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Renal arterioles in patients with type I diabetes and microalbuminuria before and after treatment with antihypertensive drugs

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Abstract Antihypertensive drugs can slow or even reverse the progression of diabetic nephropathy at the microalbuminuric stage. This study was performed to obtain quantitative data on changes in the renal arterioles in a follow-up study. Twelve patients with type I diabetes and with microalbuminuria were allocated to treatment for 3 years with either an ACE inhibitor (group I, 6 patients) or a beta blocker (group II, 6 patients). Baseline and follow-up renal needle biopsy specimens were taken and serially sectioned at 1 µm for light microscopy, enabling identification of arterioles as afferent or efferent. Thin sections for electron microscopy were made at 50-µm intervals, and micrographs were taken of arteriolar profiles. Matrix volume fraction of the media and a calculated matrix thickness were obtained. At baseline, structural parameters were higher than normal values. At follow-up all patients were normoalbuminuric. Both groups showed only minor changes in arteriolar structures over 3 years. In the afferent arterioles in group II there was a significant increase in the matrix volume fraction of the media, and there was a tendency to an increase in matrix thickness in both groups. In the efferent arterioles there were no significant changes in parameters. There were no differences between the two groups in arteriolar structural changes from baseline to follow-up. Thus, this study shows a slight but significant matrix accumulation in the afferent arterioles during treatment with antihypertensive drugs. This may have implications for the progression to overt nephropathy, which indicates a need for more long-term studies of treatment with antihypertensive drugs in incipient nephropathy in type I diabetes.

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Introduction

Several clinical studies show significant effect of blood pressure-lowering drugs, in particular ACE inhibitors, on the progression of nephropathy in type I diabetic patients with microalbuminuria [3, 11, 12, 20] when albumin excretion rate (AER) and glomerular filtration rate (GFR) are used as end-points. Hypotheses have been put forward to explain the renal protective effect, including, for example, the assumption that the glomerular capillary pressure is lowered. In experimental diabetes glomerular pressure is increased [1] and can be lowered by treatment with ACE inhibitors [22], and nonhaemodynamic effects of ACE inhibitors have also been suggested [9, 21]. Abnormalities in the renin–angiotensin system in diabetes have been much debated, and the juxtaglomerular apparatus is enlarged at the microalbuminuric stage of the renal disease [4]. So far, however, no studies on the effect of these drugs on the pathological lesions have appeared.

Regulation of glomerular perfusion is carried out via the afferent and efferent arterioles [16]. In the early phases in type I diabetes increased GFR and filtration fraction are common features [1, 19], suggesting changes in the function of the juxtaglomerular arterioles. In overt diabetic nephropathy the classic arteriolar lesion is hyalinosis, characterised by matrix accumulation in the media [2, 8, 13]. Recent electron microscopy studies in micro- and normoalbuminuric type I diabetic patients show matrix accumulation in the arteriolar media at the microalbuminuric stage [14]. This may affect the ability of the arterioles to react to humoral and nervous stimuli, thus changing the glomerular haemodynamics. This indicates a role for the renal arterioles in the progression of the diabetic renal disease.

It is therefore of considerable interest to study the effect of antihypertensive drugs on the structure of the re-

nal arterioles in microalbuminuric type I diabetic patients. This study was performed to obtain quantitative data on the arteriolar lesions in type I diabetic patients with microalbuminuria before and after treatment for 3 years with either an ACE inhibitor or a beta blocker.

Materials and methods

Baseline and follow-up biopsies after a 3-year treatment course were obtained at St. Göran's Children's Hospital, Sweden, from 12 microalbuminuric type I diabetic patients aged ≥15 years and with a more than 5-year history of diabetes. All patients had had prepubertal onset of diabetes. Informed consent had been obtained from all subjects, and the study was approved by the local ethics committee. At baseline 18 patients with microalbuminuria were included in the study, but for reasons unrelated to their renal disease 5 dropped out; 1 was later excluded because of poor fixation of the biopsy material. Clinically there were no differences between the cases lost and the group studied here.

