

# Severity of Glomerulopathy Predicts Long-Term Urinary Albumin Excretion Rate in Patients With Type 1 Diabetes and Microalbuminuria

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**OBJECTIVE** — To investigate whether the degree of glomerular structural lesions in young patients with type 1 diabetes and microalbuminuria was associated with urinary albumin excretion rate (AER) 6 years later and whether the AER level was influenced by blood glucose control, blood pressure, or glomerular filtration rate (GFR).

**RESEARCH DESIGN AND METHODS** — There were 17 young adults with type 1 diabetes and microalbuminuria, 8 men and 9 women with mean age 20 years (95% CI: 18–22) and duration of diabetes of 11 years (10–13), who participated in a 6-year prospective study. Kidney biopsies (measurements of basement membrane thickness [BMT] and mesangial and matrix volume fractions) and GFR were performed at baseline. AER and HbA<sub>1c</sub> were measured at least three times a year and blood pressure once a year.

**RESULTS** — In a multivariate analysis, baseline BMT and mean 6-year HbA<sub>1c</sub> contributed significantly to AER at the end of the study ( $R^2 = 0.69$ ,  $P < 0.01$ ). When mesangial volume fraction replaced BMT as the independent variable, this parameter and AER at baseline predicted the AER at 6 years ( $R^2 = 0.55$ ,  $P < 0.05$ ). Mesangial volume fraction and BMT (in separate analysis) contributed significantly to change in AER during the study. During the study, neither AER (30  $\mu\text{g}/\text{min}$  [19–40] to 16  $\mu\text{g}/\text{min}$  [7–90]) nor blood pressure (96 mmHg [92–102] to 95 mmHg [91–98]) changed significantly in the group. However, HbA<sub>1c</sub> was reduced from 10.3 (9.6–11.0) to 8.4% (7.8–9.1) ( $P < 0.01$ ).

**CONCLUSIONS** — In young patients with microalbuminuria, the long-term urinary AER was predicted by the degree of glomerular structural changes and associated with blood glucose control, but not with blood pressure or GFR.

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More than 10 years ago, it was shown that microalbuminuria in patients with type 1 diabetes predicted the development of clinical diabetic nephropathy (1–3). Later studies have shown that in

a substantial part of the patients with low-grade microalbuminuria, no increment is observed in urinary albumin excretion rate (AER) during a period of 4–8 years (4,5). Cross-sectional studies have revealed that

the increased AER in patients with microalbuminuria is paralleled by the degree of glomerulopathy, i.e., by basement membrane thickening and mesangial matrix expansion (6–8).

The morphological changes in early diabetic nephropathy have been linked to long-term hyperglycemia (6,7). Improved blood glucose control either by pancreas transplantation (9) or by intensified treatment (10) has resulted in amelioration of the morphological changes in renal allografts. Previously (11), we have shown in a randomized prospective 2.5-year trial in young patients with type 1 diabetes and microalbuminuria that the mesangial matrix expansion was arrested and the increment of the basement membrane thickness (BMT) was less pronounced in patients on continuous subcutaneous insulin infusion (CSII) who improved their blood glucose control, although the mean HbA<sub>1c</sub> was as high as 8.7% (i.e., 1.4 times above the upper normal limit, 6.1%).

Arterial hypertension is closely linked to diabetic nephropathy. So far, the connection between blood pressure and morphological changes in early diabetic nephropathy is not fully clarified. Two studies (7,10) found no relation at all, but in one series from 1989, structural changes in microalbuminuric patients were most pronounced in patients having either hypertension or reduced glomerular filtration rate (GFR) (12). The importance of GFR for the development of diabetic nephropathy is difficult to assess because of the well-known transient hyperfiltration observed in most patients. No relationship was found between GFR and structural changes in our study (11), but glomerular hyperfiltration preceded microalbuminuria and was associated with glomerular structural changes in a 5-year study in adolescents (8).

The aim of the present longitudinal study was to investigate whether the level of AER was associated with the degree of glomerular structural lesions found 6 years earlier and whether the development of AER was influenced by putative risk factors like blood pressure, GFR, and hyperglycemia.

