Hormonal influence on renal function with particular reference to diabetes mellitus
Hoogenberg, Klaas

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HORMONAL INFLUENCE ON RENAL FUNCTION WITH PARTICULAR REFERENCE TO DIABETES MELLITUS

Klaas Hoogenberg
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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 \(11\beta\)-hydroxysteroid dehydrogenase (\(11\beta\)-HSD) in protecting the mineral-ocorticoid receptor from activation by cortisol is briefly recapitulated.

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\(^{1}\) 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

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| 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
Introduction

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),
   - polyl 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 isoform 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 NADP⁺ 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 $\text{GFR} = k_f (\Delta P_c - \Delta \pi)$, net hydraulic pressure ($\Delta P_c$), net oncotic pressure ($\Delta \pi$), filtration surface area and hydraulic permeability ($k_f$) 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 hyperfiltrating 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 chronical 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 pathogenic 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 α-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, α-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 simulates 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{G}-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 enlargement 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 11\$\beta\$-hydroxysteroid dehydrogenase (11\$\beta\$-HSD) [224]. Two isoforms have been identified. 11\$\beta\$-HSD\textsubscript{1} is present in the liver. This enzyme is NADH/NAD\textsuperscript{+} dependent and catalyses the interconversion between cortisol and cortisone. 11\$\beta\$-HSD\textsubscript{2} is expressed in the kidney and unidirectionally catalyses the oxidation of cortisol to its inactive compound, cortisone. This isoenzyme is NADP\textsuperscript{+} dependent [225]. Thus, there is a link between cortisol metabolism and the regulation of volume and sodium homeostasis. For instance, genetic mutations in 11\$\beta\$-HSD\textsubscript{2} 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 glycerrhetinic acid ingestion [228].

It is unknown whether 11\$\beta\$-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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Chapter 1


**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?

8. 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?

9. 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?
CHAPTER 2
ABNORMAL PLASMA NOREpinePhrine RESPONSE AND EXERCISE-INDUCED ALBUMINURIA IN IDDM PATIENTS

K. Hoogenberg and R.P.F. Dullaart

Submaximal exercise provokes an abnormal elevation in albuminuria in patients with IDDM. Plasma catecholamines might be involved in this phenomenon by a renal vasoconstrictive effect. Twelve healthy subjects (Controls: albuminuria < 10µg/min), 13 normoalbuminuric IDDM patients (DNormo: albuminuria < 10µg/min) and 13 microalbuminuric IDDM patients (DMicro: albuminuria 10-200µg/min) performed a fixed bicycle workload (600 kpm for 20 min + urine collection 40 min postexercise). None of the patients suffered from autonomic neuropathy or hypertension. Fractional albumin clearance (FalbCl) rose in DNormo (p = 0.02) and DMicro (p = 0.01) but not in the Controls (p = 0.40). Basal plasma epinephrine and norepinephrine (NE) were not different in the three groups. The increments in NE were more pronounced in DNormo and DMicro than in Controls (Controls < DNormo, p < 0.05; Controls < DMicro, p < 0.01). The changes in FalbCl were significantly correlated with the changes in NE (all subjects r = 0.65, p < 0.001). The increments in epinephrine were not different in the IDDM groups compared to the controls, and were not related to the changes in FalbCl. Multiple regression analysis showed that changes in plasma NE (p < 0.002) and in mean arterial pressure (MAP, p < 0.005) independently contributed to the changes in FalbCl (multiple R = 0.73). It is concluded that the exercise-induced plasma NE response is increased in normo- and microalbuminuric IDDM patients. NE appears to contribute in the exercise-induced changes in renal protein handling, possibly by its effect on renal haemodynamics.

Introduction
Submaximal exercise has been reported to result in a marked elevation of albuminuria in patients with insulin-dependent diabetes mellitus (IDDM) with either normal or slightly elevated urinary albumin excretion at rest [1-7]. This albuminuric response is thought to be related to an increased glomerular passage of macromolecules, and possibly indicates an early impairment in the glomerular filtration barrier in IDDM [8]. Changes in tubular protein reabsorption are not considered to be of great importance during moderately strenuous exercise since the excretion of β2-microglobulin, a marker of tubular protein handling, does not increase under these circumstances [2,3].

The mechanisms by which exercise leads to a pronounced increase in albuminuria in IDDM patients are still poorly understood. During exercise the effective renal plasma flow (ERPF) decreases more than the glomerular filtration rate (GFR). Consequently, the filtration fraction i.e. the GFR divided by the ERPF increases [4,6,9]. A rise in the
intraglomerular pressure, which is assumed to be reflected by this increase in filtration fraction [9], might be an important factor that contributes to the enhanced excretion of urinary albumin. An abnormal rise in systolic blood pressure has also been implicated in the exercise induced rise in albuminuria in IDDM particularly in conjunction with microalbuminuria [5], although this has not always been reported [2,6]. Studies in the rat have shown that norepinephrine (NE) increases renal vascular resistance predominantly via efferent glomerular vasoconstriction [10]. Both epinephrine and NE increase renal vascular resistance in humans [11-13]. It has therefore been proposed that the renal haemodynamic changes during exercise are related to increased circulatory levels of plasma catecholamines [9,14]. By a renal haemodynamic mechanism, plasma catecholamines thus could possibly affect renal protein handling during exercise in IDDM patients.

The purpose of the present study was to determine whether an altered plasma catecholamine response could play a role in the exaggerated rise in albuminuria in IDDM with normo- and microalbuminuria during moderately strenuous exercise.

Subjects and methods

Subjects

All subjects consented to the procedure after explanation of the purpose of the study, which was approved by the local medical ethics committee. Three groups of subjects were investigated (Table 1): 12 healthy control subjects (urinary albumin excretion rate (Ualb.V < 10 µg/min, Controls); 13 IDDM patients with normoalbuminuria (Ualb.V < 10 µg/min, DNormo), 13 IDDM patients with microalbuminuria (Ualb.V ranging from 10 to 200 µg/min, DMicro).

Ualb.V was determined in three consecutive overnight urine collections obtained within three months prior to the study. Three urine samples were used to classify the IDDM patients because of the high variability of Ualb.V [15]. A level of Ualb.V above 10 µg/min was considered to be abnormal (above the 97.5 percentile for 50 healthy controls subjects). Urinary tract infection was excluded by bacterial culture. All IDDM patients suffered from ketosis prone diabetes mellitus and were considered to be insulin-dependent on clinical grounds. All subjects had a serum creatinine level below 120 µmol/l and were normotensive (systolic blood pressure ≤140 mmHg and diastolic blood pressure ≤90 mmHg). No medication other than insulin or oral contraceptives were allowed. The IDDM patients did not have autonomic neuropathy as determined by Valsalva manoeuvre, beat to beat variation during deep breathing and blood pressure response to standing [16]. A questionnaire showed no differences in physical activity among the three groups. As is shown in Table 1 the three groups were closely comparable in age. There were no significant differences in sex distribution. The two IDDM groups were comparable with respect to duration of disease, metabolic control and retinopathy. All subjects were studied in the fasting state while the morning insulin dose was withheld. Blood pressure was measured using a sphygmomanometer (Baumanometer, W.A. Braun Inc, N.Y., USA). Korotkoff phase 5 was taken as the diastolic blood pressure. Blood pressure and pulse rate were measured at 5 min intervals
Table 1. Clinical characteristics of the study groups.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=12)</th>
<th>DN normo (n=13)</th>
<th>DMicro (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25±3</td>
<td>26±6</td>
<td>27±5</td>
</tr>
<tr>
<td>Duration of disease (years)</td>
<td>--</td>
<td>10±4</td>
<td>12±5</td>
</tr>
<tr>
<td>Sex (males/females)</td>
<td>6/6</td>
<td>5/8</td>
<td>10/3</td>
</tr>
<tr>
<td>Ualb.V (µg/min)(^a)</td>
<td>3.1 (1.6-7.8)</td>
<td>5.1 (2.6-9.8)</td>
<td>21.3 (10.2-166.7)(^c)</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>79±8</td>
<td>76±12</td>
<td>89±17(^d)</td>
</tr>
<tr>
<td>Overnight creatinine clearance (ml/min per 1.73m(^2))</td>
<td>109±27 110±29</td>
<td>102±23(^d)</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>21.6±1.7</td>
<td>22.9±2.1</td>
<td>23.4±2.3</td>
</tr>
<tr>
<td>HbA(_1) (%)</td>
<td>5.3±10.4(^e)</td>
<td>7.7±1.6</td>
<td>7.6±1.2</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>4.6±0.4(^e)</td>
<td>11.9±4.7</td>
<td>12.1±5.2</td>
</tr>
<tr>
<td>Retinopathy (O/B/P)(^b)</td>
<td>--</td>
<td>11/1/1</td>
<td>6/4/3</td>
</tr>
<tr>
<td>Systolic blood pressure change to standing (mmHg)</td>
<td>1 (-11 to 12)</td>
<td>3 (-12 to 15)</td>
<td>1 (-13 to 12)</td>
</tr>
<tr>
<td>Beat to beat variation (beats/min)</td>
<td>35±7</td>
<td>39±13</td>
<td>32±12</td>
</tr>
<tr>
<td>Valsalva ratio</td>
<td>1.76±0.28</td>
<td>1.72±0.28</td>
<td>1.78±0.32</td>
</tr>
</tbody>
</table>

Controls: control subjects; DN normo: IDDM patients with Ualb.V<10 µg/min; DMicro: IDDM patients with Ualb.V>10 µg/min and <200 µg/min. \(^a\) Ualb.V: urinary albumin excretion rate; \(^b\) Retinopathy: O: absent; B;background; P:proliferative. Values are given in mean±SD, except Ualb.V and systolic blood pressure change to standing which are given in median(range); \(^c\) denotes \(p<0.001\) from Controls, DN normo; \(^d\) denotes \(p<0.05\) from DN normo; \(^e\) denotes \(p<0.01\) from DN normo, DMicro.

the exercise and the recordings were averaged for analysis. Mean arterial pressure (MAP) was calculated as \(\%\) diastolic + \(\%\) systolic blood pressure.

Experimental design

The exercise protocol of Mogensen [1,2] was used, except that the subjects exercised only at 600 kpm for 20 min. The subjects drank 250 ml water per 20 min throughout the test from 0800 hours until 1200 hours to promote diuresis. Before the exercise the subjects were sitting for three hours. Urine was collected at 20 min intervals from 1000 hours until 1200 hours, including two postexercise collections. The exercise was performed on a bicycle ergometer from 1100 hours until 1120 hours. Blood samples were taken at regular intervals from a cannula inserted into an antecubital vein which was kept patent with a saline drip. During the study, the fractional clearance of albumin (FalbCl), calculated as the albumin clearance divided by the creatinine clearance, was used instead of the Ualb.V. This allowed us to take account of differences in GFR between the individuals and possible changes in GFR during the test, to correct for
changes in serum albumin concentration due to exercise induced haemoconcentration [17], and for possible errors in urine collection due to incomplete bladder emptying [15]. It was assumed that urinary albumin excretion had returned to baseline levels after two hours water loading [18] and so the 20 min urine collection directly before the exercise test was used as the reference period. $\text{FabCl}$ during the 20 min of exercise until 40 min thereafter was averaged for each subject to evaluate the effect of exercise.

Laboratory methods

Urine samples were stored at -20°C for a maximum of 2 weeks until analysis. Samples from each subject were determined in one run. Urinary albumin was measured using a commercially available double-antibody radioimmunoassay (Diagnostic Products Corporation, A peldoorn, The Nether-lands, cat no KHAD2). The lower detection limit was 0.07 mg/l. Serum albumin was measured on a SMA C autoanalyzer (Technicon Instruments Inc. Tarrytown, NY, USA). Urinary and serum creatinine were measured on SMA(C) auto-analyzers. Blood glucose was measured on a Yellow Springs glucose analyser (M odel 23A, Yellow Springs Inc., Yellow Springs, Ohio, USA). HbA1c was determined by colorimetry [19]. Plasma epinephrine and NE concentrations were measured using high-performance liquid chromatography as previously described [20].

Statistical analysis

Results are expressed as mean±SD for parametrically distributed data and as median (interquartile ranges) for non-parametrically distributed data. Comparisons of variables between groups were carried out using analysis of variance for parametrically and non-parametrically distributed data as appropriate. Changes of variables within groups were assessed by paired Student's t-tests or Wilcoxon tests. Adjustment for multiple comparisons was carried out using Duncan's method [21]. Differences in prevalence of clinical variables were analyzed by chi-square statistic. Correlations were sought using Spearman's rank correlation. Multiple regression analysis was used to disclose the independent contribution of parameters. $P$-values less than 0.05 were considered to be significant.

Results

Blood glucose

Blood glucose concentrations, measured directly before and after the exercise, were 4.5± 0.4 and 4.4± 0.6; 11.2± 3.1 and 11.7± 2.8; 11.1± 3.6 and 10.7± 4.2 mmol/l in Controls, DNormo and DMicro, respectively.

Blood pressure and pulse rate

Systolic and diastolic blood pressure were similar in the three groups at rest (Table 2). During exercise systolic blood pressure was higher in DMicro than in DNormo whereas diastolic blood pressure was lower in DNormo than in the other two groups. The increments in systolic blood pressure and MAP were larger in DMicro than in the other groups. The changes in Controls and in DNormo were similar. The pulse
rate was significantly higher in the two IDDM groups compared with the controls but the increment in response to exercise were comparable in all groups (Table 2).
Table 2. Blood pressure and pulse rate response to exercise.

<table>
<thead>
<tr>
<th>Blood pressure (mmHg)</th>
<th>preexercise</th>
<th>during exercise</th>
<th>increase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>systolic/diastolic</td>
<td>systolic/diastolic</td>
<td>systolic</td>
</tr>
<tr>
<td>Controls (n=12)</td>
<td>122±11 / 79±8</td>
<td>154±16 / 73±7</td>
<td>32±8(^a)</td>
</tr>
<tr>
<td>DNormo (n=13)</td>
<td>118±10 / 73±6</td>
<td>150±17 / 66±9(^c)</td>
<td>32±15(^a)</td>
</tr>
<tr>
<td>DMicro (n=13)</td>
<td>120±10 / 76±9</td>
<td>166±14(^b) / 74±8</td>
<td>46±15(^a,d)</td>
</tr>
</tbody>
</table>

Pulse rate (beats/min)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n=12)</td>
<td>67±11(^e)</td>
</tr>
<tr>
<td>DNormo (n=13)</td>
<td>79±9(^a)</td>
</tr>
<tr>
<td>DMicro (n=13)</td>
<td>80±10(^a)</td>
</tr>
</tbody>
</table>

Controls, DNormo, DMicro: see text and Table 1. MAP: mean arterial pressure. Data are given in mean±SD. \(^a\) denotes \(p<0.001\) from preexercise; \(^b\) denotes DMicro>DNormo, \(p<0.05\); \(^c\) denotes DNormo<Controls, DMicro, \(p<0.05\); \(^d\) denotes DNormo>DNormo, \(p<0.01\); \(^e\) denotes Controls<DNormo, DMicro, \(p<0.01\).

Table 3. Effect of exercise on fractional albumin clearance.

<table>
<thead>
<tr>
<th>Fractional albumin clearance (×10(^{-6}))</th>
<th>preexercise</th>
<th>exercise+postexercise</th>
<th>% of preexercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n=12)</td>
<td>0.69 (0.54-1.44)</td>
<td>0.82 (0.51-2.19)</td>
<td>111 (70-296)</td>
</tr>
<tr>
<td>DNormo (n=13)</td>
<td>0.75 (0.33-1.53)</td>
<td>2.04 (0.79-6.69)(^{a,e})</td>
<td>272 (87-1599)(^c)</td>
</tr>
<tr>
<td>DMicro (n=13)</td>
<td>2.99 (1.77-11.97)(^{c,d})</td>
<td>10.93 (3.38-26.25)(^{b,d,f})</td>
<td>215 (104-524)(^{f,g})</td>
</tr>
</tbody>
</table>

Controls, DNormo, DMicro: see text and Table 1. Data are given in median (interquartile ranges). \(^a\) denotes DNormo>Controls, \(p<0.05\); \(^b\) denotes DMicro>DNormo, \(p<0.05\); \(^c\) denotes DMicro>DNormo, \(p<0.01\); \(^d\) denotes DMicro>Controls, \(p<0.001\); \(^e\) denotes \(p=0.02\) from pre-exercise; \(^f\) \(p=0.01\) from pre-exercise; \(^g\) % change in DMicro>Controls, \(p<0.05\).

Fractional albumin excretion

In the preexercise period no difference in FalbCl could be demonstrated between DNormo and Controls, but FalbCl was significantly higher in DNormo than in Controls during the exercise+ postexercise period (Table 3). In DMicro, FalbCl was significantly higher compared with DNormo and Controls during both periods (Table 3). During the exercise + postexercise period, FalbCl increased in DNormo (\(p=0.02\)) and in DMicro (\(p=0.01\)) but not in Controls (\(p=0.40\)). The relative change in FalbCl, expressed as a percentage of the preexercise values, was greater in DMicro than in Controls (\(p<0.05\)).

Plasma norepinephrine and epinephrine concentrations

At baseline there were no differences in plasma epinephrine and NE concentrations among the three groups (Table 4). In all groups, plasma epinephrine and NE concentrations increased significantly in response to exercise. The exercise-induced increments in
Table 4. Plasma norepinephrine and epinephrine concentrations.

<table>
<thead>
<tr>
<th></th>
<th>preexercise 1000 hours</th>
<th>exercise 1100 hours</th>
<th>exercise 1120 hours</th>
<th>postexercise 1200 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Norepinephrine (nmol/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (n=12)</td>
<td>2.3 (2.0-3.7)</td>
<td>2.5 (2.0-4.3)</td>
<td>3.5 (2.7-5.1)</td>
<td>1.9 (1.2-2.5)</td>
</tr>
<tr>
<td>DNormo (n=13)</td>
<td>2.4 (1.4-3.1)</td>
<td>2.9 (1.9-3.3)</td>
<td>5.2 (3.7-6.7)</td>
<td>2.3 (1.7-2.9)</td>
</tr>
<tr>
<td>DMicro (n=13)</td>
<td>2.1 (1.6-2.3)</td>
<td>2.2 (1.5-3.1)</td>
<td>5.8 (4.4-6.6)</td>
<td>2.1 (1.6-2.4)</td>
</tr>
<tr>
<td><strong>Epinephrine (nmol/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (n=12)</td>
<td>0.14 (0.08-0.21)</td>
<td>0.11 (0.09-0.18)</td>
<td>0.40 (0.20-0.50)</td>
<td>0.16 (0.13-0.23)</td>
</tr>
<tr>
<td>DNormo (n=13)</td>
<td>0.13 (0.10-0.21)</td>
<td>0.12 (0.10-0.16)</td>
<td>0.30 (0.18-0.43)</td>
<td>0.15 (0.06-0.25)</td>
</tr>
<tr>
<td>DMicro (n=13)</td>
<td>0.10 (0.08-0.26)</td>
<td>0.14 (0.07-0.29)</td>
<td>0.88 (0.43-0.97)</td>
<td>0.18 (0.07-0.29)</td>
</tr>
</tbody>
</table>

Controls, DNormo, DMicro: see text and Table I. Data are given in median (interquartile ranges). 

\( ^a \) denotes \( p<0.05 \) and \( ^b \) denotes \( p<0.01 \) from preexercise; \( ^c \) denotes DNormo>Controls, \( p<0.05 \); \( ^d \) denotes DMicro>Controls, \( p<0.02 \); \( ^e \) denotes DMicro>Controls and DNormo, \( p<0.05 \).

plasma NE concentration were greater in both IDDM groups (DNormo: 2.5(1.0-4.6) nmol/l; DMicro: 3.2(2.1-3.8) nmol/l) than in Controls (1.0(0.0-2.0) nmol/l) (DNormo>Controls, \( p<0.05 \); DMicro>Controls, \( p<0.01 \) (Figure 1A). The exercise induced increments in plasma epinephrine were not different in Controls (0.24(0.11-0.35) nmol/l) compared with DNormo (0.18(0.08-0.29) nmol/l) and DMicro (0.52(0.22-0.70) nmol/l), but the increments in DMicro were greater than in DNormo (\( p<0.05 \)) (Figure 1B).

Relationships between plasma catecholamines, haemodynamics and glycaemia

The relative changes in FabCl were positively related to the increase in plasma NE in all groups (All subjects \( r=0.65, p<0.001 \); Controls \( r=0.62, p<0.05 \); DNormo \( r=0.63, p<0.05 \); DMicro \( r=0.56, p<0.05 \) (Figure 2). The exercise induced changes in FabCl were also correlated with the increase in MAP in DNormo (\( r=0.64, p<0.01 \)) and in DMicro (\( r=0.60, p<0.02 \)) but not in Controls (\( r=0.25, p=0.22 \)). Multiple regression analysis demonstrated that both changes in plasma NE (\( p<0.002 \)) and in MAP (\( p<0.005 \)) independently contributed to the exercise induced alterations in FabCl (multiple \( r=0.73, n=38 \)). Multiple regression analysis in two IDDM groups showed similar independent effects of plasma NE (\( p<0.02 \)) and MAP (\( p<0.005 \)) on the changes in FabCl (multiple \( r=0.75, n=26 \)). When multiple regression analysis was carried out using the increments in systolic blood pressure instead of MAP the contribution of blood pressure rise was not significant (\( p=0.20 \)).
Figure 1. Changes in plasma norepinephrine and epinephrine concentrations in response to exercise. Controls ○, DN normo ○, DMicro ●: see text and Table 1. Bars are median values. Upper panel: Increments in plasma epinephrine concentration. * denotes p<0.01 from preexercise; ** denotes increment in DMicro> DN normo, p<0.05. Lower panel: Increments in plasma norepinephrine concentration. * denotes p<0.05 from preexercise; ** denotes increment in DN normo and DMicro> Controls, p<0.05.
Baseline plasma NE concentrations and pulse rate were not correlated. During exercise a positive relationship was observed between plasma NE and pulse rate in Controls (r = 0.77, p < 0.01) as well in the combined IDDM groups (r = 0.53, p < 0.01). No significant correlations were found between plasma epinephrine concentrations and either FbbCl, pulse rate or blood pressure during exercise in any of the groups. In the IDDM groups the changes in plasma epinephrine and NE concentrations were not related to actual glycaemia and HbA1.

Discussion

Exercise has been proposed to provoke abnormalities in renal protein handling in IDDM [1,2]. It is unclear whether plasma catecholamines are involved in this abnormal rise in albuminuria. The subjects participating in the present study did not have systemic hypertension or signs of autonomic neuropathy. Patients with an abnormal urinary albumin excretion rate above 10 µg/min were designated microalbuminuric but it should be noted that levels higher than 20 µg/min are considered to represent a risk marker for diabetic nephropathy [22].

Exercise induced an abnormal rise in albuminuria in both IDDM groups in accordance with many previous studies employing a comparable fixed workload [1-5]. The mechanisms which are responsible for this abnormal rise in albuminuria are still not precisely understood. Systemic blood pressure rise, alterations in renal haemodynamics and in glomerular perm selectivity or a combination of these factors are likely to be involved [1-9]. As earlier studies have shown [2,6] no difference in blood pressure response was observed between the normoalbuminuric IDDM and non-diabetic subjects, making it unlikely that an abnormal increase in blood pressure per se was responsible for the marked albuminuric response in this group of patients. In the microalbuminuric IDDM subjects, the increase in blood pressure was larger than in the other groups, in accordance with other data [5], suggesting that alterations in systemic haemodynamics could have contributed to the abnormal rise in albuminuria in these patients. This study showed a significant relationship between the increase in plasma NE and the albuminuric response during exercise. In addition, the rise in plasma NE was significantly greater in normo- and microalbuminuric IDDM patients than in the normal subjects, although there was a considerable overlap in individual responses. Multiple regression analysis substantiated that both the exercise induced rise in plasma NE and in MAP had an independent effect on the albuminuric response. It is noteworthy that, although used by some investigators [7], diastolic blood pressure recordings are probably underestimated when measured by the auscultatory method during exercise [23]. Several studies have shown a relationship between (change in) systolic blood pressure and albuminuria during exercise [3,5], but this has not consistently been reported [4,6]. In this investigation, excluding patients with hypertension, the contributory effect of systemic blood pressure was not significant when using systolic blood pressure instead of MAP in the multiple regression analysis.

It has been documented that the filtration fraction rises more and is higher during exercise in IDDM subjects, especially in patients with microalbuminuria [4,6]. It is therefore probable that there is a link between the increased glomerular passage of
Figure 2. Changes in plasma norepinephrine concentration and relative changes in fractional albumin clearance in response to exercise. Controls ○, DNormo ○, Dmicro ●: see text and Table 1. Relative changes in fractional albumin clearance are expressed as % of pre-exercise values. All subjects (n=38): $r=0.65$, $p<0.001$; Controls (n=12) ○:$r=0.62$, $p<0.05$ DNormo (n=13) ○:$r=0.63$, $p<0.05$ DMicro (n=13) ●:$r=0.56$, $p<0.05$

albumin and the rise in filtration fraction during exercise in IDDM patients. Experimental studies in the rat have shown that infusion of NE results in an increase in filtration fraction in parallel with directly measured increments in intraglomerular pressure [10], whereas the degree of albuminuria in response to NE is closely related to changes in filtration fraction [24]. Taken together, the previously reported renal haemodynamic changes [4,6] and the presently shown significant relationship between changes in albuminuria and changes in plasma NE concentrations during exercise would support the hypothesis that NE is involved in the increase in albuminuria during exercise [9,14]. This present observations also suggest that an altered catecholamine response could possibly contribute to an abnormal rise in albuminuria in IDDM.

Several factors might be involved in the abnormal rise in plasma NE during exercise in IDDM. A fixed workload was employed in the expectation that this would
discriminate IDDM and non-diabetic subjects with respect to their albuminuric response [1-5]. It has been suggested that the maximal working capacity is reduced in microalbuminuric but not significantly so in normoalbuminuric IDDM patients [6]. Aerobic working capacity was found to be decreased in patients with autonomic neuropathy [17,25] and microalbuminuric whereas normoalbuminuric patients showed a normal maximal oxygen uptake [26]. It seems therefore probable that the fixed workload was more stressful for the microalbuminuric patients but it is unlikely that the relative workload was larger for the normoalbuminuric IDDM patients. In addition, it has been shown that an exaggerated catecholamine response can be related to suboptimal metabolic control [27,28], but no relationship between plasma catecholamines and HbA1c or actual glycaemia could be demonstrated in this study. The expected increase in pulse rate [2,7] raises the possibility of an abnormal regulation of the autonomic nervous system in IDDM [29]. Moreover, an enhanced vasopressor responsiveness to infusion of NE has been demonstrated in IDDM patients either without overt microvascular complications [30] or with microalbuminuria [31]. Whether glomerular vascular structures exhibit such an increased sensitivity to circulatory catecholamines remains to be elucidated.

In conclusion, the present observations suggest that renal protein handling during exercise is related to both circulating plasma NE and blood pressure in IDDM. The exaggerated albuminuric response, even in the absence of an increased urinary albumin excretion at rest, was associated with abnormalities in systemic catecholamine regulation. Direct experiments are needed to demonstrate a causal relationship between these phenomena. In addition, the rise in plasma NE was significantly greater in normo- and microalbuminuric diabetic patients than in the normal subjects, although there was a considerable overlap in individual responses.

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Acknowledgments

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CHAPTER 3

INFLUENCE OF AMBIENT PLASMA NOREPINEPHRINE ON RENAL HAEMODYNAMICS IN IDDM PATIENTS AND HEALTHY SUBJECTS

K. Hoogenberg¹, A.R.J. Girbes⁴, C.A. Stegeman², W.J. Sluiter¹, W.D. Reitsma³ and R.P.F. Dullaart¹

Imbalances in renal vasodilatory and vasoconstrictive mechanisms are responsible for the renal haemodynamic changes observed in IDDM patients. Animal experiments have shown that norepinephrine (NE) infusion increases the intraglomerular pressure by predominantly efferent arteriolar vasoconstriction. The relationships between ambient plasma NE levels and renal haemodynamics were studied in 18 healthy control subjects (group C), in 17 normoalbuminuric (albumin excretion rate (Ualb.V) <20 µg/min; group D1) and in 17 microalbuminuric (Ualb.V 20-200 µg/min; group D2) IDDM patients without overt autonomic neuropathy. Supine glomerular filtration rate (GFR (ml/min per 1.73m²)) and effective renal plasma flow (ERPF (ml/min per 1.73m²)) were determined over a 2 h period using constant infusions of ¹²⁵I-iothalamate and ¹³¹I-hippuran, respectively. The subjects were studied in the fasting state. The IDDM patients were investigated during near normoglycaemia. Data are given as means±SD. In group D1, GFR and ERPF (126±15 and 538±89, respectively) were elevated as compared to group C (108±15 and 478±73; p<0.01 and p<0.05, respectively). In group D2, GFR (124±25, p<0.05) but not ERPF (515±104) was higher than in group C. GFR and ERPF were negatively correlated with venous plasma NE in C (r=-0.61, p<0.005 and r=-0.64, p<0.001, respectively), in group D1 (r=-0.54, p<0.03 and r=-0.63, p<0.005, respectively) and in group D2 (r=-0.53, p<0.03 and r=-0.60, p<0.01, respectively). Multiple regression analysis disclosed that diabetes per se, independent from plasma NE, had a positive contribution to GFR. In contrast, ERPF was only related to plasma NE levels. In conclusion, GFR and ERPF are inversely related to venous plasma NE levels both in healthy and in IDDM subjects, supporting the hypothesis that plasma NE is a vasoconstrictive substance. The independent positive effect of diabetes as a categorial variable on GFR, suggests that concomitant vasodilating mechanisms play a role in the renal haemodynamic alterations in IDDM patients.

Introduction

In patients with insulin-dependent diabetes mellitus (IDDM) the presence of microalbuminuria does not only predict the future development of diabetic nephropathy [1], but is also associated with generalized vascular damage [2]. Microalbuminuria is thought to result from an increased glomerular leakage of macromolecules. Both changes in the glomerular permselectivity properties as well as in systemic and intrarenal haemo-dynam-

¹ Departments of Endocrinology, ² Nephrology, ³ General Medicine, and ⁴ Surgical Intensive Care Unit, Groningen State University Hospital, The Netherlands

cs factors have been demonstrated to underlie elevations in urinary albumin excretion [3]. Before and during the microalbuminuric phase renal haemodynamic changes, commonly reflected by an increase in glomerular filtration rate (GFR), are frequently observed in IDDM patients [4-7]. In animal models of diabetes mellitus an increase in GFR is associated with the development of glomerulosclerosis and the progressive loss of kidney function [8]. Some clinical observations also support the notion that an elevated GFR per se might contribute to the subsequent development of diabetic nephropathy [5,9].

The elevated GFR associated with IDDM has been attributed to an increase in effective renal plasma flow (ERPF) [5-7], an increase in the glomerular filtration surface area [10] and possibly an increase in the intraglomerular pressure [5]. Many vasodilatory and vasoconstrictive substances are able to modulate renal haemodynamics by influencing the glomerular afferent and efferent arteriolar tone [6,7,11]. Glomerular arterioles contain α-adrenoreceptors which mediate vasoconstriction induced by norepinephrine (NE) released at the terminal nerve ending of the sympathetic nervous system (SNS). Intravenous infusion to reach high physiological NE levels lowers ERPF, whereas the fall in GFR is less marked [11-14]. Consequently, the filtration fraction (FF) (i.e. the quotient of GFR and ERPF) rises, reflecting a change in pressure profile along the arterioles. Recent data suggest that IDDM with microalbuminuria are hypersensitive to NE-induced vasoconstriction of dorsal hand veins which also carry α-adrenoreceptors [15]. Thus alterations in SNS activity might be implicated in the pathogenesis of diabetes-associated renal haemodynamic abnormalities. However, the relation between plasma NE and renal haemodynamic parameters is uncertain. In a small group of adolescent IDDM patients a strong negative relationship between the plasma NE level and GFR was observed [16], whereas others did not find such a relationship [17,18].

Therefore, the present cross sectional study was conducted to establish the relationships between circulatory NE levels and renal haemodynamic changes in normo- and microalbuminuric IDDM patients as compared with matched healthy subjects.

**Subjects and methods**

*Subjects*

All participants consented to the procedure after explanation of the purpose of the study, which was approved by the local medical ethics committee. Eligible subjects had an age between 21 and 50 years, a serum creatinine ≤120 µmol/l, a urinary protein excretion ≤500 mg/day, and a body mass index <27 kg/m². Subjects with hypertension (systolic blood pressure >160 mmHg and/or diastolic blood pressure >95 mmHg), using antihypertensive or anti-inflammatory drugs were excluded from participation. The IDDM were considered to be insulin-dependent since their glucagon stimulated C-peptide levels were <0.2 nmol/l. They had a disease duration of at least 5 years. Autonomic function was assessed with beat-to-beat variation (abnormal if variation less than 10 beats per minute), valsalva manoeuvre (abnormal if lower than 1.11) and systolic blood pressure response to standing (abnormal if exceeding 30 mmHg) [19]. None of the
Table 1. Clinical characteristics of the study groups.

