Inhibition of the renin-angiotensin system in normotensive type 2 diabetes:

effects and mechanisms of action

A.A.M. Zandbergen

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Inhibition of the Renin-Angiotensin System in Normotensive Type 2 Diabetes:

effects and mechanisms of action

Remming van het renine-angiotensine systeem in normotensieve type 2 diabeten:

effecten en werkingsmechanismen

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Promotor:	Prof.dr. S.W.J. Lamberts
Overige leden:	Prof.dr. A.H.J. Danser
	Prof.dr. A.J. van der Lelij
	Prof.dr. D. de Zeeuw
Copromotor:	Dr. A.H. Bootsma

CONTENTS

I	**Introduction**	7
I.1	Renal disease in diabetes mellitus	9
I.2	The IGF-I system and diabetes mellitus	17
I.3	Aims of the thesis	21
II	**Effects of short-term losartan treatment on albuminuria, the IGF-I system and insulin resistance**	29
II.1	Effect of losartan on microalbuminuria in normotensive patients with type 2 diabetes mellitus. A randomised clinical trial *Annals of Internal Medicine 2003;139(2):90-96*	31
II.2	The insulin-like growth factor I (IGF-I) system and the renal and haemodynamic effects of losartan in normotensive patients with type 2 diabetes *Clinical Endocrinology 2006;64(2):203-208*	47
II.3	The effect of losartan on urinary growth hormone in normotensive patients with type 2 diabetes and microalbuminuria *Submitted*	59
II.4	Short-term administration of an angiotensin-receptor antagonist in patients with impaired glucose tolerance improves insulin sensitivity and increases free IGF-I *European Journal of Endocrinology 2006;155(2):293-296*	65
III	**Clinical outcome of normotensive patients with type 2 diabetes mellitus and microalbuminuria**	73
III.1	Normotensive women with type 2 diabetes mellitus and microalbuminuria are at high risk for macrovascular disease *Diabetes Care 2006;29:1851-1855*	75
III.2	Change in albuminuria is predictive of cardiovascular outcome in normotensive patients with type 2 diabetes mellitus and microalbuminuria *Submitted*	87
IV	**General discussion and summary**	99
IV.1	General discussion	101
IV.2	Summary	119
IV.3	Samenvatting	125
	Dankwoord	129
	Curriculum vitae	133

I

Introduction

I.1

Renal disease in diabetes mellitus

Type 2 diabetes mellitus has become a major health problem and its prevalence increases rapidly[1]. It associates with an increased risk of microvascular and macrovascular diseases, like retinopathy, nephropathy, coronary heart disease, cerebrovascular disease and peripheral artery disease[2-6]. Renal disease represents a major cause of morbidity and mortality in patients with diabetes mellitus. About 40% of the patients with type 2 diabetes mellitus develop diabetic nephropathy, ranging from microalbuminuria to end-stage renal disease. Despite recent therapeutic advances, diabetes mellitus has become the leading cause of end-stage renal failure, and is responsible for more than 40% of all end-stage renal diseases in the Western world[7,8].

PATHOGENESIS AND NATURAL COURSE

Before the onset of microalbuminuria, the first clinical sign of diabetic nephropathy, the glomeruli of the diabetic kidney express several histologic abnormalities[9,10]. Due to increased glucose dependent matrix formation, chronic hyperglycaemia results in mesangial expansion and thickening of the glomerular basement membrane. Glucose excess also promotes glycosylation of matrix proteins, forming early glycosylation products, which eventually convert into irreversible advanced glycosylation end products (AGEs). These AGEs contribute to microvascular complications and diabetic nephropathy as a result of tissue accumulation[11]. Furthermore, hyperglycaemia increases the expression and activation of several cytokines, vascular growth factors and immune-related as well as profibrotic processes[12]. Insudation of proteins like fibrin, immunoglobulins and complement causes hyaline deposits in the arterioles of the glomeruli, thereby narrowing the vessels resulting in ischemia and renal vasodilatation. These pathophysiological mechanisms induce intraglomerular hypertension, which eventually results in glomerulosclerosis[9,10,13].

Intraglomerular hypertension plays a part in the development of glomerular hyperfiltration, an elevation of 25 to 50% in glomerular filtration rate (GFR)[14]. Besides haemodynamic changes, such as systemic hypertension and dilatation of the afferent glomerular arterioles, with increased renal blood flow and intraglomerular pressure, several other factors are considered to contribute in the pathogenesis of glomerular hyperfiltration, including angiotensin II, growth hormone (GH) and AGEs[14-16]. Glomerular hypertrophy and increased renal size accompany glomerular hyperfiltration.

In patients with type 1 diabetes mellitus, glomerular hyperfiltration mostly develops a few years after diabetes is diagnosed[17]. In patients with type 2 diabetes, it is more often present shortly after the diagnosis, because the diabetes usually exists for some time before the diagnosis is formal[18]. The appearance of abnormal amounts of albumin in the urine accompanies the decline in GFR[17,18] (*figure 1*). The rate of fall in GFR has an average of 10 to 12 ml per minute per year in untreated patients, finally resulting in end-stage renal disease[19].

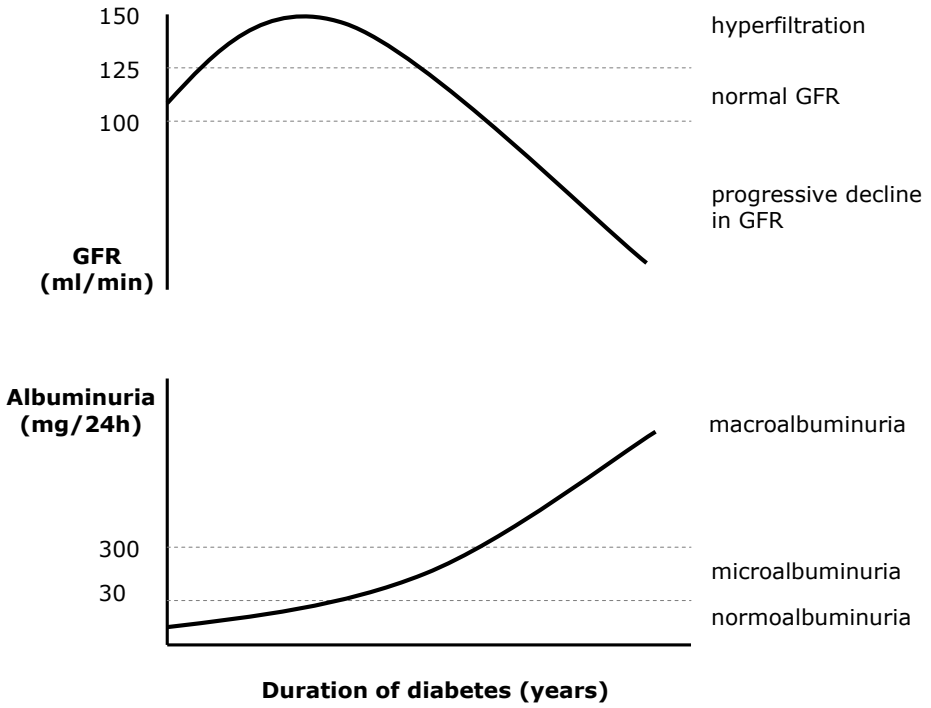

Figure 1. Association between glomerular filtration rate (GFR) and albuminuria in the course of diabetic nephropathy (based on: Mogensen CE[17])

ALBUMINURIA

The normal rate of urinary albumin excretion is less than 30 mg per 24 hour (or less than 20 µg per minute). Abnormal urinary albumin excretion in patients with diabetes mellitus was first described in 1969 by Keen et al[20]. Persistent increased urinary albumin excretion between 30 and 300 mg per 24 hours (20 to 200 µg per minute) is classified as microalbuminuria (also called incipient nephropathy), and is considered to be the first clinical sign of renal involvement in patients with diabetes mellitus[21,22]. Progression from microalbuminuria to macroalbuminuria (overt nephropathy), with urinary albumin excretion more than 300 mg per 24 hours (more than 200 µg per minute), occurs in 20 to 40 % of Caucasian type 2 diabetic patients in a period of 10 years[21,23]. Risk factors for progression of albuminuria include hypertension, worse glycaemic control, smoking, as well as ethnicity (for example, in Pima Indians, the rate of progression of albuminuria almost doubles that of Caucasians)[18,24]. Table 1 shows the definitions and classification in urinary albumin levels in patients with diabetes mellitus.

Table 1

	24h urine (mg/24h)	timed urine (µg/min)	single-use sample (mg/l)	ACR (mg/mmol)	
normoalbuminuria	<30	<20	<20	<2.5 M	<3.5 F
microalbuminuria	30-300	20-200	20-200	2.5-25 M	3.5-35 F
macroalbuminuria	>300	>200	>200	>25 M	>35 F

ACR: albumin-to-creatinine ratio M: male F: female

NEPHROPATHY AND CARDIOVASCULAR DISEASE

Renal disease is associated with an increased risk of cardiovascular morbidity and mortality. This increased risk not only applies to end-stage renal disease, but is present in any of the earlier stages as well. For example, in patients with type 2 diabetes mellitus and microalbuminuria, 40 to 50% eventually die of cardiovascular disease. In type 2 diabetic patients without nephropathy, this risk is three times as low[25,26].

Microalbuminuria is not only the first clinical sign of diabetic nephropathy, it is also considered to be an early manifestation of generalised vascular dysfunction and atherosclerosis. In both type 1 and type 2 diabetes mellitus, as well as in non-diabetic persons, microalbuminuria is a strong and independent predictor of cardiovascular diseases and all-cause mortality[21,27-30]. Still, there is no consensus on the nature of this close link[31]. At present, the most likely explanation involves common pathophysiological mechanisms, such as endothelial dysfunction and chronic low-grade inflammation, together with the possibility that both renal and cardiovascular disease factors interact and enhance each other[31-33].

There is increasing evidence that microalbuminuria is associated with endothelial dysfunction. Increased levels of indirect estimates of endothelial function, such as von Willebrand factor, soluble adhesion molecules and endothelin, are observed when microalbuminuria is present[32-34]. After adjustment for classical cardiovascular risk factors, the association between microalbuminuria and endothelial dysfunction persists. A possible mechanism is that endothelial dysfunction results in microalbuminuria by inducing glomerular hypertension and impairing the function of the basement membrane, mesangial cells and podocytes[35].

Furthermore, high-sensitivity C-reactive protein has recently been established as a sensitive marker of a chronic low-grade inflammatory state of the arterial vessel wall, which is closely linked to generalised vascular endothelial dysfunction[36]. It is predictive of cardiovascular disease, independent of the classical cardiovascular risk factors in various cohorts[37]. The correlation observed between hsCRP and microalbuminuria, although the number of studies is limited at present, supports the role of inflammation in the pathogenesis of diabetic nephropathy[38,39].

THE RENIN-ANGIOTENSIN SYSTEM AND DIABETIC NEPHROPATHY

Several studies support the role of the renin-angiotensin system (RAS) in the pathogenesis of micro- and macrovascular complications in type 2 diabetes mellitus[40]. Particularly the local, independently regulated tissue-based RASs like the intrarenal ones within the glomeruli and proximal tubuli, are thought to be activated as a result of hyperglycaemia[41,42]. Whereas the systemic RAS, which is thought to be suppressed in diabetes mellitus, involves the regulation of blood pressure and volume status, the local RASs are more involved in cell growth and development, as well as tissue repair[43].

Activation the intrarenal RAS through hyperglycaemia in patients with diabetes mellitus stimulates the local angiotensin II production. Binding of intrarenal angiotensin II to the angiotensin 1 (AT1) receptor increases arteriolar resistance and intraglomerular pressure, thereby reducing renal blood flow and facilitating urinary albumin excretion[40,41,43]. Furthermore, angiotensin II interacts with several growth factor systems, like transforming growth factor-beta (TGF-beta), vascular endothelial growth factor (VEGF), platelet derived growth

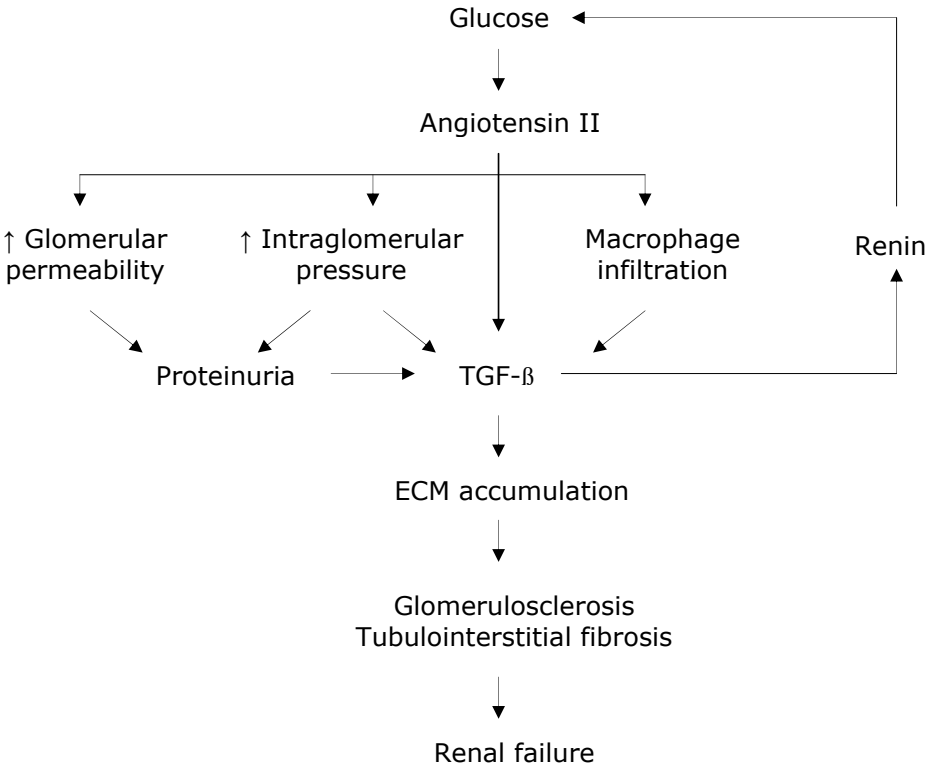

Figure 2. Angiotensin II and the pathogenesis of diabetic nephropathy (adapted from Gilbert[40])

factor (PDGF) and insulin-like growth factor I (IGF-I). This results in increased production of extracellular matrix proteins in the mesangium and tubulointerstitium, and eventually in progressive glomerulosclerosis and tubulointerstitial fibrosis[40,42] *(figure 2)*.
Angiotensin II also binds to the angiotensin 2 (AT2) receptor, which probably has opposite effects to that of AT1 receptor activation (for example inhibition of cell growth and matrix production and reduction of intraglomerular pressure)[41,44]. Recent studies suggest a reduction in the number of AT2 receptors in the diabetic kidney, thus contributing to the pathogenesis of diabetic nephropathy[41].

INHIBITORS OF THE RENIN-ANGIOTENSIN SYSTEM AND DIABETIC NEPHROPATHY

Inhibition of the RAS by means of the angiotensin I converting enzyme (ACE) inhibitors and the angiotensin-receptor antagonists has unique effects on the kidney, such as increasing renal plasma flow, reducing the filtration fraction as well as reducing urinary albumin excretion[45]. Several ACE inhibitors have been shown to preserve renal function and reduce albuminuria in normotensive and hypertensive patients with type 1 as well as type 2 diabetes mellitus[46-50]. These renoprotective and antiproteinuric effects appear to be at least partly independent of the reduction in blood pressure, resulting from treatment with ACE inhibitors[48-50].

The angiotensin-receptor antagonists, of which losartan was the first introduced for clinical use in 1995, antagonise the AT1 and AT2 receptors of many tissues. The affinity for the AT1 receptor is 10.000 times as high as for the AT2 receptor[51]. The first clinical studies showed reduction in blood pressure and albuminuria in hypertensive patients, at least similar to ACE inhibitors[52,53]. Since then, several studies have investigated the effects of angiotensin-receptor antagonists on renal function and urinary albumin excretion, in both type 1 and type 2 diabetes mellitus, mostly with hypertension[54-57].

The role of the angiotensin-receptor antagonists in the treatment of nephropathy in hypertensive type 2 diabetic patients has been further established with the publication of large clinical trials such as the Irbesartan Type II Diabetes with Microalbuminuria (IRMA) trial, the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) study and the Irbesartan Diabetic Nephropathy Trial (IDNT) in 2001[58-60]. In the IRMA trial, irbesartan reduced the progression of microalbuminuria to overt proteinuria in hypertensive patients with type 2 diabetes mellitus[58]. The results of the RENAAL and IDNT study showed that losartan and irbesartan, respectively, reduced doubling of serum creatinine, progression to end-stage renal disease, as well as death in hypertensive patients with type 2 diabetes mellitus with overt proteinuria[59,60].

Data on the renal effects of the angiotensin-receptor antagonists in patients with type 2 diabetes mellitus without hypertension still are limited.

I.2

The IGF-I system and diabetes mellitus

THE IGF-I SYSTEM

The insulin-like growth factor 1 (IGF-I) is a single chain peptide hormone, with a structure very similar to that of insulin[61]. It is produced mainly in the liver, stimulated primarily by growth hormone (GH). In return, IGF-I inhibits the secretion of GH by feedback inhibition[62]. Most IGF-I is bound in the circulation to IGF-binding proteins (IGFBP-1 to 6) and less than 1% circulates as unbound hormone, which is the biologically active form[63]. The hepatic production of IGF-I is also under the influence of other hormones, like stimulation by insulin and suppression through angiotensin II[64].

IGFBP-3, the most abundant binding protein, reversibly combines with almost 90% of the IGF-1, and an acid-labile subunit (ALS) to form a stable ternary complex[61-63,65]. The production of IGFBP-3 (mostly in endothelial cells) is partly regulated by IGF-I, whereas ALS is synthesized by hepatocytes and strictly controlled by GH. IGFBP-3 provides the intravascular transport of IGF-I, and indirectly controls its bioactivity by modulating the interaction between IGF-I and its receptor. IGFBP-3 serves as a circulating reservoir of IGF-I. Proteolysis of the complex by serine protease increases the concentrations of free, active IGF-I in response to metabolic and other needs[61-63,65,66].

THE IGF-I SYSTEM AND DIABETES MELLITUS

Besides stimulation of cell proliferation and differentiation, the GH/IGF-I system plays an important role in glucose homeostasis. Like insulin, IGF-I increases peripheral glucose uptake, stimulates glycogen synthesis and decreases hepatic glucose production. GH exerts opposite effects by increasing hepatic gluconeogenesis and glycogenolysis, together with decreasing peripheral glucose utilization by inhibition of glycogen synthesis and glucose oxidation[61,62,67].

In many patients with type 1 and type 2 diabetes mellitus, as well as insulin resistance without diabetes mellitus, the GH/IGF-I system is disturbed. In type 1 diabetes, insulin deficiency in the vena porta results in down-regulation of hepatic GH receptors and in post-GH receptor defects, which causes an impaired hepatic IGF-I production, and thus low levels of IGF-I and consequently GH hypersecretion[65,68,69]. The serum concentration of IGF-I is also reduced in insulin-resistant states and type 2 diabetes mellitus, probably because of insulin and GH resistance at the hepatic receptors[70-71]. The reduced levels of IGF-I and the GH hypersecretion in diabetes mellitus correlate with increased insulin resistance, worse metabolic control, and micro- and macrovascular complications[66,72-74]. Furthermore, the proteolysis of IGFBP-3 is increased in insulin resistance and type 2 diabetes, resulting in more free and bioactive IGF-I. This mechanism might compensate for the reduced IGF-I bioavailability at tissue levels[71,75].

THE IGF-I SYSTEM AND DIABETIC NEPHROPATHY

The role of the GH/IGF-I system in the pathogenesis of diabetic nephropathy is not exactly known. Recent studies showed an association between low serum levels of free and total IGF-I with kidney volume and microalbuminuria in children and adolescents with type 1 diabetes, independent of metabolic control[76-78]. Accordingly, increased renal concentrations of IGF-I are thought to protect diabetic kidney cells from ischemic injury and to accelerate tissue repair and recovery of renal function[62,72]. In diabetic mice models, high GH levels correlate with diabetes-induced renal changes, and selective GH blockade reduces renal hypertrophy, glomerular enlargement and urinary albumin excretion[79]. Although data in humans are limited, some studies also revealed an association between higher serum GH levels and early changes of diabetic nephropathy, such as nephromegaly and microalbuminuria[77,80].

Proteolysis of the IGFBP-3/IGF-I complex increases the bioavailability of IGF-I at the tissue level[75]. Previously, the glomeruli and interstitial cells of the diabetic kidney in rats showed an increased concentration of IGFBP-3[81]. Also in humans with diabetes mellitus, increased proteolysis of the IGFBP-3/IGF-I complex and higher levels of urinary IGFBP-3 seem to be associated with early stages of diabetic nephropathy like microalbuminuria[82,83].

I.3

Aims of the thesis

Since the majority of clinical studies on the effects of angiotensin-receptor antagonists on renal function and urinary albumin excretion have been performed in hypertensive patients with type 2 diabetes mellitus[52-60], we investigated the effects of the angiotensin-receptor antagonist losartan on urinary albumin excretion and renal function in type 2 diabetic patients with microalbuminuria, without hypertension. To further reveal the working mechanisms of angiotensin-receptor antagonists in normotensive patients, we studied the GH/IGF-I system in those patients, as well as the effects of losartan on it.

Recent studies suggest a role for RAS inhibitors in reducing the risk of developing type 2 diabetes mellitus[84,85]. Therefore, and because of the importance of the IGF-I system in glucose homeostasis[61,62,67], we studied the effects of losartan on insulin resistance, together with the effects on IGF-I metabolism in patients with impaired fasting glucose.

Finally, we performed a follow-up study of our patient population to evaluate cardiovascular outcome, the course of renal function, albuminuria and blood pressure in normotensive patients with type 2 diabetes mellitus and microalbuminuria. We focused on sex differences regarding the cardiovascular disease risk, and change in urinary albumin excretion as a predictor of cardiovascular complications and death.