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**Abbreviations:** AER, albumin excretion rate;  $\Delta$ AER, change in albumin excretion rate over 6 years; AER<sub>6years</sub>, albumin excretion rate at the end of the study; AGE, advanced glycation end product; BMT, basement membrane thickness; CSII, continuous subcutaneous insulin infusion; CT, conventional treatment; CV, coefficient of variation; GFR, glomerular filtration rate; HPLC, high-performance liquid chromatography; Vv(mat/glom), matrix volume fraction; Vv(mes/glom), mesangial volume fraction.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

**Table 1—Clinical, glomerular, and morphological characteristics of the 17 study participants at the start of the study**

Sex (M/F)	8/9
Age (years)	20 (18–22)
Diabetes duration (years)	11 (10–13)
HbA <sub>1c</sub> (%)	10.3 (9.6–11.0)
Systolic blood pressure (mmHg)	124 (119–130)
Diastolic blood pressure (mmHg)	81 (77–84)
AER ( $\mu\text{g}/\text{min}$ )	30 (19–40)
GFR [ $\text{ml} \cdot \text{min}^{-1} \cdot (1.73 \text{ m}^2)^{-2}$ ]	143 (129–157)
BMT (nm)	593 (541–644)
Mesangial/glomerular volume fraction (%)	21.0 (20.7–23.3)
Matrix/glomerular volume fraction (%)	12.8 (12.0–13.6)

Data are means (95% CI). AER is the median of at least three measurements in the year preceding the study.

## RESEARCH DESIGN AND METHODS

### Subjects

The study is a 6-year follow-up of a 24- to 36-month prospective randomized clinical trial that has been thoroughly described previously (6,11). There were 30 young patients with type 1 diabetes and persistent microalbuminuria (AER between 15 and 200  $\mu\text{g}/\text{min}$  in at least two of three overnight urine samples taken during 1 year) who were randomized to either CSII or conventional treatment (CT) (multiple injections or two to three injections per day). The previous study (11) describes the 18 patients (9 CSII and 9 CT) >18 years of age who accepted renal biopsies. During the years after the randomized trial was ended, two of the nine patients on CSII have switched to multiple injections, and one of the nine CT patients has chosen to use CSII. The idea of the original study was to test the effect of improved control and not the efficacy of different treatment modalities (CSII vs. CT). Some of the patients changed the mode of treatment after the initial 2.5–3 years, and we therefore report all the patients as one group and not according to the principle of intention to treat. Only 1 of the 18 patients was treated with an ACE inhibitor from year 5 and is therefore excluded.

The baseline characteristics of the 17 patients are shown in Table 1. They were followed by the same investigator (H.-J.B.) at the outpatient clinic at 3-month intervals (2 months the first 2 years). Renal function tests and biopsies were performed at entry and after 26–34 months. This study concerns the results of the GFR and morphological parameters at baseline and the AER after 6 years.

The protocol was approved by the Regional Ethics Committee, and the patients gave their written consent. No serious complication of the kidney biopsy procedure was observed. One patient had macroscopic hematuria for 1 day.

### Clinical and laboratory examinations

AER was measured in timed overnight urine samples at 3-month (2 months the first 2 years) intervals. The samples were kept at 4°C from 1 to 4 days, and the albumin concentration was measured by immunoturbidimetry. The interassay coefficient of variation (CV) was 4.7% in the range of 10–50  $\mu\text{g}/\text{l}$ . The individual AER values presented are the mean of three measurements.

During the first 3 years, HbA<sub>1c</sub> was analyzed by high-performance liquid chromatography (HPLC) (Diamat analyzer; Bio-Rad, Richmond, CA). The normal range was 4.3–6.1%, with an interassay CV of 3%. For the last 4 years, an immunological method, DCA (Bayer, Leverkusen, Germany), was used. The normal range was 4.1–5.9%, with an interassay CV of 3.4%. The DCA values were transformed to values corresponding to the HPLC method (multiplied by 1.04).

