Initiation, progression and remission of diabetic nephropathy

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1. INTRODUCTION AND AIMS

Diabetes is the leading cause of end stage renal disease (ESRD) in the western world, accounting for approximately 45% of patients with ESRD in the U.S.A., and 25% of the patients with ESRD in Europe. Furthermore, the number of patients diagnosed each year with ESRD due to diabetes is rising dramatically. The incidence is expected to grow from 41,000 new patients in the year 2000 to 300,000 in the year 2030 in the United States (1). A cumulative incidence of diabetic nephropathy of 25 to 40% after at least 25 years of diabetes duration has been documented in type 1 diabetic patients (2-4). Furthermore, diabetic nephropathy is a chronic progressive disease. Based on a study conducted in 1976, Kussman concluded: "Diabetic nephropathy is irreversible in humans. No cases of recovery or cure have been reported in the literature. Once the clinical signs of nephropathy have become manifest, the natural course is inexorably progressive to death" (5). Before the use of antihypertensive treatment in patients with diabetic nephropathy, the average survival time from onset of diabetic nephropathy was 5 to 7 years (2, 5). Despite a markedly improved prognosis observed after the use of antihypertensive treatment has been introduced in these patients (6), diabetic nephropathy is even now regarded as a progressive and irreversible disease. The costs of the treatment and care of patients progressing to ESRD are very high, and rapidly increasing due to the escalation in the number of patients with diabetes world wide. In addition, survival on renal replacement therapy is by no means a long-lasting solution for diabetic patients, as only 50% of the diabetic patients on dialysis is alive 2 years after onset of dialysis (7). Survival is better after kidney transplantation, but the lack of available donor kidneys is critical. In addition, renal replacement therapy is not an option at all in less developed countries. Consequently, the definitive goal in the treatment of diabetic patients must be prevention of diabetic nephropathy. However, if diabetic nephropathy develops progression to ESRD must be avoided. Prevention of development and progression of diabetic nephropathy may be achieved by early aggressive treatment of modifiable risk factors as early as possible in the disease process. In patients with overt nephropathy, ESRD can be prevented by slowing the deterioration in kidney function. If induction of remission of disease or even regression of decline in glomerular filtration rate (GFR) could be obtained, an even better prognosis could be achieved. In all cases, thorough knowledge of risk factors for disease progression is of vast importance.

In general, the aim of this thesis has therefore been to elucidate risk factors for development and progression of diabetic kidney disease, and to study whether the concept of inevitable progression towards ESRD in diabetic nephropathy can be challenged. More specific, the aims have been to evaluate recent trends in the cumulative incidence of diabetic nephropathy, and to identify risk factors for development of microalbuminuria shortly after onset of diabetes. Furthermore, to identify the impact of risk factors for progression of diabetic nephropathy, i.e. progression promoters, when this complication has evolved, and to search for new progression promoters of importance. Whether remission and regression of diabetic nephropathy is feasible has also been investigated. Lastly, the feasibility and consequences of remission of nephrotic range albuminuria for decline in kidney function and survival have been elucidated.

2. DEFINITIONS

2.1 MICROALBUMINURIA

Persistent microalbuminuria was defined as a urinary albumin excretion rate between 30 and 300 mg/24 h in at least two out of three consecutive samples, as recommended in a consensus report (8, 9). The upper boundary of microalbuminuria corresponds to dipstick (Albustix) positive urines (albumin levels are ~100 to 200 mg/l), and thus microalbuminuria can not be detected by conventional stix. Type 1 diabetic patients with an albumin excretion rate in the microalbuminuric range (from 30 to 300 mg/24 h) have long lasting preserved kidney function (10). Time of onset of microalbuminuria was defined as the first recorded positive urine sample in at least two out of three consecutive samples.

2.2 DIABETIC NEPHROPATHY

Persistent albuminuria was defined as a urinary albumin excretion rate above 300 mg/24 h in at least two out of three consecutive samples (8). Diabetic nephropathy was diagnosed clinically if the following criteria were fulfilled: persistent albuminuria, duration of diabetes of more than 10 years, presence of diabetic retinopathy, and absence of clinical or laboratory evidence of other kidney or renal tract disease (7, 8, 11). If these criteria were not fulfilled, a diagnostic kidney biopsy was performed, which was required to show only diabetic glomerulosclerosis. Time of onset of diabetic nephropathy was defined as the first recorded positive urine sample in at least two out of three consecutive samples.

2.3 NEPHROTIC RANGE ALBUMINURIA

Patients with nephrotic range albuminuria have a very poor prognosis (12, 13). Nephrotic range proteinuria has previously been defined as persisting proteinuria above 3500 mg/24 h (14). A correction factor between proteinuria and albuminuria of 0.70 was applied (unpublished data), and thus in our studies, nephrotic range albuminuria was defined as persisting albuminuria above 2500 mg/24 h in at least two out of three consecutive 24 hours urine collections.

2.4 PROGRESSION OF DIABETIC NEPHROPATHY

The decisive hard endpoint of chronic progressive kidney diseases is ESRD, where renal replacement therapy becomes essential for survival. To evaluate disease progression and therapeutic interventions in clinical practise in the predialysis state, other endpoints are required. One possibility is to study the progression of renal morphological lesions, as renal structural lesions can be detected even in the early stages of diabetic nephropathy, where GFR is still within the normal range (15). However, to evaluate structural progression of kidney disease, the primary endpoint need to be clearly defined, and requires serial biopsies over time. Due to several practical and ethical issues, this has only been done in few studies (16, 17). Even though surrogate markers of decline in GFR – such as doubling of serum creatinine – is cheap and convenient, the sensitivity is low, and the endpoint is only relevant when a sufficient number of events can be expected (18). The Food and Drug Administration in the USA have approved the rate of decline in GFR as an endpoint in kidney diseases, if GFR is measured for a period of two to three years (19). In patients with short duration of follow-up, the variability of the decline in GFR is large, with loss of precision of the outcome. Furthermore, short term changes after initiation or intensified anti-

hypertensive treatment may dilute the potential beneficial effect of intervention. Therefore, the following requirements should be fulfilled in order to make a valid determination of the rate of decline in GFR: the applied GFR method should have a good accuracy and precision, repeated measurements of GFR (approximately every 6-12 months) should be performed, and the observation period should be extended to at least 2 years (20).

Intervention that reduces the progression of diabetic nephropathy has always been associated with a reduction in proteinuria as reviewed by Parving (21). In addition, the initial reduction in albuminuria during antihypertensive treatment predict the long-term decline in kidney function in both diabetic and non-diabetic nephropathies (22-24). Therefore, in studies of shorter duration, changes in albuminuria can be used as a surrogate endpoint for the rate of decline in GFR. In our studies of progression of diabetic nephropathy, we have measured GFR with ^{51}Cr-EDTA clearance (see section 3) repeatedly at least yearly for a minimum of three years in each patient.

2.5 REMISSION

2.5.1 Regression to normoalbuminuria

After microalbuminuria has developed, some patients may revert to normoalbuminuria for a shorter or longer period of time. Especially after the introduction of early secondary prevention strategies in the treatment of diabetic nephropathy, regression of microalbuminuria occur. We defined regression of microalbuminuria to normoalbuminuria as a urinary albumin excretion rate below 30 mg/24 h in two out of three consecutive 24 hours urine collections, in addition to a decrease in urinary albumin excretion rate of at least 30% from the microalbuminuria level, sustained for at least one year. Spontaneous regression to normoalbuminuria was classified as regression observed in patients not receiving blood pressure lowering agents before regression was obtained.

2.5.2 Remission and regression of diabetic nephropathy

In our studies of remission and regression of overt diabetic nephropathy, remission of diabetic nephropathy was defined as a decrease in albuminuria to the microalbuminuric range (below 300 mg/24 h (equivalent to 200 μg/min)) (8) in at least two out of three consecutive 24 hour urine collections, and a decrease of at least 30% from pre-remission levels, sustained for at least one year during follow-up. Regression of diabetic nephropathy was defined as a rate of decline in GFR equal to or less than 1 ml/min/year during the entire observation period. This decline in GFR is equivalent to the decline in GFR due to the natural ageing process in non-diabetic patients without kidney disease (25). Spontaneous remission and regression was defined as remission or regression observed in patients not receiving blood pressure lowering agents before or during follow-up.

2.5.3 Remission of nephrotic range albuminuria

Remission of nephrotic range proteinuria has previously been classified as a reduction from nephrotic range proteinuria (proteinuria above 3500 mg/24 h) to below 1000 mg/24 h (14). Keeping the above-mentioned correction factor between proteinuria and albuminuria of 0.70 in mind, we used the following definition of remission of nephrotic range albuminuria: a reduction in albuminuria from nephrotic range albuminuria (albuminuria above 2500 mg/24 h) to below 600 mg/24 h, sustained for at least one year during the follow-up period.

3. METHODS

3.1 GLOMERULAR FILTRATION RATE

Glomerular filtration rate was measured in the supine position after a single intravenous injection of 3.7 MBq ^{51}Cr-EDTA by determination of the radioactivity in venous blood samples taken 180, 200, 220 and 240 minutes after the injection (26). Glomerular filtration rate measured with ^{51}Cr-EDTA plasma clearance underestimates GFR as compared with the gold standard – Inulin clearance – by 10% (27), and our results were multiplied by a factor 1.10. Extra renal loss was corrected for by subtracting 3.7 ml/min. Finally, results were standardised for 1.73m^2 body surface, using the patient's surface area at the start of the study throughout. The mean variability in GFR of each patient from day to day was 4%. Linear regression analysis, least square method, was used to determine the rate of decline in GFR for each patient.

3.2 STATISTICAL METHODS

Statistical analysis was performed by standard statistical methods using a commercially available program, SPSS version 8.0 to 11.0 (SPSS Inc., Chicago, Illinois, USA). More sophisticated analysis were performed by a biostatistician, and includes evaluation of non-linear effects of progression promoters on the decline in GFR (28), and Cox regression analysis with delayed entry used in the study of remission of nephrotic range albuminuria (29). These calculations were performed by the freely available software R (http://www.ci.tuwien.ac.at/R). A p-value <0.05 (two sided) was considered statistically significant in all analyses.

4. STUDY POPULATIONS

Only patients with type 1 diabetes (insulin dependent diabetes/IDDM) defined according to World Health Organisation (WHO) criteria were included in the present studies. Three cohorts were studied:

1) To evaluate the initiation of diabetic kidney disease, we performed a prospective observational follow-up study of an inception cohort, comprising all newly diagnosed type 1 diabetic patients referred to the Steno Diabetes Center between September 1st 1979 and August 31st 1984 (n=286). Data from the inception cohort were also used to evaluate the cumulative incidence of severe diabetic microangiopathy as compared with previously described prevalence cohorts (30, 31).

2) Evaluation of progression promoters, i.e. risk factors for progression of kidney disease, can take place at the start of a study, thus evaluating the predictive value of risk factors, or during the course of the study. To evaluate new baseline risk factors for progression of diabetic kidney disease, we analyzed data from a case-control study comprising all adult Caucasian type 1 diabetic patients with diabetic nephropathy, who had their GFR measured at Steno Diabetes Center during 1993, and consented to participate (n=199). A group of 188 persistently normoalbuminuric type 1 diabetic patients served as controls. Of the 199 patients with diabetic nephropathy, only patients who were followed for at least 3 years after the baseline examination with yearly GFR measurements were studied with respect to nephropathy progression (n=157).

3) For the studies of the impact of potential progression promoters during follow-up, and for evaluation of the concept of remission and regression of diabetic nephropathy, we performed another prospective cohort study. All type 1 diabetic patients with diabetic nephropathy, in whom GFR was monitored yearly for at least three years at the Steno Diabetes Center were included. This cohort consisted of 301 patients. In the studies of nephrotic range albuminuria, an additional 20 patients who initially had their GFR measured at Bispebjerg Hospital were added.

5. NATURAL HISTORY OF DIABETIC NEPHROPATHY

Diabetic nephropathy develops through several stages, and classification of the different phases has been described thoroughly (7). Initial changes in newly onset type 1 diabetic patients consist of hyperperfusion and glomerular hyperfiltration (32). These changes are functional and reversible, as elevated GFR has been demonstrated to return to near normal levels within hours after reduction in blood glucose to normal levels by continuous insulin infusion (33). Augmented levels of glucagon and growth hormone accompanies hyperglycaemia, and are capable of inducing a rise in GFR as

reviewed by Hostetter (34). Finally, changes in circulating and tissue levels of angiotensin II, catecholamines and prostaglandines may contribute to the glomerular hyperfiltration (34). No single factor seems however to account fully for the observed functional changes, and it is yet controversial whether glomerular hyperfiltration have importance as predictor of later development of overt diabetic nephropathy (35-38).

Subsequently, a phase with commencement of renal structural changes evolves. These morphological alterations includes hypertrophy of both glomerular and tubular elements, thickening of glomerular and tubular basement membranes, mesangial expansion, and modest expansion of the tubulointerstitium, hyalinosis of the efferent and afferent arterioles, and later on glomerular occlusion and tubulointerstitial fibrosis (39). Characteristic are the nodular glomerulosclerosis as described by Kimmelstein and Wilson in 1936 (40). The progression of these morphological changes in the kidneys has been demonstrated to be postponed by improved glycaemic control (16). However, in general the structural changes progress and are pursued by a phase with increase in urinary albumin excretion rate to the level of microalbuminuria or incipient diabetic nephropathy, defined as an urinary albumin excretion rate between 30 and 300 mg/24 h (8). Persistent microalbuminuria is an established risk factor for the development of overt diabetic nephropathy (41-45). Furthermore, microalbuminuria is associated with other microvascular and macrovascular complications in diabetes, such as retinopathy, neuropathy and cardiovascular disease (7). If no intervention is applied, there is a continued increase in urinary albumin excretion rate, which ranges from 6 to 14% per year as reviewed by Post Hansen (46). This eventually results in the development of macroalbuminuria with urinary albumin excretion rate persistently above 300 mg/24 h. At the onset of overt diabetic nephropathy, GFR is normal; in the prior stage of microalbuminuria hyperfiltration is often observed. GFR starts to decline when albuminuria exceeds approximately 300 mg/24 h. Albuminuria indicates defects in the filtration barrier, and is associated with a quantitative widening of the glomerular basement membrane and epithelial foot processes, but also a qualitative change of these. Thus, a loss of size and charge selectivity of the glomerular barrier develops over time (47, 48). If no intervention is applied, the disease is associated with increasing levels of arterial blood pressure and albuminuria, and a relentless mean rate of decline in GFR of approximately 10-12 ml/min/year (49-51). The structural and functional changes are closely interrelated, as decrease in GFR correlates with decreased filtration surface area due to occluded glomeruli and mesangial expansion (52). In man, the morphological changes are closely associated with declining kidney function (53, 54).The prognosis has been associated with the level of albuminuria, as patients with the highest levels of albuminuria, i.e. nephrotic range albuminuria, have the most advanced renal structural changes, the fastest decline in GFR and shortest survival time (5, 12, 13). If left untreated, diabetic nephropathy is a rapidly progressive and irreversible kidney disease with high morbidity and mortality (5, 12). The average survival time from onset of proteinuria has been demonstrated to be only 5 to 7 years (2, 5). Progression to uraemia will inevitably occur and require renal replacement therapy in order to keep the patients alive.

6. INCIDENCE OF SEVERE DIABETIC MICROANGIOPATHY

The treatment of patients with type 1 diabetes was changed radically after Banting, Best, Collip and MacLeod discovered insulin in 1921 in Toronto, Canada. However, the effective treatment of hyperglycaemia did not only prolong the lives of the patients, but also exposed the appalling long-term micro- and macrovascular damages after longer duration of diabetes. The term "diabetic angiopathy" was introduced by Lundbæk, and associations between micro – and macrovascular complications were described (55). The observation that diabetic kidney disease was strongly associated with other severe complications, such as proliferative retinopathy (56, 57), and cardiovascular mortality (58), implicated albuminuria to reflect more than renal disease. The Steno hypothesis, as proposed by Deckert, advocates albuminuria to reflect a more generalized vascular process which affects the glomeruli in the kidneys, the retina and the intima of large vessels simultaneously (59).

