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Glomerular charge selectivity and the influence of improved blood glucose control in Type 1 (insulin-dependent) diabetic patients with microalbuminuria

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Summary. We first compared glomerular charge selectivity index in two matched groups of Type 1 (insulin-dependent) diabetic patients with micro- and normoalbuminuria respectively, and secondly, investigated prospectively in a randomized clinical trial, the influence of improved metabolic control on selectivity index in diabetic patients with microalbuminuria. In Study 1, 27 patients with microalbuminuria (albumin excretion ≥ 15 μg/min in at least two out of three overnight urine samples) were matched (age, diabetes duration, mean 1-year HbA_{1c}, gender) with normoalbuminuria patients ($n = 24$), and in Study 2, 23 microalbuminuric patients were randomly allocated to either intensive (continuous subcutaneous insulin infusion) or conventional treatment. Glomerular charge selectivity index was measured as IgG/IgG_4 selectivity index, i. e. total IgG/IgG_4 clearance ratio in timed overnight urine samples. The microalbuminuric patients had a significantly reduced selectivity index compared to the normoalbuminuric patients: 1.20 (0.92–1.40) vs 1.68 (1.22–2.21), median and 95 % confidence interval ($p < 0.01$). In Study 2, the HbA_{1c} improved in the intensive-treatment group compared to the conventional-treatment group: at 2, 6 and 12 months the difference in mean percentage HbA_{1c} between the groups was 1.1, 1.2 and 1.4, respectively ($p < 0.01$). A sharp 50 % increment in IgG/IgG_4 selectivity index was seen in the intensive-treatment group during the first 6 months ($p < 0.05$ compared to the conventional group). We conclude that adolescents and young adults in an early stage of diabetic nephropathy have reduced glomerular charge selectivity, which may be improved by reducing the mean blood glucose level.

Key words: Type 1 (insulin-dependent) diabetes mellitus, nephropathy, microalbuminuria, continuous subcutaneous insulin infusion, metabolic control, glomerular charge selectivity.

Loss of glomerular charge selectivity is one among several possible explanations for the increased albumin excretion rate associated with diabetic nephropathy [1–3]. Additional factors are renal haemodynamics [4], structural changes of the glomerular basement membrane and the mesangium [5, 6], loss of size selectivity [7] and probably epithelial cell structure [8]. The relative importance of these factors probably changes with progress towards clinical diabetic nephropathy [2, 3, 9].

Strict blood glucose control has been shown to favourably influence the rate of progression of the nephropathy in passing from the microalbuminuric stage into clinical nephropathy [10, 11]. The mechanism of the beneficial effect of inducing near-normoglycaemia is unknown, thus the aim of the present study was to investigate prospectively the influence of improved blood glucose control on glomerular charge selectivity in Type 1 (insulin-dependent) diabetic patients with microalbuminuria.

Subjects, materials and methods

Subjects

Three hundred and seventy-one Type 1 diabetic patients aged between 10–30 years with diabetes duration of more than 5 years were screened for persistent microalbuminuria. The patients were non-proteinuric as demonstrated by a negative Albustix (Boehringer Mannheim GmbH, Mannheim, FRG) and came from five different out-patient clinics (282 subjects from Aker University Hospital, Paediatric and Medical Departments, and 89 from nearby paediatric departments). Persistent microalbuminuria was defined as an albumin excretion rate between 15–200 μg/min in at least two out of three overnight urine samples taken during one year. Forty-five patients (12 %) satisfied this criterion and 30 of these patients agreed to take part in a prospective 2-year study. The protocol was approved by the Regional Ethics Committee.

Design

In Study 1, the 30 patients with microalbuminuria were matched with respect to age, diabetes duration, mean 1-year HbA_{1c}, and gender, with normoalbuminuric patients ($n = 327$) from the previously

Table 1. Clinical data (median and range) of the Type 1 diabetic patients participating in Study 1

	Microalbuminuric	Normoalbuminuric
n	27	24
Female/male	13/14	11/13
Age (years)	18 (14–29)	19 (14–24)
Diabetes duration (years)	11 (5–18)	10 (7–17)
HbA_{1c} (%)[a]	10.0 (7.9–12.6)	9.4 (6.7–13.8)
Systolic BP (mm Hg)	124 (94–150)	118 (94–130)
Diastolic BP (mm Hg)	80 (56–98)	75 (66–84)
UAE (μg/min)[a]	30 (14–194)	5 (3–13)