<table>
<thead>
<tr>
<th></th>
<th>Group C (n=18)</th>
<th>Group D1 (n=17)</th>
<th>Group D2 (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30.8 ± 7.7</td>
<td>32.0 ± 8.3</td>
<td>35.8 ± 8.0</td>
</tr>
<tr>
<td>Duration of disease (years)</td>
<td>-</td>
<td>14.8 ± 6.0</td>
<td>18.8 ± 5.3</td>
</tr>
<tr>
<td>Sex (males/females)</td>
<td>13 / 5</td>
<td>15 / 2</td>
<td>14 / 3</td>
</tr>
<tr>
<td>Ualb.V (µg/min)</td>
<td>n.d.</td>
<td>9.1(6.4-11.5)</td>
<td>34.4(22.9-105.3)c</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>92 ± 9</td>
<td>95 ± 7</td>
<td>100 ± 8d</td>
</tr>
<tr>
<td>Body mass index (kg/m^2)</td>
<td>22.6 ± 2.3</td>
<td>22.5 ± 2.0</td>
<td>23.7 ± 2.0</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>n.d.</td>
<td>8.6 ± 1.4</td>
<td>8.3 ± 1.0</td>
</tr>
<tr>
<td>Urinary sodium excretion (mmol/day)</td>
<td>182 ± 76</td>
<td>156 ± 63</td>
<td>203 ± 82</td>
</tr>
<tr>
<td>Urinary urea excretion (mmol/day)</td>
<td>425 ± 108</td>
<td>435 ± 127</td>
<td>384 ± 113</td>
</tr>
<tr>
<td>Insulin dose (U/kg per day)</td>
<td>-</td>
<td>0.74 ± 0.23</td>
<td>0.79 ± 0.22</td>
</tr>
<tr>
<td>Retinopathy (O/B/P)</td>
<td>n.d.</td>
<td>11/4/2</td>
<td>8/5/4</td>
</tr>
<tr>
<td>Valsalva ratio</td>
<td>n.d.</td>
<td>1.57 ± 0.26</td>
<td>1.62 ± 0.38</td>
</tr>
</tbody>
</table>

Beat-to-beat variation during deep breathing n.d. 29 ± 13 28 ± 10

Change in systolic blood pressure in response to standing (mmHg) n.d. -5 (-10 to 5) -4 (-8 to 2)

Group C: control subjects; group D1: IDDM patients with Ualb.V <20 µg/min; group D2: IDDM patients with Ualb.V > 20 µg/min and <200 µg/min. n.d. denotes not determined; a Ualb.V: urinary albumin excretion rate; b Retinopathy: O: absent; B: background; P: proliferative. Values are given in mean ± SD, except for Ualb.V and blood pressure response to standing which are given in median (interquartile range). c denotes p<0.001 from group D1; d denotes p<0.01 from groups C and D1

Subjects suffered from overt autonomic neuropathy defined as at least 1 abnormal test. Because of the high variability of urinary albumin excretion [20], three timed overnight urine collections were obtained to measure urinary albumin excretion rate (Ualb.V). Microalbuminuria was defined as Ualb.V ranging 20 to 200 µg/min in at least two urine samples [1].

Three groups of subjects were included in the study: 18 healthy controls (group C); 17 IDDM patients with normoalbuminuria (group D1), 17 IDDM patients with microalbuminuria (group D2). Table 1 shows the clinical characteristics of the study groups and data of the autonomic function tests. The groups were closely comparable with respect to age and there was no difference in sex distribution. Mean urinary sodium and urea excretion was similar in the groups and none of the subjects used a protein (<0.8 g/day per kg bodyweight) or a sodium restricted (<50 mmol/day) diet. The two IDDM groups did not differ in diabetes duration and metabolic control. The proportion of patients with retinopathy was similar in each IDDM group.

Procedure of the renal haemodynamic measurements
All participants were studied after an overnight fast and remained fasting during the procedure. Starting at 0800 h, the subjects were studied in the supine position and were only allowed to stand on voiding. Diuresis was promoted by a waterload of 300 ml/h, and no other liquids than water were permitted to drink. The IDDM patients received their last regular insulin dose 8 to 12 h before the start of the study.

The IDDM patients were studied during near normoglycaemia to minimize the possible effects of actual glycaemia on GFR and ERPF [21]. Thus, a 5% glucose solution was initially infused at a rate 1 ml(0.28 mmol)/h per kg bodyweight together with regular acting insulin (Velosulin H.M., Novo-Nordisk, Bagsvaerd, Denmark). The amount of insulin infused was 1% of the total daily requirements per hour. Blood glucose was measured at 30 min intervals. If blood glucose exceeded 8.3 mmol/l extra insulin was given intravenously until one hour before the start of the renal haemodynamic measurements. Further insulin boluses were not given since insulin can acutely stimulate catecholamine release [22]. After a stabilisation period of at least 2 h the renal clearance studies were carried out.

GFR and ERPF were measured simultaneously during a 2 h clearance period, using primed infusions of $^{125}$I-iothalamate and $^{131}$I-hippuran, respectively [23]. $^{125}$I-iothalamate and $^{131}$I-hippuran were infused at a constant rate of 4.8µCi/h (178 kBq/h) and 12 µCi/h (444 kBq/h), respectively. The clearances were calculated using the formulas $U.V/P$ and $I.V/P$, respectively. $U.V$ represents the urinary excretion rate of the tracer, $I.V$ represents the infusion rate of the tracer, $P$ represents the mean tracer value of 2 plasma samples taken at the beginning and at the end of the clearance period. Errors in the estimation of the GFR due to incomplete bladder emptying and dead space were corrected by multiplying the clearance of $^{125}$I-iothalamate with the formula: clearance of $^{131}$I-hippuran ($I.V/P$) / clearance of $^{131}$I-hippuran ($U.V/P$). The coefficients of variation for GFR and ERPF are 2.2% and 5.0%, respectively [23]. The GFR and ERPF were corrected to 1.73m$^2$ of body-surface area. The FF was calculated as the ratio of the GFR and the ERPF.

Blood pressure was recorded every hour, using a sphygmomanometer. Korotkoff phase I and V were taken as the systolic and the diastolic blood pressure, respectively. At the beginning and the end of the clearance period venous blood was drawn from a catheter inserted into an antecubital vein (kept patent with 0.9% NaCl 20 ml/h).

**Laboratory methods**

Blood glucose was measured using a Yellow Springs glucose Analyser (Model 23A, Yellow Springs Inc., Yellow Springs, Ohio, USA). HbA1c was determined by colorimetry (24) (reference values: 4.5 to 5.8%). Serum creatinine, albumin, and urinary sodium and urea were measured on SMA(C) autoanlysers (Technicon Instruments Inc. Tarrytown, N.Y., USA). Urinary albumin was measured by radioimmuno-assay (Diagnostic Products Corporation, Apeldoorn, The Netherlands; coefficient of variation 8%). Plasma insulin and plasma C-peptide concentrations were measured by radioimmuno-assay. Plasma epinephrine and NE concentrations were determined by high-performance liquid chromatography (coefficient of variation 4%) [25].

**Statistical analysis**

Results are expressed as mean±SD for parametrically distributed data and as median
Ambient plasma noradrenaline and renal haemodynamics

(Interquartile ranges) for non-parametrically distributed data. Comparisons of variables between groups were carried out using analysis of variance for parametrically and non-parametrically distributed data as appropriate. Changes of variables within groups were assessed by paired Student's t-tests or Wilcoxon tests. Adjustment for multiple comparisons was carried out using Duncan's method [26]. Differences in prevalence of clinical variables were analysed by chi-square statistic. Correlations were studied using Spearman's rank analysis. Multiple stepwise regression analysis was used to disclose the independent contribution of the hormonal and metabolic parameters to GFR and ERPF. Two-sided p-values less than 0.05 were considered to be significant.

Results

Blood glucose, plasma insulin and renal haemodynamic parameters

During the 2-h clearance period, insulin and blood glucose concentrations were not different between the two IDDM groups and did not change significantly (Table 2). GFR was significantly higher in both IDDM groups as compared to group C (p<0.01 and p<0.05 for groups D1 and D2, respectively, Table 2), whereas ERPF was only higher in group D1 than in group C (p<0.05, Table 2). FF was significantly elevated in group D2 as compared to group C (p<0.05) (Table 2). In the combined IDDM groups, ERPF as well as FF were higher than in group C (p<0.05, for both, Table 2).

Plasma norepinephrine and epinephrine.

Plasma NE levels were higher in group C than in groups D1 and D2 at the beginning (p<0.05 for both, Table 3) but not significantly so at the end of the 2 h clearance period (Table 3). The differences in averaged plasma NE levels did not reach significance (p=0.08 for group C compared with groups D1 and D2, Table 3). Plasma epinephrine concentrations were not significantly different between the groups. No significant changes were observed in plasma NE and epinephrine during the 2-h study period.

Correlation analysis

The averaged values of plasma NE and epinephrine were used for the correlation analysis. GFR and ERPF were negatively correlated with plasma NE levels in group C (r=-0.61, p<0.005 and r=-0.64, p<0.001, respectively), in group D1 (r=-0.54, p<0.03 and r=-0.63, p<0.005, respectively) and in group D2 (r=-0.53, p<0.03 and r=-0.60, p<0.01, respectively, Figure 1A,B). In the combined IDDM groups, a positive correlation was found between FF and plasma NE (r=0.34, p<0.05, Figure 1C) which was not present in group C (r=0.11, NS, not shown). GFR was significantly related to HbA1c (r=0.34, p<0.05) in the combined IDDM groups, whereas no significant relation existed between HbA1c, ERPF (r=0.24, p=0.17) and FF (r=0.02, NS). Plasma epinephrine was not correlated with renal haemodynamics.
Table 2. Renal haemodynamics and corresponding plasma glucose and insulin levels.

<table>
<thead>
<tr>
<th></th>
<th>Group C</th>
<th>Group D1</th>
<th>Group D2</th>
<th>IDDM all</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=18)</td>
<td>(n=17)</td>
<td>(n=17)</td>
<td>(n=34)</td>
</tr>
<tr>
<td>Glomerular filtration rate (ml/min per 1.73m²)</td>
<td>108 ± 15</td>
<td>126 ± 15(^a)</td>
<td>124 ± 25(^b)</td>
<td>125 ± 20(^b)</td>
</tr>
<tr>
<td>Effective renal plasma flow (ml/min per 1.73m²)</td>
<td>478 ± 73</td>
<td>538 ± 89(^b)</td>
<td>515 ± 104</td>
<td>527 ± 96(^b)</td>
</tr>
<tr>
<td>Filtration fraction</td>
<td>0.22 ± 0.02</td>
<td>0.23 ± 0.03</td>
<td>0.24 ± 0.03(^b)</td>
<td>0.24 ± 0.03(^b)</td>
</tr>
<tr>
<td>Fractional sodium excretion</td>
<td>0.95 ± 0.53</td>
<td>0.78 ± 0.31</td>
<td>0.93 ± 0.42</td>
<td>0.88 ± 0.33</td>
</tr>
<tr>
<td>Plasma glucose (mmol/l) 08.00 h</td>
<td>4.6 ± 0.4</td>
<td>12.2 ± 5.5(^a)</td>
<td>11.6 ± 4.4(^a)</td>
<td>11.9 ± 4.9(^a)</td>
</tr>
<tr>
<td></td>
<td>begin</td>
<td>6.6 ± 1.1</td>
<td>6.9 ± 1.4</td>
<td>6.7 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>end</td>
<td>6.5 ± 1.1</td>
<td>6.5 ± 1.2</td>
<td>6.5 ± 1.1</td>
</tr>
<tr>
<td>Free plasma insulin (mU/l) begin</td>
<td>n.d.</td>
<td>28 (23 - 49)</td>
<td>26 (18 - 35)</td>
<td>28 (19 - 35)</td>
</tr>
<tr>
<td></td>
<td>end</td>
<td>n.d.</td>
<td>32 (19 - 43)</td>
<td>23 (13 - 34)</td>
</tr>
</tbody>
</table>

Group C: control subjects; group D1: IDDM with Ualb.V < 20 µg/min; group D2: IDDM patients with UalbV > 20 µg/min and < 200 µg/min; IDDM all: combined IDDM groups; n.d. not determined; Values are given in mean±SD, except for free insulin which is given in median (interquartile range). \(^a\) denotes \(p<0.01\) and \(^b\) denotes \(p<0.05\) from group C.

Table 3. Plasma norepinephrine and epinephrine concentrations during the renal haemodynamic measurements.

<table>
<thead>
<tr>
<th></th>
<th>Group C</th>
<th>Group D1</th>
<th>Group D2</th>
<th>IDDM combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=18)</td>
<td>(n=17)</td>
<td>(n=17)</td>
<td>(n=34)</td>
</tr>
<tr>
<td><strong>Plasma norepinephrine (nmol/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Begin</td>
<td>1.56 ± 0.45(^a)</td>
<td>1.32 ± 0.56</td>
<td>1.28 ± 0.40</td>
<td>1.29 ± 0.48</td>
</tr>
<tr>
<td>End</td>
<td>1.52 ± 0.53</td>
<td>1.30 ± 0.50</td>
<td>1.33 ± 0.49</td>
<td>1.31 ± 0.49</td>
</tr>
<tr>
<td>Mean</td>
<td>1.54 ± 0.50</td>
<td>1.31 ± 0.45</td>
<td>1.30 ± 0.44</td>
<td>1.31 ± 0.44</td>
</tr>
<tr>
<td><strong>Plasma epinephrine (nmol/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Begin</td>
<td>0.15 ± 0.10</td>
<td>0.20 ± 0.14</td>
<td>0.19 ± 0.10</td>
<td>0.19 ± 0.12</td>
</tr>
<tr>
<td>End</td>
<td>0.13 ± 0.10</td>
<td>0.18 ± 0.13</td>
<td>0.20 ± 0.10</td>
<td>0.19 ± 0.11</td>
</tr>
<tr>
<td>Mean</td>
<td>0.14 ± 0.11</td>
<td>0.18 ± 0.12</td>
<td>0.22 ± 0.10</td>
<td>0.19 ± 0.11</td>
</tr>
</tbody>
</table>

Group C: control subjects; group D1: IDDM patients with Ualb.V < 20 µg/min; group D2: IDDM patients with UalbV > 20 µg/min and < 200 µg/min. Values are given in mean ± SD. \(^a\) denotes \(p<0.05\) from groups D1 and D2.
Multiple regression analysis was carried out to evaluate whether renal haemodynamics were independently related to the plasma NE level and to establish if plasma NE contributed to the differences in GFR and ERPF between the IDDM and the non-diabetic subjects. In the analyses plasma NE as well age, diabetes duration, HbA1c, sodium and urea excretion, and blood pressure were the independent variables. In the combined groups (n=52), ERPF was only related to plasma NE ($r=-0.61, P<0.001$). The model was not improved by including the presence of IDDM as a categorial variable ($p=0.30$, multiple $r=0.59$). Thus IDDM per se did not influence ERPF, independently from the level of NE. GFR was related to ERPF both in the combined groups ($r=0.79, p<0.001$) as well as in IDDM groups ($r=0.86, p<0.001$). However in the combined groups, IDDM, included in the model as a categorial variable, had an independent effect on GFR ($r=0.22, p<0.01$; multiple $r=0.88$), indicating that diabetes associated vasodilation influenced GFR. The other covariates did not contribute to either ERPF or GFR in a model including plasma NE and IDDM.

**Discussion**

The elevations in GFR and ERPF associated with IDDM are considered to reflect an imbalance between glomerular vasodilatatory and vasoconstrictive mechanisms [5-7,11,17]. This study demonstrates that GFR and ERPF are inversely related to plasma NE levels in normo- and microalbuminuric IDDM patients as well as in healthy subjects. Our results extend recent observations in a small group of adolescent IDDM patients showing a negative correlation between plasma NE and GFR [16] and the possibility arises that plasma NE is a determinant of renal haemodynamics.

No differences were observed in the inverse correlations between plasma NE and ERPF in the IDDM patients as compared to the control subjects. This raises the possibility that the presently observed slightly lower plasma NE levels in the IDDM subjects could contribute to the elevations in ERPF. Clearly, our study design does not permit a conclusion with respect to potential differences in renal NE sensitivity in association with IDDM or microalbuminuria [15].

Both animal and human studies indicate that renal blood flow is an important determinant of GFR [5-7,27]. Our data support this view since GFR was strongly related to ERPF. However, the elevated GFR cannot be fully accounted for by an increase in ERPF, since the filtration fraction was higher in the IDDM patients, particularly in those with microalbuminuria. GFR is also determined by the intraglomerular pressure, the oncotic pressure and the ultrafiltration coefficient and any of these factors or a combination of them might explain the IDDM related increase in filtration fraction [5]. Indeed, the intraglomerular pressure is elevated in experimental diabetes [8] and capillary hypertension has been documented in IDDM patients [28]. In addition, the glomerular filtration surface area is increased in IDDM [10] which will influence the ultrafiltration coefficient. The present study did not differentiate between these mechanisms but multiple regression analysis disclosed an independent effect of the diabetic state per se on GFR, thus suggesting that concomitant vasodilating mechanisms are present in IDDM patients.
Figure 1. Relationships between renal haemodynamics and plasma norepinephrine levels (NE). ○ Control subjects, ○ IDDM patients with Ualb.V<20 µg/min, ● IDDM patients with Ualb.V>20 µg/min and <200µg/min. A: plasma NE and GFR in ○ $r=-0.61$, $p<0.005$; in ○ $r=-0.54$, $P<0.03$; in ● $r=-0.53$, $p<0.03$; B: plasma NE and ERPF in ○ $r=-0.64$, $p<0.001$; in ○ $r=-0.63$, $p<0.005$; and in ● $r=-0.60$, $p<0.01$; C: plasma NE and FF in the combined IDDM groups $r=0.34$, $p<0.05$. 
NE constricts renal vasculature via stimulation of α1-adrenoreceptors, which are located along afferent and efferent renal arterioles [11,14,29]. As compared with other isolated renal vessels, efferent glomerular arterioles from the rabbit have the highest sensitivity to the vasoconstrictive action of NE [30] and micropuncture studies in the rat have shown that NE lowers glomerular blood flow and increases the transglomerular pressure [13]. In humans NE infusion results in a decrease in ERPF and a rise in filtration fraction [12]. Moreover, an increase in plasma NE appears to contribute to exercise-induced changes in microalbuminuria possibly by a renal haemodynamic mechanism [31].

The pathophysiological mechanisms responsible for the relation between the basal plasma NE level and renal haemodynamics are unknown. NE, present in venous forearm blood, is derived both from local skeletal muscular nerve activity and from the arterial circulation [32]. The forearm venous plasma NE concentration is the net result of these sources minus local neuronal uptake. Thus, the peripheral venous forearm NE concentration is influenced by other factors than SNS activity alone. NE acts both locally as a neurotransmitter and distantly as a hormonal factor [33]. It cannot be discriminated whether the relationship between plasma NE and renal haemodynamics indicates a distant effect or reflects a generalized or local neurotransmitter spillover of the SNS. To study the role of SNS activity in diabetes related renal haemodynamic abnormalities it would be necessary to obtain arterial and venous renal blood samples simultaneously with GFR and ERPF determinations.

In conclusion, GFR and ERPF are inversely correlated with the venous plasma NE level in healthy subjects as well as in IDDM patients with normo- and microalbuminuria, supporting the hypothesis that circulatory NE can be a renal vasoconstrictive factor. The positive contribution of IDDM per se to GFR, independently from the negative effects of plasma NE, suggests that concomitant vasodilating mechanisms play a role in the renal haemodynamic alterations in IDDM patients.

References

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Acknowledgments

We are indebted to M. van Kammen, F. Kranenburg-Nienhuis and A. Drent-Bremer for their skilful assistance.
CHAPTER 4

EXOGENOUS NOREPINEPHRINE INDUCES AN ENHANCED MICROPROTEINURIC RESPONSE IN MICROALBUMINURIC IDDM PATIENTS

K. Hoogenberg¹, W.J. Sluiter¹, G. Navis², T.W. van Haften¹, A.J. Smit³, W.D. Reitsma⁴ and R.P.F. Dullaart¹

Exogenous norepinephrine (NE) increases intraglomerular pressure in animal experiments, but it is unknown whether NE induces a microproteinuric response in man. Moreover, it has not been studied whether possible microproteinuric and renal haemodynamic changes induced by NE are altered in IDDM complicated by microalbuminuria. Therefore, the microproteinuric and renal haemodynamics responses to exogenous NE infusions were measured in 8 matched normoalbuminuric IDDM patients (group D1), microalbuminuric IDDM patients (group D2) and control subjects (group C). As anticipated, mean arterial pressure (MAP)-NE dose response curves were significantly shifted leftwards in groups D1 and D2 compared to group C (p<0.05), indicating a higher systemic NE responsiveness in IDDM. On separate days, NE or placebo was infused at individually determined NE threshold (ΔMAP = 0mmHg, T), 20% pressor (ΔMAP = 4mmHg, 20%P) and pressor doses (ΔMAP = 20mmHg, P) with measurement of urinary albumin and IgG excretion (Ualb.V and Ulg.G.V), and of glomerular filtration rate (GFR, by 125I-iothalamate) and effective renal plasma flow (ERPF, by 131I-hippurate). At NE pressor dose Ualb.V and Ulg.G.V rose in all groups (p<0.05 to 0.01), whereas urinary β2-microglobulin was unchanged. The increases in Ualb.V and Ulg.G.V were more pronounced in the microalbuminuric group than in the other groups (p<0.05). An NE dose-dependent fall in ERPF and rise in filtration fraction (FF) was found in all groups (p<0.05 to 0.001 for all), whereas GFR did not change significantly. The renal haemodynamic dose-response relationship was similar in the groups. In conclusion, exogenous NE acutely promotes glomerular protein leakage, and it is plausible that intraglomerular NE effects contribute to this phenomenon. The microproteinuric response is enhanced in microalbuminuric IDDM despite unaltered renal haemodynamic responsiveness, which may reflect a specific NE response or a general effect of vasopressor stimuli to promote glomerular protein leakage in patients with a preexistent defect in glomerular perm selectivity.

Introduction

Microalbuminuria predicts the future development of nephropathy in insulin-dependent diabetes mellitus (IDDM) [1-3]. Both structural changes in the glomerular basement membrane and changes in systemic and renal haemodynamics are thought to be involved in the development and progression of microalbuminuria in IDDM [2-5]. Diabetes-associated glomerular hyperfiltration and elevations in intraglomerular pressure

¹ Department of Endocrinology, ² Nephrology, ³ Angiology and ⁴General Medicine, Groningen State University Hospital, Groningen, The Netherlands

may contribute to the progression to overt diabetic nephropathy [6-10]. Several neurohormonal systems, such as the renin-angiotensin system (RAS) and the sympathetic nervous system (SNS) are involved in the regulation of glomerular pressure [11]. Norepinephrine (NE) increases intraglomerular pressure in the rat [12]. In accordance with a renal vasoconstrictive effect of NE, the effective renal plasma flow (ERPF) is correlated negatively with the ambient plasma NE level in man [13], and in non-diabetic subjects exogenous NE lowers ERPF without much change in glomerular filtration rate (GFR) [14-16].

Exogenous NE may increase proteinuria in non-diabetic subjects with overt proteinuria [15], but it is unknown whether exogenous NE affects microproteinuria in IDDM. In an uncontrolled study in healthy subjects, NE did not evoke an albuminuric response [17]. Exercise, a manoeuvre that increases NE levels, is well known to increase microproteinuria in normo- and microalbuminuric IDDM patients and healthy subjects. Increases in urinary albumin excretion during exercise are related to changes in plasma NE and systemic blood pressure [18]. Systemic and intraglomerular effects of NE may, therefore, be involved in the increased microproteinuria following strenuous activities [18,19]. It has, moreover, been proposed that an exaggerated renal haemodynamic NE responsiveness could play a role in the pathogenesis of diabetic renal disease [20].

This randomized, placebo controlled study was performed to test whether NE evokes an increase in microproteinuria and if so whether such an increase is more pronounced in normo- and microalbuminuric IDDM patients. Therefore, we compared the microproteinuric and renal and systemic haemodynamic responses during stepwise exogenous NE infusions in IDDM patients with and without microalbuminuria and healthy subjects. Since an enhanced systemic blood pressure response to NE was anticipated in IDDM [21-23], the subjects were studied at individually determined NE threshold, 20% pressor and pressor doses.

Methods

Subjects

All subjects consented to participate in the study, which was approved by the local medical ethics committee. Eligible subjects had an age between 30 and 55 years, a serum creatinine <120µmol/l, a urinary protein excretion <500 mg/day, a body mass index <28 kg/m², no overt hypertension (systolic blood pressure <160 mmHg and diastolic blood pressure <95 mmHg), no signs of clinical autonomic neuropathy assessed by beat-to-beat variation during deep breathing (abnormal if difference <10 beats per min), Valsalva manoeuver (abnormal if ratio <1.1) and systolic blood pressure response to standing (abnormal if decline >20 mmHg) [24]. Diabetic patients were insulin-dependent, glucagon-stimulated plasma C-peptide level was <0.2 nmol/l in all of them. They had a disease duration of at least 15 year. Patients with untreated proliferative retinopathy, symptomatic coronary heart disease, or peripheral vascular disease were excluded. Microalbuminuria was defined as a persistently elevated urinary albumin excretion rate (Ualb.V) between 20 and 200µg/min in at least 2 of the 3 overnight urine collections obtained over 1 year [1,25]. Urinary tract infection was excluded in all subjects by bacterial culture.
Eight healthy control subjects (group C), 8 normoalbuminuric (group D1) and 8 microalbuminuric IDDM patients (group D2) were included (Table 1). They were individually matched for gender, age (within 5 yr), body mass index (within 2.5 kg/m²) and smoking habits. Metabolic control and daily insulin requirement was similar in the normo- and microalbuminuric diabetic groups. Retinopathy was more severe in the microalbuminuric group. Creatinine clearance was similar in the groups. Mean arterial pressure (MAP) was higher in patients with microalbuminuria (Table 1). Seven microalbuminuric diabetic patients used an angiotensin converting enzyme (ACE) inhibitor that was stopped 6 weeks before the study. No other medication was used.

To avoid bias due to differences in sodium and protein intake on NE sensitivity and renal haemodynamics [26,27], all participants adhered to diet containing 100 mmol sodium and 1 g protein/kg body wt per day 1 week before the studies. Protein intake was calculated from urinary urea excretion assuming nitrogen balance as described [28].

### Table 1. Prestudy clinical characteristics of control and IDDM subjects.

<table>
<thead>
<tr>
<th></th>
<th>Non-diabetic Subjects</th>
<th>Normoalbuminuric IDDM patients</th>
<th>Microalbuminuric IDDM patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group C</td>
<td>Group D1</td>
<td>Group D2</td>
</tr>
<tr>
<td>Number</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Age (years)</td>
<td>44.4 ± 3.2</td>
<td>45.5 ± 3.6</td>
<td>46.7 ± 3.5</td>
</tr>
<tr>
<td>Duration of disease (years)</td>
<td>-</td>
<td>21.6 ± 2.1</td>
<td>27.6 ± 2.2</td>
</tr>
<tr>
<td>Sex (males/females)</td>
<td>7 / 1</td>
<td>7 / 1</td>
<td>7 / 1</td>
</tr>
<tr>
<td>Cigarette smokers (n)</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Urinary albumin excretion(µg/min)</td>
<td>4.0 (2.6-6.2)</td>
<td>4.5 (2.5-8.4)</td>
<td>65.9 (40.1-108.1)</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>84 ± 4</td>
<td>83 ± 4</td>
<td>88 ± 7</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>108 ± 5</td>
<td>114 ± 7</td>
<td>116 ± 12</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>94.8 ± 2.5</td>
<td>92.4 ± 3.6</td>
<td>101.9 ± 1.5</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.2 ± 1.0</td>
<td>24.5 ± 1.1</td>
<td>25.6 ± 0.9</td>
</tr>
<tr>
<td>Glycated haemoglobin (%)</td>
<td>5.7 ± 0.2</td>
<td>8.3 ± 0.3</td>
<td>8.2 ± 0.3</td>
</tr>
<tr>
<td>Sodium excretion (mmol/day)</td>
<td>109 ± 13</td>
<td>103 ± 9</td>
<td>109 ± 7</td>
</tr>
<tr>
<td>Protein intake (g/kg per day)</td>
<td>1.11 ± 0.07</td>
<td>0.94 ± 0.07</td>
<td>1.03 ± 0.05</td>
</tr>
<tr>
<td>Insulin dose (U/kg per day)</td>
<td>-</td>
<td>0.74 ± 0.10</td>
<td>0.79 ± 0.09</td>
</tr>
<tr>
<td>Retinopathy (absent/background/proliferative)</td>
<td>-</td>
<td>5 / 2 / 1</td>
<td>0 / 5 / 3</td>
</tr>
<tr>
<td>Valsalva ratio</td>
<td>1.63 ± 0.08</td>
<td>1.59 ± 0.07</td>
<td>1.52 ± 0.07</td>
</tr>
<tr>
<td>Beat-to-beat variation</td>
<td>25 ± 3</td>
<td>24 ± 4</td>
<td>16 ± 3</td>
</tr>
<tr>
<td>Change in systolic blood pressure in response to standing (mmHg)</td>
<td>-9.4 ± 2.6</td>
<td>-6.9 ± 3.5</td>
<td>-7.3 ± 2.8</td>
</tr>
</tbody>
</table>

Values are given in mean±SEM, except UalbV which is given in geometric mean and 95% confidence interval. \(^a^p<0.001\) and \(^b^p<0.05\) from group C and D1; \(^c^p<0.001\) from group D1 and D2; and \(^d^p<0.05\) from group D1 by \(t\) analysis.
Dietary compliance was acceptable in the participants and similar in the groups (Table 1). The 3 women were studied in the luteal phase of the menstrual cycle.

**Study design**

The experiments were carried out during 3 consecutive days (see below) in a room with a controlled temperature of 22°C. The subjects were studied after an overnight fast and remained so during the experiments. Smoking was prohibited and no other liquid than water was allowed to drink. After completion of each study day meals were provided at 1700 h and 2000 h. The diabetic patients received an intravenous insulin infusion (Velosulin H.M., Novo-Nordisk, Bagsvaerd, Denmark) at a rate of 30 mU/kg per h, starting at 0600 h. Blood glucose was measured every 5 to 10 min and clamped at 5 mmol/l by varying the infusion rate of a 20% glucose solution using the euglycaemic clamp technique [29]. Potassium chloride (10 mmol per 100 g of glucose) was added to the glucose bottles. The euglycaemic insulin clamp was used to minimize effects of differences in actual glycaemia on renal haemodynamics [30] and to avoid that changes in insulin levels during the experiments would potentially influence NE sensitivity [31]. The diabetic subjects had been admitted to the hospital the evening prior to the study. Blood glucose was kept between 7 and 11 mmol/l in the evenings and nights before and between study days. The controls received a 5% glucose infusion (without insulin) to keep blood glucose unchanged from fasting levels, to prevent a decline in plasma insulin during prolonged fasting and to prevent increases in endogenous catecholamine release as a consequence of glucose counter regulation. The minimal glucose infusion rate was 30 ml/h, because a 5% glucose solution was used as vehicle for renal tracers as well as for NE and placebo.

**NE dose-response curves (day 1)**

On day 1, individual dose-response curves to exogenous NE started at 1230 h as described [32]. After a 30 min baseline period, an NE infusion (norepinephrine tartrate, Centrafarm BV, Leiden, The Netherlands, dissolved in 5% glucose) was given in the right antecubital vein. The NE infusion rate was increased at predetermined 25 min steps of 10, 20, 40, 80, 100, 150, 200, 250, 300, 400 and 600 ng/kg per min. The maximal infusion rate was reached if MAP was increased by 20 to 25 mmHg. Blood pressure was measured every 2 min at the left upper arm with a semi-automated sphygmomanometer (Dinamap® 1846, Critikon, Tampa, FL, USA). Pulse rate was recorded continuously on an ECG oscilloscope (Hewlett Packard, Boblingen, Germany). MAP was calculated as \( \frac{a}{b} \) systolic blood pressure+\( \frac{c}{d} \) diastolic blood pressure (mmHg).

The pressor dose was defined as the NE infusion rate required to induce a rise in MAP of 20 mmHg above baseline and was calculated from a semi-logarithmic plot of the NE dose vs the individual changes in MAP. The individual regression lines were constructed from the linear part of the dose-response curve [32]. For each participant the NE threshold dose (0 mmHg change in MAP), 20% pressor dose (increase in MAP of 4 mmHg) and pressor dose (increase in MAP of 20 mmHg) were calculated from the individually obtained regression equations.
Renal haemodynamic studies and proteinuria assessment (day 2 and 3)

On day 2 and 3, NE or placebo were infused in randomized order in a single-blinded fashion at threshold, 20% pressor and pressor doses. The subjects remained supine and were only allowed to stand on voiding. At 0700 h, each subject drank 600 ml water to promote diuresis. Thereafter, urinary volume loss was supplemented by oral water after subtraction of the amount of intravenous solutions. Note that urinary albumin excretion temporarily rises after a water load and that its level has returned to baseline after a 2 h period [33]. Between 0900 h and 1630 h, renal haemodynamics were measured during 10 consecutive clearance periods each lasting 45 min. Two baseline clearance periods were followed by 6 periods of NE (2 periods each at threshold, at 20% pressor and at pressor doses) or placebo. Finally, 2 determinations were obtained after cessation of NE or placebo infusion (recovery period).

