Diabetologia (1988) 31: 708-710

Diabetologia

Rapid communications

Improved metabolic control does not alter the charge-dependent glomerular filtration of albumin in uncomplicated Type 1 (insulin-dependent) diabetes

A. Kverneland[1], B. Welinder[2], B. Feldt-Rasmussen[1] and T. Deckert[1]

[1] Steno Memorial Hospital and [2] Hagedorn Research Laboratory, Gentofte, Denmark

Summary. The selectivity index, i.e. clearance of non glycated albumin/clearance of glycated albumin was studied in fourteen patients with Type 1 (insulin-dependent) diabetes and normal urinary albumin excretion. The index was increased above one in all patients, and correlated significantly to HbA_{1c}. It was, however, unaffected by 12 weeks of improved metabolic control with a mean decline in HbA_{1c} of 1.9% in seven patients. We conclude that the increased electronegative charge of the glomerular filtration barrier observed in uncomplicated diabetes is related to long term metabolic control but not reversible during twelve weeks of strict metabolic control. This indicates a slow turnover of the components responsible for the increased charge selectivity in uncomplicated diabetes.

Key words: HbA_{1c}, Type 1 (insulin-dependent) diabetes mellitus, glomerular charge, glycated albumin, albumin clearance.

Glomerular filtration of macromolecules is dependent on pore size and pore charge of the glomerular basement membrane (GBM). In diabetic animals the anionic charge of the GBM has been shown to be increased [1]. In an earlier study published in this journal we reported on charge selectivity of the glomeruli assessed in patients with Type 1 (insulin-dependent) diabetes who had normal urinary albumin excretion rates; similar changes were suggested to take place in these patients [2]. Since a significant correlation was found between charge selectivity and HbA_{1c}, it was suggested that the increase of anionic charge of the GBM in diabetic patients is associated to the quality of metabolic control [2]. We have now tested the influence of long term (months) metabolic control on charge selectivity of GBM.

Subjects and methods

Fourteen patients (7 M and 7 F) with Type 1 diabetes of two to ten years duration were studied in an open, prospective study. They had no history of non-diabetic renal disease and all had a negative bacterial culture of the urine. Only patients with normal urinary albumin excretion <30 mg/24 h in two 24-h urine collections, normal blood pressure, normal s-creatinin and no retinopathy were included in the study. All patients were offered frequent visits to the out-patient clinic and supervision by the same doctor in order to obtain better metabolic control. Insulin infusion pumps (Nordisk Infuser, Nordisk Gentofte, Denmark) for continuous subcutaneous insulin infusion were randomly distributed to 7 patients. Blood and urine samples were collected before and 4 and 12 weeks after the onset of the experimental period. After 12 weeks the patients were ranked according to the decrease of HbA_{1c} during the experiment. The 7 patients with the most pronounced fall in HbA_{1c} were sampled in Group A, the 7 patients with the least pronounced fall in HbA_{1c} in Group B. The two groups (A vs B) were well matched according to: age (years) 33 (27-45) vs 39 (27-46), diabetes duration (years) 5 (2-10) vs 5 (2-7), mean blood pressure (mm Hg) 86 (80-107) vs 86 (81-93) and insulin dosage (IU/24 h) 40 (28-53) vs 42 (36-62). All subjects gave their informed consent for participation and the study was approved by the regional Ethics Committee.

Glomerular charge selectivity was assessed by the renal clearance of two plasma proteins which were similar in size but slightly different in charge, i.e. non-glycated albumin and glycated albumin, the latter being slightly more negatively charged compared to non-glycated albumin. Glycated albumin in urine and plasma was measured using the Furosine Tyrosine method described by Schleicher and Wieland [3], modified for measurements in urine by Welinder et al. [4] and described in detail in our previous paper [2]. The molar concentration of non-glycated and glycated albumin derived lysine in urine and plasma was calculated. The ratio between non-glycated albumin-derived lysine clearance and glycated albumin-derived lysine clearance was expressed as the Selectivity Index. The interassay variation of serum and urine was 3.6% and 4.0%, respectively.