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II

Effects of short-term losartan treatment on albuminuria, the IGF-I system and insulin resistance

II.1

Effect of losartan on microalbuminuria in normotensive patients with type 2 diabetes mellitus. A randomised clinical trial

Zandbergen AAM, Baggen MGA, Lamberts SWJ, de Zeeuw D, Bootsma AH, Ouwendijk RJTh

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ABSTRACT

Background: Angiotensin-converting enzyme inhibitors have shown antiproteinuric effects in normotensive and hypertensive diabetic patients. Angiotensin-receptor antagonists reduce urinary albumin excretion and the risk of renal and cardiovascular complications in hypertensive patients with type 2 diabetes mellitus. The effect of angiotensin-receptor antagonists in normotensive diabetic patients with microalbuminuria has not yet been reported.
Objective: To assess the antiproteinuric effects of losartan in normotensive patients with type 2 diabetes and microalbuminuria.
Design: Multicenter, randomised, double-blind, placebo-controlled clinical trial.
Setting: 19 outpatient clinics in the Netherlands.
Patients: 149 normotensive patients with type 2 diabetes mellitus and microalbuminuria.
Intervention: 74 patients were randomly assigned to receive losartan and 73 were assigned patients to receive placebo for 10 weeks; 71 patients in each group completed the study. The losartan dose was 50 mg during the first 5 weeks and 100 mg during the subsequent 5 weeks.
Measurements: Change in urinary albumin excretion rate after 5 and 10 weeks, change in creatinine clearance and blood pressure, as well as safety and tolerability of losartan.
Results: A significant 25% relative reduction in the albumin excretion rate was observed after 5 weeks of losartan 50 mg, with further improvement over the subsequent 5 weeks with losartan 100 mg (relative reduction 34%). In the losartan group, creatinine clearance did not improve and blood pressure decreased slightly. Side effects did not differ between treatment groups.
Conclusions: The angiotensin-receptor antagonist losartan reduces urinary albumin excretion in normotensive patients with type 2 diabetes and microalbuminuria. In multivariate analysis, the antiproteinuric effect of losartan was independent of the associated reduction in blood pressure. Losartan was safe and well tolerated in these normotensive patients.

INTRODUCTION

Diabetes mellitus is an important cause of nephropathy, end-stage renal disease and cardiovascular events. Nephropathy occurs in about 40% of patients with type 2 diabetes, and 25 to 40% of the diabetic patients in Europe and the United States develop end-stage renal disease. Increased urinary albumin excretion, the first marker of kidney damage, carries an increased risk for renal as well as cardiovascular diseases[1-5]. Because reduction of microalbuminuria is associated with marked renal protection, the delay or retardation of the disease process is important in the management of diabetes mellitus[1].

Angiotensin-converting enzyme (ACE) inhibitors reduce urinary albumin excretion in both normotensive and hypertensive patients with type 1 or type 2 diabetes mellitus. The antiproteinuric effects appear to be at least partly independent of the blood pressure reduction caused by these agents[6-11], although some studies have not confirmed this finding[12]. Angiotensin II is thought to play a prominent role in the pathogenesis of diabetic nephropathy. Therefore, not only preventing the formation of angiotensin II by ACE inhibition, but also blockade of the angiotensin receptor might be renoprotective.

The trials of angiotensin-receptor antagonists in hypertensive patients with type 1 or 2 diabetes mellitus and microalbuminuria showed a reduction in albumin excretion, regardless of pretreatment levels[13-20]. Recently, the angiotensin-receptor antagonist irbesartan retarded progression from microalbuminuria to overt proteinuria in hypertensive patients with type 2 diabetes[21]. In RENAAL (The Reduction of Endpoints in NIDDM [non-insulin-dependent diabetes mellitus] with the Angiotensin II Antagonist Losartan study) and IDNT (Irbesartan Diabetic Nephropathy Trial), losartan and irbesartan, respectively, reduced proteinuria and slowed the progression of diabetic nephropathy in hypertensive patients with type 2 diabetes[22-24].

Therefore, therapy that interferes in the renin-angiotensin-aldosterone system should probably be initiated in a stage as early as microalbuminuria, in order to reduce albumin excretion and the associated risk of overt nephropathy. All previous studies on this subject investigated hypertensive patients. Therefore, we conducted a multicenter randomised, double-blind, placebo-controlled clinical trial with forced dose titration to investigate the effects of losartan on the urinary albumin excretion rate in normotensive patients with type 2 diabetes mellitus and microalbuminuria. Secondary objectives were to determine the time course of the antiproteinuric effect, the optimal dose of losartan, effects on creatinine clearance and blood pressure, and the safety and tolerability of losartan in normotensive patients.

METHODS

Study design

In 19 outpatient clinics in the Netherlands, we conducted a 20-week randomised, placebo-controlled trial, consisting of a 5-week screening and washout period, a 10-week double-blind treatment period, and a 5-week placebo washout period. The study was performed according to the guidelines of Good Clinical Practice, and approved by the institutional review board at each centre. All patients gave written informed consent.

Patients

From March 1999 through August 2001, all outpatients with type 2 diabetes mellitus, microalbuminuria and a sitting blood pressure of 150/90 mmHg or less were invited to participate. Type 2 diabetes mellitus was defined as diabetes diagnosed at age older than 30 years or controlled by diet or blood glucose lowering-agents for at least 6 months. Microalbuminuria was defined as a urinary albumin excretion rate of 20 to 200 µg/min.

The current definition of normotension is a blood pressure less than 140/90 mmHg, with a blood pressure-lowering target of less than 130/80 mmHg in hypertensive adults with diabetes mellitus[25,26]. However, the protocol for this study was developed in 1998, when normotension was defined as a sitting blood pressure of 160/90 mmHg or less. Because of this and the fact that the mean baseline blood pressures appeared to be 135.9/78.8 mmHg for the losartan group and 138.3/80.3 mmHg for the placebo group, our study population can be considered normotensive.

We excluded patients with previous myocardial infarction, cerebrovascular event, unstable angina pectoris or symptomatic heart failure. Patients with electrocardiographic abnormalities (atrioventricular conduction disturbances, sick sinus syndrome, atrial fibrillation or other clinically significant rhythm disturbances), acute renal failure, chronic glomerulonephritis, polycystic kidney disease or a serum creatinine level over 150 µmol/l were not eligible. Also, patients with an HbA_{1c} level of more than 10% or other relevant laboratory abnormalities were excluded. Concomitant use of antihypertensive agents, ophthalmic preparations containing β-blocking agents, steroids or lithium was not allowed.

Eligible patients were included in the screening and washout period (weeks –5 to 0). In patients not taking blood pressure-lowering or renoprotective medication, microalbuminuria was assessed by two sequential 24-hour urine collections before the randomisation visit (week 0). Patients could be randomised if the average of these two measurements confirmed microalbuminuria as defined above. Eligible patients who were receiving ACE inhibitors, angiotensin-receptor antagonists or any other antihypertensive agents first entered a 5-week washout period. In these patients, microalbuminuria was assessed after this period. If such medication had been initiated to normalise known hypertension, the patient was excluded.

Interventions

Patients who met all study criteria were randomly assigned in a double-blind fashion to receive losartan 50 mg once daily or matching placebo (week 0). After 5 weeks of treatment, patients initially assigned to receive 50 mg of losartan had a fixed dose titration to 100 mg of losartan; patients initially assigned to receive placebo continued receiving placebo. Treatment was continued at this dose for another 5 weeks, after which all patients were switched to single-blind placebo for the last 5 weeks. At the end of this washout period, patients returned for their final visit (week 15).

The pharmaceutical company provided each centre with the study medication and randomly assigned the patients in blocks of four. The investigators dispensed the study medication and provided the patient with the first available allocation number. In each centre, the allocation numbers were assigned in consecutive order. The patients had an equal probability of assignment to either group. The randomisation list was inaccessible to all investigators. No randomisation code was broken, so all investigators and participants remained blinded to treatment assignment during the entire study.

Throughout the study, patients received the standard of care for diabetes. They were instructed not to change their physical exercise patterns significantly, because doing so may influence proteinuria.

Outcomes

The primary endpoint was the change in urinary albumin excretion rate from baseline to week 10. Secondary endpoints were change in albumin excretion rate at week 5, change in creatinine clearance from baseline to week 10, and change in systolic and diastolic blood pressures at weeks 5 and 10. Tertiary endpoints were safety and tolerability of losartan.

Measurements

At each visit, the same investigator measured blood pressure and pulse. Blood pressure was measured by a sphygmomanometer, with the patient seated for 5 minutes before the first measurement. Three replicate measurements, obtained 1 minute apart, were averaged.

In each centre, microalbuminuria was measured just before every visit in two sequential 24-hour urine collections by using an immunonephelometric assay on an automated analyser (Beckman Coulter, Brea, California). The measurements of the two samples were averaged. Complete laboratory evaluations (biochemistry, haematology) were conducted at weeks 0 and 10; at weeks 5 and 15, only renal function and electrolytes were checked. A virtual central laboratory recalculated all laboratory results, including microalbuminuria, and corrected them for possible bias.

At weeks 0, 5, 10 and 15, adverse events were recorded, and medication use and compliance were checked by counting pills. Patients were considered compliant if at least 75% of the study drug had been taken.

Statistical analysis

Primary and secondary endpoints were analysed in the full-analysis study population, which consisted of all the patients randomised (intention-to-treat analysis). The statistical method used was the generalised linear mixed model for longitudinal data, which compares the differences in trends over time between both treatment groups[27]. Baseline values were used as covariates in this model, and outcomes were adjusted by using treatment, visit, centre, and the corresponding interaction terms as fixed effects.

A generalised linear mixed model was also applied to evaluate whether the observed changes in albumin excretion rate were consistent across different baseline blood pressure levels. Furthermore, an additional analysis using analysis of variance was performed to evaluate whether changes in blood pressure affected the observed changes in albumin excretion rate.

Safety data were analysed for all patients who took at least one dose of study medication and for whom at least one safety assessment was available. No statistical analysis on these data was conducted.

The study had 92% power with an α level of 0.05 to detect a 30% difference in the change in albumin excretion rate between both treatment groups. We used SAS software, version 6.12 (SAS Institute, Inc., Cary, North Carolina), for all statistical analyses.

RESULTS

Patients

Of the 236 patients enrolled in the screening period, 49 used an ACE inhibitor or angiotensin-receptor antagonist before enrolment and underwent initial washout. Of the 236 patients, 147 were randomly assigned. The main reason for exclusion before randomisation was a screening-phase albumin excretion rate less than 20 µg/min, despite levels between 20 and 200 µg/min before enrolment. Of the 147 patients (full-analysis population), 74 were allocated to losartan and 73 were allocated to placebo.

In the losartan group, 3 patients discontinued treatment before the end of the study: 1 just after week 0 because the baseline urinary albumin excretion rate was retrospectively discovered to be greater than 200 µg/min, 1 after week 5 because of a cerebrovascular event within the preceding year, and 1 just after week 5 because of dizziness and headache after ingestion of 100 mg of losartan. In the placebo group, 1 patient discontinued treatment at week 5 because of a protocol violation (medication of wrong allocation number was given), and 1 at week 10 because the baseline urinary albumin excretion rate was retrospectively discovered to exceed 200 µg/min.

The remaining 142 patients completed the entire study, 71 in each treatment group. Baseline characteristics did not significantly differ between groups (*table 1*).

Table 1. Baseline characteristics of the full-analysis study population

Characteristic	Losartan group (n=74)				Placebo group (n=73)			
Age, yr	56.9	±11.0			58.5	±12.3		
Male gender, n (%)	50	(68%)			51	(70%)		
Race or ethnic group, n (%)								
Caucasian	59	(80%)			63	(86%)		
Black	5	(7%)			3	(4%)		
Asian	2	(3%)			0	(0%)		
Other	8	(10%)			7	(10%)		
Body mass index, kg/m^2	32.0	±16.2			28.7	±4.5		
Blood pressure, mmHg								
Systolic	135.9	±10.4			138.3	±9.7		
Diastolic	78.8	±9.4			80.3	±7.5		
Pulse, bpm	79.2	±11.5			76.4	±11.3		
Biochemistry								
Serum creatinine, µmol/l (mg/dl)	87.6	±20.8	(1.0	±0.2)	87.7	±17.5	(1.0	±0.2)
Total cholesterol, mmol/l (mg/dl)	5.3	±1.0	(204.6	±38.6)	5.4	±0.9	(208.4	±34.7)
HDL cholesterol, mmol/l (mg/dl)	1.2	±0.3	(46.3	±11.6)	1.2	±0.5	(46.3	±19.3)
LDL cholesterol, mmol/l (mg/dl)	3.1	±0.9	(119.7	±34.7)	3.2	±0.8	(123.5	±30.9)
Triglycerides, mmol/l (mg/dl)	2.4	±1.7	(212.6	±150.6)	2.3	±2.0	(203.7	±177.1)
HbA_{1c}, %	8.0	±1.2			7.5	±1.1		
Urinalysis								
Creatinine, mmol/l	7.4	±2.7			7.5	±2.9		
Albumin excretion rate, µg/min	78.6	±51.3			89.4	±57.0		
Creatinine clearance, ml/s								
As measured	1.77	±0.49			1.68	±0.58		
Calculated with Cockcroft-Gault formula	1.71	±0.60			1.64	±0.57		

Data presented in SI units as means ±SD, unless otherwise noted
Traditional units in parentheses, when applicable

Albumin excretion rate

After 5 weeks of treatment with losartan 50 mg, the mean albumin excretion rate was reduced from 78.6 µg/min at baseline to 59.3 µg/min (relative reduction 24.6%) (*table 2*). In

the placebo group, the albumin excretion rate decreased from 89.4 µg/min to 83.3 µg/min (relative reduction 6.8%). The adjusted mean difference in albumin excretion rate between the losartan and placebo groups was -16.9 µg/min (95% CI –26.7 to –7.0 µg/min; p<0.001).

At week 5, patients in the losartan group had a fixed dose titration to 100 mg of losartan, and patients in the placebo group continued to receive placebo. After 5 weeks of treatment with this dose, the mean albumin excretion rate in the losartan group decreased further, from 78.6 µg/min at baseline to 51.9 µg/min at week 10 (relative reduction 34%). In the placebo group, the mean albumin excretion rate at week 10 was 97.3 µg/min (increase 10.3%). The difference in the adjusted mean albumin excretion rate between the losartan and placebo groups (-39.1 µg/min [95% CI –48.9 to –29.2 µg/min]) was statistically significant (p<0.001). After 10 weeks of treatment with losartan, 8 patients returned to normoalbuminuria (albumin excretion rate <20 µg/min). In the placebo group, the minimum urinary albumin excretion rate after 10 weeks was 21 µg/min.

The adjusted mean albumin excretion rate decreased by 8.3 µg/min (95% CI 0.04 to 16.5 µg/min; p=0.049) when the losartan dose was increased from 50 mg to 100 mg. The antiproteinuric effect of losartan was reversible after discontinuation of treatment (*figure 1, table 2*).

Figure 1. Time course of the effect of losartan and placebo on mean urinary albumin excretion rate (AER)

Table 2. Effects of losartan and placebo on urinary albumin excretion rate, creatinine clearance and blood pressure

Endpoint		Week 0 (baseline)	Week 5 (losartan 50 / placebo)	Week 10 (losartan 100 / placebo)	Week 15 (washout period)
AER, µg/min	losartan	78.6 ±51.3	59.3 ±37.0	51.9 ±36.8	78.6 ±64.8
	placebo	89.4 ±57.0	84.9 ±58.2	98.7 ±67.1	102.5 ±72.8
Creatinine clearance, ml/s	losartan	1.77 ±0.49	1.70 ±0.64	1.60 ±0.49	1.72 ±0.85
(measured)	placebo	1.68 ±0.58	1.67 ±0.64	1.67 ±0.60	1.64 ±0.64
Creatinine clearance, ml/s	losartan	1.71 ±0.56		1.65 ±0.59	
(calculated with Cockcroft-Gault formula)	placebo	1.64 ±0.57		1.69 ±0.63	
Systolic blood pressure, mmHg	losartan	135.9 ±10.4	132.4 ±13.5	131.3 ±15.6	137.6 ±10.4
	placebo	138.3 ±9.7	136.1 ±12.7	138.4 ±12.9	141.2 ±11.3
Diastolic blood pressure, mmHg	losartan	78.8 ±9.4	76.8 ±9.4	75.8 ±8.6	78.0 ±9.1
	placebo	80.3 ±7.5	79.7 ±7.9	79.8 ±7.0	80.7 ±7.8

Data depicted as means ±SD. AER denotes urinary albumin excretion rate

Secondary endpoints

Losartan was associated with a 9.7% relative reduction in measured creatinine clearance after 10 weeks of treatment, whereas this measure remained unchanged in the placebo group (adjusted difference between losartan and placebo groups -0.16 ml/s [95% CI –0.29 to –0.03 ml/s]; p=0.014). When calculated with the Cockcroft-Gault formula, creatinine clearance decreased 3.3% in the losartan group and increased 2.7% in the placebo group; this difference was not statistically significant.

There was no statistically significant difference in blood pressure at baseline between the losartan and placebo groups. After 5 weeks of treatment with losartan 50 mg, mean blood pressure was reduced from 135.9/78.8 mmHg to 132.4/76.8 mmHg; in the placebo group, blood pressure decreased from 138.3/80.3 mmHg to 136.1/79.7 mmHg. The difference in mean blood pressure reduction between losartan and placebo was not statistically significant. After 5 weeks of losartan 100 mg, mean blood pressure decreased to 131.3/75.8 mmHg. After 10 weeks of placebo use, mean blood pressure was 138.4/79.8 mmHg (adjusted difference in mean systolic blood pressure between the losartan and placebo groups –3.8 mmHg [95% CI –6.5 to –1.1 mmHg; p=0.006]; difference in diastolic blood pressure –2.7 mmHg [95% CI –4.5 to –0.8 mmHg; p=0.005]) (*table 2*). Blood pressure returned to pretreatment levels after the 5-week placebo washout period.

Influence of blood pressure on albumin excretion rate

At baseline, 62 patients had a blood pressure between >140/90 and ≤150/90 mmHg, 53 between >130/80 and ≤140/90 mmHg, and 32 of ≤130/80 mmHg. The change in mean albumin excretion rate from baseline to week 10 was independent of blood pressure at baseline in both the losartan and placebo groups ($p>0.2$ for the interaction between baseline blood pressure and effect of treatment on albumin excretion rate in the losartan group and for the interaction in the placebo group).

Furthermore, in multivariate analysis, the reduction in albumin excretion rate was independent of the reduction in blood pressure associated with losartan ($p>0.2$ for the interaction between changes in blood pressure and changes in albumin excretion rate and $p<0.001$ for the interaction between treatment group and changes in albumin excretion rate).

Compliance, tolerability and safety

Compliance was high in both treatment arms and at both doses, ranging from 94.4% in the placebo group to 100% in the losartan 50 mg group.

During the first five weeks of treatment, 10 and 12 adverse events, possibly related to the study drug, occurred in the placebo group and in the losartan group, respectively. During the subsequent five weeks, the corresponding figures were 3 and 2. All adverse events were mildly or moderately severe. No deaths were reported.

Laboratory evaluations showed no significant changes in either treatment group. Neither losartan nor placebo affected serum potassium, sodium, uric acid or HbA_{1c} levels.

DISCUSSION

To our knowledge, this study is the first randomised, placebo-controlled trial showing that the angiotensin-receptor antagonist losartan reduces urinary albumin excretion in normotensive patients with type 2 diabetes mellitus and microalbuminuria. Since 100 mg of losartan reduced the albumin excretion rate significantly more than losartan 50 mg, the present data also show the incremental benefit of a higher dose. The antiproteinuric effect was reversible upon discontinuation of losartan therapy. Losartan was well tolerated at both dose levels.

Treatment with losartan 50 mg reduced blood pressure slightly, but the effect was not significantly different from that in the placebo group. Losartan 100 mg resulted in a somewhat higher, but still modest blood pressure reduction. However, the reduction in albumin excretion rate was independent of the decrease in blood pressure associated with losartan. These findings confirm an independent renoprotective effect, as postulated in previous studies[6-8,21-23]. Furthermore, the antiproteinuric effect of losartan was consistent across different baseline blood pressure levels, including patients who are normotensive according to the current definition.

The IRMA (Irbesartan Type II Diabetes with Microalbuminuria) trial studied the effect of the angiotensin-receptor antagonist irbesartan on the progression of microalbuminuria to overt proteinuria in hypertensive patients with type 2 diabetes[21]. In this study, 150 mg and 300 mg of irbesartan were compared with placebo for the time to onset of overt nephropathy, changes in albuminuria, and creatinine clearance. The albumin excretion rate decreased by 24% with irbesartan 150 mg and by 38% with 300 mg. The steepest decrease in albumin excretion rate occurred within the first 3 months of treatment. In our study, 50 mg of losartan resulted in a 25% relative reduction in the albumin excretion rate, compared with a 34% reduction with 100 mg. Thus, the antiproteinuric effects of losartan in our study appear to be qualitatively and quantitatively comparable to those of losartan and irbesartan in previous studies in hypertensive patients with diabetes mellitus[14,16,18,19].

As stated, the IRMA trial showed a significant 14% additional reduction in microalbuminuria with a double dose of irbesartan[21], an observation similar to ours. As shown in previous studies that investigated the time course of the antiproteinuric response of angiotensin-receptor antagonists and ACE inhibitors[28-30], the incremental reduction in albumin excretion with losartan 100 mg appears attributable to a dose response rather than a time response. In patients with type 1 diabetes and microalbuminuria, the antiproteinuric response to losartan was maximal within one week of treatment, and these changes paralleled those of blood pressure and renal haemodynamics[28]. In non-diabetic patients with proteinuria and patients with type 1 diabetes mellitus and macroproteinuria, the antiproteinuric effects of losartan and enalapril were maximal within 4 weeks of treatment. Increasing the dose further reduced albuminuria, and again, maximal effect was seen within 4 weeks[29,30].