GFR and ERPF were measured simultaneously as the urinary clearances of $^{125}\text{I}$-iothalamate and $^{131}\text{I}$-hippurate, respectively, and corrected for incomplete urine collections as described previously [34]. The coefficients of variation for GFR and ERPF are 2.2% and 5.0%, respectively [34]. The GFR and ERPF were corrected to $1.73 \text{m}^2$ of body-surface area. Filtration fraction (FF) was calculated as the quotient of GFR and ERPF.

Laboratory measurements

Venous blood samples were taken at the end of each clearance period with the subjects in supine position. Blood was drawn from a needle inserted into the left antecubital vein that was kept patent with a saline drip (0.9% NaCl, 30 ml per h). Blood samples were immediately centrifuged at 4°C and the samples were stored at -20°C before assay. Breakdown and in vitro generation of angiotensin II (AII) was prevented by the addition of o-phenantroline, enalapril and neomycin. Aliquots of urine for measurement of urinary proteins were stored at -20°C and analysed within 2 weeks. For proper determination of IgG, urine was 1:1 diluted with buffered glucose (0.1 M NaCl, 40 mM phosphate, sodium azide (0.2%), 5% bovine serum albumin and 100 mM glucose adjusted to pH 7.4) [35].

Plasma insulin was determined by RIA. Plasma NE was analysed by HPLC [13,18]. Plasma total renin and plasma active renin were determined with commercially available double-antibody RIA’s (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The antibody to renin has a cross-reactivity with prorenin of 0.2%. Prorenin was calculated as the difference between total renin and active renin levels. Serum ACE activity was measured with an HPLC technique. AII was determined by RIA [36] by courtesy of P.M.H. Schippers (Dept. of Pharmacology, State University Maastricht, The Netherlands). Cross-reactivity of the AII antibody with angiotensin I is <0.1%. Serum aldosterone was determined by RIA. Serum albumin and IgG were measured using ELISA. Blood glucose was measured using a Yellow Springs glucose Analyser (Model 23A, Yellow Springs Inc., Yellow Springs, Ohio, USA).

Urinary albumin was measured with ELISA using rabbit anti-human albumin antibody (DAKO). The detection limit is 1 µg/l. Urinary IgG was measured using an in-house developed ELISA using polyclonal goat-antihuman-IgG (gammachain) (Tago, Burlingame, CA, USA, cat no 4100) and goat-antihuman-IgG peroxidase (Nordic,
Tilburg, The Netherlands) as conjugate [35]. The detection limit is 1.5 µg/l. Urinary β2-microglobulin was measured by nephelometry.

Sodium, potassium, urea and creatinine in serum and urine were measured on SMA(C) autoanalysers (Technicon Instruments Inc. Tarrytown, N.Y., USA). Glycated haemoglobin was measured by HPLC (Bio-Rad, Veenendaal, The Netherlands).

Statistical analysis

Results are expressed as mean±SEM for normally distributed data and as geometric mean (95% confidence interval, CI) for not-normally distributed data unless stated otherwise. The 2 clearance periods obtained at baseline, threshold, 20% pressor, pressor and recovery were averaged for analysis. The NE effects were analysed after subtraction of the data obtained during the placebo day. Within-group and between-group differences were analysed with one-way ANOVA and repeated measurements ANOVA, for parametrically or non-parametrically distributed data where appropriate. Adjustment for multiple comparisons was carried out using Duncan's method. Bivariate correlations were sought using Spearman's rank correlation. If correlations were significant, logistic regression lines were constructed using standard curve fitting models where appropriate. Multiple regression analysis evaluated independent contributions of parameters on the time-controlled observations during the 3 NE infusion periods. In case of not-normally distributed data, logarithmically transformed values were used. P-values less than 0.05 were considered to be significant.

Results

Blood glucose and insulin concentrations during NE and placebo administration

In the diabetic groups, blood glucose remained unchanged and was similar during NE and placebo infusions (Table 2). In group C, blood glucose rose modestly with increasing NE dose to levels that were slightly higher than in group D1 and D2 (Table 2). A reduction of glucose infusion rate was, however, not possible (see study design). In all groups, plasma insulin was stable during NE and placebo administration. As expected, plasma insulin levels were consistently higher in group D1 and D2 compared to group C (p<0.001, Table 2).

NE dose response curves, blood pressure and venous plasma NE during renal haemodynamic studies

The dose-response curves of the calculated threshold, 20% pressor and pressor doses were significantly shifted leftwards in group D1 and D2 compared to group C (p<0.05 for both, Figure 1A). As a consequence of the lower NE doses required, lower plasma NE levels were attained in group D1 and D2 compared to group C during the NE infusion day (p<0.05, Table 3). There was a close relationship between the NE infusion rate and increments in plasma NE levels in all groups (C: r = 0.93, p<0.001 (n=24); D1: r = 0.91, p<0.001 (n=24); and D2: r = 0.88, p<0.001 (n=24), Figure 1B). As this relationship was similar in the 3 groups, the averaged venous plasma NE-pressor curves were also significantly shifted leftwards in both diabetic groups compared to group C (Figure 1C).
Table 2. Blood glucose and plasma insulin during norepinephrine (NE) and placebo infusions.

<table>
<thead>
<tr>
<th>Period</th>
<th>NE (mmol/l)</th>
<th>Placebo (mmol/l)</th>
<th>NE (mmol/l)</th>
<th>Placebo (mmol/l)</th>
<th>NE (mmol/l)</th>
<th>Placebo (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood Glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>5.1±0.1</td>
<td>5.1±0.1</td>
<td>4.9±0.1</td>
<td>5.3±0.1</td>
<td>5.2±0.2</td>
<td>5.1±0.1</td>
</tr>
<tr>
<td>Threshold</td>
<td>5.8±0.2</td>
<td>5.0±0.1</td>
<td>5.0±0.1</td>
<td>4.9±0.1</td>
<td>5.2±0.2</td>
<td>5.0±0.1</td>
</tr>
<tr>
<td>20% Pressor</td>
<td>6.1±0.2</td>
<td>5.0±0.1</td>
<td>5.1±0.1</td>
<td>5.1±0.1</td>
<td>5.3±0.1</td>
<td>5.0±0.1</td>
</tr>
<tr>
<td>Pressor</td>
<td>6.8±0.4</td>
<td>4.9±0.1</td>
<td>5.1±0.1</td>
<td>5.1±0.1</td>
<td>5.3±0.1</td>
<td>5.0±0.1</td>
</tr>
<tr>
<td>Recovery</td>
<td>6.1±0.3</td>
<td>4.9±0.1</td>
<td>5.1±0.1</td>
<td>5.0±0.1</td>
<td>5.2±0.1</td>
<td>5.1±0.1</td>
</tr>
</tbody>
</table>

Plasma Insulin (mU/l)

<table>
<thead>
<tr>
<th>Period</th>
<th>NE (mU/l)</th>
<th>Placebo (mU/l)</th>
<th>NE (mU/l)</th>
<th>Placebo (mU/l)</th>
<th>NE (mU/l)</th>
<th>Placebo (mU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>10±2</td>
<td>11±2</td>
<td>30±3</td>
<td>31±3</td>
<td>31±4</td>
<td>31±3</td>
</tr>
<tr>
<td>Threshold</td>
<td>11±2</td>
<td>12±2</td>
<td>30±3</td>
<td>28±4</td>
<td>30±4</td>
<td>30±3</td>
</tr>
<tr>
<td>20% Pressor</td>
<td>12±2</td>
<td>13±2</td>
<td>31±3</td>
<td>31±3</td>
<td>30±4</td>
<td>30±3</td>
</tr>
<tr>
<td>Pressor</td>
<td>13±1</td>
<td>12±2</td>
<td>32±4</td>
<td>29±3</td>
<td>32±4</td>
<td>31±4</td>
</tr>
<tr>
<td>Recovery</td>
<td>19±4b</td>
<td>12±2</td>
<td>30±3</td>
<td>31±3</td>
<td>30±3</td>
<td>29±3</td>
</tr>
</tbody>
</table>

Values are given in mean±SEM. a) p<0.001 for all values in group C from group D1 and D2; b) p<0.05; c) p<0.001 from baseline after correction for placebo day; d) p<0.05; e) p<0.01 from group D1 and D2.

NE infused at the calculated threshold, 20% pressor and pressor doses resulted in similar changes in MAP in the 3 groups with adequate achievement of target blood pressures (Figure 1C, Table 3), while MAP was unaltered with placebo. Baseline pulse rate was similar among the groups (group C: 63±4, group D1: 62±4, group D2: 67±4 (beats/min)). In group C, pulse rate declined with each infusion step (threshold dose: -5±2, p<0.05; 20% pressor dose: -7±2, p<0.01; pressor dose: -11±2 (beats/min), p<0.001). In group D1 and D2 this fall was only present at NE pressor dose ( -7±2 and -6±2 (beats/min), p<0.01, respectively). The overall pulse rate response was less pronounced in group D1 and D2 compared to group C (p<0.05).

Plasma renin and angiotensin II

During the placebo day, plasma renin and prorenin levels were higher in group D2 than in group C and D1 (renin, see Table 3; averaged prorenin 173 (125 to 241), 281 (196 to 403) and 512 (297 to 884) mU/L (geometric mean and 95%CI in group D1, D2 and C, respectively). During NE pressor dose, renin increased in all groups (p<0.05) and remained so after cessation of NE infusion (p<0.05 for all, Table 3).
Table 3. Blood pressure, venous plasma norepinephrine (NE), plasma active renin and angiotensin II concentrations during NE and placebo infusions.

<table>
<thead>
<tr>
<th>Period</th>
<th>Non-diabetic Subjects (n=8)</th>
<th>Normoalbuminuric IDDM patients (n=8)</th>
<th>Microalbuminuric IDDM patients (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group C</td>
<td>Group D1</td>
<td>Group D2</td>
</tr>
<tr>
<td>Mean Arterial Pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>92.7±2.0</td>
<td>90.2±2.7</td>
<td>90.3±2.6</td>
</tr>
<tr>
<td></td>
<td>91.4±3.0</td>
<td>90.3±2.6</td>
<td>97.4±1.3</td>
</tr>
<tr>
<td></td>
<td>96.5±2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ at Threshold</td>
<td>2.0±0.5</td>
<td>-0.2±0.7</td>
<td>2.3±0.6</td>
</tr>
<tr>
<td></td>
<td>-1.0±0.4</td>
<td>2.5±0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1±0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ at 20% Pressor</td>
<td>4.7±1.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-1.7±0.8</td>
<td>5.7±0.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>-1.8±0.9</td>
<td>6.0±1.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-1.3±0.8</td>
</tr>
<tr>
<td>Δ at Pressor</td>
<td>20.6±1.6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>-0.6±1.0</td>
<td>18.5±1.3&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>-2.0±0.7</td>
<td>20.8±1.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-2.1±0.8</td>
</tr>
<tr>
<td>Δ at Recovery</td>
<td>-7.0±3.0</td>
<td>0.5±1.6</td>
<td>-3.3±1.8</td>
</tr>
<tr>
<td></td>
<td>-3.6±1.3</td>
<td>-2.8±2.1</td>
<td>-0.8±0.7</td>
</tr>
<tr>
<td>Venous Plasma NE (nmol/l)&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2.6(2.0-3.4)</td>
<td>2.6(1.7-3.8)</td>
<td>2.3(1.7-3.2)</td>
</tr>
<tr>
<td></td>
<td>2.3(1.6-3.2)</td>
<td>3.0(2.2-4.0)</td>
<td>2.7(2.2-3.4)</td>
</tr>
<tr>
<td>Threshold</td>
<td>7.0(4.0-12.4)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.6(1.9-3.8)</td>
<td>4.8(3.4-6.9)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2.4(1.7-3.3)</td>
<td>5.3(4.2-6.5)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.9(2.3-3.6)</td>
</tr>
<tr>
<td>20% Pressor</td>
<td>10.4(6.1-17.5)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.6(2.0-3.5)</td>
<td>5.9(3.7-9.4)&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2.5(1.8-3.6)</td>
<td>6.7(5.3-8.4)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.7(2.0-3.5)</td>
</tr>
<tr>
<td>Pressor</td>
<td>29.2(20.8-41)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.0(8.3-32.2)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.6(2.0-3.5)</td>
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<tr>
<td></td>
<td>17.3(11-27.1)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.6(2.2-3.1)</td>
<td></td>
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<tr>
<td>Recovery</td>
<td>3.6(2.7-4.9)</td>
<td>2.9(2.1-4.1)</td>
<td>3.1(2.1-4.6)</td>
</tr>
<tr>
<td></td>
<td>2.5(2.0-3.1)</td>
<td>3.6(2.6-5.1)</td>
<td>3.2(2.4-4.2)</td>
</tr>
<tr>
<td>Plasma Active Renin (mU/l)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>40 (27-60)</td>
<td>41 (30-55)</td>
<td>37 (20-69)</td>
</tr>
<tr>
<td></td>
<td>37 (16-61)</td>
<td>37 (16-61)</td>
<td>57 (36-98)</td>
</tr>
<tr>
<td></td>
<td>74 (48-113)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threshold</td>
<td>42 (30-59)</td>
<td>40 (32-49)</td>
<td>36 (19-67)</td>
</tr>
<tr>
<td></td>
<td>29 (15-59)</td>
<td>53 (33-84)</td>
<td>59 (34-103)</td>
</tr>
<tr>
<td>20% Pressor</td>
<td>46 (33-65)</td>
<td>39 (29-53)</td>
<td>40 (22-75)</td>
</tr>
<tr>
<td></td>
<td>28 (14-55)</td>
<td>61 (34-109)</td>
<td>59 (34-101)</td>
</tr>
<tr>
<td>Pressor</td>
<td>57 (38-85)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>40 (30-53)</td>
<td>52 (30-90)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>31 (16-59)</td>
<td>87 (48-159)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>66 (41-107)</td>
</tr>
<tr>
<td>Recovery</td>
<td>55 (33-99)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>37 (27-51)</td>
<td>55 (28-108)&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>31 (14-65)</td>
<td>84 (50-139)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>71 (47-107)</td>
</tr>
<tr>
<td>Plasma Angiotensin II (pmol/l)&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>13.9±2.4</td>
</tr>
<tr>
<td></td>
<td>13.6±2.1</td>
<td>16.3±2.0</td>
<td>18.1±2.2</td>
</tr>
<tr>
<td>Threshold</td>
<td>16.3±1.6</td>
<td>14.8±2.4</td>
<td>13.6±2.1</td>
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<td>14.1±2.3</td>
<td>16.1±2.0</td>
<td>16.9±2.4</td>
</tr>
<tr>
<td>20% Pressor</td>
<td>18.1±1.6</td>
<td>14.8±2.7</td>
<td>15.3±2.4</td>
</tr>
<tr>
<td></td>
<td>13.3±1.9</td>
<td>18.1±2.6</td>
<td>17.6±2.7</td>
</tr>
<tr>
<td>Pressor</td>
<td>24.6±3.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.8±2.5</td>
<td>18.3±3.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>14.6±2.6</td>
<td>25.1±4.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>17.9±2.8</td>
</tr>
<tr>
<td>Recovery</td>
<td>22.6±5.2</td>
<td>14.3±1.8</td>
<td>20.1±4.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>14.4±2.3</td>
<td>20.9±3.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.5±1.8</td>
</tr>
</tbody>
</table>

Values are given in mean±SEM, except plasma NE and plasma active renin which are given in geometric mean and 95% confidence interval. <sup>a</sup> Overall values in group C>groups D1 and D2 (<i>p</i>&lt;0.05); <sup>b</sup> overall values in group D2>groups C and D1 (<i>p</i>&lt;0.05); <sup>c</sup> <i>p</i>&lt;0.05, <sup>d</sup> <i>p</i>&lt;0.01 and <sup>e</sup> <i>p</i>&lt;0.001 from baseline, after correction for placebo day.
Norepinephrine, microproteinuria and renal haemodynamics 69

Figure 1. Relationships between norepinephrine (NE) infusion, plasma NE increment and changes in mean arterial pressure (MAP). A. Systemic pressor responses to exogenous NE infusions at threshold (T), 20% pressor (20% P) and pressor (P) dose. B. NE infusion dose and changes in plasma NE at NE threshold, 20% pressor and pressor dose. Spearman’s rank correlation coefficients: control subjects \( r_s=0.93, p<0.001 \); normoalbuminuric IDDM patients \( r_s=0.91, p<0.001 \) and microalbuminuric IDDM patients \( r_s=0.88, p<0.001 \). The logistic regression lines are constructed by curve fitting. There were no differences in the relationships. C. Actual changes in MAP vs changes in plasma NE at T, 20% P and P dose during NE infusions. Data in geometric means ± antilog SEM. ● control subjects (group C), □ normoalbuminuric (group D1) and △ microalbuminuric (group D2) IDDM patients; * denotes \( p<0.05 \) from normo- and microalbuminuric IDDM.
During placebo infusion, serum ACE activity and plasma angiotensin II were not different among the groups (Table 3). During NE pressor infusion, plasma AII levels increased similarly in all groups ($p<0.05$ for all).

**Urinary excretion of albumin, IgG and β2-microglobulin.**

Urinary albumin and IgG excretion rates (Ualb.V and UIgG.V) were higher in group D2 than in groups C and D1 at all observation periods ($p<0.01$, Table 4). During the placebo day UIgG.V fell in all groups ($p<0.05$ to 0.01). Ualb.V also fell significantly during placebo in group D2 ($p<0.05$ to 0.01) with a tendency to fall in the other groups as well (Table 4). Interestingly, during NE pressor infusion, Ualb.V and UIgG.V rose significantly in all groups ($p<0.05$ for all, Table 4) and some of these parameters were still elevated at recovery in group C and D1. The increments in Ualb.V and UIgG.V (corrected for placebo) were more pronounced in group D2 (31.3 (11.8-83.1) and 4.0 (2.0-8.1) µg/min, respectively) than in group C (4.2 (1.3-13.7) and 0.6 (0.2-2.8) µg/min, respectively) and D1 (3.8 (1.4-10.1) and 0.6 (0.2-2.0) µg/min, respectively) ($p<0.05$, Table 4 and Figure 2). Fractional albumin and IgG excretion also increased during NE pressor infusion in all groups ($p<0.05$ to 0.01, Table 4). β2-Microglobulin excretion was not different in the groups during placebo and remained stable during NE infusion (Table 4, Figure 2).

Multiple stepwise regression analysis was carried out to evaluate which parameters contributed independently to the changes in microproteinuria and renal haemodynamics in response to exogenous NE. Baseline Ualb.V, change in MAP and change in plasma NE independently contributed to 37% ($p<0.001$), 8% ($p<0.01$) and to 6% ($p<0.05$) of the variance in change in Ualb.V (multiple r=0.56, 72 data sets). The variance in change in UIgG.V resulted for 27% from baseline UIgG.V ($p<0.001$), for 7% from the change in MAP ($p<0.02$) and for 5% from the change in plasma NE ($p<0.05$, multiple r=0.48, 72 data sets). No independent effects of plasma insulin, blood glucose and angiotensin II on microproteinuria were demonstrated ($p>0.20$, for all).

When the relative increments in Ualb.V and UIgG.V at NE pressor infusion were compared, no differences in the responses were observed between the 3 groups (% increment in Ualb.V and UIgG.V at NE pressor dose in group C: 233(122-344)% and 233(104-361)% (geometric mean and 95% confidence interval), in group D1 202(127-278)% and 154(115-193)%, in group D2 166(133-202)% and 166(117-214)%, respectively). Furthermore, multiple regression analysis showed that the relative changes in Ualb.V and UIgG.V were not independently related to baseline microproteinuria ($p>0.20$ for both). Thus, the extent of baseline glomerular protein leakage was the main determinant of the absolute microproteinuric response to NE.

**Renal haemodynamic studies**

During the placebo day, GFR and ERPF were not significantly different between the 3 groups, whereas FF was slightly but significantly higher in group D2 compared to the other groups ($p<0.05$). Glomerular hyperfiltration, defined as GFR>130 ml/min per 1.73 m$^2$ [9], was present in 2 patients of group D1 and 3 patients of group D2. GFR remained essentially unaltered in the groups during NE infusion (Table 5).
Table 4. Urinary excretions of albumin, IgG and β2-microglobulin during norepinephrine (NE) and placebo infusions.

<table>
<thead>
<tr>
<th>Period</th>
<th>NE</th>
<th>Placebo</th>
<th>NE</th>
<th>Placebo</th>
<th>NE</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>4.6(3.5-6.2)</td>
<td>5.6(3.5-7.6)</td>
<td>4.2(2.9-6.0)</td>
<td>5.6(3.4-9.3)</td>
<td>47.9(23.2-98.8)</td>
<td>52.2(22.3-122.2)</td>
</tr>
<tr>
<td>T</td>
<td>4.7(2.9-7.8)</td>
<td>4.6(3.2-6.6)</td>
<td>4.3(2.9-6.6)</td>
<td>5.1(3.1-8.3)</td>
<td>47.6(23.9-94.7)</td>
<td>38.9(16.2-93.4)</td>
</tr>
<tr>
<td>S</td>
<td>5.5(3.5-8.8)</td>
<td>4.3(2.9-6.3)</td>
<td>5.6(3.7-8.6)</td>
<td>5.2(3.2-8.7)</td>
<td>50.3(24.9-101.5)</td>
<td>36.8(14.8-91.8)</td>
</tr>
<tr>
<td>P</td>
<td>9.7(4.6-20.2)</td>
<td>4.5(3.1-6.7)</td>
<td>7.3(4.8-11.2)</td>
<td>4.6(2.8-7.4)</td>
<td>58.5(33.6-101.7)</td>
<td>33.8(14.9-72.2)</td>
</tr>
<tr>
<td>R</td>
<td>8.2(4.0-16.7)</td>
<td>4.6(3.1-6.9)</td>
<td>7.4(5.1-11.0)</td>
<td>4.1(2.6-6.4)</td>
<td>43.7(24.4-78.1)</td>
<td>31.3(15.2-64.6)</td>
</tr>
<tr>
<td>IgG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1.1(0.8-1.4)</td>
<td>1.1(0.8-1.5)</td>
<td>1.0(0.8-1.4)</td>
<td>1.2(0.8-1.8)</td>
<td>11.7(4.9-28.1)</td>
<td>12.8(4.8-34.2)</td>
</tr>
<tr>
<td>T</td>
<td>1.0(0.7-1.5)</td>
<td>1.1(0.8-1.4)</td>
<td>1.0(0.8-1.4)</td>
<td>1.2(0.9-1.8)</td>
<td>11.5(4.8-27.9)</td>
<td>9.6(3.2-28.8)</td>
</tr>
<tr>
<td>S</td>
<td>1.3(0.9-1.8)</td>
<td>1.0(0.7-1.3)</td>
<td>1.3(0.9-1.8)</td>
<td>1.2(0.8-1.9)</td>
<td>12.4(5.3-29.1)</td>
<td>8.6(2.9-25.4)</td>
</tr>
<tr>
<td>P</td>
<td>2.2(1.1-4.3)</td>
<td>1.0(0.8-1.4)</td>
<td>1.7(1.2-2.6)</td>
<td>1.1(0.7-1.7)</td>
<td>15.3(7.4-31.7)</td>
<td>7.9(3.0-21.0)</td>
</tr>
<tr>
<td>R</td>
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<td>1.0(0.7-1.5)</td>
<td>1.9(1.3-2.6)</td>
<td>1.0(0.6-1.4)</td>
<td>11.7(5.6-24.3)</td>
<td>7.7(3.0-19.9)</td>
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<tr>
<td>β2-MG</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>68(53-87)</td>
<td>72(43-122)</td>
<td>62(43-82)</td>
<td>59(43-82)</td>
<td>94(53-167)</td>
<td>88(48-163)</td>
</tr>
<tr>
<td>T</td>
<td>53(31-91)</td>
<td>65(45-93)</td>
<td>52(36-75)</td>
<td>52(38-72)</td>
<td>89(54-145)</td>
<td>84(53-133)</td>
</tr>
<tr>
<td>S</td>
<td>34(20-56)</td>
<td>63(36-112)</td>
<td>43(30-62)</td>
<td>50(37-68)</td>
<td>76(45-128)</td>
<td>73(48-110)</td>
</tr>
<tr>
<td>R</td>
<td>38(22-65)</td>
<td>57(37-88)</td>
<td>39(22-42)</td>
<td>44(29-66)</td>
<td>38(18-80)</td>
<td>69(47-100)</td>
</tr>
</tbody>
</table>

Values are given as geometric mean and 95% confidence interval. B: baseline; T: threshold; S: 20% pressor; P: pressor; R: recovery. a All values in group D2>group C and D1 (p<0.01); b p<0.05, c p<0.01 from baseline (decline at placebo day); d p<0.05, e p<0.01 from baseline (increase during NE corrected for placebo day); f p<0.05 from groups C and D1 (absolute increase).
Figure 2. Relationship between plasma norepinephrine (NE) and A. urinary albumin (Ualb.V), B. IGG (UIgI.V) and C. β2-microglobulin excretion. Data in geometric mean ±antilog SEM, values corrected for placebo day. • control subjects (group C), □ normoalbuminuric (group D1) and Δ microalbuminuric (group D2) IDDM patients; * denotes $p<0.05$ and ** $p<0.01$ from baseline; *** $p<0.05$ increase in group D2 > group C and D1.
Table 5. Renal haemodynamics during norepinephrine (NE) and placebo infusions.

<table>
<thead>
<tr>
<th>Subjects (n=8)</th>
<th>Non-diabetic</th>
<th>Normoalbuminuric</th>
<th>Microalbuminuric</th>
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<tr>
<td>Group C</td>
<td>NE Placebo</td>
<td>NE Placebo</td>
<td>NE Placebo</td>
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<tr>
<td>Baseline</td>
<td>103±5</td>
<td>103±4</td>
<td>105±5</td>
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<td>Threshold</td>
<td>106±6</td>
<td>105±5</td>
<td>109±5</td>
</tr>
<tr>
<td>20%Pressor</td>
<td>104±5</td>
<td>103±4</td>
<td>109±6</td>
</tr>
<tr>
<td>Pressor</td>
<td>103±4</td>
<td>103±4</td>
<td>108±5</td>
</tr>
<tr>
<td>Recovery</td>
<td>92±5</td>
<td>102±3</td>
<td>94±5</td>
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<table>
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<th>Group D1</th>
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<tr>
<td>Baseline</td>
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<td>107±6</td>
<td>104±8</td>
</tr>
<tr>
<td>Threshold</td>
<td>108±9</td>
<td>109±8</td>
<td>109±8</td>
</tr>
<tr>
<td>20%Pressor</td>
<td>111±8</td>
<td>111±8</td>
<td>111±8</td>
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<tr>
<td>Pressor</td>
<td>106±19</td>
<td>109±8</td>
<td>108±9</td>
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<td>Recovery</td>
<td>95±8</td>
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<table>
<thead>
<tr>
<th>Group D2</th>
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<tr>
<td>Threshold</td>
<td>109±8</td>
<td>109±8</td>
<td>109±8</td>
</tr>
<tr>
<td>20%Pressor</td>
<td>111±8</td>
<td>111±8</td>
<td>111±8</td>
</tr>
<tr>
<td>Pressor</td>
<td>108±9</td>
<td>108±9</td>
<td>108±9</td>
</tr>
<tr>
<td>Recovery</td>
<td>108±8</td>
<td>108±8</td>
<td>108±8</td>
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</table>

Values are given in mean±SEM. *Overall responses at threshold, 20% pressor and pressor period in group C > groups D1 and D2 (p<0.05); b p<0.05 from group C and D1 at placebo day; c p<0.01 and d p<0.001 from baseline, after correction for placebo day.*

ERPF progressively decreased in all groups with increasing NE infusion rates (p<0.05 to 0.001). As a result, FF rose significantly with all NE infusion steps (Table 5). The overall changes in ERPF and FF during NE infusions were more pronounced in group C compared to the diabetic groups (p<0.05 and p<0.01, respectively). In each group, the changes in ERPF and FF were strongly correlated with the changes in plasma NE levels (group C: r=-0.82 and r=-0.85, n=24, p<0.001; group D1: r=-0.87 and r=0.85, n=24, p<0.001; group D2: r=-0.76 and r=0.83, n=24, p<0.001, respectively, but no differences in the relationships between the 3 groups were observed (Figure 3A-B). A similar relationship was observed with NE infusion dose (data not shown). Thus, for a given increase in plasma NE level the changes in ERPF and FF were similar in group D1 and D2 compared to group C and the differences in NE dose fully accounted for the between group differences in ERPF and FF responses. These relationships were not confounded by variation in plasma insulin and blood glucose (data not shown).
Figure 3.A. Relationships between changes in plasma norepinephrine (NE) and change in effective renal plasma flow (ERPF), and B. and filtration fraction (FF) following exogenous NE infusions. The logistic regression lines are constructed by curve fitting (3 data sets per subject). No difference in ERPF and FF responses between the groups were demonstrated. ● control subjects (group C), □ normoalbuminuric (group D1) and △ microalbuminuric (group D2) IDDM patients.
Discussion

The present study is the first to document that exogenous NE increases microproteinuria in conjunction with a systemic blood pressure rise and renal vasoconstriction in normo- and microalbuminuric IDDM patients and healthy subjects. The lack of effect on urinary β2-microglobulin indicates that NE did not affect tubular protein handling. The rises in absolute and fractional urinary albumin and IgG excretion, therefore, support the notion that NE increases glomerular protein leakage. Furthermore, in absolute terms the microalbuminuric response was more pronounced in microalbuminuric IDDM patients than in normoalbuminuric IDDM patients and healthy controls. By contrast, the renal haemodynamic responses to NE were similar in the 3 groups, as indicated by similar relationships between plasma NE increments and renal haemodynamic responses.

As systemic blood pressure is an important determinant of microproteinuria in IDDM [4,5,18,37], this study was designed to evaluate the microalbuminuric and renal haemodynamic effects of NE given in doses that induced predetermined blood pressure increments. We were thus able to avoid confounding effects of differences in blood pressure responses to NE. The enhanced blood pressure responsiveness in the diabetic groups is in keeping with earlier reports [21-23] and underscores the relevance of this approach. The less pronounced decline in heart rate in the IDDM patients, which may reflect impaired baroreflex function, could have contributed to this enhanced systemic NE responsiveness [38].

The prevailing plasma insulin and blood glucose levels can affect systemic vascular NE responsiveness, as well as ERPF and GFR [30,31,39-41]. Exogenous insulin acutely elevates renal plasma flow at levels of 90 mU/L, but not significantly so at levels of 30 to 40 mU/L [40,41], and rises in blood glucose from normal to moderately elevated levels modestly increases GFR [30]. In our study, plasma insulin was kept constant during NE infusion in all groups. Its level was higher in the IDDM patients (approximately 30 mU/L) than in the control subjects (approximately 10 mU/L). This difference was intentionally allowed since plasma insulin levels of 30 mU/L are normally encountered in IDDM, but non-physiologically high in fasting healthy subjects [13]. The normoglycaemic circumstances allowed evaluation of the NE effects unaffected by the prevailing blood glucose. The slight increase in blood glucose during NE infusion in the control subjects is very unlikely to be of relevance in the interpretation of the NE effects, as supported by the lack of effect of this parameter in statistical analyses.

The similar regression lines between NE doses and venous plasma NE increments in all groups, is in keeping with an unaltered plasma NE clearance in diabetic patients [42]. Although venous antecubital NE measurement has the disadvantage of being confounded by forearm NE spillover and extraction [43], it is thus improbable that the interpretation of our results was biased by using venous plasma NE levels. Yet, our study does not allow conclusions to potential differences in intrarenal NE handling between (microalbuminuric) IDDM patients and control subjects.

Based on observations in hand vein studies, which like renal arterioles also contain α-adrenoceptors, it has been suggested that an exaggerated renal vascular responsiveness to NE might be involved in elevations in albumin excretion rate in IDDM [20]. In this
study, we found no evidence for an altered renal vascular NE responsiveness in normo- and microalbuminuric IDDM as indicated by similar relationships between increments in plasma NE and renal haemodynamic changes in the 3 groups. In comparison, the diabetic state has been variably associated with either an increase, no change or a decrease in vascular reactivity to vasoconstrictive agents [44]. These discrepancies may be attributed to differences in the species investigated, in the blood glucose and insulin levels during the experiments, or the vascular bed under study.

Posture and diurnal rhythm affect glomerular protein leakage. Our study clearly demonstrates that urinary protein excretion falls under conditions of supine rest. Interestingly, for albumin this fall was particularly apparent in the microalbuminuric patients. This indicates that it is mandatory to include time-control data to properly evaluate the effects of acute interventions on microalbuminuria. The previous reported lack of effect of NE to increase microalbuminuria in healthy subjects may thus have been due to the uncontrolled data use in that study [17]. Two recent studies report that exogenous angiotensin II does not influence microalbuminuria in non-diabetic [45] and diabetic subjects [46], in spite of a clear-cut effect on renal haemodynamics. This could indicate that low doses of exogenous angiotensin II, unlike our findings with NE, are indeed unable to induce a rise in albuminuria in man. On the other hand, the absence of time-control data in those studies also hampers a direct comparison with our data.