The concentration of total albumin (glycated plus non-glycated) in urine and plasma was measured by ELISA, interassay variation 8.3% [5].

HbA_{1c} was measured by HPLC (Bio-Rad-Diamat, Richmond, Calif., USA) normal range 4.3-6.2%, interassay variation 3.8%.

Statistical analysis

Results are expressed as medians with ranges. The significance of differences between or within groups were tested with non-parametric statistics for paired and unpaired data.

Results

HbA_{1c} at the onset of the study were similar in the two groups (Table 1). In Group A consisting of the seven patients who improved their metabolic control HbA_{1c} was reduced significantly by a median of 1.9% during the 12-week experimental period (Table 1). Patients in Group B had unchanged metabolic control. Plasma glycated albumin was reduced after 4 weeks reflecting the shorter half-life of this protein. All patients had a Selectivity Index (SI) of more than 1 at the onset of the study reflecting a renal clearance of non-glycated albumin 4 to 6-fold larger than that of the more anionic glycated albumin. There was a significantly positive correlation between SI and HbA_{1c} ($n=14$, $r=0.56$, $2p<0.05$, Fig. 1).

The SI was unchanged in the two groups during the experimental period and unrelated to changes in metabolic control (Table 1).

Table 1. Selectivity Index (SI) in Group A (improved metabolic control) and Group B (unchanged metabolic control)

Group	Weeks	HbA_{1c} (%)	p-glycated albumin (mmol furosine/ mmol albumin)	Selectivity index
A	0	9.1[a] (8.1-9.8)	0.68 (0.50-0.91)	4.3 (3.1-6.7)
	4	8.1 (6.9-9.1)	0.55 (0.41-0.79)	4.6 (2.7-7.3)
	12	7.2[a] (6.1-7.9)	0.53[a] (0.41-0.72)	4.2 (2.1-7.3)
B	0	9.7 (7.9-11.4)	0.90 (0.41-1.23)	5.9 (2.3-7.7)
	4	9.6 (7.8-10.8)	0.77 (0.44-0.90)	5.0 (1.6-7.5)
	12	9.8 (8.1-12.7)	0.77 (0.47-0.95)	5.7 (3.1-8.5)

[a] 0 weeks versus 12 weeks: $2p<0.01$

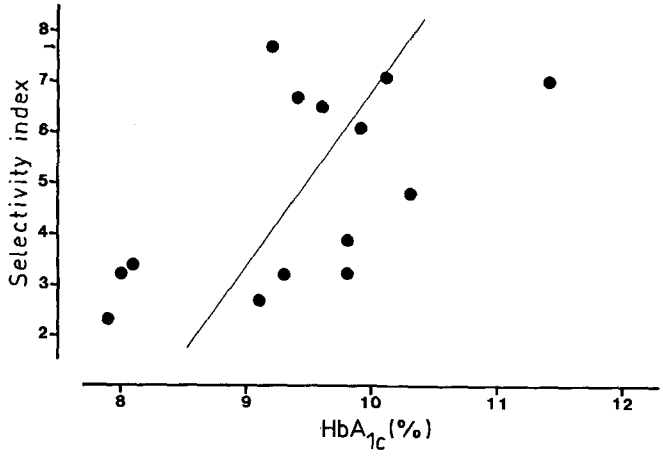

Fig. 1. Correlation between HbA_{1c} and the selectivity index (SI), clearance of non-glycated albumin/clearance of glycated albumin in 14 Type 1 diabetic patients with normal urinary albumin excretion below 30 mg/24 h ($r=0.56$, $n=14$, $2p<0.05$)