Several large observational studies have evaluated the incidence of persistent proteinuria and proliferative retinopathy (2-4, 60). These studies demonstrated the incidence of diabetic nephropathy to increase rapidly after 10 years of diabetes, peak between 10 to 20 years of diabetes, and decline hereafter. The cumulative incidence of diabetic nephropathy after 25 years of diabetes was reported to decrease between 1940 and 1950, from 40 percent to 25-30 percent (2-4). Unfortunately, no declining trend in the incidence of proliferative retinopathy was demonstrated (60). In spite of the improvement in the treatment and care of the patients, the cumulative incidence of persistent albuminuria after 25 years of diabetes has remained stable at approximately 25-30% until the 1980's, and increasing with duration of diabetes (3, 4). In 1994, Bojestig reported a dramatic decline in the cumulative incidence of diabetic nephropathy in Swedish children with type 1 diabetes diagnosed before the age of 15 years. After 20 years of diabetes the cumulative incidence of diabetic nephropathy decreased from 28 percent in patients with onset of diabetes between 1961-1965 to 5.8 percent in patients with onset of diabetes between 1971-1975 (61). The Swedish study was, however, not able to demonstrate a decline in laser treated retinopathy (62). Subsequent studies conducted by Rossing and Rossing based on a prevalence cohort of 356 type 1 diabetic patients (identified at the Hvidöre Hospital), revealed no evidence of a declining incidence of diabetic nephropathy nor proliferative retinopathy after 15 years of diabetes (30, 31). Due to the conflicting evidence of a decline in incidence of severe microvascular complications in type 1 diabetes during the last decades, we analyzed data from two long-term prospective observational studies lasting 20 years or more, including 600 Caucasian patients with onset of type 1 diabetes between 1965 and 1984, followed until death or to 2000 (63). The study populations comprised an inception cohort of all newly diagnosed type 1 diabetic patients referred to the Steno Memorial Hospital between September 1st 1979 and August 31st 1984 (n=286), and the patients from the Hvidøre 1984 cohort with extended follow-up to at least 20 years of diabetes. Our study revealed a significant decrease in the cumulative incidences after 20 years of diabetes of diabetic nephropathy (from 31.1 to 13.7%) (**Figure 1**), proliferative retinopathy

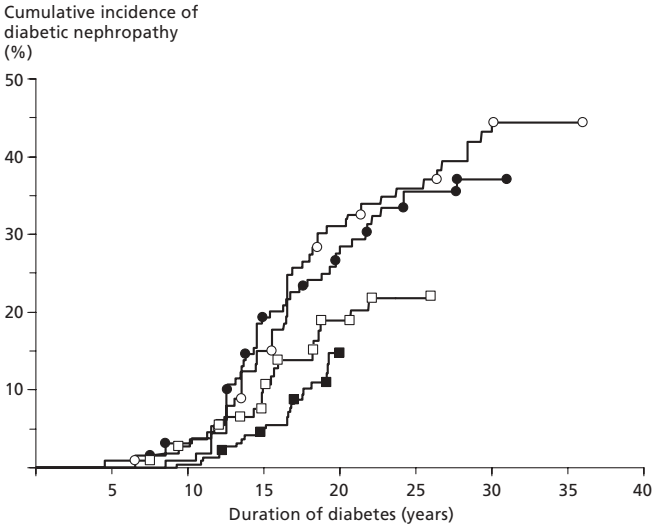

Figure 1. Cumulative incidence of diabetic nephropathy in 600 type 1 diabetic patients with onset of diabetes from 1965 to 1969, n=113, group A (white circles); 1970 to 1974, n=130, group B (black circles); 1975 to 1979, n=113, group C (white squares), and 1979 to 1984, n=244, group D (black squares). P<0.001, Log rank test, pooled over strata.

(from 31.2 to 12.5%) and diabetic maculopathy (from 18.6 to 7.4%), with increasing calendar year of diagnosis (63). Furthermore, patients from the most recent cohort had a significantly better visual acuity after 20 years of diabetes compared with the three earlier cohorts. The decline in the cumulative incidence of diabetic nephropathy – although not as stunning as the results from Bojestig – was confirmed, and we extended the findings to include vision threatening diabetic retinopathy (proliferative retinopathy as well as maculopathy) in type 1 diabetes.

To reduce selection bias only type 1 diabetic patients from the Copenhagen area were included in our study. At that time, no other treatment facilities other than Hvidøre Hospital or the Steno Memorial Hospital were available for type 1 diabetic patients. The patients were randomly referred to one of these hospitals, and treatment at both hospitals has always been free of charge. In the prevalence cohorts, selective dropout from the clinic before the baseline examination in 1984 could occur, therefore, the cumulative incidence observed in the inception cohort must be regarded as closer to the true incidence of the microvascular complications. Thus, the demonstrated decline in cumulative incidence of diabetic microangiopathy possibly reflects a conservative estimate. However, in order to determine the true incidence of diabetic late complications, population-based studies are needed. Previous prospective studies of type 1 diabetic patients have identified age at onset of diabetes, poor glycaemic control, blood pressure elevation, and smoking as risk factors for development of microvascular complications (3, 4, 64-67).

In our study, the age at onset of diabetes was ranging from 20 to 23 years, and slightly higher in patients with onset of diabetes from 1975 to 1984 as opposed to the previous cohorts. The incidence of diabetic nephropathy has been demonstrated to decrease with increasing age at onset of diabetes (4), while others have found that onset of diabetes before the age of 10 years of age is associated with delay in later development of proteinuria (3). It is unlikely, that the dramatic decrease in the incidences of both diabetic nephropathy and proliferative retinopathy solely can be explained by the slight difference in age at onset.

In the Diabetes Control and Complications Trial (DCCT) comprising 1441 type 1 diabetic patients the beneficial effect of intervention aiming at strict glycaemic control as a tool of preventing or delaying development of diabetic microvascular complications was clearly demonstrated (68). In the study by Bojestig et al, excellent glycaemic control in the patients was achieved (HbA_{1c} ~7% with a normal range of 3.2-6.0%) (61), a level comparable to the intensive treated group in the DCCT. The observed decline in the cumulative incidence of diabetic nephropathy was ascribed exclusively to improved glycaemic control, as no other intervention was applied. In comparison, our HbA_{1c} values was at a level comparable to the conventionally treated group in the DCCT (HbA_{1c}: 8.8% with a normal range of 4.1-6.4%) in the three first cohorts, with a significant reduction in long-term glycaemic control observed in the inception cohort (HbA_{1c}: 8.5%), of the same order of magnitude as demonstrated in the follow-up study of the DCCT, the Epidemiology of Diabetes Interventions and Complications (EDIC) study (69).

During the follow-up period in our study (63), two major changes were introduced in the care of the patients. Firstly, several long-term studies had demonstrated a beneficial effect of the use of angiotensin converting enzyme (ACE) inhibitors on prevention or delaying progression from microalbuminuria to overt nephropathy, as well as progression of diabetic retinopathy, even in normotensive type 1 diabetic patients (70-73). At the Steno Diabetes Center, several of the smaller studies advocating the concept of ACE inhibition in the prevention of diabetic nephropathy were performed (11, 70, 74, 75). As a consequence, this new treatment modality was early adopted at our institution. A general policy to prescribe ACE inhibitors for normotensive microalbuminuric patients was introduced in our clinic in 1994. The prevention of diabetic nephropathy through ACE inhibition has recently been associated with long lasting preservation of kidney function (10). Secondly, the criteria for diagnosis of arterial hypertension was changed in 1995 from the World Health Organization's criteria (≥160/95 mm Hg) to the American Diabetes Associations criteria (≥140/90 mm Hg), with even lower blood pressure target after initiation of antihypertensive treatment (76). It is likely, that new indication for ACE inhibitors as well as the application of more strict criteria for the diagnosis and treatment of arterial hypertension has imposed a beneficial effect on the observed decline in the cumulative incidence of microvascular complications in type 1 diabetes.

Smoking has been associated with proteinuria in cross sectional studies, whereas data on retinopathy has been conflicting (77-80). Prospective studies have however demonstrated a relationship between smoking and progression from normoalbuminuria to microalbuminuria and overt nephropathy, as well as progression of retinopathy (64, 66, 81-83), and cessation of smoking has been related to a decrease in albumin excretion rate (81). The prevalence of smokers in Denmark has declined linearly through the last decades, reaching 33% in 2000 (84). This trend is reflected in our study populations, as the prevalence of smokers was reduced from approximately 65% in the prevalence cohorts to 45% in the inception cohort. However, unfortunately the prevalence of smokers in our diabetic population still remains much higher than the background population (84). Nevertheless, the reduction in patients who smoke could contribute to the observed decline in the cumulative incidence of microvascular complications.

Recently, a significant decline in the 20-year cumulative incidence rates of ESRD in type 1 diabetic patients diagnosed between 1965 and 1969, 1970 and 1974, and 1975 and 1979 have been reported (9.1%, 4.7%, and 3.6%, respectively; $p = 0.006$) (85). Thus supporting our findings of a decrease in the individual risk of progressing to nephropathy, and even ESRD. Despite these beneficial findings, the incidence rates for ESRD in diabetic patients increases at approximately 9% per year, and diabetic nephropathy is the leading cause of ESRD in the United States (1). However, the rise in diabetic patients referred to renal replacement therapy can partly be explained by better referral of that population for kidney failure, and the ageing of the general populations. Furthermore the majority of the patients referred for renal replacement therapy are type 2 diabetic patients (1). In conclusion, the cumulative incidence of severe diabetic microangiopathy has decreased in type 1 diabetic patients during the past decades, even though ~15% of the patients still develop these severe complications after 20 years of diabetes. In concordance with the declining incidence in development of overt nephropathy, a decline in progression to ESRD has recently been reported (85). However, at present time it is not known whether the development of severe diabetic microangiopathy can be totally prevented or only postponed. Intervention studies have demonstrated that strict glycaemic control (68) as well as treatment with ACE inhibitors in microalbuminuric patients (45) can reduce the rate of progression to macroalbuminuria. The cost-effectiveness of screening and treatment of microalbuminuria with ACE inhibitors a well as strict glycaemic control and the combination of both has been evaluated (86-88). Screening for microalbuminuria followed by appropriate treatment, was found to be cost saving with improvement in life expectancy (88). The cost-effectiveness of secondary as well as tertiary prevention may vary by cost savings due to complications avoided, and counterbalanced by improved life expectancy in chronically ill patients. However, there is no doubt that delay or prevention of complications will improve quality of life. Future research will have to elucidate the impact of multifactorial secondary prevention strategies with respect to feasibility, costs and effectiveness in every day life. To increase efficacy and prevent the preventable, early detection of high risk patients even before microalbuminuria develops is of vast importance. Therefore, identification of early risk markers for subsequent development of microalbuminuria is urgently needed.

7. INITIATION OF DIABETIC NEPHROPATHY

The epidemiological studies revealed that only a fraction of type 1 diabetic patients develop diabetic nephropathy, while others seems to be protected from this complication (2-4). Thus, hyperglycaemia may be regarded as a necessary, but not a sufficient factor for the development of diabetic kidney disease. It has been demonstrated that diabetic nephropathy evolves more frequently in the male gender (2, 4). Further intensive research into the pathogenesis of the disease has been performed, and it is now generally accepted that the aetiology of the structural and functional changes of diabetic nephropathy is multifactorial. In an inception cohort of 286 patients with newly diagnosed type 1 diabetes, we evaluated baseline risk factors for the development of persistent micro- and macroalbuminuria prospectively (89). The cumulative incidence of persistent micro- and macroalbuminuria was 33.6% (95% CI 27.2-40.0%), and 14.6% (8.9-20.3), respectively after 20 years of follow-up. After initial glycaemic stabilization, urinary albumin excretion rate, male gender, mean arterial blood pressure, HbA_{1c}, and height were found to be significant predictors for the development of persistent microalbuminuria (89) (**Table 1**).The impact of increased levels of urinary albumin excretion rate has been investigated extensively, and numerous studies have documented that persistent microalbuminuria is a sensitive risk marker for development of overt diabetic nephropathy (41-43). However, even urinary albumin excretion rate within the normal range has been associated with the risk for development of micro- and macroalbuminuria in type 1 diabetes (83, 90-93). In our study, the level of urinary albumin excretion rate at onset of diabetes, and within the normal range, was found to predict development of microalbuminuria (89). The difference in renal susceptibility among individuals, as dispersion in the level of urinary albumin excretion rate within the normal range suggests, may reflect an inherited difference in renal susceptibility, which may confer abnormal glomerular haemodynamic and permselectivity (34). Furthermore, different levels of renal functional reserve capacity as recently demonstrated in patients with primary hypertension (94) may also contribute. As the prevalence of diabetes increases, it has become even more urgent to determine the pathogenetic mechanisms responsible for the development of microvascular complications. Evidence emerges of a complicated interaction between different contributors to the disease process. Genetic susceptibility, metabolic abnormalities, haemodynamic changes, upregulated growth factors and cytokines may all play a part in the development of diabetic glomerulopathy. Assessment of predictors for the development of incipient diabetic nephropathy, as done in our study (89), is central when aiming at establishing intervention strategies at the earliest possible stage. The combination of several risk markers – as performed by Rossing et al (83) – can be used when initiating primary prevention trials in order to find those patients who are most likely to benefit from intervention, thereby reducing sample size and follow-up time or both in the trials.

Table 1. Cox proportional hazard model of baseline risk factors for development of persistent microalbuminuria in all 277 patients with type 1 diabetes followed for 18 years.

	All patients (n=277)	
Variable	**Relative risk (95% CI)**	**P value**
Log10 Urinary albumin excretion rate (per 10 fold increase)	3.78 (1.57-9.13)	0.003
Sex (male versus female)	2.41 (1.43-4.06)	0.001
Mean arterial blood pressure (per 10 mm Hg increase)	1.38 (1.20-1.57)	<0.001
Glycosylated Haemoglobin A_{1c} (per 1% increase)	1.18 (1.04-1.32)	<0.01
Height (per 1 cm increase)	0.96 (0.95-0.98)	<0.001

Age at onset, weight, smoking status, serum cholesterol, and fasting plasma C-peptide were not included in the final model.

7.1 GENETIC SUSCEPTIBILITY

The fact that some diabetic patients are vulnerable, while others seems to be protected against the impact of hyperglycaemia lead to the search for non glycaemic factors modulating the risk of the renal complications in diabetes. The striking racial variation (95) in the prevalence of diabetic kidney disease supports a genetic heredity of vulnerability to the harmful effects of hyperglycaemia. Furthermore, several studies indicate a familial clustering of diabetic nephropathy as well as cardiovascular disease (96-101). The occurrence of familial clustering strongly supports the hypothesis of genetic factors being involved in the development of diabetic kidney disease. However, mutual environmental risk factors in the affected patients could in fact explain the observed familial clustering. So far, none of the established risk factors for the development of diabetic kidney disease exhibits large enough effects, and the concordance rates are most likely explained by shared genetic traits. At present time, diabetic nephropathy is considered to be a complex genetic trait with a combination of alleles at several genes in addition to environmental factors providing the increased risk of the disease. Many studies of different putative candidate genes have been carried out, including diabetes susceptibility genes, genes involved in glucose metabolism and in regulation of blood pressure, genes regulating cardiovascular risk factors, and glomerular structural as well as growth factor genes as recently reviewed by Tarnow (102). Owing to small sample sizes, differences in designs and populations studied, results have been contradictory and subsequently difficult to interpret. The ACE I/D polymorphism have functional impact of the encoded product, as level of circulating ACE is related to genotype with patients with the DD genotype having twice as high plasma ACE as those with the II genotype (103), and may therefore have pathophysiological significance due to the actions of angiotensin II. Some have reported the ACE/ID polymorphism to be related to the development of diabetic nephropathy (104, 105), while others have failed to find an association (106). In a meta analysis, a modest effect of the D-allele of the ACE/ID polymorphism as a risk factor for the development of diabetic nephropathy in Caucasians has been demonstrated (107). In our study of the inception cohort of 286 type 1 diabetic patients followed from onset of diabetes, we did not find an association between the ACE/ID polymorphism and the development of micro-macroalbuminuria (unpublished data), but the study is likely to be underpowered for this type of analysis. In order to evaluate complex genetic traits like diabetic nephropathy, strict criteria of phenotyping, and a sufficient large number (>1000) of individuals are needed. Many studies of varying size have evaluated the impact of other genes on the risk of development of diabetic nephropathy with inconsistent results. To enhance the cost benefit of screening, and early treatment of patients at high risk of developing diabetic nephropathy, early identification of these patients is of utmost importance. In spite of the challenges met, evaluation of the genetics of diabetic nephropathy remains to be a key factor in that process. Furthermore, if new candidates genes can be identified, functional abnormalities related to the genes might be detected, which may give novel insight in the pathogenesis of the development of diabetic microangiopathy.