The microproteinuria promoting effect of NE may have arisen from its systemic blood pressure elevating effects as well as from an intrarenal effect, like a rise in glomerular pressure. Animal experiments have shown that NE, irrespective of systemic blood pressure, induces a rise in glomerular pressure [12], thereby promoting glomerular protein leakage. Although glomerular pressure cannot be measured directly in man, our findings of a well-preserved GFR despite a pronounced decrease in ERPF during NE are strongly suggestive for a rise in filtration pressure. To identify the determinants of this microproteinuric response, we performed multiple regression analysis. This analysis revealed blood pressure to be an independent determinant of the microproteinuric response, suggesting that the rise in blood pressure is causally involved in the rise in microproteinuria. Microalbuminuria was also independently related to the plasma NE level, indeed suggesting a blood pressure independent effect of NE as well.

The exaggerated rise in microproteinuria in microalbuminuric IDDM could not be explained by an altered blood pressure response or renal haemodynamic responsiveness to NE nor by a difference in NE-induced angiotensin II release. Instead, the baseline level of microproteinuria appeared to be the main determinant of the microproteinuric response to NE across the groups. In accordance, no between group differences in relative increments in urinary albumin and IgG excretion were seen. This underscores that the preexistent defect in glomerular perm selectivity is crucial in the absolute microproteinuric effect of NE. Our data do not allow a conclusion whether such an effect is specific for NE, or alternatively, whether any proteinuria-promoting manoeuvre is inclined to induce a larger response when a defect in glomerular capillary permselectivity is already present. The enhanced microproteinuric response was found both for the negatively charged albumin and for the much larger and predominantly neutrally charged IgG, raising the possibility that a loss of anionic charge of the glomerular basement membrane is not the only abnormality at this early stage of diabetic nephropathy [47].
What is the clinical relevance of our findings? The present results are consistent with the concept that NE contributes to a rise in microproteinuria as for instance observed during strenuous exercise [18,19]. The larger absolute response in micro-albuminuric IDDM may implicate that this phenomenon contributes to persistent microproteinuric glomerular protein leakage. As glomerular protein leakage is presumed to be involved in the progression of diabetic nephropathy [48,49], such small but repetitive increases in glomerular protein leakage could be involved in progressive renal damage in the long run.

In conclusion, exogenous NE causes a microproteinuric response in association with a rise in blood pressure, renal vasoconstriction and possibly intraglomerular hypertension. In microalbuminuric IDDM, this microproteinuric response is enhanced, which may reflect either a specific response to NE or a general effect of vasopressor stimuli to promote glomerular protein leakage in patients with a preexistent defect in glomerular permselectivity.

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CHAPTER 5

NOREPINEPHRINE-INDUCED BLOOD PRESSURE RISE AND RENAL VASOCONSTRICTION IS NOT ATTENUATED BY ENALAPRIL IN MICROALBUMINURIC IDDM

K. Hoogenberg¹, G. Navis² and R.P.F. Dullaart¹

In non-diabetic subjects an attenuated systemic norepinephrine (NE) responsiveness may contribute to the mechanisms of action of angiotensin-converting enzyme (ACE) inhibitor treatment. We determined whether ACE-inhibitor treatment influences systemic and renal haemodynamic responsiveness to exogenous NE, as well as urinary albumin excretion during NE, in micro-albuminuric IDDM patients, representing a patient category that benefits by strict blood pressure control. In 7 microalbuminuric IDDM patients, systemic and renal responsiveness to NE, infused at individually determined threshold (Δmean arterial pressure (MAP)=0mmHg), 20% pressor (ΔMAP =4mmHg) and pressor (ΔMAP=20mmHg) doses, were compared before and after 8 weeks treatment with enalapril, 10 mg daily. Blood glucose was clamped at 5 mmol/l and insulin was infused at 30 mU/kg per h. Enalapril decreased MAP (p<0.05) and microalbuminuria (p<0.05), whereas effective renal plasma flow (ERPF) increased (p<0.01) and glomerular filtration rate (GFR) remained unaltered. Filtration fraction tended to decline (p=0.09). The ACE inhibitor-induced fall in MAP disappeared at NE pressor dose, and the overall mean increase in MAP in response to NE was even higher with than without enalapril (p<0.05). After enalapril, ERPF remained higher at all NE doses (p<0.05), but the magnitude of the NE-induced fall in ERPF was not altered by ACE inhibition treatment. Overnight urinary albumin excretion fell by ACE-inhibition (p<0.05), but this effect was not seen during NE infusion. The angiotensin II/active renin ratio and serum aldosterone levels remained lower with enalapril at all NE doses (p<0.05). In conclusion, enalapril does not attenuate systemic and renal vascular responsiveness to exogenous NE in microalbuminuric IDDM despite adequate inhibition of the renin-angiotensin-aldosterone system. These findings suggest that the effect of NE on vasoconstriction is not effectively counteracted by ACE inhibition treatment alone.

Introduction

Blockade of the renin-angiotensin-aldosterone system (RAAS) by angiotensin-converting enzyme (ACE) inhibitors lowers blood pressure, (micro)albuminuria and slows progression of diabetic and non-diabetic renal insufficiency, although such treatment is unable to completely prevent this serious complication [1-3]. The mechanism of action of these agents is still incompletely understood. Both angiotensin II-dependent and independent mechanisms are thought to be involved. The sympathetic nervous system (SNS) and RAAS are closely related [4] and ACE inhibition may influence systemic norepinephrine (NE) reactivity. Experimental studies indeed have shown that systemic

¹Department of Endocrinology and ²Nephrology, Groningen University Hospital, The Netherlands.

Nephrol Dial Transplant (in press)
vascular NE responsiveness is attenuated by ACE inhibitors [5,6]. A decrease in systemic NE responsiveness after ACE inhibition treatment has been demonstrated in hypertensive subjects [7-9]. Moreover, in healthy subjects the renal vasoconstrictive response has been found to be reduced after ACE inhibition [10]. This would suggest that attenuation of renal NE responsiveness may be involved in the renoprotective effect of ACE inhibitors in non-diabetic subjects.

In insulin-dependent and non-insulin-dependent diabetes mellitus (IDDM and NIDDM), the systemic vasopressor response to exogenous NE has been repeatedly found to be enhanced [11-13]. The effect of ACE inhibition on systemic and renal NE responsiveness is less well characterised in diabetes mellitus. In patients with NIDDM and in a heterogeneous group of NIDDM and IDDM patients, treatment with enalapril or captopril did not normalise the exaggerated systemic NE responsiveness [14,15], but this has not been studied in IDDM complicated by microalbuminuria. It is of particular relevance to evaluate the NE responsiveness after ACE-inhibition treatment in microalbuminuric IDDM patients, because control of factors that contribute to a blood pressure rise in such patients appears to be indicated to prevent progression to overt nephropathy. The purpose of our study was, therefore, to evaluate the effect of enalapril treatment on systemic and renal haemodynamic responsiveness to exogenous NE in IDDM patients with microalbuminuria.

Subjects and methods

 Subjects

The study was approved by the local medical ethics committee and all participants gave written informed consent. The patients were considered insulin-dependent, and glucagon stimulated C-peptide levels were less than 0.2 nmol/l. Seven patients (6 men, 1 women) participated. They had a mean age of 48±9 (mean±SD) years and a diabetes duration of 29±5 years. None had severe obesity (body mass index ranging from 23.5 to 27.9 kg/m²). All of the patients had persistent microalbuminuria (defined as an urinary albumin excretion rate (Ualb.V) between 20 to 200 µg/min in at least two out of three overnight urine collections for a 1-year period). All patients were treated with enalapril for elevated blood pressure related to the presence of incipient nephropathy (systolic/ diastolic blood pressure >140/90 mmHg), but none had essential hypertension before development of microalbuminuria. ACE inhibition treatment was stopped for 6 weeks before the start of the study. Without enalapril, 6 patients had a systolic blood pressure > 140 mmHg and 1 patient had a diastolic blood pressure >90 mmHg. None of the patients had untreated proliferative retinopathy, symptomatic coronary heart disease, peripheral vascular disease, or clinical autonomic neuropathy as evaluated by standard tests (beat-to-beat variation during deep breathing, Valsalva manoeuvre and systolic blood pressure response to standing). All participants were instructed by dietician to adhere to a diet containing 100 mmol sodium and 1 gram protein/kilogram body weight per day throughout the studies. Three timed overnight urine collections were obtained directly prior to the studies.
Enalapril and norepinephrine reactivity in IDDM

### Table 1. Baseline characteristics before and after 8 weeks treatment with enalapril.

<table>
<thead>
<tr>
<th></th>
<th>before ACEi</th>
<th>with ACEi</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c (%)</td>
<td>8.4 ±0.2</td>
<td>8.5 ± 0.2</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>83.2 ± 2.7</td>
<td>83.3 ± 2.7</td>
</tr>
<tr>
<td>Urinary urea excretion (mmol/day)</td>
<td>279 ±26</td>
<td>282 ± 16</td>
</tr>
<tr>
<td>Sodium excretion (mmol/day)</td>
<td>110 ±8</td>
<td>123 ± 10</td>
</tr>
<tr>
<td>Mean of 3-timed overnight urinary albumin excretion rates (µg/min)</td>
<td>71.4 (33.5 - 152.2)</td>
<td>50.9 (36.8 - 97.2)(^a)</td>
</tr>
</tbody>
</table>

Data in mean±SEM, except for urinary albumin excretion rate which is geometric mean (95% confidence interval). ACEi: angiotensin converting enzyme inhibition. \(^a\) denotes \(p<0.05\)

### Study design

NE-infusions were performed on three separate study days. On the first day, systemic MAP-NE dose response curves were made. The individual NE threshold (\(\triangle\)mean arterial pressure (MAP)=0mmHg), 20% pressor (\(\triangle\)MAP=4mmHg) and pressor doses (\(\triangle\)MAP=20mmHg) were calculated from MAP-NE dose response curves. On a separate day, the stepwise NE infusions were given with renal function measurements during 10 consecutive 45 min clearance periods. Two baseline periods were followed by 6 periods of NE (2 periods for threshold, 20% pressor and pressor doses, respectively), after which 2 determinations were obtained after cessation of the infusion (recovery period). Blood pressure was recorded every 5 min (Dinamap). These systemic and renal haemodynamic studies were repeated after 8 weeks of treatment with the ACE inhibitor enalapril (10 mg/day), given at 0800 h. The same NE doses were administered during this second evaluation. The patients also participated in another study that aimed to compare the renal haemodynamic and microproteinuric NE responses in micro- and normoalbuminuric IDDM patients and control subjects, and the pre-enalapril data were also used for that study [13]. One initially studied patient declined to participate in the second evaluation.

The experiments were carried out in a temperature-controlled room kept at 22°C. The subjects were studied after an overnight fast and remained so during the experiments. Smoking was prohibited and no liquid other than water was allowed to drink. The subjects remained in supine position and were only allowed to stand on voiding. Two hours before the start of the renal haemodynamic measurements, each subject drank 600 ml water to promote diuresis. Thereafter, urinary volume losses were suppleted by water drinking. An euglycaemic insulin clamp was used to minimise effects of differences in actual glycaemia on renal haemodynamics [16] and to avoid that changes in insulin levels during the experiments would potentially influence NE sensitivity. Insulin was infused (Velosulin H.M., Novo-Nordisk, Bagsvaerd, Denmark) at a rate of 30 mU/kg per h. Blood glucose was kept at 5 mmol/l by varying the infusion rate of a 20% glucose solution. Potassium chloride (10 mmol per 100 g of glucose) was added to the glucose vials.

GFR and ERPF were measured simultaneously using primed infusions of \(^{125}\)I-iothalamate and \(^{131}\)I-hippurate, respectively [16]. The clearances were calculated using the formula \(U.V/P\) and \(I.V/P\), respectively. \(U.V\) represents the urinary excretion rate of the
Chapter 5

tracer, I.V represents the infusion rate of the tracer, and P represents the tracer value of plasma samples taken at the end of each clearance period. Errors in the estimation of GFR due to incomplete bladder emptying and dead space were corrected by multiplying the clearance of $^{125}$I-iothalamate with the formula: clearance of $^{131}$Hippurate (I.V/P) / clearance of $^{131}$I-hippurate (U.V/P). The coefficients of variation for GFR and ERPF are 2.2% and 5.0%, respectively [16]. The GFR and ERPF were corrected to 1.73 m$^2$ of body-surface area. Filtration fraction (FF) was calculated as the quotient of $^{125}$I-iothalamate and $^{131}$I-hippurate clearances.

Analytical methods

Blood samples were taken from an intravenous catheter at the end of each clearance period with the subjects in supine position. Blood was immediately centrifuged at 4°C and samples were stored at -20°C before assay. Aliquots of urine for measurement of urinary proteins were stored at -20°C and analysed within 2 weeks. Plasma insulin was determined by RIA. Plasma NE was analysed by HPLC [17]. Active plasma renin was determined with a commercially available double-antibody RIA (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) with a cross-reactivity with prorenin of 0.2%. Serum ACE activity was measured with an HPLC technique. Angiotensin II was determined by RIA (Dept. of Pharmacology, State University Maastricht, The Netherlands). Cross-reactivity of the angiotensin II antibody with angiotensin I is < 0.1%. Serum aldosterone was determined by RIA. Blood glucose was measured using a Yellow Springs glucose Analyser (Model 23A, Yellow Springs Inc, Yellow Springs, Ohio, USA). Urinary albumin was measured with ELISA using rabbit anti-human albumin antibody (DAKO). The detection limit is 1 µg/l. Sodium, potassium, creatinine and serum albumin in serum and urine were measured on SMA(C) autoanalysers (Technicon Instruments Inc. Tarrytown, N.Y., USA).

Statistical analysis

Results are expressed as mean±SEM for parametrically distributed data and as geometric mean (95% confidence intervals) for non-parametrically distributed data unless stated otherwise. The 2 clearance periods obtained at baseline, threshold, 20% pressor, pressor and recovery were averaged for analysis. Where appropriate, repeated measurements ANOVA for parametrically or non-parametrically distributed data was used to analyse the effects of the NE-infusions before and after ACE inhibition. Paired t-tests or Wilcoxon tests were then used to establish differences between corresponding observation periods. Differences in NE effects on MAP and ERPF before and after ACE inhibition were evaluated by comparing the overall mean changes of each individual. $p$-Values less than 0.05 were considered to be significant.
Results

Metabolic control, body weight, urinary urea and sodium excretion were similar before and after treatment with enalapril (Table 1). Enalapril lowered baseline MAP by 5%, but did not change pulse rate (Table 2). GFR was unaltered, whereas ERPF increased by 9% (Table 2). FF tended to fall ($p=0.09)$. Overnight microalbuminuria fell by 28% (Table 1). Enalapril increased plasma active renin and decreased serum ACE activity and serum aldosterone levels (Table 3). Baseline plasma AII did not significantly change, but the AII/active renin ratio profoundly decreased, indicating adequate RAAS inhibition.

Plasma NE concentrations during NE infusions were similar before and after ACE inhibition (Table 2). Before enalapril, NE increased MAP at 20% pressor and pressor doses to target levels (Table 2). With enalapril, NE infusion also increased MAP. At NE pressor dose there was no difference in MAP compared to before enalapril. The overall mean increment in MAP was greater during than before enalapril ($p<0.05$, Figure 1A). At recovery, MAP was again lower with than without enalapril. ACE inhibition treatment did not influence the fall in pulse rate at NE-pressor dose and its rise at recovery (Table 2).

GFR remained essentially unaltered during NE infusions before and after enalapril, but declined at recovery (Table 2). ERPF progressively decreased at 20% pressor and at pressor dose both before and during enalapril, and corresponding rises in FF were seen. During all observation periods, ERPF remained significantly higher with than before enalapril, and the magnitude of the NE-induced decrease in ERPF was not influenced by enalapril treatment (Figure 1B). The overall urinary albumin excretion rate during NE infusion was 51.5 (24.3-109.1) µg/min before and 57.6 (26.3-126.3) µg/min after enalapril ($p>0.20$).

Before enalapril, plasma active renin, angiotensin II and serum aldosterone levels increased at NE pressor dose, whereas the angiotensin II/active renin ratio remained unaltered (Table 3). With enalapril, plasma active renin and serum aldosterone levels rose at NE-pressor dose, but plasma angiotensin II did not significantly increase. At NE-pressor dose a slight fall was seen in the angiotensin II/active renin ratio. The overall mean levels of plasma active renin, serum ACE activity, angiotensin II, the angiotensin II/active renin ratio and serum aldosterone were lower after enalapril.

Mean blood glucose and plasma insulin concentrations were 5.2±0.1 and 5.1±0.1 mmol/l and 230±22 and 251±19 pmol/l, before and after enalapril, respectively (NS).

Discussion

As expected, enalapril lowered blood pressure and overnight urinary albumin excretion, and induced renal vasodilatation in microalbuminuric IDDM patients. The systemic haemodynamic and the renal responsiveness to exogenous NE, as well as urinary albumin excretion during NE, were however not altered by ACE inhibition treatment.
### Table 3. Parameters of the renin-angiotensin II-aldosterone system during stepwise norepinephrine infusions without and with enalapril

<table>
<thead>
<tr>
<th></th>
<th>Plasma active Renin (mU/l)a</th>
<th>Angiotensin-converting enzyme (U/l)a</th>
<th>Angiotensin II (pmol/l)a</th>
<th>Angiotensin II/ plasma active renin ratio</th>
<th>Serum aldosterone (nmol/l)a</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>without enalapril</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>61 (33-111)</td>
<td>29 ± 3</td>
<td>15 ± 2</td>
<td>0.26 ± 0.05</td>
<td>360 ± 40</td>
</tr>
<tr>
<td>Threshold</td>
<td>55 (32-94)</td>
<td>28 ± 3</td>
<td>14 ± 1</td>
<td>0.28 ± 0.05</td>
<td>330 ± 40</td>
</tr>
<tr>
<td>20% Pressor</td>
<td>64 (49-187)</td>
<td>28 ± 4</td>
<td>16 ± 1</td>
<td>0.28 ± 0.06</td>
<td>420 ± 40</td>
</tr>
<tr>
<td>Pressor</td>
<td>95 (49-187)c</td>
<td>28 ± 4</td>
<td>25 ± 5c</td>
<td>0.25 ± 0.05</td>
<td>710 ± 130c</td>
</tr>
<tr>
<td>Recovery</td>
<td>91 (51-160)d</td>
<td>25 ± 3</td>
<td>21 ± 4</td>
<td>0.24 ± 0.03</td>
<td>460 ± 50</td>
</tr>
<tr>
<td><strong>with enalapril</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>121 (41-359)</td>
<td>6 ± 2b</td>
<td>14 ± 4</td>
<td>0.10 ± 0.02b</td>
<td>90 ± 20b</td>
</tr>
<tr>
<td>Threshold</td>
<td>127 (49-335)</td>
<td>6 ± 2b</td>
<td>11 ± 2</td>
<td>0.09 ± 0.02c</td>
<td>100 ± 30b</td>
</tr>
<tr>
<td>20% Pressor</td>
<td>137 (54-485)</td>
<td>7 ± 2b</td>
<td>12 ± 2</td>
<td>0.09 ± 0.02b</td>
<td>110 ± 20b</td>
</tr>
<tr>
<td>Pressor</td>
<td>201 (85-485)e</td>
<td>8 ± 2b</td>
<td>18 ± 4</td>
<td>0.08 ± 0.01b,d</td>
<td>220 ± 30b,e</td>
</tr>
<tr>
<td>Recovery</td>
<td>198 (98-402)c,d</td>
<td>9 ± 2b</td>
<td>15 ± 2</td>
<td>0.07 ± 0.01c,e</td>
<td>170 ± 40b</td>
</tr>
</tbody>
</table>

Data in mean±SEM and geometric mean (95% confidence intervals). a p<0.01 overall mean values without vs with enalapril, b p<0.05 and c p<0.01 from corresponding infusion period before enalapril, d p<0.05 and e p<0.01 from baseline values.
Figure 1(A,B). Plot comparing individual changes in mean arterial pressure (MAP, A) and effective renal plasma flow (ERPF, B) in response to stepwise norepinephrine infusions before and after ACE inhibition. The lines indicate identical changes. Overall mean change in MAP was greater with than without enalapril (n=7, p<0.05 by paired Wilcoxon test). No difference in overall ERPF response was observed.
Apart from general factors like basal vasomotor tone and vascular reactivity, sodium and volume homeostasis, as well as baroreceptor feedback control are determinants of the systemic reactivity to exogenous NE, and may thus contribute to an enhanced systemic NE responsiveness in diabetes mellitus [8,11,13,18]. Such an exaggerated responsiveness has been found to be associated with an increased exchangeable sodium and a higher extracellular volume, and this hyperreactivity decreases after diuretic treatment [11]. In addition, microalbuminuric IDDM patients have an attenuated pulse rate decline during NE infusion [13], which suggests that an altered baroreceptor feedback control may also contribute to the systemic NE hyperresponsiveness in IDDM [18].

The observation that enalapril treatment failed to diminish systemic vascular NE reactivity in microalbuminuric IDDM is in keeping with previous findings in diabetic patients [11,12]. By contrast, in essential hypertension, the systemic responsiveness to NE appears to be attenuated after ACE inhibition treatment [7-9]. Several factors could be involved in the lack of attenuation of the systemic NE responsiveness after ACE inhibition in diabetes mellitus. First, ACE inhibition therapy is unlikely to have influenced sodium and volume status in the present study since body weight remained unchanged. Indeed, captopril does not significantly decrease exchangeable sodium in diabetes mellitus [14]. In this respect, it is noteworthy that ACE inhibition treatment induces a negative sodium balance in essential hypertension [19]. Second, a similar heart rate decline during NE before and after enalapril was observed, suggesting no important alterations in baroreceptor sensitivity, in agreement with findings in hypertensive diabetic patients [14]. Third, although enalapril induced adequate RAAS suppression, it cannot be excluded that a more complete inhibition of angiotensin II formation is required to blunt systemic NE reactivity. Finally, we consider a type II error very unlikely to explain the lack of attenuation of enalapril on systemic NE reactivity, since the mean increment in MAP in response to NE was even higher after ACE inhibition.

This study is the first to document the effect of ACE-inhibition treatment on NE-induced renal vasoconstriction in microalbuminuric IDDM patients. In IDDM, ACE inhibitors increase renal plasma flow without having much effect on glomerular filtration rate. As a consequence, filtration fraction tends to decline [20]. These changes are ascribed to a predominantly postglomerular dilatation which is in part due to decreased angiotensin II formation. In our study, the renal vasodilatory effects of enalapril remained present during NE infusion, a finding thus far only documented in healthy volunteers [10]. Enalapril prevented the rise in angiotensin II levels during NE pressor infusion. It may thus be inferred that this partial RAAS blockade accounted for the ongoing renal vasodilatation of the ACE inhibitor during NE infusion as compared to pretreatment. Although difficult to demonstrate in humans, animal studies have unequivocally documented bidirectional interactions between the RAAS and the SNS in a way such that each system is able to potentiate the other [4]. ACE inhibition treatment could, therefore, induce a decrease in SNS activation. In the isolated kidney, ACE inhibitors indeed have been demonstrated to diminish renal vascular NE responsiveness [5,6], and neuronally-induced renal vasoconstriction [21], suggesting interference in the RAAS-SNS interaction. The present in vivo study does, however, not support the assumption of an intrarenal sympatholytic effect of enalapril in microalbuminuric IDDM patients, since the
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exogenous NE-induced fall in ERPF was not altered by ACE inhibition treatment.

Our findings may have clinical implications for the future design of renoprotective strategies in IDDM patients. Currently, ACE inhibition treatment is considered to be the main tool to retard the decline in renal function loss in IDDM, both by lowering systemic blood pressure and by decreasing intraglomerular pressure [1-3]. Despite this proven renoprotective potential, many patients still progress to end stage renal failure. Albeit in a small number of patients, the present study is consistent with the possibility that microalbuminuric IDDM patients are not sufficiently protected by ACE inhibition treatment against rises in systemic blood pressure due to bouts of SNS activation during daily life activities. Although the effects of exogenous NE cannot be directly extrapolated to physiological SNS stimulation, it is noteworthy that increases in urinary albumin excretion are well related to rises in blood pressure and plasma NE levels during exercise, which represents a strong endogenous SNS stimulus [17].

In conclusion, we found that enalapril does not attenuate systemic and renal vascular responsiveness to exogenous NE in microalbuminuric IDDM patients. These findings suggest that a potentially adverse effect of NE on systemic and renal vascular tone is not effectively counteracted by ACE inhibition treatment alone.

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Acknowledgments

Plasma angiotensin II was determined by courtesy of P.M.H. Schifflers, Department of Pharmacology, State University Maastricht, The Netherlands. This study was in part supported by grants from the Dutch Diabetes Foundation.
CHAPTER 6

EFFECTS OF LOW DOSE DOPAMINE ON RENAL AND SYSTEMIC HAEMODYNAMICS DURING INCREMENTAL NOREPINEPHRINE INFUSION IN HEALTHY VOLUNTEERS

K. Hoogenberg¹, A.J. Smit² and A.R.J. Girbes³

Dopamine is widely used to prevent a fall in renal function and blood flow in septic patients, especially when other pressor agents like norepinephrine (NE) are used. No human renal haemodynamic studies exist to support this. Therefore, the effects of low dose dopamine on NE-induced renal and systemic vasoconstriction were assessed in 7 normotensive healthy volunteers. On separate days, either a low dose dopamine (4 µg/kg per min) or a placebo (5% glucose) infusion, in a single blinded randomized order, was added to incremental NE infusions of 40, 80, and 150 ng/kg per min given for 60 min each. Blood pressure and heart rate were measured with a semi-automated device, glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were determined with constant infusions of ¹²⁵I-iothalamate and ¹³¹I-hippurate, respectively. NE-alone progressively increased mean arterial pressure (MAP) to pressor levels, whereas this effect was attenuated by addition of dopamine (p<0.05 vs NE-alone). GFR increased during lower nor-epinephrine doses and did not decline at the highest NE dose. Addition of dopamine further increased GFR. ERPF fell with each NE-alone infusion step, but this decline was completely prevented by concomitant dopamine infusion (p<0.01 vs NE-alone). Sodium excretion tended to decrease with NE, but increased 2 to 3 fold after addition of dopamine (p<0.01 from NE-alone). In conclusion, NE causes a large decrease in ERPF but not in GFR in healthy man. Concomitant dopamine administration prevents this fall in ERPF, increases sodium excretion, and also attenuates the NE-induced rise in MAP. These findings warrant further clinical evaluation of the effect of concomitant low-dose dopamine and NE administration in critically ill patients.

Introduction

Norepinephrine (NE) is increasingly recognized as a valuable agent in the treatment of septic shock [1-6]. By eliciting a strong vasoconstrictive response, NE has been reported to restore blood pressure and tissue perfusion more efficaciously than high dose dopamine [1,4,6]. However, the NE-induced reduction in renal blood flow [7-9] make many clinicians reluctant to use this agent in hypotensive patients since an already compromised renal function may further deteriorate under these circumstances. Intravenous dopamine at dosages from 1 to 4 µg/kg per min augments renal blood flow in healthy man [10,11] as well as in mechanically ventilated patients [12]. These low infusion rates of dopamine have been proposed to oppose the renal vasoconstrictive actions of NE - [13,14]. For this reason low dose dopamine is widely used as a renoprotective agent

¹Department of Endocrinology, ²Angiology and ³Surgical Intensive Care, University Hospital Groningen, The Netherlands.

during NE therapy. However, the renal supportive action of dopamine to overcome NE-induced renal vasoconstriction has not been proven in these situations, and the haemodynamic effects of combined infusions has received little attention. In the dog, dopamine has been documented to increase in renal blood flow during NE infusions, but these observations cannot be easily extrapolated to humans since dogs have a different renal haemodynamic profile during NE infusion from man [15]. In healthy subjects, very recent results suggested that dopamine may blunt the renal vasoconstrictive effect of NE that was given as a single pressor dose [16]. In the present study, we evaluated whether dopamine could counteract NE’s actions on renal haemodynamics by adding low dose dopamine to stepwise incremental NE doses in normotensive healthy subjects.

Materials and methods

Written informed consent was obtained after explanation of the purpose of the study which was approved by the local medical ethics committee. Eligible subjects were males with an age between 20 to 35 years, a body mass index <27 kg/m², a systolic/diastolic blood pressure <140/85 mmHg, a serum creatinine concentration< 90 µmol/l, no history of cardiovascular disease and no use of any medications. Seven healthy males with a mean of age 28 (range 22-35) years, mean body mass index of 23.8 (range 20.9-26.7) kg/m², and mean blood pressure of 123/76 (range 112-132/66-85) mmHg, participated in the studies. A 24 h urine collection revealed a normal creatinine clearance (mean 127 (range 117-161) ml/min/1.73 m² in all of them. Mean sodium excretion amounted to 255 (range 189-321) mmol/day and was considered appropriate in the studies so that all subjects were advised to adhere to their usual dietary habits. They were only requested to avoid large protein meals 24 h prior to the studies.

The experiments were carried out on 2 separate days, with a 1 wk interval, in a temperature controlled room of 22°C. The subjects were studied after an overnight fast and remained fasting during the experiments. Smoking was prohibited and water was the only liquid available to drink. Measurements were made with the subjects in the supine position, and they were only allowed to stand on voiding. To promote diuresis, each subject drank 600 ml water at 0730 h after which urinary volume losses were supplemented by oral water. A cannula was inserted in an antecubital vein for infusions of renal tracers, NE, dopamine or placebo. Venous blood was collected from the contra lateral arm. After an equilibration period of 120 min, baseline measurements were made from 1000 h to 1130 hrs. From 1130 hrs to 1430 hrs, NE was infused at 3 incremental doses of 40, 80, 150 ng/kg per min, respectively, with each infusion lasting 60 min. In a single blinded, randomized order, either dopamine (4 µg/kg/min) or placebo (5% glucose) was added to NE infusions. After cessation of the infusions at 1430 hrs, measurements were continued during a 60 min recovery period.

Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured with constant infusions of ¹²⁵I-iothalamate and ¹³¹I-hippurate, respectively, as described previously [17]. After a priming dose, the radiopharmaceuticals were allowed to equilibrate for 120 min. Thereafter, hourly clearances were calculated using the formulas U.V/P and I.V/P, respectively. U.V represents the urinary excretion rate of the tracer, I.V represents the infusion rate of the tracer, and P represents the plasma tracer
level. Values were corrected to 1.73 m² of body-surface area. Filtration fraction (FF) was calculated as the ratio of the iothalamate and hippurate clearances. Blood pressure was recorded every 5 minutes with a semiautomated device (Dinamap 1846, Critikon, Tampa, FL, USA). Mean arterial pressure (MAP) was calculated as ⅓ systolic blood pressure + ⅔ diastolic blood pressure (mmHg). Pulse rate was recorded continuously on an electrocardiography oscilloscope (Hewlett Packard, Boblingen, Germany). Renal vascular resistance (RVR) in dyn.sec/cm² per 1.73 m² was calculated as MAP×80/ (1/haematocrit) × ERPF).

Blood samples were taken in the middle and the end of each clearance period while the subjects were in supine position. Plasma samples for determination of NE and dopamine were collected in pre-chilled tubes containing 10% EDTA, immediately centrifuged at 4°C, and stored at -20°C until HPLC analysis [18]. Electrolytes and creatinine in serum and urine were measured on SMA(C) autoanalysers (Technicon Instruments Inc. Tarrytown, N.Y., USA).

Based on previous studies in healthy volunteers, ERPF was expected to decline 20-30% with the maximal NE dose [19]. The hypothesis was tested that dopamine counteracted this NE-induced decline in ERPF. A sample size of 7 subjects was large enough to demonstrate a difference between no change vs. 20-30% change in ERPF with a power of 85% and a two-sided p-value<0.05. The coefficients of variation of ERPF and GFR are <5% [17]. Results are expressed as mean±SEM for parametrically distributed data and geometric mean (95% confidence interval) for non-parametrically distributed data. Within day changes were evaluated using ANOVA for repeated measurements for parametrically and non-parametrically distributed data as appropriate. Between infusion differences were tested separately for each period with paired Student t-tests for parametrically and Wilcoxon test for non-parametrically distributed data. p-Values less than 0.05 were considered significant.

Results

On both study days, plasma NE concentrations increased similarly with the infusions. At recovery from the combined NE/dopamine infusions plasma NE was still elevated from baseline values. The dopamine infusions led to high circulating levels of plasma dopamine (Table 1).

Baseline blood pressure and heart rate were not different on both study days. Systolic blood pressure increased similarly with both infusion regimens (p<0.05 to 0.01, Table 2). Diastolic blood pressure was evidently increased during 80 and 150 ng/kg per min NE-alone infusion (p<0.01), while only a modest rise was present during the combined NE/dopamine at the 150 ng/kg per min infusion dose (p<0.05). Diastolic blood pressure was lower with NE/dopamine compared with NE-alone during all infusion periods (p<0.01, Table 2). Consequently, NE/dopamine augmented pulse pressure (difference in systolic and diastolic blood pressure) more than NE-alone (p<0.05, Table 2), and MAP was lower with NE/dopamine than with NE-alone (p<0.05, Figure 1A). Heart rate declined with all NE-alone infusions (p<0.05 to 0.01), whereas no change in heart rate was present with the NE/dopamine infusions (Table 2).
Table 1. Plasma norepinephrine and plasma dopamine concentrations.

