Effect of angiotensin converting enzyme inhibitor or beta blocker on glomerular structural changes in young microalbuminuric patients with Type I (insulin-dependent) diabetes mellitus

S. Rudberg¹, R. Østerby², H.-J. Bangstad³, G. Dahlquist⁴, B. Persson¹

¹ Department of Woman and Child Health, Paediatric Unit, Karolinska Institute, Stockholm, Sweden

² Electron Microscopy Laboratory, University Institute of Pathology, Aarhus Kommunehospital, Aarhus, Denmark

³ Department of Paediatrics, Ullevål University Hospital, Oslo, Norway

⁴ Department of Paediatrics, Umeå University Hospital, Umeå, Sweden

Abstract

Aims/hypothesis. To investigate the influence of angiotensin converting enzyme inhibitors and beta blockers on the progression of early diabetic glomerulopathy.

Methods. Thirteen patients with Type I (insulin-dependent) diabetes mellitus (mean age 18.8 years) with microalbuminuria 31 (19–160) µg/min were randomised to treatment with enalapril (group 1, $n = 7$) or metoprolol (group 2, $n = 6$). Renal biopsies were taken before and after 38 (36–48) months of treatment. Albumin excretion rate, blood pressure and HbA_{1c} were measured every third month. A reference group without antihypertensive treatment (group 3, $n = 9$), with similar age, diabetes duration and degree of microalbuminuria as group 1 and 2, had baseline and follow-up renal biopsies taken previously with an interval of 26–34 months, analysed at the same laboratory. Glomerular structures were measured by stereological methods.

Results. Measurements of basement membrane thickness, mesangial and matrix volume fractions were similar among groups at baseline. Structural variables

were only increased in group 3 at follow-up. Delta values in basement membrane thickness and diabetic glomerulopathy index per 24 months were lower in group 1 and 2 than in group 3 ($p < 0.05$). Microalbuminuria returned to normal in group 1 and 2 only. Decreased albumin excretion rate tended to inversely correlate with increased basement membrane thickness ($p = 0.08$) and diabetic glomerulopathy index ($p = 0.05$). Mean HbA_{1c} was similar between groups. Mean diastolic blood pressure was lower in group 1 and 2 than in group 3 ($p < 0.01$). Mean HbA_{1c} and mean diastolic blood pressure correlated to changes in basement membrane thickness, mesangial volume fraction and diabetic glomerulopathy index ($p < 0.05$).

Conclusion/interpretation. Contrary to findings in the group without antihypertensive treatment, no progression of glomerulopathy was seen in those treated with enalapril or metoprolol. [Diabetologia (1999) 42: 589–595]

Keywords Type I diabetes, microalbuminuria, diabetic glomerulopathy, angiotensin converting enzyme inhibitor, beta-receptor blocker.

Received: 11 November 1998 and in revised form: 21 December 1998

Corresponding author: S. Rudberg, MD, Ph.D., Department of Woman and Child Health, Astrid Lindgren's Children's Hospital/Karolinska Hospital, S-171 77 Stockholm, Sweden

Abbreviations: GFR, Glomerular filtration rate; ERPF, effective renal plasma flow; FF, filtration fraction; AER, albumin excretion rate; BMT, basement membrane thickness; Vv(mes/glom), mesangial volume fraction per glomerulus; Vv(mat/glom) matrix volume fraction per glomerulus; DGP, diabetic glomerulopathy; ACEI, angiotensin converting enzyme inhibitor; PAH, p-aminohippuric acid.

Long-term treatment with angiotensin converting enzyme inhibitors (ACEI) has a beneficial effect on the progression of microalbuminuria in patients with Type I (insulin-dependent) diabetes mellitus [1, 2]. In addition, some clinical studies but not all [3–5] have suggested a superior effect of ACE-inhibitors over beta-blockers on the progressive decline in glomerular filtration rate (GFR) in Type I diabetic patients with nephropathy.

