority of kidney recipients had end stage renal disease due to diabetes mellitus. Recall that diabetic nephropathy is the leading cause of renal failure in the United States, accounting for more than 50% of patients, but most of these patients do not receive renal transplant; they remain on dialysis.

There is one study demonstrating the efficacy of pancreas transplant for diabetic nephropathy [61]. In this study, serial renal biopsies were performed for up to 10 years in 8 patients. Over this time period the authors found there was a gradual decrease in mesangial matrix and in the size of the mesangium. There was also a significant decrease in basement membrane thickness. Despite these optimistic results, pancreas transplantation as we know it today will never be a significant form of treatment for patients with diabetic renal disease.

Drug therapy will always be the mainstay of treatment for diabetic nephropathy. We include, but shall not discuss here the importance of weight loss and lipid control. Tight regulation of blood sugar, keeping hemoglobin A_1C to 6.5 or less is also obviously paramount.

Given the strong association of hypertension in diabetes mellitus and the huge pharmacologic armamentarium available, one has a wide variety of drugs from which to choose for patient treatment. The drugs, however, should be chosen carefully in the diabetic as some which prevent progression of nephropathy may not alone provide adequate blood pressure control. Moreover, drugs that effectively control blood pressure may not be "renoprotective." And finally, it may be necessary to use antihypertensive agents in diabetic patients who, by all criteria, have a normal blood pressure. In essence, we are saying that the drug chosen for both blood pressure control and protecting the kidney from further damage of diabetes mellitus may be the same initially, but added therapy may be required to achieve a

desirable blood pressure of 120/70mmHg. We are also saying that one should treat normotensive patients with drugs that will lower blood pressure if certain markers of diabetic nephropathy are found.

The data in animals, and more importantly in humans, show that in order to prevent the progression of diabetic nephropathy and decrease microalbuminuria at the earliest clinical sign of nephropathy, drugs that inhibit the renin-angiotensin-aldosterone axis should be prescribed.

Currently we have four classes of drugs that fall into this category. They are the ACE inhibitors, the angiotensin receptor blockers (ARBs), and the anti-renin drugs. We also include the aldosterone receptor antagonists as an abnormally high aldosterone level appears to exert pathologic actions on certain cardiovascular/renal tissues, including the mesangium [62-65].

Very soon after Brenner's Laboratory published their results that controlling glomerular capillary pressure in rats with experimental diabetes, many small clinical studies appeared demonstrating that proteinuria and the rate of progression of diabetic nephropathy was ameliorated by the administration of ACE inhibitor therapy to patients. Brenner [44] has summarized some of them recently. Anecdotally, we know of a number of nephrologists who began using these drugs as first line therapy early in the 1980s in all their diabetic patients.

There are now many major clinical trials, both here and in Europe, examining the role of drug therapy in the prevention of the progression of diabetic nephropathy (two early landmark studies, one here and the other in the United Kingdom looked at the results of long term tight glycemic control) [66, 67]. While the role glucose control and renal disease continue to be debated there is no question it is important [68]. The initial large scale clinical trial using pharmacologic intervention was performed in over 400 patients with proteinuria (>500mg/24hr) and Type 1 diabetes mellitus [69]. The patients received the ACE inhibitor, captopril, or a "placebo". Blood pressure control in each group could be achieved with any therapies other than calcium channel blockers. The study was to last 5 years and the end points were time to doubling of serum creatinine, initiation of renal replacement therapy or death. The Collaborative Study Group reported their dramatic and efficacious findings of ACE inhibitor therapy in the *New England Journal of Medicine* in 1993 [69].

Subsequently, a 7 year follow up was reported in almost 100 patients with Type 2 diabetes given the ACE inhibitor, enalapil. The study showed all of the patients had microalbuminuria, a normal serum creatinine, and they were normotensive. In the placebo group, urine microalbumin doubled over the time period studied, but in the patients treated with ACE inhibitor, it did not. Patients in the EUCLID study (Type 1 diabetic nephropathy), the HOPE, BENEDICT, and ADVANCE studies (Type 2 diabetic nephropathy), numbering well over 12,000, all showed decreased microalbumin excretion with ACE inhibitors over the time period studied (see Ref 70 for original citations) [70-73].

The ACE inhibitors were developed in 1970 to be used in the therapy of hypertension. Their mechanism of action is specifically to inhibit the conversion of AngI to AngII, thereby interrupting the renin-angiotensin-aldosterone cascade in the kidney and adrenal cortex. Thus, these patients have low AngII levels and high AngI levels (they also have low aldosterone levels). At the time these drugs were synthesized AngII was well known to be a potent vasoconstrictor of vascular smooth muscle; the salt retaining properties of aldosterone were also well known. All forms of hypertension have either a volume or vasoconstrictor component (or both) and a class of drugs targeted to attack either component should be a very

useful form of therapy. Thus, it was felt the ACE inhibitors would alter the vasoconstrictor component of hypertension. Forty years ago we did not know just how efficacious this class of drugs would be in the prevention of diabetic nephropathy, nor did we know of the many diverse actions this class of drugs seems to have. In the kidney, the ACE inhibitors appear to preserve renal function primarily by decreasing glomerular capillary pressure secondary to a marked fall in efferent arteriolar resistance.