<table>
<thead>
<tr>
<th>Period</th>
<th>Baseline</th>
<th>Norepinephrine infusion rate</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>40 ng/kg</td>
<td>80 ng/kg</td>
</tr>
<tr>
<td>Plasma norepinephrine (nmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE-alone</td>
<td>1.7(1.1-2.8)</td>
<td>5.1(3.3-7.9)</td>
<td>9.6(6.2-14.9)</td>
</tr>
<tr>
<td>NE+dopamine</td>
<td>1.4(0.9-2.1)</td>
<td>5.4(4.5-6.5)</td>
<td>9.7(7.2-13.2)</td>
</tr>
<tr>
<td>Plasma dopamine (nmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data in geometric mean and 95% confidence interval. NE: norepinephrine, n.d. not detectable. a denotes p<0.05 and b denotes p<0.01 vs. NE-alone.

Table 2. Systolic and diastolic blood pressure, pulse pressure and heart rate.

<table>
<thead>
<tr>
<th>Period:</th>
<th>Baseline</th>
<th>Norepinephrine infusion rate</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>40 ng/kg</td>
<td>80 ng/kg</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE-alone</td>
<td>123 ± 3</td>
<td>133 ± 3a</td>
<td>145 ± 4b</td>
</tr>
<tr>
<td>NE+dopamine</td>
<td>121 ± 3</td>
<td>137 ± 2a</td>
<td>148 ± 4b</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE-alone</td>
<td>76 ± 4</td>
<td>80 ± 3d</td>
<td>84 ± 4bd</td>
</tr>
<tr>
<td>NE+dopamine</td>
<td>75 ± 3</td>
<td>73 ± 3</td>
<td>74 ± 3</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE-alone</td>
<td>47 ± 3</td>
<td>53 ± 3</td>
<td>60 ± 3a,</td>
</tr>
<tr>
<td>NE+dopamine</td>
<td>46 ± 4</td>
<td>64 ± 3bc</td>
<td>74 ± 5bc</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE-alone</td>
<td>59 ± 3</td>
<td>54 ± 3c</td>
<td>51 ± 3ac</td>
</tr>
<tr>
<td>NE+dopamine</td>
<td>58 ± 4</td>
<td>61 ± 3</td>
<td>61 ± 3</td>
</tr>
</tbody>
</table>

Data in mean±SEM. NE: norepinephrine a denotes p<0.05 and b denotes p<0.01 vs. NE+dopamine; c denotes p<0.05 and d denotes p<0.01 vs. NE-alone.
Renal effects of combined norepinephrine and dopamine

Table 3. Urine flow and fractional sodium excretion.

<table>
<thead>
<tr>
<th>Period</th>
<th>Baseline</th>
<th>Norepinephrine infusion rate</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine flow (ml/min)</td>
<td></td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>40 ng/kg</td>
<td>80 ng/kg</td>
<td>150 ng/kg per min</td>
</tr>
<tr>
<td>NE-alone</td>
<td>6.4 ± 0.8</td>
<td>9.4 ± 1.1</td>
<td>8.1 ± 1.2</td>
</tr>
<tr>
<td>NE+dopamine</td>
<td>6.0 ± 0.7</td>
<td>10.0 ±1.1</td>
<td>8.1 ± 1.1</td>
</tr>
<tr>
<td>Fractional sodium excretion (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE-alone</td>
<td>0.98±0.35</td>
<td>0.91±0.23</td>
<td>0.69±0.09</td>
</tr>
<tr>
<td>NE+dopamine</td>
<td>0.84±0.17</td>
<td>2.29±0.17</td>
<td>2.44±0.43</td>
</tr>
</tbody>
</table>

Data in mean±SEM. NE: norepinephrine, $^a$ denotes $p<0.05$ and $^b$ denotes $p<0.05$ from baseline values; $^c$ denotes $p<0.05$ and $^d$ denotes $p<0.01$ vs NE-alone

Baseline renal haemodynamic parameters were similar on both study days. With NE-alone, GFR rose slightly but significantly at the 40 and 80 ng/kg per min infusion dose ($p<0.05$), whereas no change from baseline values was present at the 150 ng/kg per min dose (Figure 1B). The combined NE/dopamine infusions increased GFR progressively ($p<0.01$) to values that exceeded the NE-alone infusions ($p<0.05$, Figure 1B). ERPF declined in a dose dependent fashion with each NE-alone infusion step ($p<0.05$ to 0.01, Figure 1C), and was accompanied by increases in FF ($p<0.01$, Figure 1D). These alterations were opposed by simultaneous dopamine infusion, which in fact induced a modest rise in ERPF at the 40 and 80 ng/kg per min NE-dose ($p<0.05$, Figure 1C). FF remained unchanged with NE/dopamine infusion, and was significantly lower compared with the NE-alone infusions ($p<0.01$, Figure 1D). RVR progressively increased with NE-alone ($p<0.01$), but changed only marginally with the combined NE/dopamine infusion. Consequently, RVR reached higher levels with NE-alone compared with NE/dopamine ($p<0.05$ to .01, Figure 1E).

Urine flow did not change with NE-alone, while a slight increase was seen during NE/dopamine at 40 and 150 ng/kg/min dose ($p<0.05$, Table 3). Fractional sodium excretion was similar at the start of both infusions days. A tendency towards a decrease in fractional sodium excretion was noted with the highest NE dose ($p<0.10$). In contrast, addition of dopamine stimulated fractional sodium excretion ($p<0.01$), reaching 2 to 3 fold higher levels than during NE-alone ($p<0.05$ to 0.01, Table 3).

Discussion

In the present study, we demonstrated that a 4 µg/kg/min dopamine infusion blunted the renal vasoconstrictive and overridden the sodium preserving effects of exogenous NE infusions in normotensive healthy subjects. The dopamine infusion also diminished the rise in blood pressure, enlarged pulse pressure and blunted the fall in heart rate which suggests that dopamine also influenced systemic haemodynamics during the NE infusions.
Figure 1.A: Mean arterial pressure; B: glomerular filtration rate; C: effective renal plasma flow; D: filtration fraction; E: renal vascular resistance at baseline, at the incremental nor-epinephrine (NE) infusions of 40 (NE:40), 80 (NE:80) and 150 (NE:150) ng/kg per min, - and at recovery from the infusions. ●NE-alone infusion, ○ NE in combination with low dose dopamine (4 µg/kg per min). * denotes $p<0.05$ and † denotes $p<0.01$ from baseline; ‡ denotes $p<0.05$ and § denotes $p<0.01$ vs NE-alone infusion.
Exogenous NE is well known to decrease ERPF and to increase FF without substantially altering GFR, and its administration is generally associated with increases in tubular sodium reabsorption [7-9]. In contrast, dopamine enhances ERPF and increases GFR to a lesser extent leading to a fall in FF. Moreover, dopamine strongly augments sodium excretion [10-12]. These renal effects are observed with dopamine doses from 1 to 4 µg/kg/min. In this study, a 4 µg/kg/min dopamine dose was given with the objective to produce maximal antagonistic action on the renal NE responses. Dopamine prevented the decrease in ERPF, increased GFR and fractional sodium excretion during the NE infusions. When our study was in progress a report in healthy subjects appeared showing that dopamine at a somewhat lower infusion rate of 3 µg/kg/min blunted the decline in ERPF and increased sodium excretion when added to a single NE pressor infusion [16]. Except for this slight difference in study design, the main outcome is remarkably similar. Since hippurate clearances have been found to reliably measure both NE- and dopamine-induced changes in renal blood flow [20], our findings as well as those in the aforementioned study [16] are unlikely to be biased by the methods used. Data from animal studies have shown that both substances influence renal vascular resistance on the level of the glomerular arterioles via stimulation of α- and DA-adrenoreceptors, respectively [21,22]. While NE constricts, dopamine dilates pre- and postglomerular vessels concomitantly. Since these vessels are the major sites of resistance to blood flow in the kidney, NE is supposed to reduce ERPF via glomerular arteriolar vasoconstriction, whereas dopamine changes vessel tone and flow in the opposite direction.

Apart from direct renal vasodilation, indirect effects by changes in cardiac output could also be involved. Cardiac output and heart rate increase by β1-adrenergic receptor stimulation at dopamine doses as low as 3 µg/kg/min [23]. In this study, dopamine attenuated the rise in MAP, enlarged pulse pressure and blunted the decline in heart rate during the NE infusions, and these effects may have resulted from β1-adrenoreceptor stimulation. If true, an increase in cardiac output could have stimulated ERPF. We [12] previously suggested such a mechanism in postoperative mechanically ventilated patients in whom the ratio increase in ERPF/increase in cardiac output was not altered by 4 to 8 µg/kg/min dopamine infusions. Apart from direct renal haemodynamic or systemic haemodynamic effects of dopamine and/or NE on intraglomerular pressure, other changes in e.g. oncotic pressure, filtration area and the diffusion coefficient [24] and a combination of them, could have caused alterations in GFR during the infusions. Elevations in intraglomerular pressure have been demonstrated in rat studies with NE infusions when blood pressure was allowed to increase [25]. Renal vasodilation by dopamine could have resulted in an increased transmission of systemic blood pressure to the renal vascular bed and consequently raised intraglomerular pressure to higher levels than NE alone. Redistribution of renal blood flow and increases in filtration area are other mechanisms by which dopamine may increase GFR [26].

The finding that dopamine increased natriuresis during NE illustrates another important renal action of this substance. The well documented natriuretic effects [7-9] have been ascribed to stimulation of specific dopamine-receptors at the proximal tubule [27] and may occur independent of renal vasodilation [28]. Conversely, NE generally produces antinatriuresis by promoting tubular sodium reabsorption [7-9], but in this study the number of subjects may have been too small to demonstrate such an effect on renal
sodium handling. Dopamine’s potential to counteract any possible antinatriuretic effect of NE, is probably due to inhibition of the high energy-dependent sodium reabsorption process in the proximal tubule [27].

NE has many characteristics to fulfill treatment goals in septic shock. It quickly restores perfusion pressure and thereby oxygen supply to hypoperfused tissues. It is, therefore, not surprising that NE is a valued treatment option in this condition [1-6]. However, fear of renal function loss with NE monotherapy is widely distributed, but has in fact only been demonstrated with suprapharmacological doses in experimental studies [29]. Nonetheless, low-dose dopamine is often proposed to conserve renal function during NE therapy [13,14]. The present results, although obtained in healthy volunteers and thus difficult to extrapolate to septic patients, support these assumptions to variable extent: NE reduced ERPF, but not GFR, and addition of dopamine was indeed able to restore both GFR and ERPF above baseline. Although little direct evidence is known about the effects of NE and dopamine on renal haemodynamics in septic patients, some of the tubular effects have been studied in these patients.

A recent clinical study reported a loss of effectiveness of dopamine to stimulate diuresis and natriuresis in patients with sepsis syndrome whereas no effects could be elicited in patients with septic shock [30]. These findings contrast with our recent observations that dopamine (2, 4 or 6 µg/kg/min) on top of a constant background NE infusion, induced a dose-dependent increase in urine output and fractional sodium excretion in septic patients [31]. Moreover, dopamine increased diuresis in oliguric resuscitated surgical intensive care patients [32] and critically ill patients considered at risk for renal insufficiency [33], but was not effective in patients after major abdominal surgery [34]. That human studies addressing renal haemodynamics and not only diuresis and natriuresis in such patients are warranted, is supported by a recent study which reported disappointing effects of the benefit of dopamine on prevention of renal failure [35].

In conclusion, in healthy volunteers low-dose dopamine prevented the NE-induced fall in renal blood flow, and it increased sodium excretion. The so often invoked unfavourable renal effects of NE were not manifested by a fall in GFR. Future studies have to extend these findings in patients with sepsis, and should in particular assess whether or not low-dose dopamine improves clinical outcome in these patients.

References
Renal effects of combined norepinephrine and dopamine


Acknowledgments

We are indebted to mrs. A. Drent-Bremer and mrs. M. van Kammen from the department of nephrology, for their skilful assistance while performing renal haemodynamic assessments. Dr. Hoogenberg is supported by a grant from the Dutch Diabetes Foundation.
CHAPTER 7

EFFECT OF GROWTH HORMONE (GH) AND INSULIN-LIKE GROWTH FACTOR-I ON URINARY ALBUMIN EXCRETION: STUDIES IN ACROMEGALY AND GH DEFICIENCY

K. Hoogenberg¹, W.J. Sluiter¹ and R.P.F. Dullaart¹

Glomerular hyperfiltration is a characteristic feature of acromegaly, but it is uncertain whether albuminuria is elevated in this disease. To investigate the role of abnormal growth hormone (GH) and insulin-like growth factor I (IGF-1) levels on urinary protein excretion we measured overnight urinary albumin excretion rate (Ualb.V) and creatinine clearance in 14 acromegalic patients with metabolically active disease (fasting GH>5 µg/l) and IGF-1>2.2 kU/l), 8 GH deficient patients and 20 control subjects. Ualb.V was higher in the acromegalic patients (8.4 (4.2-68.2) µg/min; median (range)) than in the GH deficient patients (2.0 (0.9-5.9) µg/min, p<0.001) and control subjects (3.3 (1.0-7.8) µg/min, p<0.01). Five acromegalic patients had Ualb.V levels above the normal upper normal limit of 10 µg/min. Only one patient with concomitant untreated hypertension had persistent microalbuminuria. Creatinine clearance was also higher in the acromegalic patients (p<0.05) and lower in the GH deficient patients (p<0.05) than in the control subjects. In 11 of these acromegalic cases the lowering of GH by 63% and of IGF-1 by 48%, following treatment with the somatostatin analogue (n=10) or spontaneous pituitary infarction (n=1), reduced Ualb.V by 29% to 4.9 (3.1-45.2) µg/min (p<0.01). Among the acromegalic patients (25 observations) Ualb.V was related to GH (r=0.61, p<0.01), IGF-1 (r=0.57, p<0.01) and creatinine clearance (r=0.54, p<0.01). In conclusion, circulatory GH and IGF-1 levels influence albuminuria. Since persistent microalbuminuria is uncommon in acromegaly, it is unlikely that GH elevations alone predispose to clinically important glomerular damage.

Introduction

The appearance of microalbuminuria is an early and characteristic sign of renal involvement in patients with (insulin-dependent) diabetes mellitus (IDDM) [1]. Renal haemodynamic factors are thought to play an important role in the development of glomerular damage in experimental diabetes mellitus, renal ablation and intrinsic renal disease [2,3]. Although not consistently reported, an increased glomerular filtration rate (GFR) has also been found to precede the development of microalbuminuria, overt proteinuria and the subsequent decline in renal function in IDDM patients [4-6]. Glomerular hyperfiltration is a feature of only few other human diseases. In acromegaly an increased renal function is well recognized [7-10]. Despite similarities in renal haemodynamic changes between acromegaly and IDDM [10,11], renal insufficiency

¹ Department of Endocrinology, State University, Groningen, The Netherlands

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seems uncommon in acromegaly [12], whereas in contrast 30 to 40% of diabetic patients develop nephropathy [13].

Growth hormone (GH) stimulates renal haemodynamics indirectly through an effect on insulin-like growth factor 1 (IGF-1) [14,15]. The effects of circulatory GH and IGF-1 on urinary albumin excretion are not well studied. In acromegaly no correlation could be demonstrated between urinary GH and albumin excretion [9], but recent observations show that IGF-1 infusion increases albuminuria in healthy subjects [16].

To investigate the role of abnormal GH and IGF-1 levels in urinary protein excretion we measured albuminuria in patients with active acromegaly, severe GH deficiency and control subjects. In acromegalic patients the influence of effective GH lowering therapy on albuminuria was also studied.

### Subjects and methods

#### Subjects

All subjects consented to participate in the study which was approved by the local medical ethics committee. Fourteen adult patients with active acromegaly, 8 adult patients with severe GH deficiency and 20 control subjects were included. The diagnosis of active acromegaly was based on a fasting serum GH concentration >5µg/l that did not decrease to <2µg/l after 100 g glucose, and a plasma IGF-1 level >2.2 kU/l [17]. Two potentially eligible acromegalic patients were not studied because of renal tract abnormalities. The pretreatment acromegalic group was composed of 6 patients who had not undergone any previous treatment for acromegaly and 8 patients in whom surgery, irradiation, bromocriptine or a combination of them had failed to normalize GH and IGF-1 levels. None of the participants used GH lowering medical treatment at the start of the study.

### Table 1. Clinical and biochemical characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Acromegalic patients</th>
<th>GH deficient patients</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pretreatment (n=14)</td>
<td>post-treatment (n=11)</td>
<td>change (n=11)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>42(24-67)</td>
<td>42(25-67)</td>
<td>40(26-56)</td>
</tr>
<tr>
<td>Female/male</td>
<td>6/8</td>
<td>6/5</td>
<td>4/4</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>93(72-130)</td>
<td>92(77-20)</td>
<td>-4(-10 to 3)</td>
</tr>
<tr>
<td>Hypertension(n)</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>CrCl (ml/min per 1.73m²)</td>
<td>125(85-182)</td>
<td>112(87-138)</td>
<td>-8(-16 to -1)</td>
</tr>
<tr>
<td>GH (µg/l)</td>
<td>16.8(6.5-69.5)</td>
<td>5.2(0.5-13.9)</td>
<td>-12(-26 to -7)</td>
</tr>
<tr>
<td>IGF-1 (kU/l)</td>
<td>4.8(2.2-13.8)</td>
<td>2.1(0.2-6.7)</td>
<td>-2(-7 to -1)</td>
</tr>
</tbody>
</table>

Data in median(range); changes within acromegalic group in median(95%confidence interval).
GH: growth hormone; IGF-1: insulin-like growth factor-I; MAP: mean arterial pressure; CrCl: creatinine clearance; nd: not determined; a p<0.05 from control subjects; b p<0.01 and c p<0.001 from GH deficient patients; d p<0.05 and e p<0.01 from pretreatment.
Eleven of these 14 patients could be prospectively studied and were included in the post-treatment acromegalic group. They were reevaluated 3 to 4 months after effective reduction of GH and IGF-1 levels. In 10 patients this was accomplished by subcutaneous injections of the somatostatin analogue, octreotide (Sandostatin®, Sandoz, Basel, Switzerland), in doses of 300 to 600 µg daily. In one patient pituitary infarction shortly prior to planned therapy obviated the need for octreotide treatment. Severe GH deficiency was considered to be present if peak GH after insulin-induced hypoglycaemia was <1.5 µg/l and if IGF-1 was <0.34 kU/l [18]. GH deficiency was due to primary hypopituitarism in 6 patients and to non-functioning pituitary adenoma in 2 patients. The acromegalic and GH deficient patients received supplementation doses of thyroxine, cortisone acetate and sex steroids if necessary.

Clinical and laboratory measurements

In the acromegalic and GH deficient patients fasting venous blood was obtained to assay GH (2 occasions) and IGF-1. Precisely timed overnight urine samples were collected under sterile conditions to measure urinary albumin excretion rate (Ualb.V) and creatinine clearance. Three urine samples were obtained from each acromegalic and GH deficient patient. The control subjects collected one specimen. Creatinine clearance was used as a parameter of renal function. Blood pressure was measured using the auscultatory method. Mean arterial pressure (MAP) was calculated as ¼ systolic pressure+ ¾ diastolic pressure. In the post-treatment acromegalic group the procedures were repeated at follow-up. Overnight Ualb.V has been previously established to range from 1.6 to 10.0 µg/min (2.5 to 97.5th percentile) in 50 healthy subjects in our clinic. Micro-albuminuria was defined as Ualb.V>20 µg/min [1]. Hypertension was defined as systolic blood pressure >160 mmHg and/or diastolic blood pressure >95 mmHg.

Serum GH was measured by radioimmunoassay (Farmos Diagnostica, Turku, Finland). The lower detection limit was 0.5 µg/l. Plasma IGF-1 was assayed with a kit purchased from Nichols Institute of Diagnostics (San Juan Capistrano, CA, USA). Reference values for adults range from 0.34 to 2.2 kU/l. Urinary albumin was measured by radioimmunoassay (Diagnostics Products Corporation, Apeldoorn, The Netherlands). The intra- and interassay coefficients of variation were 2.3% and 7.7% for urinary albumin concentrations between 0.07 and 60 mg/l [19]. The lower detection limit was 0.07 mg/l. Serum and urinary creatinine and serum albumin were measured on a SMAC II autoanalyzer (Technicon Instruments Inc., Tarrytown, NY, USA).

Statistical analysis.

Data are expressed as median (range). Within group changes in variables are given as median (95% confidence interval (CI)). Parameters from each individual were averaged for analysis. Between group differences in parameters were assessed by Kruskal-Wallis analysis of variance. Adjustment for multiple comparisons was carried out using Duncan's method. Paired Wilcoxon tests were used to analyse within group changes. Spearman's rank correlation was performed to calculate correlations. Multiple regression analysis was applied to evaluate the independent contribution of parameters. A two-sided p-value<0.05 was taken as significant.
### Table 2. Parameters of urinary albumin excretion.

<table>
<thead>
<tr>
<th></th>
<th>Acromegalic patients</th>
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<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pretreatment (n=14)</td>
<td>post-treatment (n=11)</td>
<td>change (%)</td>
</tr>
<tr>
<td>Ualb.V (µg/min)</td>
<td>8.4(4.2-68.2)</td>
<td>4.9(3.1-45.2)</td>
<td>-29(-46 to -14)</td>
</tr>
<tr>
<td>Ualb.V (µg/min/1.73m²)</td>
<td>7.1(3.2-57.8)</td>
<td>4.4(2.8-50.3)</td>
<td>-23(-44 to -11)</td>
</tr>
<tr>
<td>FalbCl (10⁻⁶)</td>
<td>1.3(0.6-10.8)</td>
<td>0.9(0.5-6.7)</td>
<td>-27(-39 to -3)</td>
</tr>
</tbody>
</table>

Data in median (range); % change within acromegalic group in median (95% confidence interval). Ualb.V denotes urinary albumin excretion rate. FalbCl denotes fractional albumin clearance.  

- †* p<0.01 from control subjects; ‡* p<0.05 and ‡‡* p<0.001 from GH deficient patients;  
- † p<0.05 and †† p<0.01 from pretreatment.

### Results

Figure 1. Urinary albumin excretion rate (Ualb.V) in □ acromegalic patients pretreatment (n=14), □ acromegalic patients post-treatment (n=11), ○ control subjects (n=20) and ● growth hormone (GH) deficient patients (n=8). The bars represent the median levels. The shaded area represents the normal range. †† †p<0.01 from control subjects; †* p<0.05 and †‡ p<0.001 from GH deficient patients; ‡§ p<0.01 from pretreatment.
The clinical and biochemical characteristics of the study participants are given in Table 1. The groups were comparable regarding age and sex distribution. Median blood pressure levels were comparable, although 2 acromegalic patients had hypertension. During the study hypertension was treated with a calcium-entry blocker in one patient, mild hypertension being untreated in the other. One acromegalic patient had concomitant diabetes mellitus that was adequately controlled with insulin. Creatinine clearance was higher in the untreated acromegalic patients and lower in the GH deficient patients as compared with the control subjects. In the post-treatment acromegalic patients serum GH was reduced by 63(58-77)% and IGF-1 was reduced by 48(32-69)% ($p<0.01$ for both). Circulatory GH and IGF-1 levels decreased to $<5$ µg/l and to $<2.2$ kU/l in 5 and 6 patients, respectively. The reduction in GH and IGF-1 levels was accompanied by a fall in creatinine clearance (Table 1). Blood pressure (Table 1) and body weight (change -1.0 (-3.8 to 1.5) kg) were unaltered at follow-up.

Ualb.V was elevated in the group of untreated acromegalic patients and tended to be lower in the group of GH deficient subjects as compared with the control subjects (Figure 1, Table 2). Ualb.V was above the upper normal limit of 10µg/min in 5 acromegalic patients (36%). One acromegalic patient with concomitant untreated hypertension had persistent microalbuminuria (Ualb.V of 68.2µg/min), whereas Ualb.V was 7.6 and 9.1µg/min in the treated hypertensive and the diabetic patient, respectively. In the acromegalic patients urinary albumin excretion was still elevated after correction for body-surface area and when expressed as fractional albumin clearance (Table 2). At follow up Ualb.V was lower in 10 of 11 patients. Ualb.V was also reduced in the patient with pituitary infarction. In this post-treatment acromegalic group both Ualb.V and fractional albumin clearance were significantly decreased to a median level comparable with that in the control subjects (Figure 1, Table 2).

Among the acromegalic patients (25 observations) Ualb.V was significantly correlated with serum GH ($r=0.61$, $p<0.01$, Figure 2A), plasma IGF-1 ($r=0.57$, $p<0.01$, Figure 2B) and creatinine clearance ($r=0.54$, $p<0.01$, Figure 3A). Multiple regression analysis confirmed that Ualb.V was independently related to serum GH ($p<0.02$), or alternatively to plasma IGF-1 ($p<0.03$), and creatinine clearance ($p=0.05$). Ualb.V was not related to blood pressure (Figure 3B), age and body-surface area. Fractional albumin clearance was also independently related to circulatory GH ($p=0.01$) and IGF-1 ($p<0.01$).

**Discussion**

This study demonstrates that urinary albumin excretion rate is elevated in acromegaly and tends to be reduced in GH deficiency. Furthermore, urinary albumin excretion was consistently reduced after GH and IGF-1 lowering. The positive relations between GH, IGF-1 and albuminuria supports that these hormonal factors are involved in urinary protein excretion. However, the elevations in albuminuria that occurred with GH excess were minor and only 1 of 14 patient had microalbuminuria according to the cut-off level of 20µg/min, proposed to define incipient nephropathy in IDDM [1].
Figure 2. Relationships between serum growth hormone (GH), plasma insulin-like growth factor I (IGF-1) and urinary albumin excretion rate (Ualb.V) in □ acromegalic patients pretreatment (n=14), □ acromegalic patients post-treatment (n=11) and ● growth hormone (GH) deficient patients (n=8). The shaded area represents the normal range. A: GH and Ualb.V in acromegalic patients (25 observations) $r=0.61$, $p<0.01$; B: IGF-1 and Ualb.V in acromegalic patients (25 observations) $r=0.57$, $p<0.01$. 
Figure 3. Relationships between creatinine clearance, mean arterial blood pressure (MAP) and urinary albumin excretion rate (Ualb.V) in □ acromegalic patients pretreatment (n=14), □ acromegalic patients post-treatment (n=11) and ● growth hormone (GH) deficient patients (n=8).

A: creatinine clearance and Ualb.V in acromegalic patients (25 observations) \( r=0.54, \ p<0.01 \) and in GH deficient patients \( r=0.26, \ NS \); B: MAP and Ualb.V in acromegalic patients (25 observations) \( r=0.07, \ NS \) and in GH deficient patients \( r=-0.18, \ NS \).
Previous studies have also shown that microalbuminuria is infrequent in acromegaly [9,10]. These observations would agree with the contention that the elevated urinary albumin excretion in acromegaly represents a functional rather than a structural phenomenon.

The mechanisms responsible for the stimulatory effects of GH and/or IGF-1 on albuminuria are not precisely understood. Haemodynamic factors and permselectivity properties of the glomerular filtration barrier govern the glomerular passage of albumin [20]. The presence of IGF-1 receptors on all type of glomerular cells represents a structural basis for the in vivo effects of IGF-1 on glomerular function [21]. IGF-1 infusion rapidly increases GFR, renal plasma flow and albuminuria in human subjects [16]. Glomerular vasodilation and a rise in the ultrafiltration coefficient are probably responsible for these stimulatory effects of IGF-1 on renal haemodynamics [22]. The impaired renal vasodilatory response to amino acid infusion in already hyperfiltering patients corroborates the presence of abnormal glomerular vasodilation in acromegaly [10]. In this study the expected increase in renal function could be demonstrated using creatinine clearance as a parameter of glomerular filtration. The positive relation between creatinine clearance and albuminuria supports that renal haemodynamic factors play a contributory role in urinary albumin excretion. The present observations also show that fractional albumin excretion was elevated in acromegaly. In accordance, IGF-1 infusion enhances fractional albumin clearance as well [16]. Thus, the elevated urinary albumin excretion cannot be fully explained by an increase in renal function per se. Experimental evidence indicating a direct effect of IGF-1 on glomerular protein handling is not yet available, but IGF-1 could alter glomerular permselectivity properties by relaxing the mesangium [21]. It cannot be excluded that the observed reduction in albuminuria that followed GH and IGF-1 lowering was to some extent directly caused by the somastatin analogue, octreotide. However, albuminuria was also decreased after spontaneous GH and IGF-1 normalization and octreotide does not acutely reduce albuminuria in IDDM patients, despite a rapid decrease in renal function [23]. It is evident that systemic blood pressure can influence albuminuria. In patients with IDDM a rise in blood pressure and progression of albuminuria develop concomitantly [24]. Microalbuminuria has also been observed in essential hypertension, albeit at higher blood pressure levels [25]. In our study systemic blood pressure did not have an independent effect on albuminuria in acromegaly, which could have been due to the fact that most studied patients were normotensive. In this respect it is noteworthy that the only patient with persistent microalbuminuria had untreated hypertension. Moreover, we cannot exclude that alterations in tubular protein reabsorption contributed to the increased albuminuria, but the normal β2-microglobulin excretion suggests that tubular function is not impaired in acromegaly [9].

In a retrospective histopathological survey no severe glomerular lesions were documented in 20 acromegalic cases studied at autopsy [26], consistent with the presently observed minor elevations in albuminuria. Besides acromegaly and IDDM long-standing glomerular hyperfiltration is a feature of subjects with one kidney and patients with type 1 glycogen storage disease [27,28]. Microalbuminuria is rare in subjects with a single kidney [27], but glomerulosclerosis, overt proteinuria and renal insufficiency are common complications of type 1 glycogen storage disease [28]. Taken together, these observations suggest that glomerular hyperfiltration does not result in important glomerular injury in
humans, unless additional, yet to be defined factors, are present. In contrast, experimental animal studies have shown that mice transgenic for bovine GH and rats bearing GH-producing tumours develop considerable glomerular enlargement, glomerulosclerosis and proteinuria, whereas less severe abnormalities have been observed in experimental models of IGF-1 hypersecretion [29,30]. In such animals, GH is extremely elevated to levels not encountered in human subjects with acromegaly. Between species differences in susceptibility to glomerular lesions may possibly also account for these apparently contradictory findings.

In conclusion, circulatory GH and IGF-1 levels influence urinary albumin excretion. In active acromegaly slightly elevated levels of albuminuria are observed that can be reduced with GH-lowering measures. Since persistent microalbuminuria is uncommon in acromegaly, it is unlikely that GH elevations alone predispose to clinically important glomerular damage.

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CHAPTER 8

INSULIN-LIKE GROWTH FACTOR I AND ALTERED RENAL HAEMODYNAMICS IN GROWTH HORMONE DEFICIENCY, ACROMEGALY AND DIABETES MELLITUS

K. Hoogenberg, P.M. ter Wee, A.G. Lieverse, W.J. Sluiter and R.P.F. Dullaart

Growth hormone (GH) influences renal haemodynamics indirectly by enhancing the synthesis of insulin-like growth factor I (IGF-I). In patients with acromegaly increases in glomerular filtration rate (GFR) are well correlated with IGF-I levels. Circulatory GH-levels have also been implicated in the elevated GFR of IDDM patients, but IGF-I levels are often low in these patients. In the present study, we found IGF-I levels to be highly correlated with renal haemodynamics parameters in GH-deficient, acromegalic and healthy subjects, whereas this relationship was not present in normo-hyperfiltering IDDM groups. In contrast, in all groups amino acid stimulated renal function was inversely correlated with baseline renal haemodynamics, indicating similar renal haemodynamic abnormalities in hyperfiltering IDDM and acromegalic patients, despite large differences in plasma IGF-I levels. It is suggested, therefore, that the lack of a relationship between plasma IGF-I and renal function in IDDM does not exclude the role of elevated GH levels in diabetic glomerular hyperfiltration.

Introduction

Evidence has accumulated that glomerular hyperfiltration plays an important role in the development of glomerular damage in experimental diabetes mellitus [1] and possibly in human diabetes as well [2]. An increased glomerular filtration rate (GFR) is also a characteristic feature of acromegaly [3]. Growth hormone (GH) influences renal haemodynamics indirectly by enhancing the synthesis of insulin-like growth factor I (IGF-I), which has a direct stimulatory effect on renal function [4]. Since circulatory levels of GH are frequently elevated in patients with insulin-dependent diabetes mellitus (IDDM), especially during poor metabolic control, it is possible that circulatory GH plays a pathogenetic role in diabetic glomerular hyperfiltration. However, no differences in diurnal GH-profile could be demonstrated between normo- and hyperfiltering diabetic patients [5]. In contrast, recent studies show a positive correlation between GH-releasing hormone-stimulated [6] or exercise-stimulated GH levels [7] and renal haemodynamics in Type 1 diabetes mellitus, independently of metabolic control.

In the present study we therefore investigated the relationship between plasma IGF-I

1 Departments of Internal Medicine, State University Hospital, Groningen; 2 Free University of Amsterdam, The Netherlands.