[a] Mean values of 2–4 measurements in the year preceeding the study. BP, Blood pressure; UAE, urinary albumin excretion rate

Table 2. Clinical data (median and range) of the Type 1 diabetic patients participating in Study 2

	CSII	CT
n	12	11
Female/male	6/6	4/7
Age (years)	18 (14–29)	18 (17–29)
Diabetes duration (years)	10 (5–18)	12 (8–16)
HbA_{1c} (%)[a]	10.0 (8.1–12.3)	9.2 (7.9–11.0)
Systolic BP (mm Hg)	121 (94–142)	124 (114–150)
Diastolic BP (mm Hg)	79 (56–92)	84 (74–98)
UAE (μg/min)[a]	19 (14–58)	30 (15–50)

[a] Mean values of 2–4 measurements in the year preceeding the study. BP, Blood pressure; UAE, urinary albumin excretion rate; CSII, continous subcutaneous insulin infusion; CT, conventional treatment

mentioned screened population. Twenty-seven normoalbuminuric patients were included. Three patients within each group were excluded because of unmeasurable, low IgG_4 values in the urine. Clinical data of the patients are given in Table 1. The patients had neither clinical nephropathy nor proliferative retinopathy. All except one patient had normal blood pressure (< 140/90 mm Hg). This patient was initially hypertensive (140/98 mm Hg), but the blood pressure declined to 135/85 mm Hg in the course of 6 months without antihypertensive treatment.

Study 2 was a randomized, prospective, clinical trial evaluating the importance of improved metabolic control in patients with microalbuminuria. Thirty patients with microalbuminuria were block-randomized (age, diabetes duration, mean 1-year HbA_{1c}, and mean 1-year urinary albumin excretion rate [UAE]), Table 2) to either intensive treatment (CSII = continuous subcutaneous insulin infusion) or conventional treatment (CT = multiple injections in 12 patients and three injections in three patients). Patient follow-up was conducted by means of regular visits to the out-patient-clinic every second month. This report deals with the first 12 months and the 23 patients from whom we obtained repeated IgG/IgG_4 measurements.

Methods

Charge selectivity index was measured as IgG/IgG_4 selectivity index, i.e. total IgG/IgG_4 clearance ratio:

$$\frac{\text{clearance of IgG}}{\text{clearance of IgG}_4} = \frac{U - IgG/S - IgG}{U - IgG_4/S - IgG_4} = \frac{U - IgG \times S - IgG_4}{U - IgG_4 \times S - IgG}$$

in timed overnight urine samples (U = urine, S = serum). Serum and urine IgG and IgG_4 concentrations were measured by enzyme linked immunosorbent assays as previously described [12, 13], including pre-storage dilution of urine samples (1:5 in phosphate buffered saline supplemented with 1 % bovine serum albumin). Samples were stored at −70 °C and assayed within 7 weeks. Intra- and inter-assay coefficients of variation were below 5 and 10 %, respectively, in both the IgG and the IgG_4 assay.

UAE rate was measured in at least two timed overnight urine samples in the year preceding the study, and then every second month in Study 2. The albumin concentration was measured by immunoturbidimetry in samples kept at 4 °C from 1 to 3 days. The inter-assay coefficient of variation was 4.7 % in the range of 10–50 mg/l. The urine samples were negative for leucocytes, nitrite, albumin and ketones as demonstrated in the dipstick test.

Retinol binding protein (RBP) was measured by an ELISA-method [14].

HbA_{1c} was analysed by an HPLC method ("Diamat" analyser; Biorad, Richmond, Calif., USA). Normal range was 4.3–6.1 %, with an inter-assay coefficient of variation of 3 %.

Glomerular filtration rate (GFR) was measured by inulin-clearance (Inulin; Laevosan, Linz, Austria) after oral water loading. High concentrations of interfering glucose were removed by glucose oxidase. The GFR was corrected for body surface area (1.73 m^2).

Blood pressure was measured by conventional mercury sphygmomanometer with patients sitting after a 10 min rest, and diastolic pressure was measured according to the Korotkoff V sounds. Measurements in the microalbuminuric patients represent the mean values of four measurements during a 2-month period, in the normoalbuminuric patients, the mean of two measurements.