In the losartan-treated patients, the measured creatinine clearance decreased compared with baseline levels as well as compared with placebo recipients. The difference between the measured and calculated creatinine clearance can be explained by inaccuracy of urine collection; the Cockcroft-Gault formula corrects for this discrepancy. As known from other studies of ACE inhibitors and losartan in early diabetic nephropathy, creatinine clearance stabilises after prolonged treatment[6,10,11,14,17,21-23,31]. The initial decrease is thought to result from renal haemodynamic factors and is reversible after withdrawal of medication[10,17]. The extent of the reduction in creatinine clearance in our study is comparable to that observed in other studies.

Losartan at both dose levels was well tolerated with a safety profile similar to that of placebo. These findings parallel observations made by others studying the nephroprotective effect of losartan in hypertensive diabetic patients[13,14,23]. Importantly, our study shows that normotensive patients can be treated safely with the antihypertensive drug losartan.

A possible side of action of angiotensin-receptor antagonists in normotensive diabetic patients is the vascular endothelium. Endothelial dysfunction has been associated with increased urinary albumin excretion, as well as with an increased risk of cardiovascular events in type 2 diabetes[32,33]. Inhibition of urinary albumin excretion may reflect recovery of endothelial function, and may predict a reduction in the risk of complications.

One of the main limitations of this study is the brief follow-up period. However, the study was established to investigate whether losartan reduced microalbuminuria in normotensive patients with type 2 diabetes, since to our knowledge it is the first trial reported in this patient population. Furthermore, this study evaluated the effect of losartan on surrogate outcomes (albumin excretion rate), and therefore cannot be extrapolated to hard clinical endpoints; however, microalbuminuria is considered to be predictive of renal and cardiovascular morbidity[2-5,32].

A comparison with an ACE inhibitor or other antihypertensive drug would be of interest. However, when we developed the protocol, antihypertensive treatment of patients with type 2 diabetes mellitus and a blood pressure of 150/90 mmHg or less was not the standard of care, nor was ACE inhibitor treatment of normotensive patients with type 2 diabetes and microalbuminuria. Accordingly, there was no reason to include a comparison group beyond placebo. Furthermore, investigating whether angiotensin-receptor antagonists have an advanced antiproteinuric effect over ACE inhibitors in this population would require an extremely large study because of the expected similar effects of both agents.

In conclusion, the present data demonstrate that the angiotensin-receptor antagonist losartan reduces urinary albumin excretion in patients with type 2 diabetes mellitus and microalbuminuria without hypertension. The antiproteinuric effects of ACE inhibitors and angiotensin-receptor antagonists in hypertensive diabetic patients decrease the risk of overt nephropathy, end-stage renal disease and cardiovascular events[9,11,14,21-24]. Therefore, a long-term, large-scale clinical trial, preferably comparing losartan with an ACE inhibitor and other antihypertensive agents, is needed to investigate the effects of losartan on hard clinical endpoints in normotensive patients with type 2 diabetes, with treatment started at a stage as early as microalbuminuria.

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APPENDIX

The following centres (investigators) in the Netherlands participated in the study:

Sint Franciscus Hospital (J.W.F. Elte), Vlietland Hospital, Vlaardingen (S.G.T. Hulst), Rotterdam Eye Hospital (Th.L.J.M. van der Loos and F.J.M. Klessens-Godfroy), Erasmus University Medical Centre (S.W.J. Lamberts, A.H. Bootsma), University Hospital Maastricht (T.W.A. de Bruin), Albert Schweitzer Hospital, Zwijndrecht (R.J.M van Leendert), Diabetes Centre Bilthoven (G.E.M.G. Storms), Isala Clinics, location Sophia (J. Lambert), Atrium Medical Centre Brunssum (W.J.J.R. Venekamp), Medical Centre Alkmaar (W. Bronsveld), Gelre Hospitals, location Lukas (R.P. Verhoeven), University Medical Centre St Radboud, (L.D. Elving), Hospital Walcheren (W.L. Blok), Albert Schweitzer Hospital, location Dordwijk (B.M. van Ouwerkerk), Ruwaard van Putten Hospital (M.H. Silbermann), Máxima Medical Centre, location Veldhoven (R.J. Erdtsieck), Hofpoort Hospital (J.W. van der Beek-Boter), Rode Kruis Hospital (G. Schrijver), Ikazia Hospital (A.A.M. Zandbergen, M.G.A. Baggen, R.J.Th. Ouwendijk).

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II.2

The insulin-like growth factor I (IGF-I) system and the renal and haemodynamic effects of losartan in normotensive patients with type 2 diabetes mellitus. A randomised clinical trial

Zandbergen AAM, Lamberts SWJ, Baggen MGA, Janssen JAMJL, Boersma E, Bootsma AH

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ABSTRACT

Objective: Losartan has shown to protect the diabetic kidney, at least partly independent of changes in blood pressure. Imbalances in the IGF-I system are associated with the development of diabetic nephropathy. We investigated whether renal as well as haemodynamic effects of losartan are associated with changes in the IGF-I system in normotensive patients with type 2 diabetes mellitus.

Design and patients: This randomised, double-blind placebo-controlled clinical trial involved 74 normotensive patients with type 2 diabetes mellitus and microalbuminuria. 38 patients were assigned to receive losartan and 36 patients were assigned to receive placebo for 10 weeks.

Measurements: Serum levels of total and free IGF-I, IGFBP-3, creatinine and HbA_{1c}, as well as urinary albumin excretion rate, creatinine clearance and blood pressure were measured prior to the start of treatment and after 10 weeks of treatment.

Results: At baseline, serum levels of IGFBP-3 were elevated and serum levels of free IGF-I were reduced. Losartan tended to reduce IGFBP-3 levels and to raise free IGF-I levels, although not statistically significant. These effects were more pronounced in a subanalysis of 18 losartan-treated patients with stable metabolic parameters (from 133.2 nmol/l to 122.6 nmol/l; p=0.006 for IGFBP-3, and a rise in free IGF-I levels by 8% (ns)). Serum levels of total IGF-I were unaffected. The change in IGFBP-3 levels was inversely correlated to the change in creatinine clearance (r=-0.4; p=0.02). Total and free IGF-I levels inversely correlated to systolic blood pressure (r=-0.46; p=0.007 and r=0.26; p=0.14 respectively). Furthermore, changes in total IGF-I and IGFBP-3 levels correlated to changes in serum creatinine levels in the metabolically stable patients (r=0.58, p=0.02 and r=0.6, p=0.01, respectively). Changes in IGF-I system were unrelated to reduction in microalbuminuria associated with losartan.

Conclusions: Losartan lowered the elevated levels of IGFBP-3, although only significantly in the metabolically stable patients. A tendency towards a rise in free IGF-I levels was observed as well, but this change was small and not statistically significant. These changes were not related to reduction in microalbuminuria, but might contribute to effects of losartan on creatinine clearance and blood pressure of losartan in normotensive patients with type 2 diabetes mellitus.

INTRODUCTION

Recently, we have published a clinical trial investigating the effects of the angiotensin-receptor antagonist losartan on urinary albumin excretion rate in normotensive patients with type 2 diabetes mellitus[1]. A significant reduction in urinary albumin excretion rate by 35% was observed after 10 weeks of losartan treatment. This reduction could not be explained by changes in blood pressure or creatinine clearance, suggesting other losartan-mediated mechanisms being involved. One potential other mechanism might be direct effects of losartan on the GH/IGF-I system, which has also been implicated in the development of diabetic nephropathy[2-7].

The GH/IGF-I system is often unbalanced in type 2 diabetic patients, with reduced serum IGF-I levels that result in GH hypersecretion[8-11]. The exact role of these changes in the pathogenesis of diabetic nephropathy is still not fully understood. Serum concentrations of GH correlate with early changes of diabetic nephropathy[12]. Increased renal concentrations of IGF-I are thought to protect diabetic kidney cells from ischemic injury and to accelerate tissue repair and recovery of renal function[11,13]. Some studies showed an association between low serum IGF-I levels and microalbuminuria in diabetic adolescents[14,15], although others could not confirm this finding[12,16]. Furthermore, Park et al showed that the concentration of IGFBP-3 is elevated in the glomeruli and interstitial cells of the diabetic kidney[17]. Increased proteolysis of the IGFBP-3/IGF-I complex and higher levels of urinary IGFBP-3 seem to be associated with early stages of diabetic nephropathy like microalbuminuria, both in type 1 and type 2 diabetic patients[15,18].

The IGF-I system interacts with angiotensin II in several ways. Little is known about the interaction between IGFBP-3 and angiotensin II. IGF-I seems to have growth promoting effects synergistic to angiotensin II in rat aortic smooth muscle cells[19,20], but in myocytes of diabetic mice, the production and function of angiotensin II is reduced by IGF-I[21]. Circulating plasma levels of IGF-I in rats are reduced by angiotensin II infusion through reduction of the hepatic IGF-I mRNA levels, which is reversed by losartan[20]. To our knowledge, no clinical trials have been published investigating the effect of inhibitors of the renin-angiotensin system (RAS) on the IGF-I system in individuals with diabetes mellitus.

In the present study, we studied the IGF-I system in normotensive patients with type 2 diabetes mellitus and the effects of losartan on this system. We also investigated whether changes in the IGF-I system were associated with the reduction in microalbuminuria, or other changes in renal function and haemodynamic parameters observed during losartan treatment.

METHODS

Study design

This study involved 74 patients from a randomised, double-blind, placebo-controlled multicenter clinical trial, that investigated the effects of losartan on urinary albumin excretion rate, creatinine clearance and blood pressure in normotensive patients with type 2 diabetes mellitus and microalbuminuria. The study design, methods and results have been published previously[1]. Patients living in the city of Rotterdam and surrounding areas also participated in the substudy described here. The study was reviewed and approved by the institutional review board of each centre. Informed consent was obtained from each patient prior to participation in the trial.

Patients and treatment

The eligibility criteria have been described in detail[1]. Briefly, patients with type 2 diabetes mellitus, microalbuminuria and a sitting blood pressure of 150/90 mmHg or less were randomised. Type 2 diabetes mellitus was defined as diabetes diagnosed at age older than 30 years or controlled by a diet or blood glucose-lowering agents for at least 6 months. Microalbuminuria was defined as a urinary albumin excretion rate of 20 to 200 µg/min.

The current definition of normotension is a blood pressure less than 140/90 mmHg, with a blood pressure-lowering target of <130/80 mmHg in hypertensive adults with diabetes mellitus[22,23]; however, the protocol for this study was developed in 1999, when normotension was defined as a sitting blood pressure 160/90 mmHg or less. Because of this and the fact that the mean baseline blood pressures appeared to be 134.9/79.5 mmHg for the losartan group and 136.3/80.1 mmHg for the placebo group, our study sample can be regarded as normotensive.

The main exclusion criteria included previous myocardial infarction or cerebrovascular events, unstable angina pectoris and symptomatic heart failure. Also, patients with ECG abnormalities, acute renal failure, chronic glomerulonephritis, polycystic kidney disease, a serum creatinine level >150 µmol/l, an HbA_{1c} level >10% or concomitant use of antihypertensive agents, steroids or lithium were not eligible.

Eligible patients were randomly assigned in a double-blind fashion to losartan treatment 50 mg once daily or matching placebo (week 0). After 5 weeks of treatment, patients initially receiving 50 mg of losartan had a fixed dose-titration to 100 mg of losartan; patients initially assigned to receive placebo continued receiving placebo. Treatment was continued at this dose level for another 5 weeks, after which all patients were switched to single-blind placebo for the last 5 weeks. At the end of this wash-out period, patients returned for their final visit (week 15).

Measurements

Prior to start of treatment (week 0) and after 10 weeks of treatment with either losartan or placebo, blood samples were collected in the morning via venipuncture and stored at -70°C until analysis. Serum levels of total IGF-I, free IGF-I and IGFBP-3 were all measured in the same laboratory, using radioimmunoassay (Diagnostic System Laboratories Inc., Webster, Texas, USA)[24].

Microalbuminuria was measured just before (week 0) and after 10 weeks of treatment in two sequential 24-hour urine collections with an immunonephelometric assay on an automated analyzer (Beckman Coulter, Brea, California). The presented value for microalbuminuria is the average of two 24-hour urine samples. Serum levels of creatinine, HbA_{1c} and urinalysis including creatinine level were also measured at weeks 0 and 10. Creatinine clearance was measured in the two sequential 24-hour urine collections at weeks 0 and 10, and again, the results of the two samples were averaged.

At every visit, blood pressure was measured by a sphygmomanometer, with the patient in sitting position for 5 minutes before the first measurement. Three replicate measurements, obtained 1 minute apart, were averaged.

Statistical analysis

Intention-to-treat analysis was performed. Results are presented as means and standard deviation, unless otherwise noted. The paired Student's *t*-test was used for comparisons in the same treatment group before and after intervention. The unpaired Student's *t*-test was used for analysing differences in changes between the two groups. Associations between the variables were quantified using the Pearson correlation coefficient; results are given including p-value. We used SAS software, version 6.12 (SAS Institute, Inc., Cary, North Carolina), for all statistical analyses.

RESULTS

Patients

The study population consisted of 74 patients, of whom 38 patients were allocated to losartan treatment and 36 patients were allocated to placebo. All patients completed the intended treatment period. Baseline characteristics of the study population did not differ significantly between groups (*table 1*).

Baseline variables and correlations

The baseline levels for both treatment groups separately are presented in table 1. Before treatment started (week 0), the mean level of total IGF-I of the whole study population (n=74) was 19.2±7.7 nmol/l (age-adjusted normal range: 11-35 nmol/l). The mean level of free IGF-I

Table 1. Baseline characteristics of the study population

Characteristic	Losartan group (n=38)		Placebo group (n=36)	
Age, yr	55.8	±10.0	55.6	±10.0
Male gender, n (%)	24	(63%)	28	(78%)
Insulin use, n (%)	26	(68%)	25	(69%)
Body mass index, kg/m^2	30.3	±4.4	28.0	±4.0
Blood pressure, mmHg				
Systolic	134.9	±8.8	136.1	±8.6
Diastolic	79.5	±7.7	80.1	±5.5
Biochemistry				
Total IGF-I, nmol/l	18.7	±7.0	20.4	±6.1
Free IGF-I, pmol/l	53.6	±18.3	58.8	±26.1
IGFBP-3, nmol/l	131.5	±25.4	133.3	±21.5
Serum creatinine, µmol/l	80.9	±17.0	79.7	±13.4
HbA_{1c}, %	8.3	±1.0	7.8	±0.9
Urinalysis				
Albumin excretion rate, µg/min	84.5	±57.4	95.8	±46.5
Measured creatinine clearance, ml/min	105.2	±26.2	106.5	±29.3

Data presented in SI units as means ±SD, unless otherwise noted

was reduced compared to normal healthy controls, which is 56.2±26.1 pmol/l (age-adjusted normal range: for men 92.8-631.3 pmol/l, and for women 91.5-754.1 pmol/l[25]). Mean levels of IGFBP-3 were elevated: 132±28 nmol/l (age-adjusted normal range: 97-108 nmol/l).

We have studied the well-known associations between baseline levels of total and free IGF-I and IGFBP-3, using the results of both treatment groups together. Total IGF-I correlated to free IGF-I (Pearson correlation coefficient r=0.52; p<0.0001), as well as to IGFBP-3 (r=0.54; p<0.0001). Free IGF-I also correlated to IGFBP-3 (r=0.25; p=0.03).

In the complete group of 74 individuals, free IGF-I was related to creatinine clearance (r=0.25; p<0.04). For total IGF-I and IGFBP-3, respectively, no correlation was found. There were no significant associations between total or free IGF-I or IGFBP-3 and albumin excretion rate or HbA_{1c} level at baseline.

Effects of losartan on the IGFBP-3/IGF-I system

In either treatment group, total IGF-I levels were not significantly different from baseline after the 10-week treatment period. The mean levels of free IGF-I tended to increase non-significantly by 6%, from 53.6 pmol/l at baseline to 56.8 pmol/l, whereas in the placebo group, the mean levels of free IGF-I tended to decrease non-significantly from 58.8 pmol/l to 55.5 pmol/l. In the losartan group, serum IGFBP-3 concentration had reduced from 131.5 nmol/l at baseline to 126.6 nmol/l after 10 weeks (ns). In the placebo group, no effects on IGFBP-3 levels were observed (*table 2*).

Effects of losartan on the IGFBP-3/IGF-I system related to the renal and haemodynamic effects associated with losartan

Ten weeks of losartan treatment reduced albumin excretion rate from 84.5 µmol/l to 48.8 µmol/l (relative reduction 42%; p=0.002). In multivariate analysis, the reduction in albumin excretion rate was not associated with the reduction in creatinine clearance or changes in

Table 2. Effects of losartan and placebo after 10 weeks of treatment on total and free IGF-I, IGFBP-3, serum creatinine, AER, HbA_{1c}, creatinine clearance and blood pressure

Endpoint		Week 0		Week 10		P value
Total IGF-I, nmol/l	losartan	18.7	±7.0	18.7	±9.1	0.9
	placebo	20.4	±6.1	20.4	±7.6	1
Free IGF-I, pmol/l	losartan	53.6	±18.3	56.8	±27.7	0.2
	placebo	58.8	±26.1	55.5	±30	0.4
IGFBP-3, nmol/l	losartan	131.5	±25.4	126.6	±35.3	0.3
	placebo	133.3	±21.5	132.3	±29.2	0.6
Serum creatinine, µmol/l	losartan	80.9	±17.0	82.1	±19.4	0.3
	placebo	79.7	±13.4	79.5	±17.5	0.9
AER, µg/min	losartan	84.5	±57.4	48.8	±27.6	0.002
	placebo	95.8	±46.5	106.8	±73.3	0.2
HbA_{1c}, %	losartan	8.3	±1.0	8.0	±0.9	0.3
	placebo	7.8	±0.9	7.8	±1.1	0.7
Creatinine clearance, ml/min (measured)	losartan	105.2	±26.2	98.7	±33.2	0.1
	placebo	106.5	±29.3	111.4	±38.6	0.4
Systolic blood pressure, mmHg	losartan	134.9	±8.8	131.3	±15.6	0.02
	placebo	136.1	±8.6	135.6	±11.1	0.5
Diastolic blood pressure, mmHg	losartan	79.5	±7.7	76.8	±8.7	0.04
	placebo	80.1	±5.5	79.8	±7.1	0.7

Data depicted as means (SD). AER denotes urinary albumin excretion rate

blood pressure[1]. Creatinine clearance decreased non-significantly by 6% from 105.2 ml/min to 98.7 ml/min (p=0.1) (*table 2*). The changes in levels of serum creatinine and HbA_{1c} after losartan treatment were small and not statistically significant.

The change in IGFBP-3 was inversely correlated to the change in creatinine clearance (r=-0.4; p=0.02) in the losartan treated patients. Changes in total or free IGF-I and IGFBP-3 were not correlated to changes in albumin excretion rate or HbA_{1c} level.

After 10 weeks of losartan treatment, mean blood pressure had reduced from 134.9/79.5 mm Hg to 131.3/76.8 mm Hg (p=0.02 for the reduction in systolic blood pressure, and p=0.04 for the reduction in diastolic blood pressure). In the placebo group blood pressure did not change (*table 2*). Total IGF-I levels inversely correlated to systolic blood pressure (r=-0.46, p=0.007). Free IGF-I levels inversely correlated non-significantly to systolic blood pressure (r=-0.26, p=0.14). No associations were found between diastolic blood pressure and total or free IGF-I levels.

When pooling the data of both treatment groups, the above-mentioned associations remained. Moreover, the correlation between free IGF-I levels and systolic blood pressure became significant (r=-0.24, p=0.04).

Metabolically stable subgroup

In type 2 diabetes mellitus, the production and bioactivity of IGF-I are influenced by changes in diabetes treatment and metabolic control, among other factors[8,26]. In an attempt to eliminate those factors, we studied a subgroup in the losartan treated group, consisting of 18 patients with stable metabolic parameters during the 10-week treatment period. A patient was defined stable when HbA_{1c} levels varied by less than 0.4%, while their diabetes medication had not to be adjusted during the 10 weeks of follow-up.

After 10 weeks of losartan treatment, IGFBP-3 had significantly reduced from 133.2 nmol/l to 122.6 nmol/l (p=0.006). Free IGF-I tended to increase non-significantly by 8%. Total IGF-I levels were within the normal range and had only increased non-significantly. Losartan had increased creatinine clearance, and had decreased serum levels of creatinine, but these changes were small and did not reach statistical significance.

The change in total serum IGF-I, as well as the change in IGFBP-3, correlated significantly to the change in serum level of creatinine (r=0.58, p=0.02 and r=0.6, p=0.01, respectively). In this metabolically stable subgroup, total IGF-I correlated with systolic blood pressure as in the whole losartan treated group (r=-0.5, p=0.03).

Compared to the metabolically stable patients, the reduction of IGFBP-3 and the rise in free IGF-I were smaller and failed to reach statistical significance in the losartan treated patients without stable metabolic parameters.

DISCUSSION

In this study, we investigated the effects of the angiotensin-receptor antagonist losartan on the IGF-I system in patients with type 2 diabetes mellitus, also relating these effects to the previously described effects of losartan on renal function and blood pressure in this population. After 10 weeks of losartan treatment, a trend towards reduction of the elevated serum levels of IGFBP-3, as well as towards increase of the reduced serum levels of free IGF-I was observed, but not to statistical significance. Losartan treatment did not affect serum levels of total IGF-I. These changes in the IGF-I system were not related to reduction in microalbuminuria, but correlated inversely to creatinine clearance.

The GH/IGF-I system plays an insulin-like role in regulating glucose metabolism and is often unbalanced in insulin-resistant states and type 2 diabetes mellitus[26]. Low levels of IGF-I with consequent GH hypersecretion reflect the disturbed IGF-I system, and are associated with increased insulin resistance and premature atherosclerosis and microangiopathy[2,3,13,27]. In our population, baseline serum total IGF-I levels were within normal ranges, but baseline levels of free IGF-I were highly reduced. It was previously hypothesised that the unbound fraction of IGF-I is the biologically active hormonal form, involved in regulation mechanisms and biological effects, and might therefore be of more clinical relevance than total IGF-I levels[10,11].