In the early 1990s, a second class of drugs was introduced which also disrupted the renin-angiotensin-aldosterone axis, but by a different mechanism. These drugs are collectively known as "sartans" and are blockers of the AT1 receptor, hence the name ARBs, or angiotensin receptor blockers [73]. Patients who take these drugs have a high Ang II levels but it is unable to exert its action because the tissue receptors are blocked. In the kidney, these drugs lower glomerular capillary pressure by a mechanism similar to that described above for the ACE inhibitors. While they lower systemic blood pressure only modestly, their therapeutic window is very narrow. There are really only two doses one can use, the lower dose being at about the threshold of the dose-response curve, while the higher dose is at the plateau. This is rather unusual in the pharmacology of drug action and therefore, in our view, the drugs are of limited usefulness. Note that any other effect of AngII not mediated by the AT1 receptor (i.e., mediated by AT2 receptor, calcium, PKC, etc) will not be altered by ARB administration and indeed, could even be made worse because AngII levels rise significantly following the use of these drugs.

Now a number of studies clearly show that the ARBs are renoprotective and/or decreasing all cause mortality [74-79]. The combination of ACE inhibitors and ARB may be beneficial [80-84], but there clearly are hazards [85].

As regards the third class of drugs, there is currently only one anti-renin agent on the market in the United States. Aliskiren (Rasilez®) is the first of a new class of antihypertensives that inhibits renin formation. The drug is orally active and patients receiving this drug should have low plasma renin activity, low AngII, and low aldosterone levels. It was thought this drug would be a breakthrough in blood pressure therapy and be renoprotective. Whether it provides long term protection in diabetic nephropathy is not yet known. Recent studies suggest that, at least in the short term, it does decrease albuminuria and that when combined with an ARB it is mosre efficacious [85-87].

One troubling finding of aliskiren is the observation that it stimulates *renal* renin secretion to a greater extent than either ACE inhibitors or ARBs especially when the drug is given at higher doses. We shall wait to see the role this class of drugs as regards the preservation of renal function both in diabetes mellitus and other proteinuric renal diseases.

The final group of drugs which affect the renin-angiotensin-aldosterone axis are the aldosterone antagonists. Here we have spironolactone, eplerenone and canrenone. These agents act by blocking the aldosterone mineralocorticoid receptors in various tissues, including the kidney. For years the drugs were given as a mild diuretic, primarily in salt-retaining states. In the last several decades it became apparent that these drugs were also useful in the treatment of heart failure because of their beneficial effects on cardiovascular remodeling [88, 89]. These effects are separate and distinct from the effects of ACE inhibitors possibly by decreasing cardiac and vascular smooth muscle fibrosis. Whether they are of value in decreasing the progression of diabetic renal disease is now being examined [90].

Hyporeninemic Hypoaldosteronism

In the late 1970s and early 1980s our laboratory at the University of Illinois College of Medicine in Chicago, identified patients who were admitted to hospital (either the University Hospital or the West Side VA) with hyperkalemia. We would scan the admitting lab work and if the patients had hyperkalemia, regardless of other diagnoses, we would further evaluate a number of aspects of renal function [91-94].

While there were many patients with hyperkalemia, a subset were those with diabetes mellitus and some degree of chronic renal insufficiency [88]. It now appears that fully two-thirds of patients with hyporeninemic hypoaldosteronism have diabetes mellitus, predominately Type 2.

Why this syndrome is seen in diabetes mellitus is not known but it has been suggested that prorenin is nonezymatically glycated, thereby rendering it inactive. Consequently, plasma and intrarenal renin release would be lowered. This would then decrease the conversion of AngI to AngII causing a fall in aldosterone release. These patients would develop hyperkalemia, a powerful and direct stimulus to aldosterone release. But there is no rise in aldosterone when these patients develop hyperkalemia. Why they don't respond is unknown. It's possible that either there is atrophy of the zona glomerulosa or that the vessels supplying the adrenal cortex and medulla are atherosclerotic such that the usual stimuli can no longer bind to their receptors and exert their actions.

Aldosterone is needed under normal conditions to allow the kidney to excrete adequate amounts of potassium when a subject is on a low sodium high potassium diet, i.e. the diet on which our species evolved. When on a high salt diet, non-aldosterone dependent potassium and acid excretion is sufficient to maintain homeostasis. Thus, aldosterone deficiency becomes clinically manifest under conditions of reduced distal sodium delivery. Thus, this disease requires both heart and renal disease to be expressed.

In general, the resulting hyperkalemia is not life threatening in most patients unless some intercurrent event such as heart failure develops. Treatment with the aldosterone analogue (Florinef®) is usually not required in patients with diabetes mellitus. The other feature of hyporeninemic hypoaldosteronism which may occur is Type IV distal renal tubular acidosis. This typically does require a small amount of bicarbonate therapy. Alternatively, the combination of increase salt intake with generous doses of loop diuretics can effectively treat the hyperkalemia and metabolic acidosis associated with this syndrome.

Integrating the pathophysiology of this interesting syndrome in our understanding of diabetic nephropathy is difficult. We eagerly await studies addressing this.

Summary

The pathogenesis of diabetic nephropathy is doubtless multifactorial. Hyperfiltration secondary to a rise in glomerular capillary pressure is key in increasing SN_{GFR} in early disease. It appears that physical factors, (i.e., stretch or sheer stress) lead to events at the cellular level which increase certain growth factors including epidermal growth factor, TGF-β1, AngII, and VEGF. All of these stimulate an increase in mesangial matrix either by stimulating collagenous or non-collagenous protein synthesis or both. This enlargement of the

mesangium affects the glomerular podocytes and their negative charge on the glomerular basement membrane, thus allowing proteins to be excreted in the urine. The cascade for diabetic nephropathy is now set in motion. Identifying patients early so that regression of the renal abnormalities, not progression, is key [95].

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