Statistical analysis

The change over time within each group was tested by paired *t*-test. Differences between groups were tested by unpaired non-parametric rank sum test (Mann-Whitney). Correlations were expressed by Pearsson's correlation coefficient, *r*. Results are presented as either median or mean of changes expressed as percentage of baseline values (Study 2) with 95 % confidence intervals. In case of missing data (urine samples not obtained, $n = 3$), linear interpolation was used. Statistical significance was defined as $2p < 0.05$.

Results

Study 1

The patients with microalbuminuria had a significantly decreased IgG/IgG_4 selectivity index compared to the patients with normoalbuminuria 1.20 (0.92–1.40) vs 1.68 (1.22–2.21), $2p < 0.01$ (Fig. 1).

Study 2

Metabolic control improved in the CSII-group during the 12 months compared to baseline and the difference ($\Delta HbA_{1c}/HbA_{1c\ baseline}$) between the CSII- and the CT-group was statistically significant at 2, 6, 9, and 12 months (4 months, NS) as shown in Figure 2.

Table 3 shows the individual selectivity indices. The median intra-individual coefficient of variation was 26 % (range 12–74 %). IgG and IgG_4 data, on which the selectivity index is based, are depicted in Table 4.

A sharp 50 % increment in IgG/IgG_4 selectivity index was seen in the CSII-group during the first 6 months of good metabolic control ($p < 0.05$ compared to the CT-group, Fig. 3, Table 5). No further increase in selectivity index was seen from 6 to 12 months, but the change from

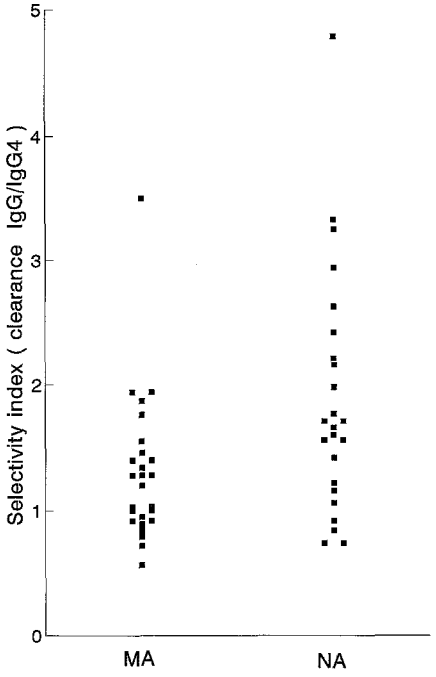

Fig. 1. IgG/IgG_4 selectivity index. Patients with microalbuminuria (MA, $n = 27$), Patients with normoalbuminuria (NA, $n = 24$)

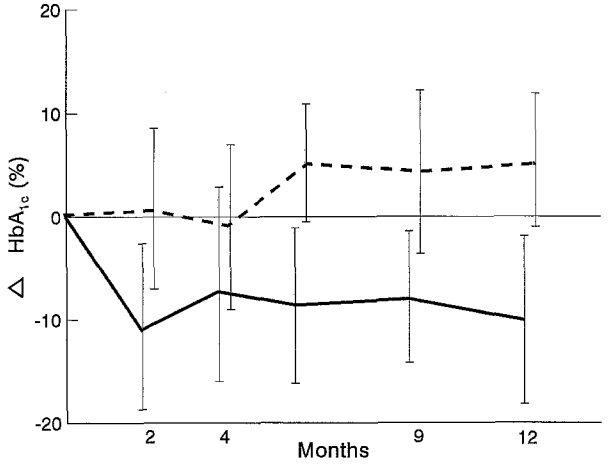

Fig. 2. Change in HbA_{1c} (% from baseline) in patients with microalbuminuria. Mean with 95% confidence interval. Continuous subcutaneous insulin infusion (CSII):— ($n = 12$). Conventional treatment (CT): ------ ($n = 11$)

baseline to 12 months in the CSII-group was near significant ($2p = 0.051$).

UAE remained unchanged during the study period (Table 5). There was no correlation between changes from baseline in selectivity index and UAE rate.

The timed urinary excretion of retinol binding protein was not statistically different in the groups with micro- and normoalbuminuria respectively [143 (65–212) vs 150 (95–275) ng/min]. In Study 2 no significant change was observed from baseline to 12 months, in either of the two treatment groups [CSII: 143 (21–261) to 124 (88–306) and CT: 192 (41–420) to 154 (54–323) ng/min].

