CHAPTER 1
INTRODUCTION

Background

In patients with (insulin-dependent) diabetes mellitus (IDDM)\(^1\), the development of nephropathy, hallmarked by the presence of proteinuria in excess of 0.5 g/day, is a serious complication. Diabetic nephropathy (DN) leads to progressive deterioration in kidney function. Moreover, DN is associated with a highly increased incidence of cardiovascular disease. Renal failure and cardiovascular disease are in fact competing risks in these patients, making renal replacement therapy only necessary in those who survive cardiovascular complications. The natural course of DN has changed over the last decades. Early and aggressive treatment has been shown to retard renal function loss and, in particular, to improve survival in these patients. Despite the large progress in the treatment of DN, its etiology is yet incompletely understood and it is not possible to prevent this complication. There is evidence that both hereditary, as well as metabolic and haemodynamic factors contribute to its pathogenesis. Knowledge of these factors will identify patients at risk for developing nephropathy. The concept of microalbuminuria, i.e. a urinary albumin excretion rate between 20 and 200 µg/min or 30 to 300 mg/day, as an early clinical sign of diabetic renal involvement, has greatly improved our understanding of the natural course of diabetic renal disease and has enabled the development of early intervention and prevention strategies.

This thesis aims to evaluate the influence of norepinephrine (NE) and the growth-hormone insulin-like growth factor-I (GH-IGF-I) axis on renal function. Both substances belong to hormonal systems that control renal function in opposite directions: NE causes renal vasoconstriction, whereas stimulation of the GH-IGF-I-system induces renal vasodilation. The early stages of diabetic renal involvement are characterised by imbalances in glomerular vasodilatation and vasoconstriction. Against this background, the possible role of these hormonal factors in DN is investigated.

This chapter outlines the epidemiology, the functional stages, the pathogenesis and the therapeutic aspects of renal disease in IDDM. Several aspects of the pathogenesis of DN are more extensively overviewed in sections on the effects of NE and the GH-IGF-I-system on kidney function. Abnormalities in sodium and volume homeostasis in IDDM, and the role of 11β-hydroxysteroid dehydrogenase (11β-HSD) in protecting the mineralocorticoid receptor from activation by cortisol is briefly recapitulated.

\(^1\) Abbreviations: IDDM: insulin-dependent diabetes mellitus; DN: diabetic nephropathy; ERPF: effective renal plasma flow; GBM: glomerular basement membrane; GFR: glomerular filtration rate; GH: growth hormone; IGF-I: insulin-like growth factor-I; NE: norepinephrine; RAAS: renin-angiotensin-aldosterone system; SNS: sympathetic nervous system
Epidemiology of diabetic renal disease

In the early cohorts of IDDM patients, diagnosed between 1933 to 1959, the cumulative incidence of nephropathy amounted to 41-43% after 25 years of diabetes duration [1,2]. In these cohorts, nephropathy was associated with a 10-year mortality rate of 50 to 77% [1,2]. Comparing the 25 years cumulative incidence of nephropathy in IDDM diagnosed between 1933-1942 and 1953-1962, a remarkable decrease was noted from 41% to 27% [3]. A very spectacular decline up to 28%, 8.9% and 5.8% in IDDM patients diagnosed between 1961-1965, 1966-1970, and 1971-1975 has been reported in metabolically well-controlled Swedish patients [4], but in Danish cohorts a 35% incidence is still observed [5].

Survival in IDDM with proteinuria has improved dramatically. In Denmark, IDDM patients with onset of proteinuria between 1957 and 1973 had a mortality rate that was 40 times higher than in patients without proteinuria [6]. After onset of proteinuria, the 8 years survival rate of such patients was only 48%. In comparison, 8 years survival in IDDM patients with onset of proteinuria between 1974-1978 and 1979-1983 had increased to 82% and 87%, respectively [7,8].

The decline of incidence in overt proteinuria and cardiovascular mortality has been attributed to both improved blood pressure regulation and metabolic control [3-5]. In the older epidemiological studies [1,2,6], arterial hypertension was not treated since it was not recognised to have prognostic significance. Glycaemia could also not be strictly controlled since home-based blood glucose and glycosylated haemoglobin measurements were not yet available [9]. Remarkably, the peak incidence in proteinuria (10 to 15 years after the onset of IDDM) has remained unchanged over the last decades [1-6].

It is noteworthy that there is also a decline in the incidence of progression from microalbuminuria to overt proteinuria in IDDM. Previously, 80 to 90% of microalbuminuric IDDM patients progressed to overt nephropathy [10,11]. Recent estimates revealed that during the last 10 to 15 years only 30% of microalbuminuric patients progressed to clinical proteinuria [12-16]. This suggests that better metabolic control and blood pressure regulation are currently achieved in many microalbuminuric IDDM patients. The Diabetes Control and Complication Trial (DCCT) indeed showed that intensive insulin treatment reduces the progression of microalbuminuria in IDDM [17,18]. Furthermore, several trials unequivocally demonstrated that early treatment with antihypertensive drugs can arrest or delay the progression of microalbuminuria in normotensive microalbuminuric IDDM [19-22]. Only 10% of microalbuminuric patients treated with ACE-inhibitors developed overt proteinuria over an 8 years period, which is remarkably different from the 40% incidence of microalbuminuria in patients not treated with ACE-inhibition [22]. Longer follow-up will clarify whether intensive insulin treatment [17,18] and early ACE-inhibition treatment [19-22] will really prevent or only postpone DN. From an optimistic point of view, the aforementioned estimates suggest a decline in incidence and prevalence of nephropathy in the next decades.

Functional stages of diabetic renal involvement
The renal changes in IDDM patients are classically divided into 5 functional stages [23] (Table 1). An increased kidney size due to glomerular enlargement and tubular hypertrophy and hyperplasia (renal hypertrophy/hyperplasia), with concomitant increases in glomerular filtration rate (GFR) and renal blood flow (glomerular hyperfiltration/ hyperperfusion) are typical for stage 1 diabetic renal involvement. Transient increases in urinary albumin excretion can be seen at diagnosis of IDDM and often reverse after institution of insulin therapy. Physical exercise testing is associated with abnormal albumin excretion rates at this stage of renal involvement. In a subset of patients, renal hyperfunction persist for years, especially during poor metabolic control.

Stage 2 is characterised by early histologic alterations such as glomerular basement membrane (GBM) thickening and mesangial expansion, that are generally present after 2 years of disease duration. Except for the aforementioned exercise provocation, albumin excretion rate is normal, so that the glomerular filtration barrier against the loss of macromolecules is assumed to be intact. However, some studies have found an increased urinary excretion of the much larger and neutrally charged IgG in normoalbuminuric IDDM patients [24]. In the context of an intact GBM this finding is not well understood. GFR is either normal or elevated, comparable to stage 1 involvement. There are no reliable methods to diagnose this stage of renal involvement and the early histologic abnormalities have been found to correlate poorly with future progression to DN [25]. Also, the increase in urinary albumin excretion induced by exercise lacks any predictive value on the future development of nephropathy.

