Low-Molecular-Mass Proteinuria as a Marker of Proximal Renal Tubular Dysfunction in Normo- and Microalbuminuric Non-Insulin-Dependent Diabetic Subjects

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We determined the urinary excretion, expressed as the protein/creatinine ratio (morning urines), of albumin (a marker of glomerular dysfunction) and retinol-binding protein (RBP; a low-molecular-mass protein marker of tubular proteinuria) in 102 non-insulin-dependent diabetic patients. There was a statistically significant ($P < 0.0001$) correlation ($\rho = 0.38$) between the urinary excretion values of the two proteins. The population could be divided into four subgroups: 32 with normal excretion values, 15 with above-normal urinary excretion of RBP, 24 with above-normal urinary excretion of albumin, and 31 patients with above-normal urinary excretion of both proteins. No patients had above-normal serum creatinine concentrations or above-normal serum RBP concentrations. This seems to exclude "tubular overflow proteinuria" as the cause of the increased urinary excretion of RBP seen in some patients with non-insulin-dependent diabetes. Our data suggest the presence of a state of proximal tubular dysfunction in these patients.

Indexing Terms: albuminuria · kidney function

It is now well-established that a slight increase in the urinary excretion of albumin ("microalbuminuria") is an early predictor of glomerular dysfunction in diabetes mellitus ($1$, $2$). We and other investigators have also reported ($3$–$5$) a significant increase in the urinary excretion of the low-molecular-mass protein retinol-binding protein (RBP; $21$ kDa) in insulin-dependent (Type I) diabetics in the absence as well as in the presence of microalbuminuria. Similar observations were made in a study ($6$) of unselected diabetic patients that included Type I and Type II (non-insulin-dependent) diabetes. The latter findings raised two questions: Is proximal tubular dysfunction with impaired reabsorption of low-molecular-mass proteins the first stage in development of some types of nephropathy in insulin-dependent diabetes? And, can the microalbuminuria observed in some insulin-dependent diabetic subjects be accounted for, at least to some extent, by a state of proximal tubular dysfunction leading to impairment of the tubular reabsorption of filtered albumin?

In the present study we have extended these investigations on the urinary excretion pattern of glomerular and tubular proteins to a population of non-insulin-dependent diabetic patients.

Materials and Methods

The concentration of RBP in serum and urine was determined by a two-site enzyme-linked immunosorbent assay (ELISA) as previously described ($7$). Albumin in serum and urine was assessed by an immunonephelometric technique we previously reported ($8$). Creatinine in serum and urine was measured by the routine Jaffé method (SMA-II; Technicon, Tarrytown, NY).

The study groups comprised non-insulin-dependent diabetic subjects with a median age of 70 years (range 38–91), a median body weight of 75 kg (range 51–127), and a median diabetes duration of 11 years (range 6–49). We excluded eight patients with above-normal ($\geq 120$ $\mu$mol/L) serum creatinine concentrations.

Venous blood samples and single-voided urine specimens (morning) were obtained from all patients.

Urinary excretion rates were expressed as the protein/creatinine ratios. The upper limit of the observed range for a group of 22 apparently healthy persons (laboratory personnel with a median age of 40 years, range 26–52) was used as cutoff limit. The control group was not comparable with the patient population with regard to age. This is, however, not important, inasmuch as the urinary excretion of 10 proteins including albumin and low-molecular-mass proteins in the elderly (ages $\geq 70$ years) reportedly do not differ significantly ($9$) from the excretion in younger subjects (ages 10–69 years).

Results

We determined the urinary excretion of albumin and RBP, expressed as protein/creatinine ratios, in 102 non-insulin-dependent diabetic patients. There was a statistically significant ($P < 0.0001$) correlation ($\rho = 0.38$) between the urinary excretion values of the two proteins (Spearman rank correlation).

The relationship between the urinary excretion values of albumin and RBP is shown in Figure 1. We used the cutoff limits for normal albumin ($\leq 29$ $\mu$mol/mol creatinine) and RBP excretion ($\leq 1.37$ $\mu$mol/mol creatinine) to divide the population studied into four groups. Group I (lower left quadrant) consisted of 32 normalalbuminuric diabetic patients with a normal urinary excretion of RBP. Group II (upper left quadrant) was 15 normoalbuminuric patients with above-normal urinary excretion of RBP. Group III (lower right quadrant) comprised 21 microalbuminuric and 3 macroalbuminuric (albumin $\geq 290$ $\mu$mol/mol creatinine) patients with normal urinary excretion of RBP. Group IV (upper right quadrant) comprised 31 patients with above-normal
mass proteinuria, as indicated by above-normal urinary excretion of RBP. These findings are similar to those observed in Type I diabetic subjects (3–5) and in a mixed population of Type I and Type II diabetic subjects (6).