A reduction in median GFR was observed in the CSII-group during the 12-month study period [145 (123–166) vs 127 (103–144) $ml \cdot min^{-1} \cdot 1.73\ m^{-2}$], but the change did not reach statistical significance. No reduction was found in the CT-group [127 (97–188) vs 121 (101–165) $ml \cdot min^{-1} \cdot 1.73\ m^{-2}$].

Discussion

Applying fractional clearance of IgG/IgG_4 as a charge selectivity index, we have in the present study found reduced selectivity index in a group of Type 1 diabetic patients with low-grade microalbuminuria compared to a well-matched group with normoalbuminuria. Additionally, it was found that the improved metabolic control over a 12-month period was related to an increment in the selectivity index.

Previous studies have used different kinds of glomerular charge selectivity indices [2, 3, 15, 16]. Viberti et al. [2] used relative clearances of IgG/albumin. They suggested that in patients with UAE less than 60 μg/min, increased intraglomerular pressure was primarily responsible for the increased albumin excretion and that only in patients with UAE greater than 60 μg/min and in patients with proteinuria and unreduced GFR, did a charge selectivity defect contribute to the increased UAE. However, a more ideal method for investigating the glomerular charge selectivity theory in a clinical setting is by comparing the urinary clearance of two endogenous molecules with identical size and configuration, but different isoelectric points [9]. Total IgG and IgG_4 satisfy this criterion, having similar molecular weight (155,000) while IgG_4 is clearly more anionic than IgG (isoelectric points = 5.5–6.0 vs 5.8–7.3 [3]). The resemblance of structure and size between IgG and

Table 3. Individual IgG/IgG_4 selectivity indices during 12 months of continous subcutaneous insulin infusion (CSII) or conventional treatment (CT)

Patients		No. of months			
		0	2	6	12
CSII	1	1.3	1.6	2.2	3.3
	2	1.4	1.0	0.9	1.7
	3	1.9	2.1	1.6	1.5
	4	0.9	1.8	1.5	1.5
	5	0.8	1.0	0.6	0.9
	6	1.3	1.2	1.7	3.2
	7	1.5	1.1	1.1	1.0
	8	1.0	0.9	1.5	1.0
	9	1.3		1.4	1.1
	10	1.0	2.3	2.1	2.9
	11	0.6	1.0	3.2	2.1
	12	1.0	1.2	3.6	1.1
CT	13	1.3	0.9	1.1	1.0
	14	0.9		1.3	1.7
	15	1.2	1.3	1.4	1.6
	16	0.9	0.6	0.6	0.8
	17	1.9	1.2		1.2
	18	1.0	0.6	0.7	0.8
	19	1.9	1.4	0.7	0.8
	20	0.8	1.0	0.8	1.2
	21	1.0	0.7	0.9	2.2
	22	0.7	1.1	1.4	1.3
	23	1.8	1.5	1.1	2.0

Table 4. Urinary IgG and IgG_4 excretion during 12 months of continuous subcutaneous insulin infusion (CSII) or conventional treatment (CT). Median and 95% confidence interval

	IgG (ng/min)				IgG_4 (pg/min)			
No. of months	0	2	6	12	0	2	6	12
CSII	2.3 (1.5–4.1)	3.2 (1.6–5.1)	2.6 (1.8–3.7)	2.8 (2.2–6.6)	114 (38–161)	97 (68–166)	117 (68–166)	100 (65–325)
CT	2.7 (0.4–14.8)	3.2 (0.5–5.4)	2.7 (1.8–7.0)	3.5 (1.8–12.2)	89 (33–343)	94 (62–249)	88 (42–236)	94 (41–302)

Table 5. IgG/IgG_4 selectivity index (SI) and urinary albumin excretion (UAE), during 12 months of continuous subcutaneous insulin infusion (CSII) or conventional treatment (CT). Median and 95% confidence interval

	SI				UAE (μg/min)			
No. of months	0	2	6	12	0	2	6	12
CSII	1.1 (0.9–1.4)	1.2 (1.0–1.8)	1.6 (1.1–2.3)	1.5 (1.0–2.9)	16 (11–89)	12 (7–28)	12 (6–52)	14 (8–42)
CT	1.0 (0.8–1.9)	1.0 (0.6–1.4)	1.1 (0.7–1.4)	1.2 (0.8–2.0)	25 (5–54)	25 (6–70)	14 (6–25)	13 (7–77)

IgG_4 makes it very unlikely that haemodynamic factors or blood pressure will influence the glomerular filtration of the IgG-molecules differently.

