CHAPTER 12
SUMMARY AND CONCLUSIONS

Chapter 1 outlines the epidemiology, the functional stages, the pathogenesis and therapeutic aspects of renal disease in patients with insulin-dependent diabetes mellitus (IDDM). Several aspects of the pathogenesis of diabetic nephropathy (DN) are more extensively overviewed in sections on 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 haemodynamics in opposite ways: NE causes renal vasoconstriction, the GH-IGF-I-axis induces renal vasodilatation. Since the early stages of diabetic renal involvement are characterised by an imbalance in glomerular vasodilation and vasoconstriction, the possible role of these humoral systems in diabetic nephropathy (DN) is discussed. The role of 11β-hydroxysteroid dehydrogenase (11β-HSD) in protecting the mineralocorticoid receptor from activation by cortisol is briefly recapitulated in the context of abnormalities in sodium and volume homeostasis in IDDM.

Effects of NE on renal protein handling and renal haemodynamics.

In chapter 2, the relationships between plasma NE and the rise in albuminuria after a fixed exercise test is evaluated in normo-and microalbuminuric IDDM patients and healthy subjects. Physical exercise provides a strong sympathetic stimulus and can lead to an increase in urinary albumin excretion. NE is both a neurotransmitter and a hormonally active substance spilled over from the sympathetic nervous system. Plasma NE levels may influence renal function by vasoconstriction mediated via \( \alpha \)-adrenoceptors, which have been located along glomerular arterioles. The albuminuric response after exercise is thought to result from an enhanced glomerular passage of macromolecules in conjunction with rises in systemic blood pressure and alterations in renal haemodynamics.

Moderate strenuous exercise was found to induce an exaggerated rise in albuminuria in both IDDM groups, in keeping with earlier reports. Blood pressure rose to higher levels in the microalbuminuric IDDM patients. The rise in plasma NE levels was significantly greater in normo- and microalbuminuric IDDM patients than in healthy subjects. Multiple regression analyses revealed that both elevations in blood pressure and stimulated plasma NE levels independently contributed to the albuminuric response.

Exercise and exogenous NE induce comparable renal haemodynamic changes in humans. Both are associated with a decrease in effective renal plasma flow (ERPF) without much change in glomerular filtration rate (GFR). Consequently, filtration fraction rises which suggests a change in pressure profile along glomerular vessels, in favour of an increase in intraglomerular pressure. Indeed, in experimental studies NE increases intraglomerular pressure. Thus, the relationship between changes in plasma NE concentrations and changes in albuminuria during exercise support the hypothesis
that NE is involved in the albuminuric response by a renal haemodynamic mechanism, like a rise intraglomerular pressure. The observations also suggest that an enhanced catecholamine response may contribute to an exaggerated rise in albuminuria in microalbuminuric IDDM patients. From these experiments no conclusion can be drawn whether an altered renal vascular responsiveness to NE in IDDM is involved in this phenomenon.

In chapter 3, the possible relationships between circulatory NE levels and renal haemodynamic parameters are investigated in normo- and microalbuminuric IDDM patients and in healthy subjects. Both GFR and ERPF were higher in IDDM patients compared to healthy subjects. GFR and ERPF were found to be inversely correlated with venous plasma NE levels. No differences were observed in the relationships between plasma NE and ERPF between the IDDM and healthy subjects. The slightly lower plasma NE levels in the IDDM patients could thus contribute to the elevations in ERPF. GFR was negatively related to plasma NE and positively with the presence of IDDM. This supports the notion that concomitant vasodilating mechanisms play a role in the elevations of GFR in IDDM patients. These results suggest that circulatory NE is a determinant of renal haemodynamics both in IDDM patients and healthy subjects.