Stage 3 diabetic renal involvement, also designated as incipient diabetic nephropathy, is characterised by a persistently raised albumin excretion rate (micro-albuminuria), which typically develops after 5 to 15 years of diabetes duration in a subset of IDDM patients. The raised albumin excretion is ascribed to an early impairment of the glomerular filtration barrier against the loss of macromolecules. A decrease in the negatively charged heparan sulfate proteoglycan (HS-PG) of the GBM is one of the biochemical alterations that is likely to be responsible for the increased loss of albumin, but haemodynamic factors may be involved as well [26]. GFR is remarkably unaltered or may be elevated in

| Table 1. Functional stages of diabetic renal involvement |
|----------------------------------|---|---|---|
| **GFR** ||**Ualb.V** ||**MAP** |
| **Stage 1 Hyperfunction** | ↑1 | = (1)* | Nephromegaly |
| **Stage 2 Silent phase** | ↑/= | = (1)* | Early histological changes |
| **Stage 3 Microalbuminuria** | ↑ (11)** | = (1)** | Albumin excretion 20-200 µg/min (30-300 mg/day) |
| **Stage 4 Nephropathy** | ↓ | ↑1 | ↑ | Dipstick positive (albuminuria > 200 µg/min, proteinuria > 500 mg/day) |
| **Stage 5 End stage renal failure** | ↓↓ | ↑1 | ↑1 | Dialysis support or transplantation |

GFR: glomerular filtration rate, Ualb.V urinary albumin excretion rate, MAP: mean arterial pressure. * can be present during poor metabolic control, ** aggravates during exercise.
subgroups of patients. Blood pressure may still be below the normal range, although slight increases in night and day-time blood pressure have been reported with 24-hour ambulatory blood measurements [27]. Exercise causes an exaggerated blood pressure rise, further indicating abnormalities in blood pressure regulation at this stage of renal involvement.

The clinical hallmark of stage 4 involvement is the presence overt proteinuria in excess of 0.5 g/day. Arterial hypertension is almost always present and contributes importantly to the loss of kidney function [28]. Unless arterial hypertension is treated, GFR declines at a rate of approximately 1 ml/min per month. Besides more outspoken thickening of the GBM and mesangial expansion due to increased formation of extracellular matrix, histologic examination now shows arteriolar hyalinosis and an increased number of sclerosed glomeruli, appearing in a diffuse and nodular pattern, as first described by Kimmelstiel and Wilson [29]. The progressive nature of nephropathy results in generalised glomerulosclerosis ultimately leading to end-stage renal disease. Stage 5 diabetic renal involvement represents end stage renal failure requiring dialysis or renal transplantation.

Microalbuminuria: a predictor of DN and cardiovascular disease

Under normal circumstances small amounts of albumin pass through the glomerular filtration membrane. Most of the filtered albumin undergoes tubular reabsorption, so that the final urinary albumin excretion rate is very low [30]. With the introduction of sensitive assays in the early seventies it became possible to detect urinary albumin at these low concentrations [31,32]. It was soon established that elevations in urinary albumin excretion (microalbuminuria) were typically present in the years preceding DN [10,11]. Moreover, it has been established that the presence of microalbuminuria carried an increased risk on cardiovascular complications [33,34]. These findings led to the concept that microalbuminuria represents the incipient stage of diabetic renal disease [23,35-39]. The association with cardiovascular complications suggested that the presence of microalbuminuria could also be an indicator of generalised vascular damage [37]. At present, microalbuminuria is the first detectable clinical sign of an increased risk of DN and of cardiovascular disease in IDDM [36,38,39].

Despite the great advance to measure proteinuria at low levels with assays that have low coefficients of variation [31], measurement of urinary albumin excretion rate is complicated by a large biological variability [38-40]. Day-to-day variation in albuminuria is as high as 30-50%, and there is considerable chance that a random urine sample will show supra normal values. Thirty-eight % of patients with IDDM experience sporadic episodes of microalbuminuria without developing persistent microalbuminuria [41]. Recent diagnosis, worsened metabolic control, systemic illness, urinary tract infection and physical exercise are potential confounding factors that temporarily raise albumin excretion [10,38,39]. While single urine measurements suffice for screening purposes, there is general agreement that multiple urine collections are required to diagnose microalbuminuria reliably [36,38,39,42]. An albumin/creatinine ratio in a random or early morning urine sample of >3.5 mg/mmol is highly predictable for the presence of microalbuminuria [38,40], although a lower cut-off level of 2.5 mg/mmol has been proposed for firstly voided morning urine [39]. For definite evaluation of
microalbuminuria, three timed overnight urine collections can be used to avoid the effects of daytime physical activities, but 2-4 hour daytime or 24 hour urine collections also give reliable results [43]. Using overnight or timed day-time urine collections, albumin excretion is expressed in µg/min and microalbuminuria is defined as levels between 20 µg/min and 200 µg/min [36,38,39]. In 24 hour urine collections an albumin excretion rate between 30 to 300 mg/day indicates microalbuminuria. The lower level of 20 µg/min (or 30 mg/day) is clearly above the upper normal limit of 10 to 12 µg/min found in healthy subjects [38,39,44]), but the cut-off level of 20 µg/min has been chosen because of its predictive value to discriminate patients at risk to develop nephropathy [36,38,39]. Thus there is a grey area between 10 and 20 µg/min (15-30 mg/day). The upper value of 200 µg/min (300 mg/day) corresponds to a total protein excretion of 0.5 g per day.

If microalbuminuria is correctly diagnosed, 30% of these patients will progress to overt proteinuria in 10 years [12-16,19-22,36,38]. A normal albumin excretion rate excludes progression to nephropathy with a 99.5% chance [41]. It has been reported that patients with an albumin excretion rate between 70 to 200 µg/day are particularly likely to progress, whilst patients with a lower albumin excretion rate between 20 to 70 µg/day are more likely to remain stable [12,42]. Thus, microalbuminuria is a very sensitive but not specific measure to identify patients at risk of progression to nephropathy.

The increased risk of cardiovascular morbidity and mortality in microalbuminuric IDDM patients indicates that elevations in urinary albumin excretion have much broader consequences than representing a risk marker for the development of nephropathy [33,35,37-39]. This has been brought into a wider perspective by demonstrating that microalbuminuria also predicts early mortality in non-insulin dependent diabetes mellitus (NIDDM) as well as in the general population [45-47]. Apart from other well established risk factors, microalbuminuria appears to be a powerful indicator of cardiovascular disease [48]. The association between microalbuminuria and ischaemic heart disease is intriguing, and may be part of the metabolic syndrome of which alterations in blood pressure regulation are an important component [49]. The clear association between elevations in blood pressure and microalbuminuria in patients with IDDM as well as in patients with essential hypertension, and the fact that albumin excretion acutely falls after blood pressure lowering, support a haemodynamic basis in the genesis of micro-albuminuria [9,42,50]. On the other hand, an atherogenic lipoprotein profile [51,52], increased plasma concentrations of clotting factors and decreased fibrinolysis [51,54], endothelial dysfunction [53-55] and insulin resistance [56] are other manifestations of the metabolic syndrome that have been documented in microalbuminuric IDDM patients.

