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# INCREASED ALBUMIN EXCRETION RATE DURING A STANDARDIZED EXERCISE-TEST IN DIABETICS WITH LOWERED BLOOD FILTERABILITY

# Excrétion urinaire d'albumine accrue lors d'un test d'effort standardisé chez des diabétiques présentant une filtrabilité sanguine abaissée

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ABSTRACT :

**Increased albuminin excretion rate during a standardized exercise-test in diabetics with lowered blood filterability.**

Albumin excretion rate in urine is a marker of early, reversible stages of diabetic nephropathy. Does abnormal blood rheology represent an additional risk factor in this multifactorial process ? We investigated a possible link between red cell filterability and microalbuminuria during an exercise test (exercise is supposed to improve the detection of excessive microalbuminuria). 77 diabetics (27 females, 50 males, age : 15-60 yr) underwent a 20 min inframaximal progressively increasing workload on cycloergometer, rising heart rate up to 200 minus the age. Filterability of whole blood and washed red cells were measured on 5 $\mu$m polycarbonate sieves reused after ultrasonic cleaning. Whole blood filterability was found to be impaired in 35 subjects (group A) and normal in 41 (group B). Groups A and B were matched for age, sex, blood pressure, glycemic equilibrium, and duration of disease. Microalbuminuria was higher in A at rest (39.79 $\pm$ 13.83 $\mu$g/min vs 12.9 $\pm$ 3.21, $p<0.01$) and after exercise (91.80 $\pm$ 20.79 vs 42.23 $\pm$ 7.85, $p<0.01$). The slopes of regression lines between resting Microalbuminuria and blood pressure were greater in group A than in group B ($p<0.01$). No relationship betwen microalbuminuria and washed red cell filterability was detected. This study confirms on a larger scale a previous report of our team. Some hemorheologic disorders detectable with whole blood filterability (but not with washed red cell filtration) are associated with an increase in resting and postexercise microalbuminuria.

*Key-words :* Diabetes. Microalbuminuria. Erythrocyte deformability. Hemorheology.

RÉSUMÉ :

**Excrétion urinaire d'albumine accrue lors d'un test d'effort standardisé chez des diabétiques présentant une filtrabilité sanguine abaissée.**

L'excrétion urinaire d'albumine est un marqueur des stades précoces, réversibles de la néphropathie diabétique. L'existence d'anomalies de la rhéologie sanguine représente-t-elle un facteur de risque supplémentaire dans cette maladie multifactorielle ? Nous avons recherché une relation entre la filtrabilité érythrocytaire et la microalbuminurie au cours d'un test d'effort (l'effort sensibilisant la détection des microalbuminuries excessives). 77 diabétiques (27 femmes, 50 hommes, âge : 15-60 ans) ont réalisé 20 min d'exercice triangulaire inframaximal sur cycloergomètre, élevant la fréquence cardiaque à 200 moins l'âge. La filtrabilité du sang total et d'érythrocytes lavés a été mesurée sur filtres Nuclepore de 5 $\mu$m réutilisés après lavage ultrasonique. Un abaissement de la filtrabilité du sang total est retrouvé chez 35 patients (groupe A), les 41 autres (groupe B) ayant des valeurs normales.

Les groupes A et B étaient appariés pour l'âge, le sexe, la pression artérielle, l'équilibre glycémique et la durée de la maladie. La microalbuminurie était plus élevée dans le groupe A au repos (39,79 $\pm$ 13,83 $\mu$g/min contre 12,9 $\pm$ 3,21, $p<0.01$) et après effort (91,80 $\pm$ 20,79 contre 42,23 $\pm$ 7,85, $p<0.01$). La pente des droites de corrélation entre microalbuminurie de repos et pression artérielle est plus importante dans le groupe A que dans le groupe B ($p<0.01$). Aucune relation entre microalbuminurie et filtrabilité d'hématies lavées n'est retrouvée. Cette étude confirme sur un plus grand échantillon nos précédents travaux. Certaines anomalies hémorhéologiques détectables par la filtrabilité du sang total (mais pas avec la filtration d'hématies lavées) s'accompagnent d'une microalbuminurie accrue au repos et à l'effort.