In chapter 4, a randomized, placebo controlled NE infusion experiment is undertaken in matched groups of normo- and microalbuminuric IDDM patients and healthy subjects. Microproteinuria, renal and systemic haemodynamic responses were measured during stepwise exogenous NE infusions at individually determined NE threshold, 20% pressor and pressor doses. The study addressed the following questions. First, does exogenous NE induce a microproteinuric response? Second, is there a difference in microproteinuric response in normo- and microalbuminuric IDDM patients and healthy subjects? Third, what are the determinants of such a microproteinuric response? Fourth, are there differences in renal haemodynamic NE-responsive ness between these groups?

Exogenous NE was found to increase microproteinuria in conjunction with a rise in systemic blood pressure and renal vasoconstriction in all groups. NE increased urinary albumin and IgG excretion, but no effect was seen on urinary β2-microglobulin excretion. This indicates that NE increases glomerular protein leakage. Furthermore, the absolute microproteinuric response was more pronounced in microalbuminuric IDDM patients than in normoalbuminuric IDDM patients and healthy subjects. Multiple regression analysis showed that the increase in microproteinuria was not only related to the rise in systemic blood pressure induced by the NE infusions, but also to the increase in plasma NE level itself. The renal haemodynamic NE responsiveness (i.e. a fall in ERPF and rise in filtration fraction) was similar in the groups.

This study is the first to demonstrate that a vasopressor agent causes an increase in microproteinuria. These results disagree with previous studies using angiotensin II. Our findings support the hypothesis that an intrarenal mechanism contributes to the NE-induced increase in microproteinuria, and demonstrate that a low dose of NE causes glomerular vasoconstriction. This is in accord with the role of circulatory NE in the albuminuric response following exercise, and with the relationship of plasma NE with renal haemodynamics as outlined in the preceding chapters. The enhanced microproteinuric response in microalbuminuric IDDM is likely to be the result of a glomerular
permselectivity defect. Plasma NE rises during strenuous daily life activities and this may contribute to the perpetuation of microproteinuria. Proteinuria itself is a determinant of future loss of renal function, although it is still unknown if this also holds true for the microalbuminuric phase of diabetic renal disease. It can, therefore, be argued that protection against the renal NE effects may be of clinical benefit.

In chapter 5, the possibility that treatment with the ACE-inhibitor, enalapril, attenuates systemic and renal haemodynamic NE responsiveness in microalbuminuric IDDM is investigated. Such an effect would be of particular relevance in microalbuminuric IDDM patients, since systemic NE responsiveness has been found to be exaggerated, and strict blood pressure control has been shown to prevent or delay progression of albuminuria in these patients.

Enalapril was found to lower systemic blood pressure and overnight urinary albumin excretion, and to increase ERPF. The blood pressure lowering effect of enalapril disappeared with NE pressor infusion. The overall mean increase in blood pressure in response to NE was even higher with than without enalapril. ERPF remained elevated during NE infusion with enalapril treatment, and the NE-induced fall in ERPF was unaltered by enalapril. Urinary albumin excretion was similar during the NE infusions before and after enalapril treatment. These results are in keeping with earlier reports in patients with non insulin-dependent diabetes mellitus, but contrast with findings in patients with essential hypertension. The lack of effect of ACE inhibition treatment on the systemic and renal effects of NE may have clinical implications for the design of renoprotective strategies in IDDM patients.

In chapter 6, the possibility that low dose dopamine infusion counteracts NE-induced renal vasoconstriction is investigated. Although low dose dopamine is widely used to attenuate the decrease in renal haemodynamics during NE infusion therapy, this effect has not been proven in humans. Dopamine (4 µg/kg per min) was added to incremental doses of NE in normotensive healthy subjects. This dose of dopamine was shown to prevent the fall in ERPF during NE infusion. Dopamine also attenuated the rise in blood pressure, enlarged pulse pressure, blunted the fall in heart rate, and induced a large natriuretic response. Thus, dopamine is indeed able to oppose NE-induced renal vasoconstriction. They also indicate that dopamine influences systemic haemodynamics during NE infusion. Further studies are warranted to confirm these findings in critically ill patients, and to establish whether low dose dopamine infusion is able to improve their clinical outcome. The latter is of particular relevance since a recent study reported disappointing effects of low dose dopamine on prevention of renal failure in this patient category.

