

Plasma disappearance of glycated and non-glycated albumin in Type 1 (insulin-dependent) diabetes mellitus: evidence for charge dependent alterations of the plasma to lymph pathway

L. Bent-Hansen, B. Feldt-Rasmussen, A. Kverneland and T. Deckert

Steno Diabetes Center, Gentofte, Denmark

Summary. The fractional plasma escape rates of glycated and non-glycated albumin have earlier been measured in groups of Type 1 (insulin-dependent) diabetic patients and control subjects. The escape of non-glycated albumin was similar in control subjects and normoalbuminuric patients, but elevated in patients with micro or macroalbuminuria. In all groups the escape rate of glycated albumin was lower than that of non-glycated albumin. Glycation increases the anionic charge of albumin. To assay for charge-dependent alterations of transport a selectivity index (non-glycated albumin/glycated albumin transport ratio) was determined from the disappearance data. The index was high in control subjects (1.021 ± 0.0057 (SEM)). This reflects a mean difference between the two escape rates of 2.1% per hour (for comparison the mean of the fractional escape rate of non-glycated albumin of the normal control subjects was 4.7% per

hour). The index was numerically even higher in normoalbuminuric patients (1.031 ± 0.0047 (SEM)), but reached significantly lower levels in patients with microalbuminuria (1.013 ± 0.0030 (SEM), $p < 0.02$). Patients with clinical nephropathy had very low levels indicating loss of selectivity (1.002 ± 0.0068 (SEM), $p < 0.001$). This pattern accords well with measurements of renal clearance selectivity indices, suggesting a general, progressive deterioration of anionic perivascular barrier components in diabetic microangiopathy. The structural target for these changes is likely to be the glycosaminoglycans of the glomerular basal membrane and the interstitial matrix.

Key words: Albumin, capillary permeability, glycated albumin, diabetes mellitus.

Plasma protein disappearance is elevated in Type 1 (insulin-dependent) diabetic patients with microalbuminuria, reflecting a general deterioration of the microvascular barrier function when entering this stage of disease [1, 2]. However, the specificity of the lesion and the relation to structural or hydrodynamic alterations is unknown.

In 1987 we studied the plasma disappearance of glycated and non-glycated albumin in Type 1 diabetic patients with normal or elevated urinary albumin excretion rates [3]. The primary aim was to investigate potential metabolic differences, but crude transport data were included as well. We observed a selective restriction of the plasma disappearance of the more electronegative glycated albumin compared with non-glycated albumin in healthy control subjects compared with Type 1 diabetic patients. There were no significant differences between the three diabetic subgroups which were established on the basis of their urinary albumin excretion rates. Based on the recent demonstrations of increased charge dependent glomerular transport in the presence of microal-

buminuria [4–6] we recalculated our previous data in order to assay the charge specificity of increased whole body plasma protein disappearance in Type 1 diabetic patients with or without signs of increased glomerular protein sieving.

Subjects and methods

The experiment has been described before [3]. Twenty-five male Type 1 diabetic patients were divided into groups according to their urinary albumin excretion in three or more 24-h urine collections. Group 1 had normal albumin excretion (< 30 mg/24 h), group 2 had microalbuminuria (30–300 mg/24 h) and group 3 had clinical nephropathy (> 300 mg/24 h). Six normal male subjects, matched for age with the diabetic groups served as the control group (age mean: 32 ± 8 years).

At time zero 80 kBq of ^{131}I -albumin and 80 kBq of ^{125}I -glycated albumin (5.5 mol glucose/mol albumin) was injected in one arm. Plasma samples were taken every 10 min for the first hour following injection.

The plasma samples were assayed for the content of ^{131}I and ^{125}I activity in a dual channel scintillation counter.