Microalbuminuria predicts overt diabetic renal disease [6]. Still around 30% of patients with low-

grade microalbuminuria may revert to normoalbuminuria without intervention [7]. Further, the degree of structural glomerular changes differs among young diabetic patients within the low grade microalbuminuric range [8]. It is probable that those with the most advanced structural changes in this early stage of the disease are at the highest risk for progression to clinical nephropathy. As the reduction of AER does not necessarily reflect a prohibited progression of renal injury [9], it is important to investigate the influence of pharmacological intervention, e.g. antihypertensive treatment, not only on functional variables but also on the progression of renal structural changes. Experimental studies have shown a reduced basement membrane thickness (BMT) [10] and prevention of late renal structural changes in ACEI-treated diabetic rats [11]. In a longitudinal study of a mixed group of patients with either Type I diabetes or Type II (non-insulin-dependent) diabetes mellitus and with nephropathy and hypertension no differences in renal morphology changes, using light microscopy, were seen between patients treated with ACEI or other antihypertensive agents [12]. Up to date no electron microscopy data in microalbuminuric Type I diabetic patients dealing with the effect of ACE-inhibitors or other antihypertensive treatments on diabetic glomerulopathy changes have been presented.

The aim of our study was to investigate whether treatment with ACEI or beta blocker for 3–4 years could retard the progression of glomerular structural changes in Type I diabetic adolescents with microalbuminuria compared to those without antihypertensive treatment.

Patients and methods

Subjects. All adolescents included in the study were 15 years old or older with more than 5 years duration of Type I diabetes and with an albumin excretion rate (AER) over 15 µg/min in at least 2/3 consecutive overnight urine samples during 1 year. They were identified from medical records in three different departments of paediatrics in the south and north-west of Stockholm. We traced 20 patients with microalbuminuria (15–200 µg/min) and 2 with macroalbuminuria (> 200 µg/min) and invited them to participate in the study. The study was approved by the local ethics committee. Two patients refused to participate. The other 18 patients (with microalbuminuria and 2 with macroalbuminuria) and their parents gave their informed consent to participate in the study and all these patients had an initial renal biopsy taken. Subjects were thereafter randomly allocated to treatment with either the ACE-inhibitor enalapril (20 mg/day, group 1) or the beta blocker metoprolol (100 mg/day, group 2). The results in this report deals only with the patients with microalbuminuria. Of the 18 microalbuminuric patients 13 completed the study (7 with enalapril and 6 with metoprolol) and had a follow-up renal biopsy taken after 36–48 months of treatment. Five patients (three females and two males) dropped out due to pregnancy (two patients), social reasons (two patients) and severe hypertension that required multiple antihypertensive drugs (one patient).

Table 1. Clinical data at entry of study

	Enalapril Group 1	Metoprolol Group 2	Reference Group 3
<i>n</i>	7	6	9
Age (years)	17.7 (1.5)	20.2 (2.1)	19.9 (3.5)
Sex (male/female)	2/5	3/3	6/3
Diabetes duration (years)	9.9 (3.4)	12.5 (2.9)	11.2 (1.6)
BMI (kg/m ²)	25.0 (3.4)	24.0 (1.8)	23.5 (2.4)
Insulin dose (U · kg ⁻¹ · day ⁻¹)	0.93 (0.17)	1.10 (0.15)	0.89 (0.13)
Mean (SD)			

Microalbuminuria had been present for less than 2 years prior to the study. All participants had a fundus photography taken during the year prior to the study. Simplex retinopathy (defined as one or more microaneurysms or haemorrhages) was present in ten patients. None of the microalbuminuric patients had proliferative changes. No patient was on a low protein diet or other continuous medication other than insulin at the start of the study. All patients were on 4–5 s.c. daily insulin injections.

The ethics committee did not permit us to take renal biopsies in an untreated control group. Thus to obtain a reference group without antihypertensive treatment we used a group of nine patients on conventional insulin treatment (i.e. two or multiple insulin injections daily) with similar age, duration of Type I diabetes and degree of microalbuminuria as the antihypertensive treated groups. These patients had previously participated as a control group (on conventional insulin treatment) in a trial at Aker Diabetes Centre in Oslo with baseline and follow-up renal biopsies taken at 26 and 34 months intervals. In this study the effect of continuous subcutaneous insulin infusion had been compared with conventional insulin treatment on the progression of diabetic glomerulopathy [13]. Renal biopsies were taken in the same way as in the antihypertensive treated groups and all morphometric analyses were carried out in the same laboratory in Århus. Clinical data on study participants are presented in Table 1.