Pathogenesis of diabetic renal disease

The pathogenesis of structural and functional abnormalities in DN is likely to be multifactorial (Table 2). In this section metabolic and haemodynamic abnormalities that
Table 2. Mechanisms and factors implicated in the pathogenesis of diabetic nephropathy

1. Metabolic consequences of hyperglycaemia:

   **Features:** microcirculatory changes, glomerular basement membrane (GBM) thickening, decreased heparan-sulfate-proteoglycan content of GBM, mesangial cell proliferation and extracellular matrix production

   **Possible pathways:**
   - upregulation of diacylglycerol (DAG) and protein kinase-C (PKC)
   - nonenzymatic glycosylation: production advanced glycosylation products (AGE’s),
   - polyol pathway: sorbitol accumulation, altered cellular redox state

   **Implicated intrarenal growth factors and cytokines:**
   - angiotensin II, endothelin, insulin-like growth factor I
   - platelet-derived growth factor-β, vascular endothelial growth factor,
   - transforming growth factor-β and other cytokines as IL-1β, IL-6, IL-8, TNF, IFN-γ

2. Altered renal haemodynamics:

   **Features:** increases in glomerular blood flow, intraglomerular pressure, filtration surface

   **Mechanisms:** diminished arteriolar resistance, imbalances in afferent/efferent tone, mesangial dysfunction

   **Contributing factors:**
   - hyperglycaemia and insulin
   - activated growth-hormone-insulin-like-growth-factor-I-axis
   - hyperglucagonaemia
   - inadequate suppressed renin-angiotensin II system, increased angiotensin II reactivity
   - altered sympathetic tone, increased norepinephrine reactivity
   - increased induction of nitric oxide versus disturbed endothelial function
   - abnormal prostaglandin metabolism, increased levels of atrial natriuretic factor, upregulation of kinins
   - augmented tubular sodium reabsorption, increases in total exchangeable sodium and extracellular volume

3. Elevated lipid levels: glomerular lipid accumulation in glomerulosclerosis resembling atherosclerosis

4. Genetic Predisposition: genes involved yet unknown, only polymorphism in the ACE-gene identified as a marker of progression

are considered to be implicated in the pathogenesis of DN are outlined. Furthermore, alterations in lipoprotein metabolism and genetic factors that may influence the development of DN are briefly described. Particular attention is paid to the renal effects of NE and the GH-IGF-I-system.

**Metabolic factors**

Chronic hyperglycaemia is an inevitable consequence of IDDM and may induce alterations in many cellular and molecular functions. The metabolic theories address mechanisms by which elevated blood glucose levels may be causally involved in the development of microvascular complications.

First, the injurious effects of hyperglycaemia could be mediated via its effects on the microcirculation. Elevated blood glucose levels induce arteriolar vasodilatation, increase
blood flow and raise hydrostatic pressure, impair vasoregulation and thereby fail to protect target organs from increases in blood pressure. By such effects on the microcirculation, particularly on capillary pressure, hyperglycaemia may be responsible for leakage of plasma proteins and deposition of proteins in the walls of arterioles and capillaries and thus induce damage to the kidneys [57,58].

A second pathway stresses the direct role for blood glucose to induce structural glomerular abnormalities. Under experimental conditions, glucose has been demonstrated to cause GBM thickening and mesangial cell proliferation [59,60], to increase extracellular matrix production and synthesis of type IV collagen [61,62], and to decrease GBM density of the negatively charged HSPG [63]. These effects may in part be mediated by the expression of a matrix-producing cytokin, transforming-growth-factor-β (TGF-β) [64]. More recently, it has been shown that upregulation of intracellular signal transduction via stimulation of diacylglycerol (DAG) and protein kinase C (PKC), as present in diabetic patients, can raise TGF-β and other growth factors, like vascular endothelial growth factor, angiotensin II and endothelin [65]. Interestingly, inhibition of this system by an orally active PKC-β isomorph inhibitor has been shown to reverse the expression of TGF-β, to decrease the production of type IV and VI collagen and to restore haemodynamic abnormalities in diabetic rats [66]. These findings are in favour of an important role of the PKC-transduction system through which elevated levels of blood glucose may be involved in the pathogenesis of DN.

Third, hyperglycaemia is also associated with an increased non-enzymatic glycosylation of long-lived proteins that may undergo Amadori rearrangement and thereby lead to the irreversible formation of advanced glycosylation endproducts (AGE’s). AGE’s have been demonstrated to induce mesangial expansion and increase type IV collagen synthesis [67-69]. These effects may be mediated via specific AGE receptors [69], and are prevented by neutralising antibodies or by aminoguanidine in experimental diabetes [69,70].

Finally, hyperglycaemia causes an increased substrate delivery into the polyol pathway that results in the accumulation of sorbitol and changes the cytosolic redox state. In the polyol pathway, glucose is reduced to sorbitol by the enzyme aldose-reductase and sorbitol is oxidised to fructose by the enzyme sorbitol dehydrogenase (Figure 1). Under conditions of hyperglycaemia increased amounts of sorbitol are produced, as for instance documented in diabetic kidneys. The accumulation of this compound has been proposed to cause damage of renal tissue [71]. Another consequence of increased substrate delivery into the polyol pathway is the accumulation of NADPH and NADH, leading to an increased cytosolic cell ratio of free NADH/NAD⁺ [72]. Such changes in redox state are also present in hypoxic tissues. Since vasodilation and increased blood flow are characteristic early vascular responses of tissue hypoxia and are also seen during hyperglycaemia, the so-called pseudohypoxia theory argues that an altered cytosolic redox state may be involved in the haemodynamic alterations and subsequent microvascular complications of IDDM [72].
Figure 1. Reduction of glucose to sorbitol and oxidation of sorbitol to fructose in the sorbitol pathway. Reduction of glucose to sorbitol by aldose reductase (AR) is coupled to oxidation of NADPH to NADP. NADP is reduced to NADPH by the hexose monophosphate pathway. Oxidation of sorbitol to fructose by sorbitol dehydrogenase (SDH) is coupled to reduction of NAD to NADH. The cytosolic ratio of free NADH/NAD is in equilibrium with lactate and pyruvate. G6P: glucose-6-phosphate; 6PG: 6-phosphogluconate, and R5P: ribulose-5-phosphate.

Renal haemodynamic factors

Elevations in GFR have long been recognised in patients with IDDM and can persist for many years after the onset of diabetes [73-75]. The early stages of experimental diabetes are also characterised by a state of glomerular hyperfiltration. Its possible pathogenetic role became apparent when glomerular hyperfiltration, consequently to experimental renal ablation, was found to induce glomerulosclerosis [76]. In a similar way, a chronic increase in single nephron glomerular filtration (SNGFR) was associated with the development of glomerulosclerosis and renal function loss in diabetic rats [77].