IGFBP-3 reversibly combines with almost 90% of the IGF-1 and indirectly controls bioactivity of IGF-1[10,11]. The production of IGFBP-3 is positively regulated by GH. In response to metabolic needs, proteolysis of the IGFBP-3/IGF-I complex increases the concentrations of free IGF-I, probably compensating for reduced IGF-I bioavailability at tissue levels[8-10,28]. Increased IGFBP-3 proteolysis has been described in patients with insulin resistance and diabetes mellitus, as well as in a variety of kidney diseases, including diabetic nephropathy[16,19]. The assay we used to assess IGFBP-3 levels measures IGF-I bound to BP-3, as well as the disintegrated fragments of the IGFBP-3 complex that originate from proteolysis of the complex.

In our patients, baseline IGFBP-3 levels were elevated, possibly because of increased IGFBP-3 proteolysis, as well as increased IGFBP-3 production because of GH hypersecretion. Losartan influenced IGFBP-3 levels in our study, but it is still unknown whether blocking RAS may have direct effects on the proteolysis or production of IGFBP-3. The slight, although non-significant rise in IGF-I levels might have resulted by negative feedback in a diminution of GH secretion and thereby IGFBP-3 production, contributing to the reduced IGFBP-3 levels[10,11,20,21,28]. Whether improvement of insulin resistance through losartan treatment reduced IGFBP-3 proteolysis remains unclear, because insulin resistance was not evaluated in this study.

The production and bioactivity of different variables of the IGF-I system in type 2 diabetes are influenced by many factors, like insulin resistance, endogenous insulin production, insulin levels, metabolic control, diabetes treatment and obesity[8,9]. The baseline levels of HbA_{1c}, insulin use and BMI did not significantly differ between the treatment groups, but the study protocol, of which this is a substudy, did not include assessment of insulin levels or insulin

resistance (other than HbA_{1c}), nor required that diabetes treatment was consistent during follow-up. In the losartan treated group, the effects of losartan on the IGF-I system were more pronounced in the metabolically stable group, with reduction of IGFBP-3 levels becoming statistically significant, and a stronger, although still non-significant, increase of free IGF-I levels.

Our study did not reveal an association between changes in circulating total and free IGF-I or IGFBP-3 concentrations and the reduction of urinary albumin excretion rate, suggesting involvement of other mechanisms for this reduction, such as improvement of renal haemodynamics, or changes in the renal levels of variables of the IGF-I system.

The changes in the IGFBP-3/IGF-I system associated with losartan were related to improvement of creatinine clearance. Lower IGFBP-3 levels were correlated to improvement of creatinine clearance in the whole losartan treated group, as well as to lower levels of serum creatinine in the metabolically stable group. Furthermore, a positive relation between mean serum levels of free IGF-I and mean levels of creatinine clearance was observed at baseline. After 10 weeks of losartan treatment, the change in total serum IGF-I concentration correlated positively to the change in serum creatinine levels in the metabolically stable group, meaning higher levels of total serum IGF-I were associated with lower serum levels of creatinine. Obviously, the significance of the observed correlations should be viewed with the limitations germane to the non-significant changes in serum levels of IGF-I and aspects of renal function. However, these observations are in concordance with previous studies, showing that IGF-I enhances renal plasma flow, glomerular filtration rate and creatinine clearance[5,11,13,29]. Since increased levels of IGFBP-3 are probably involved in the pathogenesis of diabetic nephropathy and renal dysfunction[14,18], reduction of IGFBP-3 might contribute to improvement of renal function.

The interactions between the GH/IGF-I axis, insulin resistance and blood pressure have been studied previously. IGF-I appears to be independently associated with insulin resistance and correlates inversely to blood pressure in subjects with insulin resistance as well[30,31]. Similarly, our data showed inverse correlations between both total and free IGF-I levels and systolic blood pressure. In our normotensive population, the reduction in blood pressure was only small. The changes in the IGF-I system, associated with losartan, probably contributed to its beneficial effect on blood pressure.

In conclusion, the angiotensin-receptor antagonist losartan tends to lower the elevated serum levels of IGFBP-3 in type 2 diabetic patients, although only significantly in our metabolically stable patients. This reduction is associated with improvement of creatinine clearance. The tendency towards raising the circulating levels of IGF-I after losartan treatment is small and non-significant; however, it is associated with improvement of creatinine clearance and reduction of blood pressure. Our data show no relation between changes in the IGF-I system and reduction in microalbuminuria. Further research would be of interest, involving more patients with type 2 diabetes and stable metabolic parameters, to investigate to what extent changes in the IGF-I system contribute to the renal and haemodynamic effects of losartan.

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II.3

The effect of losartan on urinary growth hormone in normotensive patients with type 2 diabetes mellitus and microalbuminuria

Zandbergen AAM, Lamberts SWJ, Janssen JAMJL, Bootsma AH

Submitted

ABSTRACT

Objective: Previously, we reported that losartan tended to reduce the elevated levels of IGFBP-3 and to raise the reduced levels of free IGF-I in normotensive patients with type 2 diabetes mellitus and microalbuminuria. These changes might contribute to effects of losartan on creatinine clearance and blood pressure in these patients. We investigated whether these observations can be explained by changes in GH levels.

Design, patients and measurements: This randomised, double-blind placebo-controlled clinical trial involved 74 normotensive patients with type 2 diabetes and microalbuminuria, of whom 38 received losartan and 36 received placebo for 10 weeks. Before and after the treatment period, urinary GH levels were measured, in addition to serum levels of total and free IGF-I, IGFBP-3, creatinine and HbA_{1c}, albuminuria, creatinine clearance and blood pressure.

Results: The baseline level of urinary logGH was 0.8±0.1 pg/ml in the losartan group and 0.9±0.1 pg/ml in the placebo group (ns). After 10 weeks of losartan treatment, logGH tended to reduce to 0.7±0.1 pg/ml, but not to statistical significance. LogGH did not change in the placebo group. Urinary logGH levels correlated significantly with serum free IGF-I levels (r=0.34, p=0.009 at baseline, r=0.40, p=0.003 at week 10, age-adjusted). Urinary logGH was not significantly associated with serum IGFBP-3, albuminuria or creatinine clearance.

Discussion: The unbalances in the GH/IGF-I system tended to improve, but did not thoroughly clarify the reduction in serum IGFBP-3 after losartan treatment. Other mechanisms, like improved insulin sensitivity through other pathways than the GH/IGF-I system, probably contributed to this reduction as well.

Previously, we reported the results of a randomised, double-blind placebo-controlled clinical trial, that investigated the insulin-like growth factor I (IGF-I) system in normotensive patients with type 2 diabetes mellitus and the effects of short-term treatment with the angiotensin-receptor antagonist losartan on this system[1]. In that study, we observed a tendency towards reduction in the elevated baseline serum levels of insulin-like growth factor binding protein 3 (IGFBP-3) after 10 weeks of losartan treatment, as well as a tendency towards a rise in free IGF-I, of which the serum concentration was reduced at baseline. These changes were not correlated with the observed reduction in microalbuminuria after losartan treatment, but were related to improvement of creatinine clearance and reduction of blood pressure.

Many patients with diabetes mellitus show a well-known unbalance in the growth hormone (GH)/IGF-I system, with reduced levels of circulating IGF-I and consequently GH hypersecretion[2]. As the production of IGFBP-3 depends on GH, GH hypersecretion might have contributed to the elevated baseline levels of IGFBP-3 of our population. We also hypothesised that increased proteolysis of the IGF-I/BP-3 complex, which has been described in insulin resistance, diabetes mellitus as well as in a variety of kidney diseases, possibly contributed to the elevated IGFBP-3 concentration. Thus, reduction in IGFBP-3 levels after losartan treatment might result from reduction in GH hypersecretion due to increased hepatic IGF-I production after blocking angiotensin II, and/or from improvement of insulin resistance[3].

To elucidate underlying mechanisms, we measured urinary GH levels in our patients, before and just after 10 weeks of treatment. Urine samples from 24-h urine collections were used, which had been stored at -70° until analysis. Urinary GH levels all were measured using enzyme-linked immunosorbent assays (ELISAs) (Mediagnost Wachstumhormon EIA E022, Mediagnost, Germany). Although only 0.01% of circulating GH appears in the urine and physicochemical characteristics (pH, salt concentration and ionic composition) show more variation in urine than in blood, urinary GH excretion rate measured with the current ultrasensitive immunoassays in 24-h urine collections is assumed to accurately reflect integrated 24-h plasma GH levels[4].

Because of skewed distribution, GH data were log-transformed. The baseline level (mean±SEM) of urinary log GH was 0.8±0.1 pg/ml in the losartan treated group (n=38) and 0.9±0.1 pg/ml in the placebo group (n=36) (ns). After 10 weeks of losartan treatment, log GH tended to reduce to 0.7±0.1 pg/ml, but not to statistical significance. Log GH did not change at all in the placebo group. As previously described in more detail, serum free IGF-I levels increased from 53.6 pmol/l at baseline to 56.8 pmol/l (ns) in the losartan treated group, and decreased from 58.8 pmol/l to 55.5 pmol/l (ns) in the placebo group[1]. Urinary log GH levels correlated significantly with serum free IGF-I levels (Pearson's correlation coefficient r=0.34, p=0.009 at baseline, r=0.40, p=0.003 at week 10, adjusted for age) (*figure 1*). Urinary log GH was not significantly associated with serum IGFBP-3 levels, urinary albumin excretion or creatinine clearance.

Because of reduced insulin action, either through portal insulin deficiency or insulin resistance, hepatic IGF-I production is reduced in type 1 diabetes, as well as in type 2 diabetes and states of insulin resistance without diabetes. This results in GH hypersecretion, reflecting an increase in pituitary secretion[2]. Accordingly, we observed a significant correlation in our study population between urinary GH and serum free IGF-I, reflecting an intact feedback mechanism, before as well as after losartan treatment. Recent studies suggest that inhibitors of the renin-angiotensin system (RAS), like losartan, reduce the risk of developing new-onset type 2 diabetes, probably as a result of reduced insulin resistance[5]. Previously, we reported that short-term administration of losartan improved insulin resistance and increased serum levels of free IGF-I in subjects with impaired fasting glucose[5]. In the present study, urinary GH levels showed a tendency to reduce with losartan treatment, although not statistically significant, which was probably caused by the small number of patients together with the variation in urinary GH levels. However, the decrease in GH levels was accompanied by an increase of serum free IGF-I levels, as shown in figure 1.

The reduction in urinary GH levels was not significantly correlated with the reduction in serum IGFBP-3, possibly because of our small sample size. Furthermore, reduction in IGFBP-3 might also result from reduction in proteolysis of the IGF-I/BP-3 complex because of improvement of insulin sensitivity associated with losartan, besides reduction of GH dependent pro-

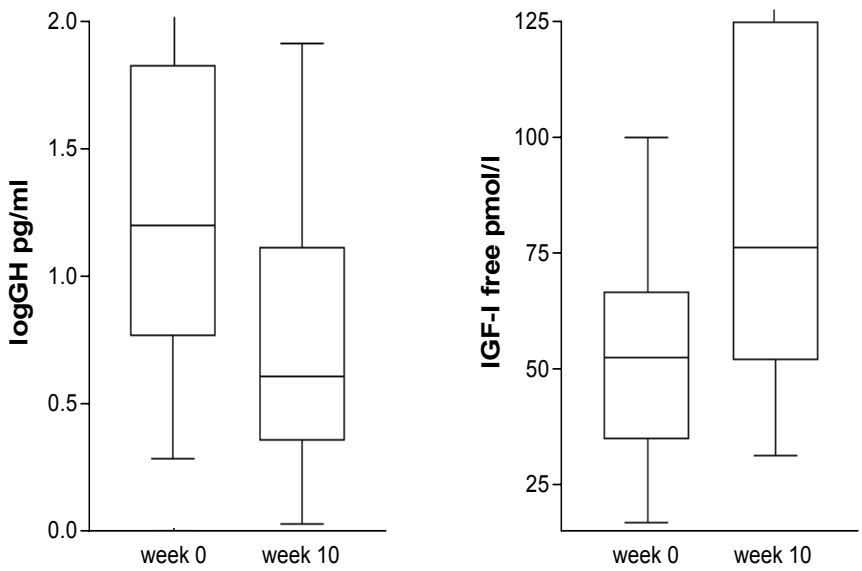

Figure 1. Effect of 10 weeks of losartan treatment on urinary logGH and serum free IGF-I levels

duction of IGFBP-3. The improved insulin sensitivity in our patients probably derived from other mechanisms than improvement of the unbalances in the GH/IGF-I system. The system might be too disturbed to be normalised in this short treatment period, and the rise in free IGF-I, caused by either increased hepatic IGF-I production after blocking angiotensin II or improvement of hepatic insulin sensitivity itself, too small to reduce GH hypersecretion to great extent. Conclusively, the unbalances in the GH/IGF-I system tended to improve, but did not thoroughly clarify the reduction in serum IGFBP-3 levels after losartan treatment. Other mechanisms, like improved insulin sensitivity through other pathways than the GH/IGF-I system, probably contributed to this reduction as well in our patient population.

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II.4

Short-term administration of an angiotensin-receptor antagonist in patients with impaired fasting glucose improves insulin sensitivity and increases free IGF-I

Zandbergen AAM, Lamberts SWJ, Janssen JAMJL, Bootsma AH

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ABSTRACT

Objective: Blocking the renin-angiotensin system (RAS) may reduce the risk of developing type 2 diabetes, but data are inconclusive and mechanisms involved unclear. RAS and RAS inhibition also influence the IGF-I system, which is important in glucose homeostasis. We investigated the effects of the angiotensin-receptor antagonist losartan on insulin resistance and IGF-I levels.

Design and methods: In this hypothesis-generating study, five individuals with impaired fasting glucose received losartan 100 mg during 8 weeks. Before and after the treatment period, insulin sensitivity was assessed using HOMA and 2-h CIGMA. Furthermore, serum levels of free and total IGF-I, IGFBP-3, lipids and HbA_{1c} were measured.

Results: After the treatment period, the HOMA score for insulin resistance had decreased from 5.3±1.1 to 3.7±0.9 (p=0.004), and the 2-h CIGMA score from 23.4±3.1 to 15.9±2.1 (p=0.07). Serum levels of free IGF-I had increased from 57±18.8 to 134±31.3 pmol/l (p=0.04). The difference, in terms of percentage, in decrease of HOMA related with the difference in increase in free IGF-I levels (Pearson's correlation coefficient r= -0.8; p=0.07). A trend into the same direction was observed with 2-h CIGMA. No differences were observed in lipids, total IGF-I, IGFBP-3 or HbA_{1c}.

Conclusions: Losartan raised serum levels of free IGF-I, which might contribute to the improvement of insulin resistance associated with losartan treatment. These observations, if confirmed in broader studies, will help our understanding of the pathogenesis of type 2 diabetes mellitus, as well as of the role of angiotensin-receptor antagonists in its prevention.

INTRODUCTION

Type 2 diabetes mellitus is a major health burden, associated with excess morbidity and mortality[1]. The prevalence of type 2 diabetes mellitus is increasing rapidly, and interventions to prevent or delay its onset are becoming more important. Preventive measures should address insulin resistance, as this is the major identifiable defect in subjects at risk for type 2 diabetes.

Recent publications suggest that blocking the renin-angiotensin system (RAS) may reduce the risk of developing type 2 diabetes[2]. Development of diabetes in these trials was considered as a secondary endpoint or studied in post-hoc analysis, and large-scale prospective placebo-controlled clinical trials are necessary, designed to assess the protective benefits of RAS inhibition in subjects at risk of developing type 2 diabetes. The mechanisms involved in the effects of RAS inhibition on glucose metabolism and insulin sensitivity are still unclear[3].

IGF-I is important in glucose homeostasis, and might play a protective role in the development of glucose intolerance[4]. Interactions between RAS and the IGF-I system are complex and not fully clarified. It was previously hypothesised that production of IGF-I is reduced by angiotensin II, which is reversed by losartan[5]. RAS inhibition seems to have metabolic and growth promoting effects, which could be mediated by increased IGF-I levels[6].

We performed a proof of principle study in subjects with impaired fasting glucose, to investigate the effects of short-term administration of the angiotensin-receptor antagonist losartan on insulin resistance, assessed by using the homeostasis model assessment of insulin resistance (HOMA), as well as the 2-h continuous infusion of glucose with model assessment (2-h CIGMA). Furthermore, serum levels of total and free IGF-I, IGFBP3, HbA_{1c} as well as lipids were measured, to investigate the effect of losartan on these parameters. Finally, possible correlations between changes in insulin resistance and changes in the IGF-I system were studied.

PATIENTS

Five subjects (one man and four women; mean age 53.4±1.3 yr) participated in the study protocol. They had no relevant medical history, nor were taking medication. The individuals had impaired fasting glucose by current WHO criteria (mean fasting glucose levels 6.5±0.1 mmol/l, mean HbA_{1C} 6.6±0.3 %)[7]. They were normotensive (mean systolic blood pressure 137.2±4.6 mmHg, mean diastolic pressure 84.6±1.9 mmHg), and obese (mean BMI 30.6±2.1 kg/m^2).

The study was performed according to the guidelines of good clinical practice and was approved by the institutional review board. All persons gave written informed consent prior to participation.

METHODS

Insulin resistance was assessed using HOMA and 2-h CIGMA. Previous studies showed that the insulin resistance score, based on these methods, strongly correlates with insulin resistance assessed by the glucose clamp technique in both diabetic and nondiabetic subjects. Furthermore, the 2-h CIGMA can be considered as a test of glucose tolerance, analogous to the oral glucose tolerance test[8].

HOMA uses mathematical modelling of fasting plasma glucose and insulin levels to estimate insulin resistance: fasting serum insulin (μU/ml) x fasting plasma glucose (mmol/l) / 22.5[8]. Three baseline samples, taken at 5 minute intervals, are averaged for the mean levels of glucose and insulin. High HOMA scores denote low insulin sensitivity. The 2-h CIGMA consists of a 180 $mg/min^{-1}/m^2$ glucose infusion for 120 minutes. Three blood samples are taken afterwards, at 120, 125 and 130 minutes, for measurement of glucose and insulin levels. Again, the means of these three samples are used to estimate the insulin resistance score, using the above-mentioned formula.

During the 8-week study period, the subjects received losartan 100 mg orally, once daily. They were instructed not to change their diet or exercise pattern during this period. Blood pressure and body weight were measured at weeks 0 and 8.

Just before start of treatment, we performed the HOMA and 2-h CIGMA. Furthermore, venous blood samples were taken for measurement of free and total IGF-I, IGFBP-3, lipids and HbA_{1c}. Serum levels of total and free IGF-I were measured using direct immunoradiometric assay (Diagnostic System Laboratories Inc., Webster, Texas, USA)[9,10]. All samples were measured at the same time. After 8 weeks of losartan treatment, insulin sensitivity was measured again, as well as serum levels of total and free IGF-I, IGFBP-3, lipids and HbA_{1c}.

Statistics

Results are presented as means±SEM, unless otherwise noted. The paired Student's *t*-test was used for comparisons before and after losartan treatment. Associations between the variables were quantified using the Pearson correlation coefficient; results are given including p-value. We used GraphPad Prism software, version 3.00 (GraphPad Software Inc., San Diego, California, USA), for all statistical analyses.

RESULTS

After 8 weeks of treatment with losartan 100 mg, the HOMA score for insulin resistance had decreased from 5.3±1.1 to 3.7±0.9 (p=0.004), and the 2-h CIGMA score had decreased from 23.4±3.1 to 15.9±2.1 (p=0.07). Serum levels of free IGF-I had increased from 57±18.8 to 134±31.3 pmol/l (p=0.04) (*table 1*). Normal values of free IGF-I at this age vary between

Table 1. Effects of losartan after 8 weeks of treatment on total and free IGF-I, IGFBP-3, HOMA, 2-h CIGMA, HbA1c , and blood pressure

Endpoint	Week 0	Week 8	P value
Total IGF-I, nmol/l	20.7±5.3	15.6±3.1	0.1
Free IGF-I, pmol/l	57.4±18.8	134.0±31.3	0.04
IGFBP-3, nmol/l	4.2±0.2	4.3±0.2	0.7
HOMA	5.3±1.1	3.7±0.9	0.004
2-h CIGMA	23.4±3.1	15.9±2.1	0.07
HbA_{1c}, %	6.6±0.3	6.7±0.3	0.3
Systolic blood pressure, mm Hg	137.2±4.6	127.0±5.3	0.09
Diastolic blood pressure, mm Hg	84.6±.1.9	82.6±2.2	0.2

Data depicted as means ±SEM

87.1-660.4 pmol/l for men, and 76.7-770.9 pmol/l for women[11]. The difference, in terms of percentage, in decrease of HOMA correlated with the difference in increase in free IGF-I levels (Pearson's correlation coefficient r= -0.8; p=0.07) (*figure 1*). A trend into the same direction was observed in the correlation between the difference in decrease of CIGMA and the difference in increase in free IGF-I levels (r=-0.8; p=0.1). No differences were observed in lipid levels, total IGF-I, IGFBP-3 or HbA_{1c}.