Table 1. Results

	FER _{non-glycated albumin} (% · h ⁻¹)	Selectivity index (h ⁻¹)
Control subjects (n = 6)	5.74 ± 0.68	1.021 ± 0.0057
Normoalbuminuria (n = 9)	5.60 ± 0.67	1.031 ± 0.0050
Microalbuminuria (n = 8)	7.57 ± 0.72 ^{a,b}	1.014 ± 0.0030 ^b
Clinical nephropathy (n = 8)	9.14 ± 0.88 ^{a,b}	1.004 ± 0.0068 ^{a,b}

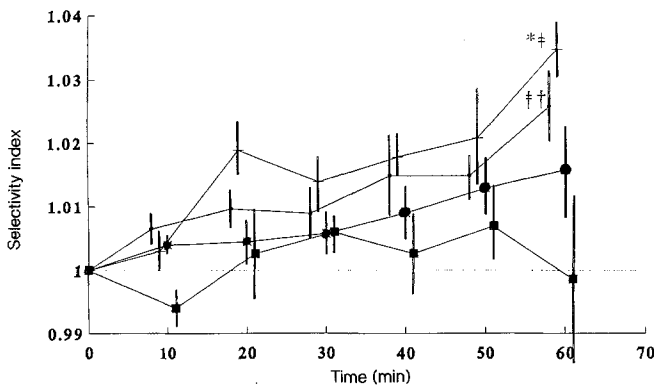
^a *p* < 0.05 vs control subjects^b *p* < 0.05 vs normoalbuminuric patientsFER_a, Fractional escape rate of non-glycated albumin

Fig. 1. The ratio of plasma non-glycated albumin/glycated albumin in the three patient groups and in control subjects (mean ± SEM). • normal control subjects, patients with: + normal urinary albumin excretion rate, ● microalbuminuria, ■ diabetic nephropathy. * *p* < 0.001 † *p* < 0.05 vs patients with microalbuminuria; ‡ *p* < 0.001 vs patients with nephropathy

Calculations

In order to compensate for small changes in plasma water during the experiment the plasma activity was divided with the total protein of the sample. The disappearance curves were drawn in a semilogarithmic scale, and the fractional escape rate (FER_{albumin}) was determined as the time constant of the ¹³¹I curves from 10 to 60 min. The ¹³¹I/¹²⁵I ratio was calculated at each point, and correlated with time (method of least squares). From these correlation lines the selectivity index (SI) was calculated by dividing the zero ratio of each line with the 60-min value. Thus the SI of each subject was determined from all six measured plasma ratios, denoting the increase of FER_{non-glycated albumin} over FER_{glycated albumin}. The direct comparison of the simultaneously determined plasma activities instead of the calculated FER_{non-glycated albumin} and FER_{glycated albumin} [3] yields a much more sensitive analysis, cancelling some of the considerable variation in the calculation of FER.

Statistical analysis

Results are given as mean ± SEM. The Student's *t*-test was used for comparison between the groups. A *p* value of < 0.05 (two-tailed) was considered significant.

Results

Results are shown in Table 1 and Figure 1. The FER_{non-glycated albumin} as originally calculated is given in the first column, and the calculated SI in the second. The figure 1.021

denotes an absolute difference between the two FER values (% per hour) of 2.1 % per hour. It appears that only in group 3 does the SI approach 1, indicating similar transport of the two albumins. In all other groups the transport of albumin was faster than that of glycated albumin (SI > 1, *p* < 0.005).

It should be noted, that the calculation of FER_{non-glycated albumin} and SI only describes the plasma disappearance curves from 10 to 60 min. To see if there were any charge-dependent effects in the initial part of the curve from zero to 10 min, the zero ratio of the correlation lines was divided by the standard ratio of the injectate. However, no significant changes were found.