Albumin excretion rate (AER) was within the microalbuminuric range: 15–200 µg/min in at least two out of three timed overnight urine samples collected consecutively in the last year before the baseline biopsy was taken. Microalbuminuria had been present for a mean duration of 2 years. Prior to treatment the patients had not received antihypertensive drugs or a low-protein or low-salt diet.

At baseline 6 patients were randomised to treatment with an ACE inhibitor (enalapril 20 mg/day; group I), and 6 to treatment with a beta blocker (metoprolol 100 mg/day; group II). The median (range) interval between baseline and follow-up biopsy was 42 (36–46) months in group I and 37.5 (36–54) months in group II (P=0.74).

Table 1 presents clinical data in the two groups at baseline and at follow-up. Blood pressure was measured with an automatic device (DINAMAP, Critikon Johnson-Johnson, Tampa, Calif.) after rest; the means of two measurements are given. GFR was measured at the time of the biopsies using continuous infusion of inulin [10]. Timed overnight AER was obtained using an immunoturbidimetric method [18], and the values shown are the medians of three values taken before the biopsies. HbA_{1C} was analysed by high-pressure liquid chromatography (Auto-A, Kyoto-Daiichi, Kagaku, Japan) with reference level 4–6%. The HbA_{1C} presented in Table 1 relates to the time of the biopsies.

Renal needle biopsies using ultrasound guidance were performed with an 18-G needle (PrecisionCut AB, BD, New Jersey, USA). They were fixed in 2% glutaraldehyde in buffer and mailed to the laboratory in Aarhus for dehydration and embedding into Epon in small blocks.

The blocks were serially sectioned in 1-µm-thick sections, which were picked up and stained with toluidine blue for light microscopy. At 50-µm intervals thin sections covering the full extent of the biopsies were made for electron microscopy. The arterioles were identified as afferent or efferent by following their course in the 1-µm serial sections by light microscopy. At 7,430× magnification all afferent arteriolar profiles on the thin sections were photographed, making sampling unbiased. When necessary, photomontages were made to cover the extent of an arteriolar profile. Photographing a calibration grid with 2160 lines/mm at the same time as the biopsy checked the final magnification.

The efferent profiles were only included in the study if the profile was less than 100 µm from the glomerular vascular pole. This distance was calculated from the measured horizontal and vertical distance using Pythagoras' theorem.

On average, 16 (7–22) afferent profiles and 12 (7–20) efferent profiles were obtained per biopsy.

An arteriolar profile consists of the lumen, an endothelial layer and the media (Fig. 1), the latter consisting of smooth muscle cells and extracellular matrix. The main parameter describing the arteriolar structure was the matrix volume fraction of the media, V_V (matrix/media). It was obtained by point counting, the distance between points corresponding to 3 µm. To obtain another estimate of the actual amount of matrix the matrix thickness, matrix-T, was also calculated. It is the arbitrary thickness of the matrix if it is spread in an even layer with a basal area corresponding to the outer circumference of the arteriole. It is calculated from the volume fraction of the matrix, V_V (matrix/media), divided by the surface density of the arteriole, S_V (arteriolar circumference/media), the latter found by counting intersections between test lines and the outer circumference of the arteriole. Finally the radius of the lumen, including the endothelium, in the arteriole was calculated assuming a circular lumen. It was calculated, as for matrix-T, from the volume/surface ratio using the volume and surface densities.

Glomerulopathy parameters, previously published for baseline biopsies [17], were estimated by electron microscopy using standard stereological methods: basement membrane thickness, matrix volume fraction of the mesangium, mesangial volume fraction of the glomerulus and matrix star volume were the main parameters used (follow-up data unpublished). For estimates of mesangial volume fraction three levels in each of three glomeruli (i.e. 9 glomerular cross sections) were used per biopsy. The remaining parameters were measured at a higher magnification at one level in each of three glomeruli.

All measurements on the follow-up biopsies were performed without knowledge of which treatment the patient had received.

Student's t-test was used to test differences between the two treatment groups at baseline, at follow-up, and when changes in parameters were considered. Student's paired t-test was used to test differences between baseline and follow-up within groups.