Study protocol for antihypertensive treated groups. Prior to the random selection to either enalapril or metoprolol an ultrasound guided transcutaneous renal biopsy was taken using an automated biopsy device with an 18 gauge needle (Precision Cut AB, Becton Dickinson, N.J., USA). Tissue was fixed in 2% glutaraldehyde in modified Tyrodes buffer containing 22.5 g/l dextrane T40 [14] and delivered to the Electron Microscopy Laboratory in Århus where it was sectioned into smaller blocks, dehydrated and embedded into epon. Prior to the renal biopsy a continuous inulin- and p-aminohippuric acid (PAH)-clearance was undertaken for measurement of GFR and effective renal plasma flow (ERPF) and the calculation of filtration fraction (FF).

Renal biopsies and measurements of GFR and ERPF were repeated after median 38 (36–48) months of follow-up.

Prior to treatment with an antihypertensive drug and every third month thereafter during follow up, HbA_{1c}, AER timed overnight, blood pressure, serum-ACE activity, weight and height were measured.

Clinical and laboratory methods for antihypertensive groups. Blood samples were collected between 8 and 9 am after an overnight fast and HbA_{1c} was analysed by HPLC (Auto-A, Kyoto-Diaciich, Kagaku, Kobe, Japan). Reference levels were 4–6% and the interassay coefficient of variation 3%.

We analysed ACE-activity in serum using a RIA method (Buhlman Laboratories, Basel, Switzerland). The reference range was 18–55 U/ml.

Systolic and diastolic blood pressures were measured using an automatic device (Dinamap, Critikon, Johnson-Johnson, Tampa, Fla., USA) with the patient in the supine position after 5 min rest, using an appropriately sized cuff.

Body mass index (BMI) was calculated as body weight divided by the height (m^2) and AER was analysed by an immunoturbidimetric method [15] on fresh specimens (Instrumentation Lab, Lexington, Mass., USA). The interassay coefficient of variation was 4.7 % in the range 10–50 mg/l.

The GFR and ERPF were measured during oral hydration by giving a continuous infusion of inulin (Inulin, Laevosan, Linz, Austria) [16] and p-aminohippuric acid (PAH, Merck Sharp and Dohme, Rahway, N.J., USA) [17]. The FF was calculated as GFR/ERPF.

Clinical and laboratory methods for reference group. The AER, blood pressure and HbA_{1c} were measured at baseline and thereafter every second month. The AER was analysed from urine samples timed overnight using an immunoturbidimetric method. Blood pressure measurements were by a conventional mercury sphygmomanometer. The HbA_{1c} was analysed by HPLC (reference level 4.3–6.1 %). The GFR was analysed by a continuous inulin-infusion at baseline and follow-up [13].

Morphometric methods. The same morphometric methods were used for antihypertensive treated and reference groups and all renal biopsies were analysed at the Electron microscopy Laboratory in Århus. In the reference group biopsies were embedded in Vestopal (Serva Feinbiochemica, Heidelberg, Germany), whereas in the antihypertensive groups Epon 112 (TAAB Laboratories, Berkshire, England) was used. The investigator who carried out the quantitative morphometry was not included in any other part of the study and was unaware of the patients' group category and all other clinical data until the final measurements had been completed.

Quantitative structural data were obtained by electron microscopy using strictly systematic independent sampling of glomeruli; serial 1 μm thick sections were cut, picked up on slides and stained with toluidine blue. The first section of the block having full tissue was the baseline section. Thin sections for electron microscopy were cut in new appearing glomeruli in the block, i.e. sampling was independent of glomerular size and morphology. The levels for thin sectioning were predetermined to be at 50 μm and multiples thereof from the baseline

section, i.e. with a random independent position within the glomeruli. In each of three new appearing glomeruli the first three levels were used for electron microscopy.

Quantitation by electron microscopy. Two levels of magnification were used: at low magnification ($2300\times$) the entire glomerular profile was photographed to produce photomontages, i.e. three complete cross sections were obtained in each of three glomeruli. On the montages the mesangial volume fraction $V_v(\text{mes}/\text{glom})$ was estimated with an 8:1 grid with a point distance between fine points of about 8 μm .

At high magnification ($9800\times$) a sub-sample of the area ($\sim 24\%$) was photographed in the largest of the three profiles in each glomerulus. On the micrographs the BMT was estimated by orthogonal intercepts [18], and the matrix volume fraction within the mesangium $V_v(\text{mat}/\text{mes})$, estimated with a 2:1 grid. Finally, matrix star volume was measured by classifying point sampled intercepts in a predetermined direction [19].