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and GFR as well as effective renal plasma flow (ERPF) among GH-deficient, acromegalic, normo- and hyperfiltering IDDM patients and healthy control subjects. As the assessment of renal reserve filtration capacity using amino acids (AA) infusion has been demonstrated to provide insight into the presence of glomerular vasodilation associated with abnormal GH secretion [3,8] and diabetes mellitus [9], the AA-induced changes in renal haemodynamics in these patients were compared.

Subjects and methods

The study was approved by the local medical ethics committee and all participants consented to the procedure. Fasting GFR and ERPF were determined simultaneously, using $^{125}$I-iothalamate and $^{131}$I-hippuran, respectively [9]. On the second day, renal reserve filtration capacity was measured as the % increment of GFR and ERPF after 17-hours of amino acids infusion (Vamin-N®, 7% weight/volume, Kabi Vitrum, Limoges, France; infusion rate 83 ml/h). All subjects had an age between 21 and 55 years. Only subjects without hypertension (systolic blood pressure <160 mmHg and diastolic blood pressure <95 mmHg) and without blood pressure lowering or non-steroidal anti-inflammatory medication participated. The IDDM patients did not have clinical proteinuria and had a disease duration of at least 5 years.

The relationships between plasma IGF-I and baseline GFR and ERPF were determined in 8 GH-deficient patients [8], 7 patients with active acromegaly who were studied before and after treatment with the somatostatin analogue octreotide [3], 17 normo- and 9 hyperfiltering Type 1 diabetic patients (GFR >130 ml/min per 1.73m$^2$) and 6 healthy controls. Renal reserve filtration capacity was compared among these GH deficient and acromegalic patients, another 6 normo- and 6 hyperfiltering diabetic patients and 12 healthy controls [9]. Serum GH and plasma IGF-I concentrations were measured by radioimmunoassays. Data are given as mean ±SEM. Correlation coefficients were calculated with linear regression analysis. A $p$-value <0.05 was considered significant.

Results

Serum GH levels were higher in the normo- and hyperfiltering IDDM patients than in the control subjects (4.1±1.2 and 5.9±2.9 µg/l vs 0.8±0.2 µg/l, $p<0.001$ for both). In contrast, plasma IGF-I was significantly lower in both IDDM groups as compared to controls (0.37±0.02 and 0.36±0.03 kU/l vs 1.08±0.20 kU/l, $p<0.02$ for both). In the combined groups of GH-deficient, acromegalic and control subjects, positive correlations were observed between plasma IGF-I, GFR ($r=0.87$, $p<0.001$, Figure 1A) and ERPF ($r=0.77$, $p<0.001$, Figure 1B). In the combined IDDM groups, these renal haemodynamics parameters were not related to plasma IGF-I ($r=0.08$ for GFR and $r=0.11$ for ERPF).

AA infusion significantly increased GFR in GH-deficient patients ($p<0.001$), healthy subjects ($p<0.01$), normofiltering IDDM ($p<0.01$) and treated acromegalic patients ($p<0.02$) but not in patients with active acromegaly and hyperfiltering IDDM patients (Figure 2A). ERPF was stimulated in GH-deficient patients ($p<0.001$), healthy subjects ($p<0.05$) and treated acromegalic patients ($p<0.02$), whereas there was no
Figure 1. Relationships of plasma IGF-I with GFR (A) and ERPF (B) in healthy subjects ⊥, GH-deficient patients ⊙, acromegalic patients before ⋄ and after ⌂ treatment, normofiltering □ and hyperfiltering △ diabetic patients.
Figure 2. Relationships of baseline renal haemodynamics with AA-induced increments in GFR (A) and ERPF (B) in healthy subjects ◀, GH-deficient patients ○, acromegalic patients before ● and after ○ treatment, normofiltering □ and hyperfiltering □ diabetic patients.

increase in patients with active acromegaly as well as in normo- and hyperfiltering IDDM
patients (Figure 2B). Taken all groups together, significant inverse relationships could be demonstrated between mean baseline renal haemodynamics and the mean AA-induced responses (GFR: $r=-0.95$, $p<0.005$; ERPF: $r=-0.90$, $p<0.02$).

**Discussion**

This study showed that baseline GFR and ERPF are positively related to the plasma level of IGF-I in GH-deficient, acromegalic and control subjects, but not in diabetic patients. Both hyperfiltering diabetic and acromegalic patients had an abolished response to AA infusion. Thus it appears that hyperfiltering IDDM and acromegalic patients share comparable renal haemodynamic abnormalities, despite large differences in plasma IGF-I levels.

The stimulatory effects of IGF-I on renal haemodynamics are ascribed to glomerular vasodilation and a rise in the ultrafiltration coefficient [4]. The enhanced response of GFR and ERPF to AA in GH deficiency, in contrast to acromegaly, supports the notion that IGF-I is an important determinant of these renal haemodynamic parameters. The renal vasodilatory effects of IGF-I could be mediated by nitric oxide, whereas prostaglandins may have a permissive role [4]. These factors are also thought to play a role in the multifactorial process that leads to diabetic glomerular hyperfiltration [10].

Plasma IGF-I was low in the diabetic patients, despite elevated GH levels. This paradox is in accordance with other observations [11], but does not exclude the potential contributory role of abnormalities in the GH-IGF-I-system in diabetic glomerular hyperfiltration. For example, increases in IGF binding protein I facilitate the action of IGF-I on aortic smooth muscle cells [12]. Thus alterations in IGF-I binding proteins in diabetes mellitus may influence its biological activity. Furthermore, augmented accumulation of IGF-I [11] and increased expression of kidney IGF-I receptors [13] have been found in the diabetic kidney, possibly resulting in an enhanced renal haemodynamic effect of IGF-I in the diabetic state. Therefore, the lack of a relationship between plasma IGF-I and renal function in IDDM does not contradict that elevated circulatory GH levels could play a contributory role in diabetic glomerular hyperfiltration. Obviously, further experiments are needed to clarify the impact of abnormalities in the GH-IGF-I-axis on diabetes-related changes in renal function.

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CHAPTER 9

CONTRIBUTORY ROLES OF CIRCULATORY GLUCAGON AND GROWTH HORMONE TO INCREASED RENAL HAEMODYNAMICS IN IDDM PATIENTS


The stimulatory effects of growth hormone (GH) and glucagon on renal function are well known, but it is uncertain whether these hormones are involved in the increase in renal function, characteristic of IDDM patients. Therefore, the circulatory levels of GH and glucagon were measured in 10 IDDM patients with an elevated glomerular filtration rate (GFR > 130 ml/min per 1.73m²) and in 20 age and sex matched normofiltering patients (GFR ranging from 90 to 130 ml/min per 1.73m²). In the patients, fasting glomerular filtration rate and effective renal plasma flow (ERPF) were determined using 125I-iothalamate and 131I-hippuran, respectively, during near-normoglycaemia. On a separate day, the levels of glucagon and GH were determined in the fasting basal state and after exercise. Multiple regression analysis disclosed that GFR was positively correlated with HbA1c ($r^2=0.18$, $p<0.01$), glucagon ($r^2=0.14$, $p<0.03$) as well as exercise-stimulated GH ($r^2=0.10$, $p<0.05$). ERPF was independently associated with HbA1c ($r^2=0.24$, $p<0.005$) and glucagon ($r^2=0.18$, $p<0.01$), whereas renal vascular resistance (RVR) was negatively correlated with stimulated GH ($r^2=0.18$, $p<0.02$). Kidney volume was positively correlated with HbA1c ($r^2=0.26$, $p<0.001$) and inversely with RVR ($r^2=0.16$, $p<0.01$), but not with glucagon or stimulated GH. The present study suggests that circulatory GH and glucagon play a contributory role in the renal haemodynamic changes in IDDM.

Introduction

Elevations in glomerular filtration rate (GFR) are well recognized in insulin-dependent diabetes mellitus (IDDM) and can persist for many years after onset of the disease [1-4]. In experimental diabetes mellitus glomerular hyperfiltration appears to be involved in the pathogenesis of glomerulosclerosis and the progressive loss of kidney function [5]. Some clinical observations also support the suggestion that an elevated GFR might contribute to the subsequent development of diabetic nephropathy [6], but in other studies no such role could be assigned to glomerular hyperfiltration [7].

Moderate hyperglycaemia has been found to increase GFR [8,9], whereas improved metabolic control reduces renal haemodynamics as well as kidney volume [2,10-12]. Plasma levels of growth hormone (GH) and glucagon, which have a renal vasodilatory effect [13,14], are frequently elevated under circumstances of suboptimal metabolic control [15-18]. However, no differences in the diurnal profiles of GH and glucagon could

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1Department of Endocrinology and Nephrology, and Department of Radiology, Groningen State University Hospital, Groningen, The Netherlands.

be demonstrated between normo- and hyperfiltering IDDM patients [19], although recently an increased responsiveness to GH-releasing hormone has been observed in IDDM patients with glomerular hyperfiltration [20]. Thus, the contributory roles of these hormones in the maintenance of diabetic glomerular hyperfiltration are still controversial. Therefore, we employed an exercise test to stimulate GH physiologically and related GH as well as glucagon levels to renal function and kidney size in IDDM patients without clinical nephropathy.

**Patients and methods**

*Patients*

The study was approved by the local medical ethics committee and all patients consented to the procedure. Inclusion criteria to participate were an onset of disease before 30 years of age, glucagon-stimulated plasma C-peptide concentration <0.2 nmol/l, diabetes duration for at least 5 years, no arterial hypertension (systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥95 mmHg), no orthostatic hypotension, serum creatinine ≤120 µmol/l, urinary albumin excretion rate (Ualb.V) ≤200 µg/min on three consecutive overnight urine samples, and no use of anti-inflammatory and antihypertensive drugs.

**Table 1.** Clinical characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Group H (n=10)</th>
<th>Group N (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>39 ± 14</td>
<td>40 ± 12</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>9 / 1</td>
<td>18 / 2</td>
</tr>
<tr>
<td>Duration of disease (years)</td>
<td>24 ± 13</td>
<td>21 ± 10</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.0 ± 2.4</td>
<td>23.8 ± 2.3</td>
</tr>
<tr>
<td>Retinopathy (O/B/P)³</td>
<td>2 / 4 / 4</td>
<td>6 / 8 / 6</td>
</tr>
<tr>
<td>Ualb.V (µg/min)²</td>
<td>25 (13 - 44)</td>
<td>15 (12 - 44)</td>
</tr>
<tr>
<td>Ualb.V&gt;20 µg/min</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>93 ± 9</td>
<td>95 ± 9</td>
</tr>
<tr>
<td>HbA₁ (%)</td>
<td>8.0 ± 1.5</td>
<td>7.2 ± 0.9</td>
</tr>
<tr>
<td>Blood glucose (mmol/l)⁴</td>
<td>9.3 ± 2.3</td>
<td>7.7 ± 1.7</td>
</tr>
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</table>

H: IDDM patients with glomerular filtration rate >130 ml/min per 1.73m²; N: IDDM patients with glomerular filtration rate ranging from 90 to 130 ml/min per 1.73m². Data are given as mean±SD, except for Ualb.V which is given in median and interquartile ranges. ³ Retinopathy: O: absent; B: background; P: proliferative; ² Ualb.V: urinary albumin excretion rate; ⁴ Blood glucose: mean of three 8-sample-based 24-h blood glucose profiles

The study groups were comprised of 10 patients with glomerular hyperfiltration, defined as a GFR>130 ml/min per 1.73m² [21], and 20 normofiltering patients (GFR between 90 and 130 ml/min per 1.73m²) (Table 1). The subjects from these groups, designated H for the hyperfiltering and N for the normofiltering patients, were individually matched with respect to sex, age and diabetes duration (within 5 years) to exclude any
influence due to differences in clinical parameters. The proportion of patients with microalbuminuria, as well as retinopathy was not significantly different (Table 1). HbA1c values and mean blood glucose concentrations (obtained from the three 8-sample-based 24-h blood glucose profiles) tended to be higher in H than in N, but this difference did not reach statistical significance (p=0.14 and p=0.08, respectively; Table 1). None of the patients consumed a protein or sodium-restricted diet. Besides insulin, no other medication was used.

Renal haemodynamics and kidney volume

The patients were studied after an overnight fast, both when measuring kidney function and during the exercise procedure to eliminate the confounding effects of recent nutrient intake. Supine GFR and effective renal plasma flow (ERPF) were determined simultaneously using $^{125}$I-iothalamate and $^{131}$I-hippuran, respectively [22]. To minimize the possible effect of actual glycaemia on GFR and ERPF the patients were studied during near-normoglycaemia (blood glucose between 4.4 and 8.3 mmol/l [9]. Starting at 0800 h, a 5% glucose solution was infused intravenously at a rate 1 ml (0.28 mmol)/kg per h to which regular acting insulin (Velosulin H.M., Novo-Nordisk, Bagsvaerd, Denmark) was added in a dose of 1% of the total daily requirements per hour. After a 4 h period to normalize and stabilize blood glucose levels no further corrections of blood glucose were made and GFR and ERPF were measured over a 2 h clearance period from 1200 to 1400 h. The renal vascular resistance (RVR, in dynes.s/cm² per 1.73m²) was calculated by dividing the mean arterial blood pressure by the renal blood flow (RBF) multiplied by 80, where RBF was estimated from the ERPF and the haematocrit (HTC) using the formula: 

\[ \text{RBF}(\text{l/min per 1.73m}^2) = \frac{\text{ERPF}(\text{l/min per 1.73m}^2)}{(1-\text{HTC})} \]

Kidney volume was determined by summation of the volumes of both kidneys, obtained by ultrasonography [23].

Exercise test

On a separate day following the renal haemodynamic studies, the patients performed a bicycle-exercise test with a fixed workload of 600 kpm for 20 min. The morning insulin dose was withheld. After 2 h rest, blood samples were taken at 1000, 1100, 1120 (i.e., directly at the end of exercise) and 1200 h from a cannula inserted into an antecubital vein.

Laboratory methods

Blood glucose was measured using a Yellow Springs glucose Analyzer (Model 23A, Yellow Springs Inc., Yellow Springs, Ohio, USA). HbA₁ was determined by col-orationmetry [24].
Figure 1. Metabolic assessments during the exercise. Hyperfiltering ○, normofiltering ● IDDM patients: see text and Table 1. Measurements at 1000, 1100, 1120 and 1200 h: 1 h before, at the start, directly after and 40 min after the exercise. Data are given in mean ± SEM. Differences in both IDDM groups: * denotes $p<0.005$ for growth hormone from preexercise, ** denotes $p<0.01$ for insulin from baseline.

Urinary albumin was measured by radioimmunoassay (Diagnostic Products Corporation, Apeldoorn, The Netherlands). Serum growth hormone (GH) concentrations were measured by radioimmunoassay (Farmos Diagnostica, Turku, Finland). Insulin-like growth factor-I (IGF-I) was measured by radio-immunoassay (Nichols Institute of Diagnostics, San Juan Capistrano, Calif., USA). Plasma glucagon was measured by radioimmunoassay, using antibody 30K (obtained from Professor RH Unger, Dallas, Tex., USA [25]). Plasma free insulin and plasma C-peptide concentrations were measured by radioimmunoassays.

Statistical analysis

Results are expressed as mean±SD or mean±SEM (Figures) for parametrically distributed data and as median (interquartile ranges) for non-parametrically distributed data. Data were compared with unpaired $t$-tests. Within group changes of parameters were evaluated using analysis of variance. Duncan's method [26] was used to adjustment for multiple comparisons. Multiple regression analysis was used to disclose the independent contribution of the various metabolic parameters to GFR, ERPF, RVR and kidney volume. $P$-values less than 0.05 were considered to be significant.
Results

Renal haemodynamic parameters and kidney volume

The renal haemodynamics and kidney volume measurements are given in Table 2. By selection, GFR was higher in H than in N, whereas ERPF was also clearly elevated in group H compared with N (p<0.001). The FF was similar in the groups H and N, but RVR was lower in group H than in group N (p<0.001). The difference in kidney volume was not significant. The mean blood glucose and insulin concentrations during the renal haemodynamic assessments were comparable between the groups (Table 2).

Table 2. Renal haemodynamics, corresponding plasma glucose and insulin levels, and kidney volume.

<table>
<thead>
<tr>
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<th>Group H (n=10)</th>
<th>Group N (n=20)</th>
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<tbody>
<tr>
<td>Glomerular filtration rate</td>
<td>160 ± 22ᵃ</td>
<td>109 ± 12</td>
</tr>
<tr>
<td>Effective renal plasma flow</td>
<td>670 ± 142ᵃ</td>
<td>470 ± 74</td>
</tr>
<tr>
<td>Filtration fraction</td>
<td>0.24 ± 0.04</td>
<td>0.24 ± 0.03</td>
</tr>
<tr>
<td>Renal vascular resistance</td>
<td>6654 ± 1422ᵃ</td>
<td>9195 ± 1697</td>
</tr>
<tr>
<td>Kidney volume</td>
<td>332 ± 99</td>
<td>291 ± 54</td>
</tr>
<tr>
<td>Plasma glucose</td>
<td>6.7 ± 1.4</td>
<td>6.1 ± 1.1</td>
</tr>
<tr>
<td>Insulin</td>
<td>24.4 ± 11.5</td>
<td>23.9 ± 11.1</td>
</tr>
</tbody>
</table>

Group H: IDDM patients with glomerular filtration rate >130 ml/min per 1.73m²; group N: IDDM patients with glomerular filtration rate ranging from 90 to 130 ml/min per 1.73m². Data are given as mean±SD.ᵃ denotes P<0.001 from group N.

Metabolic assessments during exercise

As expected, the circulatory level of GH increased in response to the exercise (p<0.005, Figure 1A). Plasma glucagon did not significantly change during the procedure (Figure 1B). Plasma insulin levels decreased (p<0.01, Figure 1C). Blood glucose ranged between 4.9 and 15.4 mmol/l and tended to increase at the end of the exercise (Figure 1D). No significant differences were found between the two groups in the absolute levels of growth hormone, glucagon, insulin and glucose neither at the separate measurements nor in the averaged values of the test (p>0.10 for all comparisons). Baseline plasma IGF-I levels were 0.35 ± 0.08 kU/l and 0.36 ± 0.07 kU/l in the groups H and N, respectively (NS).

Correlation analyses

For GH the averaged baseline and exercise-stimulated levels were used separately in the regression analysis. For glucagon the averaged values of the whole procedure were taken since glucagon was not stimulated by exercise.

Simple regression analysis showed significant correlations between stimulated GH as well as glucagon and GFR (Figure 2A and B). The correlation between GFR and HbA1c did not reach statistical significance (r=0.32, p=0.054). ERPF was correlated with HbA1c and glucagon (Figure 2C and D), but not with GH (r=0.28, p=0.13).
Figure 2. Relationships between renal haemodynamics and metabolic parameters. Hyperfiltering ○, normofiltering ● IDDM patients. A: exercise stimulated-serum growth hormone and GFR, $r=0.40$, $p<0.05$; B: mean plasma glucagon and GFR, $r=0.40$, $p<0.05$; C: HbA1c and ERPF, $r=0.42$, $p<0.02$; D: mean plasma glucagon and ERPF, $r=0.36$, $p<0.05$; E: exercise-stimulated serum growth hormone and RVR, $r=-0.43$, $p<0.01$; F: HbA1c and kidney volume, $r=0.61$, $p<0.001$. 
Table 3. Determinants of renal haemodynamics and kidney volume as assessed by stepwise multiple regression analyses.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Partial correlation coefficient</th>
<th>Contribution to variance</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Determinants of glomerular filtration rate.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1c</td>
<td>0.42</td>
<td>18%</td>
<td>( p&lt;0.01 )</td>
</tr>
<tr>
<td>Glucagon</td>
<td>0.38</td>
<td>14%</td>
<td>( p&lt;0.03 )</td>
</tr>
<tr>
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<td>0.32</td>
<td>10%</td>
<td>( p&lt;0.05 )</td>
</tr>
<tr>
<td>Multiple-r</td>
<td>0.67</td>
<td>44%</td>
<td>( p&lt;0.002 )</td>
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<tr>
<td><strong>Determinants of effective renal plasma flow.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1c</td>
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<td>24%</td>
<td>( p&lt;0.005 )</td>
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<tr>
<td>Glucagon</td>
<td>0.43</td>
<td>18%</td>
<td>( p&lt;0.01 )</td>
</tr>
<tr>
<td>Multiple-r</td>
<td>0.62</td>
<td>39%</td>
<td>( p&lt;0.002 )</td>
</tr>
<tr>
<td><strong>Determinants of renal vascular resistance.</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Stimulated GH</td>
<td>0.43</td>
<td>18%</td>
<td>( p&lt;0.01 )</td>
</tr>
<tr>
<td><strong>Determinants of kidney volume.</strong></td>
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<td></td>
</tr>
<tr>
<td>HbA1c</td>
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<td>( p&lt;0.001 )</td>
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<tr>
<td>Renal vascular resistance</td>
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<td>( p&lt;0.01 )</td>
</tr>
<tr>
<td>Multiple-r</td>
<td>0.72</td>
<td>52%</td>
<td>( p&lt;0.001 )</td>
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</tbody>
</table>

RVR was negatively related to GH (Figure 2E). Basal GH levels were not correlated with renal haemodynamic parameters. Kidney volume was correlated with HbA1c (Figure 2F), but not with glucagon or GH.

Multiple regression analysis disclosed that HbA1c \( (r^2=0.18, \ p<0.01) \), glucagon \( (r^2=0.14, \ p<0.03) \) and stimulated GH \( (r^2=0.10, \ p<0.05) \) independently contributed to the GFR (multiple \( r=0.67, \ p<0.002 \), Table 3). Also, HbA1c \( (r^2=0.24, \ p<0.005) \) and glucagon \( (r^2=0.18, \ p<0.01) \) showed independent relations with the ERPF (multiple \( r=0.62, \ p<0.002 \), Table 3). RVR only showed a negative relation with stimulated GH \( (r^2=0.18, \ p<0.01) \) (Table 3). Kidney volume was positively related HbA1c \( (r^2=0.26, \ p<0.001) \) independent from the inverse relation with RVR \( (r^2=0.16, \ p<0.01) \) (multiple \( r=0.72, \ p<0.001 \), Table 3). Blood glucose levels, IGF-I concentrations and clinical parameters such as age, diabetes duration, body mass index, retinopathy and Ualb.V were not related to renal function or kidney size.

Discussion

In this study an exercise test was performed to stimulate GH levels physiologically and on both study days the patients were kept fasting to eliminate the effects of recent nutrient intake on hormone levels and renal function. Under these circumstances renal haemodynamic parameters were significantly related to circulatory levels of plasma glucagon, exercise-stimulated GH levels as well as HbA1c, whereas the absolute levels of these hormonal and metabolic factors were not significantly different between the hyper- and normofiltering IDDM patients. Possible between group differences in these parameters
could have been obscured because of the arbitrarily employed definition of glomerular hyperfiltration. A GFR above the upper normal limit of 130 ml/min per 1.73m² was designated as glomerular hyperfiltration, comparable to a cut-off value of 135 ml/min per 1.73m² used by others [10]. However, such a ‘control population-based’ definition has the limitation that subtle renal haemodynamic alterations in apparently normofiltering patients cannot be discriminated and is likely to represent a minimal estimation of the hyperfiltration phenomenon [27].

In the multiple regression analysis with the renal haemodynamic parameters as dependent variables, glucagon contributed to 14% and 18% of the variances in GFR and ERPF, respectively. The contributory effect of stimulated GH to GFR was 10% and amounted to 18% of the variance in RVR. In other studies plasma glucagon and GH concentrations were not different in normo- and hyperfiltering patients and were not correlated with renal haemodynamic parameters [1,4,19]. These negative results could possibly be explained by differences in study design, since stimulatory tests were not used [1,4,19] or single basal hormone levels were obtained [4]. Recently, an augmented GH response to GH-releasing hormone has been documented in hyperfiltering patients [20] supporting an association between an abnormal GH release and renal haemodynamic changes in IDDM. Long-term metabolic control, as determined by HbA1c levels, contributed to 18, 24 and 26% of the variances in GFR, ERPF and kidney volume, respectively. In accordance, other cross-sectional and intervention studies showed a relation of metabolic control with renal function and kidney size [4,10,11].

Several lines of evidence support that glucagon and GH are important humoral determinants of renal function in man. Infusion of glucagon to reach levels as encountered in poorly controlled IDDM acutely increases both GFR and ERPF in IDDM patients [14]. Conversely, the rapid decrease in renal function during the administration of the somatostatin analogue octreotide is closely related to reductions in plasma glucagon [28] and can be completely prevented by concomitant low-dose glucagon administration [29]. Furthermore, renal haemodynamics have been shown to be more responsive to increments in plasma glucagon in well-controlled IDDM patients than in normal subjects [14]. The role of circulatory GH in the maintenance of renal function is illustrated by the fact that acromegalic and GH-deficient patients show differences in GFR and ERPF of about 50% [30,31]. The abolished renal-stimulatory effects of amino acids in acromegaly as opposed to the enhanced response in GH-deficiency further supports that GH is involved in renal vasodilation [30,31]. GH increases GFR and ERPF indirectly by stimulating IGF-I synthesis [32,33]. In spite of the high concentrations of GH, plasma IGF-I levels are frequently found to be reduced in IDDM [34,35]. In agreement, plasma IGF-I levels were low in both groups of IDDM patients. In the interpretation of these data it should be noted that plasma IGF-binding proteins could have interfered with the presently used radioimmunoassay [36] and that free IGF-I concentrations were not measured. Disturbances in IGF-I binding proteins, modulating its biological activity [36,37], or alterations in renal IGF-I accumulation [35] might possibly explain why, in accordance with other observations [4,38], the low circulatory IGF-I levels were not significantly correlated with renal haemodynamics in these IDDM patients. Moreover, an altered or variable renal haemodynamic responsiveness to circulatory IGF-I in IDDM cannot be excluded. Indeed, in vitro studies have shown increased expression of IGF-I receptors in
cultured mesangial cells from diabetic mice [39].

In conclusion, the present results suggests that circulatory glucagon as well as GH play a contributory role in the increase in renal haemodynamics in IDDM patients.

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CHAPTER 10

INCREASED URINARY IGG/ALBUMIN INDEX IN NORMOALBUMINURIC IDDM PATIENTS: A LABORATORY ARTEFACT

K. Hoogenberg¹, P. Visser², J. Marrink², W.J. Sluiter¹ and R.P.F. Dullaart¹

The previous observation that urinary IgG excretion is increased in normoalbuminuric IDDM patients is unexplained and could possibly be related to a laboratory phenomenon. When untreated urine samples were stored at -20°C for 2 to 4 weeks, the IgG/albumin index (IgG clearance divided by albumin clearance) was higher in normoalbuminuric IDDM patients than in control subjects (0.91(0.68-1.54), n=27 vs 0.72(0.55-0.79), n=15 (median(interquartile range)), p<0.05). In normo- and microalbuminuric IDDM patients the IgG/albumin index was higher in urine samples with glucose than without glucose (1.16(0.93-1.68), n=11 vs 0.73(0.50-0.91), n=16; p<0.05, and 0.33(0.23-0.60), n=17 vs 0.15(0.10-0.26), n=14, p<0.02 for normo- and microalbuminuric patients, respectively). We, therefore, evaluated the preserving effects of glucose and bovine serum albumin (BSA) on urinary IgG after 1 hour to 16 weeks of freezing at -20°C in 4 non-diabetic subjects (proteinuria ranging from 0.05 to 8.0 g/day). Urine samples were either stored without precautions or treated with addition of phosphate buffer, BSA (1%) and glucose (100 and 300 mM). The weekly decline from 1 to 16 weeks of IgG in the urine aliquots diluted 1:1 with buffered glucose 300 mM and glucose 300 mM + BSA 1% was insignificant, whereas urinary IgG declined with all other storage regimes (p<0.05). These results suggest that glucose in urinary specimens of IDDM patients prevents at least in part the loss of urinary IgG and may thus explain the higher urinary IgG/albumin index when unprocessed urine is stored frozen before assay. Laboratory precautions are necessary when urinary IgG cannot be measured immediately.

Introduction

The glomerular filtration of macromolecules is dependent on their charge, size and structure, on the properties of the glomerular filtration barrier which govern charge and size selectivity, as well as on renal haemodynamic factors [1]. In the microalbuminuric stage of diabetic renal disease an important role is attributed to a loss of negative charge of the glomerular basement membrane, which is probably due to a decrease in the negatively charged proteoglycan, heparansulphate [2-5]. This impaired charge selectivity leads to enhanced glomerular passage and urinary excretion of negatively charged molecules like albumin and IgG4 [1,6,7]. In more advanced stages of diabetic nephropathy a concomitant defect in glomerular size selectivity is thought to result in a grossly increased urinary excretion of neutrally charged IgG [1]. Therefore, the observation that the urinary IgG excretion [3,8] or the IgG/albumin clearance ratio [9] is elevated in

¹Department of Endocrinology and ²Immunoochemistry, State University Hospital, Groningen, The Netherlands.

Diabetic Medicine 1996; 13: 651-655
normoalbuminuric diabetic patients compared to healthy subjects is puzzling and awaits further explanation. This phenomenon could possibly be related to laboratory conditions of urine storage before IgG assay. In this respect it is important to note that urinary IgG concentration decreases within several weeks after freezing of samples at -20°C and that addition of bovine serum albumin (BSA) to the urine specimens and buffering urine to neutral pH diminishes this decline in IgG concentration [10].

In the present study we describe the apparent increasing effect of glucosuria on urinary IgG measurement when aliquots were stored at -20°C without precautions. Laboratory experiments were carried out to determine the effect of different storage conditions, including addition of glucose and BSA, on urinary IgG measurement.

Patients and methods

Study A: Urinary IgG (UIgG.V) and albumin (Ualb.V) excretion were determined in overnight and in day-time urine collections of 15 healthy controls (age 25±5 years, group C), in 27 normoalbuminuric (age 30±9 years, diabetes duration 15±10 years, group D1) and 31 microalbuminuric insulin-dependent diabetic patients (IDDM) (age 43±11 years, diabetes duration 22±9 years, group D2). Microalbuminuria was defined as an Ualb.V>20 µg/min in an exactly timed overnight urine collection. On the following morning, directly after completion of the overnight collection, a one hour urine collection was made and venous blood was taken for measurements of blood glucose, serum albumin and IgG at the beginning of this period. In the overnight samples, the IgG/albumin index was calculated as the IgG clearance divided by the albumin clearance.

Study B: The effects of laboratory storage conditions on urinary IgG measurement was evaluated in 4 non-diabetic subjects with a total urinary protein excretion of 0.05, 0.2, 2.0 and 8.0 g/day.

In all participants of both studies urinary tract infection was excluded by Dipslide tests. Urine samples for IgG measurement were stored at -20°C without precautions for 2 to 4 weeks in study A. Freshly collected samples were processed in study B (see below).

Urinary IgG was measured by an in-house developed enzyme linked immunosorbent assay (ELISA), essentially according to Fomsgaard et al. [11]. The following materials were used: coating: polyclonal goat-antihuman-IgG (gammachain) (Tago, Burlingame, CA, cat no 4100), as conjugate: goat-antihuman-IgG peroxidase (GAHu/IgG (H+L)/PO, Nordic, Tilburg, The Netherlands), enzyme substrate: o-phenylenediamine di-HCl (Eastman Kodak, Rochester, NY, USA). The IgG standard (standard human serum ORDT 06/07, Behringwerke AG, Marburg, FRG) was diluted providing a range from 1.5 to 200 µg/l. Incubations were carried out at 37°C. The colour development was stopped by adding 100 µl H₂SO₄ (0.5 mol/l). The absorbance was measured at 492 nm using a Titertek Multiscan (Flow Laboratories, Irvine, UK). Results were computed according to Rodbard et al. [12]. The intra- and interassay coefficients of variation (CV) were 5% and 15%, respectively. The lower detection limit was 1.5 µg/l.

To evaluate the effect of laboratory storage conditions on urine IgG measurement (study B) the following experiments were carried out: within 4 hours after voiding urine aliquots were treated (A) undiluted; (B) diluted 1:1 with 40 mM phosphate buffer, 0.1 M NaCl adjusted to pH 7.4 and sodium azide (NaN₃ 0.2%); (C) diluted 1:1 with buffer and
Glucose and urinary IgG in IDDM.

BSA 1% (10 g/l); (D) diluted 1:1 with buffer and glucose 100 mM; (E) diluted 1:1 with buffer and glucose 300 mM; (F) diluted 1:1 with buffer, glucose 100 mM and BSA 1%; (G) diluted 1:1 with buffer, glucose 300 mM and BSA 1%. IgG was measured in these samples before freezing and after 1 hour, and 1, 2, 4, 8 and 16 weeks of storage at -20°C. All IgG measurements were performed in quadruplicate.