According to the equation GFR= kf(ΔPc-Δπ), net hydraulic pressure (ΔPc), net oncotic pressure (Δπ), filtration surface area and hydraulic permeability (kf) determine glomerular ultrafiltration [30]. Since GFR changes linearly with glomerular blood flow under conditions of pressure equilibrium in the rat [30] and is highly correlated with effective renal plasma flow (ERPF) in man [78], renal blood flow is considered a determinant of GFR. Theoretically, a change in any of these factors could be involved in diabetic hyperfiltration. Using the micropuncture technique, increases in glomerular blood flow, intraglomerular pressure and ultrafiltration coefficient have been documented in hyperfiltering diabetic rats [79-81]. Of these factors, the rise in intraglomerular capillary pressure was shown to play a key role as pharmacological amelioration of intraglomerular hypertension with ACE-inhibitors could be prevent glomerulosclerosis in these animals [82-83]. The rise intraglomerular capillary pressure in diabetes has been attributed to imbalances in afferent and efferent glomerular arteriolar tone, and to an increase in systemic arterial pressure [80,81]. A diminished glomerular afferent tone and an increased glomerular efferent constriction are vascular abnormalities that have been implicated in the glomerular hypertension associated with diabetes [80,81].

The original assumption that intraglomerular hypertension directly causes glomerular damage appears to be an oversimplification since later studies have shown that haemodynamic and non-haemodynamic factors are involved in the process of
glomerulosclerosis [84-87]. Nevertheless, an important role is still attributed to glomerular hypertension, either as an initiator or as a conditional factor in a cascade of cellular events that leads to glomerular damage. Several mechanisms have been proposed. First, chronic pressure overloading of the capillary endothelial cell layer may lead to cell detachment, GBM denudation, collagen exposure and consequently to platelet aggregation, fibrin accumulation and intracapillary microthrombosis. Second, capillary dilation may disrupt the attachment of podocytes to the GBM with the subsequent formation of subendothelial deposits. Third, continuous stretching may induce mesangial cell proliferation and extracellular matrix production by stimulating cytokin expression [85-87]. As a result, either of these mechanisms may impair the glomerular filtration barrier and enhance glomerular protein passage, which, in turn, might be toxic and accelerate glomerular damage [88]. In a similar way, glomerular accumulation of atherogenic lipoproteins could contribute to the process of glomerulosclerosis [89].

The neurohumoral stimulus for the hyperfiltration phenomenon in diabetes is unknown, although many factors could play a contributory role [81]. Moderate hyperglycaemia increases GFR [90,91] and chronic lowering of blood glucose reduces GFR in hyperfiltering IDDM patients [92]. The effect of glucose on GFR may result from a decrease in afferent glomerular arteriolar tone, mediated via the tubulo-glomerular feedback (TGF) loop [90,91]. Insulin at doses that raise its plasma level 4 to 8 fold acutely elevates ERPF [93,94], and its vasodilating properties are probably mediated via nitric oxide that directly affects glomerular arteriolar tone and mesangial function [95]. Another relevant action of insulin is an increased tubular sodium reabsorption, leading to increases in total exchangeable sodium and extracellular volume, which have been implicated in diabetic glomerular hyperfiltration [96,97]. Alterations in contra-regulatory hormones, GH and glucagon, which are well known renal vasodilators [98-100], could also play a role since these hormones are often elevated during suboptimal metabolic control [101-104]. It is possible that intrarenal accumulation of IGF-I, as part of the GH-IGF-I-system, may induce the early functional and morphological renal changes in diabetes [105]. Renal haemodynamics in diabetes may also depend on alterations in neurohormonal systems that regulate glomerular tone, such as the renin-angiotensin-aldosterone-system (RAAS) and the sympathetic nervous system (SNS) [81]. The presence of diabetes obviously affects the SNS, although various changes have been reported [106]. Furthermore, systemic blood pressure responses to pressor agents like angiotensin II and NE are increased in diabetes mellitus [107-110]. Among other factors, vasoactive substances, such as prostaglandins, nitric oxide, kinins and atrial natriuretic peptide, have also been implicated in diabetic glomerular hyperfiltration [81]. Currently, none of these factors has been found to fully account for the increase in GFR in IDDM patients. For instance, exogenous infusions of glucose, insulin, GH, IGF-I and glucagon all increase GFR, but not to levels as generally encountered in diabetic hyperfiltration.

Although animal studies provided an experimental basis for an increased GFR and elevated intraglomerular pressure as pathogenetic factors involved in the development of DN, it should be stressed that there are caveats in extrapolating these data to the human situation. First, glomerular pressure cannot be measured in man and it is thus unknown whether glomerular hyperfiltration in human IDDM is accompanied by an increase in intraglomerular pressure. The only indirect proof stems from fingernail micropuncture
studies that demonstrated capillary hypertension in human IDDM, but no differences were found between normo- and microalbuminuric patients [111]. Second, it is clinically difficult to measure glomerular hyperfiltration in IDDM patients. Many clinical studies defined glomerular hyperfiltration as a GFR above the upper normal limit of a control population. Such an arbitrary definition does not discriminate subtle intrarenal haemodynamic abnormalities in apparently normofiltering patients and has, therefore, the disadvantage to underestimate the hyperfiltration phenomenon. This could explain why some [112], but not all [113,114] clinical observations found an elevated GFR to be implicated in DN. Finally, in man renal insufficiency is an uncommon consequence of long-standing glomerular hyperfiltration per se, since subjects with one kidney [115-117] and patients with acromegaly [118,119] are not at high risk of renal failure. This strongly suggests that glomerular hyperfiltration alone does not result in important glomerular injury in humans.

Lipoproteins

Higher serum levels of low-density lipoprotein (LDL) cholesterol, apolipoprotein B, triglycerides and lipoprotein (a), and lower levels of high-density lipoprotein (HDL) cholesterol have been observed in IDDM patients with nephropathy and even in patients with microalbuminuria [51,52,120-122]. Such atherogenic lipoprotein changes are likely to explain in part the increased cardiovascular risk in these patients. There exists much controversy whether lipoprotein abnormalities are also involved in the pathogenesis of DN [123]. An independent association between elevated LDL cholesterol levels and progression of microalbuminuria [124] and overt nephropathy [125] has been observed. This suggests a pathophysiological role of hyperlipidaemia analogous to atherosclerosis. Indeed, the lipid depositions and mesangial cell proliferation of glomerulosclerotic lesions show a remarkable resemblance with the lipid filled monocytes and vascular smooth muscle cell proliferation in atherosclerotic plaques. Furthermore, experimental studies showed hypercholesterolaemia to aggravate glomerulosclerosis, which was prevented by cholesterol lowering therapy [126,127]. However, clinical support for the benefit of cholesterol lowering therapy on progression of nephropathy is lacking. Short-term simvastatin treatment did not decrease albuminuria in IDDM with nephropathy [128]. Lovastatin, another HMG CoA reductase inhibitor, attenuated the rate of renal function loss in NIDDM patients with nephropathy, but the lack of intergroup comparison in this study has been criticised [129].