7.2 METABOLIC CHANGES

Hyperglycaemia

In vitro, hyperglycaemia induces basement membrane thickening, increases the synthesis of extracellular matrix, and vasoconstriction (108-110). Furthermore, hyperglycaemia is associated with disturbed cell cycle and accelerated death of endothelial cells (111). On the molecular level, four mechanisms have been implicated in this glucose mediated vascular damage: increased polyol pathway flux, increased advanced glycation end product (AGE) formation, activa-

tion of protein kinase C (PKC), and increased hexosamine pathway flux (112). The contribution of the polyol pathway may be very much species specific, and negative clinical trials with pharmacological inhibition of aldose reductase have questioned the relevance of this mechanism in humans (113). Glucose form early, non enzymatically, reversible glycosylation products with proteins (Schiff bases), which in turn rearrange into Amadori products. Through complex chemical rearrangements, irreversible AGE's are formed, which are capable of forming covalent bonds with other proteins (114). Recent studies have demonstrated Amadori products to modulate mesangial cell growth and collagen gene expression in vitro (115), and to be correlated with markers of endothelial dysfunction, and independently associated to diabetic nephropathy in humans (116). Advanced glycosylation end products can damage target cells in at least three different ways. 1) Intracellular proteins can be modified by AGE formation; thereby the function of these proteins can be altered. 2) Extracellular matrix components can be altered by AGE precursors, and subsequently interact abnormally with other matrix proteins, induce cross link formation, and interact with receptors for matrix proteins on cells. 3) Plasma proteins can be changed by AGE formation, bind to AGE receptors on endothelial and mesangial cells and macrophages, inducing production of reactive oxygen species (112). In animal models, the importance of AGE formation on the pathogenesis of microvascular complications have been demonstrated, as treatment with an AGE inhibitor (Aminoguanidine) is associated with reduced renal AGE accumulation, retardation in the development of albuminuria, and attenuation of mesangial expansion (117). A large randomized trial in type 1 diabetic patients with this compound, were discontinued prematurely due to toxicity. Meanwhile, other AGE inhibitors (118) as well as the role of specific AGE receptors (119) are under investigation.

Protein kinase C isoforms may be activated directly by hyperglycaemia (120) or indirectly through AGE receptors, and increased activity of the polyol pathway (112). Various actions of cytokines, e.g. vascular endothelial growth factor (VEGF) (121) and transforming growth factor-β (TGF-β) (122) are shown to be PKC dependent. Animal models have demonstrated inhibitors of PKC-β to attenuate glomerular hyperfiltration, reduce albuminuria, and decrease expression of TGF-β (122, 123). Pharmacological inhibition of PKC-β in clinical trials in diabetic retinopathy are ongoing (124), although recent results have been disappointing (Abstract: Milton et al, Diabetes, Vol. 52, suppl. 1, page A127, 2003). Lastly, activation of the hexosamine pathway by hyperglycaemia may result in altered gene expression and protein function contributing to the pathogenesis of diabetic complications (112).

A common link between the four basic mechanisms of microvascular damage induced by hyperglycaemia has been suggested to be overproduction of superoxides by the mitochodria (125, 126). This implies that disruption of the superoxide overproduction potentially could normalize all four mechanisms at one time. In clinical practice, this is however hardly possible by conventional antioxidants, as attempted in the Heart Outcomes Prevention Evaluation trial (HOPE) and the Heart Protection Study (HPS), where low dose vitamin supplementation did not succeed in reducing neither risk for cardiovascular nor renal disease in diabetic patients (127, 128).

In man, the relation between glycaemic control on one side, and risk of development of microangiopathy on the other was reported by Pirart (129). A close correlation between level of hyperglycaemia and development of microalbuminuria in type 1 diabetic patients has been firmly established in subsequent observational studies (83, 90, 92, 130). In a meta-analysis on 16 smaller randomized trials of intensive therapy aiming at tight blood glucose control, Wang et al reported that intensive treatment was associated with reduced risk of nephropathy and retinopathy progression (131). A finding confirmed and extended by the DCCT trial comprising 1441 type 1 diabetic patients, where the beneficial effect of intervention aiming at strict glycaemic control as a means of preventing or delaying development of diabetic microvascular complications was clearly demonstrated (68). Intensive blood glucose control during 6.5 years reduced the occurrence of microalbuminuria (>40 mg/24 h) by 39% as compared to conventional therapy (68). The time, effort, and cost required in the DCCT were however considerable in order to achieve good glycaemic control, and the incidence of severe hypoglycaemia was three times higher in the intensive treated group (68). Whether the benefits from the DCCT persisted after the trial ended, was evaluated in the EDIC study (69, 132). The reduction in risk of microvascular disease in intensive group persisted eight years after end of trial; however the difference in glycaemic control narrowed during follow-up (during DCCT 7.2 vs. 9.1%, during EDIC 8.0 vs. 8.2%). The latter emphasize that substantial effort is needed to obtain and maintain excellent glycaemic control.

In our prospective study of an inception cohort, we demonstrate poor glycaemic control at onset of diabetes to be an important predictor for later development of microalbuminuria (89). This observation strongly support the conclusion from the EDIC study (69, 132), and implies that vigorous efforts to normalize glycaemia should be implemented from diagnosis of diabetes, and maintained as long as possible.

Dyslipidaemia

Abnormalities in the lipid metabolism may be of importance in the pathogenesis of glomerulosclerosis. Experimental induced hypercholesterolaemia in rabbits induces glomerular lesions (133), and pharmacologic reduction of serum lipids in Zucker rats did not only reduce serum cholesterol and fasting triglyceride levels, but was also associated with concomitant reduction in urine albumin excretion as well as mesangial matrix expansion and cellularity (134). Dyslipidaemia is a common feature even early in the course of diabetic nephropathy (135, 136). Histologic data in humans support the concept of a relation between dyslipidaemia and diabetic kidney disease, as glomerular lipid deposits have been demonstrated in kidney biopsies from patients with diabetic nephropathy (137). Type 2 diabetic patients with the metabolic syndrome including dyslipidaemia, have a higher prevalence microalbuminuria or macroalbuminuria as compared to type 2 diabetic patients without the metabolic syndrome (23 vs. 7%, $p = 0.003$) (138). Even in patients with type 1 diabetes, there is a strong, positive, and independent correlation between urinary albumin excretion rate and lipoprotein profile in the DCCT cohort (139), as well as in the European Diabetes Prospective Complications Study (EURODIAB) (140). In a short term observational study, low-density lipoprotein was found to be a significant independent predictor of development of microalbuminuria (130). Further longitudinal studies with repeated concomitant measurements of lipoprotein profile and urinary albumin excretion rate before and after development of microalbuminuria are needed to establish whether dyslipoproteinaemia is a predictor of development – or a consequence – of diabetic kidney disease. The association between dyslipidaemia and development of microalbuminuria has not yet been fully clarified.

7.3 HAEMODYNAMIC ABNORMALITIES

Glomerular filtration rate is determined by four factors; glomerular plasma flow, systemic oncotic pressure, glomerular transcapillary hydraulic pressure, and the glomerular capillary ultrafiltration coefficient (141). In animal models, renal vasodilatation causes an increase in single nephron GFR due to increase in glomerular plasma flow and glomerular transcapillary hydraulic pressure. In progressive kidney disease, the initial hit is suggested to be intraglomerular hypertension, soon followed by altered permselective properties of the glomerulus, increased protein filtration, protein accumulation in the mesangium, proliferation of mesangial cells, and matrix accumulation leading to glomerulosclerosis (34). In accordance, the two kidney Goldblatt hypertensive model indicates that systemic and local, intrarenal haemodynamic changes can induce the characteristic

structural changes in diabetic animals (142). In humans, glomerular hyperfiltration occurs early in the course of diabetes, thus renal haemodynamic alterations associated with hyperglycaemia could initiate the process of glomerulosclerosis (34). Furthermore, the cardiac output and peripheral vasodilatation is increased during hyperglycaemia in diabetic patients1 (43, 144).The landmark studies performed by Brenner and co-workers did not only provide evidence of the importance of haemodynamic factors in the development of diabetic glomerulosclerosis. Moreover, the important role of reducing intraglomerular capillary pressure was emphasized using low protein diet and inhibitors of the renin angiotensin system (RAS) (145-147). The role of the RAS in the development and progression of diabetic nephropathy has been extensively evaluated during the last decades. However, plasma components of the RAS are low or normal in diabetes, except for the precursor of renin: prorenin. In normotensive type 1 diabetic patients, increase in prorenin has been reported even before development of microalbuminuria, and level of serum prorenin was found to be a predictor of development of microalbuminuria and diabetic retinopathy (148). In overt nephropathy, prorenin as well as renin have been shown to be elevated in plasma (149). All components of the RAS, including enzymes and receptors are present within the kidney, and most likely the RAS is activated locally within the kidney. The end product of the RAS, angiotensin II, has haemodynamic effects which include contraction of the efferent arteriole in the glomeruli. In addition, non haemodynamic effects of angiotensin II with importance for the development and progression of diabetic kidney disease, such as cell growth, proliferation and apoptosis through a range of pathways have been demonstrated (150, 151). Angiotensin II has also been demonstrated to induce extracellular matrix accumulation through stimulation of TGF-β (152). The haemodynamic and non haemodynamic effects can be hard to separate from each other, and a link between the deleterious effects of hyperglycaemia, haemodynamic changes with intraglomerular hypertension, and cytokines has now been established. The elastic and compliant property of the glomeruli allows pressure to regulate volume, and implies cyclic changes in the glomerular volume. These cyclic alterations may be aggravated by impaired autoregulation, which are often observed after longstanding diabetes (153), leading to downstream transmission of systemic pressure leaving end organs unprotected from the higher systemic blood pressure. Glomeruli will undergo expansive cycles of distension-contraction under conditions of systemic hypertension and impaired autoregulation. Cyclic stretch in tissue culture induces accumulation of extracellular matrix through stimulation of TGF-β, and extracellular matrix accumulation is markedly enhanced in presence of hyperglycaemia (154). Therefore, restriction of glomerular distension may be of utmost importance in preventing development and progression of glomerulosclerosis, however no effective treatment can improve glomerular pressure autoregulation (155). In contrast, it has recently been demonstrated that dihydropyridine calcium channel blockers may impair or even abolishes autoregulation in type 2 diabetic patients (156). In order to prevent and retard development of micro- and macroalbuminuria, reduction of systemic blood pressure and intraglomerular pressure is necessary, and agents reducing intraglomerular pressure may be advantageous in the prevention of diabetic nephropathy.

In humans, arterial hypertension is observed frequently in patients with diabetic nephropathy (157). Whether elevated blood pressure has a causative role and initiates development of nephropathy, or the development of nephropathy causes blood pressure to rise is not fully clarified. In cross sectional studies, some have demonstrated blood pressure to be a risk marker for development of microalbuminuria (92), while others have failed to find an association (83, 90). Our study suggests that systemic blood pressure elevations even at the onset of diabetes and within the normal range, play an important role in the development of persistent microalbuminuria (89). This is in accordance with the concept of the critical role of glomerular hypertension in the initiation of diabetic kidney disease (34), and further supported by the studies that demonstrate a familial predisposition to hypertension increases the risk of development of diabetic nephropathy (158, 159). Recently, nocturnal hypertension has emerged as a new risk factor for development of microalbuminuria (160). Suggesting that either changes in systemic blood pressure are very small, which makes their detection difficult unless carefully validated and precise methods are used, or arterial blood pressure has been measured at the wrong time (160), i.e. at daytime and not during the night.

In normotensive microalbuminuric type 1 diabetic patients a beneficial effect of treatment with an ACE inhibitor was originally demonstrated by Marre (161), and confirmed by others as reviewed by Mogensen (9). A meta-analysis of secondary prevention studies of diabetic nephropathy using ACE inhibitors conclude that in normotensive type 1 diabetic patients, ACE inhibitors significantly reduces progression to macroalbuminuria, and increases chances of regression to normoalbuminuria (45). The reduction in systemic blood pressure could not entirely explain the beneficial findings. Recently, two very important issues have been reported. Firstly, prevention of diabetic nephropathy through ACE inhibition has been demonstrated to be associated with long lasting preservation of kidney function (10), and secondly implementation of the knowledge acquired from the treatment trials of ACE inhibition in microalbuminuric patients is feasible in everyday life in a tertiary referral center (46). However, primary prevention using ACE inhibitors in normotensive normoalbuminuric patients have not yet been convincingly shown in trials. In a randomised placebo controlled trial in normotensive type 1 diabetic patients with normoalbuminuria or microalbuminuria, the ACE inhibitor Lisinopril was compared to placebo. At inclusion, more than 80% (n=440) of the patients had normoalbuminuria, and the study failed to show a statistical significant treatment difference (12.7% 95% CI: –2.9 to 26.0, p=0.1) (162). In a similar population of 89 type 1 diabetic patients, it was demonstrated that urinary albumin excretion can be significantly reduced by treatment with an ACE inhibitor with lowering of systemic blood pressure (163). However, no information of the impact of ACE inhibition on primary prevention of diabetic nephropathy, i.e. development of persistent microalbuminuria, was reported (163).

7.4 GROWTH FACTORS AND CYTOKINES

Growth hormone and Insulin-like growth factor-1

The growth hormone (GH) hypothesis suggest that GH is one causal factor in the development of diabetic microangiopathy (164). The hypothesis was based on the findings that hypophysectomy improved diabetic retinopathy, that 24 hour levels of GH was found to be elevated in diabetic patients, and returned to normal levels after blood glucose normalization (164). Pituitary GH induce insulin-like growth factor-1 (IGF-1) in various organs. In diabetic animals, the rapid increase in kidney size and function after induction of diabetes is preceded by an increase in renal tissue IGF-I concentration, and strict glycaemic control through insulin treatment abolishes both the increase in kidney IGF-I and renal hypertrophy (165). In dwarf rats with isolated GH deficiency, a smaller degree of renal and glomerular enlargement was observed, and an attenuated rise in albuminuria was observed as compared to diabetic rats with normal functioning pituitary gland (166). The role of GH and IGF-1 in the early changes of diabetic glomerulopathy is further supported by animal studies, where treatment with a long acting somatostatin analogue for 6 months was associated with significant reductions of increase in kidney weight, kidney IGF-1 concentrations and urinary albumin excretion rate compared with untreated diabetic rats (167). Treatment with a GH antagonist in diabetic mice showed comparable results (168). A cross sectional study of 155 children and adolescents with type 1 diabetes demonstrated a relationship between kidney weight and IGF-1 (169). In addition, the 14 patients with

microalbuminuria had significantly higher levels of urinary IGF-1 and GH as compared to the normoalbuminuric patients (169). In diabetic patients with pre proliferative retinopathy, a long-term multicenter trial on the effect of the somastatin analogue Sandostatin on progression to proliferative retinopathy is at present time ongoing. Long-term prospective studies to investigate the role of GH in humans on development and progression of diabetic nephropathy are lacking, as well as treatment trials to establish the effect of intervening in the GH/IGF-1 system on development and progression of diabetic kidney disease.