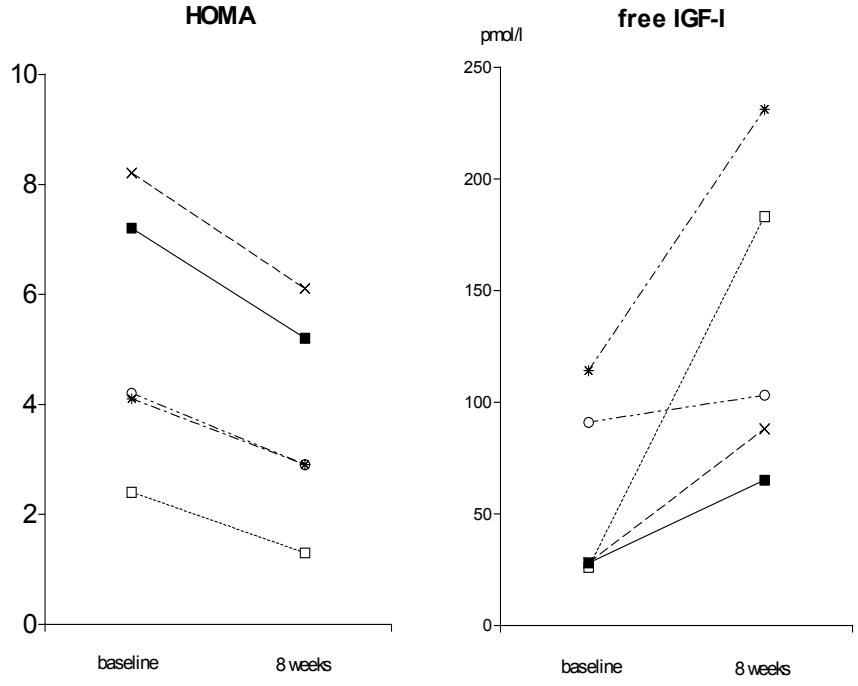

Figure 1. Effect of 8 weeks of losartan treatment on HOMA and serum levels of free IGF-I

Systolic blood pressure had reduced from 137.2±4.6 mmHg to 127.0±5.3 mmHg (ns) after 8 weeks of losartan, and diastolic blood pressure from 84.6±1.9 mmHg to 82.6±2.2 mmHg (ns). Body weight did not differ significantly. In these normotensive subjects, losartan 100mg was well-tolerated, no side effects were reported.

DISCUSSION

In this proof of principle study, we investigated the effects of short-term administration of the angiotensin-receptor antagonist losartan on insulin resistance and variables of the IGF-I system in individuals with impaired fasting glucose. Insulin resistance was reduced after the 8 week treatment period. At baseline, plasma levels of free IGF-I were reduced compared to healthy subjects, and had normalised during the treatment period. This increase might contribute to the improvement in insulin sensitivity, as suggested by the close to significant correlation between the decrease in insulin resistance scores and the increase in serum levels of free IGF-I.

Previous studies showed that the incidence of new-onset diabetes was reduced by RAS inhibitors, suggesting active positive effects of these drugs on long-term glucose metabolism. As stated in the introduction section, prospective studies are required to confirm these observations as well as to elucidate the underlying mechanisms. We observed a significant reduction of the insulin resistance score, measured using HOMA; when measured using 2-h CIGMA, the reduction of the insulin resistance score just failed to reach significance. After this short-term treatment period, no differences in mean HbA_{1c} levels were noticed. Since insulin resistance is one of the main predictors for the development of type 2 diabetes mellitus, improvement of insulin sensitivity might be the underlying mechanism in delaying or preventing this disease.

The IGF-I system plays an important contributory role in the regulation of glucose metabolism. IGF-I, like insulin, increases peripheral glucose uptake and decreases hepatic glucose production. Free IGF-I is the unbound, biologically active form. Serum levels of free IGF-I are often reduced in diabetic patients, which associates with increased insulin resistance and worse metabolic control. Furthermore, increasing circulating IGF-I levels by means of recombinant human (rh)IGF-I is associated with improvement of insulin sensitivity and reduced insulin requirements in both type 1 and type 2 diabetes[4,12]. The association we observed between the decrease in insulin resistance and the increase in free IGF-I is in accordance with these data.

The RAS interacts with the IGF-I system in several ways. These interactions are complex and not fully clarified. A previous study in rats showed that circulating levels of IGF-I are reduced by angiotensin II infusion through reduction of the hepatic IGF-I mRNA levels, which is reversed by losartan[13]. Only few clinical trials have been performed investigating the effects of

RAS inhibition on the IGF-I system in humans[5,6]. A previously published study showed metabolic and growth promoting effects of an ACE-inhibitor, that might result from an increased IGF-I concentration[6].

An important limitation of our study is the short follow-up period. Although insulin resistance was reduced significantly, we cannot ascertain from these results that the onset of new-diabetes will be prevented or delayed. Obviously, the significance of our observations should be viewed with the limitations germane to the small sample size. The results are promising in these subjects, but are preliminary and need to be confirmed in a large-scale and long-term study. Furthermore, the limited number of patients restricts our analysis to a univariate one.

In conclusion, short-term treatment with losartan raises serum levels of free IGF-I, which might contribute to the improvement of insulin resistance associated with losartan treatment. The results of this hypothesis-generating study form a basis for further studies that improve our understanding of the pathogenesis of type 2 diabetes mellitus, which ultimately might result in new preventive interventions.

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III

Clinical outcome of normotensive patients with type 2 diabetes mellitus and microalbuminuria

III.1

Normotensive women with type 2 diabetes mellitus and microalbuminuria are at high risk for macrovascular disease

Zandbergen AAM, Sijbrands EJG, Lamberts SWJ, Bootsma AH

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ABSTRACT

Objective: The excess risk of macrovascular disease and death, associated with diabetes mellitus, seems higher in women than in men. The pathogenesis for this risk difference has not been fully elucidated. We investigated whether female gender was associated with macrovascular disease and death, independently of the known risk factors related to type 2 diabetes mellitus, or nephropathy or retinopathy, in normotensive patients with type 2 diabetes and microalbuminuria.

Design and methods: We conducted a prospective, prolonged follow-up study of a subgroup of 67 diabetic patients (46 men, 21 women) without established cardiovascular disease, who participated in a larger clinical trial. Data were collected on current and past health, medication use, blood pressure, renal function and HbA_{1c} during the follow-up period of 4.7±0.1 (±SEM) years. The endpoint was a composite of death, cardiovascular disease, cerebrovascular events, and peripheral artery disease.

Results: Of the women, 8 (38.1%) met the endpoint, compared with 6 (13.4%) of the men (p=0.02 for difference in event free survival). The Hazard ratio of the women relative to the men was 3.19 (95% CI; 1.11 to 9.21), which further increased after adjusting for age, systolic blood pressure, body mass index, smoking, total cholesterol/HDL-cholesterol ratio and urinary albumin excretion and retinopathy.

Conclusions: In our study population of normotensive patients with type 2 diabetes mellitus and microalbuminuria, female gender was associated with increased risk of fatal and non-fatal cardiovascular disease, independent of the classical cardiovascular risk factors, the severity of nephropathy or presence of retinopathy, or health care utilization.

INTRODUCTION

Type 2 diabetes mellitus, with its rapidly growing prevalence, has become a major public health problem[1]. It associates with an increased risk of coronary heart disease (CHD), cerebrovascular disease and peripheral vascular disease[2,3]. Strikingly, this excess risk for cardiovascular disease seems relatively higher for female diabetic patients than for male diabetic patients[3-6]. Type 2 diabetes contributes substantially to the annually 500.000 women dying of cardiovascular disease in the USA. It is unclear whether the difference in cardiovascular risk between the sexes is caused by known cardiovascular risk factors (like duration of diabetes and systolic blood pressure) or by lower health care utilization by women compared to men[7,8].

In the present study, we extended the prospective follow-up of normotensive patients with type 2 diabetes mellitus and microalbuminuria, who participated in a double-blind, randomised, placebo-controlled trial[9]. The original study excluded patients with a history of cardiovascular disease and the prolonged follow-up study removed differences in health care utilization among the participants.

In this study, we investigated whether female gender was associated with cardiovascular disease, cerebrovascular disease, peripheral artery disease and death, independently of the classical cardiovascular risk factors related to type 2 diabetes, nephropathy, and retinopathy.

METHODS

Study design

The present study is a prolonged follow-up study of a subgroup of patients (n=67) who participated in a larger clinical trial. The study design, methods, and results of this randomised, double-blind, placebo-controlled multicenter clinical trial have been published previously[9]. In the original trial, we investigated the effects of short-term treatment with the angiotensin-receptor antagonist losartan in normotensive patients with type 2 diabetes mellitus and microalbuminuria. After the 20 week study period, the 67 patients, who had been recruited from hospitals in the city of Rotterdam and surrounding areas, received standard medical care. During follow-up, we collected the data on current and past health, medication use, blood pressure, and laboratory results of assessment of renal function and HbA_{1c}. The study was performed according to the guidelines of good clinical practice and was approved by the institutional review board. All persons gave written informed consent prior to participation.

Patients

The eligibility criteria have been described in detail[9]. Briefly, patients with diabetes mellitus type 2, urinary albumin excretion rate from 20 to 200 µg/min, and office measurement

of blood pressure equal to or below 150/90 mm Hg were randomised. Type 2 diabetes was defined as diabetes diagnosed at age older than 30 years or controlled by a diet or blood glucose-lowering agents for at least 6 months. The current definition of normotension is a blood pressure less than 140/90 mm Hg, with a blood pressure lowering target equal to or below 130/80 mmHg in adults with diabetes mellitus[10,11]. However, the protocol of this study was designed in 1999, when normotension was defined as blood pressure equal to or below 160/90 mmHg during office measurement. Our criteria resulted in a mean baseline blood pressure of 135/81 mmHg, which can be regarded as normotensive at present.

The main exclusion criteria included a history of myocardial infarction or cerebrovascular events, unstable angina pectoris and heart failure. Patients with ECG abnormalities including left ventricular hypertrophy, acute renal failure, chronic glomerulonephritis, polycystic kidney disease, a serum creatinine level above 150 µmo/l, an HbA_{1c} level above 10% or concomitant use of antihypertensive agents, steroids or lithium were excluded as well.

Endpoints

The endpoint of the present study was a composite of death, acute myocardial infarction, unstable angina pectoris, coronary interventions, heart failure, cerebral ischemic stroke or transient ischemic attack, and peripheral artery disease (peripheral arterial bypass graft, peripheral percutaneous transluminal angioplasty or other percutaneous invasive intervention, intermittent claudication defined as classical symptoms in combination with at least one unequivocal result of one of the following: (a) ankle/arm index <0.9 or (b) a stenosis (>50%) on an angiogram or duplex scan). Patients with a history of cardiovascular disease were excluded from the original trial restricting our observations to incident events.

Statistical analyses

Continuous variables are presented as means with standard error of mean (SEM), discrete variables as frequencies and percentages. The independent Student's *t*-test was used for comparisons between men and women. Cumulative event free survival was analyzed with the Kaplan–Meier method and the log rank test, as well as with Cox regression. The 95% CI of the Hazard ratio was calculated as the exponent of the regression coefficient and its standard error.

As we questioned whether female gender was an independent risk factor for macrovascular outcome in our study population, we used clinical variables, important with regard to macrovascular complications in type 2 diabetes, which were significantly different between the sexes at baseline in our multiple Cox regression models. We also adjusted for other classical cardiovascular risk factors. Two Cox regression models were performed, to investigate whether level of baseline albuminuria, respectively presence of retinopathy, could explain for possible sex differences, thereby able to serve as risk indicator of those patients with the highest cardiovascular risk.

Statistical significance was assessed at the 5% level of probability. We used SPSS 12.0.1 for Windows (SPSS Inc., Chicago, Illinois) for all analyses.

RESULTS

Table 1 shows characteristics according to sex at baseline and after a mean follow-up of 4.7±0.1 (±SEM) years. The women had a higher baseline systolic blood pressure and a higher body mass index compared to the men. Age, duration of diabetes, HbA_{1c} levels, diastolic blood pressure, urinary albumin excretion rate, frequency of current smoking and frequency of retinopathy did not differ between men and women. At baseline, 46 (68.7%) of the patients were treated with an insulin-based regime, the remaining 21 (31.3%) with oral glucose-lowering agents only. As a result of the inclusion criteria, no antihypertensive treatment was used at baseline. At the end of the follow-up period, 93% of the patients used antihypertensive

Table 1. Characteristics of men and women at baseline and after 5 year follow-up

	Men (n=46)	Women(n=21)	P value
Baseline			
Age (y)	56.0 (± 1.8)	54.0 (± 2.8)	0.5
Duration of diabetes mellitus (y)	14.3 (± 1.5)	15.7 (± 1.7)	0.5
HbA1c (%)	8.1 (± 1.3)	8.0 (± 1.0)	0.7
Body mass index (kg/m^2)	27.7 (± 0.7)	31.6 (± 1.2)	0.008
Systolic blood pressure (mm Hg)	133.9 (± 1.7)	139.2 (± 1.9)	0.04
Diastolic blood pressure (mm Hg)	79.4 (± 1.1)	81.6 (± 1.6)	0.3
Total cholesterol/HDL-C ratio	5.38 (± 0.3)	4.6 (± 0.3)	0.06
Current smoking (%)	30	38	0.6
Serum creatinine (µmol/l)	84.8 (± 2.3)	74.0 (± 4.7)	0.05
UAE (mg/l)	70.4 (± 6.1)	81.8 (± 11.3)	0.3
Retinopathy (%)	27	48	0.1
5 year follow-up			
HbA1c (%)	8.6 (± 1.4)	8.3 (± 1.0)	0.4
Systolic blood pressure (mm Hg)	139.5 (± 2.6)	140.3 (± 3.5)	0.9
Diastolic blood pressure (mm Hg)	79.9 (± 6.5)	77.2 (± 7.8)	0.2
Serum creatinine (µmol/l)	89.0 (± 3.8)	81.3 (± 4.5)	0.2
UAE (mg/l)	133.7 (± 40.1)	124.4 (± 34.2)	0.9
Antihypertensives (%)	89	100	0.03
Aspirin (%)	23.3	28.6	0.7
Statin use (%)	55	48	0.6

Values are given as means ± standard error of the mean (SEM)

UAE denotes urinary albumin excretion

treatment, of which 98% consisted of blockers of the renin-angiotensin system (RAS), in minority in combination with any of the other classes of antihypertensive medication.

During 85.9 person years, 8 (38.1%) women had a cardiovascular event (cardiovascular disease: 3; cerebrovascular disease: 2; peripheral artery disease: 3; no deaths were reported) and during 198.5 person years 6 (13.4%) men also met the composite endpoint (cerebrovascular disease: 2; peripheral artery disease: 2; death: 2; -one because of cerebral haemorrhage, one because of cardiac arrest-). One man who first developed peripheral artery disease, afterwards developed cerebrovascular disease as well as cardiovascular disease, and one man who first developed cerebrovascular disease, also developed peripheral artery disease. The time to the first event in those two men counted for the event-free survival time. The Kaplan-Meier showed a significant difference in event free survival between women and men (p=0.02) (*figure 1*).

Table 2 shows that women had 3.19 (95% confidence interval; 1.11 to 9.21) times higher risk of cardiovascular disease risk in univariate analysis relative to men. In this study, we questioned whether female gender was an independent risk factor for macrovascular outcome in normotensive patients with type 2 diabetes mellitus and microalbuminuria. As demonstrated in table 1, body mass index (BMI) and systolic blood pressure were significantly different between both men and women. In our multiple Cox regression models, we put in these variables to exclude that the observed increased risk in women is due to higher baseline systolic

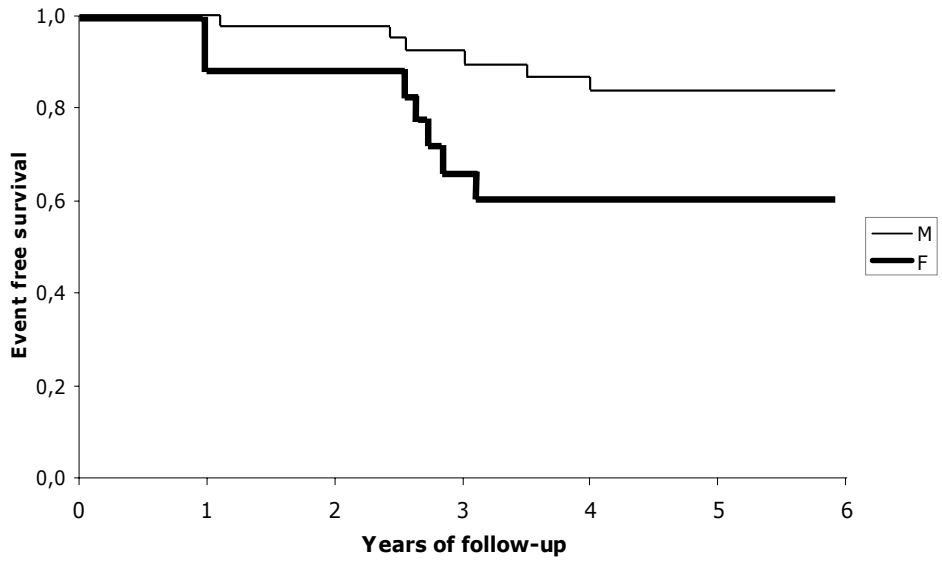

Figure 1. Kaplan-Meier estimates of event-free survival in women and men with type 2 diabetes. Women had significant shorter event-free survival compared to men (Log rank test; p=0.02)

Table 2. Hazard ratios for composite cardiovascular endpoint of women relative to men

Variables	Hazard ratio	(95% CI)	P value
Univariate			
Women relative to men	3.19	(1.11 to 9.21)	0.03
Multiple Cox regression: model 1			
Women relative to men (adjusted for age, systolic blood pressure, body mass index, total cholesterol/HDL-C ratio, current smoking, urinary albumin excretion)	6.40	(1.24 to 32.93)	0.02
Multiple Cox regression: model 2			
Women relative to men (adjusted for age, systolic blood pressure, body mass index, total cholesterol/HDL-C ratio, current smoking, retinopathy)	8.23	(1.81 to 37.62)	0.009

blood pressure or BMI in women. Furthermore, we adjusted for other classical cardiovascular risk factors, like age, smoking, and total cholesterol over HDL cholesterol ratio.

We have made two multiple Cox regression models to analyse the effect of nephropathy and retinopathy, respectively model 1 and 2 in table 2. In model 1, after adjustment for age, systolic blood pressure, BMI, total cholesterol over HDL-cholesterol ratio, smoking and urinary albumin excretion, the Hazard ratio of the women relative to the men increased to 6.40 (95% confidence interval; 1.24 to 32.93). In model 2, the point estimate of the cardiovascular risk of women relative to men increased to 8.23 (95% confidence interval 1.81 to 37.62).

DISCUSSION

In the present prospective follow-up study, we observed that female gender was an independent and important cardiovascular risk factor in normotensive patients with type 2 diabetes mellitus and microalbuminuria. Adjustment for the classical cardiovascular risk factors like age, systolic blood pressure, BMI, smoking, total cholesterol over HDL cholesterol ratio, as well as the presence of urinary albumin excretion or retinopathy further increased the excess risk of the women. Hence, we could not have identified the women at baseline who had an event afterwards. The women entered the present study with a significantly higher mean systolic blood pressure than the men, although without differences between the sexes in signs of organ damage. During follow-up, significantly more women were treated with antihypertensive drugs resulting in similar systolic blood pressure in both sexes. This showed that the risk difference between the sexes was also not the result of differences in access to health care.

Previous studies showed that women with diabetes mellitus lose their usual relative protection (compared to men) against cardiovascular disease[12]. Estimates of cardiovascular

mortality in diabetic women range from 2- to 5 fold the rate in nondiabetic women, whereas in diabetic men, estimates vary from 1- to 3-fold the rate in nondiabetic men[13]. Data about the reasons for this excess risk of cardiovascular disease in diabetic women are conflicting. In a number of studies, differences in the distributions of other major risk factors explained the sex difference in cardiovascular disease risk to a great extent[7,8,14]. A recently published meta-analysis established an estimate of the odds ratio for fatal and nonfatal cardiovascular disease due to diabetes in both women and men. The authors concluded that after adjusting for the well established, modifiable cardiac risk factors, the difference in risk between women and men is modest and not statistically significant[8]. To our knowledge, our study is the first prospective follow-up study that investigated normotensive type 2 diabetic patients who had microalbuminuria, without previously established cardiovascular diseases.

The women of our study population had a higher baseline systolic blood pressure as well as a higher body mass index compared to the men. However, adjustment for these differences did not change the cardiovascular risk between the sexes. Levels of blood pressure and HbA_{1c} did not reach the current treatment goals during follow-up, but were similar in both men and women and did not change the cardiovascular risk between the sexes in multivariate Cox analysis. Moreover, our results during follow-up indicate that women and men were according to the same protocol, achieving identical treatment targets.

Microalbuminuria has been considered as an indicator of endothelial dysfunction, as well as the first clinical sign of diabetic nephropathy[15,16]. It is associated with cardiovascular morbidity and all cause mortality, in both type 1 and type 2 diabetes mellitus, as well as in non diabetic individuals[17,18]. In the present study, the level of urinary albumin excretion did not explain the sex difference observed in our study population. Moreover, adjustment for the presence of retinopathy at baseline, an important microvascular complication of type 2 diabetes mellitus and a sign of arterial atherosclerosis, also did not explain the high risk among our women with type 2 diabetes.

The strength of the present study lies in its prospective design and the absence of prevalent cardiovascular disease at baseline, in spite of the presence of microalbuminuria. Moreover, the diabetes was equally complicated with retinopathy and nephropathy in women and men. An important limitation of our study is the small sample size. Nonetheless, in concordance with previous studies, we observed statistically significant differences between women and men with regards to cardiovascular risk, which further increased after multivariate regression analyses. Furthermore, as our patients did not meet the treatment targets, it would be interested to investigate whether the event rates and sex differences still exist when the current more aggressive treatment goals for blood pressure lowering as well as HbA_{1c} are achieved.

In summary, we conclude that female gender is an independent risk factor for macrovascular disease and death in our study population of normotensive patients with type 2 diabetes and microalbuminuria. This excess risk of the women relative to the men could not be explained by differences in other classical cardiovascular risk factors, the severity of ne-

phropathy or presence of retinopathy, nor health care utilization. In contrast to recent studies our observations suggest that the cardiovascular risk of women with type 2 diabetes can easily be underestimated when additional risk factors are made mandatory. Further research is needed to elucidate the pathogenesis of this excessive risk in women with type 2 diabetes, because at this point we did not find risk indicators that discriminate between women with high and low risk of cardiovascular disease.