Table 1 Clinical data. Median (range)

	Baseline		Follow-up	
	Group I	Group II	Group I	Group II
n	6	6		
Sex	5F/1 M	3F/3 M		
Age (years)	18 (15–20)	20 (18–23)		
Diabetes duration (years)	10 (6–15)	12.5 (9–16)		
Systolic blood pressure (mmHg)	122.5 (105–135)	122.5 (110–140)	126 (105–149)	125 (99–139)
Diastolic blood pressure (mmHg)	77.5 (70–95)	81.5 (65–95)	73 (60–85)	78 (62–90)
AER (µg/min)	31.5 (23–160)	27 (19–41)	11.5 (10–14)[a]	5.5 (4–12)[b]
HbA_{1C} (%)	9.4 (7.5–11.5)	8.6 (5.9–10.8)	8.3 (7.2–11.2)	7.7 (4.7–10.6)
GFR (ml min^{-1} 1.73 m^{-2})	110.5 (88–151)	121 (95–138)	113 (90–153)	118.5 (106–148)

[a] P=0.003 vs. baseline values
[b] P=0.0001 vs. baseline values

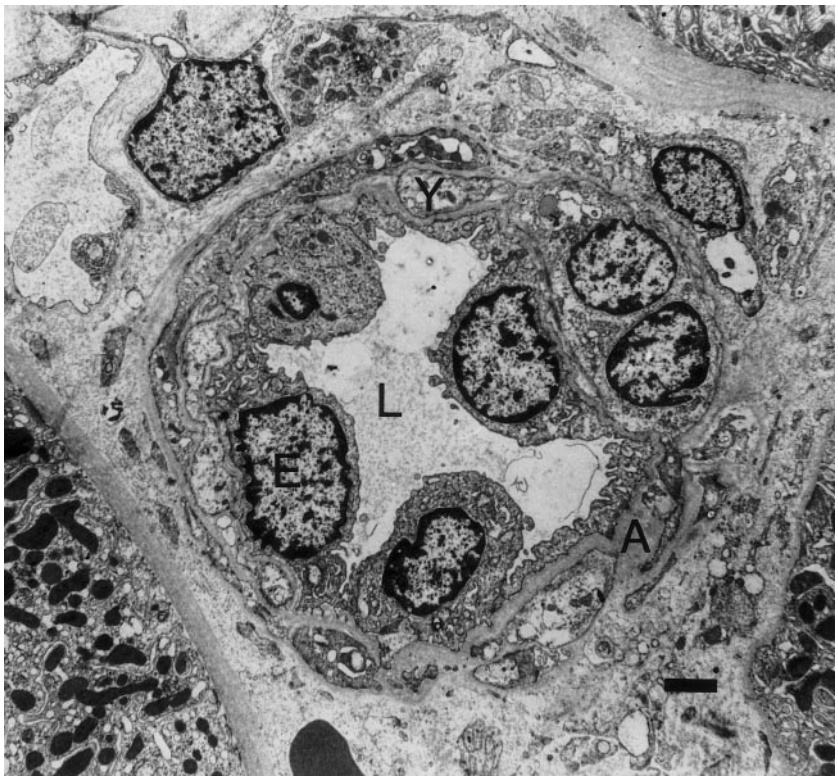

Fig. 1 Micrograph showing a cross section of a profile of an efferent arteriole. In this case the whole profile could be covered in one micrograph. (*L* lumen, *E* endothelium, *Y* myoepithelial cell, *A* extracellular matrix) *Bar* 1 µm

Correlations were tested with simple least-squares regression. Statistical significance was defined as $P<0.05$.

Owing to the variation in time before the follow-up biopsy was taken the structural changes were standardised corresponding to 36 months [Δ values (36 months)] assuming linear change of the pathology during the treatment period.

Results

Clinical data are shown in Table 1. The results did not differ between the two groups at baseline or at follow-up. Blood pressure remained almost constant, with only a minimal decrement in the diastolic blood pressure seen in both groups. In both groups a significant decrease in AER was noted (Table 1). A tendency to decreased HbA_{1C} was also seen in both groups (Table 1). Mean HbA_{1C} during the study was similar in both groups [9.1% (SD 1.7%) in group I and 8.2% (SD 2.2%) in group II] in measurements taken every 6 months ($P=0.43$). GFR remained stable in the two groups throughout the study period.