*Mots-clés :* Diabète. Microalbuminurie. Déformabilité érythrocytaire. Hémorhéologie.

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Conventional test strips, as well as usual chemical techniques, cannot detect reduced quantities of albumin below 200 mg/l. When giving positive results, these methods detect only massive losses of albumin ("macroalbuminuria") which are related to glomerular diseases. Nevertheless, more sensitive assays for albumin have been developped, allowing the study of lower excretion rates ("microalbuminuria"), which can be normal ("normoalbuminuria") or excessive. In diabetics, excessive microalbuminu-

ria has been shown to be a marker of increased risk of renal disease. Overt nephropathy is preceded by reversible functional disorders associated with an increase in albumin excretion rate (6).

Therefore, microalbuminuria in diabetes mellitus is a subject of intensive research, since early treatment of these preliminary stages may prevent the onset of irreversible renal disease. The mechanism of these early stages of diabetic nephropathy remains uncompletely understood. Microalbuminuria has been shown to correlate with blood pressure and to be increased during periods of insufficient glycemic control. Other putative determinants of microalbuminuria, like circulating anti-insulin antibodies (3), have been described.

A hemorheologic explanation for microalbuminuria has been proposed by Simpson. This author suggests that blood hyperviscosity results in raised intraglomerular pressure, and thus in increased albumin leakage (8).

Conflicting results have been published concerning this topic. A first study by Hill and coworkers (5) was unable to find any definite relationship between microalbuminuria and blood rheology in type I diabetic children. Those data, put together with the findings of Ditzel (4) concerning a lack of correlation between blood viscosity and glomerular hyperfiltration at the beginning of diabetic disease, suggested that blood rheology was not an important factor in the pathogenesis of early stages of diabetic nephropathy.

Nevertheless, recent papers by Solerte and coworkers (9) are consistent with Simpson's theory : these investigators reported (a) that microalbuminuria was correlated to blood viscocity and negatively correlated to blood filterability and (b) that pentoxifylline, a drug enhancing red cell deformability, reduced microalbuminuria. Therefore, the possible involvement of hemorheologic abnormalities in the pathophysiology of early renal dysfunction remains controversial. In a previous study, we investigated a possible relationship between hemorheological status and microalbuminuria during a standardized exercise-test. Our results suggested that the increase in microalbuminuria induced by exercise is not related to the hemorheologic effects of muscular activity. Furthermore, baseline values of microalbuminuria were higher in patients with impaired blood filterability ($p<0.03$). In this study, we aimed at confirming those preliminary results on a larger group of diabetics.

## MATERIALS AND METHODS

### Patients

We have been studying 77 type I (insulin dependent) diabetics by evaluating together microalbuminuria during a standardized exercise-provocation test and filterability of whole blood and washed erythrocytes.

Patients (50 males, 27 females, age 15-60 yr, duration of disease 1-27 yr) were all routinely treated diabetics receiving 2 or 3 daily insulin injections. They had their normal breakfast and did not change their treatment on the day of the investigation. They underwent between 9 and 11 a.m. a 2 hr standardized test as previously described (3). Briefly, they remained recumbent during 60 min and drank during this period 600 ml of water. At the onset of the following hour they performed a 20 min strenuous inframaximal exercise on cycloergometer. A triangular increase in workload (every 5 min) was used to rise heart rate up to 200/min minus the age. Heart rate was monitored on electrocardioscope. The final step was maintained 5 min. After exercise, the patients, remained recumbent until the end of the second hour. They urinated before starting the test and at the end of both the first and the second hour, so that two samples of urine were collected, corresponding to resting and postexercice conditions.