RBP (21 kDa) circulates in plasma, where 90% of it is bound to transthyretin (55 kDa). The unbound fraction is filtered freely through the glomeruli and catabolized after reabsorption in the proximal tubules (11–14). Ordinarily, very small amounts of RBP escape into urine, but in proximal tubular dysfunction large amounts are excreted. RBP has, therefore, been used as a sensitive marker of proximal tubular dysfunction (11–14). Watts et al. (15) observed a correlation between the urinary excretion of the low-molecular-mass proteins β2-microglobulin and RBP in insulin-dependent diabetic patients. The former protein seemed to be more sensitive than RBP in detecting an abnormality of the renal proximal tubule, but alkalization of the patients was necessary because of the low stability of β2-microglobulin at urinary pH <6.0 (11). Low-molecular-mass proteinuria is also a typical finding in chronic renal failure (16), for which a severely reduced glomerular filtration rate is accompanied by a parallel significant increase in the plasma concentration of creatinine and unbound RBP (16). The filtered load of RBP in the remaining functional nephrons exceeds the reabsorptive capacity of the proximal tubules, resulting in urinary excretion of large amounts of RBP: "tubular overflow proteinuria" (16).

No patients had above-normal concentrations of serum creatinine, and all had serum RBP concentrations below the upper limit of the normal range. These findings seem to exclude tubular overflow proteinuria as the mechanism responsible for the low-molecular-mass proteinuria observed in the present population of diabetic patients. We conclude that proximal tubular dysfunction with impaired reabsorption of low-molecular-mass proteins seems to occur not only in insulin-dependent but also in non-insulin-dependent diabetes.

The mixed type of proteinuria, i.e., glomerular and tubular proteinuria, observed in one-third of the diabetic patients raises some interpretative problems. High urinary concentrations of albumin, by affecting the absorptive function of the proximal renal tubules, could contribute to development of low-molecular-mass proteinuria. In fact, high concentrations of albumin seemed to interfere with the absorption of the low-molecular-mass proteins myoglobin and β2-microglobulin in the perfused rat kidney (17, 18). Conversely, if low-molecular-mass proteinuria reflects a state of tubular malfunction in which the tubular reabsorption of filtered proteins (e.g., albumin) is impaired, this might contribute to the development of microalbuminuria. The latter hypothesis agrees with studies showing that the tubular damage associated with hypokalemia produced an increased urinary excretion not only of typical tubular markers, e.g., low-molecular-mass proteins and tubular enzymes, but also of the glomerular marker albumin (19). We emphasize, however, that findings of this type may just as well indicate the coexistence of tubular and

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**Figure 1.** Urinary excretion (protein/creatinine ratio) of albumin and retinal-binding protein (RBP) plotted on log scales (but data not log-transformed) in 102 non-insulin-dependent (Type II) diabetic patients.

- **Dotted line:** upper limits of the observed protein ranges for apparently healthy persons. Median values with ranges (μmol/mol creatinine) for each group: I (lower left quadrant), albumin 2.9 (1.5–27.8), RBP 0.47 (0.17–1.24), n = 32; II (upper left quadrant), albumin 14.1 (1.2–25.9), RBP 2.91 (1.38–255.04), n = 18; III (lower right quadrant), albumin 73.1 (51.4–1204.8), RBP 0.82 (0.11–1.29), n = 24; IV (upper right quadrant), albumin 104.1 (29.9–3284.1), RBP 4.46 (1.99–716.15), n = 31
glomerular lesions in the kidneys of hypokalemic and (or) diabetic patients (19).

By contrast to albumin, the urinary excretion of RBP seems to correlate to the metabolic control of Type II diabetes. Prospective trials should deal with a possible correlation between metabolic control, urinary RBP excretion, and micro/macrovacular complications in non-insulin-dependent diabetes. Another point of interest would be to test the validity of urinary RBP excretion as a predictor of mortality rates in Type II diabetes.

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References


Testosterone Concentration Is Increased in Whole Saliva, but Not in Ultrafiltrate, after Toothbrushing

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The concentration of testosterone in whole saliva is significantly increased (by 9%) after toothbrushing. In ultrafiltrates of saliva collected at the same time as the whole saliva, testosterone concentrations after toothbrushing were unchanged. In 88% of the 162 whole-saliva specimens, but not in the ultrafiltrates, we also measured higher hemoglobin concentrations after toothbrushing. We conclude that the increase of testosterone in whole saliva after toothbrushing can be attributed to a protein-bound fraction. For analytes that are bound to serum proteins, salivary measurements can give spurious results. This problem can be avoided by using as a diagnostic medium an ultrafiltrate of saliva collected directly in the mouth.

Indexing Terms: saliva, ultrafiltrate; free steroid; gingival exudate; hemoglobin; variation, source of

Salivary testosterone has been used in many studies during the past decade for the diagnostic evaluation of androgenicity (for an overview, see I, 2). Circulating testosterone is substantially bound to three proteins: sex-hormone-binding globulin (SHBG)1 and, with lower avidity, albumin and corticosteroid-binding globulin.

1 Nonstandard abbreviations: SHBG, sex-hormone-binding globulin; s, whole saliva after toothbrushing; s, whole saliva before toothbrushing; u, ultrafiltrate of saliva after toothbrushing; and u, ultrafiltrate of saliva before toothbrushing.