Structural changes are shown in Table 2 and results in the individual patients are shown in Figs. 2–5. There were no differences in any of the structural parameters between the two treatment groups at baseline, except for a lower matrix volume fraction of the media in the efferent arteriole in group I than in group II ($P=0.05$). When changes in the parameters from baseline to follow-up were considered there were no differences between the groups. In the afferent arterioles there was an increase in the matrix volume fraction of the media in both groups, but it only reached significance in group II ($P=0.0063$;

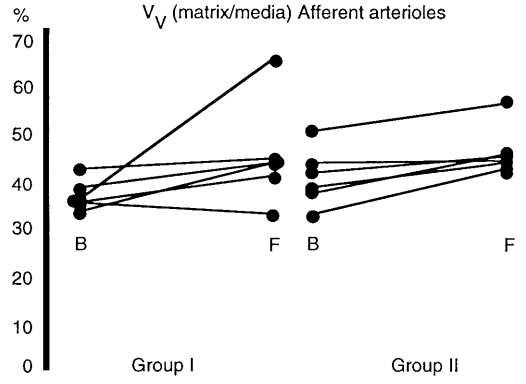

Fig. 2 Matrix volume fraction of the media in afferent arterioles in group I and group II. Mean values in each subject are indicated, as are *lines* connecting values at baseline (*B*) and at follow-up (*F*)

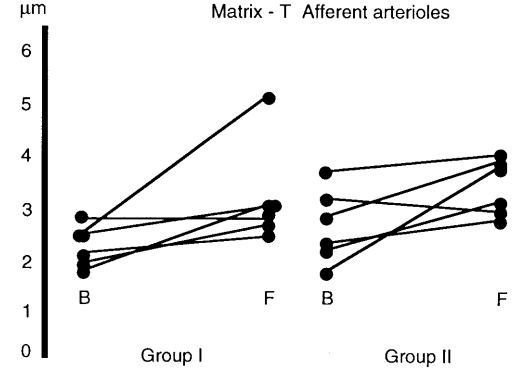

Fig. 3 The estimated matrix thickness in the afferent arterioles

Table 2 Structural data. Mean (SD). Baseline and follow-up values and structural changes standardised to 36 months (last two columns) assuming linear changes in the lesions during the study period

		Baseline		Follow up		ΔValues (36 months)	
		Group I	Group II	Group I	Group II	Group I	Group II
V_V (matrix/media) (%)	Afferent arteriole	38.0 (3.23)	42.2 (5.96)	46.3 (10.6)	48.3 (4.99)a	6.81 (9.36)	5.47 (2.95)
	Efferent arteriole	48.1 (4.62)	56.9(8.26)b	59.6 (9.50)	62.2 (6.22)	9.61 (9.33)	4.68 (6.44)
Matrix-T (μm)	Afferent arteriole	2.37 (0.39)	2.81 (0.67)	3.25 (0.91)	3.47 (0.60)	0.76 (0.70)	0.58 (0.75)
	Efferent arteriole	1.87 (0.45)	2.60 (0.98)	2.39 (0.66)	2.84 (0.57)	0.46 (0.48)	0.19 (0.78)
Luminal radius (μm)	Afferent arteriole	3.46 (0.89)	2.93 (0.50)	3.21 (0.57)	3.41 (0.63)	–0.19 (1.01)	0.50 (0.78)
	Efferent arteriole	2.91 (0.48)	3.20 (0.23)	3.02 (0.48)	3.16 (0.71)	0.12 (0.25)	–0.27 (0.69)

[a] P=0.0063 vs baseline.
[b] P=0.05 vs group I at baseline.