In both studies urine aliquots for albumin measurement were stored at 4°C and analyzed within 24 hours. Urinary albumin was measured in duplicate using a commercially available double antibody radioimmunoassay (Diagnostic Products Corporation, Apeldoorn, The Netherlands, cat no KHAD). The intra- and interassay CV’s were 2.3% and 7.7%, respectively. The lower detection limit was 0.07 mg/l. Serum IgG was measured by nephelometry (Behring Nephelometer Analyzer™, Behringwerke AG, Marburg, FRG). The intra- and interassay CV’s were 2.4 to 3.6% and 2.5% respectively. Serum albumin was measured on a SMAC-II autoanalyzer (Technicon Instruments Inc., Tarry Town, NY, USA). The intra- and interassay CV were 1.9% and 2.5%, respectively. Blood glucose was measured using a Yellow Springs glucose Analyzer (Model 23A, Yellow Springs Inc., Yellow Springs, Ohio, USA). Urinary glucose was measured using a Polarimeter Type 243 (Thorn Automation, Nottingham, UK).

Statistical analysis

Results are expressed as mean±SD or as median (interquartile range) for parametrically and non-parametrically analysed data, respectively. In study A, differences in urinary albumin excretion, urinary IgG excretion and the albumin/IgG index were evaluated by Kruskal-Wallis oneway analysis of variance with Duncan’s method to correct for multiple comparisons. Correlation coefficients between urinary and blood glucose and protein excretion were calculated using Spearman’s rank analysis. In study B, mean (±SD) recovery of IgG at each interval was calculated as percentage of the freshly analysed samples for each of the four urine collections. The overall means and their common SD's are given in the Table, and the data are compared by Student t-tests. Using linear regression analysis, the weekly percentage decline (±SD) from 1 to 16 weeks was calculated for each collection. The overall mean declines (± SD) are given in the Table. A two-sided p-value less than 0.05 was considered to be significant.

Results

Urinary albumin and IgG excretion (study A)

Overnight Ualb.V and urinary IgG excretion (UIgG.V) were significantly higher in group D2 (54.6(41.6-109.5) and 3.10(2.35-8.81) µg/min, respectively) than in groups D1 and C (7.1(4.6-10.1) and 4.6(3.0-7.2) for Ualb.V; 1.64(1.02-2.37) and 0.46(0.35-0.66) µg/min for UIgG.V, p<0.01 for all comparisons). Ualb.V was similar in group D1 and group C, but UIgG.V was higher in group D1 compared to group C (p<0.05). Since no differences were present in serum albumin and IgG concentrations, the overnight IgG/albumin index was higher in group D1 (0.91(0.68-1.54)) than in group C (0.72(0.55-0.79), p<0.05). This index was lower in group D2 (0.27(0.14-0.40), p<0.01) than in group C. As shown in Figure 1, in both diabetic groups the IgG/albumin index was higher in urine samples with glucose as compared to those without glucose (1.16(0.93-1.68) (n=11)
vs 0.73(0.50-0.91) (n=16), p<0.05, for group D1; 0.33(0.23-0.60) (n=17) vs 0.15(0.10-0.26) (n=14), p<0.02, for group D2). In group D1, the IgG/albumin index in samples without glucose was not different from group C (Figure 1). In the day-time urine collections similar differences were present (not shown). In the combined diabetic patients (n=58), the day-time IgG/albumin index was positively correlated with urinary glucose concentration ($r = 0.33$, $p<0.05$), urinary glucose excretion ($r=0.44$, $p<0.01$) and blood glucose ($r = 0.56$, $p<0.01$).

**Figure 1.** The overnight IgG/albumin index in non-diabetic control subjects (group C, ○), in normoalbuminuric IDDM patients (group D1) without (□) and with glucosuria (●), and in microalbuminuric IDDM patients (group D2) without (▲) and with glucosuria (◆). Bars represent median values. * denotes $p<0.05$ from group C and group D1 without glucosuria; † denotes $p<0.01$ from groups C and D1 with and without glucosuria ‡ denotes $p<0.02$ from group D2 without glucosuria.

**Effects of laboratory storage condition on urinary IgG measurement (study B)**

In the 4 non-diabetic patients the urinary IgG concentration was 0.68, 1.52, 12.1 and 109 mg/l in the immediately processed samples. The IgG concentrations after storage at
Table 1. Recovery of urinary IgG after different storage conditions during variable intervals at -20°C in 4 non-diabetic subjects with proteinuria.

<table>
<thead>
<tr>
<th>Dilution (1:1)</th>
<th>Storage time</th>
<th>%</th>
<th>%</th>
<th>%</th>
<th>%</th>
<th>%</th>
<th>%</th>
<th>weekly decline %</th>
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<tr>
<td>A</td>
<td>undiluted urine</td>
<td>mean</td>
<td>100</td>
<td>98</td>
<td>83*</td>
<td>86*</td>
<td>81*</td>
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<td>B</td>
<td>buffer alone</td>
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<td>98</td>
<td>73*</td>
<td>73*</td>
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<td>60*</td>
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<td></td>
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<td>7.7</td>
<td>4.6</td>
<td>5.8</td>
<td>8.9</td>
</tr>
<tr>
<td>C</td>
<td>+BSA 1%</td>
<td>mean</td>
<td>100</td>
<td>97</td>
<td>83*</td>
<td>85*</td>
<td>80*</td>
<td>73*</td>
</tr>
<tr>
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<td>7.4</td>
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<tr>
<td>D</td>
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<td>86*</td>
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<td>12.5</td>
<td>10.7</td>
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</table>

Data are given as overall mean and common SD in % of measurements in fresh urine and percentage decline per week (from 1 to 16 weeks) and common SD. * denotes $p<0.05$ as compared to fresh urine or as compared to zero-decline.

-20°C are given as percentage recovery of the initial value (Table 1). No immediate effect of 1 hour of freezing could be demonstrated. In contrast, the IgG content after 1 week of storage declined significantly in the untreated ($p<0.01$, A), buffered ($p<0.005$, B), buffered and BSA 1% diluted ($p<0.01$, C), buffered and glucose 100 mM diluted ($p<0.05$, D) urine specimens (Table 1). Moreover, this decline was progressive, since the weekly decline from 1 to 16 weeks of storage was significant in these storage regimes ($p<0.01$ for A,B,C and D). In glucose 300 mM diluted (E), glucose 100mM and BSA 1% diluted (F) and glucose 300 mM and BSA 1% diluted (G) urine specimens a small but significant decline in IgG was noted after 16 weeks. The weekly decline in IgG was not significant in the urine specimens diluted with glucose 300 mM ($p<0.20$, E) or glucose 300 mM and BSA 1% ($p<0.20$, G), but significant if diluted with glucose 100 mM and BSA 1% ($p<0.05$, F).

Discussion

In addition to documentation of microalbuminuria, measurement of IgG with sensitive assays will gain insight in the glomerular permelectivity defects underlying the development and evolution of diabetic renal disease. In a cross-sectional study we confirmed recent unexplained findings showing that normoalbuminuric IDDM patients...
have a higher urinary IgG excretion and a higher IgG/albumin index compared to controls [3,8,9]. Of importance, urine samples for IgG measurement were stored at -20°C without precautions in the study by Deckert et al [3] and in the present study, whereas in the other reports [8,9] the urinary storage conditions were not mentioned. The present data showed that among both normo- and microalbuminuric IDDM patients the IgG/albumin index was higher in samples with glucose compared to samples without glucose. Moreover, the IgG/albumin index was positively correlated with glucosuria. This unexpected finding prompted us to evaluate the effect of laboratory storage conditions on IgG measurement. In agreement with other data, storage at -20°C without precautions resulted in a pronounced loss of IgG [10], but in contrast with that study, addition of BSA alone did not prevent the decline in IgG. Only addition of 300 mM glucose (preferably in combination with BSA 1%) to the specimens prevented a rapid decline in IgG. Moreover, such high urinary glucose concentrations can be encountered in vivo during periods of severe hyperglycaemia. It is, therefore, plausible that the apparently increasing effect of glucose on IgG excretion in IDDM is attributable to a diminished or absent loss of IgG during storage without precautions. An alternative explanation, such as a general abnormality in glomerular or tubular IgG handling in diabetes mellitus, is difficult to envisage, since the glomerular permselectivity is considered to be intact [13] and β2-microglobulin excretion is unchanged [1] in normo-albuminuric IDDM patients. Indeed, we were unable to reproduce an elevated IgG/albumin index in normoalbuminuric IDDM patients when using adequate storage procedures [unpublished observations]. Deckert et al. were also unable to find a difference in the IgG/albumin index in normoalbuminuric IDDM patients compared to controls if urine samples were stored at -40°C after predilution with 5% BSA in phosphate buffer [14, personal communication B. Feldt-Rasmussen].

A gradual loss in urinary IgG during storage at -20°C was observed, without an immediate effect of freezing and thawing. Denaturation or aggregation of globulins into multimers of 2 to 4 molecules at low temperatures, which might interfere with epitope recognition in the enzyme-linked immunosorbent assay, can possibly explain this loss in IgG. The employed glucose concentrations of 100 to 300 mM in the specimens were very high compared with the measured IgG concentrations that ranged from 0.0045 to 0.716 mM. This excess of glucose molecules could prevent IgG denaturation or aggregation during prolonged freezing. In contrast to IgG, urinary albumin does not decline after freezing for 1 week [15], although a period of longer storage is also associated with a loss of albumin [16]. Of note, addition of glucose does not influence the albumin measurement in samples assayed within one week after collection [15].

In conclusion, the presence of glucose in urine samples of IDDM patients most likely prevents, at least in part, the loss of urinary IgG when aliquots are frozen without precautions. This may explain the apparently higher IgG/albumin index in normoalbuminuric IDDM patients as compared to healthy subjects. Addition of glucose 300 mM plus BSA 1% (1:1) to the urine samples is an appropriate measure to prevent a substantial decline in IgG. It is, therefore, necessary to use such precautions when it is not possible to assay IgG immediately.

**References**
CHAPTER 11

ALTERATIONS IN CORTISOL METABOLISM IN IDDM PATIENTS: RELATIONSHIP WITH METABOLIC CONTROL AND ESTIMATED BLOOD VOLUME AND EFFECT OF ANGIOTENSIN CONVERTING ENZYME-INHIBITION

R.P.F. Dullaart¹, F.L. Ubels², K. Hoogenberg¹, A.J. Smit², J.J. Pratt⁵, J.H.J. Muntinga³, W.J. Sluiter¹ and B.G. Wolthers⁴

11β-hydroxysteroid dehydrogenase (11β-HSD) catalyses the interconversion of cortisol and its inactive metabolite, cortisone, and protects the mineralocorticoid receptor from activation by cortisol. Sodium and fluid retention is a well documented phenomenon in IDDM patients, but it is unknown whether alterations in cortisol metabolism contribute to its pathogenesis. Therefore, we evaluated some aspects of cortisol metabolism by measuring urinary metabolites of cortisol and cortisone in 8 microalbuminuric and 8 normoalbuminuric IDDM patients and 8 matched control subjects. In both IDDM groups, the overnight excretion of tetrahydrocortisol (THF), allo-tetrahydrocortisol (allo-THF) and tetrahydrocortisone (THE) was lower than in the control group (p<0.05 to p<0.01). Both the allo-THF/THF ratio, a parameter of 5α/5β reduction of cortisol, and the cortisol to cortisone metabolite ratio (THF+allo-THF/THF), which reflects the overall direction of the cortisol to cortisone interconversion, were lower in the IDDM groups (p<0.05 to p<0.01). In the combined subjects (n=24), allo-THF, allo-THF/THF and THF+allo-THF/THF were inversely correlated with HbA1c (r=-0.69, p<0.001, r=-0.61, p<0.01 and r=-0.58, p<0.01, respectively). Upper arm segmental blood volume, estimated by an electrical impedance technique, was positively correlated with the cortisol to cortisone metabolite ratio both in the control subjects (r=0.77, p<0.05) and in the IDDM patients in whom it was measured (r=0.56, p<0.05, n=13), whereas the regression line was shifted leftwards in IDDM (i.e. a lower ratio at the same blood volume, p<0.03 by analysis of covariance). In 7 microalbuminuric IDDM patients, the angiotensin converting enzyme-inhibitor, enalapril (10 mg daily for 6 to 12 weeks) resulted in a moderate further lowering of the cortisol to cortisone metabolite ratio (p<0.05). The present data suggest a chronic hyperglycemia-related impairment in the reduction of corticoids to tetrahydro metabolites and an imbalance in 11β-HSD. Altered 11β-HSD activity is unlikely to be primarily responsible for the sodium and fluid retention in IDDM. Moreover, an additional mechanism of action of angiotensin-converting enzyme inhibition might be provided by an effect on 11β-HSD activity.

Introduction

Corticosteroid hormones play a central role in extracellular sodium and fluid homeostasis. The mineralocorticoid receptor has an equal affinity for cortisol and aldosterone in vitro, but the distal renal tubule binds almost exclusively mineralocorti-coi-
ds in vivo [1-4]. Recently, it has become clear that the mineralocorticoid receptor is protected from excess circulating cortisol by the enzyme 11ß-hydroxysteroid dehydrogenase (11ß-HSD) [3]. This enzyme, of which several isoforms have been identified, catalyses the interconversion of cortisol and its inactive metabolite, cortisone [4]. These observations have provided a link between cortisol metabolism and the regulation of volume and sodium homeostasis, and there is increasing evidence to underscore the clinical importance of 11ß-HSD. The type 1 syndrome of apparent mineralocorticoid excess (AME), characterized by sodium retention and hypokalaemic hypertension despite suppressed plasma aldosterone, has been shown to be caused by impaired 11ß-HSD activity [5,6]. Similarly, glycyrrhetinic acid, the main metabolite of glycyrrhizic acid present in licorice, inhibits 11ß-HSD, thus allowing more cortisol to gain access to the mineralocorticoid receptor [7]. In these situations defective dehydrogenase activity of 11ß-HSD is reflected by an abnormal proportion of urinary cortisol to cortisone metabolites [5-8].

In diabetes mellitus an increase in exchangeable sodium accompanied by extracellular volume expansion has been well documented and might contribute to the blood pressure rise associated with microalbuminuria [9-15]. The mechanisms responsible for this abnormal sodium retention are still incompletely understood, although enhanced renal tubular sodium reabsorption most probably plays an important role [15-16]. Plasma aldosterone levels are usually found to be within normal limits in insulin-dependent diabetic (IDDM) patients [14,15]. Mild hypercortisolism, possibly associated with the presence of complications, has been found in diabetic patients [17-19], but it is unclear in how far the diabetic state leads to alterations in cortisol metabolism and whether possible changes in the cortisol to cortisone conversion influence volume homeostasis.

Against this background we measured urinary cortisol and cortisone metabolites in normo- and microalbuminuric IDDM patients and in control subjects. Parameters of cortisol metabolism were related to metabolic control and upper arm segmental blood volume, estimated by an electrical impedance technique. Since recent experimental data indicate that 11ß-HSD can be stimulated by angiotensin converting enzyme (ACE)-inhibition [20], the effect of this treatment was studied in microalbuminuric patients.

**Subjects and methods**

**Subjects**

The protocol was approved by the local medical ethics committee and written informed consent was obtained from all participants. The subjects also participated in another study (investigating the effects of catecholamines on renal function) which will be reported elsewhere. That study was started after completion of the data collection and does not interfere with the present results. The IDDM patients had ketosis-prone diabetes mellitus and glucagon-stimulated C-peptide levels were <0.2 nmol/l in all of them. None of the participants had autonomic neuropathy. Microalbuminuria was defined as urinary albumin excretion rate (Ualb.V) between 20 and 200 µg/min in at least 2 of 3 overnight urine collections obtained over a one year period [21]. Ualb.V was assessed in the absence of urinary tract infection as indicated by a sterile urine culture. Three groups of subjects were studied: 8 IDDM patients with normoalbuminuria, 8 IDDM patients with
microalbuminuria and 8 healthy control subjects, individually matched for sex and age. Except for IDDM the participants did not have other diseases. In particular, all subjects were clinically and biochemically euthyroid, as indicated by a normal TSH level (0.3 to 5.0 mU/l). None of the participants had severe obesity, defined as body mass index (BMI) >30 kg/m². The mean±SD BMI was 23.2±2.9, 24.5±3.0 and 25.6±2.6 kg/m² for the control subjects, the normo- and the microalbuminuric IDDM patients, respectively (p>0.10). There were 7 men and one woman in each group. Mean age was 44±9, 46±10 and 47±10 yr in these three groups, respectively (p>0.40). The mean diabetes duration was 22±6 and 28±6 yr in the normo- and the microalbuminuric IDDM patients (p<0.08). In the normoalbuminuric group 5 patients had no retinopathy, 2 had background retinopathy and one had proliferative retinopathy, whereas in the microalbuminuric group 5 patients had background and 3 patients had proliferative retinopathy (p<0.05). None of the control and normoalbuminuric IDDM subjects had arterial hypertension, defined as systolic blood pressure ≥160 mm Hg and/or diastolic blood pressure ≥95 mm Hg. An ACE-inhibitor was used by 7 microalbuminuric IDDM patients. This treatment was withheld for a 6 week period before the study.

Clinical procedures

The participants were instructed by a dietician to adhere to a diet containing 100 mmol sodium/day starting 1 week before the study to avoid possible confounding effects of differences in sodium intake on gluco- and mineralocorticoid status. At the end of this period urinary sodium excretion was 109±36, 103±26 and 109±19 mmol/24 h in the control subjects and the normo- and microalbuminuric IDDM patients (p>0.40), indicating acceptable diet compliance. An exactly timed overnight urine collection was then obtained for measurement of steroid metabolites, free cortisol, aldosterone, Ualb.V and creatinine clearance. None of the IDDM patients had symptomatic nocturnal hypoglycaemia and blood glucose at 0300 h varied between 4–9 mmol/l. On the following morning, fasting venous blood was drawn at 0900 h, while the subjects were in the supine position. Supine blood pressure was measured at 5 min intervals during one hour using an automated device (Dinamap®) and the results were averaged for analysis. Initial blood volume, estimated by a previously described electrical impedance technique [22] was used as an index of upper arm segmental blood volume. In brief, a blood pressure cuff is placed over a 2 cm wide segment of the left upper arm. With a computer-assisted model the segment’s initial blood volume (expressed in ml/cm) is calculated from the increase in impedance during rapid inflation of the cuff at a period of constant suprasystolic cuff pressure. In healthy subjects the coefficient of variation of the initial blood volume measurement determination is 6% (derived from Ref. 23). This technique can also be used to calculate other vascular parameters, like arterial blood volume and static arterial compliance. The validity of the mathematical model and its derived parameters has been demonstrated by a close correlation of the diameter of the brachial artery as estimated by this technique with its diameter as assessed by high-resolution echography (r=0.93 in 11 healthy subjects, Van Leeuwen JTM, unpublished results). The impedance technique was available to all control subjects, to 7 normoalbuminuric and to 6 microalbuminuric IDDM patients.

The 7 microalbuminuric IDDM patients in whom ACE-inhibition treatment was stopped before the study consented to be reevaluated 6-12 weeks after resumption of ACE
inhibition therapy (enalapril, 10 mg/day). Venous blood samples were then taken 30-60 min after enalapril administration. On this second evaluation the electrical impedance technique was not available.

Laboratory measurements

Blood samples for measurement of cortisol, aldosterone, ACTH and renin were collected in EDTA-anticoagulated tubes and placed on ice immediately. The plasma specimens and aliquots of overnight urine collections were stored at -20 C until assay. Plasma cortisol and aldosterone were measured by RIA’s as described [24,25]. Plasma ACTH was assayed with a commercially available IRMA (Nichols Institute of Diagnostics, Wijchen, The Netherlands). PRA was measured by RIA (Du Pont, Billerica, MA, USA). Urinary steroid metabolites were determined by gas chromatography [26]. β-glycyrrhetinic acid in the urine specimens was analysed by gaschromatography/mass spectrometry (GC/MS) using α-glycyrrhetinic acid as internal standard. The detection limit of the assay is approximately 5µg/l. Urinary free cortisol and free aldosterone were measured after extraction and chromatography by RIA’s. Aldosterone-18-glucuronide was assayed by RIA after hydrolysis at pH 2.0 followed by chromatography. Serum and urinary electrolytes and creatinine were measured on SMAC and SMA-II autoanalyzers, respectively (Technicon Instruments, Tarrytown NY, USA). HbA1c was measured by high-performance liquid chromatography (HPLC, Bio-Rad, Veenendaal, The Netherlands; normal values 4.6 to 6.1%). Blood glucose was determined on a YSA glucose analyser (model 23A, Yellow Springs, Yellow Springs OH, USA). Urinary albumin was measured by RIA (Diagnostic Products Corporation, Apeldoorn, The Netherlands).

Evaluation of cortisol metabolism

5β tetrahydrocortisol (THF), 5α tetrahydrocortisol (allo-THF) and 5β tetrahydrocortisone (THE) are major metabolites of cortisol and cortisone which are excreted in the urine [27]. These metabolites are primarily produced in the liver by the irreversible ring A reduction of glucocorticoids to dihydro metabolites and the subsequent keto reduction to tetrahydro compounds. The first step is catalysed by 5α and 5β reductases. The urinary allo-THF/THF and androsterone (A, 5α-androstan-3α-ol-17-on)/etiocholanolone (E, 5β-androstan-3α-ol-17-on) ratios reflect the proportion of 5α/5β reduced C-21 and C-19 steroids, respectively [27,28]. According to Ulick et al [5,8], the urinary cortisol to cortisone metabolite ratio (THF+allo-THF/THE) is an index of 11β-HSD activity and this ratio reflects the set-point of the overall direction of the cortisol to cortisone interconversion. Since 11β-HSD activity may be influenced by food intake [20], we used overnight instead of 24-h urine collections. It has also been suggested that one calculates the urinary THF+allo-THF/free cortisol ratio as a measure of the efficiency of the ring A reduction of cortisol [8]. As urine was collected overnight, we did not use this parameter but instead calculated the excretion of cortisol metabolites and free cortisol separately.

Statistical analysis

Parameters are expressed as mean±SD, or as median and range. Differences in parameters between the control group, the normoalbuminuric IDDM group and the
Cortisol metabolism in IDDM

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microalbuminuric IDDM group were evaluated by Kruskal-Wallis analysis of variance using Duncan's method to correct for multiple comparisons. Bivariate correlations were assessed by linear regression analysis. Analysis of covariance was used to evaluate independent relationships between variables. In the microalbuminuric IDDM group the effect of ACE-inhibition therapy on the various parameters was expressed as mean or median change with 95% Confidence Interval and was analysed by the paired Wilcoxon rank test. A two-sided \( p \)-value < 0.05 was taken as significant.

Results

Table 1 shows some clinical and laboratory data of the study groups. Systolic blood pressure was higher in the untreated microalbuminuric IDDM patients compared to the normoalbuminuric IDDM and the control subjects. No differences in diastolic blood pressure were observed. Overnight UaIb.V was >20 µg/min in each micro-albuminuric patient and <20 µg/min in all other subjects. Overnight creatinine clearance and serum potassium concentration were not different among the groups. Initial blood volume was higher in the normoalbuminuric IDDM than in the control group, but the difference between the microalbuminuric IDDM and the control subjects was not significant. Mean fasting blood glucose and HbA1c levels were similarly elevated in the IDDM groups.

As shown in Table 2 the urinary excretion of the major metabolites of cortisol, THF and allo-THF, as well as of cortisone, THE, were lower in both IDDM groups than in the control group. When expressed per mmol of creatinine, the urinary excretion of these glucocorticoid metabolites was similarly decreased in the IDDM groups (not shown). Of importance, the excretion of these metabolites was not equally decreased. Consequently, the THF+allo-THF/THE ratio, i.e. the cortisol to cortisone metabolite ratio, was lower in both IDDM groups compared to controls. The allo-THF/THF ratio, which reflects \( 5\alpha/5\beta \) reduction of cortisol, was also lower in the IDDM groups. This change in \( 5\alpha/5\beta \) reduction was not restricted to cortisol, since the A/E ratio, representing \( 5\alpha/5\beta \) reduction of C-19 steroids, was again lower, especially in the microalbuminuric IDDM group. \( \beta \)-glycyrrhetinic acid was detected in the urine samples of 2 control subjects and 1 normoalbuminuric IDDM patient. Exclusion of the steroid data of these subjects from the analysis did not change the statistical results.

The overnight urinary excretion of cortisol and aldosterone, as well as the fasting plasma levels of these corticosteroids and of ACTH and PRA are given in Table 3. Urinary free cortisol, but not plasma cortisol, was lower in the normoalbuminuric IDDM group than in the other groups. In the microalbuminuric IDDM group the plasma level of cortisol was higher, but urinary free cortisol was not different compared to the control group. No differences in plasma ACTH were observed. Urinary free aldosterone, aldosterone-glucuronide and plasma aldosterone were not different in the diabetic groups compared to the control group, whereas urinary free aldosterone was higher in the
Table 1. Clinical and laboratory data of study subjects

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (n=8)</th>
<th>Normal Ualb.V (n=8)</th>
<th>Micro Ualb.V (n=8)</th>
<th>Change with ACE inhibition (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic BP (mmHg)</td>
<td>127±9</td>
<td>127±12</td>
<td>144±11(^{a,b})</td>
<td>-12(-22 to -2)(^d)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>79±7</td>
<td>75±8</td>
<td>80±7</td>
<td>-8(-11 to -4)(^d)</td>
</tr>
<tr>
<td>Ualb.V (µg/min)</td>
<td>4(1-11)</td>
<td>5(2-14)</td>
<td>56(32-174)(^{a,b})</td>
<td>-8(-55 to 25)</td>
</tr>
<tr>
<td>Creatinine clearance (ml/sec per 1.73m(^2))</td>
<td>1.68±0.50</td>
<td>1.84±0.28</td>
<td>1.75±0.50</td>
<td>0.02(-0.44 to 0.46)</td>
</tr>
<tr>
<td>Initial blood volume (ml/cm)</td>
<td>3.53±1.14(^c)</td>
<td>4.81±1.07(^c)</td>
<td>4.25±1.48</td>
<td>ND</td>
</tr>
<tr>
<td>Blood glucose (mmol/l)</td>
<td>5.0±0.5</td>
<td>9.7±4.1(^a)</td>
<td>8.9±2.5(^a)</td>
<td>1.1(-4.3 to 6.5)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.7±0.5</td>
<td>8.3±1.0(^a)</td>
<td>8.2±0.9(^a)</td>
<td>0.2(-0.5 to 0.9)</td>
</tr>
<tr>
<td>Serum potassium (mmol/l)</td>
<td>4.2±0.2</td>
<td>4.4±0.4</td>
<td>4.2±0.3</td>
<td>0.1(-0.3 to 0.5)</td>
</tr>
</tbody>
</table>

Data are the mean±SD or the median range (in parenthesis) changes in the mean or median (95% confidence interval). BP: blood pressure; Ualb.V: albumin excretion rate; ND: not determined. \(^a\) p<0.01 vs. control subjects; \(^b\) p<0.01 vs. normoalbuminuric IDDM patients (by selection); \(^c\) p<0.05 vs. control subjects; \(^d\) p<0.02 vs without ACE inhibition

Significant relationships were present between the HbA1c level and the urinary excretion of steroid metabolites (Figure 1). In the combined groups (n=24), urinary allo-THF (Figure 1A, r=0.69, p<0.001), THF+allo-THF/THE (Figure 1B, r=0.58, p<0.01), allo-THF/THF (Figure 1C, r=0.61, P<0.01), A/E (Figure 1D, r=-0.59, p<0.01), THF (r=0.40, p=0.05, not shown) and THF+allo-THF+THE (r=-0.48, p<0.05, not shown) were inversely correlated with HbA1c. When analysing the data from the IDDM groups (n=16) separately, the relationships between HbA1c, THF + allo-THF/THE (r=-0.52, p<0.05) and A/E (r=-0.54, p<0.05) remained significant. No significant correlations were found between fasting blood glucose and urinary steroid parameters. Figure 2 shows the positive relationship between the urinary cortisol to cortisone metabolite ratio and initial blood volume in the control subjects (n=8, r=0.77, P<0.05) and in the IDDM patients (n=13, r=0.56, p<0.05). Remarkably, the bivariate relationship between the cortisol to cortisone metabolite ratio and initial blood volume was different between IDDM patients and control subjects, as depicted by the leftward shift of the regression line in the IDDM patients (i.e. a lower ratio at the same blood volume). Analysis of covariance disclosed that initial blood volume was independently related to the cortisol to cortisone metabolite ratio (p<0.01) and to the presence of IDDM (as a categorical covariate, p<0.03), but not to the HbA1c level (p=0.76). Thus, the leftward shift in this relationship in the IDDM patients remained significant after controlling for the HbA1c level. Except for possible bivariate correlations of diastolic...
Table 2. Urinary excretion of steroid metabolites in the study subjects

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (n=8)</th>
<th>IDDM patients</th>
<th>Change with ACE inhibition</th>
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<tbody>
<tr>
<td></td>
<td>Normal Ualb.V (n=8)</td>
<td>Micro Ualb.V</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>(n=8)</td>
<td>(n=7)</td>
</tr>
<tr>
<td>THF (nmol/min)</td>
<td>4.2±1.4</td>
<td>3.1±1.0</td>
<td>3.0±1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5(-1.3 to 2.2)</td>
<td></td>
</tr>
<tr>
<td>Allo-THF (nmol/l)</td>
<td>3.4±1.0</td>
<td>1.7±0.9</td>
<td>1.3±0.7</td>
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<tr>
<td></td>
<td></td>
<td>0.3(-0.4 to 0.9)</td>
<td></td>
</tr>
<tr>
<td>THE (nmol/min)</td>
<td>7.5±2.2</td>
<td>5.8±1.6</td>
<td>5.3±2.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.3(-1.4 to 4.0)</td>
<td></td>
</tr>
<tr>
<td>THF+allo-THF+THE</td>
<td>15.1±4.1</td>
<td>10.6±1.6</td>
<td>9.6±4.4</td>
</tr>
<tr>
<td>(nmol/min)</td>
<td></td>
<td>2.0(-2.9 to 7.0)</td>
<td></td>
</tr>
<tr>
<td>THF+allo-THF/THE</td>
<td>1.02±0.14</td>
<td>0.82±0.19</td>
<td>0.86±0.15</td>
</tr>
<tr>
<td>(µmol/µmol)</td>
<td></td>
<td>-0.08(-0.19 to 0.0)</td>
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</tr>
<tr>
<td>Allo-THF/THF</td>
<td>0.86±0.26</td>
<td>0.55±0.19</td>
<td>0.46±0.21</td>
</tr>
<tr>
<td>(µmol/µmol)</td>
<td></td>
<td>0.04(-0.08 to 0.16)</td>
<td></td>
</tr>
<tr>
<td>A/E (µmol/µmol)</td>
<td>1.35±0.39</td>
<td>1.04±0.34</td>
<td>0.91±0.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0.04(-0.23 to 0.14)</td>
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</tbody>
</table>

Data are the mean±SD or changes in the mean (95% confidence interval). Ualb.V: urinary albumin excretion rate. Allo-THF/THF and A/E reflect 5α/5β-reduction of C-21 and C-19 steroids, respectively. *p<0.01 and **p<0.01 vs. control subjects; *p<0.05 vs without ACE inhibition

Table 3. Urinary and plasma measurements of cortisol, aldosterone, ACTH and PRA.

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (n=8)</th>
<th>IDDM patients</th>
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<tbody>
<tr>
<td></td>
<td>Normal Ualb.V (n=8)</td>
<td>Micro Ualb.V</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n=8)</td>
<td>(n=8)</td>
<td>(n=7)</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
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</tr>
<tr>
<td>Free cortisol (pmol/min)</td>
<td>68.3±29.1</td>
<td>35.1±11.1</td>
<td>61.0±30.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.6(-53.8 to 71.1)</td>
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<tr>
<td>Free aldosterone (pmol/min)</td>
<td>0.83±0.44</td>
<td>0.72±0.42</td>
<td>1.26±0.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0.72(-1.28 to -0.17)</td>
<td></td>
</tr>
<tr>
<td>Aldosterone glucuronide (pmol/min)</td>
<td>21.5±15.9</td>
<td>17.3±7.6</td>
<td>26.5±15.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-16.5(-30.7 to -23)</td>
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<tr>
<td>Plasma</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Cortisol (nmol/l)</td>
<td>381±119</td>
<td>401±154</td>
<td>551±117</td>
</tr>
<tr>
<td></td>
<td></td>
<td>39(-73 to 152)</td>
<td></td>
</tr>
<tr>
<td>ACTH (pmol/l)</td>
<td>5.5±4.2</td>
<td>7.5±3.5</td>
<td>7.5±4.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.1(-5.5 to 19.7)</td>
<td></td>
</tr>
<tr>
<td>Aldosterone (nmol/l)</td>
<td>0.45±0.27</td>
<td>0.64±0.42</td>
<td>0.55±0.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0.17(-0.58 to -0.01)</td>
<td></td>
</tr>
<tr>
<td>PRA (ng/l.s)</td>
<td>0.30±0.22</td>
<td>0.32±0.20</td>
<td>0.40±0.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.52(0.02 to 1.82)</td>
<td></td>
</tr>
</tbody>
</table>

Data are the mean±SD or changes in the mean (95% confidence interval). Ualb.V: urinary albumin excretion. *p<0.01 vs. control subjects and **p<0.05 vs. normoalbuminuric IDDM; *p<0.02 and **p<0.05 vs without ACE inhibition
Figure 1. Relationships among HbA1c, urinary allo-THF (A), urinary THF+allo-THF/THE (B), urinary allo-THF/THF (C), and urinary A/E (D). ● Control subjects, □ normoalbuminuric and □ microalbuminuric IDDM patients. A: $r=-0.69$, $p<0.001$; B: $r=-0.58$, $p<0.01$; C: $r=-0.61$, $p<0.01$; D: $r=-0.59$, $p<0.01$ (in the combined groups).

blood pressure with initial blood volume and with the urinary cortisol to cortisone metabolite ratio in the control group (n=8, $r=0.63$ and $r=0.63$, $p<0.10$ for both), no further interrelationships between blood pressure, initial blood volume, BMI, HbA1c, steroid metabolites and aldosterone could be demonstrated.