In a cross-sectional study [9] a correlation between this IgG/IgG_4 selectivity index and albumin clearance was found. In our comparison between patients with micro- and normoalbuminuria (Study 1) the patients were well matched regarding possibly confounding factors such as age, diabetes duration and long-term hyperglycaemia. By using relative clearances of IgG/IgG_4 (as used by Pietravalle et al. [9], but not the same assay) we confirmed a significantly reduced selectivity index in patients with microalbuminuria compared to normoalbuminuric patients.

In our 12-month prospective study including only patients with microalbuminuria, we addressed the importance of improved blood glucose control on the IgG/IgG_4 selectivity index. The CSII-group, which obtained significant improvement in mean HbA_{1c}, showed an increased IgG/IgG_4 selectivity index compared to the CT-group. The increment was most pronounced after 6 and 12 months. One previous study investigated prospectively the influence of strict metabolic control during 12 weeks using non-glycated/glycated albumin as a selectivity index [17]. Their subjects were normoalbuminuric and no effect of strict metabolic control was observed. The improvement of metabolic control observed in our CSII-group could theoretically influence different factors responsible for the negative charge of the basement membrane of which heparan sulphate proteoglycan is the most important. The activity of N-acetylheparosan deacetylase, a key enzyme in heparan sulphate biosynthesis in rat, is negatively correlated to the blood glucose level [18]. Accordingly improved glycaemic control could lead to an increment of the amount of heparan sulphate proteoglycan and partly restore the charge conditions of the glomerular basement membrane. Secondly, non-enzymatic glycation alters the isoelectric point [19] of albumin and thereby the passage over the glomerular filtration barrier. A similar glycation affecting IgG and IgG_4 differently cannot be excluded. If this was the case, the tubular reabsorption could also be modified [20]. However, in our study the tubular function assessed by retinol binding protein, was not different in the group of patients with micro- and normoalbuminuria respectively. In the prospective study no significant changes were found over the 12-month period, either within the group with improved control, or between the two groups.

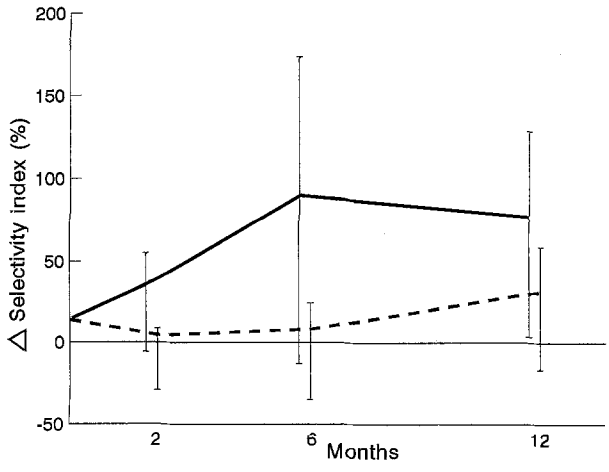

Fig. 3. Change in IgG/IgG_4 selectivity index (% from baseline) in patients with microalbuminuria. Mean with 95% confidence interval. Continuous subcutaneous insulin infusion (CSII): — ($n = 12$). Conventional treatment (CT): ------ ($n = 11$)

In a clinical setting dealing with Type 1 diabetic patients with normal or supranormal GFR, a relationship between long-term change in selectivity index and UAE is of main interest. However, our findings did not show a negative correlation between changes from baseline in selectivity index and UAE. This may indicate that the impact of charge alterations (glomerular or tubular) on the UAE during a 12-month period, is quite modest. Confounding factors, the significant day-to-day variation (within patients) seen both in UAE and IgG/IgG_4 selectivity index, and the great variation between patients in charge selectivity index, might possibly hide a relationship between these two parameters. Further it has to be emphasized that most of our patients were in the low microalbuminuric range.

We conclude that by applying a method which eliminates the possible influence of changes in size selectivity and GFR, patients with microalbuminuria have decreased glomerular charge selectivity compared to normoalbuminuric patients, and we believe that this is the first study to show that improved blood glucose control may at least temporarily restore some of the charge selectivity loss.

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