Genetic factors

The fact that only a proportion of IDDM patients eventually develop overt proteinuria and that this complication has a peak incidence 10 to 15 years after the onset of diabetes, supports the notion that specific susceptibility factors are involved in the pathogenesis of DN [1-3,5]. The observation that DN clusters in affected families further strengthens the involvement of genetic factors [130-132]. IDDM siblings belonging to families with a first-degree relative suffering from nephropathy have a life long risk of 70% to develop nephropathy, whereas this risk is only 20% when there is no family history of DN [132], and it has been suggested that one or two major genes determine
susceptibility to DN. Since a familial predisposition to essential hypertension is associated with an increased risk of DN [133-135], candidate genes have been sought among loci involved in the regulation of blood pressure.

Recent attention has been given to polymorphisms in genes encoding for RAAS components. In this respect, the ACE gene polymorphism seems to be of relevance. This polymorphism consists of an 287 basepair insertion (I) or deletion (D) of intron 16 of the ACE gene. The DD genotype has been shown to be associated with an increased cardiovascular risk in non-diabetic populations [136,137], in non-insulin-dependent diabetic (NIDDM) patients [138,139] and in IDDM patients with nephropathy [140]. DD homozygotes have elevated serum [141] and tissue ACE levels [142], causing an increased vascular conversion of angiotensin I to angiotensin II [143] and an increased pressor response to angiotensin I [144]. It is, therefore, hypothesised that increased angiotensin II formation is involved in the increased cardiovascular risk in conjunction with the DD genotype. The issue whether the DD genotype is also associated with DN is controversial. Some cross-sectional studies showed the DD genotype to be more prevalent among IDDM and NIDDM patients with nephropathy [145,146], while in other reports no association of the ACE gene polymorphism with DN could be demonstrated [147-149]. Recently it was shown that in IDDM patients treated with an ACE inhibitor, the rate of decline in GFR is greater in patients with the DD genotype compared to patients with the ID or II genotype [150,151] Similar findings have been reported in non-diabetic subjects with nephropathy [152], and that study also demonstrated a less effective antiproteinuric effect of ACE inhibition treatment in subjects with the DD genotype. Thus, the ACE gene polymorphism is more likely to be a marker of progression of DN than a susceptibility factor for DN.

The number of candidate genes for DN is growing and evaluation of their putative roles will require large numbers of subjects, including sib-pairs discordant for DN [132].

**Renal effects of norepinephrine**

After its release from the terminal nerve endings of the SNS, NE acts in an autocrine fashion on local \( \alpha \)-adrenoceptors, while at the same time small amounts leak into the circulation [153,154]. This spilled-over NE is not an inert circulating neurotransmitter, but a hormonally active substance [155,156]. In the kidney, \( \alpha \)-adrenoceptors are located along the interlobular, afferent and efferent glomerular arterioles, mesangial cells and tubular segments [157-159]. This distribution pattern suggests that NE may control glomerular blood flow, glomerular capillary pressure and renal sodium handling [153,154]. Studies on renal sympathetic nerves have shown that low frequency stimulation results in sodium retention and renin release and high frequency stimulation in a fall in ERPF and some decline in GFR [160,161]. Exogenous NE infusions markedly reduce ERPF without much change in GFR in animals [162-165]. The NE-induced renal haemodynamic changes are likely mediated via afferent and efferent glomerular arterioles [160-165]. These vessels are the major sites of flow resistance in the kidney and importantly determine renal blood flow, whereas they are also involved in the control of intraglomerular pressure [30]. Micropuncture studies in the rat have indeed documented that NE causes a fall in renal blood flow by afferent and efferent glomerular vasoconstriction, and that NE evidently increases intraglomerular pressure [162]. Interestingly, the prevailing blood pressure was found to determine the glomerular vessel response. There was a predominant increase in
efferent tone when blood pressure was kept unchanged, whereas both afferent and efferent glomerular resistance increased when blood pressure was allowed to increase [162]. The lack of change in GFR during NE infusion was explained by an increase in intraglomerular pressure offsetting the fall in glomerular blood flow [162]. Although, the precise intrarenal effects of NE are unknown in man, there are obvious similarities with animal data. Indeed, intravenous infusions of NE lower ERPF but have little effect on GFR [166, 167]. Consequently the filtration fraction (FF) rises which may reflect a change in pressure profile along the arterioles. It is therefore plausible that NE also increases intraglomerular pressure in man, as supported by the finding that NE augmented proteinuria in nephrotic patients [168].

The diabetic state has variably been associated with an increased, unchanged, or even a decreased SNS activity and/or vascular reactivity to NE [81,106]. These inconsistencies may be attributed to differences in the species investigated, in blood glucose and insulin levels, or in the vascular bed under study [81,106,169-171]. There is only one study in kidney tissue taken from severely hyperglycaemic rats that has addressed the putative role of NE in DN. In this study, afferent glomerular arteriolar responsiveness was attenuated in experimental diabetes [165]. Most evidence, however, points towards an increased vascular responsiveness in diabetes [81,106]. For instance, systemic blood pressure responsiveness to exogenous NE has repeatedly been found to be exaggerated diabetic patients [108-110]. Furthermore, the responsiveness to NE-induced vasoconstriction of dorsal hand veins, which contain α-adrenoceptors like glomerular arterioles, is increased in moderately hyperglycaemic microalbuminuric IDDM patients [172]. These findings raise the possibility that glomerular vessels in IDDM are also hyperresponsive to NE. Such an exaggerated renal responsiveness could, therefore, contribute to the elevations in intraglomerular pressure and albumin excretion rate, and thus play a pathogenesis role in DN [81,172,173]. However, no human study has evaluated renal NE responses in IDDM, and has established whether NE has the ability to increase microproteinuria.

The growth hormone-insulin-like growth factor-I system and kidney function

IGF-I is a small peptide hormone (MW 7.6 kDa), which production is under pituitary GH control [174]. The pituitary GH product of 21.5 kDa (191 amino acids) is secreted in pulsatile fashion in approximately 13 surges per day and has a short half-life of 20 minutes that is prolonged after binding to GH-binding protein. GH is a strong secretagogue of IGF-I. GH stimulates IGF-I gene transcription and increases IGF-I synthesis in many tissues. Most IGF-I present in the circulation originates from the liver [175]. IGF-I, in turn, inhibits pituitary GH release by a negative feedback mechanism. The biological activity of IGF-I depends on the plasma levels of several binding proteins that interfere with IGF-I receptor interaction, as well as on IGF-I receptor expression [174]. The IGF-I shares 70% homology with proinsulin and binds with high affinity to the IGF-I-receptor and with lower affinity to the insulin receptor. The plasma levels of IGF-I range from 10-125 nmol per liter, which is much higher than insulin with fasting levels in the picomolar range. Excessive stimulation of the insulin receptor is, however, prevented by the fact that more than 99% of IGF-I is bound to specific IGF-binding proteins (IGFBP), and only a small amount of IGF-I is present its free form [174]. About 85% of IGF-I is
bound to IGFBP-3 and forms a 150 kDa ternary complex after association with the acid-
labile subunit (ALS), that does not pass through the capillary barrier [176]. The binding
to IGFBP-3 has such a high affinity that competition between IGFBP’s and the IGF-I
receptor occurs. The 150 kDa complex can thus be viewed as a circulating IGF-I reservoir
[174]. Both cleavage by proteases and phosphorylation impair the formation of the ternary
complex, and enhance binding of IGF-I with its receptor. Another 20% of serum IGF-I is
found in smaller (±45 kDa) complexes, containing IGFBP-1, IGFBP-2, IGFBP-3 or
IGFBP-4, which can pass through capillary endothelial membranes and deliver IGF-I to
specific tissue-binding sites.