### Measurements

Albumin excretion rate was determined by immunnoephelometry on a Behring laser nephelometer. This method, which is used together with radioimmunoassay in our department, provides closely similar results (correlation on 24 values : $r = 0.979$ $p<0.01$) and has a lowest limit of sensitivity of 1.3 mg/l. Intra-assay coefficient of variation ranges between 3.2 and 6.14 %. Glycated hemoglobins HbA1 were assayed with the kit "fast hemoglobins" (Eurobio). Blood and erythrocyte filterability were measured on 5 $\mu$m Nuclepore sieves (kindly offered by Hoechst Pharmaceuticals). Sieves were reused after ultrasonic cleaning as previously reported (2). They were all from the batch No 54 P4 B5.

Whole blood filterability was measured according to Reid (7), and expressed as a flow rate of red cells passing through the sieve under 200 mm negative pressure. Red cell filterability was determined under atmospheric pressure with the technique described by Weill and coworkers (10). Hematocrit (packed cell volume) was measured by microcentrifugation.

### Satistics

Values are given as mean ± SEM. Statistical comparisons were performed by nonparametric tests : Wilcoxon rank sum test for paired data and Mann-Whitney test for unpaired data. Correlations (linear regressions) were calculated by least square fitting. Comparisons of correlation slopes were performed by calculating at value (différence between the two slopes divided by the square root of the sum of standard deviations of the slopes) for (n-4) degrees of freedom. Statistical significance was defined as $p<0.05$.

## RESULTS

All the patients completed the test. *Table I* shows the correlations between microalbuminuria (at rest and postexercise) and some other parameters. The only significant correlation was found between resting microalbuminuria and blood pressure. Surprisingly, HbA1 failed to be correlated with microalbuminuria on the whole group. A correlation between those two parameters was found only in a subgroup of patients who had a duration of diabetes ranging between 10 and 20 yr ($r = 0.727$, $p<0.01$). Neither whole blood filterability nor washed erythrocyte filterability were correlated with microalbuminuria.

The control values for the same batch of sieves were measured in 20 nondiabetic healthy individuals. Normal values of blood filterability were 1.334 ± 0.029 so that the lower limit of normality (mean - 2 SD) was 1 ml/min. This limit was used to divide our group of 77 patients into two subgroups A and B. *Table II* shows that these two subgroups are mat-

TABLE I. — Correlations in the 77 patients of the study.
*Corrélations chez les 77 patients.*

| | resting $^{UV}$Alb | post exercise $^{UV}$Alb |
|---|---|---|
| Blood filterability | r = 0.168 n.s. | r = 0.149 n.s. |
| Diastolic blood pressure | r = 0.423 p<0.0001 | r = 0.138 n.s. |
| Systolic blood pressure | r = 0.351 p<0.01 | r = 0.144 n.s. |
| Mean blood pressure | r = 0.444 p<0.0001 | r = 0.165 n.s. |
| Glycosylated hemoglobin $HbA_1$ | r = 0.154 n.s. | r = 0.180 n.s. |

TABLE II. — Comparison of the two subgroups of patients.
*Comparaison des deux sous-groupes de patients.*

| | Group A VRBC <1 ml/min (n = 41) | Group B VRBC ⩾ 1 ml/min (n = 36) | Statistical comparison |
|---|---|---|---|
| Age (Years) | 35.10 ± 2,3 | 32.24 ± 2.29 | n.s. |
| Duration of diabetes (years) | 15.79 ± 1.39 | 12.31 ± 1.71 | n.s. |
| Systolic blood pressure (cm Hg) | 11.98 ± 0.27 | 11.85 ± 0.21 | n.s. |
| Diastolic blood pressure (cm Hg) | 8.94 ± 0.29 | 9.39 ± 0.31 | n.s. |
| Hb A1 (%) | 9.43 ± 0.27 | 10.01 ± 0.35 | n.s. |
| Sex ratio (male/female) | 17/25 | 10/25 | n.s. |
| Resting microalbuminuria (µg/mn) | 39.79 ± 13.83 | 12.90 ± 3.21 | p<0.01 |
| Postexercise microalbuminuria (µg/mn) | 91.80 ± 20.79 | 42.23 ± 7.85 | p<0.01 |
| Delta microalbuminuria (µg/mn) | 74.9 ± 18.13 | 42.23 ± 7.85 | n.s. |
| Anti insulin antibodies (%) | 3.85 ± 0.80 | 3.61 ± 1.31 | n.s. |