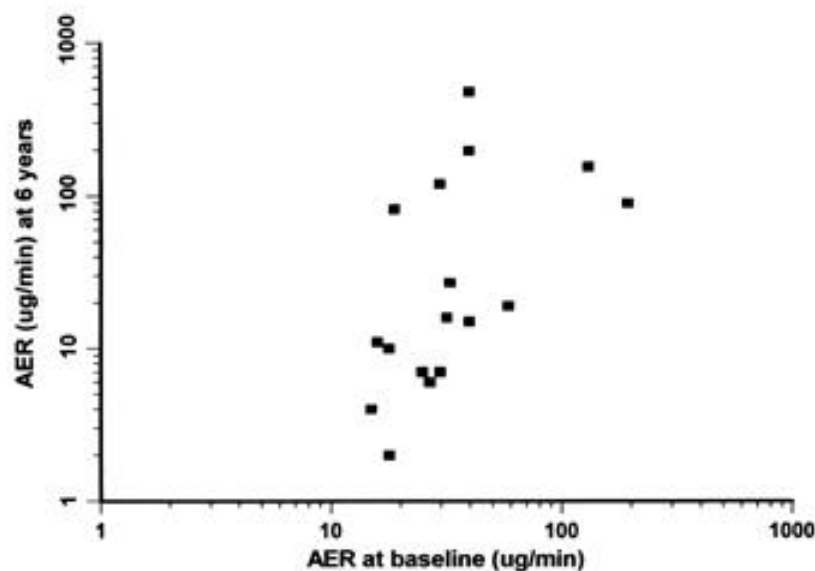
GFR was measured by inulin clearance (Inulin; Laevosan, Linz, Austria) after oral water loading. Charge selectivity index was measured as IgG/IgG<sub>4</sub> minus index, i.e., total IgG/IgG<sub>4</sub> minus clearance ratio (13), and serum advanced glycation end products (AGEs) were measured with a method described very recently (14). Blood pressure was measured once a year by conventional mercury sphygmomanometer with patients sitting after a 10-min rest.

### Renal biopsies

Ultrasound guided biopsies were taken with an 18-gauge needle-integrated automatic gun-biopsy system (Biopty; Bard, Covington, GA). The tissue was immediately immersed into the fixative, a 2% glutaraldehyde solution in modified Tyrode buffer, mailed in the fixative to the laboratory in Århus, and embedded in Vestopal.

### Measurement of structural parameters

The methods for obtaining quantitative structural parameters in the glomerulus have



**Figure 1—AER at baseline and 6 years later in 17 young adults with type 1 diabetes and microalbuminuria.**

**Table 2—Correlation coefficients between morphological changes at baseline [BMT, patients (41%) were no longer microalbuminuric (Fig. 1). In the total patient group, the median AER fell from 30 (19–40) to 16 (7–90)  $\mu\text{g}/\text{min}$  (NS). The mean arterial blood pressure remained unchanged during the study: at baseline it was 96 (92–100) mmHg, and after 6 years, 95 (91–98) mmHg. From baseline to the end of the study,  $\text{HbA}_{1c}$  was reduced from 10.3 (9.6–11.0) to 8.4% (7.8–9.1) ( $P < 0.01$ ). Mean  $\text{HbA}_{1c}$  during the 6-year study was 8.9% (8.4–9.5).**

	BMT	Vv(mes/glom)	Vv(mat/glom)	Systolic blood pressure	Diastolic blood pressure	GFR	$\text{HbA}_{1c}$
<b>AER<sub>6years</sub></b>							
<i>r</i>	0.68	0.61	0.53	0.26	0.05	0.17	0.13
<i>P</i>	0.003	0.009	0.03	0.32	0.85	0.51	0.60
<b><math>\Delta\text{AER}</math></b>							
<i>r</i>	0.52	0.76	0.42	0.32	0.05	0.20	-0.10
<i>P</i>	0.03	0.0004	0.09	0.20	0.85	0.43	0.71

been described previously (6,11). Three glomeruli from each biopsy were sampled independently of size and structure, except for one biopsy in which only two glomeruli were obtained. The structures in question are peripheral basement membrane, the mesangial region, and the mesangial matrix.