In man, renal haemodynamic parameters covary with endogenous GH and IGF-I
levels. GFR and ERPF are elevated in acromegaly, decline after GH lowering treatment
with octreotide, and are decreased in GH deficiency [177-182]. GH stimulates renal
haemodynamics after a lag period allowing IGF-I levels to increase [183,184], whereas
rhIGF-I infusion induces an immediate rise in GFR and ERPF [185-187]. Thus, GH seems
to increase renal haemodynamics indirectly via stimulating IGF-I synthesis.

IGF-I receptors as well as mRNA encoding for the different IGFBP’s are expressed
in various structures of the kidney including glomerular arterioles [188]. In contrast, GH
receptors are not present on human glomerular vessels. Contradictory results have been
presented with respect to IGF-I production in human nephrons [174,188], and it is
unknown whether the GH receptor is expressed in other glomerular structures [174].

Based on micropuncture studies in the rat, rhIGF-I has been shown to decrease
efferent glomerular arteriolar resistance with a trend towards a reduction of afferent
arteriolar resistance [185]. Exogenous rhIGF-I does not increase glomerular capillary
pressure. The rises in SNGFR and in whole kidney GFR are fully accounted for by
increments in glomerular blood flow and in the filtration coefficient [185]. The IGF-I-
induced renal changes are likely mediated via nitric oxide (NO), because IGF-I has been
shown to increase NO synthesis in cultured vascular endothelial cells [189], and the NO
synthase inhibitor, N\textsuperscript{\textomega}-nitro-l-arginine methyl ester, abolishes renal vasodilation by IGF-I
[190]. Moreover, IGF-I could also be involved in mesangial cell relaxation [191].

Several experimental studies indicate that an enhanced GH-IGF-I-axis could be
involved in diabetes-associated hyperfiltration and plays a pathogenetic role in the
development of glomerulosclerosis. Following unilateral nephrectomy in the rat, IGF-I has
been found to accumulate in hyperfiltering nephrons and the increase in SNGFR was
inhibited by anti-IGF-I-antibody administration [192]. It is of interest, that IGF-I has been
found to accumulate in kidneys of diabetic rats during the initial phases of renal enlarge-
ment and renal hyperperfusion [193]. Concomitant increases in renal IGF-I receptor
expression and receptor binding activity have also been documented in kidney tissue from
streptozotocin-induced diabetic rats [194]. This renal IGF-I accumulation has been shown
to be GH dependent since it is diminished in hypophysectomised diabetic rats and is in
part restored after GH replacement [195]. Thus, these findings suggest that renal IGF-I is
involved in renal hypertrophy and raise the possibility that GH is necessary for this effect.

A possible role of the GH-IGF-I-system in the development of glomerulosclerosis
is supported by observations in hGH-transgenic mice and in rats bearing GH-producing
tumours which develop albuminuria, mesangial cell proliferation and premature
glomerulosclerosis [196,197]. However, IGF-I alone may not be completely responsible
for GH-IGF-I-induced glomerulosclerosis, since mice transgenic for IGF-I do not develop glomerulosclerosis [198]. These negative findings may be due to lower IGF-I levels in the transgenic animals expressing IGF-I as compared to those expressing high levels of GH, but it is also possible that concomitant elevations in GH are necessary for IGF-I to induce glomerulosclerosis. Pituitary GH deficiency or GH lowering treatment with octreotide indeed modified the renal alterations after streptozotocin-induced diabetes in rats, as supported by inhibition of glomerular hypertrophy and lower albumin excretion rates in these animals [199,200]. Of interest, high glucose levels increased IGF-I, IGF-I mRNA, and IGF-I receptor expression in cultured mesangial cells, which, in conjunction with raised TGF-β1 levels, enhanced extracellular matrix production [201]. Taken together, it is possible that abnormalities in the GH-IGF-I-system can contribute to the development of glomerulosclerosis in diabetes mellitus.

During poor metabolic control both glomerular hyperfiltration and elevated circulating GH levels have been documented [75,92,101-103]. However, no difference in diurnal GH profile between normo- and hyperfiltering IDDM patients was observed [202]. Nevertheless, an exaggerated GH-responses to GH-releasing hormone has been shown in hyperfiltering IDDM patients [203], indicating that glomerular hyperfiltration is indeed related to abnormalities in GH-release. Despite high circulating GH-levels, serum IGF-I levels are often low, again in relation to poor metabolic control [204]. This has been ascribed to GH-resistance at the hepatic level [205,206]. Lower circulating IGF-I levels, in turn, may contribute to GH-hypersecretion by insufficient inhibition at the pituitary. This may cause an adverse sequence of events: GH may worsen metabolic control and poor control may elevate GH-levels. Intensive insulin treatment, and more importantly, restoration of hepatic insulinisation, have been shown to increase IGF-I levels [207,208] and reverse GH-hypersecretion [102]. This indicates that in IDDM patients relative insulinopenia may cause GH hypersecretion and lower the IGF-I level.

How can elevated GH and lower IGF-I levels be implicated in diabetic glomerular hyperfiltration? The association of increased renal haemodynamics with abnormalities in the GH-IGF-I system, as shown in diabetic rats, results from intrarenal IGF-I accumulation, an increased IGF-I receptor expression and alterations in IGFBP’s [105,193-195, 199,200]. Such alterations covary with insulin levels since they are outspokenly present during insulinopenia and partly prevented by insulin treatment [193,210]. Thus, despite impaired (hepatic) IGF-I synthesis, poor metabolic control could in fact enhance renal IGF-I accumulation. A maximally stimulated intrarenal IGF-I accumulation may, therefore, explain why GH administration does not increase GFR and ERPF in poorly controlled IDDM patients, whereas it augments GFR and ERPF in well-controlled IDDM patients [99]. The mechanisms underlying renal IGF-I accumulation are not precisely known, but are conceivably due to an increased trapping of IGF-I from the circulation [195]. Local production of IGFBP’s, increased IGF-I receptor expression [193-195] and increased IGFBP-3 protease activity that impairs the formation of the ternary complex [211] could all be involved in intrarenal IGF-I accumulation in IDDM.