Cytokines

The cytokines constitute a group of small potent regulatory peptides with multiple and often overlapping biological activities. They have been implicated in numerous biological processes in the kidney including cell hypertrophy, proliferation, regulation of matrix synthesis and degradation, inflammatory responses, and regulation of vascular tone (170). Cytokine production is usually transient and tight regulated, and the half life of cytokines in the circulation ranges from hours to a few days. High affinity receptors are expressed on target cells in many organs, and the various cytokines interacts in a complex and not fully clarified way. The investigation of the role of cytokines in development and progression of diabetic glomerulopathy is of interest since these peptides potentially could serve as early markers of development of disease, be targets for intervention, and markers used for monitoring efficacy of other treatment modalities. Transforming growth factor β (TGF-β) is a prosclerotic cytokine family, the major isoform TGF-β1 is considered to be the major mediator of collagen formation in the kidney (171). In addition, TGF-β1 inhibits the production of matrix metalloproteases, stimulates the synthesis of metalloprotease inhibitors, thereby having an effect on matrix degradation pathways (171). The TGF-β expression increases as a response to a wide range of stimuli including high glucose concentration, AGE's, oxidative stress and superoxide overproduction, cyclic stretch, and high levels of intrarenal angiotensin II as reviewed by Ziyadeh (172). These factors are all relevant for the development and progression of diabetic nephropathy. TGF-β has been found to be over-expressed in the kidneys of animals as well as humans with diabetic nephropathy (173), and treatment with TGF-β antibodies reduces extracellular matrix production in diabetic mice (174). TGF-β therefore seems to be involved in the progressive matrix accumulation and fibrosis observed in diabetic glomerulopathy, and moreover to be an important link between the haemodynamic and metabolic pathways in the initiation of diabetic nephropathy. The synthesis of another prosclerotic cytokine, connective tissue growth factor (CTGF) is stimulated by TGF-β, hyperglycaemia and cyclic mechanical stretch (175). In experimental diabetes, CTGF is increased in glomeruli of diabetic animals, and CTGF has been reported to be elevated early in the course of diabetic glomerulopathy (175). A correlation between level of urinary albumin excretion rate and CTGF has been shown in humans (176). However, the role of CTGF in the development and progression of diabetic nephropathy has not yet been studied to a large extent in humans, and prospective studies are lacking.

Vascular endothelial growth factor (VEGF) is a potent cytokine family that induces angiogenesis and markedly increases endothelial permeability (121). In diabetic microangiopathy, the hallmark of endothelial dysfunction is an increase in permeability, and VEGF has been found to be elevated in ocular fluid in diabetic proliferative retinopathy, with the level of VEGF declining after successful laser photocoagulation (177). In experimental diabetic kidney disease, VEGF expression is increased, predominantly in the glomerular podocytes (178). In 110 type 2 diabetic patients, plasma VEGF was higher among patients with micro- and macroalbuminuria as compared with the normoalbuminuric patients, with a tendency towards increasing levels of VEGF with increasing levels of albuminuria (179). In a large case-control study of type 1 diabetic patients with and without diabetic nephropathy, we found that plasma VEGF was significantly elevated early in the course of diabetic nephropathy in male subjects (180). Since a large inter-individual variability in plasma levels of VEGF was observed in our study, it is likely that VEGF and other cytokines might act predominantly as paracrine hormones in diabetic renal disease. In accordance, elevated VEGF levels in the vitreous fluid in eyes of patients with proliferative diabetic retinopathy has been reported to be produced locally by the ischemic retina, whereas circulating VEGF was not elevated (181). Furthermore, platelet derived growth factor have been implicated in the development of glomerulopathy and tumor necrosis factor-α have recently been suggested to play a role for the development of microalbuminuria (182). In the latter case, only kidney tissue and urine samples showed positive results, emphasizing the need for sampling of material close to the organs of interest. In order to establish new early markers for development of diabetic nephropathy in high risk patients, long-term studies with repeated measurement of urine samples during the course of the disease are needed. Only hereby, evaluation of the complex interplay of the various cytokines and the development of diabetic nephropathy can be performed.

7.5 OTHER FACTORS ASSOCIATED WITH DEVELOPMENT OF DIABETIC NEPHROPATHY

Glomerular hyperfiltration (35, 38), low birth weight (183), short stature (184), presence of retinopathy (64, 83), gender and onset of diabetes at young age (185) have been demonstrated to be associated with development of diabetic nephropathy. A thoroughly discussion of these factors is beyond the scope of this thesis.

8. PROGRESSION OF DIABETIC NEPHROPATHY

Once diabetic nephropathy has evolved and macroalbuminuria is persistent, arterial blood pressure increases, and an unremitting mean GFR decline is observed if no antihypertensive treatment is commenced (49-51). However, the beneficial impact of antihypertensive treatment on progression of diabetic nephropathy is now well established (11, 186-191). Despite a reduced variability and slower deterioration of renal function during antihypertensive treatment, the inter-individual rate of decline in GFR is highly variable ranging from nearly no loss in kidney function to a rapid decline (~20 ml/min/year) inexorably towards renal replacement therapy or death. Hypertension and proteinuria are well established progression promoters, i.e. risk factors for accelerated loss of kidney function (192), while the contribution of for instance glycaemic control and dyslipidaemia to the progressive decline in GFR, are still debatable. In order to create new powerful treatment modalities postponing or preventing the development of ESRD, assessment of progression promoters is of vast importance. Several important questions have arisen: Which factors promote progression? Is there evidence of a certain threshold effect on progression of these variables? Can identification of progression promoters yield new information of the pathogenesis of the disease, and be an additional tool in predicting the course of the disease? To address these questions, in 1983, a prospective observational study was initiated, resulting in a consecutive cohort of 301 type 1 diabetic patients with diabetic nephropathy, who had serial measurements of GFR and at least 3 years follow up (28). In this population, the impact of several putative progression promoters on the rate of decline in GFR, and potential threshold effects of the variables, were evaluated. Subsequently, the impact of smoking on progression of diabetic nephropathy was assessed, analyzing data from the same cohort. The search for new potential progression promoters are discussed in section 8.2.

8.1 RISK FACTORS FOR PROGRESSION

8.1.1 Genetics

Along with the extensive investigation of genetic risk factors for development of diabetic kidney disease, an understanding of a genetic

influence on progression of established diabetic and non diabetic nephropathy has evolved. In a prospective follow-up study of 35 type 1 diabetic patients with diabetic nephropathy, a significant steeper decline in GFR was found in patients with the DD genotype of the ACE I/D polymorphism during antihypertensive treatment with an ACE inhibitor (193). Despite similar arterial blood pressure and albuminuria during follow-up, the patients with the DD genotype had a rate of decline in GFR of 5.7 vs. 2.6 ml/min/year in patients with the II/ID genotypes. In a three years study where patients and investigators were blinded to ACE genotypes, the effect of blocking of the angiotensin II receptor with losartan was investigated on decline in kidney function in a similar population (194). In contrast to the study using ACE inhibitors, treatment with losartan had similar beneficial renoprotective effects on progression of diabetic nephropathy in patients with ACE II and DD genotypes (194). Recently, we have demonstrated a deleterious impact of increasing number of D alleles on time to doubling of serum creatinine or ESRD during ACE inhibitor treatment, with a risk ratio of 1.81 (95% CI: 1.09 to 3.03) per additional D allele (195). Furthermore, a model of several so-called "bad alleles" within the RAS yielded further evidence of gene-gene interaction on renal endpoints. Although many non genetic factors may influence the effects of medications, there is now growing evidence for genetic factors, as well as the frequency of side effects, being responsible for individual responses to therapy. The concept of pharmacogenetics, defined as the study of the role of genetics in drug response is by no means a new idea (196). Inter-individual differences in response to medication are dependent on variability in pharmacokinetics, i.e. absorption of the drug, distribution within different compartments, metabolism and excretion, and differences in pharmacodynamics. It is estimated that genetics can account for 20 to 95 percent of variability in drug deposition and effects (197). As reviewed by Evans, there are now examples of inter-individual differences in drug responses due to sequence variants in genes encoding drug-metabolizing enzymes, drug transporters, or drug targets (198). In most cases however, interplay between several gene products are involved in the response to a certain drug, and the determinants of this complex drug response are polygenic (198). Genetic factors of importance for the progression of diabetic nephropathy could possibly differentiate between responders and non responders. One of the main objectives of pharmacogenetics is to establish a more individualized drug therapy, beyond the "one size fits all" approach (199). Unfortunately, DNA for genotyping was only available in a subset of the investigated patients in our studies. With increasing knowledge, genotyping of study participants may be routinely carried out as an extension of the usual baseline information in clinical studies to adjust for these as covariates, to reduce selection bias and increase the effect of randomized treatment. In the future, relevant testing for genetic variations may be used to individualize drug therapy and thereby select the best drug at the optimal dose for each individual patient.

The influence of genetic susceptibility on progression of diabetic nephropathy seems at present to be very complex, and is complicated by gene-gene and gene-environment interactions. To assess progression of nephropathy, new large scale prospective studies are needed. Information from the new techniques with high throughput genotyping may help to shed light on this important issue. Identification of specific genotypes, or more likely a combination of these, that predispose to a rapid decline in GFR may help to target early aggressive intervention of modifiable risk factors in susceptible patients.

8.1.2 Blood pressure

Increased intraglomerular capillary hydraulic pressure plays a major role in the development of diabetic nephropathy. Does the increased intraglomerular pressure only initiate glomerulopathy as a necessary initial hit, or continuously enhance the deterioration of kidney function during the disease? The importance of intraglomerular pressure on progression of glomerulopathy was emphasized when prevention of progression was achieved by reducing the systemic and intraglomerular pressure in diabetic animals (200). Long-term treatment with an ACE inhibitor kept intraglomerular pressure normal and prevented a rise in albuminuria as opposed to conventional antihypertensive treatment, where progression in urinary albumin excretion rate was only postponed.

In one of the studies of the natural history of diabetic nephropathy, arterial blood pressure at the end of follow-up was correlated to the preceding decline in GFR before antihypertensive treatment was commenced (49). In an observational study in type 1 diabetic patients with diabetic nephropathy on antihypertensive treatment, Rossing et al found arterial blood pressure to be correlated to the decline in GFR (192), and a retrospective analysis of 58 type 1 diabetic patients with nephropathy reported an independent role of arterial blood pressure on decline in kidney function (201). We evaluated progression promoters in 301 consecutive type 1 diabetic patients with diabetic nephropathy, using annual measurement of plasma clearance of ^{51}Cr-EDTA. In our long-term prospective observational study with 7 years of follow-up, we confirm and extend the observations that arterial blood pressure is a progression promoter independent of albuminuria, glycaemic control expressed as HbA_{1c}, and serum cholesterol (28). The relationship between arterial blood pressure and diabetic nephropathy seems to be rather complex. Elevated blood pressure play a major role in the initiation and acceleration of progression of kidney disease, and nephropathy is closely associated with the increase in blood pressure observed in these patients. Impaired autoregulation in long-standing diabetes may aggravate the intraglomerular hypertension and thereby promote progression, as systemic blood pressure can be transmitted without restraint downstream to the glomerulus (202).

Antihypertensive treatment

Arterial hypertension is indeed very prevalent in patients with diabetic nephropathy (157), and antihypertensive treatment was applied in 90% of our patients. The initial studies of the effect of antihypertensive treatment in patients with overt nephropathy clearly demonstrated a beneficial effect of antihypertensive treatment with diuretics and beta blockers on decline in GFR, albuminuria and of course arterial blood pressure (11, 186, 187). These studies were however neither randomized nor placebo controlled, as patients served as their own controls. The evidence of a renoprotective effect of aggressive antihypertensive treatment in patients with diabetic nephropathy is now overwhelming. Apart from their blood pressure lowering effects, the majority of the antihypertensive agents have also the ability to impose a beneficial impact on the level of albuminuria. Not only have treatment with antihypertensive agents been shown to lower the level of albuminuria. A reduction in the rate of decline in GFR, postponed progression to ESRD, and improved survival in type 1 diabetic patients suffering from diabetic nephropathy have also been demonstrated (11, 186-191, 203, 204). Among all 301 patients, we found a much slower mean rate of decline in GFR of 4 ml/min/year as opposed to studies performed before the use of antihypertensive treatment, **Table 2**.

The question is no longer if – but rather how – to treat the diabetic patients with antihypertensive therapy. The first studies using ACE inhibitors in type 1 diabetic patients, showed that GFR is not dependent of angiotensin II, furthermore albuminuria was reduced (205, 206). The studies were however self-controlled, non randomized studies. In a randomized trial, Björck et al soon after demonstrated a renoprotective effect above and beyond the effect of lowering blood pressure of the ACE inhibitor enalapril in type 1 diabetic patients. Enalapril did not only reduce albuminuria, the rate of decline in GFR was significantly lower in the ACE inhibitor treated group as compared to patients treated with metoprolol during 2.2 years of follow-up (189). These observations were confirmed and extended by the Collaborative Study Group of Angiotensin Convert-

Table 2. Observational studies and clinical trials on progression of diabetic nephropathy in type 1 diabetic patients with and without antihypertensive treatment.

Authors	Study Design	n	Follow-up (years)	ΔGFR (ml/min/year)			Mean arterial blood pressure (mmHg)		
				No AHT	Non ACE-I	ACE-I	No AHT	Non ACE-I	ACE-I
Mogensen et al (49)	Observational, retrospective	10	2.8	10.9	–	–	121[a]	–	–
Parving et al (50)	Observational, prospective	14	2.2	9.0	–	–	111	–	–
Viberti et al (51)	Observational, retrospective	13	2.5	14.4	–	–	109[b]	–	–
Björck et al (189)	Randomized trial	40	2.2	–	5.6	2.0	–	103	102
Lewis et al (190)	Randomized trial	409	3.0	–	13.4[c]	9.2[c]	–	100	96
Elving et al (191)	Randomized trial	29	2.0	–	3.7	4.9	–	101[d]	100[d]
Tarnow et al (226)	Randomized trial	48	4.0	–	5.5	6.5	–	103	100
Mulec et al (294)	Observational, retrospective	158	8.0	–	3.8[e]		–	102[e]	
Hovind et al (28)	Observational, prospective	301	6.7	1.9	3.8	4.5	95	102	103

Glomerular filtration rate (GFR) determination was based on renal or plasma clearance of filtration markers. Blood pressure measurements were based on mean of values during follow up. a) MABP at end of the study, b) MABP at entry to the study. c) Cr-Clearance, decline in GFR calculated from percentage decrease per year from baseline clearance. d) MABP during last 6 months of follow up. e) Antihypertensive treatment varied throughout the follow up period.

ing Enzyme inhibition with Captopril in Diabetic Nephropathy, where a risk reduction was obtained in the captopril treated patients for doubling of serum creatinine of 48% (95% CI: 16 to 69%) (190). The beneficial effects of inhibition of the RAS has recently been demonstrated in patients with type 2 diabetes and nephropathy as well (207, 208), and blockade of the RAS is now recommended in all diabetic patients with micro- and macroalbuminuria irrespective of blood pressure (209). As albuminuria is an independent progression promoter during antihypertensive treatment, and the reduction in albuminuria after commencement of antihypertensive treatment at present is the best clinical guideline predicting a beneficial effect on the long-term decline in kidney function (22, 23), therapy should be titrated against optimal reduction in arterial blood pressure as well as reduction in albuminuria. In animal studies, the use of supra maximal doses of ACE inhibitors have additional effects on renal structural lesions (210, 211). Furthermore, the optimal dose for blood pressure response and renal effects may differ as demonstrated during treatment with an angiotensin II receptor antagonist in type 2 diabetic patients by Rossing et al (212). The optimal renoprotective doses of ACE inhibitors have not been studied in type 1 diabetic patients with diabetic nephropathy. Even though blockade of the RAS reduces albuminuria, many patients will have inadequately controlled blood pressure. The major focus in these patients is a reduction in arterial blood pressure which may require additional antihypertensive treatment.