Photomontages of the entire glomerular profile were made at a magnification of  $\times 2,350$  at three levels in each glomerulus. The set of three sections, separated by 60  $\mu\text{m}$ , had a random position along the glomerular diameter and was used for the estimate of mesangial volume fraction. A systematic subsample of the largest of the three sections was photographed at a magnification of  $\times 9,900$  and used for the measurements of BMT and mesangial matrix.

Concerning the peripheral BMT, measuring points were sampled independently of the basement membrane appearance with a line grid, with measurements taken at intersections between grid lines and the endothelial–basement membrane interface. The true BMT was estimated (15), classifying only at the sampling places, where the distinctness of the epithelial cell membrane showed that the section was perpendicular to the surface of the basement membrane.

Mesangial volume fraction, Vv(mes/glom), was estimated by point counting (on an 8:1 grid) using the circumscribed polygon as the reference space (16). Mesangial matrix was estimated as volume fraction of mesangium, Vv(mat/mes), using a 2:1 grid. The product of the two volume fractions gives matrix as a fraction of glomerular space, Vv(mat/glom).

**Statistical analysis**

Comparisons of changes over time were done by two-tailed paired Student's *t* test and univariate linear correlations by least-square regression (Number Cruncher Sta-

tistical System, Kaysville, UT). Stepwise multivariate analysis was performed to evaluate the relative importance of the baseline morphological changes, long-term hyperglycemia, GFR, and blood pressure for the AER at the end of the study and also for change in AER during the study. AER values were log-transformed before analysis because of the skewed distribution. The results are, unless otherwise stated, reported as mean (95% CI).

**RESULTS**

**Clinical investigations**

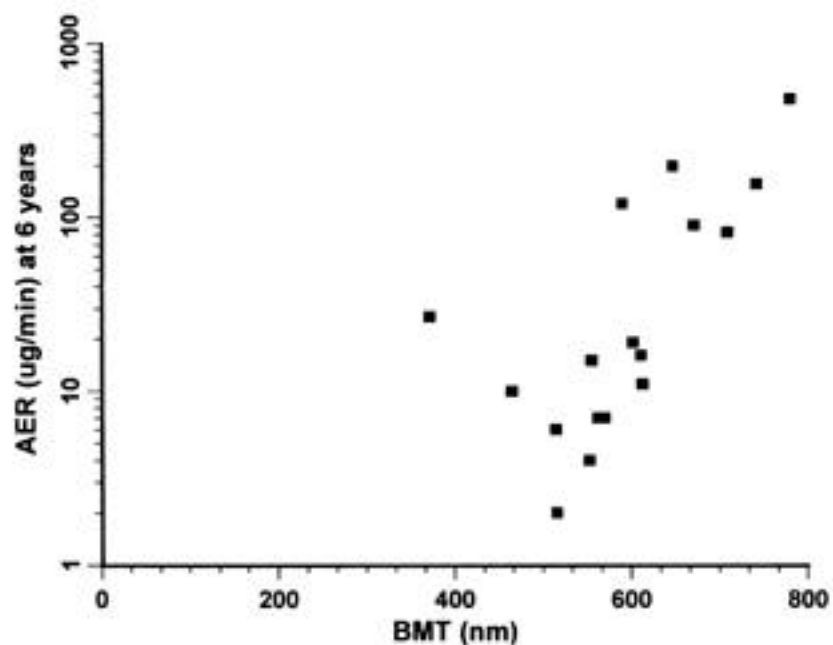
Of the patients, 12 (71%) did not show an increase in AER during the 6-year observation period. At the end of the study, seven