Statistical methods. Comparisons between groups were by one-way ANOVA. Single correlation analyses were by the Pearson method. Prior to comparisons between groups delta-values of morphometric measures were adjusted for treatment time and calculated per 24 months assuming a linear increase. Comparisons between baseline and follow-up data were with paired *t*-test. Power calculations were based on previous morphological results [13] and calculated according to Neyman's solution [20]. To detect a 25 % increase with a power of 80 % at the significance level of 5 %, six patients in each group were required. Due to the skewed distribution of AER, these values were log transformed when used in the statistical calculations. Data are presented as means $\pm$ SD except for AER where median and range is given.

Results

Clinical results. Baseline and follow-up data for all three groups on HbA_{1c} , systolic and diastolic blood pressures, GFR and AER are given in Table 2. A decrease in AER was seen in group 1 ($p = 0.01$) and 2 ($p < 0.001$) but not in group 3. The AER returned to normal in all patients in group 1 and 2. The HbA_{1c}

Table 2. Clinical results at the time for base-line and follow-up renal biopsies

	Enalapril Group 1		Metoprolol Group 2		Reference Group 3	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
HbA_{1c} (%)	9.1 (1.7)	8.2 (2.9)	8.6 (1.8)	7.7 (2.1)	10.1 (2.3)	9.7 (1.3)
Blood pressure, systolic	125 (11)	120 (11)	123 (11)	122 (15)	127 (13)	129 (16)
diastolic (mm Hg)	80 (8)	71 (8)	80 (13)	77 (10)	84 (7)	84 (9)
Albumin excretion rate (ug/min), (median, range)	31 (23–160)	12 ^a (8–16)	29 (19–41)	5 ^b (4–12)	33 (18–194)	35 (6–187)
Glomerular filtration rate ($ml \cdot min^{-1} \cdot 1.73 m^{-2}$)	118 (22)	117 (23)	119 (16)	122 (15)	136 (31)	143 (25)
Effective renal plasma flow ($ml \cdot min^{-1} \cdot 1.73 m^{-2}$)	568 (72)	585 (124)	585 (99)	589 (114)	–	–
Filtration fraction (%)	20.9 (3.7)	20.6 (2.6)	20.3 (1.4)	21.2 (2.8)	–	–

^a $p = 0.01$, ^b $p < 0.001$ vs baseline
Mean (SD)

Fig. 1A-D. Measurements taken every 6th month during 36 months of follow-up in enalapril- (group 1 ■), metoprolol (group 2 ●) and 24 months of follow-up in reference (group 3 ▲) groups of **A** Albumin excretion rate (median, range), **B** systolic- and **C** diastolic blood pressures and **D** HbA_{1c} (mean,SD)

was not significantly changed in any group. Systolic and diastolic blood pressures, GFR and filtration fraction (only measured in group 1 and 2) were unchanged between baseline and follow-up (Table 2). Serum ACE activity was suppressed in group 1 (24.1 ± 7.7 vs 13.4 ± 7.9 U/ml, $p < 0.001$) but not in group 2 (24.7 ± 4.5 vs 23.5 ± 4.4 U/ml).

Data on AER, systolic and diastolic blood pressure measured every 6 months during the first 36 months of follow-up in groups 1 and 2 and the first 24 months in group 3 (i.e. while all patients in each group still remained in the study) are given in Figure 1. Mean HbA_{1c} and mean systolic blood pressure during study did not differ between the 3 groups. Mean diastolic blood pressure was higher in group 3 than in group 1 ($p < 0.001$) and 2 ($p = 0.01$). The changes in HbA_{1c} or in systolic blood pressure between baseline and follow-up did not differ be-

tween the groups. A decrease in diastolic blood pressure was seen only in group 1 compared with group 3 ($p = 0.05$).

Renal structural results. Baseline structural data including basement membrane thickness (BMT), mesangial volume fraction [Vv(mes/glom)], matrix volume fraction [Vv(mat/glom)], matrix star volume and overall diabetic glomerulopathy index, i.e. index DGP [BMT/10 + Vv(mat/glom)% + matrix star volume] did not differ among groups (Table 3). The BMT, matrix star volume and index DGP (Figs. 2–4) were increased at follow-up in group 3 only. Mesangial volume fraction was not changed in any group (Table 3).

