The increase in albuminuria induced by exercise is not associated with preferential excretion of glycated albumin in Type 1 (insulin-dependent) diabetic patients

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Summary. The increase in urinary albumin excretion induced by acute exercise in Type 1 (insulin-dependent) diabetic patients is associated with the urinary excretion of cationic proteins. To test whether the renal excretion of glycated albumin (more anionic than non-glycated albumin) is affected by exercise, we submitted seven normoalbuminuric (albumin excretion rate < 30 mg/24 h) Type 1 diabetic patients and six well-matched healthy subjects to an exercise test (600 kpm/min for 20 min) on a bicycle ergometer, preceded and followed by a 1-h resting period. The selectivity index (renal clearance of non-glycated/glycated albumin) was not

significantly different among the pre-exercise, exercise and post-exercise periods, either in the normal subjects (1.01 ± 0.03 vs 1.08 ± 0.06 vs 1.08 ± 0.05) or in the diabetic patients (1.25 ± 0.09 vs 1.20 ± 0.07 vs 1.20 ± 0.06), whereas it was significantly higher ($p < 0.05$) in diabetic patients compared to healthy subjects during pre-exercise. These results are not consistent with the hypothesis that acute exercise may induce a preferential excretion of glycated albumin.

Key words: Renal clearance, exercise, glycated albumin, non-glycated albumin.

Glycation involves both circulating proteins, such as albumin [1], and structural proteins, such as the components of glomerular basement membrane (GBM) [2]. The effect of glycation on the GBM charges has not been completely clarified, though glycated albumin has been reported to be more anionic than unmodified plasma albumin at physiological pH [3, 4]. Preferential urinary excretion of glycated albumin in diabetic subjects during normal life conditions (i.e. not during exercise) has been confirmed by some authors [5, 6], but refuted by others [7, 8]. Under short-term (hours) experimental conditions, it is reasonable to assume that the degree of protein glycation remains constant, since non-enzymatic reactions are very slow and take place during a period of several days or weeks [9]. On the other hand, the increase in albumin excretion rate (AER) induced by exercise in Type 1 (insulin-dependent) diabetic patients is associated with the urinary excretion of cationic proteins [10]. Microalbuminuria in diabetes is thought to be partly dependent on progressive loss of the anionic charges of the GBM as an expression of a reduced proteoglycan turnover [11]. Exercise could affect glomerular permeability and/or tubular reabsorption of proteins conditioning the renal handling of molecules with different charge or conformation.

The aim of the present study is to evaluate the renal excretion of non-glycated and glycated albumin in nor-

moalbuminuric diabetic patients during the increased AER induced by acute exercise.

Subjects and methods

Two groups of subjects were studied. One group consisted of seven Type 1 diabetic patients each receiving three insulin injections per day and with reasonable metabolic control ($HbA_{1c} < 8.5\%$ during the last year). None had either somatic or autonomic neuropathy, as assessed by standard clinical methods. Retinopathy was assessed on eye-ground examination through dilated pupils: two of seven patients had mild background retinopathy. Patients were selected on the basis of: a) AER < 30 mg/24 h in three 24-h urine collections; b) absence of post-voiding residual urine in the bladder (echographic evaluation); and c) normal working and physical activity and usual moderately strenuous non-combative sports. The control group consisted of six healthy subjects, matched for age, sex, body mass index, blood pressure and level of physical fitness. None of the subjects (patients or control subjects) had a history of renal disease or first-degree family history of diabetes or hypertension; all had sterile urine and normal values for creatinine clearance; all were normotensive and were receiving no drug other than insulin. Further characteristics of the patients and control subjects are given in Table 1. All the procedures followed were in accordance with the Helsinki Declaration of 1975. All the subjects gave their informed consent to participate in the study.