Discussion

Glycation of albumin takes place at the free lysine amino terminals, thereby increasing the net anionic charge of the molecule [7]. The size increases to a lesser degree, making the simultaneous transport index i.e. the SI of the two albumins a sensitive tool for charge-dependent permeability analysis. In the kidney, the glomerular basement membrane functions not only as a simple molecular sieve, but also as an electrostatic barrier [4–6, 8, 9]. When applying SI clearance measurements in groups of diabetic patients and control subjects a complicated pattern appears [5]. Patients with normal urinary albumin excretion have a higher SI (non-glycated albumin clearance/glycated albumin clearance) than control subjects, patients with microalbuminuria have an SI comparable to control subjects, and patients with clinical nephropathy have a low SI. In concert with other relative clearance data [6, 9] these findings indicate a basic decrease of charge-dependent transport in normoalbuminuric diabetic patients. This may be caused by increased glycation of membrane structures, increasing the charge density of the filter in normoalbuminuric patients. In microalbuminuria the normal electrostatic barrier function starts to degenerate, and is followed by more extensive structural lesions in overt nephropathy. The electrostatic properties of the glomerular membrane are partly due to a high content of strongly anionic glycosaminoglycans (GAGs), and an impaired synthesis of these compounds may initiate the development of diabetic nephropathy [8].

In the present study a similar pattern could be demonstrated in whole body plasma disappearance SI, suggesting that similar mechanisms operate not only in the kidney, but also in the entire peripheral circulation.

To calculate the SI we normally use the FER of the more electronegative molecule as the denominator i.e. SI = FER_{non-glycated albumin}/FER_{glycated albumin}. By this calculation the: (SI – 1) × 100 equals the relative difference between the FER values; the unit is: “percent of the FER_{glycated albumin}”. (In the original paper [3] the SI was given as the reciprocal value). The equation in that paper was based on the individually estimated FER values which resulted in a high risk for type 2 error due to the high variability of each of the two estimates in combination with the small numbers studied. When using our original calculations we found that normal control subjects had a significantly

higher index (a more electronegative barrier) than diabetic patients. The SI of normal control subjects was 1.25 indicating that the FER of the glycosylated albumin was increased by 25% compared with the non-glycosylated albumin. The corresponding value in the patients was 1.07 ($p < 0.05$). There was no significant difference between the patient groups. In the present paper we calculated for each time point the ratio between the two albumins. The SI for each patient was given as the 60-min value estimated on the basis of the SI-correlation line of the patient. By this calculation the $(SI - 1) \times 100$ equals the difference in absolute terms between the FER values; the unit is: “% per hour”. The differences between the SI values in the patient groups are significant only after 60 min of study. However, the general trend in each of the curves seems to support the conclusion obtained on the basis of the correlation line estimates.

As recently shown [10], the plasma disappearance of albumin includes two phases: one fast in the interval from zero to about 10 min, and one slow in the interval from 10 to 60 min (FER). The first phase is believed to mainly denote the tracer passage of capillary walls, and the latter the interstitial transport. However, the increased permeability in diabetic patients with albuminuria [2, 6, 9] as well as the changes of SI seem limited to the second phase, and may thus reflect interstitial rather than endothelial alterations. Similar to the glomerular basement membrane, a major resistance component of extracellular space to macromolecules is the content of anionic GAGs. These are chemically closely related to the renal GAGs, and although they do not have a firm structure and differ in composition and charge, they probably share the same metabolic pathways. Thus, a generalized defect synthesis of highly charged GAGs in susceptible patients is a likely basic step in the formation of malignant diabetic microangiopathy. Apart from the barrier function, GAGs have strong anticoagulative and anti-precipitating capabilities, and participate in microvascular as well as macrovascular repair processes. A defect synthesis of GAGs might therefore explain aspects of increased hyalinization, fibrosis, and atherosclerosis in albuminuric patients.

In conclusion, the peripheral SI of albumin/glycosylated albumin disappearance produces a similar pattern as in the kidney. This indicates, that the peripheral barrier to macromolecules is also a charged barrier, and that an early

step in the development of diabetic microangiopathy is the break-down of this barrier.

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Dr. L. Bent-Hansen
Cardiovascular Laboratory (2014)
Medical Department B
Rigshospitalet
DK-2100 Copenhagen Ø
Denmark