Discussion

We have earlier [2] demonstrated that compared to non-diabetic patients, SI is significantly increased in Type 1 diabetic patients with normal urinary albumin excretion. Since the increase of the SI was positively correlated to HbA_{1c}, we suggested that the increase of anionic charge of the glomerular basement membrane in diabetic patients was due to poor metabolic control affecting the glomerular structural proteins. Increased SI in patients with uncomplicated Type 1 diabetes was found also in the present study, and so was the correlation between SI and HbA_{1c}. The increased anionic charge (SI) was, however, not reduced during 12 weeks of improved metabolic control in spite of the observed correlation to HbA_{1c}. So far these observations are unexplained. A number of factors may play a role. First: Non-enzymatic glycation of structural proteins of the GBM - tending to increase the anionic charge of these substances - has been demonstrated by several groups [6, 7], but such processes are expected to be reversible during three month periods of improved metabolic control as in this study. Second: The formation of advanced glycation end products may lead to an increase of negative charge within the GBM [8] and it is not expected that significant changes in the concentration of advanced glycation end product within the GBM would take place during twelve weeks of strict metabolic control due to the very slow metabolic turnover of such products. Our results are in accordance with such products playing a role. Other anionic components of GBM, i.e. Heparan Sulphate Proteoglycane and sialic acid are decreased in diabetic animals and man and can therefore not explain the observed increase of negative charge of the GBM in uncomplicated diabetic patients [9]. Finally, it has been shown in diabetic animals and man that clogging of anionic plasma proteins takes place within the GBM [10]. It cannot be excluded that such alterations would contribute to the increase of negative charges within the glomerular filtration barrier. If so, this clogging was not reversed during 12 weeks of improved metabolic control.

In conclusion, SI is increased in Type 1 diabetic patients with normal urinary albumin excretion, indicating increased negative charge of GBM. These

changes are related to long term metabolic control but are not reversible during twelve weeks of strict metabolic control. These results are not in contradiction to the hypothesis that the primary event in the development of diabetic microangiopathy is a loss of the electronegative charge of basal membranes due to a reduction of their content of heparane sulphate proteoglycane [9].

Acknowledgement. The study was supported financially by Diabetesforeningen and Nordisk Insulinlaboratorium, Denmark.

References

1. Michels LD, Davidman M, Keane WF (1982) Glomerular permeability of neutral and anionic dextrans in experimental diabetes. Kidney Int 21: 699-705
2. Kverneland A, Feldt-Rasmussen B, Vidal P, Welinder B, Bent-Hansen L, Søegaard U, Deckert T (1986) Evidence of changes in renal charge selectivity in patients with Type 1 (insulin-dependent) diabetes mellitus. Diabetologia 29: 634-639
3. Schleicher E, Wieland OH (1981) Specific quantitation by HPLC of protein (Lysine) bound glucose in human serum albumin and other glycosylated proteins. J Clin Chem Clin Biochem 19: 81-87
4. Welinder BS, Vidal P, Deckert T, Hansen B (1985) Estimation of glycosylated albumin in serum and urine. Diabetes Res Clin Pract [Suppl 1]: 599
5. Feldt-Rasmussen B, Dinesen B, Deckert M (1985) Enzyme immunoassay: an improved determination of urinary albumin in diabetics with incipient nephropathy. Scand J Clin Lab Invest 45: 539-544
6. Schleicher E, Wieland E (1984) Changes of human glomerular basement membrane in diabetes mellitus. J Clin Chem Clin Biochem 22: 223-227
7. Cohen MP, Urdanivia E, Surma M, Wu V-Y (1980) Increased glycosylation of glomerular basement membrane collagen in diabetes. Biochem Biophys Res Commun 95: 765-769
8. Brownlee M, Vlassara H, Cerami A (1984) Nonenzymatic glycosylation and the pathogenesis of diabetic complications. Ann Int Med 101: 527-537
9. Deckert T, Feldt-Rasmussen B, Djurup R, Deckert M (1988) Glomerular size and charge selectivity in insulin-dependent diabetes mellitus. Kidney Int 33: 100-106
10. Kanwar YS, Rosenzweig LJ (1982) Clogging of the glomerular basement membrane. J Cell Biol 93: 489-494

Received: 7 July 1988

Dr. T. Deckert
Steno Memorial Hospital
Niels Steensensvej 2
DK-2820 Gentofte
Denmark