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APPENDIX

Participating investigators:

R.J.Th. Ouwendijk and M.G.A. Baggen, Ikazia Hospital
Th.L.J.M. van der Loos and F.J.M. Klessens-Godfroy, Rotterdam Eye Hospital
J.W.F. Elte, Sint Franciscus Hospital
S.W.J. Lamberts and A.H. Bootsma, Erasmus University Medical Centre
R.J.M. van Leendert, Albert Schweitzer Hospital location Zwijndrecht
S.G.T. Hulst, Vlietland Hospital location Vlaardingen
J.W. van der Beek-Boter, Hofpoort Hospital

III.2

Change in albuminuria is predictive of cardiovascular outcome in normotensive patients with type 2 diabetes mellitus and microalbuminuria

Zandbergen AAM, Vogt L, de Zeeuw D, Lamberts SWJ, Ouwendijk RJTh, Baggen MGA, Bootsma AH

Submitted

ABSTRACT

Background: Microalbuminuria is associated with cardiovascular morbidity and all-cause mortality. Reducing albuminuria with antihypertensive treatment appears to reduce the risk of cardiovascular complications in hypertensive patients with diabetes mellitus. The question remains whether this risk reduction is associated with blood pressure changes or with albuminuria changes separately.

Objective: To investigate whether sustained change in microalbuminuria independently predicts cardiovascular complications in patients with type 2 diabetes mellitus and microalbuminuria, without hypertension.

Design, setting and patients: A multicenter prospective follow-up study of 67 normotensive patients with type 2 diabetes and microalbuminuria who participated in a short-term study on the albuminuria lowering effects of the angiotensin-receptor antagonist losartan.

Measurements: During follow-up (4.7 (0.8) years), data were collected on cardiovascular complications, medication use, blood pressure, renal function and albuminuria. The endpoint was a composite of death, cardiovascular disease, cerebrovascular events, and peripheral artery disease.

Results: After one year of follow-up, three groups could be discerned: one with rapid progression of albuminuria, one with stable albumin excretion levels and one with reduction of albuminuria. Patients with rapid progression of albuminuria were at highest risk to reach the composite endpoint, whereas patients with reduction in albuminuria of 30% or more were at lowest risk (p=0.02). Importantly, the three groups showed a similar blood pressure course. After adjustment for the classical cardiovascular risk factors, change of albuminuria remained an independent predictor of risk (HR 5.1; 95% CI, 1.5-18.1; p=0.01).

Conclusion: In non-hypertensive patients with type 2 diabetes mellitus and microalbuminuria, sustained reduction in albuminuria reflects reduction in risk of cardiovascular complications. Urinary albumin excretion during treatment reflects therapeutic responsiveness in these patients, and therefore is useful as modifiable treatment goal.

INTRODUCTION

Diabetes mellitus is associated with a well-known increased risk of cardiovascular morbidity and mortality[1,2]. Independent risk markers, next to the well established cardiovascular risk factors, are useful in identifying those patients with higher risk of cardiovascular events and could serve as therapeutic goal in the treatment of these patients.

At present, higher levels of urinary albumin excretion are considered to be an early manifestation of generalized vascular dysfunction and atherosclerotic damage[3]. Microalbuminuria is thus associated with cardiovascular morbidity and all cause mortality, in both type 1 and type 2 diabetes mellitus, as well as in non-diabetic individuals[4-6].

Inhibitors of the renin-angiotensin system, the angiotensin-converting enzyme (ACE) inhibitors and angiotensin-receptor antagonists, are effective in reducing blood pressure as well as albuminuria and protect renal and cardiac function in patients with diabetes mellitus. These effects appear to be at least partly independent of the associated reduction in blood pressure[7-10]. Recently, a few studies have been published which showed that reduction in albuminuria in hypertensive diabetic patients reduces the risk of subsequent cardiovascular events[11-13]. Again, the question remains whether this risk reduction is not fully explained by the reduction of a too high blood pressure. No data are available of diabetic patients with normal blood pressures. In the present study, we investigated whether rate of change in albuminuria, from baseline over year, is an indicator for adverse cardiovascular outcome in patients with type 2 diabetes mellitus and microalbuminuria, without hypertension.

METHODS

Study design

Previously, we published the results of a randomised, double-blind, placebo-controlled, Dutch multicenter trial, that investigated the short-term effects of the angiotensin-receptor antagonist losartan on urinary albumin excretion rate in normotensive patients with type 2 diabetes mellitus[10]. A significant reduction in albuminuria by 35% was observed after 10 weeks of losartan treatment, which could not be explained by changes in blood pressure or creatinine clearance.

The study described here is a prolonged follow-up study of a group of patients (n=67) from that trial. They were selected on the basis of their address, which was the city of Rotterdam and surrounding areas. After the original 20 week study period, all patients received medical care from their own physician in the outpatient clinics, according to the Dutch standards at that time (not mandated by study protocol). During the follow-up period with a mean of 4.7 (0.8) years, data were collected on current and past health, medication use, blood pressure as well as annual laboratory results of assessment of renal function and urinary albumin excre-

tion. The study was performed according to the guidelines of good clinical practice and was approved by the institutional review board. All persons gave written informed consent prior to participation.

Patients

Microalbuminuric patients with type 2 diabetes mellitus and normal blood pressure were selected for the study. Microalbuminuria was defined as urinary albumin excretion of 20 to 200 µg/min, normotension as a blood pressure less than 140/90 mm Hg[14].

The main exclusion criterion was a history of cardiovascular disease. Patients with ECG abnormalities, acute renal failure, chronic glomerulonephritis, polycystic kidney disease, serum creatinine level of more than 150 µmo/l, HbA_{1c} level of more than 10% or concomitant use of antihypertensive agents, steroids or lithium were excluded as well.

Measurements

Baseline microalbuminuria was measured in two sequential 24-hour urine collections using an immunonephelometric assay on an automated analyser (Beckman Coulter, Brea, California). The presented value for baseline microalbuminuria is the average of two 24-hour urine samples. During follow-up, albuminuria was measured each year in morning spot-urine samples, using the same immunonephelometric assay[15].

Blood pressure was measured by a sphygmomanometer, with the patient in sitting position for 5 minutes before the first measurement. Three replicate measurements, obtained one minute apart, were averaged.

Endpoints

The primary endpoint of the present study was a composite of death, acute myocardial infarction, unstable angina pectoris, coronary interventions, heart failure, cerebrovascular events, and peripheral artery disease. In patients who had multiple endpoints of different types, the time to the first event was counted for the event-free survival time. Secondary endpoints were changes in serum creatinine, glomerular filtration rate, albuminuria and blood pressure.

Statistical analyses

Continuous variables are presented as means and standard deviations (mean (SD)), discrete variables as frequencies and percentages. The paired Student's *t*-test was used for comparisons in the same variables between baseline and during follow-up. Multivariate Cox regression analyses were performed to investigate correlations between baseline variables and the composite cardiovascular endpoint. With regards to the rate of change in urinary albumin excretion from baseline over year, the cohort was divided into three groups: reduction of albuminuria of 30% or more, change of less than 30% (slow reduction or regression), or rapid

progression of albuminuria of 30% or more. The correlation between rate of change in albuminuria from baseline over year and cumulative event free survival was analyzed with the Kaplan–Meier method and the log rank test as well as multivariate Cox regression. The 95% CI of the Hazard ratio was calculated as the exponent of the regression coefficient. Statistical significance was assessed at the 5% level of probability. We used SPSS 12.0.1 for Windows (SPSS Inc., Chicago, Illinois) for all analyses.

RESULTS

Endpoints

Table 1 shows the baseline characteristics of the study population, divided in three groups by change in urinary albumin excretion at one year. The patients were normotensive at baseline, without any antihypertensive treatment. As follows from the inclusion criteria of the original study, they had no history of macrovascular disease. They all had microalbuminuria, with a mean urinary albumin excretion of 73.9 (44.0) mg/l. During the follow-up period of 4.7 (0.8) years, 14 (21%) experienced one of the composite cardiovascular endpoints. Among these 14 patients, two deaths were reported (one because of cerebral haemorrhage, one because of cardiac arrest).

Table 1. Baseline characteristics of the study population, divided in three groups by change in urinary albumin excretion at 1 year

	Stable UAE* (n=16)	**Reduction in UAE (n=36)**	**Progression in UAE (n=15)**
Male sex (n(%))	13 (81)	23 (64)	10 (67)
Age (y)	58.2 (12.0)	54.7 (12.1)	47.2 (10.1)‡
Systolic blood pressure (mm Hg)	137.2 (12.8)	134.3 (11.3)	134.8 (12.3)
Diastolic blood pressure (mm Hg)	78.1 (9.5)	80.9 (6.8)	79.6 (7.4)
Total cholesterol/HDL-C ratio	5.4 (1.7)	5.0 (1.8)	6.0 (1.4)
Current smoking (%)	46	24	45
Serum creatinine (µmol/l)	84.8(20.0)	79.6 (19.4)	73.9 (14.5)
MDRD†	74.1 (33.6)	85.1 (31.3)	92.2 (37.7)
UAE (mg/l)	56.7(32.1)	69.3 (40.0)	84.3(53.1)
Use of RAS§ inhibition in first year of follow-up (%)	94	94	100

Values are given as means (SD) unless otherwise noted

*UAE denotes urinary albumin excretion

†MDRD denotes Modification of Diet in Renal Disease equation

‡p=0.02 for difference in age between Stable UAE and Progression in UAE groups. Other differences in characteristics between groups: ns

§RAS denotes renin-angiotensin system

After finishing the study protocol, 63 (95%) of the 67 patients continued the use of RAS inhibitors because of microalbuminuria. 3 patients did not receive any antihypertensive treatment directly following the trial, and of one patient, the medication used in the first year of follow-up is unknown. At the end of the follow-up period, 62 (93%) patients still used antihypertensive treatment, consisting of inhibitors of the renin-angiotensin system in 98%, in minority in combination with any of the other classes of antihypertensive medication. The mean systolic blood pressure was raised from 135.6 (11.1) mm Hg at baseline to 138.8 (14.9) mm Hg after one year (ns). At the end of the follow-up period, the systolic blood pressure was further raised to 139.8 (15.5) mm Hg (p=0.02 compared to baseline value). Mean diastolic blood pressure did not change significantly (baseline 80.1 (7.3) mm Hg, after one year 79.2 (6.7) mm Hg, end of follow-up period 79.1 (7.0) mm Hg).

Serum creatinine levels were raised from 80.1 (18.3) µmol/l at baseline to 86.5 (22.9) µmol/l at the end of the follow-up period (p<0.001). Estimated glomerular filtration rate, determined with the Modification of Diet in Renal Disease equation, had decreased from 83.3 (32.2) to 67.3 (42.7) (p<0.001). Albuminuria increased from 73.9 (44.0) mg/l at baseline to 81.6 (100.0) mg/l at one year (ns), and further to 130.9 (210.0) mg/l at the end of the follow-up period (p<0.001 compared to baseline). During follow-up, 11 (16.4%) of the patients developed macroalbuminuria (urinary albumin excretion >200 mg/l), while 17 (25.4%) converted from microalbuminuria to normoalbuminuria (urinary albumin excretion <20 mg/l).

The three groups, divided by the change in albuminuria after one year, did not differ significantly in baseline characteristics except for age, which we have corrected for in our multivariate analyses (*table 1*). In the group with stable urinary albumin excretion, levels of albuminuria were 56.7 (32.1) mg/l at baseline, 57.5 (32.7) mg/l after one year and 65.4 (72.4) mg/l at the end of the follow-up period (ns). Mean levels of albuminuria reduced from 69.3 (40.0) mg/l at baseline to 39.3 (26.8) mg/l after one year (p<0.0001) in the group with reduction of urinary albumin excretion, and rose again to 62.0 (76.2) mg/l at the end of the study. For the group with rapid progression of urinary albumin excretion, albuminuria levels were 84.3 (53.1) mg/l at baseline, 223.3 (143.3) mg/l after one year (p<0.01) and 354.1 (352.8) mg/l at the end of follow-up (*figure 1*). For the three groups separately, neither systolic nor diastolic blood pressures changed significantly (*figure 1*).

Correlations change in albuminuria from baseline over year

The Kaplan-Meier curves in figure 2 show a significant difference in event free survival between the three groups of change in albuminuria from baseline over year (p=0.02). Patients with rapid progression of albuminuria were at highest risk to reach the composite endpoint, whereas patients with reduction in albuminuria of 30% or more were at lowest risk. After adjustment for sex, age, systolic blood pressure, total cholesterol over HDL-cholesterol ratio and current smoking in a multivariate Cox regression model, change of albuminuria remained an independent, significant predictor (HR 5.1; 95% CI, 1.5-18.1; p=0.01). Further correction for

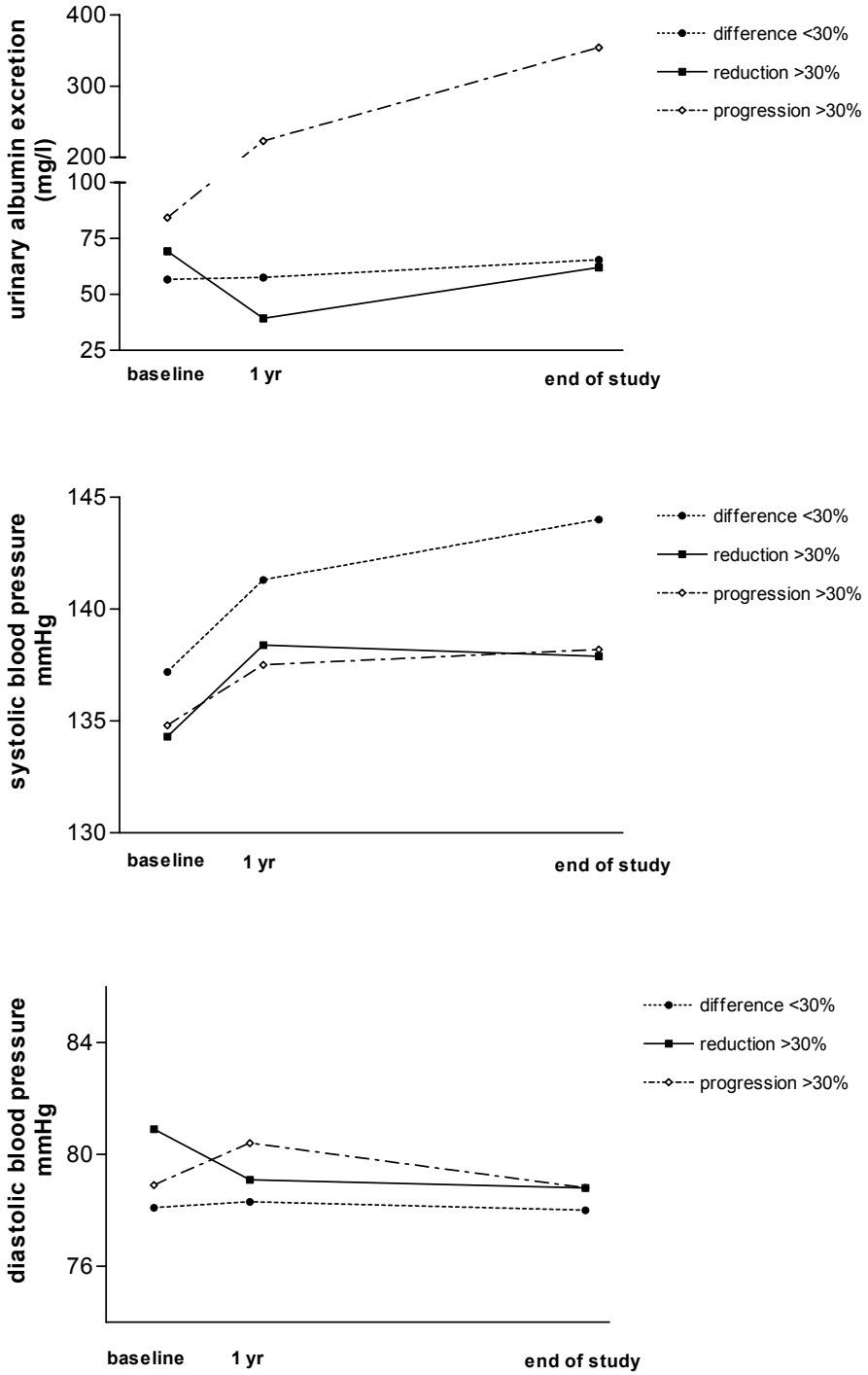

Figure 1. Course of urinary albumin excretion and blood pressure during follow-up in the three groups of change in albuminuria from baseline over year

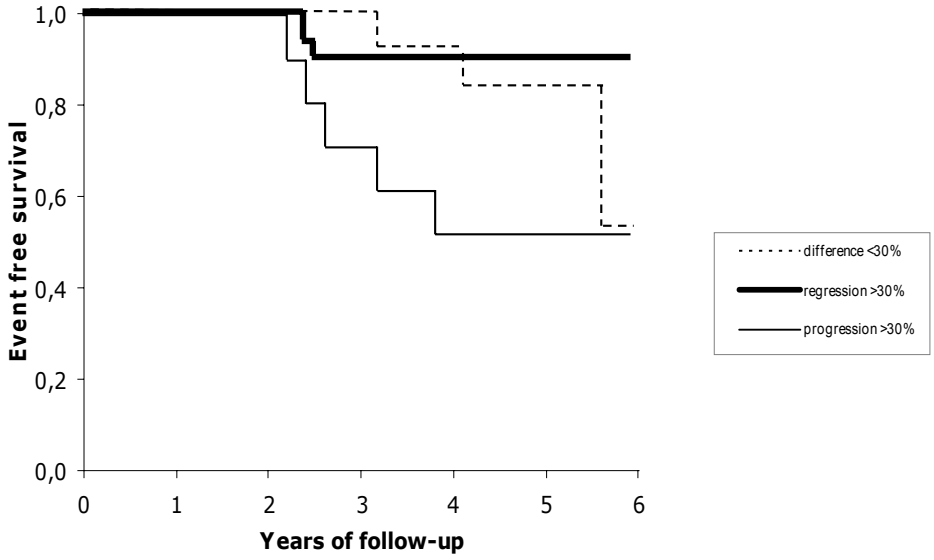

Figure 2. Kaplan-Meier estimates of event-free survival in the three groups of change in albuminuria from baseline over year

baseline albuminuria levels slightly reduced the HR to 4.1 (95% CI 1.2-14.7; p=0.03).

The association between albuminuria at baseline and the incidence of the composite endpoint was no longer significant after adjustment for sex, age, systolic blood pressure, total cholesterol over HDL-cholesterol ratio and smoking (HR 2.6; 95% CI, 0.77-9.04; p=0.1).

DISCUSSION

The results of this study show that normotensive microalbuminuric type 2 diabetic patients run a marked risk for progressive disease despite adequate treatment with inhibition of the renin-angiotensin system. The cardiovascular risk does not depend on changes in classical risk parameters such as blood pressure, but does on the rate of one year change in urinary albumin excretion. Patients with rapid progression of albuminuria had the highest cardiovascular risk, whereas patients with regression of albuminuria of at least 30% had the lowest risk. This association persisted after adjustment for sex, age, systolic blood pressure, total cholesterol over HDL-cholesterol ratio and current smoking. Further adjustment for baseline levels of microalbuminuria only slightly reduced this increased risk. This clearly urges more aggressive cardioprotective treatment in normotensive patients with rising levels of albuminuria.

Microalbuminuria is the first clinical sign of nephropathy in patients with diabetes mellitus[16]. Furthermore, it is also an established strong and independent predictor of macrovascular diseases and all-cause mortality in hypertensive as well normotensive persons, with or

without diabetes mellitus[4-6]. There is still no consensus on the pathophysiological mechanisms of this observation. However, albuminuria is thought to reflect generalized vascular dysfunction, with transvascular leakiness, atherosclerotic damage and thrombosis. All of these mechanisms might contribute to development of cardiovascular and renal diseases[3].

Inhibition of the renin-angiotensin system by means of the ACE inhibitors or angiotensin-receptor antagonists, is effective in reducing blood pressure as well as albuminuria at least partly independent of this blood pressure reduction[7-10]. The average reduction in urinary albumin excretion of these drugs is up to 40%, which is significantly more than with other classes of antihypertensive drugs. In patients with diabetes mellitus type 2, angiotensin-receptor antagonists have shown antiproteinuric and renoprotective effects at all stages of nephropathy.

In addition to these renoprotective effects, it is important to investigate whether therapeutic interventions aimed at lowering albuminuria result in reduction of cardiovascular event risk as well[17,18]. Recently, a few studies on this subject were published. In a population of patients with hypertension (and a subgroup of diabetics) and left ventricular hypertrophy (the LIFE study), reduction in albuminuria explained one-fifth of the reduction in risk of fatal and nonfatal cardiovascular events during losartan-based treatment, compared to atenolol-based treatment[11,19]. Furthermore, the PREVEND IT trial investigated whether treatment with the ACE inhibitor fosinopril effected the cardiovascular event rate in a cohort of patients with microalbuminuria from a general population[20,21]. In that study, fosinopril treatment was associated with a significant reduction in urinary albumin excretion and a non-significant trend in reducing cardiovascular events. These studies still leave the possibility of blood pressure lowering being the driving parameter of cardiovascular risk reduction, and albuminuria changes just being an innocent bystander. To our knowledge, no studies have been published investigating the association between reduction in microalbuminuria and cardiovascular outcome in type 2 diabetic patients without hypertension. This study indeed shows that even with no appreciable changes or even rises in blood pressure, the change in albuminuria differentiates the cardiovascular outcome of the normotensive group of patients. This clearly urges for a more aggressive approach of albuminuria, when it remains high.