In cardiovascular disease, the controversy of the existence of a J-shaped relationship between blood pressure and cardiovascular mortality has been extensively debated. Increasing mortality from coronary ischemia as blood pressure is reduced below some critical level that is necessary to preserve perfusion of the myocardium has been suspected, assessed but not confirmed in a clinical trial (213). The aggressive approach towards reduction of arterial blood pressure in diabetic patients with nephropathy has raised similar considerations. In our prospective observational study of 301 type 1 diabetic patients with diabetic nephropathy, we found a threshold for the association between the level of albuminuria and the rate of decline in GFR, while no threshold for the level of arterial blood pressure was demonstrated (28). In nine intervention studies in patients with diabetic and non diabetic renal disease, the relationship between attained arterial blood pressure and the rate of decline in GFR was found to be linear with no apparent J-curve (214). This suggest that aggressive antihypertensive treatment in terms of renoprotection should aim at "the lower the better", as no lower threshold for a beneficial effect of lowering blood pressure on deterioration in kidney function is evident.

8.1.3 Albuminuria

As previously discussed, the initial hit in progressive kidney disease is suggested to be intraglomerular hypertension leading to altered permselective properties of the glomerulus (34). The mechanisms responsible for the abnormal urinary excretion of proteins in glomerular diseases are first of all a loss of charge and size selectivity of the glomerular capillary wall, leading to the transglomerular passage of albumin and other high molecular weight proteins, that usually do not cross the glomerular barrier (47, 215). Due to the increased workload and/or the toxic effect derived from the increased load of the abnormally amount of filtered proteins in the tubular lumen, the reabsorption will subsequently be impaired leading to augmented albuminuria (215). The quantity as well as the molecular weight and radius of the proteins that reach the tubular lumen increase progressively with increasing severity of the disruption of the glomerular capillary wall. Whether proteinuria is a simple marker of the extent of the glomerular damage or may by itself promote progression of glomerulosclerosis has not been fully clarified. However, animal studies have found that proteinuria may precede the structural glomerular epithelial cell changes (216). Other experimental studies have provided evidence for pathways where increased protein traffic across the glomerular capillary wall may promote glomerulosclerosis (217). Recently, it has been suggested that proteins are degraded during renal passage and excreted in protein fragments even in healthy persons (218). A contributing factor to increased levels of intact albumin in urine observed in kidney diseases may therefore be promoted by metabolic events that affect the degradation of proteins during the passage through the kidney, a cellular defect distal to the glomerular basement membrane. These observations needs to be confirmed, and thorough evaluation of the clinical relevance are required. At present, it is not known whether some proteins or protein fragments are more harmful than others, but future analyses of urine samples of proteins and their degradation products – so called proteomic analyses – may shed light on this matter.

Watkins originally demonstrated that diabetic patients with the highest level of albuminuria have the poorest prognosis (12).

In non-diabetic kidney disease, the MDRD study evaluated target blood pressure for renoprotection in relation to the level of albuminuria (219). It was demonstrated that the severity of proteinuria determined the optimal blood pressure target for renoprotection. In patients with 1 to 3 grams of proteinuria per day a target of mean arterial blood pressure of 98 mm Hg was suggested, whereas additional benefit in patients with >3 grams proteinuria per day of lowering mean arterial blood pressure to 92 mm Hg was found (219). Differentiated targets for antihypertensive treatment based on the level of albuminuria is now reflected in some (220), but not all (221-223) of the latest guidelines on treatment of hypertension. The renoprotective effect of lowering of albuminuria was confirmed in the Ramipril Efficacy In Nephropathy trial in non diabetic kidney disease (224). In diabetic nephropathy, intervention that has ameliorated the progression of diabetic nephropathy has always been associated with a reduction in proteinuria in type 1 diabetes (74, 186, 187, 189, 225, 226). We have confirmed and extended these observations in our study (28). Dividing the 301 patients in quin-

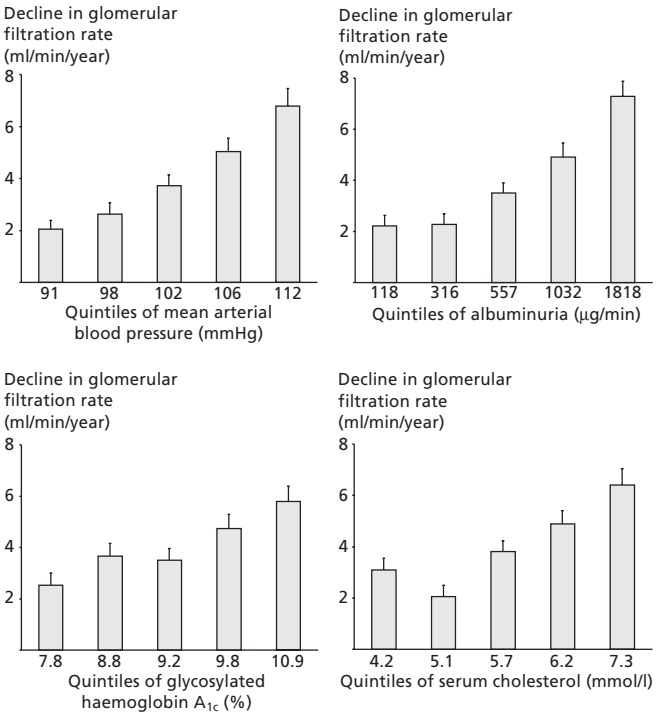

Figure 2. Impact of mean arterial blood pressure, glycosylated haemoglobin A_{1c}, albuminuria and serum cholesterol on the decline in glomerular filtration rate, P<0.001 for each factor. Copyright© 2001 Kidney International.

tiles based on independent progression promoters revealed a linear relationship between rate of decline in GFR and arterial blood pressure, HbA_{1c}, and serum cholesterol (**Figure 2**). However, the correlation between rate of decline in GFR and albuminuria seemed to be curve linear. A threshold for albuminuria on the rate of decline in GFR was found at 436 µg/min, p<0.01 (approximately 600 mg/24 h), whereas none of the other progression promoters showed a significant threshold (28).

As we used mean of all values of albuminuria during follow-up, it is however not possible to evaluate whether patients with low level of albuminuria per se have a slow deterioration in kidney function in our study population or that a reduction in albuminuria in fact will lead to an improved prognosis. However, it has previously been demonstrated that the initial reduction in albuminuria is a useful clinical guideline predicting a beneficial effect of antihypertensive treatment on the long-term decline in kidney function in diabetic and non-diabetic nephropathies (22-24).

Our findings in type 1 diabetic patients are in accordance with the concept of deleterious effects of increased protein trafficking through the mesangium. However, the beneficial outcome in patients with lower levels of albuminuria might well be explained by improvement in the filtration barrier or lowering of the intracapillary hydraulic pressure. The effect of a reduction in albuminuria during follow-up has been analyzed thoroughly in three of our subsequent studies and will be discussed in section 9.

8.1.4 Metabolic alterations

Hyperglycaemia

Hyperglycaemia plays a crucial role in the development of diabetic kidney disease, and strict glycaemic control has been demonstrated to prevent or postpone the onset of overt nephropathy. When diabetic nephropathy has evolved, and kidney function begins to decline, there has been a general belief that the degree of glycaemic control had little or no impact on the progression to ESRD. However, this assumption is based on studies on very small study populations, some with inappropriate methods for measuring kidney function (using serum creatinine), or glycaemic load over time (using blood glucose measurements in stead of HbA_{1c}), and importantly a limited duration of follow-up (3, 227-230). It has furthermore been the common experience that improvement in glycaemic control can be hard to obtain in type 1 diabetic patients with overt diabetic nephropathy, as many of the patients have reduced or even abolished awareness of hypoglycaemia due to autonomic neuropathy. The concept of "a point of no return" has been challenged by several observational studies. Nyberg et al found a correlation between the rate of decline in GFR and HbA_{1c} in 18 hypertensive type 1 diabetic patients followed for 18 months (231). During 10 years of follow-up of the same number of patients from the Steno Diabetes Center, Parving et al found that the variation in HbA_{1c} and arterial blood pressure explained two thirds of the variation in the rate of decline in GFR in patients treated with ACE inhibitors (232). In our long-term study we confirm the association between rate of decline in GFR and glycaemic control, as we identified HbA_{1c} to be an independent progression promoter (28). However, whether improvement in glycaemic control may merit a diminished rate of decline in GFR is not known, since long-term studies of sufficient size does not exist. Recent data from the EDIC study shows a long-term beneficial effect of strict glycaemic control on development of microvascular complications (69), what may be referred to as glycaemic memory. This glycaemic memory may work both ways. An assumption supported by the fact that pancreas transplantation can reverse the lesions of diabetic glomerulopathy in patients with type 1 diabetes, but reversal requires more than five years of normoglycaemia (233).

The achieved levels of glycaemia in this unique study can hardly be accomplished in all type 1 diabetic patients with nephropathy, as they often suffer from several diabetic late complications including autonomic neuropathy, and not seldom have reduced or totally loss of hypoglycaemic awareness. In conclusion, the level of glycaemic control is clearly associated with the rate of decline in GFR, and the renal structural lesions can be reversed by improvement in the level of glycaemia. Strict glycaemic control has impact on development of microvascular complications. In contrast, the effect of strict glycaemic control on progression of overt diabetic nephropathy has not been adequately evaluated in long term clinical trials. Whether this ever will be done is however questionable.

Dyslipidaemia

Hyperlipidaemia has been suggested to be involved in the initiation and progression of diabetic nephropathy. Once the glomerular capillary wall have been damaged by increased intraglomerular pressure and/or hyperglycaemia, the passage of lipids and lipoproteins into the mesangium could be feasible as proposed by Moorhead et al (234). The increased flux of lipoproteins in a state of hyperlipidaemia could thereby promote progression in chronic renal diseases, in a process comparable to atherosclerosis with formation of foam cells from macrophages. The damaging effect of oxidized lipoproteins, and the increased intraglomerular pressure through release of renal eichosanoids might also contribute. In our study, elevated serum cholesterol act as an independent progression promoter in diabetic nephropathy (28). Dyslipidaemia is a common feature in patients with albuminuria, and reduction in albuminuria is assocated with a decrease in plasma lipids. In animal studies, lowering of plasma lipids reduces intraglomerular pressure, albuminuria, and development of glomerulosclerosis (134, 235). A role independent of the lipid lowering effects of statins (3-hydroxy-3-methylglutaryl coenzyme A inhibitors) on progressive renal disease via effects on prosclerotic cytokines has been suggested in rats (236). In humans, several short-term studies dealing with a limited number of diabetic patients have investigated the potential renoprotective effect of treatment with statins as reviewed by Jandeleit-Dahm (236). Most studies demonstrates no or only a minimal effect on proteinuria over weeks to months. Neither have a beneficial effect of lipid lowering treatment on rate of decline in GFR been demontrated (237, 238). A meta-analysis of 13 smaller studies, hereof more than half

conducted in diabetic patients, have evaluated the effect of lipid lowering treatment mainly with statins on progression of renal disease in 362 patients (239). While no effect on albuminuria was found, a beneficial effect of lipid lowering treatment was found on rate of decline in GFR. Treatment effect on rate of decline in GFR: 0.156 ml/min/month (95% CI: 0.026 to 0.285), p=0.008 (239). Even though being a meta-analysis, the number of studies and patients under study was relatively small, the reasons for renal impairment heterogeneous and different methods for measurement of GFR and albuminuria were applied. The Heart Protection study comprising 5963 diabetes patients (10% with type 1 diabetes) recently reported that allocation to simvastatin was associated with a significantly smaller increase in serum creatinine during follow-up, reflecting a beneficial effect on renal function (240). No information regarding the effect on urinary albumin excretion rate is yet available. Patients with diabetic nephropathy have a markedly increased risk of cardiovascular disease, and lipid lowering treatment have been demonstrated to reduce the risk of cardiovascular events and death in patients with diabetes and cardiovascular disease (241). In addition, patients with diabetes assigned to simvastatin in the Heart Protection Study, had a 22% reduction (95% CI: 13 to 30) in the event rate of major cardiovascular disease. The reduction in risk was similar across all LDL sub categories (240). A post hoc analysis of the Cholesterol and Recurrenct Event study (CARE) have demonstrated that statin treatment reduces cardiovascular morbidity in patients with chronic renal insufficiency (242). Thus, the clustering of atherogenic risk factors in patients with diabetes and renal disease reinforces the need of a very aggressive approach towards therapy, including lipid lowering agents.

In conclusion, the role of lipid lowering treatment as renoprotective agents remains controversial. Long-term trials using pharmacological lipid lowering treatment and applying a principal endpoint such as decline in GFR rate are non-existing. Such a trial would indeed cause critical ethical considerations, since diabetic patients with renal disease have such a high risk of cardiovascular disease that lipid lowering treatment in this population must be considered mandatory. A recent positional statement from the American Diabetes Association imposes treatment with hypolipidaemic drugs if LDL cholesterol is >130 mg/dl (~3.4 mmol/l) in the absence of vascular complications, and in presence of vascular complications, treatment should be initiated at LDL values >100 mg/dl (~2.6 mmol/l) (243).

8.1.5 Protein intake

In our study of progression of diabetic nephropathy, the patients had a diabetic diet containing 45-55% carbohydrates, 30-35% fat, and 15-20% protein, and no sodium or protein restrictions were applied during the study (28). The protein intake was not assessed in our study, but other observational studies in type 1 diabetic patients with diabetic nephropathy have not found dietary protein intake to be associated with the rate of decline in GFR (232, 244). Protein restriction in diabetic rats leads to normalisation of intraglomerular hydraulic pressure, prevents development of albuminuria, and glomerular damage normally observed in these animals1 (46, 245). The beneficial effects of a low protein diet found in diabetic animal models, have not been reproduced convincingly in humans with various kidney diseases. During 2.2 years of follow-up in the MDRD study of predominantly non diabetic kidney diseases including 585 patients with baseline GFR between 25 and 55 ml/min/year/1.73 m^2, the mean rate of decline in GFR was 1.2 ml/min/year lower in the low protein diet group as compared to the normal protein diet group (p=0.30) (246). In a meta-analysis by Pedrini et al, several smaller studies including type 1 diabetic patients were summarized (247). This meta-analysis demonstrated that a low protein diet significantly slowed the increase in urinary albumin level or the decline in GFR or creatinine clearance (247). However, due to dubious designs, methodology, and insufficient adjustment of other progression promoters in the five studies including 108 type 1 diabetic patients included in the meta-analysis, the results should be interpreted with caution. In a four year prospective controlled trial of 82 type 1 diabetic patients with progressive diabetic nephropathy assigned to either low or normal protein diet, the rate of decline in GFR was reduced significantly from pre study progression rates in both the low and the normal protein diet (248). No difference in the rate of decline in GFR between groups during the study (3.8 vs. 3.9 ml/min/year, NS) was found, and albuminuria remained stable in both groups which might be ascribed to the modest difference in protein intake obtained during follow-up: 0.89 (95% CI: 0.83 to 0.95) in the low protein diet group vs. 1.02 (95% CI 0.95 to 1.10) g/kg/24 h in the normal protein diet group, p=0.005 (248). A relative risk of the combined endpoint of ESRD or death was found to be 0.23 (95% CI: 0.07 to 0.72), p=0.01 in patients in the low protein diet group as compared to the normal protein diet group (248). Even though the mechanisms for this favourable outcome of protein restriction is yet unknown, similar findings have been reported in non diabetic nephropathies (249). In conclusion, low protein diet is associated with improved survival free of ESRD in patients with diabetic nephropathy.