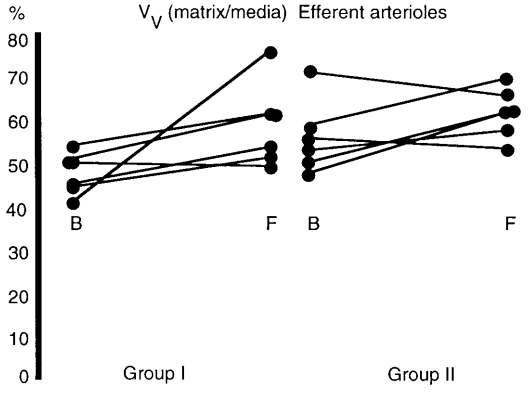

Fig. 4 Matrix volume fraction of the media in efferent arterioles

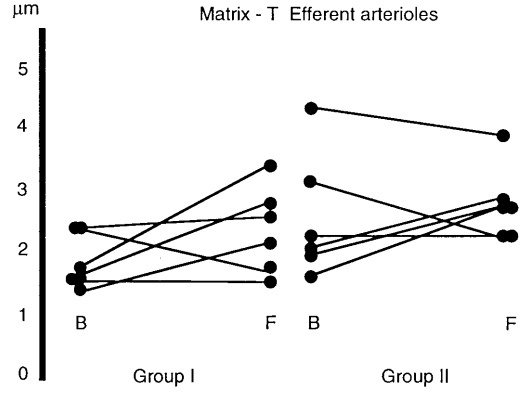

Fig. 5 The estimated matrix thickness in the efferent arterioles

P=0.15 in group I). An increase was also seen in the matrix thickness, but this failed to reach significance (P=0.065 in group I and P=0.098 in group II). In the efferent arterioles a slight increase was noted in the matrix volume fraction of the media (P=0.065 in group I and P=0.12 in group II) and no changes in the matrix thickness (P=0.181 and P=0.50 in groups I and II). Estimated luminal radius in the arterioles remained constant in the groups in both the afferent and the efferent arterioles (Table 2).

Coefficients of variation (CV) among arteriolar profiles in the matrix volume fraction within biopsies were 0.12–0.37 (median 0.19) at baseline for afferent arterioles and 0.13–0.29 (median 0.19) for efferents. CVs among subjects at baseline, with the two groups considered together, were 0.13 for afferent arterioles and 0.15 for efferent arterioles.

There were no significant correlations between the arteriolar structural parameters and diabetes duration, age, blood pressure or HbA_{1C} at baseline. No correlations existed between the structural changes versus changes in AER, changes in HbA_{1C} or mean HbA_{1C} throughout the study period.

Several correlations existed between arteriolar pathology and glomerulopathy parameters. At baseline a significant positive correlation was seen between the matrix thickness in both afferent and efferent arterioles and the basement membrane thickness in the glomerulus (r=0.64 and P=0.025, r=0.64 and P=0.026 respectively) in the whole series. At follow-up, the two groups were considered separately, and in the same parameters a significant correlation was noted in the efferent arterioles in group II (r=0.87 and P=0.024), whereas the correlation was not significant in group I (r=0.79 and P=0.061). In the afferent arterioles the same correlation did not reach significant levels in either group (r=0.76 and P=0.079 in group I, r=0.57 and P=0.24 in group II). There were no correlations between the changes in the glomerulopathy and arteriolar parameters.

In the combined groups there was a strong correlation between the matrix thickness in the afferent and efferent arterioles at baseline (r=0.90 and P=0.0001). Also, changes in matrix thickness in afferent arterioles correlated with the changes in the efferent arterioles (r=0.78 and P=0.026).

Discussion

Clinical studies suggest that antihypertensive treatment is of importance in the microalbuminuric stage of the diabetic renal disease, as shown by the lowering of AER [3, 11, 12, 20]. However, the mechanisms of action are not fully known, and little, if any, information is available on the effects of these important drugs at the structural level in the human kidney.

This 3-year follow-up study shows, in accordance with earlier studies, that AER was lowered significantly in both groups and that all the patients reached normoalbuminuria. HbA_{1C} tended to decrease in both groups. There were no differences in any of the other clinical parameters in the two groups at baseline or at follow-up. It should be noted that 24-h ambulatory blood pressure measurements were not performed [6, 7].