A potential weakness of this study is the small sample size. Nonetheless, the association we observed between changes in albuminuria and cardiovascular outcome were statistically significant in multivariate analysis. Furthermore, albuminuria was measured in morning spot-urine samples during follow-up, whereas baseline albuminuria was measured in two sequential 24-hour urine collections. Although urinary albumin levels may exhibit considerable intraindividual variability, previous studies revealed a close correlation between these two methods[15]. The strength of our study lies in the fact that we studied type 2 diabetic patients with microalbuminuria, but without hypertension at baseline, in a prospective design. Clearly, this study needs further follow-up in larger cohorts.

In summary, non-hypertensive patients with type 2 diabetes mellitus and microalbuminuria, run a risk for cardiovascular and renal morbidity and mortality, despite adequate treatment. Interestingly, the reduction in urinary albumin excretion predicted the cardiovascular risk for these patients. Our results confirm and extend previous observations that urinary albumin excretion can and should be used as modifiable treatment goal.

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IV

General discussion and summary

IV.1

General discussion

EFFECTS OF LOSARTAN ON ALBUMINURIA AND RENAL FUNCTION IN NORMOTENSIVE PATIENTS WITH TYPE 2 DIABETES MELLITUS AND MICROALBUMINURIA

The majority of clinical trials, investigating the effects of angiotensin-receptor antagonists on the diabetic kidney, have focused on hypertensive patients, thereby leaving the possibility that reduction of the systemic blood pressure explains for (most of) the renoprotective effects[1-5]. Therefore, we studied the effects of the angiotensin-receptor antagonist losartan on urinary albumin excretion, renal function and blood pressure in normotensive patients with type 2 diabetes mellitus and microalbuminuria. The significant reduction in urinary albumin excretion rate by 35%, which we observed after 10 weeks of losartan treatment, is quantitatively comparable to the antiproteinuric effects of losartan in hypertensive diabetic patients[1,2,4,6,7]. In our normotensive patients, this reduction could not be explained by changes in systemic blood pressure or creatinine clearance, suggesting the involvement of other losartan-mediated mechanisms.

First, antagonising the intrarenal angiotensin II leads to favorable intraglomerular haemodynamics, like reduction in arteriolar resistance and intraglomerular pressure, thereby increasing renal plasma flow, reducing the filtration fraction and thus urinary albumin excretion[8,9]. These changes mainly account for the early rapid component of albuminuria reduction, which parallels the time-course of changes in renal haemodynamics and is thought to be maximal within the first weeks of treatment[10,11]. Our results of the order of magnitude of the antiproteinuric effect after 5 respectively 10 weeks of losartan treatment, as well as the fact that urinary albumin excretion returned to pretreatment levels in the 5-week placebo washout period, are in concordance with these observations and thus probably result mostly from renal haemodynamic factors.

Secondly, losartan treatment probably results in slower, non-haemodynamic renal changes as well, contributing to the well-established long-term renoprotective effects of angiotensin II antagonists in patients with diabetes mellitus[3-5]. These changes possibly involve improvement of endothelial function and reduction of the chronic low-grade inflammatory state[12,13]. However, we have no data on this in our patient population to support this assumption in our studies. Furthermore, the interference of losartan with various growth factor systems is thought to reduce the production of extracellular matrix proteins, finally resulting in slowing down the progression of glomerulosclerosis and tubulointerstitial fibrosis[14].

Concerning the above-mentioned growth factor systems and diabetic nephropathy, we focused on the GH/IGF-I system in this thesis, and studied the effects of losartan on this system in order to further clarify the working mechanisms of the renoprotective effects of losartan in normotensive patients. After 10 weeks of losartan treatment, we observed a tendency towards reduction in the IGFBP-3 levels, which were elevated at baseline. In the subanaly-

sis of patients with stable metabolic parameters, this reduction was statistically significant. Furthermore, serum levels of free IGF-I showed a tendency to rise in the losartan treated patients, and urinary GH levels tended to decrease. These changes were not related to the reduction in microalbuminuria, confirming our hypothesis that renal haemodynamics play the most important role in this reduction.

However, the changes in the IGF-I system after losartan treatment correlated with creatinine clearance and serum creatinine levels. Lower IGFBP-3 levels were associated with improvement of creatinine clearance in the whole losartan treated group, as well as with lower levels of serum creatinine in the metabolically stable group. Besides, the changes in total serum IGF-I concentrations correlated positively with the changes in serum creatinine levels in the metabolically stable group, meaning lower serum levels of creatinine were associated with higher levels of total serum IGF-I. We could not demonstrate a significant correlation between urinary GH levels and creatinine clearance or serum creatinine levels. Although we treated our patients for only 10 weeks, these observations implicate a role for the IGF-I system in the long-term renoprotective effects and stabilization of creatinine clearance, associated with losartan treatment[1,3-5].

LOSARTAN AND THE IGF-I SYSTEM

The RAS interacts with the IGF-I system in several ways. These interactions are complex and not fully clarified. Most of the literature on this subject studied cell cultures or animal models. Importantly, angiotensin II has been shown to inhibit IGF-I and IGFBP-3 expression in cultured liver cells[15]. This observation was confirmed in a study in rats, demonstrating that circulating levels of IGF-I were reduced by angiotensin II infusion through reduction of the hepatic IGF-I mRNA levels, which was reversed by losartan[16,17]. Limited clinical trials have been performed investigating the effects of RAS inhibition on the IGF-I system in humans. A study in patients with congestive heart failure showed lowered plasma levels of IGF-I, which were restored during chronic ACE inhibition therapy, most likely by reducing angiotensin II[18]. Furthermore, a few studies demonstrated improvement of muscle strength and exercise tolerance in patients with congestive heart failure, treated with a variety of ACE inhibitors or losartan, which hypothetically might have been the result from an increase in IGF-I levels (not assessed in the studies)[19,20]. A recent study focused on the relationship between the use of ACE inhibitors and serum levels of total IGF-I in individuals aged over 65 years. This study showed significantly higher levels of total IGF-I in participants treated with ACE inhibitors for less than 3 years compared with the rest of the study population. Serum levels of free IGF-I or IGFBP-3 were not studied[21].

To our knowledge, no clinical trials investigated the effects of RAS inhibition on the IGF-I system in individuals with diabetes mellitus. In accordance with the well-known disturbances

in the IGF-I system in patients with diabetes mellitus[22], we observed reduced levels of free IGF-I and elevated levels of IGFBP-3 before start of treatment. Total IGF-I was within normal ranges, which is in agreement with previous observations that the unbound fraction of IGF-I is of more clinical relevance and involved in regulatory mechanisms and biological effects[23,24]. As expected, strong mutual correlations existed between serum levels of total IGF-I, free IGF-I and IGFBP-3. Furthermore, urinary log GH correlated significantly with serum free IGF-I, reflecting an intact feedback mechanism. Losartan tended to raise serum levels of free IGF-I in our patients, probably by increased hepatic IGF-I production caused by improvement of hepatic insulin sensitivity (see below) and/or by discontinuing the inhibition of hepatic IGF-I production through blocking angiotensin II. These raised free IGF-I levels might have contributed to the tendency towards reduction in urinary GH levels after losartan treatment, because of the negative feedback of IGF-I at the pituitary level on GH production. Serum levels of total IGF-I were unaffected by losartan.

However, our hypothesis must be interpreted with the fact that the increase in free IGF-I and the reduction in urinary GH during losartan treatment both were not statistically significant in our diabetic patients. Predominantly, the small sample size in combination with the large variation in free IGF-I and GH values probably accounts for this, since the production and bioactivity of different variables of the GH/IGF-I system are influenced by many factors, including the degree of insulin resistance and the amount of body fat, endogenous insulin production, the portal levels of insulin, the kind of diabetes treatment and the degree of metabolic control[22,25]. The studies described in chapter II.2 and II.3 were substudies from the randomised clinical trial investigating the effects of losartan on microalbuminuria (chapter II.1), and that study did not assess insulin resistance (other than HbA_{1c}) or insulin levels, nor required that diabetes treatment was maintained stable during follow-up. This probably affected the effects on the different variables of the GH/IGF-I system that were measured. In the subgroup of patients with metabolically stable parameters, the rise in free IGF-I was more pronounced and the reduction in IGFBP-3 became statistically significant, which confirms the influence of metabolic parameters on the GH/IGF-I system.

Furthermore, we investigated the effects of short-term treatment with losartan on serum IGF-I levels and insulin resistance in individuals with impaired fasting glucose, who were not taking other medication, and therefore formed a metabolic more homogenous study group (chapter II.4). After 8 weeks of losartan treatment, serum levels of free IGF-I had raised significantly. Unfortunately, we did not measure GH levels in these patients. The results of this study confirm the hypothesis that blocking angiotensin II increases the production of IGF-I. The effects we observed on insulin resistance in these patients are discussed in more detail below.

Losartan reduced after 10 weeks of treatment the elevated serum levels of IGFBP-3 in our diabetic patients, which related to improvement in creatinine clearance. We have tried to elu-

cidate the underlying mechanisms of this observation. First, since the production of IGFBP-3 depends on GH, GH hypersecretion is likely to have contributed to the elevated baseline levels. Subsequently, we hypothesised that the reduction in IGFBP-3 levels after losartan partly resulted from the reduced GH levels. However, urinary GH did not significantly correlate to serum IGFBP-3 in our study, again probably due to the small sample size combined with the variation in urinary GH levels.

Furthermore, in patients with insulin resistance and diabetes mellitus, as well as in a variety of kidney diseases including diabetic nephropathy, IGFBP-3 proteolysis was found to be increased in order to improve IGF-I bioavailability[22,25,26]. We used a radioimmunoassay to assess IGFBP-3 levels which measures IGF-I bound to BP-3, as well as the disintegrated fragments of the IGFBP-3 complex derived from proteolysis of the complex. Therefore, the decrease in IGFBP-3 levels that we observed during losartan treatment might originate from reduced proteolysis as well, for example resulting from improvement of insulin sensitivity associated with losartan.

Finally, it is unknown whether RAS inhibition has direct effects on the production or proteolysis of IGFBP-3. There is no literature on this subject, and we did not investigate it in our studies.

LOSARTAN AND INSULIN RESISTANCE

Because type 2 diabetes mellitus represents a substantial and growing health burden, with a rapidly increasing prevalence, interventions to prevent or delay its onset are of major interest. Lifestyle interventions by means of caloric restriction and regular exercise have well-established favourable effects and reduce the progression to diabetes by more than 50% in high-risk individuals[27,28]. However, since maintaining adherence to these interventions has shown to be exceptionally difficult, the use of pharmacologic agents to prevent or delay new-onset diabetes is becoming more important. In this perspective, different antihypertensive drug classes are being investigated, since they affect the glucose homeostasis in several ways and are frequently used in patients at high risk for developing type 2 diabetes mellitus.

First, thiazide diuretics and ß-blockers seem to aggravate glycaemic control through reduction of pancreatic insulin secretion and peripheral insulin sensitivity[29]. Moreover, ß-blockers diminish peripheral blood flow and stimulate α2-receptor mediated glycogenolysis, thereby contributing to further impairment of glucose homeostasis[29,30]. The class of the calcium channel blockers is thought to have neutral metabolic effects[29]. Recent studies suggest beneficial metabolic effects of several ACE inhibitors as well as angiotensin-receptor antagonists, which might result in a reduced risk of developing type 2 diabetes[29-38]. For example, the Captopril Prevention Project (CAPPP) trial showed a relative risk reduction of 14% in the incidence of type 2 diabetes in hypertensive patients after treatment with the ACE inhibitor captopril,

compared with thiazide diuretics or ß-blockers (mean follow-up: 6.1 years)[31]. In the Heart Outcomes Prevention Evaluation (HOPE) trial, ramipril reduced the risk of new-onset diabetes by 34% compared with placebo in patients with known cardiovascular disease (mean follow-up 4.5 years)[32]. Moreover, the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) showed a relative risk reduction of 30% with lisinopril compared with placebo (mean follow-up 4 years)[33]. Concerning the angiotensin-receptor antagonists, the relative risk reduction of new-onset diabetes averaged 25% in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE) with losartan compared with placebo (mean follow-up 4.8 years)[34], and 23% in the Valsartan Antihypertensive Long-term Use Evaluation (VALUE) trial with candesartan versus amlodipine (mean follow-up 4.2 years)[35].

Importantly, in all of the abovementioned trials, development of diabetes was considered as a secondary endpoint or studied by post-hoc analysis, which necessitates toning down the results. Furthermore, a part of the studies compared RAS inhibitors to diuretics or ß-blockers, thereby possibly overestimating the effects of RAS inhibition. Large-scale prospective placebo-controlled clinical trials are necessary, designed to assess the protective benefits of RAS inhibition in subjects at risk of developing type 2 diabetes[36,37]. However, the results of our prospective, hypothesis-generating study, investigating the effect of short-term administration of losartan on insulin sensitivity in patients with impaired fasting glucose (chapter II.4), are in accordance with the previous observations. In that study, insulin resistance, measured using HOMA and 2-h CIGMA, decreased after 8 weeks of losartan treatment. Obviously, the small sample size is an important limitation of our study. In addition, although insulin resistance is one of the main predictors of the development of type 2 diabetes mellitus, a long follow-up period is warranted to investigate whether losartan truly delays or prevents this disease.

Recently, the first large-scale, double-blind randomized clinical (DREAM) trial was published, that investigated the effects of the ACE inhibitor ramipril, compared with placebo, on the incidence of newly diagnosed diabetes in patients with impaired fasting glucose or impaired glucose tolerance without previous cardiovascular disease[39,40]. After the mean follow-up of 3.0 years, ramipril appeared not to prevent diabetes in these patients. Nonetheless, significantly more patients on ramipril treatment returned to normal fasting glucose levels and normal glucose tolerance, than did the patients who received placebo. Additional prospective trials investigating the role of RAS inhibition in preventing or delaying type 2 diabetes mellitus are presently ongoing (the ONTARGET and TRANSCEND trials, which study the effects of the angiotensin-receptor antagonist telmisartan as well as of ramipril, and the NAVIGATOR trial, which studies the angiotensin-receptor antagonist valsartan)[41].

The mechanisms involved in the effects of RAS inhibition on glucose metabolism and insulin sensitivity are complex and still not fully elucidated. Several physiological pathways are included. First, peripheral vasodilatation results in improved blood flow to the skeletal muscles and other tissues, thereby facilitating insulin and glucose delivery as well as insu-

lin-mediated glucose utilisation[42,43]. Besides, the increased microcirculation in the endocrine pancreas potentially improves ß-cell function and insulin secretion[43]. It has also been suggested that endothelial function improves with treatment with ACE inhibitors and angiotensin-receptor antagonists, resulting in increased vascular sensitivity to insulin[44]. Previously, an association has been suggested between overactivity of the sympathetic nervous system and hypertension, insulin resistance and subsequently hyperinsulinemia[45]. Since RAS inhibition has been shown to reduce the levels of circulating catecholamines, reduction in activity of the sympathetic nervous system possibly contributes to improvement of insulin resistance with RAS inhibitors[43,46].

Angiotensin II also has several effects on fat tissue and adipocytes. Increased levels of angiotensin II appear to inhibit the differentiation of adipocytes, thereby impairing their fat-storage capacity. This results in a shift towards fat-storage in the liver, the skeletal muscles and the pancreas and thus aggravation of insulin resistance[36,47]. Additionally, angiotensin II seems to reduce plasma levels of adiponectin, an adipocytokine known to enhance insulin action[43]. Inhibition of the RAS is likely to modulate these metabolic disturbances in a favourable way. Furthermore, some of the angiotensin-receptor antagonists seem to induce peroxisome proliferator-activated receptor-gamma (PPARγ) activity in the adipocytes, which increases insulin sensitivity[48,49]. Finally, RAS inhibitors have shown to improve insulin-signalling pathways and cellular insulin action[43].

Improvement of the disturbed GH/IGF-I system in patients with insulin resistance and type 2 diabetes mellitus might be another contributory mechanism whereby inhibitors of the RAS improve insulin sensitivity and probably interfere in the development of type 2 diabetes. In our hypothesis-generating study (chapter II.4), the reduction in insulin resistance after short-term losartan treatment did just not significantly correlate with the increase in serum levels of free IGF-I. This corresponds with the well-known insulin-like involvement of IGF-I in stimulating peripheral glucose uptake and glycogen synthesis, as well as decreasing hepatic glucose production[50].

Although we did not measure GH levels in those patients with impaired glucose tolerance, it can be hypothesised that reduced GH production, resulting from the increased IGF-I concentration, contributes to the improvement of insulin sensitivity, regarding the opposite effects of GH on glucose homeostasis[50]. After all, in our studies described in chapter II.2 and II.3, in which we investigated the losartan associated changes in serum IGF-I, urinary GH and serum IGFBP-3, the rise in free IGF-I levels was accompanied by a reduction in urinary GH.

Serum levels of free IGF-I correlated significantly with serum levels of IGFBP-3, but the reduction in urinary GH did not significantly correlate with the reduction in serum IGFBP-3 after losartan treatment. Our small sample size possibly contributed to this. However, as stated previously, the reduction in IGFBP-3 might originate from reduced proteolysis of the IGF-I/IGFBP-3 complex, possibly as a result of improvement of insulin sensitivity associated

with losartan, besides reduction of GH dependent IGFBP-3 production. In addition to the improved balance in the GH/IGF-I system, losartan-mediated effects on above-mentioned haemodynamic as well as other non-haemodynamic pathways are likely to be involved in the improvement of insulin sensitivity.

CLINICAL OUTCOME AND IMPORTANCE OF INFLUENCING ALBUMINURIA

In addition to microvascular complications like retinopathy and nephropathy, type 2 diabetes mellitus is associated with an increased risk of coronary heart disease (CHD), cerebrovascular disease and peripheral artery disease[51-58]. A previously published population-based study revealed a 7-year incidence of first myocardial infarction or death in 20% of the patients with type 2 diabetes, whereas this incidence was 3.5% in comparable individuals without diabetes[52]. Furthermore, this and other studies showed that patients with type 2 diabetes without previous coronary events have a risk of developing CHD as high as patients without diabetes but with previously encountered coronary disease[52-54]. Type 2 diabetes mellitus and insulin resistance have shown to increase the risk of stroke by 2- to 3-fold in men and by 4- to 5-fold in women, compared with non-diabetic persons[55-57]. In these patients, hypertension, duration of diabetes and metabolic control were important risk factors for development of stroke[56]. Also in the case of peripheral artery disease, type 2 diabetes is associated with at least a 2- to 4-fold increased risk[51,58]. In addition to the higher incidence of macrovascular diseases, diabetes associates with worse disease manifestation and poorer outcome and prognosis[51-58].

In general, type 2 diabetes mellitus increases the risk to encounter macrovascular diseases at least 2 to 4 times. Markedly, type 2 diabetes seems to confer a higher risk in women as compared to men[59-63]. Several studies showed estimates of cardiovascular morbidity and mortality in diabetic women ranging from 2- to 5 fold the rate in nondiabetic women, whereas in diabetic men, estimates vary from 1- to 3-fold the rate in nondiabetic men[59-61]. The pathogenesis for this excess risk in women with type 2 diabetes is uncertain. In a number of studies, differences in the distribution of other well-known risk factors (such as hypertension or dyslipidemia) explained the sex difference in cardiovascular disease risk to a great extent[64,65]. For example, a previously published meta-analysis established an estimate of the odds ratio for fatal and nonfatal cardiovascular disease due to diabetes in both women and men. The authors concluded that after adjusting for the classical, modifiable cardiac risk factors, the difference in risk between women and men is modest and not statistically significant[65]. However, prospective data on this subject are limited.

Chapter III.1 describes the results of the prospective follow-up study, which we performed in 67 of the normotensive patients with type 2 diabetes mellitus and microalbuminuria that participated in the earlier randomised trial (chapter II.1). During the follow-up period

of 4.7±0.1 (±SEM) years, 14 (21%) patients experienced one of the composite cardiovascular endpoints. This observation is in accordance with the predicted risk at baseline, namely 20.0±1.6%, when calculated using the Framingham risk score[66].

In our study population of normotensive patients with type 2 diabetes mellitus and microalbuminuria, female gender was associated with an significant increased risk of fatal and non-fatal cardiovascular diseases: 8 (38.1%) of the women had a cardiovascular event during 85.9 person years, whereas 6 (13.4%) of the men experienced one of the composite endpoints during 198.5 person years. Adjustment for the classical cardiovascular risk factors in multivariable analyses further increased the excess risk of the women. Moreover, the severity of nephropathy or presence of retinopathy, or health care utilisation could not explain the sex difference. Therefore, we were not able to unravel the underlying pathogenesis for the excessive cardiovascular risk in women with type 2 diabetes, while it was also not possible to indicate at baseline those patients with the highest risk. Hopefully, future research will succeed in doing this.

In the prolonged follow-up study of our patient population, we also investigated the course of renal function, albuminuria and blood pressure. We demonstrated that normotensive microalbuminuric type 2 diabetic patients, especially the women, run a marked risk for progressive disease and complications. Therefore, independent risk markers, besides the well known cardiovascular risk factors, are desired in identifying high-risk patients and could serve as therapeutic goal in the treatment of these patients. Because of the strong correlation between microalbuminuria and cardiovascular diseases and all-cause mortality[67-70], we focused on the usefulness of the rate of change in urinary albumin excretion at one year as predictor of cardiovascular complications and death in our follow-up study. The results showed that patients with rapid progression of albuminuria at one year had the highest cardiovascular risk, whereas patients with regression of albuminuria of at least 30% had the lowest risk. This association persisted after adjustment for sex, age, systolic blood pressure, lipids and smoking.

ACE inhibitors and angiotensin-receptor antagonists are effective in reducing blood pressure, as well as in preserving renal and cardiac function in patients with diabetes mellitus[3,4,71-73]. In addition, they appeared to reduce urinary albumin excretion up to 40%, which is more than the other classes of antihypertensive drugs[71]. Therefore, since albuminuria is strongly associated with cardiovascular outcome, changes in albuminuria during treatment might reflect changes in cardiovascular disease risk. Recently, a few studies showed that reduction in albuminuria in hypertensive patients with diabetes reduces the risk of subsequent cardiovascular events[74-76]. However, since they studied hypertensive patients, these studies still leave the possibility of blood pressure lowering being the explanation for the cardiovascular risk reduction, and albuminuria changes just being an innocent bystander.