Renal effect of the GH-IGF-I-system

In chapter 7, it is investigated whether abnormal GH and IGF-I levels influence urinary albumin excretion. GH deficient patients, patients with (un)treated acromegaly and healthy subjects were compared. Urinary albumin excretion rate was shown to be elevated in acromegalic patients and tended to be reduced in GH deficient patients as compared to healthy subjects. Moreover, GH and IGF-1 lowering by treatment with the
somatostatin analogue, octreotide, reduced albuminuria in the acromegalic patients. The level of albuminuria was positively correlated with the GH and IGF-I level. These findings support the notion that the GH-IGF-I system is involved in urinary protein excretion. Since renal insufficiency is uncommon in acromegaly, it is unlikely that GH and IGF-I elevations alone predispose to clinically important glomerular damage.

In chapter 8, baseline and amino acid-stimulated GFR and ERPF are compared in GH deficient, acromegalic and normo- and hyperfiltering IDDM patients as well as in healthy subjects. Moreover, the possible relationship between plasma IGF-I levels and renal haemodynamics were evaluated across these groups.

Baseline GFR and ERPF were shown to covary with GH status: the lowest values were found in the GH deficient patients followed by higher levels in the healthy subjects, treated and untreated acromegalic patients. The amino acid-induced increase in GFR and ERPF was enhanced in the GH deficient patients and was abolished in the acromegalic and hyperfiltering IDDM patients. Taken all groups together, an inverse relationship was found between baseline GFR and ERPF and the amino acid-induced increment in GFR and ERPF. This indicates the renal reserve filtration capacity is exhausted in glomerular hyperfiltration, and suggest that hyperfiltering IDDM and acromegalic patients share comparable renal haemodynamic abnormalities.

The plasma level of IGF-I was a determinant of GFR and ERPF across the GH deficient, acromegalic and healthy subjects, but not in the IDDM groups. The latter does not exclude a role of abnormalities in the GH-IGF-I system in glomerular hyperfiltration associated with IDDM. Enhanced glomerular IGF-I accumulation due to increased IGF-I receptor expression, alterations in local production of IGF-binding proteins and in IGF-binding protein 3 protease activity, could affect renal haemodynamics in IDDM. Obviously, IGF-I infusion experiments are required to evaluate whether renal haemodynamic sensitivity to IGF-I is enhanced in IDDM. The very limited availability to clinical use of IGF-I currently does not enable us to carry out such experiments.

In chapter 9, an exercise test is used to stimulate GH physiologically in IDDM patients with a normal and elevated GFR. GFR and ERPF were measured under standardised conditions, and IDDM patients with glomerular hyperfiltration (GFR>130 ml/min per 1.73m²) were individually matched with IDDM patients with a normal GFR (90 to 130 ml/min per 1.73m²). Kidney size was measured by ultrasonography. The circulatory levels of glucagon and GH were determined on a separate day in the fasting state and after exercise.

The mean levels of these hormones were not significantly different in the hyper- and normofiltering IDDM patients. However, multiple regression analysis showed that exercise-stimulated GH levels, circulatory plasma glucagon as well as HbA1c were significantly related to renal haemodynamic parameters and kidney size. These findings support the hypothesis that stimulated levels of GH and circulating glucagon contribute to glomerular hyperfiltration in IDDM. In contrast, previous studies showed that diurnal GH and glucagon profiles were not different in normo- and hyperfiltering IDDM patients. These discrepancies may due to the lack of use of stimulated GH levels in those studies, and to the definition of glomerular hyperfiltration.
From the studies described in the chapters 7, 8 and 9, we conclude that the GH-IGF-I system plays a role in renal haemodynamics and in glomerular protein handling in various disease states in humans, including GH deficiency and GH excess, as well as in IDDM. From a therapeutic point of view, it will be of interest to determine whether somatostatin analogues prevent progression of albuminuria and loss of renal function in IDDM patients. Such intervention could have a place as an adjunct to or as an alternative for blood pressure lowering therapy. The availability to clinical use of long acting somatostatin analogues will facilitate the evaluation of such treatment.