The change in BMT adjusted for 24 months of follow-up ($\Delta\text{BMT}/24$ month) was lower in groups 1 and 2 ($p = 0.01$) than in group 3 (overall $p = 0.008$). Similarly, $\Delta\text{index DGP}/24$ month was lower in group 1 ($p = 0.03$) and group 2 ($p = 0.04$) than group 3 (overall $p = 0.03$).

Correlations between structural and clinical data. When taking all patients from the three groups into account a decrease in AER tended to be inversely correlated to an increase BMT ($r = -0.38$, $p = 0.08$) and to $\Delta\text{index DGP}$ ($r = -0.41$, $p = 0.05$).

Table 3. Basement membrane thickness (BMT), mesangial and matrix volume fractions (Vvmes/glom; Vvmat/glom), matrix star volume, overall diabetic glomerulopathy index (index DGP + matrix star volume) at baseline and follow-up

	Enalapril Group 1		Metoprolol Group 2		Reference Group 3	
	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up
BMT (nm)	570 (120)	593 (94)	610 (93)	602 (84)	562 (90)	702 (173) ^b
Vv(mes/glom) (%)	19.6 (5.0)	19.3 (5.3)	21.3 (2.5)	23.8 (2.8)	21.2 (2.9)	28.2 (7.2)
Vv(mat/glom) (%)	10.7 (2.6)	11.2 (4.0)	11.2 (1.2)	12.9 (1.6)	12.2 (1.3)	13.1 (1.9)
Matrix star volume (μm^3)	26.8 (9.5)	30.8 (14.7)	29.5 (5.6)	35.7 (10.1)	28.2 (7.2)	37.1 (15.7) ^a
Index DGP	94.4 (22.6)	101.2 (27.1)	101.7 (13.2)	108.8 (17.1)	96.6 (13.2)	120.6 (30.9) ^b

^a $p = 0.04$, ^b $p = 0.007$ vs baseline

Mean (SD)

Mean diastolic blood pressure during the study was correlated to: ΔBMT ($r = 0.44$, $p = 0.04$), $\Delta\text{index DGP}$ ($r = 0.66$, $p < 0.001$) and Vv(mes/glom) ($r = 0.47$, $p = 0.03$). Mean systolic blood pressure was not correlated to any structural changes. Mean HbA_{1c} during the study was also correlated to ΔBMT ($r = 0.51$, $p = 0.02$), $\Delta\text{index DGP}$ ($r = 0.60$, $p < 0.001$) and marginally to $\Delta\text{Vv(mes/glom)}$ ($r = 0.42$, $p = 0.05$).

No correlations between changes in clinical and structural variables were seen when taking all patients into account. Similarly, baseline values of GFR, ERPF or FF were not correlated to any structural changes.

Discussion

It is obvious from several clinical studies that antihypertensive treatment retards the progression of diabetic nephropathy [1, 3–5]. This is the first study which also shows a retarded progression of diabetic glomerulopathy in young Type I diabetic patients

treated with either ACEI or beta blockers. Although the number of patients fulfilling the study protocol was small, and minor morphological changes thus may have been missed due to a type II error, we would have been able to detect a 25 % increase in BMT and overall diabetic glomerulopathy index with a power of 80 %. As previous results in non-antihypertensive treated young Type I diabetic patients with microalbuminuria [13] showed approximately 25 % increase in BMT during 2 years of follow-up, this magnitude of change was what we were interested in.

An equivocal proposal has been that ACEI, in contrast to conventional antihypertensive drugs such as beta blockers, can carry special renal protective properties, i.e. an effect independent of blood pressure reduction [21, 22]. We could not confirm any different effect on glomerular structures between treatment with enalapril or metoprolol. On the other hand, the increases in BMT and overall diabetic glomerulopathy index were lower during follow-up in both the group treated with enalapril and that treated with metoprolol, than in the non-antihypertensive-treated control group. Taken together, this indicates that antihypertensive treatment as such, irrespective of whether ACEI or beta blockers are used, may retard the progression of diabetic glomerulopathy in the very early phase of diabetic nephropathy. This is possibly further supported by the clinical observation

Fig. 2. Individual data of BMT at baseline and follow-up in enalapril (group 1)-, metoprolol (group 2)-, and untreated (group 3) patients

Fig. 3. Individual data of matrix star volume at baseline and follow-up in enalapril (group 1)-, metoprolol (group 2)-, and untreated (group 3) patients

that microalbuminuria returned to normal in the group treated with enalapril as well as the one treated with metoprolol but not in the control group. In addition, the decrease in AER was associated with less progression of BMT and overall diabetic glomerulopathy changes.