To our knowledge, our study described in chapter III.2 is the first one demonstrating that change in urinary albumin excretion differentiates the cardiovascular outcome in type 2 diabetic patients without hypertension. In that study, sustained reduction in albuminuria reflected reduction in risk of cardiovascular complications. Hence, urinary albumin excretion during treatment seems to be a sign of therapeutic responsiveness independent of blood pressure changes, and thus can be used as modifiable treatment goal. These observations advocate more aggressive approach of albuminuria as well as more aggressive cardioprotective treatment in normotensive diabetic patients with remaining raised levels of albuminuria.

PERSPECTIVES FOR FUTURE RESEARCH

New research questions are raised by the studies described in this thesis. We have demonstrated the beneficial effects of RAS inhibition on urinary albumin excretion in normotensive patients with type 2 diabetes mellitus and microalbuminuria, as well as the importance of albuminuria reduction regarding cardiovascular outcome. Recently, the Bergamo Nephrologic Diabetes Complication Trial (BENEDICT) showed that in patients with type 2 diabetes and arterial hypertension, but with normoalbuminuria, ACE-inhibitor therapy prevented or delayed the onset of microalbuminuria[77]. It would be interesting to investigate whether RAS inhibition can prevent microalbuminuria in diabetic patients without hypertension, and if so, whether this reflects reduction in cardiovascular morbidity and mortality as well.

The role of the IGF-I system in the development of diabetic nephropathy and in the long-term renoprotective effects of RAS inhibition needs further investigation. Moreover, large-scale prospective placebo-controlled clinical trials are necessary to assess the possible protective benefits of RAS inhibition in subjects at risk of developing type 2 diabetes. Further clarification of the interactions between the IGF-I system and type 2 diabetes will improve our understanding of the pathogenesis of this disease, which ultimately might result in new preventive interventions.

Finally, the underlying pathogenesis for the sex differences in cardiovascular outcome needs to be elucidated. Normotensive women with type 2 diabetes mellitus require more aggressive cardioprotective treatment, of which the effects on risk reduction need further evaluation.

CONCLUSIONS

1. Short-term treatment with losartan reduces urinary albumin excretion in normotensive patients with type 2 diabetes mellitus and microalbuminuria, independent of changes in

systemic blood pressure or creatinine clearance, and most likely resulting from improved renal haemodynamic factors.

2. The correlation between the effects of losartan on the different variables of the IGF-I system (rise in serum free IGF-I, reduction of urinary GH and serum IGFBP-3) and creatinine clearance implicates a role for the IGF-I system in the long-term renoprotective effects of losartan treatment.
3. The changes in the IGF-I system seem to result from improved insulin sensitivity (partly because of reduced unbalances in the GH/IGF-I system and partly because of other mechanisms), and from increased hepatic IGF-I production and subsequently reduced GH production, associated with losartan treatment.
4. Short-term administration of losartan improves insulin sensitivity in patients with impaired fasting glucose. The effects of losartan on the GH/IGF-I system probably contribute to this improvement, most likely in addition to effects of losartan on haemodynamic as well as other non-haemodynamic pathways involved in insulin sensitivity. Whether losartan delays or prevents new-onset type 2 diabetes needs to be further investigated.
5. Female gender is an important risk factor for the development of macrovascular morbidity and mortality in normotensive patients with type 2 diabetes mellitus, independent of the classical cardiovascular risk factors. Furthermore, neither the severity of nephropathy, the presence of retinopathy nor health care utilization explains this observation.
6. In patients with type 2 diabetes mellitus and microalbuminuria without hypertension, sustained reduction in urinary albumin excretion reflects reduction in risk of cardiovascular complications. Urinary albumin excretion during treatment reflects therapeutic responsiveness in these patients, and therefore can be used as modifiable treatment goal.

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IV.2

Summary

The prevalence of type 2 diabetes mellitus increases rapidly. Type 2 diabetes is associated with an increased risk of microvascular and macrovascular diseases, like retinopathy, nephropathy, coronary heart disease, cerebrovascular disease and peripheral artery disease, thereby comprising a major health problem. About 40% of the patients with type 2 diabetes mellitus develop diabetic nephropathy, ranging from microalbuminuria to end-stage renal disease. Diabetic renal disease represents a significant cause of morbidity and mortality in patients with diabetes mellitus.

In the **Introduction** section of this thesis, the pathogenesis and natural course of diabetic nephropathy are described, thereby focusing on urinary albumin excretion as first clinical sign of diabetic renal disease as well as predictor of subsequent cardiovascular morbidity and mortality. Furthermore, the role of the renin-angiotensin system (RAS) and the growth hormone/insulin-like growth hormone-I (GH/IGF-I) system in the pathogenesis of diabetic nephropathy and glucose homeostasis are outlined.

Inhibition of the RAS, by means of the angiotensin I converting enzyme (ACE) inhibitors and the angiotensin receptor antagonists, has been shown to preserve renal function and reduce albuminuria in patients with diabetes mellitus. The majority of these studies, in particular those investigating the angiotensin receptor antagonists, were performed in hypertensive patients with type 2 diabetes mellitus, thereby leaving the possibility that reduction of the systemic blood pressure explains for (most of) the renoprotective effects. Therefore, we studied the effects of the angiotensin receptor antagonist losartan on urinary albumin excretion and renal function in patients with type 2 diabetes and microalbuminuria, without hypertension. As described in **chapter II.1**, we observed a significant reduction in urinary albumin excretion rate by 35% after 10 weeks of losartan treatment, which is quantitatively comparable to the antiproteinuric effects of losartan in hypertensive diabetic patients. In our normotensive patients, this reduction could not be explained by changes in systemic blood pressure or creatinine clearance. In this short-term treatment period, the antiproteinuric effects of losartan most likely result from improved renal haemodynamic factors.

To further reveal the working mechanisms of angiotensin receptor antagonists in normotensive patients with type 2 diabetes, we studied the GH/IGF-I system in our study population, as well as the effects of losartan on this system (**chapter II.2** and **II.3**). Losartan reduced the serum levels of IGFBP-3, which were elevated at baseline. Furthermore, serum levels of free IGF-I (which were reduced at baseline) tended to raise in the losartan treated patients, and urinary GH levels tended to decrease. These changes were not related to the reduction in microalbuminuria, confirming our hypothesis that renal haemodynamics play the most important role in this reduction. However, the changes in the IGF-I system did correlate with changes in creatinine clearance and serum creatinine levels, implicating a role for the IGF-I system in the long-term renoprotective effects and stabilisation of creatinine clearance, associated with losartan treatment. As also outlined in **chapter II.2** and **II.3**, we hypothesised that

the observed changes in the IGF-I system might result from increased hepatic IGF-I production and subsequently reduced GH production, as well as from improved insulin sensitivity (partly because of reduced unbalances in the GH/IGF-I system and partly because of other mechanisms) associated with losartan treatment.

Recently, RAS inhibition has been suggested to play a role in reducing the risk of developing type 2 diabetes mellitus. **Chapter II.4** describes a study in which we investigated the effects of losartan on insulin resistance, together with the effects on IGF-I metabolism, because of the importance of the IGF-I system in glucose homeostasis. In individuals with impaired fasting glucose, we demonstrated that short-term losartan treatment was associated with reduction in insulin resistance. This reduction correlated close to significantly with the observed increase in serum levels of free IGF-I, associated with losartan treatment. The effects of losartan on different variables of the GH/IGF-I system probably contribute to improvement of insulin sensitivity, most likely in addition to effects of losartan on haemodynamic as well as other non-haemodynamic pathways involved in insulin sensitivity (**chapter II.4** and **General discussion**). Whether RAS inhibition actually delays or prevents new-onset type 2 diabetes needs further investigation in large-scale, long-term, prospective clinical trials.

Type 2 diabetes mellitus increases the risk of developing cardiovascular complications. Markedly, type 2 diabetes seems to confer a higher risk in women as compared to men. The pathogenesis for this excess risk in women in uncertain. **Chapter III.1** reports the results of the prospective follow-up study (mean follow-up 4.7±0.1 (±SEM) years), which was performed in a portion of the normotensive type 2 diabetic patients with microalbuminuria, that participated in the earlier randomised trial (**chapter II.1**). We observed that in our population, female gender was associated with an increased risk of cardiovascular morbidity and mortality. This increased risk was independent of the classical cardiovascular risk factors, the severity of nephropathy or presence of retinopathy, or health care utilization. Therefore, we were not able to unravel the underlying pathogenesis for the excessive cardiovascular risk in women with type 2 diabetes, while it was also not possible to indicate at baseline those patients with the highest risk.

As stated above, microalbuminuria is associated with cardiovascular morbidity and all-cause mortality, in addition to being the first clinical sign of diabetic nephropathy. Recently, a few studies showed that reducing albuminuria reduces the risk of cardiovascular complications in hypertensive patients with diabetes mellitus. Whether this risk reduction is associated with blood pressure changes or with albuminuria changes separately, still needs elucidation. Therefore, we focused on the usefulness of the rate of one year change in urinary albumin excretion as predictor of cardiovascular outcome in the follow-up study of our normotensive patients in **chapter III.2**. The results showed that patients with rapid progression of albuminuria at one year had the highest cardiovascular risk, whereas patients with regression of albuminuria of at least 30% had the lowest risk. Consequently, urinary albumin excretion dur-

ing treatment reflects therapeutic responsiveness independent of blood pressure changes, and therefore can be used as modifiable treatment goal.

Chapter IV.1 contains a general discussion in which our results are placed into broader perspective. Several aspects of the underlying mechanisms of the effects of losartan on renal function in normotensive patients with type 2 diabetes are discussed, as well as of the interaction between losartan and the GH/IGF-I system. Furthermore, we outlined potential physiological pathways that are involved in the effects of RAS inhibition on glucose metabolism and insulin sensitivity. We discussed clinical outcome as well the importance of influencing albuminuria in normotensive patients with type 2 diabetes mellitus and microalbuminuria. Finally, perspectives for future research subjects were described.

IV.3

Samenvatting

De prevalentie van type 2 diabetes mellitus stijgt snel. Type 2 diabetes is geassocieerd met een verhoogd risico op micro- en macrovasculaire complicaties, zoals retinopathie, nefropathie, cardiovasculaire en cerebrovasculaire ziekten, alsmede perifeer vaatlijden. Het vormt dan ook een groot en groeiend gezondheidsprobleem. Ongeveer 40% van de patiënten met type 2 diabetes mellitus ontwikkelt diabetische nefropathie, in ernst variërend van microalbuminurie tot terminale nierinsufficiëntie. Diabetische nefropathie is een belangrijke oorzaak van morbiditeit en mortaliteit bij patiënten met diabetes mellitus.

In de **Introductie** van dit proefschrift wordt de pathogenese en het natuurlijk beloop van diabetische nefropathie beschreven. De nadruk ligt hierbij op verhoogde eiwituitscheiding in de urine (albuminurie), de eerste klinische uiting van nierschade ten gevolge van diabetes, en tevens voorspeller van cardiovasculaire ziekte en mortaliteit. Daarnaast wordt de rol van het renine-angiotensine systeem (RAS) en van het groeihormoon/insulin-like growth factor-I (GH/IGF-I) systeem besproken in de pathogenese van diabetische nefropathie en glucose homeostase.

Remming van het RAS, door middel van de angiotensine I converting enzyme (ACE) remmers en de angiotensine receptor antagonisten, heeft een nierfunctie beschermende werking en reduceert albuminurie bij patiënten met diabetes mellitus. De meerderheid van deze studies (met name de studies met angiotensine receptor antagonisten) is uitgevoerd bij hypertensieve patiënten met type 2 diabetes mellitus. Omdat daling van de systemische bloeddruk in deze studies (een deel van) de renoprotectieve effecten zou kunnen verklaren, hebben wij de effecten onderzocht van de angiotensine receptor antagonist losartan op eiwitexcretie in de urine en nierfunctie in patiënten met type 2 diabetes mellitus en microalbuminurie, zonder hypertensie. Na 10 weken behandeling met losartan werd een significante reductie van 35% in eiwitexcretie aangetoond (**hoofstuk II.1**). Dit is kwantitatief vergelijkbaar met het antiproteinurische effect van losartan in hypertensieve patiënten met diabetes. Deze reductie in eiwitexcretie kon in onze normotensieve populatie niet worden verklaard door veranderingen in systemische bloeddruk of kreatinineklaring, en werd zeer waarschijnlijk veroorzaakt door verbetering van de renale haemodynamiek in deze korte-termijn studie.

Wij bestudeerden het GH/IGF-I systeem in onze studiepopulatie, alsmede de effecten van losartan op dit systeem, om het werkingsmechanisme van angiotensine receptor antagonisten in normotensieve patiënten met type 2 diabetes nader te onderzoeken (**hoofdstuk II.2 en II.3**). De serum concentratie IGFBP-3 was verhoogd aan het begin van de studie, en daalde na behandeling met losartan. Daarnaast vertoonde de serum concentratie vrij IGF-I (die verlaagd was aan het begin van de studie) een tendens tot stijgen in de met losartan behandelde patiënten, en de urine concentratie van GH een tendens tot dalen. Deze veranderingen waren niet gerelateerd aan de reductie in microalbuminurie, hetgeen de rol van verbeterde renale haemodynamiek onderschrijft in het antiproteinurische effect van losartan. De veranderingen in het GH/IGF-I systeem waren wel gecorreleerd met veranderingen in kreatinineklaring en serum kreatinine spiegels, en zouden dan ook een rol kunnen spelen in

de lange termijn renoprotectieve effecten en stabilisatie van kreatinineklaring door losartan. In **hoofdstuk II.2** en **II.3** wordt de hypothese beschreven dat de veranderingen in het IGF-I systeem door losartan het gevolg kunnen zijn van een toegenomen IGF-I productie door de lever, waardoor de GH productie vermindert. Verbetering van de insulinegevoeligheid (deels ten gevolge van een betere balans in het GH/IGF-I syteem en deels door andere mechanismen) zou eveneens kunnen bijdragen.

Recente studies suggereren dat remming van het RAS het risico op het ontwikkelen van type 2 diabetes mellitus vermindert. Tevens is bekend dat het IGF-I systeem belangrijk is in de glucose homeostase. **Hoofdstuk II.4** beschrijft een studie naar het effect van losartan op insulineresistentie en verschillende variabelen van het IGF-I systeem. Kortdurende behandeling met losartan verminderde de insulineresistentie bij personen met gestoorde nuchtere glucose waarden. De serum concentratie van het vrije IGF-I was gestegen na behandeling met losartan, en deze stijging correleerde met de afname in insulineresistentie. De effecten van losartan op de verschillende variabelen van het GH/IGF-I systeem dragen dan ook mogelijk bij aan de verbetering van insulinegevoeligheid, zeer waarschijnlijk naast andere bekende effecten van losartan op haemodynamische en niet-haemodynamische mechanismen die hierbij betrokken zijn (**hoofdstuk II.4** en **Algemene discussie**). Of remming van het RAS werkelijk het ontstaan van type 2 diabetes mellitus kan vertragen of voorkomen, moet verder onderzocht worden in grotere, prospectieve studies met langere follow-up.

Type 2 diabetes mellitus verhoogt het risico op het ontwikkelen van cardiovasculaire complicaties. Het is opvallend dat vrouwen met type 2 diabetes een groter risico lijken te lopen dan mannen. De oorzaak voor dit verhoogde risico van vrouwen ten opzichte van mannen is onduidelijk. **Hoofdstuk III.1** beschrijft de resultaten van een prospectieve studie met een gemiddelde follow-up duur van 4.7 jaar, die is uitgevoerd bij een deel van de normotensieve patiënten met type 2 diabetes mellitus en microalbuminurie die eerder deelnamen aan de gerandomiseerde studie, beschreven in **hoofdstuk II.1**. In onze studiepopulatie was vrouwelijk geslacht geassocieerd met een verhoogd risico op cardiovasculaire morbiditeit en mortaliteit. Dit verhoogde risico was onafhankelijk van de klassieke cardiovasculaire risicofactoren, de ernst van nefropathie, de aan- of afwezigheid van retinopathie, en het gebruik van of de toegang tot medische zorg. In onze studie was het dan ook niet mogelijk de oorzaak voor het verhoogde cardiovasculaire risico van de vrouwen aan te tonen, dan wel die patiënten te identificeren met het hoogste risico.

Zoals hierboven beschreven is microalbuminurie niet alleen de eerste klinische uiting van diabetische nefropathie, maar is het tevens geassocieerd met cardiovasculaire morbiditeit en overlijden. Enkele studies hebben recent aangetoond dat daling van albuminurie het risico op cardiovasculaire complicaties reduceert bij hypertensieve patiënten met diabetes mellitus. Het is in deze studies onduidelijk of deze risicoreductie veroorzaakt wordt door veranderingen in de systemische bloeddruk, met veranderingen in mate van albuminurie als gevolg hiervan. Om dit nader te bestuderen onderzochten we in onze normotensieve patiënten of

de verandering in albuminurie na een jaar voorspellend is voor cardiovasculaire complicaties (**hoofdstuk III.2**). Het bleek dat patiënten met snelle progressie van albuminurie na een jaar het hoogste risico hadden op cardiovasculaire complicaties, terwijl patiënten met regressie van albuminurie van tenminste 30% het laagste risico. Eiwituitscheiding in de urine kan dan ook beschouwd worden als maat voor effectiviteit van therapie, onafhankelijk van bloeddruk verandering, waarop de therapie kan worden getitreerd.

Hoofdstuk IV.1 bevat een algemene discussie, waarin de resultaten in breder perspectief worden geplaatst. Mogelijke werkingsmechanismen van het effect van losartan op de nierfunctie bij normotensieve patiënten met type 2 diabetes mellitus worden besproken, alsmede van de interactie tussen losartan en het GH/IGF-I systeem. Vervolgens worden verschillende fysiologische mechanismen beschreven die betrokken kunnen zijn bij de effecten van remming van het RAS op glucose metabolisme en insulinegevoeligheid. De klinische uitkomst van normotensieve patiënten met type 2 diabetes mellitus en microalbuminurie, alsmede het belang van behandeling gericht op albuminurie, worden besproken. Tenslotte worden suggesties gedaan voor toekomstig onderzoek.

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Desiderius Erasmus schetste in Lof der Zotheid (1515) het eenzame leven van een promovendus, '..die zich eindeloos aftobt, wat ten koste gaat van zijn gezondheid, waardoor hij humeurig, bijziende of zelfs blind wordt, tot armoede vervalt, bij een ieder uit de gunst valt en alle genoegens moet verzaken..'. Menig promovendus zal zich zo nu en dan, met mij, herkennen in deze beschrijving. Echter, een promotieperiode is bij uitstek ook een unieke en intensieve samenwerking tussen veel mensen, onder wie patiënten, artsen, wetenschappers en vele anderen vanuit verschillende disciplines, dit alles gesteund en omringd door de onontbeerlijke persoonlijke achterban van de promovendus. Gelukkig krijg ik hier de gelegenheid velen van hen te bedanken.

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CURRICULUM VITAE

De auteur van dit proefschrift werd geboren op 16 juli 1972 te Gilze en Rijen. Zij behaalde in 1990 haar Gymnasium B diploma aan het Theresia Lyceum te Tilburg. Van 1990 tot 1994 studeerde zij Geneeskunde aan de Erasmus Universiteit te Rotterdam. Het artsexamen werd behaald in 1997 (cum laude), waarna zij als arts-assistent niet in opleiding (AGNIO) ging werken op de afdeling Interne Geneeskunde van het Ikazia Ziekenhuis te Rotterdam. In 2000 begon zij in dit ziekenhuis haar opleiding tot internist (opleider Dr. R.J.Th. Ouwendijk). Deze opleiding werd vanaf 2003 voortgezet in het Erasmus Medisch Centrum te Rotterdam (opleiders Prof. dr. H.A.P. Pols en Dr. J.C.L.M. van Saase). Op 1 september 2005 vond de registratie plaats als internist. Zij specialiseerde zich in het aandachtsgebied Vasculaire Geneeskunde in het Erasmus Medisch Centrum (opleider Dr. A.H. van den Meiracker), en op 1 juli 2006 vond de registratie plaats als internist-vasculair geneeskundige. In 1999 begon zij met de voorbereidingen van het onderzoek, waar zij gedurende haar opleiding aan werkte, en dat uiteindelijk heeft geleid tot dit proefschrift. Sinds 1 juli 2006 is zij werkzaam als internist-vasculair geneeskundige op de afdeling Interne Geneeskunde, sectie Vasculaire Farmocologie en Metabole Ziekten, van het Erasmus Medisch Centrum.

Rijen, zondag 16 juli 1972 (0.56 uur), geboorte van Adrienne Anne Marie Zandbergen, tweede dochter van Marianne en Huub, zusje van Nicole

Adrienne, zondagskind,
Nadat je in Rijen zowel de kleuterschool (de 'papschool' zoals je die altijd noemde) als de lagere school had doorlopen, ging je naar het Theresialyceum in Tilburg, waar je na zes jaar je Gymnasium B diploma behaalde. Vervolgens ging je Geneeskunde studeren aan de Erasmus Universiteit in Rotterdam, waar je na zes jaar studeren en werken op 24-jarige leeftijd afstudeerde als basisarts. Al die tijd werd je daarbij onder meer gesteund door een grote groep medestudenten en vrienden, die je vanaf het begin van je studie rondom je had verzameld, een groep vrienden die nog steeds bestaat, ook al zijn zij intussen over het hele land en zelfs over andere werelddelen uitgezworven. Wij waren erg trots op je toen de voorzitter van de examencommissie aan de zaal van genodigden bij de uitreiking van je artsenbul extra applaus vroeg voor mevrouw Zandbergen, omdat jij als enige van die groep nieuwe artsen 'cum laude' was geslaagd. Toch niet gek voor een vrouw, wier eerste 'bul' bestond uit het strikdiploma van de papschool!
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