Both patients and healthy subjects underwent the exercise test at about 10.00 hours. On the morning of the test, waterloading to stable

Table 1. Characteristics of control subjects and Type 1 (insulin-dependent) diabetic patients. Mean $\pm$ SEM

	Control subjects	Diabetic patients
Sex (Male/Female)	3/3	4/3
Age (years)	24 $\pm$ 1	25 $\pm$ 2
BMI (kg/m ²)	21.3 $\pm$ 0.4	20.7 $\pm$ 0.5
Diabetes duration (years)	–	6 $\pm$ 1
HbA _{1c} (%)	4.9 $\pm$ 0.4	7.8 $\pm$ 0.7
Plasma albumin (g/l)	49.2 $\pm$ 2.3	47.8 $\pm$ 2.6
Plasma glycosylated albumin (% total albumin)	9.8 $\pm$ 0.3	19.2 $\pm$ 0.8
Systolic blood pressure (mm Hg)	117 $\pm$ 4	123 $\pm$ 6
Diastolic blood pressure (mm Hg)	78 $\pm$ 2	81 $\pm$ 4
Creatinine clearance (ml/s $\cdot$ 1.73 m ²)	2.03 $\pm$ 0.19	1.99 $\pm$ 0.30

Table 2. Albumin and beta-2-microglobulin excretion rate, renal clearance of non-glycosylated and glycosylated albumin, and selectivity index (clearance of non-glycosylated/glycosylated albumin) in six normal subjects and seven Type 1 (insulin-dependent) diabetic patients during periods of: pre-exercise (A), exercise (B) and post-exercise (C).

		Control subjects	Diabetic patients
Albumin excretion rate (μ g/min)	A	7.65 $\pm$ 0.55	8.90 $\pm$ 1.87
	B	35.73 $\pm$ 8.16 ^a (p < 0.01)	431.51 $\pm$ 207.19 ^a
	C	7.72 $\pm$ 1.94 (p < 0.01)	95.13 $\pm$ 49.78
Beta-2-microglobulin excretion rate (ng/min)	A	42.33 $\pm$ 3.17	57.71 $\pm$ 9.38
	B	40.12 $\pm$ 3.32	32.85 $\pm$ 5.39 ^a
	C	40.33 $\pm$ 4.57 (p < 0.05)	25.28 $\pm$ 8.58
Clearance of non-glycosylated albumin (ml/min $\cdot$ 10 ⁻⁴)	A	1.60 $\pm$ 0.15	2.09 $\pm$ 0.38
	B	7.37 $\pm$ 1.56 ^b (p < 0.05)	109.29 $\pm$ 52.96 ^a
	C	1.72 $\pm$ 0.41 (p < 0.005)	23.55 $\pm$ 12.28
Clearance of glycosylated albumin (ml/min $\cdot$ 10 ⁻⁴)	A	1.61 $\pm$ 0.18	1.72 $\pm$ 0.32
	B	6.64 $\pm$ 1.28 ^b (p < 0.05)	82.96 $\pm$ 38.41 ^a
	C	1.58 $\pm$ 0.36 (p < 0.005)	18.05 $\pm$ 8.84
Selectivity index	A	1.01 $\pm$ 0.03 (p < 0.05)	1.25 $\pm$ 0.09
	B	1.08 $\pm$ 0.06	1.20 $\pm$ 0.07
	C	1.08 $\pm$ 0.05	1.20 $\pm$ 0.06

Mean $\pm$ SEM. One-way ANOVA and Student's *t*-test on log₁₀ scale values: ^a p < 0.01 vs A and C; ^b p < 0.05 vs A and p < 0.01 vs C

diuresis [12] was employed to offset the antidiuretic effect of exercise. Blood samples were collected for the measurement of plasma glucose (Glucose Analyzer II, Beckman Instruments, Fullerton, Calif., USA) and HbA_{1c} (BioRad kit, BioRad Labs, Richmond, Calif., USA). Urine samples were collected over the following three periods, according to the protocol of Viberti et al. [13]: 1) a 1-h pre-exercise resting period in the sitting position, immediately preceded and followed by a 300 ml drink of tap water; 2) a 20-min exercise period (600 kpm/min) on a bicycle ergometer; 3) a 1-h post-exercise period in the sitting position. Systolic blood pressure and blood glucose were monitored every 10 min during the test, and at the end of each period, respectively. Plasma albumin concentration was measured by autoanalyser on blood samples collected before the test and at the end of each period. The volume of each urine sample was measured and aliquots from each collection were stored at -80°C until determination of albumin (RIA, Albumin kit, Sclavo, RIA Department, Cinisello Balsamo, Milan, Italy) and beta-2-microglobulin (RIA, Beta-2-MG Radioimmunoassay kit, Eiken Chemical Co., Tokyo, Japan). Glycosylated albumin was measured by HPLC ac-