**Relationship between AER<sub>6years</sub>,  $\Delta\text{AER}$ , and putative risk factors**

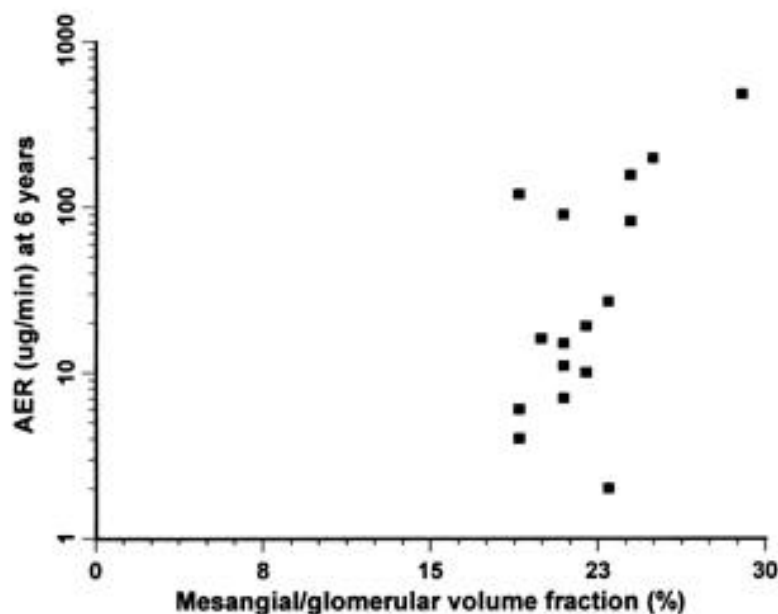
AER at the end of the study ( $\text{AER}_{6years}$ ) was used as a surrogate end point, since it was the best assessment of kidney impairment at this stage of diabetic nephropathy with considerable hyperfiltration. In the univariate correlation analysis of BMT at baseline, Vv(mes/glom) and Vv(mat/glom) correlated significantly with  $\text{AER}_{6years}$  (Table 2, Figs. 2 and 3). None of the patients with BMT  $< 590$  nm showed an increase in AER during the 6 years (Fig. 4).

The change in AER during the 6-year study ( $\Delta\text{AER}$ ) was significantly associated with both BMT and, in particular, Vv(mes/glom) (Table 2), but not with Vv(mat/glom).

The mean  $\text{HbA}_{1c}$  during the 6 years showed no significant linear relationship with  $\text{AER}_{6years}$  (Fig. 5).



**Figure 2—BMT at the start of the study and AER 6 years later in 17 young adults with type 1 diabetes and persistent microalbuminuria at baseline ( $0.68, P = 0.003$ ).**



**Figure 3**—The mesangial/glomerular volume fraction at the start of the study and AER 6 years later in 17 young adults with type 1 diabetes and persistent microalbuminuria at baseline ( $P = 0.009$ ).

### Predictors of AER

Stepwise regression analyses were first performed with  $AER_{6\text{years}}$  as the dependent variable. BMT and mean 6-year  $HbA_{1c}$  contributed significantly to the explained variation of  $AER_{6\text{years}}$  (Table 3), whereas the baseline systolic blood pressure, GFR, and AER did not. When  $Vv(\text{mes}/\text{glom})$  replaced BMT as the independent variable, only this parameter and AER at baseline influenced the  $AER_{6\text{years}}$  level significantly (Table 4).

When  $\Delta AER$  was used as the dependent variable, only BMT and  $Vv(\text{mes}/\text{glom})$ , in separate analyses, contributed significantly ( $R^2 = 0.26$ ,  $P = 0.03$  and  $R^2 = 0.58$ ,  $P = 0.0004$ , respectively).

Diabetes duration, serum levels of AGEs, or selectivity index ( $IgG/IgG_4$  in urine) at baseline were unrelated to the level of AER 6 years later.