8.1.6 Smoking

Several prospective observational studies have found an association between smoking tobacco and progression from normoalbuminuria to microalbuminuria and overt nephropathy (64, 66, 81-83). The mechanisms for the nephrotoxic influence of smoking on the kidneys are not well understood. The smoking induced renal damage have been suggested to be caused by sympathetic activation influencing systemic arterial blood pressure and renal haemodymics, endothelial dysfunction due to reduced nitric oxide availability, and diminished vasodilatation. Activation of the RAS through induction of increased renin production stimulated by activation of the sympathetic nervous system and release of catecholamines have also been implied as one of the pathogenic mechanisms (250). Smoking of tobacco is a well established risk factor for cardiovascular, respiratory and cancer diseases, and the evaluation of smoking as a risk factor for progression of overt diabetic kidney disease seem somewhat academic, and without consequences in the counselling and the care of the diabetic patients in everyday life. However, when evaluating potential risk factors for progression of kidney disease in order to be able to explain the huge variability in the rate of decline in GFR among patients, evaluation of smoking as a progression promoter is highly relevant. Some prospective studies have suggested cigarette smoking to be a risk factor for progression of diabetic nephropathy. In a one year study of 93 type 1 diabetic patients, the impact of smoking on progression of kidney disease was assessed using creatinine clearance, and smoking was suggested to worsen kidney function (251). However, since arterial blood pressure (143/84 vs. 137/85 mm Hg) as well as the level of albuminuria (2547 vs. 1592 mg/24 h) was higher in smokers as compared with non smokers, a causal role can not be established. Others have found that type 1 diabetic patients with diabetic nephropathy smoking more than 10 cigarettes per day (n=8 patients), have a higher rate of decline in creatinine clearance than non smokers, 14.9 vs. 10.3 ml/min/year (252). Again, arterial blood pressure was significantly higher in smokers compared to non smokers, 159/89 vs. 141/82 mm Hg, and levels of albuminuria was not presented (252). In a study by Mühlhauser et al, only 26 patients (4% of the study population) had macroproteinuria or elevated serum creatinine at baseline and no separate analyses on progression of diabetic kidney disease in these patients was performed (66). The studies performed so far are characterized by inadequate methods for measuring rate of decline in GFR, short duration of follow-up in small number of patients, and insufficient adjustment for other well established progression promoters (66, 251, 252). Therefore, we analyzed data from our large long-term prospective observational study of 301 type 1 diabetic patients with dia-

betic nephropathy (253). In our study, we followed the patients for at least three years with annually GFR measurements with a valid method (253). Patients were classified as smokers, ex smokers and non smokers. Smokers being defined as patients smoking more than 1 cigarette per day during a part or the whole observation period. No difference in the rate of decline in GFR was demonstrated between non smokers (n=94): mean (95% CI) 4.5 (3.7 to 5.4), ex smokers (n=31): 3.1 (1.6 to 4.7), and smokers (n=176): 3.9 (3.3 to 4.5) ml/min/year respectively (NS) (253). During follow-up, the smokers had significantly lower blood pressure as compared to the non and ex smokers, while no difference in the level of albuminuria, HbA_{1c}, or serum cholesterol was apparent. Adjustment for the difference in arterial blood pressure among groups did not alter the results significantly, with rate of decline in GFR: 4.1 ml/min/year in non smokers, 3.1 ml/min/year in ex smokers, and 4.1 ml/min/year in the smoking group respectively, NS (**Figure 3**). The definition of smoking status varies between studies. Some use a cut-off at ten cigarettes per day (252), other have used the definition of pack years ([mean number of cigarettes smoked per day/20]×number of smoking years) (66, 251). Due to assessment of smoking habits using questionnaire with the possibility of underreporting, we used the most conservative definition of smoking: smoking more than 1 cigarette per day. However, we analyzed the impact of heavy smoking versus light smoking by dividing our patients according to the median number of cigarettes smoked per day (median number of cigarettes smoked was 20). We did not find a difference in rate of decline in GFR between heavy and light smokers (253). In conclusion, data from our long-term prospective observational cohort study do not support that deterioration in kidney function in type 1 diabetic patients suffering from diabetic nephropathy is associated with tobacco smoking. The relative mortality risk for smoking diabetic patients are substantially higher than for non smoking patients (254, 255). Even in the absence of convincing data supporting the concept that smoking accelerates renal disease progression in overt diabetic kidney disease in type 1 diabetes, cigarette smoking remains a hazard to health. The results from our study can by no means be taken in to account for a general advice to diabetic patients with overt nephropathy who currently smoke to carry on with this risky habit. Conversely, our data can indeed be used to urge for the continued search for new yet unknown progression promoters, as we at present are not able to explain all the variation in renal disease progression from the current knowledge.

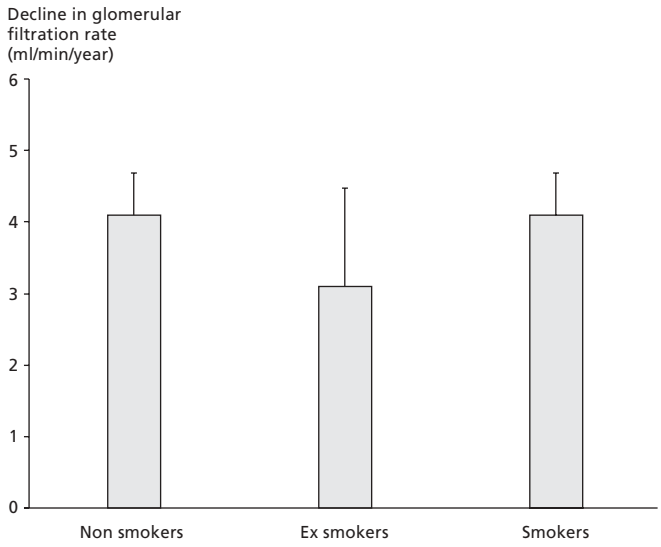

Figure 3. Impact of smoking status on the decline in glomerular filtration rate in 301 type 1 diabetic patients with diabetic nephropathy, adjusted for differences in blood pressure between groups, NS (ANOVA). Error bars represents 95% confidence intervals. Copyright © 2003 American Diabetes Association.

In summary, several potential modifiable risk factors for decline in kidney function have been identified. To illustrate the impact of a combination of risk factors for loss of renal function, a two hit model with mean arterial blood pressure (an established modifiable progression promoter) combined with albuminuria, HbA_{1c}, and serum cholesterol is shown in **Figure 4**.

Patients with diabetic nephropathy encounter a continuing loss of renal function and have in addition an increasing risk of cardiovascular disease. The main risk factors for micro- as well as macrovascular disease are closely interrelated in the diabetic patients. For some treatment modalities the evidence for a renoprotective effect is lacking, whereas the effect on macrovascular disease is beyond doubt. Clearly, to improve the prognosis in the diabetic patients with nephropathy, the treatment and care of the patients must deal with all aspects of the disease.

8.2 NEW PREDICTORS OF PROGRESSION OF DIABETIC NEPHROPATHY

The rate of loss in renal function is extremely variable once overt diabetic nephropathy has evolved. Information of the impact of progression promoters during the course of the disease is essential in

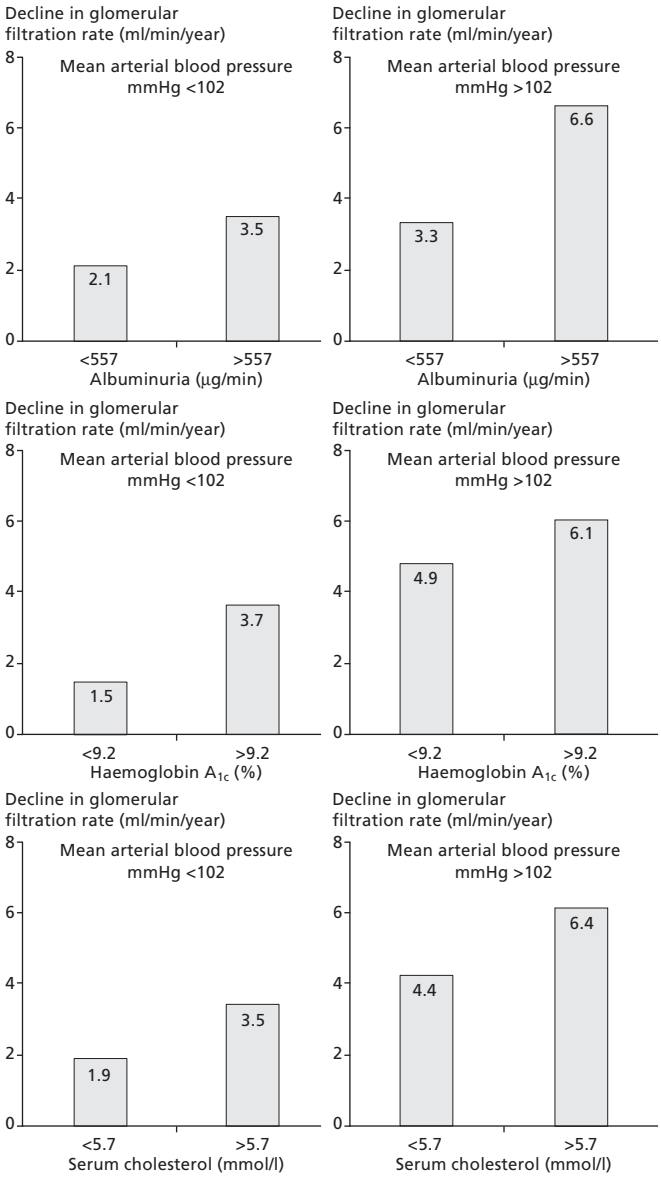

Figure 4. The decline in glomerular filtration rate divided according to median of mean arterial blood pressure combined with median albuminuria, glycosylated haemoglobin A_{1c}, and serum cholesterol. Copyright © 2001 Kidney International.

every day treatment of the patients to postpone, or even prevent, the progression to ESRD. In a study of 18 type 1 diabetic patients with diabetic nephropathy followed for 10 years and treated with ACE inhibitors, two thirds (66%) of the variation of the decline in kidney function was explained by albuminuria and HbA_{1c} during follow-up (232). In the Captopril Collaborative study with a median follow-up time of three years including 409 type 1 diabetic patients with diabetic nephropathy, baseline progression promoters were evaluated (256). Onset of diabetes later in life, parental diagnosis of type 1 diabetes, abnormal electrocardiogram, elevated mean arterial blood pressure, high blood glucose, increased serum creatinine, enhanced proteinuria and presence of oedema were found to predict doubling of serum creatinine independently (256). In our study of 301 type 1 diabetic patients with kidney disease, we were only able to explain 29% of the variation of the rate of decline in GFR by the impact of arterial hypertension, albuminuria, glycaemic control, and dyslipidaemia (28). Inclusion of genetic risk markers from the RAS as risk factors for decline in renal function in the statistical analysis in 169 type 1 diabetic patients with diabetic nephropathy, treated with ACE inhibitors and followed for 6 years, did not improve the degree of explanation of the variation considerably (195). In the search for new potentially modifiable risk factors for progression of diabetic nephropathy, we have analyzed the impact of plasma VEGF, plasma total homocysteine (tHcy), and plasminogen activator inhibitor-1 (PAI-1), measured at a defined baseline (180, 257). Of the 199 type 1 diabetic patients enrolled in the Steno case-control study, 157 were followed for at least 3 years with yearly GFR measurements from baseline examination in 1993 until 2001. The three years of follow-up was required to be able to make a valid determination of the rate of decline in GFR in the individual patient. Vascular endothelial growth factor has been suggested to play a role in development and progression of diabetic microangiopathy, and it was therefore an apparent candidate as a risk factor for nephropathy progression. Vascular endothelial growth factor was found to be elevated in men with nephropathy, but quite disappointing, no correlation was found between VEGF and the rate of decline in GFR (180). Hyperhomocysteinaemia is an independent risk factor for atherosclerosis, and evidence is emerging that homocysteine induces endothelial dysfunction by promoting oxidative damage (258). Since there are pathophysiological similarities between glomerulosclerosis and atherosclerosis, risk factors for these processes may be identical. Plasma tHcy has been positively correlated to the level of albuminuria in both type 1 and type 2 diabetes (259-262), although an independent association has been questioned (263). Homocysteine becomes elevated when kidney function is impaired, however it remains unclear whether the elevated tHcy is due to impaired metabolism or to reduced excretion in these patients (258). In non diabetic rats, elevated tHcy has been implicated in the pathogenesis of glomerular damage independent of arterial blood pressure (264). In our study of progression of diabetic nephropathy, a borderline significant correlation between tHcy and the rate of decline in GFR was demonstrated (257). A significant association between tHcy and decline in GFR was found when analyzing tHcy divided in tertiles, suggesting a non-linear relationship between tHcy and the outcome (257). However, tHcy was not found to be an independent predictor of the rate of decline in GFR (257). In accordance with our findings, studies in non diabetic nephropathies have failed to establish an association between the level of tHcy and decline in kidney function (265, 266).

Plasminogen activator inhibitor-1 has a critical inhibitory role in the regulation of intravascular fibrinolysis in addition to involvement in tissue repair and remodelling, and is directly induced by angiotensin II (267). A shift in the delicate balance of proteases and their inhibitors occurs during matrix expansion in the kidneys, where PAI-1 is suggested to be one of several modulators. In an animal study by Fogo et al, mRNA overexpression of PAI-1 was found to be localized to injured mesangial and endothelial areas, levels of PAI-1 were increased in sclerotic glomeruli, and the upregulation of PAI-1 was inhibited by treatment with drugs blocking the RAS (268). In type 1 diabetes, we have previously demonstrated that plasma PAI-1 is elevated in men with diabetic nephropathy as compared with normoalbuminuric diabetic men (269). However, no association between PAI-1 and decline in kidney function was established, and neither tHcy nor PAI-1 were however demonstrated to be independent predictors of decline in GFR (257). Thus emphasizing the interrelationship between many risk factors often confounds the relative significance of individual risk factors, and that the "classical" well established risk factors are relatively powerful as such, and always must be taken into account when evaluating new risk factors for nephropathy progression. The lack of evidence in diabetic patients for plasma tHcy or PAI-1 levels as predictors of progression of renal disease can have several explanations. Firstly, it is not clear whether circulating markers, in our study tHcy and PAI-1 levels, in fact does reflect the local expression within the kidney. Secondly, in our study the majority of patients receiving antihypertensive treatment were treated with ACE inhibitors, which may inflect on the level of PAI-1. Furthermore, it is possible that PAI-1 expression play a major role in the initiation of diabetic glomerulosclerosis, whereas the effect on progression of overt diabetic kidney disease could be more modest. Lastly, it must be recognized that results obtained from in vitro studies or animal models can not always be reproduced in man.

Meanwhile, the search for new powerful predictors of progression of diabetic kidney disease continues. Anaemia is frequently observed when renal function deteriorates, and have recently been suggested to be involved in the progressive decline in GFR in type 2 diabetic patients (270). Furthermore, the role of inflammation (271), asymmetrical dimethylarginine which is an endogenous inhibitor of nitric oxide synthase (272), elevated uric acid (273), and increasing levels of aldosterone during RAS blockade (Abstract: Schjoedt et al, Journal of the American Society of Nephrology, Vol. 14, page 7A, 2003) has been brought in to focus.