Urinary IgG excretion in normoalbuminuric IDDM

Chapter 10 describes the artefacts that can be encountered when urinary IgG is measured at low concentrations. It has been reported that urinary IgG excretion is increased in normoalbuminuric IDDM patients, but this phenomenon is not well understood. In normo- and microalbuminuric IDDM patients and healthy subjects, urinary IgG was measured in samples that were kept frozen for 2 to 4 weeks. Urinary IgG excretion was higher both in normo- and microalbuminuric IDDM patients compared to healthy subjects. Furthermore in IDDM patients, the IgG clearance divided by the albumin clearance was found to be higher in urine collections that contained glucose as compared to samples without glucose. This raised the possibility that glucose influences urinary IgG concentration, possibly by a preserving effect of glucose during storage.

In a laboratory experiment, the effects various storage procedures were evaluated in urine samples with different amounts of protein. Urinary IgG declined when samples were frozen for several weeks without precautions. The best results were obtained when urine was stored frozen with addition of bovine serum albumin, phosphate buffer and high concentrations of glucose. These results indicate that glucose in urinary specimens of IDDM patients can in fact prevent the decrease in IgG, and may thus explain the apparently higher urinary IgG excretion in normoalbuminuric IDDM patients when unprocessed urine is stored frozen before assay. This study indicates that precautions should be taken when urinary IgG cannot be measured immediately.

Sodium and volume homeostasis in IDDM and the role of $11\beta$-HSD

In chapter 11, urinary cortisol and cortisone metabolites are evaluated in normo- and microalbuminuric IDDM patients and in healthy subjects. The primary objective was to establish whether possible abnormalities in cortisol metabolism as a consequence of an altered $11\beta$-HSD enzyme activity, are involved in the abnormal sodium and fluid retention in IDDM patients. $11\beta$-HSD catalyses the interconversion of cortisol and its inactive metabolite, cortisone, and thereby protects the mineralocorticoid receptor from being activated by cortisol. A change in the so-called cortisol-cortisone shuttle towards cortisol could lead to sodium retention and volume expansion in IDDM. Alternatively, a change towards cortisone could attenuate sodium retention.

Lower urinary excretion rates of cortisol and cortisone metabolites were found in normo- and microalbuminuric IDDM patients suggesting that the diabetic state influences cortisol metabolism via an impaired reduction of glucocorticoids. Furthermore, the urinary cortisol to cortisone metabolite ratio was lower in IDDM. This indicates
that the set-point of overall direction of the 11ß-HSD catalysed cortisol to cortisone inter-conversion is shifted towards cortisone. In the IDDM patients, the urinary cortisol to cortisone metabolite ratio was inversely related to the HbA1c level. A positive relation between the urinary cortisol to cortisone metabolite ratio and initial blood volume, as a measure of whole body extravascular and blood volume, was found both in healthy subjects and in IDDM patients. Interestingly, the regression line was between these parameters was shifted leftwards in the IDDM patients. This indeed suggests that the cortisol to cortisone ratio is a determinant of volume homeostasis, but essentially excludes the possibility that the an abnormal 11ß-HSD activity is primarily responsible for an abnormal fluid and sodium retention in IDDM. Finally in microalbuminuric IDDM patients, ACE-inhibition treatment was shown to induce a modest further lowering of the cortisol to cortisone metabolite ratio.

These results raise the possibility that altered cofactor availability as consequence of chronic hyperglycaemia influences glucocorticoid reduction and 11ß-HSD activity in humans. Improved metabolic control could induce a backward shift of the cortisol-cortisone shuttle towards cortisol in IDDM patients, which would accentuate volume and sodium homeostasis. Moreover, this study suggests that stimulation of 11ß-HDS activity may be an additional mechanism whereby ACE inhibitors promote saliuresis.