cording to the protocol of Cecchini et al. [14]. Urinary proteins were precipitated by trichloroacetic acid, redissolved, and dialyzed against 0.154 mmol/l NaCl to prevent spurious elevation of glycosylated albumin due to free glucose. A concentration procedure was necessary for samples where the albumin concentration was less than 50 μ g/ml. Samples were then stored at -80°C until assay. Urinary and plasma albumin separation by affinity chromatography on classic Affi-Gel Blue was confirmed by electrophoresis. Recovery was measured using glycosylated and non-glycosylated human serum albumin. After separation from other proteins, albumin was purified by gel chromatography on a Protein Pak 125 column (Waters, Milford, Mass., USA) using a Waters 204 HPLC apparatus (ultraviolet detector set at 280 nm). Finally, glycosylated albumin was separated from the non-glycosylated by cation-exchange chromatography on an SP5 PW Toyo Soda typical column. HPLC separation gives two peaks representing the glycosylated and the non-glycosylated fraction. The amount of the two fractions was measured as a percentage of total internal standard of albumin. The thiobarbituric acid procedure revealed glucose in the glycosylated fraction only. Glycosylated and non-glycosylated albumin were expressed as a percentage of total albumin with an automatic integrator. The standard curve for this assay was linear (range 5–500 mg/l) according to the linearity of the ultraviolet detector (total area vs concentration). The inter- and intra-assay plasma and urine variations were 4.2 and 4.7%, and 5.5 and 6.1% respectively. The correlation between RIA and HPLC determination of total albumin was statistically significant ($r = 0.96$, $p < 0.001$). The ratio between the renal clearance of non-glycosylated and glycosylated albumin, as calculated from the plasma and urine measurements, was expressed as the "selectivity index".

The creatinine clearance rate was calculated after creatinine was measured in serum and urine according to an automated kinetic method that used the Jaffe reaction [15].

Statistical analysis

For statistical calculations the albumin and beta-2-microglobulin rates, and the albumin (non-glycosylated and glycosylated) clearances were converted to log₁₀ values because of their skewed distribution. Comparisons between resting, exercise and post-exercise values on log₁₀ scale were performed by the Student's *t*-test when significant differences were found by one-way analysis of variance. All values are expressed as means $\pm$ SEM.

Results

Mean values of albumin and beta-2-microglobulin excretion rate, of renal clearance of non-glycosylated and glycosylated albumin, and of selectivity index are reported in Table 2. During exercise, the increase in AER on basal values was significantly higher in diabetic patients than in control subjects ($p < 0.01$). During the post-exercise period, AER values were similar to the pre-exercise levels in control subjects, whereas they were significantly higher ($p < 0.05$) than pre-exercise levels in diabetic patients. Urinary beta-2-microglobulin excretion rate did not increase from the pre-exercise to exercise and post-exercise period in either control subjects or in diabetic patients.

The mean value of the selectivity index was significantly higher in diabetic patients than in control subjects only during the pre-exercise period ($p < 0.05$), but it did not change significantly from the pre-exercise, exercise and post-exercise period in either group of subjects.