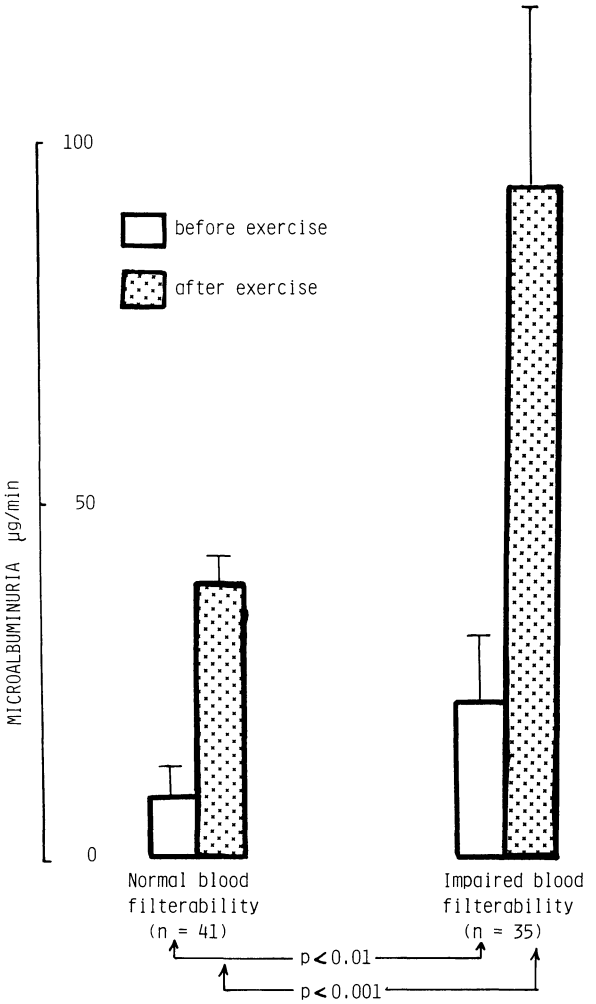


FIG. 1. — *Comparison of albumin excretion rates at rest and after exercise in the 77 patients of this study divided into two subgroups (left ; blood filterability within the normal range ; right : reduced blood filterability). Both resting and postexercice microalbuminuria are more than twofold increased when blood filterability is abnormal.*
Comparaison des excrétions urinaires d'albumine au repos et à l'effort chez les 77 patients de l'étude selon qu'ils présentent une filtrabilité sanguine entrant dans la gamme des valeurs témoins (à gauche) ou abaissée par rapport aux valeurs témoins (à droite). La microalbuminurie de repos et la microalbuminurie d'effort (index amplifié des anomalies observées au repos) sont plus que doublées en cas de filtrabilité sanguine abaissée.

ched for age, duration of disease, sex ratio, blood pressure and HbA1.

In contrast, as shown on *figure. 1*, a marked difference between the two subgroups can be detected for the values of albumin excretion rate. Group A had higher values of both resting ($p<0.01$) and postexercise ($p<0.001$) microalbuminuria. However, exercise-induced increases in microalbuminuria (delta values) did not differ *(table II)*.

The correlation between blood pressure and microalbuminuria was found to be significant in both subgroups. However, highly significant differences in slopes of correlations were found *(fig. 2)*. The slope was greater in patients of group A.