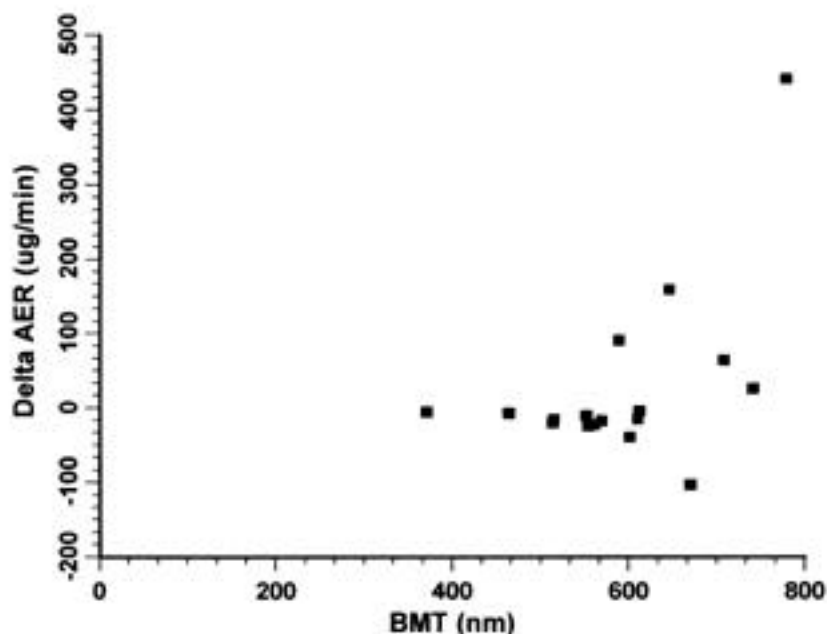
**CONCLUSIONS**— This prospective study shows that in a group of young patients with type 1 diabetes and microalbuminuria, the histological changes, namely basement membrane thickening and mesangial expansion, are predictors of the AER measured 6 years after the kidney biopsies were investigated.

Previous cross-sectional studies (6–8) and cross-sectional measurements in prospective investigations (11) have shown a direct relationship between structure and function, i.e., morphological parameters like BMT, mesangial/glomerular and

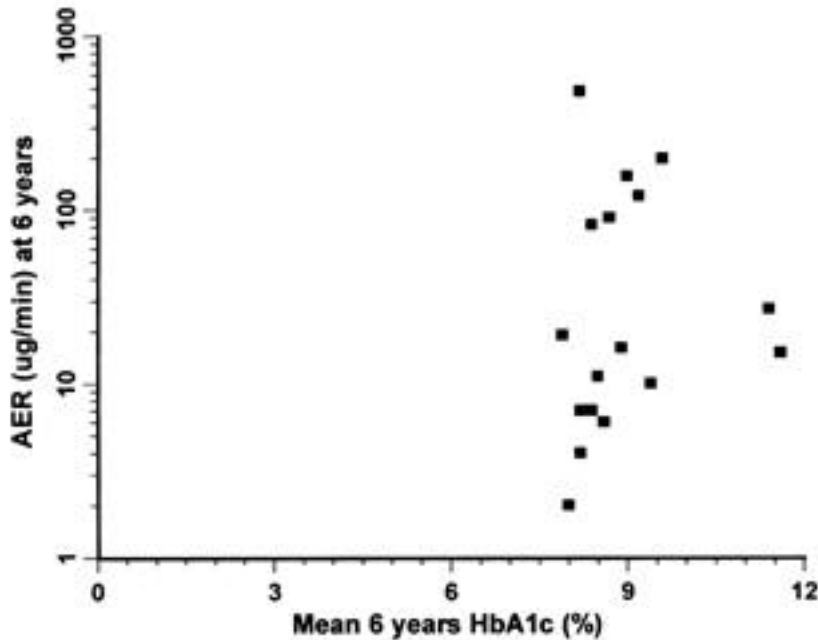
matrix/glomerular volume fractions, and AER. In the present follow-up study, we show that in type 1 diabetic patients with microalbuminuria, there is a close relationship between BMT after 10 years of diabetes duration and AER 6 years thereafter. The association between the mesangial and matrix volume fractions and AER at the end of the study was not that close. This is in accordance with the recent studies that

underscore that in the early phases of glomerulopathy, the thickening of the BMT is the most important feature (17,18). However, an alternate explanation of the weaker association between the glomerular volume fractions of the mesangium and the matrix and AER at the end of the study may also be a lower precision for the mesangial and matrix indices than for the BMT measurements (19).