9. REMISSION AND REGRESSION

Based upon studies in type 1 diabetic patients conducted before treatment with antihypertensive agents, diabetic nephropathy was considered to be a rapidly progressing, irreversible disease (49-51). We now know, that with the available treatment, the progressive decline in GFR can be reduced (28), and ESRD can thereby be postponed in many patients with diabetic nephropathy. However, for a subset of patients, diabetic kidney disease is still an irreversible and inexorable progressive disease. In those who progress to overt nephropathy, the optimal goal is remission of disease and regression of structural and functional abnormalities.

9.1 REGRESSION OF MICROALBUMINURIA TO NORMOALBUMINURIA

In order to prevent overt diabetic nephropathy, investigators have searched for early predictors for development of the disease. During the early 1980s, urinary albumin excretion rate below the range detectable by standard laboratory methods was introduced as an early and robust predictor of subsequent risk for the development of overt diabetic nephropathy. In a consensus report, the term microalbuminuria was defined as urinary albumin excretion rate ranging from 30 to 300 mg/24 h, equal to 20 to 200 μg/min (8). A suitable predictor for development of diabetic nephropathy should be identifiable early enough in the disease process for timely and effective intervention to be applied. Furthermore, those subjects at high risk for development of renal complications should be identified. In type 1 diabetic patients, the level of microalbuminuria, and the risk of development of overt nephropathy, can be modified by strict glycaemic control and the use of ACE inhibitors (45, 91).

In the initial observational studies, development of overt nephropathy in microalbuminuric type 1 diabetic patients was found to

occur in approximately 80% of the patients during 6 to 14 years of follow-up (41-43). However, the validity of microalbuminuria as a predictor for development of macroalbuminuria has recently been questioned, and regression from microalbuminuria to normoalbuminuria suggested to be a frequent phenomenon (274-276).

In a review, Caramori (274) has estimated the rate of progression from microalbuminuria to overt nephropathy to be 30% during the following 5 to 10 years. However, among several inclusion criteria for studies to be included in this review, a follow-up time from baseline examination of more than five years in the studies were required. Thus, many randomized trials lasting from 2 to 4 years were excluded, introducing selection bias (274). Except for one trial (277), the rate of progression to macroalbuminuria in the placebo groups in the excluded trials were considerably higher, ranging from 5.5 to 13%/year (70-72, 162, 278, 279). Despite the use of ACE inhibitors as secondary prevention strategy in the majority of the microalbuminuric patients in our inception cohort, nearly half of the patients progressed further from microalbuminuria to overt nephropathy during 7.5 years (89), a rate approaching that of the short term trials.

Spontaneous regression of microalbuminuria, i.e. without secondary intervention aiming at reducing the level of urinary albumin excretion rate, has recently been reported to occur frequently in type 1 diabetic patients (275, 276). A cumulative incidence of regression of microalbuminuria, defined as reduction of 50% or more in the urinary albumin excretion from one two-year period to the next, was reported to be 58% during 6 years of follow-up (276). Since the use of ACE inhibitors was not associated with regression to normoalbuminuria, the data suggests spontaneous normalization. This is based on a study of 386 type 1 diabetic patients with microalbuminuria followed for a median of 4.4 years with a drop out rate of 43% (276). Urinary albumin excretion rate was measured in random spot urine samples and recalculated to mg of albumin excreted per minute. In our inception cohort of type 1 diabetic patients followed from onset of diabetes, 79 patients developed microalbuminuria (89). The median follow-up time after onset of microalbuminuria was 7.5 years, the drop out rate was 14%, and data until last visit in the clinic were ascertained. To reduce the confounding influence of variability in urinary albumin excretion rate, we defined regression to normoalbuminuria from microalbuminuria as an urinary albumin excretion rate below 30 mg/24 h in two out of three consecutive 24 hour urine collections, and a decrease of at least 30% from the microalbuminuria level, sustained for at least one year (89.) Regression to normoalbuminuria occurred in 35% of the microalbuminuric patients, with more than half of these subsequently relapsing to persistent microalbuminuria. The 6 year cumulative incidence of regression was 31% (95% CI: 20-42) (89). Regression occurred in 28 patients of the 79 patients, and only 13% developed spontaneous permanent regression to normoalbuminuria, i.e. without antihypertensive treatment (89). None of the patients developing macroalbuminuria spontaneously regressed to microalbuminuria or normoalbuminuria. The difference in the proportion of patients who regress in the two studies may partly be explained by differences in design, as the study by Perkins et al (276) evaluated a prevalence cohort, introducing the possibility of inclusion, exclusion and survival bias. In conclusion, microalbuminuria is at present the best documented non invasive predictor of development of overt diabetic nephropathy in type 1 diabetes. The presence of microalbuminuria in most cases does imply an unalleviated progressive kidney affection.

9.2 REMISSION AND REGRESSION OF OVERT DIABETIC NEPHROPATHY

Implicit in the term ESRD is the understanding of a sequence of injuries on the kidney, starting with an initial insult, and ending with total loss of kidney function. The historical studies suggest that progression is inevitable, but major changes in the treatment of the patients have been implemented since then (49-51). Are the processes involved in the deterioration of kidney function in diabetic kidney disease strictly progressive or can the course of the disease be reversed? In spontaneously hypertensive 5/6 nephrectomized rats, tight blood pressure control decreased the apoptosis rate, mainly in tubular cells (280). In other animal studies using high doses of ACE inhibitors, remodelling of vascular sclerosis, tubulointerstitial fibrosis, and existing glomerulosclerosis is possible in chronic kidney diseases (210, 211). Thus regression of biopsy proven glomerulosclerosis can be achieved in animal models of chronic nephropathies. Could the same be the case in humans? In patients with non diabetic nephropathies, long-term ACE inhibition induced preservation and even improvement in GFR (281). When normalizing the diabetic milieu, reversal of morphological lesions of glomerulopathy can be achieved, as demonstrated by performing transplantation of kidneys with diabetic structural lesions in to non diabetic recipients with subsequent resolution of mesangial expansion (282). Furthermore, approximating normalization of blood glucose levels over a long period of time by pancreatic transplantation could reverse renal morphological lesions in a small number of patients with urinary albumin excretion ranging from normal to clearly abnormal levels (233). The latter two studies in humans must be regarded as casuistic and as a "proof of concept", as these treatment modalities are not widely applicable in everyday life. Systemic blood pressure as well as local intraglomerular hydraulic pressures are key factors in the vicious circle of progressive renal damage and progressive injury. However, many other components have been implicated in the initiation and progression of diabetic nephropathy, for instance proteinuria, glycaemic control, reactive oxygen species and cell-specific injury, growth factors and cytokines. To achieve regression of prevailing sclerosis, the dynamic control of cell proliferation, apoptosis and regeneration must be altered, and matrix degradation exceeds matrix synthesis. Theoretically, endothelial cells must regenerate, and mesangial cells and new capillary loops within the glomeruli must regrow. Finally, the shift in balance of proteases and their inhibitors occurring during matrix expansion must be reverted to induce reabsorption of excess matrix deposits. The data from experimental studies have shown that this is feasible in animal models, and casuistic reports in humans are available. We have evaluated the concept of remission and regression in our long-term prospective study of 301 type 1 diabetic patients suffering from diabetic nephropathy (283). Since no general accepted definitions for remission and regression of diabetic nephropathy yet had been established, we applied very conservative and strict criteria. Remission of diabetic nephropathy was defined as going towards normalization of the disease process: decrease in albuminuria to the microalbuminuric range. Regression was defined as normalization in the rate of decline in GFR: equal to or less than 1 ml/min/year during the entire observation period, equivalent to the natural ageing process in non-diabetic patients without kidney disease (25). When applying these definitions in our study, 31% obtained remission, and 22% achieved regression (283). In patients obtaining remission, the rate of decline in GFR was significantly lower (2.2 ml/min/year) as compared with the no remission group (4.8 ml/min/year), $p<0.001$. The association between prevalence of remission and arterial blood pressure is shown on **Figure 5**, and the association between prevalence of regression and arterial blood pressure is shown on **Figure 6**. Besides the expected lower rate of decline in GFR, mean arterial blood pressure, albuminuria, HbA_{1c}, and serum cholesterol during the observation period were lower in patients achieving regression of diabetic nephropathy. Arterial blood pressure, albuminuria, and HbA_{1c} were independently associated with regression: adjusted odds ratio [95% CI] for a 10 mm Hg decline in blood pressure: 2.14 (1.33-3.44), a tenfold lowering of albuminuria: 2.79 (1.35-5.69), and a reduction of 1% in HbA_{1c}: 2.00 (1.46-2.73). In the study population were 30 so-called genuine normotensive patients, i.e. normotensive patients not prescribed any antihypertensive medication. These patients had

a significantly lower rate of decline in GFR as compared to the hypertensive patients, 1.9±0.5 versus 4.3±0.3 ml/min/year respectively, p<0.01. We did not find any association between the use of ACE inhibitors and remission or regression of diabetic nephropathy. In clinical trials, an additional non haemodynamic renoprotective effect of ACE inhibitors has been demonstrated (189, 190). However, in observational studies such as ours, confounding by factors associated with both treatment and outcome can not be excluded (284). In addition, the dosing of ACE inhibitors was titrated on the basis of arterial blood pressure, since no optimal dosing for renoprotection of ACE inhibitors has yet been ascertained. In conclusion, aggressive antihypertensive treatment in type 1 diabetic patients can induce remission and regression in a sizeable fraction of patients with diabetic nephropathy. Several risk factors for progression of diabetic nephropathy also seem important for regression of diabetic nephropathy in type 1 diabetic patients. Thus, we have demonstrated that the functional changes can be reversed; however, kidney biopsies were unfortunately not available from our study population. In modern chemotherapeutic strategies aiming at eradication of malignancy, multiple agents are used to bring the patients "in remission". A similar comprehensive renoprotective approach in the treatment of diabetic nephropathy may be necessary to prevent patients from ever progressing to ESRD.

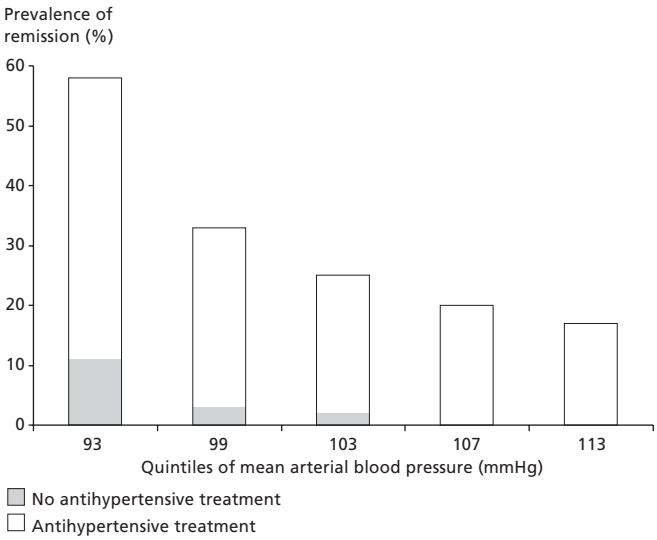

Figure 5. Prevalence of remission, defined as urinary albumin excretion rate < 200 μg/min sustained for at least one year during follow-up, in 301 type 1 diabetic patients with diabetic nephropathy. Copyright © 2001 Kidney International.

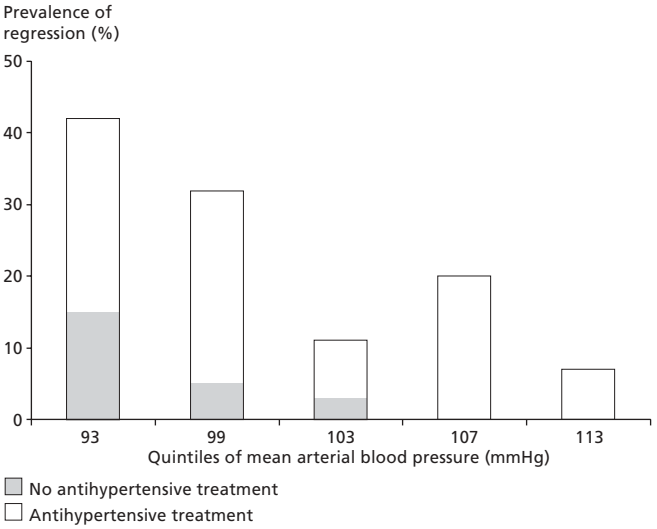

Figure 6. Prevalence of regression, defined as rate of decline in GFR≤1 ml/min/year, in 301 type 1 diabetic patients with diabetic nephropathy. Copyright © 2001 Kidney International.

9.3 REMISSION OF NEPHROTIC RANGE ALBUMINURIA

In 1972, Watkins et al (12) identified the subset of diabetic patients with proteinuria above 3000 mg/24 h to carry a very poor prognosis, as all these patients died between 2 to 6 years of follow-up (12). Albuminuria can therefore be used as an indicator of severity of disease, as patients with the highest level of albuminuria – nephrotic range albuminuria – are unfortunate with the poorest prognosis (5, 12, 13). We have confirmed this finding, as patients with the highest level of albuminuria had the steepest decline in kidney function in our study of progression of diabetic nephropathy (28). The beneficial effect of reduction in albuminuria during antihypertensive treatment on decline in kidney function in diabetic and non diabetic chronic kidney diseases (22-24), implies that reduction in albuminuria may change the prognosis, even in patients with nephrotic range albuminuria. The feasibility of strikingly reductions in proteinuria in diabetic patients with nephrotic range proteinuria was originally demonstrated by Hebert (14). Remission, defined as reduction of proteinuria from above 3500 mg/24 h to below 1000 mg/24 h, was demonstrated in 8 out of 108 patients with nephrotic range proteinuria participating in the Collaborative Study Group Multicenter Controlled Trial of Captopril Therapy in Patients with Type 1 Diabetes Mellitus and Nephropathy (14). A follow-up study of these patients demonstrated long-term remission of nephrotic range proteinuria with a remission rate of 16.7% in patients assigned to captopril treatment, and 1.5% in the patients treated with conventional antihypertensive treatment (285). To evaluate the cumulative incidence of nephrotic range albuminuria in a large population of type 1 diabetic patients, we extended our cohort of 301 type 1 diabetic patients with diabetic nephropathy to include 321 consecutive patients (286). The frequency of remission, and the impact of remission on rate of decline in GFR, was evaluated in the subset of patients with nephrotic range albuminuria. As ~40% of patients with diabetic nephropathy progressed to nephrotic range albuminuria (>2500 mg/24 h) during follow-up, the progressive nature of diabetic kidney disease was confirmed in our study (286). By applying comparable criteria (285) of remission of nephrotic range albuminuria – reduction of albuminuria from above 2500 mg/24 h to below 600 mg/24 h, we demonstrated a remission rate of 22% of nephrotic range albuminuria (286). Remission did not occur spontaneously, but was induced by antihypertensive treatment. Apart from this treatment modality, no other major target organ saving procedure was introduced during the follow-up period. The frequency of remission must, however, be regarded as conservative, since only 33% of the patients in the no remission group achieved a blood pressure below 140/90 mm Hg. As opposed to the initial findings (285), we did not find ACE inhibitors to be superior in inducing remission, although the majority of the patients obtaining remission were treated with ACE inhibitors in our study. As our study was not designed to evaluate effects of different drug regimens, no firm conclusion on this topic can be drawn from our results. Patients obtaining remission of nephrotic range albuminuria had a rate of decline in GFR during follow-up of ~50% of the patients not obtaining remission (3.8 vs. 7.5 ml/min/year, p<0.001). Furthermore, during the entire follow-up period, patients in the remission group had significantly lower arterial blood pressure and serum cholesterol (**Figure 7**). In those patients where the rate of decline in GFR could be assessed before and during remission of nephrotic range albuminuria, a significantly lower decline in GFR was found during remission. With the exception of gender, no differences in known risk factors for progression of kidney disease at onset of nephrotic range albuminuria was apparent (286). The various reasons for some patients responding better to treatment than others is of vast interest to improve the treatment and prognosis for all patients.