In a recent study significant matrix accumulation in renal arterioles in microalbuminuric type I diabetic patients compared with healthy nondiabetic controls was observed [14]. We applied the same methods for the calculations of matrix volume fraction of the media and the arbitrary matrix thickness. Thus, the results, with a slight error margin due to inter-personal variation in identification of structures, are applicable to this study and show that at baseline, the subjects in this series had significantly increased matrix accumulation in both the afferent and the efferent arterioles compared with healthy nondiabetic controls [14].

This study shows that in the afferent arterioles both groups demonstrated a slight increase in matrix accumulation, although statistical significance was seen only in V_V (matrix/media) in group II. In the efferent arterioles nonsignificant increases were noted in V_V (matrix/media) and no increase in matrix-T, indicating that perhaps a discrete atrophy of smooth muscle cells, rather than a further matrix accumulation, had occurred in the efferent arterioles. The results on arteriolar parameters are paralleled by the observation on glomerulopathy where neither group showed significant increase in glomerulopathy during the study period (unpublished data).

Owing to a decision by the ethical committee, there was no comparable untreated group in this study, and there are no studies indicating the rate of progression of arteriolar lesions in such a group. The natural course of arteriolar lesions must be progressive over time, since gross arteriolar hyalinosis is usually present at the stage of overt nephropathy [2, 15]. However, whether an untreated group would have presented a more extensive matrix accumulation cannot be substantiated.

An ACE inhibitor is usually the first drug of choice in incipient diabetic nephropathy. However, this study does not show a superior effect of this drug over a beta blocker on the progression of renal arteriolar lesions. However, owing to the small number of patients in each group the negative results may be due to a type II error. Larger studies are necessary to confirm the observation.

The correlations between afferent, efferent and glomerular lesions indicate parallel progression of lesions in the kidney. Only in the afferent arterioles was a significant progression seen in the present series. The metabolic factors caused by the diabetic state are definitely very important in the development of the pathological lesions, but an added haemodynamic factor also seems likely [1].

Hyalinisation of the afferent arteriole is a well-known concomitant of essential hypertension. Involvement of the efferent arteriole, however, is characteristic of diabetes [2], probably pointing to a metabolic pathogenesis. Clearly efferent arterioles are downstream of afferents and are therefore likely to be better protected from changes in systemic blood pressure.

It might a priori be expected that blood pressure in particular would be associated with matrix accumulation in the arterioles, but no significant correlations were seen. However, the range of blood pressure was narrow and correlations may still be found if groups with a wider range are studied or more extensive blood pressure monitoring is performed [6, 7].

The lack of correlation between HbA_{1C} and arteriolar structure may partly be due to the small groups and the narrow ranges of HbA_{1C}; our data do not exclude the possibility of a correlation.

Coefficients of variation were fairly high, both within groups and within biopsies, indicating the focal nature of the lesions. Unbiased sampling, as is achieved with the sectioning protocol, was mandatory. Serial sectioning also allowed identification of arteriolar profiles independent of orientation, which was required for the estimates of matrix thickness and luminal radius [5]. The focal nature of the lesions and the problem of orientation necessitated extensive sampling in the biopsies.

There was no difference in the luminal radius of the arterioles between or within the groups. One effect of ACE inhibitors, as seen in animal experiments, is dilatation of the efferent arteriole. However, an increase in the efferent luminal radius was not seen in group I at follow-up. Fixation artefacts inducing changes compared with the in vivo situation cannot be excluded. Also, a type 2 error may be present.

In conclusion, this study has shown a slight increase in matrix accumulation in the afferent arterioles of microalbuminuric type I diabetes patients during 3 years' treatment with antihypertensive drugs, a protocol that led to a significant decrease in AER. At baseline the arterioles showed pathologic amounts of matrix and treatment did not completely stop or revert this progression. The continued matrix accumulation may be important for the long-term prevention of progression from incipient to overt diabetic nephropathy and suggest the need for more long-term studies of antihypertensive treatment in microalbuminuric type I diabetic patients.