**Sodium and volume homeostasis in IDDM and the role of 11β-hydroxysteroid dehydrogenase**
An increase in exchangeable sodium accompanied by extracellular volume expansion is a well-documented feature of diabetic patients and might contribute to elevations in GFR, as well as to rises in blood pressure in association with microalbuminuria [96,110, 212-217]. The mechanisms responsible for this abnormal sodium retention are incompletely understood. An enhanced renal tubular sodium reabsorption, possibly mediated by insulin, may be involved [97]. In diabetic patients sodium excretion is attenuated following head out water immersion [218] and saline infusion [219]. Elevated plasma levels of sodium retaining hormones, like angiotensin II, aldosterone en NE, are not encountered in IDDM and are unlikely to explain the tendency towards sodium retention [212,213,216,220,221].

Mineralocorticosteroids play a central role in extracellular sodium and fluid homeostasis. Interestingly, the mineralocorticoid receptor has equal affinity for cortisol and aldosterone in vitro, but, in contrast, the renal tubules exclusively bind aldosterone in vivo [222-225]. Recently, it has become clear that the mineralocorticoid receptor is protected from being activated by cortisol by the intracellular enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD) [224]. Two isoforms have been identified. 11β-HSD₁ is present in the liver. This enzyme is NADH/NAD⁺ dependent and catalyses the interconversion between cortisol and cortisone. 11β-HSD₂ is expressed in the kidney and unidirectionally catalyses the oxidation of cortisol to its inactive compound, cortisone. This isoenzyme is NADP⁺ dependent [225]. Thus, there is a link between cortisol metabolism and the regulation of volume and sodium homeostasis. For instance, genetic mutations in 11β-HSD₂ that impair its activity cause hypokalaemic hypertension despite undetectable aldosterone levels [226,227]. This so-called apparent mineralocorticoid excess syndrome can also be acquired as a consequence of glycerrhethinic acid ingestion [228].

It is unknown whether 11β-HSD activity is altered in IDDM. Changes in the cortisol-cortisone shuttle towards cortisol could contribute to abnormal sodium retention in IDDM. Alternatively, a shift towards cortisone could attenuate sodium retention.

**Treatment of diabetic nephropathy**

**Arterial hypertension**

Arterial hypertension importantly contributes to the progression of proteinuria and the loss of renal function in IDDM patients with nephropathy, and many studies have demonstrated that blood pressure lowering reduces proteinuria and slows the rate of decline in GFR [28,229-233]. Even slight elevations in blood pressure have been shown to increase microalbuminuria in IDDM patients [9,39,42,112,234]. These findings have resulted in the recommendation to start antihypertensive treatment in microalbuminuric IDDM patients when blood pressure values exceed 140/90 mmHg [235,236] or even 130/85 mmHg [238]. It is, however, controversial whether one specific class of antihypertensive agent is more effective than another. β-blockers [229,230,232], peripheral vasodilators [229,230,302], diuretics [229,230,232] and ACE-inhibitors [231,233] have all been shown to effectively retard the decline in GFR in patients with overt nephropathy. Trials that compared the effectiveness of β-blockers and ACE inhibitors have shown inconsistent results [238,239]. ACE-inhibition was found to be more effective
than β-blockade, but in this study blood pressure reduction was better with the ACE-inhibitor [238]. Another study could not demonstrate a benefit of ACE-inhibition over β-blockade, but in that study baseline proteinuria was lower in the β-blocker group [239]. Similar studies in non-diabetic renal disease either showed an increased [240] or no difference in effectiveness of ACE-inhibitors as compared to β-blockers [152]. Interestingly, the latter study showed that the I/D polymorphism of the ACE gene was a determinant of the renal protective outcome.

There are theoretical advantages of ACE inhibitors that are attributable to additional renoprotection as these agents have been documented to lower intraglomerular pressure in animal studies [82, 83]. Also, such a role for ACE inhibitors has been favoured in IDDM patients with DN by demonstrating that captopril added to other antihypertensive medications reduced the rate of renal function loss that could not be attributed to its blood pressure lowering effects alone [241]. Furthermore, several meta-analyses comparing ACE-inhibition treatment with other antihypertensive therapies pointed out that ACE-inhibitors induced larger reductions in proteinuria beyond their blood pressure lowering effect [242, 243].

So, should drugs interfering with the RAAS such as ACE-inhibitors and angiotensin II-antagonists be preferred, or will any rigorous blood pressure reduction, irrespective of the choice of drug, be sufficient to treat DN? It is important to realise that a clinical trial comparing the effects on renal failure and mortality of these drugs has never been performed, but it is highly questionable whether such a study will ever be undertaken. The lesson so far learned is that blood pressure should be rigidly lowered, since patients with the largest blood pressure reduction will benefit the most [9]. ACE inhibitors have unequivocally demonstrated to be effective and have gained an important place in the treatment of DN [231, 233, 238, 241]. As these drugs have also been shown to arrests or delay progression of microalbuminuria [19-22], these patients should also be treated. Addition of diuretics may be useful to oppose the abnormal sodium retention in IDDM [213, 244]. However, the potential advantage of β-blockers should not be overlooked because of proven secondary prevention of cardiovascular disease in non-diabetic subjects. Theoretically, calcium-antagonist may also have some advantages by modifying vascular reactivity to various endogenous pressor agents [245].

Although hypertension has been recognised as important prognostic indicator of renal function loss and blood pressure lowering therapy has proven renoprotective effectiveness, many patients still progress to end stage renal disease [229-233, 241, 246, 247]. A number of post-hoc analyses on clinical trials in patients with renal disease of various origin, showed that apart from blood pressure lowering, the severity of proteinuria was correlated with a worse prognosis with respect to long-term renal function [246, 247]. Similar findings have been reported in patients with DN, in whom a relative large initial fall in albumin excretion after β-blockers [248] and ACE-inhibitors [249] predicted a slower rate of decline in GFR. These studies suggest that clinical proteinuria is not only a marker of renal disease, but could also be a pathogenetic factor in the process of glomerulosclerosis [250]. If it is true that insufficient reduction in proteinuria and substantial residual proteinuria indicate a poorer prognosis, a more aggressive antiproteinuric therapy may possibly increase long-term renal function outcome in patients with renal disease. The advocated stringent blood pressure targets [235-237] should then be individualised on the initial decline in
proteinuria [251,252]. It is, however, unknown whether this is also true for protein excretion rates in the microalbuminuric range.

**Metabolic control**

Although not being the primary objective, the DCCT unequivocally showed that intensive insulin treatment reduces the occurrence of microalbuminuria in IDDM patients [17,18]. In short, 1441 IDDM patients were allocated to either intensive or conventional insulin treatment. The goal of intensive therapy was to achieve near normal blood glucose levels. The mean HbA1c level reached was 7.2%, which was approximately 2% lower than that in the conventionally treated IDDM patients. Mean follow-up was 6.5 years (range 3 to 9 years). Most patients (n=1365) had a normal albumin excretion rate at baseline. In these patients, the estimated 9 year cumulative incidence in persistent microalbuminuria was 10% and 26% in the intensive and conventional insulin treatment groups, respectively, indicating a reduction in risk of development of microalbuminuria of 60%. In 6% and 15% of these patients, respectively, microalbuminuria progressed to levels above 70 µg/min. In all patients, including those with microalbuminuria at baseline, the calculated 9-year cumulative incidence in clinical proteinuria was 3% and 7% with intensive and conventional treatment, respectively. This indicates a risk reduction in the development of overt nephropathy of 51% [17]. In a separate analysis of the 73 patients with microalbuminuria at baseline, of which 38 were assigned to intensive therapy and 35 to conventional therapy, no difference in progression to clinical proteinuria was seen, which occurred in 8 patients of each group [17].