Since the diabetic group was heterogeneous in its response to exercise (large standard errors), individual

Table 3. Individual values of albumin excretion rate, renal clearance of non-glycated and glycated albumin in Type 1 (insulin-dependent) diabetic patients during periods of pre-exercise (A), exercise (B) and post-exercise (C)

Patient	Albumin excretion rate ($\mu\text{g}/\text{min}$)			Clearance of non-glycated albumin ($\text{ml}/\text{min} \cdot 10^{-4}$)			Clearance of glycated albumin ($\text{ml}/\text{min} \cdot 10^{-4}$)		
	A	B	C	A	B	C	A	B	C
1	4.70	120.01	22.83	1.08	27.30	5.34	1.04	27.12	5.13
2	17.21	20.49	20.97	3.03	3.79	3.53	2.65	3.31	3.13
3	7.62	20.00	23.06	1.88	5.10	5.55	2.00	5.35	5.94
4	10.39	292.97	58.35	2.70	76.15	14.99	1.91	55.05	11.33
5	7.38	1594.00	19.89	1.91	405.50	4.83	1.23	295.80	3.64
6	12.55	448.02	145.01	3.35	120.24	39.17	2.74	97.24	30.90
7	2.45	525.01	374.99	0.65	127.29	91.44	0.45	96.84	66.29

values of the AER and the clearances of non-glycated and glycated albumin of diabetic patients are reported in Table 3.

There was a positive correlation between HbA_{1c} and plasma glycated albumin concentration ($r = 0.791$, $p < 0.001$). There was no significant correlation between the selectivity index and HbA_{1c} or plasma glycated albumin values.

In diabetic patients, mean values of plasma glucose in the three periods of observation were: 7.1 ± 1.1 , 6.3 ± 0.9 and 5.9 ± 0.4 mmol/l respectively. Systolic blood pressure during exercise did not rise in the diabetic patients (from 122 ± 5 to 151 ± 6 mmHg) more than in the control subjects (from 118 ± 4 to 153 ± 3 mmHg).

Discussion

During exercise, the AER values increased by about five-fold compared to the basal values in control subjects and about 45 fold compared to the basal values in diabetic patients. The lack of increase in beta-2-microglobulin excretion suggests that the increase in AER induced by exercise is mainly due to increased glomerular permeability [13], even though a reduced tubular reabsorption of albumin cannot be excluded.

The large standard errors of the AER and the clearances of non-glycated and glycated albumin indicate that the diabetic group is heterogeneous in its response to exercise. Such heterogeneity has been previously reported by other authors [13] under experimental conditions similar to ours, and could depend on different degrees of initial glomerular abnormalities unmasked by exercise, possibly in relation to the individual's susceptibility to diabetic nephropathy [2].

The mean value of the renal clearances of non-glycated and glycated albumin during exercise was about four times that of the pre-exercise period in normal subjects, and about 50 times in the diabetic patients. However, the increase in the clearance of these two proteins during exercise was parallel, resulting in a mean selectivity index not significantly different from that of the pre-exercise period in both groups of subjects. This finding has, to our knowledge, not yet been reported and suggests that the ability of the kidney to differentiate between non-gly-

cated and glycated albumin is not modified by exercise. One explanation would be that albumins with relatively close isoelectric point values are not handled significantly differently by the barrier or that the difference in the charge of the two albumins is offset by the difference in their conformation [16]. However, since clearance values give no information about the extent of glomerular filtration or tubular reabsorption defects, the possibility that the difference in the charge of the two albumins is offset by a difference in tubular reabsorption must also be considered.

Even though the renal charge selectivity is apparently unaffected by exercise, it is altered in normoalbuminuric diabetic patients under resting conditions (pre-exercise). The renal clearance of non-glycated albumin was quite similar to that of glycated albumin in normal subjects resulting in a mean selectivity index of about 1. In contrast, diabetic patients showed a 30% higher clearance of non-glycated albumin combined with a slightly higher clearance of glycated albumin, resulting in a mean selectivity index significantly higher than in normal subjects. This finding indicates that in normoalbuminuric diabetic patients the renal excretion of glycated albumin is relatively lower than that of non-glycated albumin, as previously documented by Kverneld et al. [7, 8] on 24-h urine collection during normal physical activity. On the other hand, our results and those of Kverneld et al. disagree with the preferential urinary excretion of glycated proteins reported by others [5, 6] using a method considered as poorly specific for glycated albumin [17].

The mechanism behind the increased selectivity index in normoalbuminuric diabetic patients cannot be evaluated under our experimental conditions, but the results of the present study are not consistent with the possibility that physical exercise may induce a preferential excretion of glycated albumin.

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