A stable GFR has been described during 2 years in patients with nephropathy undergoing intensified therapy achieving a tight blood pressure control with either a beta blocker, ACEI or calcium antagonist [5]. In our study, blood pressure levels were not significantly reduced in any group. This could partly be because the patients were normotensive at the start of the study. Mean diastolic blood pressure during

follow-up was, however, clearly lower in both antihypertensive-treated groups than in the control group. It can thus be speculated that maintenance of a lower blood pressure, irrespective of type of antihypertensive treatment, may provide the main protection against progression of diabetic glomerulopathy. In addition, the finding of a correlation between progression of BMT, $V_v(\text{mes}/\text{glom})$ and index DGP on the one hand and mean diastolic blood pressure during follow-up on the other hand supports this assumption.

The protective role of improved glycaemic control on glomerular structural changes in Type I diabetes has been reported previously [13]. In accordance with this, we found a correlation between the change in BMT and in DGP-index on the one hand and mean HbA_{1c} during follow-up on the other hand, when taking all subjects into account. Still, when comparing mean HbA_{1c} between all three groups no statistically significant difference was seen. In the group receiving beta-receptor blockers, HbA_{1c} tended, however, to be lower at follow-up than at baseline. This tendency was present for the last 6 months of follow-up and it cannot be excluded that it had an additional impact on the lack of progression of diabetic glomerulopathy in this group.

Fig. 4. Individual data on diabetic glomerulopathy index (Index DGP) at baseline and follow-up in enalapril (group 1)-, metoprolol (group 2)-, and untreated (group 3) patients

Albeit this is a small study, the clear progression in diabetic glomerulopathy seen in patients without antihypertensive treatment was not seen in our patients on either ACEI or beta blockers. The groups with and without antihypertensive treatment were similar in age, duration of diabetes and degree of microalbuminuria. The reference group was not optimal, however, because the preparations and measurements were not done at the same time as those in group 1 and 2. On the other hand, the preparations of renal biopsies were similar at baseline and follow-up within all groups. Thus comparisons between absolute values between groups should be made with some caution, whereas comparisons between the changes in morphometric measures are more reliable. Different clinical and chemical laboratories were used but analyses were by the same laboratory methods, except for blood pressure measurements. Diastolic blood pressure was higher in the reference group, and it cannot be excluded with certainty that blood pressure measurement using a conventional sphygmomanometer in the reference group may have overestimated the values received by an automatic device. More important though, the same clinical and laboratory methods were used within each group and the change in HbA_{1c} did not differ between groups, whereas a decrease in diastolic blood pressure was seen in the ACEI-treated compared with the reference group.

We conclude from this study that less progression of early diabetic glomerulopathy is seen in both ACEI or beta-blocker-treated Type I diabetic young microalbuminuric patients than in patients who did not receive antihypertensive treatment during approximately 3 years and that this effect is possibly due to maintenance of a normal or low blood pressure. Whether the protection would remain over longer time-intervals, during which changes in mesangial volume fraction could also occur, cannot be deduced. The mesangial expansion is a dominant feature of advanced stages of diabetic glomerulopathy and thus associated with the demise in renal function.

Acknowledgements. This study was supported by the Swedish Diabetes Association, the Swedish Diabetes foundation, the First of May Flower Annual Campaign for Health, The Swedish Medical Association, the Research Funds of Karolinska Institute, the Novo Nordisk Research Fund, the Danish Diabetes Association, the Danish Medical Research Council, the Aarhus University Research Foundation, the Aage Louis-Hansens Foundation and the Norwegian Diabetes Association.

Thanks to K. Schultztz, G-M. Taube, T. Jansson, B. Iversen, L.Lysgaard and B. Saugbjerg for technical and laboratory assistance.