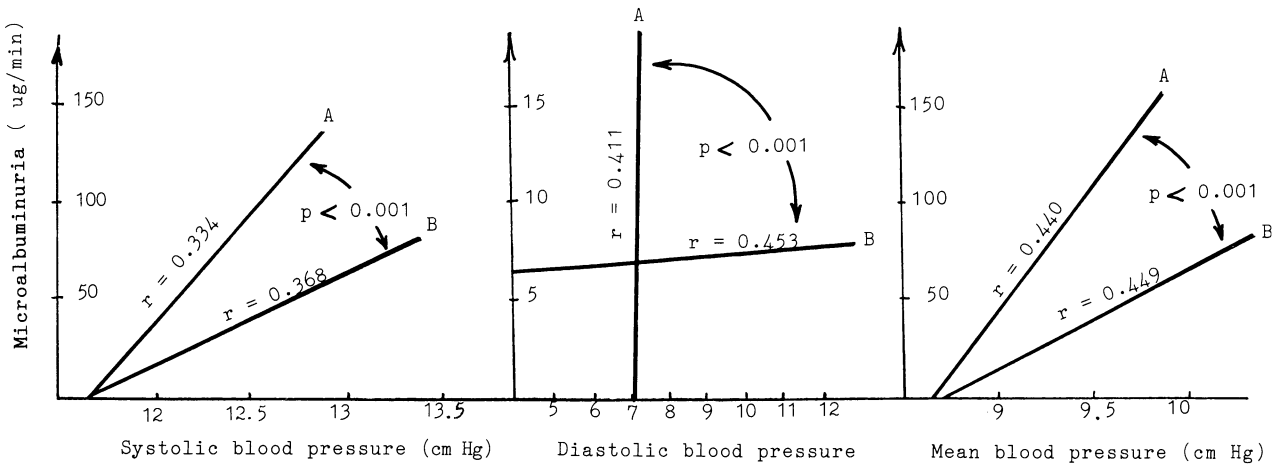


FIG. 2. — *Comparison of the correlation slopes between blood pressure and microalbuminuria in the two subgroups of patients classified according to their blood filterability. A stronger slope is found in the patients with lowered blood filterability, suggesting that hemorheologic abnormalities amplify the effect of blood pressure on glomerular microcirculation.*

Comparaison des pentes de corrélation indiquant la corrélation entre pression artérielle et microalbuminurie selon que la filtrabilité sanguine est normale ou abaissée. Un abaissement de ce paramètre s'accompagne d'une pente de régression significativement accrue, semblant indiquer que l'effet de la pression artérielle sur la microcirculation glomérulaire est amplifié en cas d'anomalies hémorhéologiques.

## DISCUSSION

These results are in agreement with our previous report (1) and with the recent papers of Solerte (9). Diabetics who have low values of blood filterability have significantly ($p<0.01$) higher microalbuminuria. Moreover, the correlation between microalbuminuria and blood pressure has a greater slope, suggesting a stronger influence of blood pressure on renal albumin leakage.

Impaired blood rheology, as hypothesized by Simpson (8), may result in microcirculatory functional abnormalities, reducing the precapillary drop in blood pressure, and therefore resulting in an increase in intraglomerular pressure. Thus, even when blood pressure is within a "normal" range, it can result in exaggerated albumin leakage if blood rheology is abnormal.

A weak point in our study is that the only hemorheological parameter which gives significant results is the nonspecific measurement of whole blood filterability, the relevance of which is a matter of controversy. When using washed red cell preparations, we were been unable to find any definite relationship with microalbuminuria. In many other clinical studies of hemorheology, whole blood filtration has been shown to detect subjects with abnormal blood rheology. However, the lack of specificity of this method makes such measurements difficult to interpret. Further studies will be needed for determining the kind of rheologic abnormality which is responsible for the results reported above : disorders of red cell deformability or aggregation, leukocyte activation, plasma viscosity ?

We think that the rheologic theory of microalbuminuria remains attractive and cannot be ruled out. Possible therapeutic approaches with rheo-active drugs like pentoxifylline (9) may provide an alternative to the antihypertensive treatment which is the most usually proposed for preventing diabetic nephropathy.

In conclusion, blood pressure and glycemic control are the main determinants of the progression of early stages of diabetic nephropathy, but additional parameters may be also involved in this complex process. This study on 77 patients suggests that abnormalities of blood rheology detectable by whole blood filtration are associated with a lower tolerance of the glomerulus to blood pressure. We hypothesize that hemorheologic disorders may be a potentiating factor in the multifactorial mechanism of this glomerular disease.

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