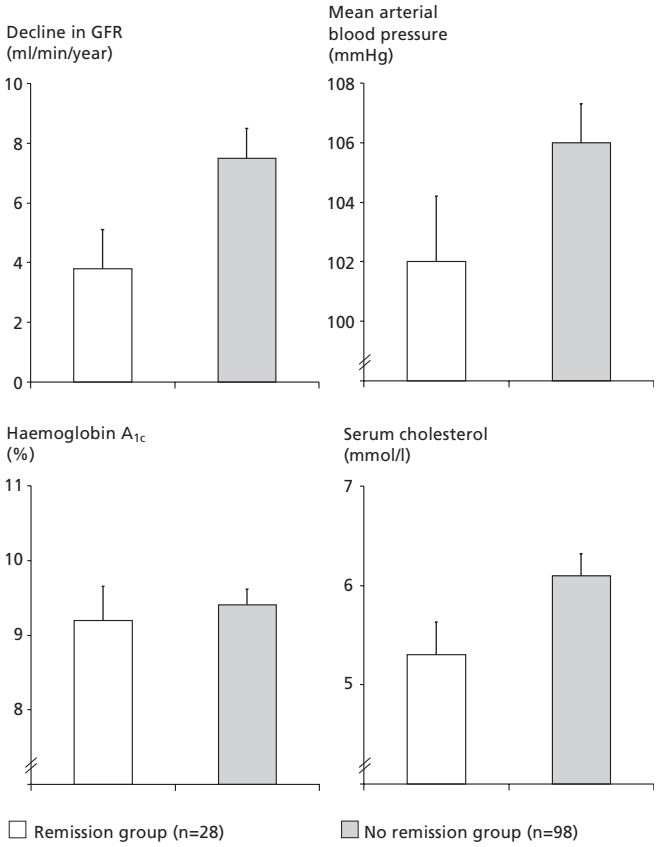

Figure 7. Decline in GFR, mean arterial blood pressure, glycosylated haemoglobin A_{1c} and serum cholesterol during follow-up in 126 type 1 diabetic patients with diabetic nephropathy – obtaining or not obtaining remission. Error bars represent 95% confidence intervals.
Statistical significant differences were found between groups in decline in GFR ($p<0.001$), mean arterial blood pressure ($p<0.01$), and serum cholesterol ($p<0.01$).

Numerous factors may be implicated, e.g. genetic factors, physiological as well as psychological patient related factors. Patient compliance with drug therapy has been recognized as one of the major reasons why antihypertensive therapy fails in the United States and elsewhere (287), and subsequently non compliers may be misclassified as non responders. The probability of poor compliance increases with increasing number of daily doses as well as number of different drugs. To control blood pressure in patients with diabetic nephropathy, antihypertensive treatment with several different antihypertensive agents is however often necessary. Poor adherence to antihypertensive treatment can be observed in patients with uncontrolled hypertension as well as in well controlled blood pressure (288), which implies that even at the same blood pressure level, non adherence to e.g. ACE inhibitor therapy will render the patients without the non haemodynamic beneficial effects of these compounds (189, 190). In conclusion, aggressive antihypertensive treatment can induce long lasting remission in a sizeable fraction of type 1 diabetic patients with nephrotic range albuminuria. The patients obtaining remission have a slow mean decline in GFR and improved cardiovascular risk profile.

9.4 REMISSION OF NEPHROTIC RANGE ALBUMINURIA AND IMPROVED SURVIVAL

Before the general use of antihypertensive treatment in patients with diabetic nephropathy, the disease was associated with an extremely high mortality, the average survival time from onset of proteinuria being only 5 to 7 years (2, 5). In studies where antihypertensive treatment was used randomly and infrequent, the mortality rate was 50 to 77% after 10 years with proteinuria (2, 3, 289). As compared to the background population, the relative mortality was ~40 times higher in type 1 diabetic patients with proteinuria, as opposed to only ~2 times higher in patients with normoalbuminuria (290). Historically, the high relative mortality in these patient was mainly due to ESRD, which was the cause of death in 66% of the patients (2). The remarkable reduction in the average rate of decline in GFR (11), has additionally been associated with improvement in median survival time, and accompanied by a change in the pattern in causes of death. Comparing two groups of type 1 diabetic patients with proteinuria without and with the use of antihypertensive treatment have showed that after introduction of this treatment modality, the 8 year mortality declined from 52% to 13% (291). In a prospective observational study including 263 patients with diabetic nephropathy, Rossing et al found a median survival time after onset of nephropathy of 14 years (6). The main causes of death were ESRD (35%), and cardiovascular disease (33%). The reduction in decline in kidney function is not surprising central in postponing time to ESRD, but a change in pattern of causes of death have also been observed. Antihypertensive treatment in these studies was primarily conventional in the sense of the use of agents other than ACE inhibitors. The Collaborative Study Group of Angiotensin Converting Enzyme inhibition with Captopril in Diabetic Nephropathy demonstrated a risk reduction in the captopril treated patients of the combined endpoint of death, dialysis or transplantation of 61% (95% CI: 26 to 80%) in patients with serum creatinine levels >133 µmol/l versus placebo. In patients with serum creatinine <133 µmol/l, the risk reduction was 46% (95% CI: –22 to 76%) (190). Albuminuria itself is associated with increased cardiovascular mortality (58), and the relative risk for cardiovascular death has recently been reported to be 2.97 in patients with proteinuria as compared to normoalbuminuric patients (6). Furthermore, a 10 fold in increase in urinary albumin excretion rate increased the risk for all cause mortality with 45% (6). Others have confirmed that higher levels of albuminuria is associated with increased mortality, as mortality ratios have been reported to be 1.5 (95% CI: 1.1 to 2.0) in type 1 diabetic patients with "light" albuminuria, and 2.9 (95% CI: 2.2 to 3.9) in patients with "heavy" proteinuria as compared to patients with no albuminuria (292). Thus, confirming a higher risk of mortality with increasing levels of albuminuria.

Even though the Food and Drug Administration in the USA have approved the rate of decline in GFR as an endpoint in kidney diseases (19), deterioration in kidney function may be regarded as a surrogate endpoint for the ultimate endpoint: ESRD in terms of dialysis or kidney transplantation. We have demonstrated that remission of nephrotic range albuminuria not only is feasible, it is also associated with a diminished rate of decline in GFR in these patients. In addition, remission coincided with an improved cardiovascular risk profile (286). Prevention or at least postponing the progression to ESRD would be an expected and positive consequence of the reduced rate of decline in GFR. However, evidence for the assumption that remission of nephrotic range albuminuria in type 1 diabetic patients will transfer in to a better long-term prognosis and survival has not been provided yet. To assess the outcome of remission of nephrotic on the hard endpoints death, dialysis or transplantation, we analyzed data from our long-term prospective observational study of type 1 diabetic patients with nephrotic range albuminuria due to diabetes (29). The patients were followed until the 31st of December 2003 or to death, dialysis or transplantation. During the median follow-up of 12.4 years, 25% of the patients in the remission group progressed to ESRD or died, whereas 74% of the patients in the no remission group progressed to the composite endpoint. Cardiovascular disease was the major cause of death in the no remission group (64% of deaths). Cox proportional hazard regression analysis with delayed entry revealed that obtaining remission was associated with a decrease in risk of reaching the composite endpoint (relative risk 0.28 (95% CI:0.13 to 0.59)) (29). Analyzing death separately resulted in a relative risk of this endpoint of 0.37 (95% CI: 0.14 to 0.94) in the remission group (29). The effect of remission of neph-

rotic range albuminuria was strong, with a reduction of both ESRD and mortality by ~60%.

In type 2 diabetes, the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan study (RENAAL), the baseline levels as well as the initial reduction in albuminuria has been demonstrated to be a powerful predictor of ESRD and cardiovascular morbidity. Every 50% reduction in proteinuria within the first six months halved the risk for cardiovascular endpoints and heart failure during follow-up (293). No other studies have been published concerning the impact of reduction in albuminuria on all cause mortality and cardiovascular events in diabetic patients.

In summary, remission of nephrotic range albuminuria is not only associated with a slower progression in diabetic nephropathy. In addition a substantially improved survival in these patients was demonstrated (29). Even though uremia is still prevalent, cardiovascular disease has become increasingly more frequent as cause of death. Early detection and treatment of cardiovascular disease in the diabetic patients have therefore become increasingly urgent as the kidney prognosis has improved during the last decades.

10. CONCLUSIONS AND FUTURE PERSPECTIVES

Diabetic nephropathy is a chronic progressive disease which develops in 25 to 40% of type 1 diabetic patients after at least 25 years of diabetes duration, and is associated with a poor prognosis including an excessive increase in cardiovascular mortality. Due to conflicting evidence for a decline in the cumulative incidence of severe microvascular complications (diabetic nephropathy and proliferative retinopathy), we analyzed data from two long-term prospective observational studies including 600 Caucasian type 1 diabetic patients. We found a decline in the cumulative incidence of severe diabetic microangiopathy during the past decades, even though ~15% of type 1 diabetic patients still have developed these severe complications after 20 years of diabetes. Whether the development of severe diabetic microangiopathy can be totally prevented or only postponed is not known at present time. However, early detection of high risk patients, even before development of microalbuminuria, is of substantial importance in order to target early intervention or even prevention of diabetic nephropathy.

Spontaneous regression of microalbuminuria, i.e. without intervention aiming at reducing the level of urinary albumin excretion rate, has been reported to occur frequently in type 1 diabetic patients. Recently, a cumulative incidence of regression of microalbuminuria was reported to be 58% during 6 years. In our inception cohort of type 1 diabetic patients followed from onset of diabetes, 79 patients developed microalbuminuria, and the 6 year cumulative incidence of regression was 31%. However, spontaneous permanent regression to normoalbuminuria was rare in our study (13% of the 79 microalbuminuric patients), and microalbuminuria is at present the best documented non invasive predictor of development of overt diabetic nephropathy in type 1 diabetes. Therefore, we have evaluated predictors of development of microalbuminuria in an inception cohort of 286 type 1 diabetic patients with newly diagnosed diabetes. Even shortly after onset of diabetes, high urinary albumin excretion rate, male gender, elevated mean arterial blood pressure, increased HbA_{1c}, and short stature were found to be significant predictors for the development of persistent microalbuminuria. Of these factors, several can be modified by treatment, introducing the option of primary prevention of diabetic nephropathy. The risk of development of microalbuminuria can be reduced by strict glycaemic control, whereas the feasibility of primary prevention by antihypertensive treatment or blocking the renin angiotensin system has not yet been convincingly demonstrated. Future research may shed light on whether early modification of blood pressure and urinary albumin excretion rate may increase efficacy of primary prevention.

Despite a reduced variability and slower deterioration of renal function after treatment with antihypertensive agent has become a part of routine care for patients with diabetic nephropathy, a substantial inter-individual rate of decline in glomerular filtration rate (GFR) remains, ranging from nearly no loss in kidney function to ~20 ml/min/year. To be able to postpone the development of end stage renal disease (ESRD) even further and to create new powerful treatment modalities, assessment of progression promoters, i.e. risk factors for progression of kidney function deterioration, is of vast importance. In our long-term prospective observational study with 7 years of follow-up of 301 type 1 diabetic patients with diabetic nephropathy, the impact of several putative progression promoters on the rate of decline in GFR was evaluated. We identified arterial blood pressure, albuminuria, glycaemic control expressed as HbA_{1c}, and serum cholesterol to be progression promoters. A threshold for albuminuria on the rate of decline in GFR was found at ~600 mg/24 h, whereas none of the other progression promoters showed a significant threshold. Furthermore, data from our long-term prospective observational cohort study did not support the suggestion that tobacco smoking is associated with deterioration in kidney function in type 1 diabetic patients with nephropathy. Even in the absence of convincing data supporting the concept that smoking accelerates renal disease progression, cigarette smoking remains a hazard to health, and the relative mortality risk for diabetic patients smoking are substantially higher than for non smoking diabetic patients. In the search for new progression promoters, 157 type 1 diabetic patients with overt nephropathy were followed from baseline examination in 1993 and for at least three years with yearly measurement of GFR. Even though a significant association between total homocysteine and decline in GFR was found, neither total homocysteine, vascular endothelial growth factor nor plasminogen activator inhibitor-1 were found to be independent predictors of decline in kidney function when adjusting for other known progression promoters. Thus emphasizing that the interrelationship between different risk factors often confounds the relative significance of individual risk factors, and well known progression promoters always must be taken into account when evaluating new risk factors for nephropathy progression.

We have furthermore studied whether the concept of inevitable progression towards ESRD in diabetic nephropathy can be challenged. In our study of 301 type 1 diabetic patients with overt diabetic nephropathy, 31% obtained remission (urinary albumin excretion rate < 300 mg/24 h) and 22% achieved regression (rate of decline in GFR ≤1 ml/min/year). In patients obtaining remission, the rate of decline in GFR was significantly lower (2.2 ml/min/year) as compared with the no remission group (4.8 ml/min/year). Thus, we have demonstrated that the functional changes can be reversed in overt diabetic nephropathy. This suggests that aggressive renoprotective treatment may prevent a substantial proportion of the patients from progression to ESRD. Several modifiable risk factors for progression of diabetic nephropathy also seem important for regression of diabetic nephropathy in type 1 diabetic patients.

Albuminuria can be used as an indicator of severity of disease, as patients with the highest level of albuminuria – nephrotic range albuminuria – are unfortunate with the poorest prognosis. We have evaluated whether this is a point of no return, or if remission at this progressive stage of diabetic nehropathy can be achieved. In the ~40% of the patients who progressed to nephrotic range albuminuria, we found that in one fifth of the patients aggressive antihypertensive treatment induced remission of nephrotic range albuminuria (urinary albumin excretion rate <600 mg/24 h). The patients obtaining remission had a slow mean decline in GFR and an improved cardiovascular risk profile.

We furthermore assessed the outcome of remission of nephrotic range albuminuria on the hard endpoints: death, dialysis or transplantation. In patients obtaining remission, 25% progressed to ESRD or died, whereas 74% of the patients in the no remission group progressed to the composite endpoint. Remission of nephrotic range albuminuria was associated with a decrease in risk of reaching the composite endpoint (relative risk 0.28 (95% CI: 0.13 to

0.59)). Analyzing death separately resulted in a relative risk of this endpoint alone of 0.37 (0.14 to 0.94) in the remission group. Thus, remission of nephrotic range albuminuria is feasible, associated not only with a slower progression in diabetic nephropathy, but also a substantial improved survival.

In conclusion, the main risk factors for development, progression, and remission of microvascular disease in type 1 diabetic are much alike and closely interrelated. The improved kidney prognosis in type 1 diabetic patients reveals an urgent need for targeting early detection and treatment of cardiovascular disease. To improve the prognosis and the quality of life in the diabetic patients even further, the treatment and care of patients must deal with all aspects of the disease, and thus prevention and treatment of micro- and macrovascular complications becomes vital.

ABBREVIATIONS

ACE:	Angiotensin converting enzyme
AGE:	Advanced glycation end product
CTGF:	Connective tissue growth factor
DCCT:	The Diabetes Control and Complications Trial Research Group
EDIC:	The Diabetes Control and Complications Trial Research Group/Epidemiology of Diabetes Interventions and Complications Research Group
ESRD:	End stage renal disease
EDTA:	Ethylenediaminetetraacetic acid
GFR:	Glomerular filtration rate
GH:	Growth hormone
IGF-1:	Insulin-like growth factor
HbA_{1c}:	Glycosylated haemoglobin A_{1c}
MDRD:	The Modification of Diet in Renal Disease study
PAI-I:	Plasminogen activator inhibitor-I
PKC:	Protein kinase C
TGF-β:	Transforming growth factor β
tHcy:	Total plasma homocysteine
RAS:	Renin angiotensin system
VEGF:	Vascular endothelial growth factor

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