References

- Mathiesen ER, Hommel E, Giese J, Parving H-H (1991) Efficacy of captopril postponing nephropathy in normotensive insulin-dependent diabetics with microalbuminuria. *BMJ* 330: 81–87
- The EUCLID study group (1997) Randomised placebo-controlled trial of lisinopril in normotensive patients with insulin-dependent diabetes and normoalbuminuria or microalbuminuria. *Lancet* 349: 1787–1792
- Björck S, Mulec H, Johnsen SA, Norden G, Aurell M (1992) Renal protective effect of enalapril in diabetic nephropathy. *BMJ* 304: 339–342
- Elving LD, Wetzels JMF, van Lier HJJ, de Nobel E, Berden JHM (1994) Captopril and atenolol are equally effective in retarding progression of diabetic nephropathy. *Diabetologia* 37: 604–609
- Sawicki PT, for the Diabetes Teaching and Treatment Programmes Working Group (1997). Stabilization of glomerular filtration rate over 2 years in patients with diabetic nephropathy under intensified therapy regimens. *Nephrol Dial Transplant* 12: 1890–1899
- Mogensen CE, Chacati A, Christensen CK et al. (1985–1986) Microalbuminuria: An early marker of renal involvement in diabetes. *Uremia Invest* 9: 85–95
- Rudberg S, Dahlquist G (1994) Determinants of progression in microalbuminuria in IDDM adolescents. *Diabetes Care* 19: 369–371
- Rudberg S, Österby R (1997) Decreased glomerular filtration rate – an indicator of more advanced diabetic glomerulopathy in the early course of microalbuminuria in IDDM adolescents? *Nephrol Dial Transplant* 12: 1149–1154
- Morelli E, Loon N, Meyer T, Peters W, Myers BD (1990). Effects of converting enzyme inhibition on barrier function in diabetic glomerulopathy. *Diabetes* 39: 76–82
- Cooper ME, Allen TJ, Macmillan PA, Clarke BE, Jerums G, Doyle AE (1989) Enalapril retards basement membrane thickening and albuminuria in the diabetic rat. *Diabetologia* 32: 326–328
- Perico N, Amuscastegui CS, Malanchini B, Bertini T, Remuzzi G (1994) Angiotensin converting enzyme inhibition and calcium channel blockade normalise early hyperfiltration in experimental diabetes, but only the former prevents late renal structural damage. *Exp Nephrol* 2: 220–228
- Taft JL, Nolan CH, Yeung SP, Hewitson TD, Martin IR (1994) Clinical and histological correlations of decline in renal function in diabetic patients with proteinuria. *Diabetes* 43: 1046–1051
- Bangstad H-J, Österby R, Dahl-Jørgensen K, Berg KJ, Hartmann A, Hanssen KF (1994) Improvement of blood glucose control in IDDM patients retards the progression of morphological changes in early diabetic nephropathy. *Diabetologia* 37: 483–490
- Maunsbach AB (1966) The influence of different fixatives and fixation methods on the ultrastructure of rat kidney proximal tubule cells. Effects of varying osmolality, ionic strength, buffer system and fixative concentration of glutaraldehyd solutions. *J Ultrastruct Res* 15: 283–309
- Teppo AM (1982) Immunoturbidimetry of albumin and immunoglobulin in urine. *Clin Chem* 28: 1359–1361
- Hilger HH, Klumper SD, Ullrich J (1958) Mikroanalytische Untersuchungen über die Wasser- und Natriumrückresorption aus den Sammelrohrzellen der Säugetierrniere. *Pflugers Arch* 267: 218–237
- Smith HW, Finkelstein N, Aliminosa L, Crawford B, Graber M (1945) The renal clearances of substituted hippuric acid and derivatives and other aromatic acids in dog and man. *J Clin Invest* 24: 388–401
- Hirose K, Österby R, Nozawa M, Gundersen HJ (1982) Development of glomerular lesions in experimental long-term diabetes in the rat. *Kidney Int* 21: 889–895
- Walker JD, Close CF, Jones SL et al. (1992). Glomerular structure in type 1 (insulin-dependent) diabetic patients with normo- and microalbuminuria. *Kidney Int* 41: 741–748
- Snedecor GW, Cochran WG (1980) Comparisons of two samples. In: *Snedecor and Cochran (ed) Statistical Methods*. 7th edn. Ames, Iowa State U.P. pp 111–114
- Rudberg S, Aperia A, Freyschuss U, Persson B (1990). Enalapril reduces microalbuminuria in young normotensive type 1 (insulin-dependent) diabetic patients irrespective of its hypotensive effect. *Diabetologia* 33: 470–476
- Lewis EJ, Hunsicker LG, Bain RP, Rohde RD (1993) The effect of angiotensin converting enzyme inhibition on diabetic nephropathy. *N Engl J Med* 329: 1456–1462