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References

1. Anderson S, Brenner BM (1988) Pathogenesis of diabetic glomerulopathy: hemodynamic considerations. Diabetes Metab Rev 4:163–177
2. Bell ET (1953) Renal vascular disease in diabetes mellitus. Diabetes 2:376–389
3. Cooper ME (1998) Pathogenesis, prevention, and treatment of diabetic nephropathy. Lancet 352:213–219
4. Gulmann C, Rudberg S, Nyberg G, Østerby R (1998) Enlargement of the juxtaglomerular apparatus in insulin-dependent patients with microalbuminuria. Virchows Arch 433:63–67
5. Gundersen HJG (1984) Stereology and sampling of biological surfaces. In: Echlin P (ed) Analysis of organic and biological surfaces. Wiley, pp 477–506
6. Hansen KW, Christensen CK, Andersen PH, Pedersen MM, Christiansen JS, Mogensen CE (1992) Ambulatory blood pressure in microalbuminuric type 1 diabetic patients. Kidney Int 41:847–854
7. Hansen KW, Poulsen PL, Mogensen CE (1995) 24-h blood pressure recordings in type I diabetic patients. J Diabetes Complications 9:237–240
8. Harris RD, Steffes MW, Bilous RW, Sutherland DE, Mauer SM (1991) Global glomerular sclerosis and glomerular arteriolar hyalinosis in insulin dependent diabetes. Kidney Int 40:107–114
9. Hasslacher C, Kempe HP, Bostedt KA (1993) ACE inhibitors and diabetic nephropathy: clinical and experimental findings. Clin Invest 71:S20–S24
10. Hilger HH, Klumper SD, Ullrich J (1958) Mikroanalytische Untersuchungen über die Wasser- und Natriumrückreresorption aus den Sammelrohrenden der Säugetierniere. Pflügers Arch 267:218–237
11. Marre M, Chatellier G, Leblanc H, Guyene TT, Menard J, Passa P (1988) Prevention of diabetic nephropathy with enalapril in normotensive diabetics with microalbuminuria. BMJ 297:1092–1095
12. Microalbuminuria Captopril Study Group (1996) Captopril reduces the risk of nephropathy in IDDM patients with microalbuminuria. Diabetologia 39:587–593
13. Olsen S (1997) Light microscopy of diabetic glomerulopathy: the classical lesion. In: Mogensen CE (ed) The kidney and hypertension in diabetes mellitus. Kluwer Academic, Boston Dordrecht London, pp 173–183
14. Østerby R, Bangstad HJ, Nyberg G, Walker JD, Viberti G (1995) A quantitative ultrastructural study of juxtaglomerular arterioles in IDDM patients with micro- and normoalbuminuria. Diabetologia 38:1320–1327
15. Parving HH, Østerby R, Anderson PW, Hsueh WA (1996) Diabetic nephropathy. In: Brenner BM (ed) The kidney. Saunders, Philadelphia London Toronto, pp 1864–1892
16. Rosivall L (1990) Morphology and function of the distal part of the afferent arteriole. Kidney Int Suppl 30:S10–S15
17. Rudberg S, Østerby R, Dahlquist G, Nyberg G, Persson B (1997) Predictors of renal morphological changes in the early stage of microalbuminuria in adolescents with IDDM. Diabetes Care 20:265–271
18. Teppo AM (1982) Immunoturbidimetry of albumin and immunoglobulin G in urine. Clin Chem 28:1359–1361
19. Viberti G, Walker JD (1991) Natural history and pathogenesis of diabetic nephropathy. J Diabet Complic 5:72–75
20. Viberti G, Mogensen CE, Groop LC, Pauls JF (1994) Effect of captopril on progression to clinical proteinuria in patients with insulin-dependent diabetes mellitus and microalbuminuria. European Microalbuminuria Captopril Study Group (see comments). JAMA 271:275–279
21. Wolf G, Ziyadeh FN (1997) The role of angiotensin II in diabetic nephropathy: emphasis on nonhemodynamic mechanisms. Am J Kidney Dis 29:153–163
22. Zatz R, Dunn BR, Meyer TW, Anderson S, Rennke HG, Brenner BM (1986) Prevention of diabetic glomerulopathy by pharmacological amelioration of glomerular capillary hypertension. J Clin Invest 77:1925–1930