Long-standing hyperglycemia is a necessary, but not a sufficient, factor for the progression of diabetic nephropathy. Recently, in a 5-year follow-up study,  $HbA_{1c}$  was shown to be an independent risk factor for the increment of BMT and overall glomerulopathy (8). We reported in the original study, which the present one is based upon, that there was a close association between  $HbA_{1c}$  during the preceding year and the most important glomerular structural parameters (6), and later on that improved blood glucose control retarded the thickening of the basement membrane and arrested the matrix expansion (11). In the present study, when a multivariate analysis was applied and the putative risk factors for  $\Delta AER$  were investigated, mean 6-year  $HbA_{1c}$  had no apparent importance. A type 2 error could not be excluded because of the small number of subjects. However, the mean 6-year  $HbA_{1c}$  clearly influenced the level of AER at the end of the study. It should be emphasized that this group consisted of young adults with rela-



**Figure 4**—BMT at the start of the study and the change in AER during 6 years in 17 young adults with type 1 diabetes and persistent microalbuminuria at baseline ( $P = 0.03$ ).



**Figure 5**—The mean 6-year HbA<sub>1c</sub> and AER at the end of the study in 17 young adults with type 1 diabetes and persistent microalbuminuria at baseline (0.35, NS).

tively short durations of diabetes. They were included in the study because they had microalbuminuria but they also had poor blood glucose control.

Most of the 17 patients in the current study had AER in the low microalbuminuric range. During the study, only five of them in fact increased their AER, and seven ended up having normoalbuminuria. This may partly be due to our inclusion criterion (microalbuminuria was defined as AER >15 µg/min in overnight urine, comparable with 20 µg/min in 24-h urine), improved blood glucose control, and regression toward the mean. There is a growing body of reports of microalbuminuric patients who do not show the expected increase in AER (20,21). This may challenge the proposed guidelines concerning the use of ACE inhibitors in microalbuminuric patients without hypertension (22). An interesting observation connected to this is that none of the patients in our study with BMT <590 nm (Fig. 3) increased their AER during the 6 years.

Patients with microalbuminuria usually have blood pressure in the normal range, but their blood pressure is still found to be higher than in patients with normoalbuminuria. Chavers et al. (12) claimed that microalbuminuric normotensive patients with normal GFR did not differ from normoalbuminuric patients regard-

ing glomerular structure. In our multivariate analysis, the BP at the beginning of the study did not contribute significantly to the explanation model either for the AER at the end of the study or for the change in AER during the study.

The importance of glomerular hyperfiltration for the development of diabetic nephropathy is under debate. Decreasing GFR has been found to be an indicator of more advanced glomerulopathy in type 1 diabetic adolescents (23). In our study, GFR at baseline had no impact on the 6-year level of AER or the change in AER, indicating that hyperfiltration did not have a harm-

ful effect. However, the implication of GFR is rather difficult to assess in this group of partly hyperfiltrating young patients.

The pathophysiological mechanisms behind the increased albumin leakage and the interplay between the structure and function in diabetic nephropathy is still poorly understood. One of several factors involved is probably the loss of anionic sites in the basement membrane. We have reported earlier a strong negative correlation (cross-sectional) between a selectivity index (urinary IgG/IgG<sub>4</sub>) and BMT (13). In the present study, no predictive value of the selectivity index for the development of AER was found.

Long-term hyperglycemia leads to nonenzymatic glycation of proteins named advanced glycation end products (24). Infusion of AGEs in experimental diabetes induces increased AER (25). We have recently reported that serum AGEs were predictive for increment of BMT over 2.5 years (14), but in the present study this was not the case for the AER-level after 6 years or changes in AER during the study.

In conclusion, the degree of glomerulopathy in young type 1 diabetic patients with 10 years' duration of diabetes and microalbuminuria is highly predictive for kidney impairment measured as AER 6 years later.

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**Table 3**—Stepwise multiple regression analysis with AER<sub>6years</sub> as the dependent variable

	R <sup>2</sup> increment	T value	P value
BMT	0.67	5.5	0.00009
BMT + mean 6-year HbA <sub>1c</sub>	0.22	2.7	0.007

R<sup>2</sup> = 0.69.

**Table 4**—Stepwise multiple regression analysis with AER<sub>6years</sub> as the dependent variable

	R <sup>2</sup> increment	T value	P value
Vv(mes/glom)	0.27	3.0	0.01
Vv(mes/glom) + AER <sub>baseline</sub>	0.20	2.5	0.02

R<sup>2</sup> = 0.55.

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