In the microalbuminuric IDDM group ACE-inhibition treatment resulted in a fall in systolic and diastolic blood pressure, whereas overnight Ualb.V and creatinine clearance did not change significantly (Table 1). Mean HbA1c and fasting blood glucose levels remained unaltered (Table 1). Overnight urinary sodium excretion rose by 42 (95%CI, 18 to 66) µmol/min from 69±41 to 111±47µmol/min at this second evaluation ($p<0.02$). As
presented in Table 2 the absolute urinary excretion of THF, allo-THF and THE did not change significantly, but there was a modest further decrease in the cortisol to cortisone metabolite ratio. No changes were observed with respect to the allo-THF/THF ratio and the A/E ratio. Urinary and plasma cortisol, as well as plasma ACTH did not change significantly following ACE-inhibition treatment (Table 3). As expected, urinary and plasma aldosterone decreased and PRA increased after administration of the ACE-inhibitor (Table 3).

**Figure 2.** Relationships between the urinary cortisol to cortisone metabolite ratio (THF+allo-THF/THE) and initial blood volume an index of blood volume. ● Control subjects ($r=0.77, p<0.05; n=8$), □ normo- and □ microalbuminuric IDDM patients ($r=0.56, p<0.05; n=13$). The regression line is shifted to the left in the IDDM patients ($p<0.03$, by analysis of covariance).

**Discussion**

The lower urinary excretion of tetrahydro metabolites of cortisol and cortisone in normo- and microalbuminuric IDDM patients strongly suggest that the diabetic state influences cortisol metabolism via an impaired reduction of glucocorticoids. It was also found that the urinary allo-THF/THF ratio as well as the A/E ratio was lower, indicating that the proportion of 5α/5β reduced corticoids of both the C-21 (glucocorticoids) and the C-19 (adrenal androgens) series was decreased. In addition, the urinary cortisol to cortisone metabolite ratio was lower in IDDM. Since this metabolite ratio reflects the set-point of overall direction of the 11β-HSD-catalysed cortisol to cortisone interconversion [5-8], the present findings support the notion that IDDM is associated with an imbalance in 11β-HSD activity. Opposite changes in the urinary allo-THF/THF ratio and in the cortisol to cortisone metabolite ratio have been demonstrated in type 1 AME syndrome and after ingestion of licorice, conditions associated with impaired dehydrogenase activity of 11β-HSD [5-8, 28, 29].

It is uncertain whether the presently observed lower urinary excretion of tetrahydro metabolites of glucocorticoids reflects decreased cortisol secretion in IDDM, since urine was collected overnight and cortisol production rate was not measured. Previous studies have shown mild hypercortisolaemia in IDDM and a diminished
activation of the hypothalamic-pituitary adrenal axis is unlikely [17-19]. Fasting plasma cortisol was indeed higher in the microalbuminuric IDDM patients. Overnight urinary free cortisol excretion was within normal limits in both IDDM groups, albeit that its level was lower in normoalbuminuric patients compared to controls. Obviously, diabetes mellitus is associated with multiple alterations in the regulation of cortisol metabolism and further study is indicated to document their exact nature.

5α and 5β reductases as well as 11β-HSD are widely distributed enzymes [4,27,30]. In rat liver 5α reductase is found in the endoplasmatic reticulum, whereas 5β reductase is a cytosolic enzyme [30]. Both reductases require NADPH as a cofactor and are capable of metabolising different classes of steroids, including C-21 and C-19 compounds [27]. 11β-HSD is predominantly concentrated in the microsomal cell fraction and thus far two functional isoforms have been identified [4,31]. 11β-HSD₁, isolated from a variety of tissues including liver and vascular smooth muscle, exhibits both dehydrogenase and reductase activity and catalyses the dehydrogenation of cortisol to cortisone as well as the reduction of cortisone to cortisol. The direction of the reaction is dependent on the availability of its cofactor NAD⁺/NADPH, the ambient cortisol/cortisone concentration and the glycosylation status of the protein [4]. 11β-HSD₂ is present in placenta and in the distal nephron. This isoform only shows dehydrogenase activity and requires NAD⁺ as a cofactor [4,32-34]. In rat kidney both NADP⁺ and NAD⁺ availability affect dehydrogenase activity of 11β-HSD, suggesting the expression of both isoforms [31]. In human renal tissue the 11β-HSD₂ isoform is preferentially expressed and 11β-HSD activity is largely NAD⁺-dependent [33,34]. Experimental studies have demonstrated a diabetes-induced accumulation of NADP⁺ and NADH in the cytosolic cell compartment as a result of increased reduction of glucose to sorbitol and oxidation of sorbitol to fructose via the sorbitol pathway [35]. The present study showed inverse correlations of urinary allo-THF, the allo-THF/THF and the A/E ratio as well as THF + allo-THF/ THE ratio with the HbA1c level. These relationships would fit the hypothesis that chronic hyperglycemia induces a decrease in intracellular NADPH which impairs apparent 5α/5β reductase activity. The data might also support the concept that a higher NADP⁺/NADPH ratio enhances dehydrogenase activity and inhibits reductase activity of 11β-HSD₁, but it seems very improbable that a lower NAD⁺/NADH stimulates dehydrogenase activity of 11β-HSD₂. In the interpretation of the current results it should be noted that the urinary THF + allo-THF/ THE ratio is only an index of the overall in vivo direction of the cortisol to cortisone interconversion. Therefore, it is not possible to direct the imbalance in 11β-HSD activity to a specific tissue or to a specific isoform.

In IDDM patients without nephropathy extracellular volume tends to be increased, whereas plasma volume is normal or elevated [9-15]. We found that initial blood volume was elevated in the normoalbuminuric IDDM group, but our technique which estimates upper arm segmental blood volume cannot be easily compared with other methods to measure whole body extravascular and blood volume. In both the control group and the combined diabetic groups a positive correlation was found between the urinary cortisol to cortisone metabolite ratio and initial blood volume. Of note, the regression line was significantly shifted leftwards in the diabetic patients, thus essentially excluding that the
overall change in \(11\beta\)-HSD activity is primarily responsible for an abnormal fluid and sodium retention in IDDM. \(11\beta\)-HSD could also play a role in blood pressure regulation [36]. Inhibition of dehydrogenase activity of \(11\beta\)-HSD enhances the vasoconstrictor potency of cortisol and impaired cortisol to cortisone conversion is a salient feature in a subgroup of patients with essential hypertension [37,38]. The similar decrease in the urinary cortisol to cortisone metabolite ratio in the microalbuminuric compared to the normoalbuminuric IDDM group makes it unlikely, however, that the blood pressure rise associated with incipient nephropathy is related to altered dehydrogenase activity of \(11\beta\)-HSD.

Plasma aldosterone as well as urinary free aldosterone and aldosterone-glucuronide excretion were unchanged in the IDDM groups compared to control subjects. Normal to low levels of circulating aldosterone have been previously shown in heterogeneous groups of diabetic patients [15,39] and in IDDM patients irrespective of the presence of microalbuminuria [14], whereas discrepant data have been reported with respect to PRA and plasma active renin [11-15,40,41]. Our data support the contention that circulating aldosterone might be insufficiently suppressed in relation to the altered volume homeostasis, although it is not likely that the sodium and fluid retention in IDDM is caused by a stimulated plasma aldosterone per se [14,15].

During ACE-inhibition therapy urinary and plasma cortisol remained unchanged, in agreement with data in healthy subjects [42]. ACE-inhibition resulted in a modest further lowering of the cortisol to cortisone metabolite ratio, without an effect on the proportion of \(5\alpha/5\beta\) reduced corticoids. Since the HbA1c level was unaltered, this effect was not attributable to a change in metabolic control. In the rat, ACE-inhibitor administration stimulates \(11\beta\)-HSD activity in renal tissue and this influence is partly antagonized by angiotensin II [20]. The presently observed moderate decrease in urinary cortisol to cortisone metabolite ratio is in keeping with enhanced dehydrogenase activity of \(11\beta\)-HSD in vivo. It is possible that, in addition to inhibition of angiotensin II and aldosterone, stimulation of \(11\beta\)-HSD could contribute to the saluretic effect of ACE-inhibition treatment [20]. Indeed, urinary sodium excretion was increased during treatment, but our study design does not allow a conclusion with respect to the responsible mechanism.

In conclusion, IDDM patients show multiple abnormalities in urinary corticoid metabolite excretion, suggestive of impaired corticoid reduction and altered \(11\beta\)-HSD activity. It seems very unlikely that a change in \(11\beta\)-HSD activity is primarily responsible for the sodium and fluid retention in IDDM. Since ACE-inhibition resulted in a further decrease in the urinary cortisol to cortisone metabolite ratio, an additional mechanism of action of this treatment might be provided by an effect on \(11\beta\)-HSD activity.

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Chapter 11


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Addendum

Scheme representing metabolic pathways of cortisol.
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 α-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 circulating 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-responsiveness 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 abolish 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 standardized 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 11β-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 11β-HSD enzyme activity, are involved in the abnormal sodium and fluid retention in IDDM patients. 11β-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-HSD activity may be an additional mechanism whereby ACE inhibitors promote saliuresis.
Inleiding.

In hoofdstuk 1 worden het voorkomen, de stadia, de theorieën over het ontstaan en de behandelingsmethoden van diabetische nierziekte (nefropathie) besproken. Naar schatting treedt nefropathie op bij 30% van de patiënten met van insuline-afhankelijke diabetes mellitus (IADM). Diabetische nefropathie wordt gekenmerkt door een verhoogde eiwituitscheiding in de urine (proteïnurie) van meer dan 0.5 gram per dag en een geleidelijk verlies van nierfunctie. Nefropathie is een ernstige complicatie van diabetes mellitus, omdat naar verloop van tijd terminaal nierfunctieverlies kan optreden, zodat nierfunctie vervangende behandeling met dialyse of transplantatie noodzakelijk wordt. Daarnaast is gebleken dat IADM patiënten met proteïnurie een sterk verhoogd risico hebben op hart- en vaatziekten. Verbeterde behandelingenmethoden kunnen het beloop van de nefropathie gunstig beïnvloeden. Met name agressieve bloeddrukverlaging vermindert de snelheid van het nierfunctieverlies en heeft naar alle waarschijnlijkheid de vermindering van het overlijden ten gevolge van hart- en vaatziekten tot gevolg.

Het identificeren van patiënten met een verhoogde kans op het ontwikkelen van een diabetische nefropathie is mogelijk gemaakt door de introductie van gevoelige laboratoriummethoden waarmee lage concentraties van eiwit, albumine, in de urine gemeten kunnen worden. Onder normale omstandigheden passeert een kleine hoeveelheid albumine het glomerulaire filter in de nieren. De glomerulus is de kleinste functionele eenheid van de nieren. Hier wordt de urine gevormd uit het bloed dat onder druk gefilterd wordt over een poreuze membraan, de glomerulaire basaal membraan. De glomerulaire basaal membraan vormt een effectieve barrière tegen verlies van grote eiwitten, zoals albumine. Gebleken is dat bij IADM patiënten een geringe verhoging van de albumine uitscheiding in de urine (microalbuminurie) van prognostische betekenis is voor het later ontwikkelen van nefropathie. Deze verhoogde doorlatbaarheid van albumine kan wijzen op vroege veranderingen in het filtratie proces ten gevolge van een hogere filtratie druk in de glomeruli en veranderingen in de glomerulaire basaal membraan.

Het ontstaan van diabetische nefropathie is waarschijnlijk multifactorieel bepaald. Men veronderstelt dat veranderingen in de bloedsomloop door het lichaam en de nieren (haemodynamische factoren) en veranderingen in de stofwisseling (metabole factoren) bij IADM patiënten bijdragen aan het ontstaan van diabetische nefropathie. Immers, zowel hoge bloeddruk als onvoldoende diabetes regulatie zijn risicofactoren voor het ontstaan van deze complicatie. Daarnaast spelen erfelijke factoren een rol bij de predispositie voor diabetische nefropathie.

Het is thans nog niet mogelijk om het ontwikkelen van diabetische nefropathie te voorkomen. Onderzoek naar factoren, die een rol spelen bij het ontstaan van diabetische nefropathie, is van belang om de behandelingsmogelijkheden te kunnen optimaliseren. In dit proefschrift is getracht meer inzicht te krijgen in de werking van noradrenaline (NA) en de groei hormoon (GH)-insulin-like growth factor-I (IGF-I)-as op de nieren. NA is een niervaatverdichtende stof (renale vasoconstrictor) die de doorstroming in de nier vermindert en de filtratiedruk kan verhogen. GH veroorzaakt indirect niervaatverwijding (renale vasodilatatie) door stimulatie van IGF-I synthese. De vroege veranderingen in de nierfunctie bij IADM patiënten worden gekenmerkt door een dysbalans tussen vasodilatatie
van de vaten die de glomerulus van bloed voorzien (preglomerulaire arteriolen) en vasoconstrictie van de vaten die het bloed afvoeren (postglomerulaire arteriolen). Deze veranderingen leiden waarschijnlijk tot een toegenomen filtratiedruk. Een apart hoofdstuk is gewijd aan methoden om urine ingevroren te bewaren alvorens kleine hoeveelheden eiwit, in casu immunoglobuline-G, te bepalen. In een ander hoofdstuk wordt de rol van de balans tussen de hormonen, cortisol en cortison, bij zoutretentie en volume expansie bij IADM patiënten belicht.

**Effecten van noradrenaline op de nierdoorbloeding en het eiwit verlies in de urine.**

NA is de neurotransmitter van het sympathische zenuwstelsel, die vrijkomt uit de zenuwvezels. NA lekt voor een deel naar de circulatie. Dit circulerende NA is als vaso-actieve stof werkzaam. NA veroorzaakt vasoconstrictie na binding aan zogenaamde α-adrenerge receptoren. In de nieren zijn α-adrenerge receptoren gelokaliseerd in de vaten van de glomerulaire arteriolen. Deze bepalen in belangrijke mate de nierdoorstroming en reguleren de filtratiedruk. Fysieke inspanning stimuleert het sympathische zenuwstelsel en kan een stijging van de albuminurie veroorzaken. Deze toename van albuminurie na fysieke inspanning wordt toegeschreven aan een verhoogde glomerulaire passage voor grote moleculen, in combinatie met een stijging van de systemische bloeddruk en glomerulaire filtratiedruk.


In hoofdstuk 3 werd de mogelijke relatie tussen het in het bloed aanwezig NA en renale haemodynamische parameters, zoals de klaring (glomerulaire filtratie rate: GFR) en de nierdoorbloeding (effectieve renale plasma flow: ERPF), geëvalueerd in normo- en microalbuminurische IADM patiënten en controle personen. De GFR en de ERPF werden bepaald met standaard infusie methoden. De in rust afgenomen plasma NA spiegel was iets lager bij de IADM patiënten dan bij de controle personen. De IADM patiënten hadden een hogere GFR en ERPF dan de controle personen, wijzend op glomerulaire vasodilatatie. De ERPF bleek in alle groepen negatief gecorreleerd met te zijn met de
plasma NA spiegel. Tussen de IADM en de controle personen bleek geen verschil in deze relatie te bestaan. Dus de lagere plasma NA spiegels zouden kunnen bijdragen aan een verhoogde ERPF bij IADM patiënten. De GFR was eveneens negatief gecorreleerd met de plasma NA concentratie. Bovendien bleek dat IADM patiënten een hogere GFR hadden, onafhankelijk van de plasma NA spiegel. Dit impliceert dat additionele vasodilaterende mechanismen, naast de invloed van een lagere NA spiegel, een rol spelen bij de verhoogde GFR bij IADM patiënten.

Hoofdstuk 4 geeft de resultaten weer van een gerandomiseerde, placebo gecontroleerde NA infusie studie bij gematchte normo- en microalbuminurische IADM patiënten en niet-diabetische controle personen. Dit onderzoek werd uitgevoerd om de volgende vragen te beantwoorden. Ten eerste, kan NA toediening een toename van de eiwituitscheiding in de urine (microproteïnurie) induceren bij de mens? Ten tweede, zijn er verschillen in een door NA-geïnduceerde microproteïnurische respons tussen normo- en microalbuminurische IADM patiënten en controle personen? Ten derde, wat zijn de determinanten van een door NA-geïnduceerde toename van microproteïnurie? Ten vierde, bestaan er verschillen in de renale haemodynamiek tijdens NA infusie tussen de onderzochte groepen? In dit onderzoek werden de microproteïnurie (uitscheiding van albumine en immunoglobuline-G), de systemische en de renale haemodynamiek gemeten tijdens 3 opeenvolgende NA infusie doses, die respectievelijk een bloeddrukstijging van 0, 4 en 20 mmHg veroorzaakten. De NA infusie dosis werd per deelnemer van te voren bepaald.

De systemische bloeddruk gevoeligheid voor NA was toegenomen bij beide groepen IADM patiënten, zoals eerder is gerapporteerd voor heterogene groepen diabetes patiënten. NA, gegeven in een dosis die de bloeddruk met 20 mmHg deed stijgen, veroorzaakte een toename van de microproteïnurie in combinaties met renale vasoconstrictie in alle onderzochte groepen. NA verhoogde zowel de uitscheiding van albumine als immunoglobuline-G, maar niet van β2-microglobuline. Dit impliceert dat NA de glomerulaire passage van eiwitten doet toenemen. In absolute zin was de toename van de microproteïnurie groter in de microalbuminurische IADM patiënten ten opzichte van de normoalbuminurische IADM patiënten en controle personen. Multipole regressie analyse toonde aan dat de stijging van de bloeddruk en de toename van de plasma NA spiegels onafhankelijk bijdroegen aan de door NA geïnduceerde toename van microproteïnurie. NA veroorzaakte een uitgesproken daling van de ERPF en stijging van de filtratie fractie (het quotiënt van GFR en ERPF), maar had geen effect op de GFR. De relatie tussen de plasma NA spiegel en de veranderingen in renale haemodynamiek verschield niet tussen de onderzochte groepen.

Bovengenoemde studie is het eerste acute infusie experiment dat een toename van microproteïnurie als reactie op de vasopressor, NA, aantoont. In eerdere, niet met placebo gecontroleerde, studies met de vasopressor, angiotensine II, werd een dergelijk effect op de microproteïnurie niet gevonden. Onze resultaten geven steun aan de hypothese dat NA via een renaal mechanisme de glomerulaire eiwitlekkage in de nieren doet toenemen. Dit is in overeenstemming met de in de voorgaande hoofdstukken veronderstelde rol van circulerende plasma NA op de toename van albuminurie bij fysieke inspanning, en de beschreven relatie met renaal haemodynamische parameters. De versterkte microproteïnurische respons bij de microalbuminurische IADM patiënten is waarschijnlijk het gevolg van een preëxistent permeabiliteitsdefect van de glomerulaire
basaal membraan. Plasma NA spiegels stijgen tijdens dagelijkse inspanningen en zouden aldus kunnen bijdragen aan eiwitekkage. De aanwezigheid van proteïnurie wordt als een ongunstige indicator beschouwd voor toekomstig nierfunctieverlies, alhoewel het niet zeker is of dit ook geldt voor de microalbuminurische fase van diabetische nefropathie. Desalniettemin zou bescherming van de nier tegen renale NA effecten van klinische belang kunnen zijn bij microalbuminurische IADM patiënten.

In hoofdstuk 5 is onderzocht of behandeling met een angiotensine-converting enzyme (ACE) remmer de NA-geïnduceerde stijging van de bloeddruk en renale vasoconstrictie beïnvloedt bij IADM patiënten met microalbuminurie. Bij patiënten met essentiële hypertensie is gebleken dat ACE-remmers inderdaad de door NA-geïnduceerde stijging van de bloeddruk verminderen, hetgeen als een additioneel werkingmechanisme van deze medicamenten kan worden gezien. Een studie bij gezonde vrijwilligers heeft bovendien aangetoond dat dit ook geldt voor de door NA-geïnduceerde ERPF daling. Zeven microalbuminurische IADM patiënten werden voor en 6 weken na behandeling met de ACE-remmer, enalapril, onderzocht. Conform de verwachting trad er tijdens enalapril behandeling een daling op van de bloeddruk en de albuminurie, en bleek de ERPF te zijn toege- nomen. De bloeddruktijging onder invloed van NA was echter meer uitgesproken tijdens enalapril behandeling. De ERPF daling en de albuminurie tijdens NA infusie werd niet door enalapril beïnvloed. Deze bevindingen suggereren dat enalapril onvoldoende bescherming biedt tegen systemische en renale NA effecten.

In hoofdstuk 6 is onderzocht in hoeverre intraveneus toegediend dopamine in staat is het renale vasoconstrictieve effect van NA infusie te antagoneren. Dopamine wordt veel toegepast om bij haemodynamisch instabiele patiënten de nierfunctie te ondersteunen tijdens NA behandeling. Bij 7 gezonde vrijwilligers werd in gerandomiseerde volgorde dopamine en placebo toegevoegd aan 3 verschillende NA infusie doses. Dopamine bleek de door NA-geïnduceerde ERPF daling zeer effectief te antagoneren. Er was een minder uitgesproken bloeddruk stijging en een sterke toename van de zout uitscheiding na toevoeging van dopamine aan de NA infusen. Deze studie bevestigt de veronderstelling dat lage dosis dopamine bij de mens de vasoconstrictieve effecten van NA in de nier tegengaat. Of dit ook geldt voor instabiele patiënten zal nader onderzocht moeten worden. De vermindering van de door NA-geïnduceerde bloeddruktijging en de toegenomen zoutexcretie onder invloed van dopamine kunnen bij haemodynamisch instabiele patiënten echter minder gewenst zijn.

Effecten van groeihormoon en insulin-like growth factor-I op de nier.

De GH-IGF-I-as wordt bij de mens beschouwd als een determinant van de nierfunctie. Exogeen toegediend GH en IGF-I stimuleren zowel de GFR als de ERPF. Het effect van de GH-IGF-I-as op microproteïnurie is niet goed bekend. In hoofdstuk 7 is onderzocht of de albumine uitscheidingsnelheid in de urine gerelateerd is aan GH en IGF-I spiegels in het bloed. In deze studie werden patiënten met GH deficiëntie, patiënten met (on)behandelde acromegalie ten gevolge van een GH producerend hypofyse adenoom en gezonde controle personen met elkaar vergeleken. De albumine uitscheidings bleek hoger
bij de patiënten met acromegalie en was iets lager bij de patiënten met GH deficiëntie ten opzichte van de controle personen. Bij de acromegalie patiënten resulteerde medicamenteuze verlaging van de GH en IGF-I spiegels met octreotide in een dalings van de albumine uitscheidingsnelheid. Er bestond een positieve relatie tussen de GH en IGF-I spiegels en de albumine uitscheidingsnelheid, onafhankelijk van de kreatinine klaring. Deze bevindingen bevestigen dier experimentele studies dat verhoogde GH en IGF-I spiegels microproteïnurie kunnen induceren, en zijn in overstemming met de toename van albuminurie na IGF-I infusie bij gezonde vrijwilligers.

In hoofdstuk 8 werden de parameters voor de nierfunctie (GFR en ERPF) basaal en na stimulatie door middel van aminozuren infusie gemeten. Vergeleken werden GH deficiënte patiënten, (on)behandelde acromegalie patiënten, IADM patiënten met een normale en een verhoogde GFR (normo- versus hyperfiltratie) en gezonde controle personen. Ook werden de IGF-I spiegels in relatie tot de gemeten nierfunctieparameters vergeleken.

De basale GFR en ERPF waarden correspondeerden met de mate van GH secretie: de laagste GFR en ERPF waarden werden gevonden bij de GH deficiënte patiënten, gevolgd door een hogere GFR en ERPF bij de controle personen, behandelde en onbehandelde acromegalie patiënten. Er bleek in alle groepen een inverse relatie te bestaan tussen de hoogte van de basale GFR en ERPF en de toename van GFR en ERPF na stimulatie met aminozuren infusie. De GFR en ERPF waren bij de acromegalie en hyperfiltrerende IADM patiënten niet meer stimuleerbaar. De GFR en ERPF correleerden met de plasma IGF-I spiegel bij de GH deficiënte patiënten, (on)behandelde acromegalie patiënten en de controle personen, maar niet bij de IADM patiënten.

Een verhoogde GFR (glomerulaire hyperfiltratie) is een fenomeen dat bij IADM en acromegalie wordt gevonden. Bij patiënten met acromegalie is deze het gevolg van de hoge GH en IGF-I spiegels, maar de rol van de GH-IGF-I-as bij diabetsche glomerulaire hyperfiltratie is niet duidelijk. De afwezige GFR en ERPF respons na aminozuren infusie suggerereert dat de renale reserve capaciteit bij glomerulaire hyperfiltratie maximaal benut is. Deze opmerkelijke overeenkomst tussen de acromegalie en hyperfiltrerende IADM patiënten werd echter niet weerspiegeld in een verhoogde plasma IGF-I concentratie bij de hyperfiltrerende IADM patiënten. Bij diabetsche ratten is accumulatie van IGF-I in de nier aangetoond. Men veronderstelt dat toegenomen renale IGF-I receptor activiteit en verandering in lokale productie van IGF-I bindende eiwitten hieraan ten grondslag liggen. Op een dergelijke wijze zou de GH-IGF-I-as een rol kunnen spelen bij glomerulaire hyperfiltratie bij IADM patiënten, ondanks het feit dat de plasma IGF-I spiegel niet verhoogd is. Deze veronderstelling zou bij IADM patiënten met een IGF-I infusie experiment onderzocht kunnen worden. De beperkte beschikbaarheid van IGF-I maakt een dergelijke studie momenteel niet mogelijk.

In hoofdstuk 9 werden de door inspanning gestimuleerde GH en glucagon spiegels geëvalueerd in IADM patiënten met normale en verhoogde GFR. Glucagon heeft evenals GH een vasodilatierende werking op de nier. Glucagon spiegels zijn dikwijls verhoogd bij IADM patiënten. Onderzocht werd of deze hormonen zouden kunnen bijdragen aan de verhoging van GFR, ERPF en niergrootte bij IADM patiënten. Door middel van gestandaardiseerde GFR en ERPF metingen werden IADM patiënten met glomerulaire hyperfiltratie geselecteerd. Deze werden individueel gematcht met normo-filterende IADM
patiënten.
De gemiddelde concentratie van deze hormonen was niet significant verschillend tussen de normo- en hyperfiltrerende IADM patiënten. Echter, multipel regressie analyse toonde dat de door inspanning gestimuleerde GH spiegel, de glucagon concentratie en het HbA1c gehalte als maat voor diabetes regulatie, positief correleerden met de GFR, ERPF en niergrootte.

Uit de hoofdstukken 7, 8 en 9 wordt geconcludeerd dat de GH-IGF-I-as de renale haemodynamiek en proteïnurie beïnvloedt bij diverse patiënten categorieën, waaronder GH deficiëntie, acromegalie en IADM. Vanuit een therapeutisch perspectief, bestaat de vraag of somatostatine analogen de progressie van albuminurie en nierfunctieverlies bij IADM patiënten zouden kunnen tegengaan. Een dergelijke interventie met GH en IGF-I concentratie verlaagende middelen zou mogelijk een waardevolle aanvulling kunnen zijn op bloeddruk verlagende behandeling. Met het beschikbaar komen van langwerkende somatostatine analogen zal dit binnen afzienbare tijd onderzocht kunnen worden.

Het effect van glucose op de immunoglobuline-G concentratie in de urine.
Het immunoglobuline-G is een groter eiwit dan albumine. Het meten van de immunoglobuline-G uitscheidingsnelheid in de urine verschafte informatie over de veranderingen in de poriën grootte van het glomerulaire basaal membraan. Bij normoalbuminurische IADM patiënten is gerapporteerd dat ook immunoglobuline-G in verhoogde mate wordt uitgescheiden. Deze bevinding is onbegrepen omdat de poriën-grootte van de glomerulaire basaal membraan bij deze patiënten geacht wordt onveranderd te zijn.
In hoofdstuk 10 vonden ook wij dat normoalbuminurische IADM patiënten een verhoogde mate van immunoglobuline-G excretie in de urine hadden ten opzichte van controle personen. Tevens bleek de immunoglobuline-G uitscheidingshoger te zijn in urine verzamelingen van IADM patiënten die glucose bevatten dan in die van patiënten zonder glucosurie. Aangezien het immunoglobuline-G gehalte in de urine kan verminderen bij het bewaren van de monsters bij -20°C gedurende enkele weken, veronderstelden wij dat de aanwezigheid van glucose mogelijk een conserverend effect op het immunoglobuline-G had. In een laboratorium experiment werd deze hypothese getoetst. Getest werden diverse bewaarregimes op de houdbaarheid van immuno-globuline-G in urine monsters van niet-diabetes patiënten met een verschillende mate van proteïnurie. De immunoglobuline-G concentratie daalde in de monsters die onbehandeld bij -20°C waren ingevroren. Daarentegen bleek een voorbehandeling met fosfaatbuffer, koeienalbumine en glucose te beschermen tegen het verlies van immuno-globuline-G. Het minste verlies na 12 weken invriezen bij -20°C werd gevonden bij die urine monsters, waaraan een hoge concentratie van glucose was toegevoegd. Deze bevinding impliceert dat de aanwezigheid
van glucose in de urine van IADM patiënten preserverend werkt op het urine immunoglobuline-G gehalte, wanneer urine verzamelingen zonder voorzorgsmaatregelen worden bewaard bij -20°C. Een dergelijk effect leidt tot de een schijnbare verhoging van de immunoglobuline-G uitscheiding bij normoalbuminurische IADM patiënten ten opzichte van niet diabetische personen. Er dienen dus voorzorgsmaatregelen getroffen te worden wanneer de urine immunoglobuline-G bepaling niet onmiddellijk kan worden uitgevoerd.

De rol van 11β-hydroxysteroid dehydrogenase bij de water- en zouthuishouding bij IADM patiënten.


Bij normo- en microalbuminurische IADM patiënten werd een lagere excretie van cortisol en cortison metaboliëten in de urine gevonden in vergelijking met de controle personen. Dit wijst erop dat de reductie van glucocorticoïden tot tetrahydrometabolieten bij IADM patiënten gestoord is. De urine cortisol/cortison metaboliet ratio was lager in de IADM groepen. Dit impliceert een verschuiving in het evenwicht tussen cortisol en cortison ten gunste van cortison. Bij IADM patiënten bleek een inverse relatie tussen het HbA1c gehalte en de urine cortisol/cortison metaboliet ratio te bestaan. Zowel bij IADM patiënten als bij de controle personen was de urine cortisol/cortison metaboliet ratio positief gerelateerd met het via een impedantie techniek gemeten extravasculaire- en bloedvolume. Deze relatie was in de IADM groepen naar links verschoven. Dit suggereert dat de 11βHSD activiteit zoals weerspiegelt in de urine cortison/cortisol metaboliët ratio, een determinant is van volume homeostase. De linksverschuiving in deze relatie bij de IADM patiënten maakt het echter onwaarschijnlijk dat een veranderde 11β-HSD activiteit verantwoordelijk is voor de toegenomen zoutretentie en volume expansie. De behandeling van de microalbuminurische IADM patiënten met een ACE-remmer induceerde een verdere verlaging van de urine cortisol/cortison metaboliet ratio.

Op basis van deze resultaten wordt de hypothese naar voren gebracht dat de gestoorde reductie van glucocorticoïden en de 11β-HSD gemediëerde verschuiving in het cortisol/cortison evenwicht bij IADM patiënten het gevolg kan zijn van een veranderde cofactor beschikbaarheid ten gevolge van chronische hyperglycaemie. De bevindingen suggereren ook dat verbeterde metabole controle tot een verandering in het evenwicht van cortisol/cortison ten gunste van cortisol kan leiden en aldus de zoutretentie en volume expansie bij IADM patiënten kan doen toenemen. De stimulatie van 11β-HSD
door ACE-remmers suggereert een additioneel werkingsmechanisme waardoor deze farmaca zoutexcretie kunnen bevorderen.
CURRICULUM VITAE

Name Klaas Hoogenberg
Address Middelhorsterweg 34
9751 TG Haren (Gn), The Netherlands
Date of birth 14 February 1962
Place of birth The Hague
Gender Male
Civil state Married, 1 son 2 years of age
Nationality Dutch
Education Highschool Atheneum B, graduated June 1980
Study Psychology for one year, 1981
Medical School, accomplished August 1987
Graduated as general physician, August 1989
Educated in Internal Medicine 1990-1996
Previous position 1990 General physician Diabetes Center, Beatrixoord, Haren (Gn)
1990-1996 Training in Internal Medicine in University Hospital, Groningen
Present position 1996 October assigned as internist for training in endocrinology
and diabetes (supported by a grant from the Dutch Diabetes
Foundation) at the department of Endocrinology, University
Hospital Groningen, P.O. Box 30.001, 9700 RB Groningen,
The Netherlands

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