The DCCT demonstrates that intensified insulin treatment reduces the risk of development of microalbuminuria, and supports the role of hyperglycaemia in the pathogenesis of incipient nephropathy [58-72]. Accordingly, a meta-analysis of 16 studies showed that lowering blood glucose levels retarded progression urinary albumin excretion in normo- and microalbuminuric IDDM patients [253]. It should be noted that the lack of effect of intensive insulin therapy on the progression of microalbuminuria to overt nephropathy in the DCCT [17], was also found in a British collaborative study [254]. Moreover, it is controversial whether improved metabolic control slows the rate of decline in GFR in patients with established DN [255-257]. Some reports have suggested that glycaemic control loses its significance as a risk factor in established nephropathy [255,256], but these result have been challenged by the positive correlation between HbA1c levels and the rate of decline in GFR in patients with clinical nephropathy [257]. It is conceivable that patients with overt DN will also benefit from strict metabolic control, although this will not reverse established renal disease [258,259]. Obviously, substantial longer observation periods are required to determine whether sustained blood glucose lowering as achieved in the DCCT will prevent or delay renal failure over 10 to 20 years.

What degree of glycaemic control should be aimed for to minimise the risk of complications? The major burden in managing IDDM patients to maintain blood glucose in the normal range is the risk of hypoglycaemia is [260,261]. This is illustrated by the 3 fold increase in severe hypoglycaemic episodes as well as more nocturnal hypoglycaemia and hypoglycaemia unawareness in the intensive insulin treated group of the DCCT [16,17]. The investigators of the DCCT recommended HbA1c levels of 7 to 8% since such
a treatment goal would achieve a maximum benefit-to-risk ratio. Another report postulated, in contrast, that no clinical benefit results from a decrease of the HbA1c level below 8.1%, since only higher HbA1c levels were associated an exponential increase in the risk of developing microalbuminuria [262]. These authors proposed the presence of a glycaemic threshold for the risk of nephropathy. However, a post-hoc analysis of the DCCT did not support the existence of a glycaemic threshold, but in fact showed a curvilinear relationship between the HbA1c level and the logarithm of the risk of microalbuminuria development [263]. Nonetheless, inherent to such a logarithmic relation, the reduction in risk is greatest when the highest HbA1c values are reduced. Weighing this risk of microalbuminuria against that of hypoglycaemia, and aware of the view that elevated blood glucose levels alone do not cause nephropathy, but that haemodynamic and genetic factors are also involved, it has been proposed that achievement of an HbA1c level below 8.1% is a reasonable primary prevention strategy [264]. It was argued that continuous monitoring will identify those patients in whom, despite improved glycaemic control, progression of microalbuminuria takes place. These patients might then benefit from early aggressive blood pressure lowering therapy.

Dietary intervention

Protein intake modulates renal function [265]. In humans, GFR and ERPF acutely increase after an amino acid infusion and an oral protein load [265,266]. Experimental studies have shown that a high dietary protein intake contributes to a rise in the intraglomerular pressure and GFR, and have documented that protein restriction ameliorates -glomerular hypertension and hyperfiltration, and retards the progression of renal function impairment [77,267]. These studies have led to the suggestion that protein restriction could be of clinical benefit in IDDM patients with nephropathy.

Only a few clinical studies addressed the effects of protein restriction in IDDM patients with (incipient) nephropathy [234,268-271]. These studies demonstrated that a low protein diet decreased albuminuria, but variably affected the rate of decline in GFR which was either found to be unchanged [234,268,271] or indeed retarded by protein restriction [269, 270]. A meta-analysis of these studies showed that the relative risk of progression of albuminuria and decline in GFR was evidently lower in IDDM patients using a low protein diet compared with patients consuming a usual-protein diet [272]. The analysis also revealed that these favourable effects were not confounded by differences in blood pressure or metabolic control [272]. Accordingly, it is likely that IDDM patients benefit from a restriction in their daily protein intake. For this reason, the American Diabetes Association has proposed to reduce dietary protein intake to 0.8 g/kg per day. It should be noted that such a protein restriction is difficult to maintain. In fact, a protein intake of 0.8 g/kg per day was on average achieved in Dutch IDDM patients following intensive dietary counselling [234]. Thus, the effectiveness of a low protein diet may be limited by suboptimal compliance. Clearly, there is currently no place to recommend -protein rich diets to IDDM patients.

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Introduction


**Purpose of the thesis**

This thesis aims to answer to following questions:

1. Does an increase in plasma NE levels contribute to the exercise-induced rise in albuminuria and are there differences between normo- and microalbuminuric IDDM patients and healthy subjects?
2. Are ambient plasma NE levels related to renal haemodynamic parameters in normo- and microalbuminuric IDDM patients and in healthy subjects?
3. Do exogenous NE infusions, given at doses that induce predetermined rises in blood pressure, cause a microproteinuric response in normo- and microalbuminuric IDDM patients and healthy subjects? What are the determinants of such a putative microproteinuric response? Are differences in renal haemodynamic NE responsiveness responsible for possible differences in NE-stimulated microproteinuria among these subjects?

4. Does ACE-inhibition treatment attenuate the NE-induced blood pressure rise and renal vasoconstriction in microalbuminuric IDDM patients.

5. Does low dose dopamine oppose NE-induced renal vasoconstriction in healthy subjects?

6. Are abnormal GH-IGF-I levels, as encountered in (un)treated acromegalic patients and GH deficient patients, associated with differences in urinary protein excretion as compared to healthy subjects?

7. Is renal functional reserve as assessed by amino acid infusion inversely related to baseline renal haemodynamic parameters among GH deficient patients, healthy subjects, patients with (un)treated acromegaly, and normo- and hyperfiltering IDDM patients? Are basal renal haemodynamic parameters positively related to the plasma IGF-I levels in these subjects?

7. Do IDDM patients with an elevated GFR have an exaggerated GH-response after exercise and a higher plasma glucagon level and as compared with IDDM patients with a normal GFR? Is exercise-stimulated GH secretion and the glucagon level related to GFR, ERPF and kidney size in IDDM patients?

8. Is an increased urinary IgG excretion a genuine feature of normoalbuminuric IDDM patients or are laboratory artefacts responsible for this thus far unexplained observation?

9. Are there differences in urinary cortisol and cortisone metabolites in normo- and microalbuminuric IDDM patients compared to healthy subjects? Does this indicate a shift in the so-called cortisol-cortisone shuttle towards cortisol that could be involved in sodium retention and volume expansion in IDDM? Does ACE inhibition treatment influence the cortisone-cortisol shuttle in microalbuminuric IDDM patients?