

## Aspects on Tubular Proteinuria

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### ABSTRACT

Tubular proteinuria, or low molecular weight (LMW) proteinuria, is less common than glomerular proteinuria, but is often of clinical significance. Both qualitative analysis of the urinary protein pattern by electrophoresis and quantitative estimations of various LMW-proteins are now usually available in the clinical routine laboratories. It is thus possible to look for LMW-proteinuria, as a sign of damage to the function of proximal renal tubules. For this purpose  $\beta_2$ -microglobulin may be used as marker protein. Chronic cadmium poisoning and the Balkan nephropathy are examples of conditions which have been extensively studied by the use of LMW-protein determinations. In this context also some rare hereditary disorders deserve to be specially mentioned. Toxic injury to the kidneys, by drugs or other agents, often affect the kidney tubules early in the course and may be identified by LMW-protein analysis. Also in other clinical situations LMW-proteins in the urine may be used for investigative or research purposes.

### INTRODUCTION

Hippocrates seems to have observed the occurrence of foamy urine in patients with renal disease but the direct demonstration of proteins in the urine is a late event in the history of clinical medicine. First in the 18th century it was shown by Cotugno of Naples that the urine from a patient with generalized edema coagulated, after acidification and heating. It was then thought to be a favourable sign " as the coagulable edema fluid was coming away". In 1798 Cruickshank observed the phenomenon of proteinuria in diabetes. The famous concept of "Bright's disease" (glomerulonephritis) belongs to the time period around 1830.

For a long time proteinuria was synonymous with albuminuria. A different proteinuric pattern was described by Friberg 1950 (6). In industrial workers, exposed to cadmium, urinary proteins were present which were not detectable by precipitation with trichloroacetic acid and, sometimes, not with the boiling

test. This type of proteinuria, with low molecular weight proteins, became known as tubular proteinuria (Butler and Flynn, 1958) as it was found to be connected with a variety of tubular renal disorders (2). Paper electrophoresis proved that  $\alpha_2$ - and  $\beta_2$  components were prominent features in this type of proteinuria. A typical protein,  $\beta_2$ -microglobulin was isolated by Berggård 1968 (3).

#### CLINICAL TYPES OF PROTEINURIA

Proteinuria may occur through many different mechanisms, some of which are listed in Table I.

Table I. Mechanisms of proteinuria

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1. Abnormal passage by plasma proteins through the glomerular membranes.
  2. Defective reabsorption of filtered plasma proteins.
  3. "Over-flow" of p-proteins, present at abnormally high concentrations.
  4. Origin of protein in the urinary pathways (e.g. Tamm-Horsfall prot.)
  5. Loss of lymphatic fluid into the urine.
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The common type, which is due to an increased glomerular permeability, is usually called glomerular proteinuria. The dominating component is albumin. Various attempts, of doubtful clinical value, have been made to subdivide glomerular proteinuria into e.g. selective (presumably reflecting a less serious damage to the glomerular membranes) and unselective proteinuria (when larger proteins than albumin are also abundant in the urine).

It is to-day preferred to use the term low molecular weight (LMW)-proteinuria for proteinuria which is due to a defective tubular reabsorption of filtered proteins (M.W. <40.00 Daltons). This will be further discussed below.

The best known type of over-flow proteinuria is the presence of Bence-Jones proteins in myeloma patients. Sometimes there is also a concomitant damage to renal tubular function with a simultaneous general LMW-proteinuria.

When progressive renal disease, of any kind, has reached a low level of glomerular filtration, about 25-30 ml/min GFR, a typical mixed proteinuric pattern occurs which is best designated "nephron loss proteinuria"(16). Both albumin and LMW-proteins are invariably found and it becomes almost impossible to obtain useful information from the pattern of proteins at electrophoretic separation or by the quantitative determination of individual proteins.

In patients with a reasonably well preserved GFR, >50 ml/min, it is, however, possible to evaluate the presence of mixed glomerular and LMW

**proteinuria.** As they reflect quite different pathophysiological mechanisms, quantitative determinations of albumin and an LMW-protein should be evaluated separately and not as the ratio between the protein concentrations.

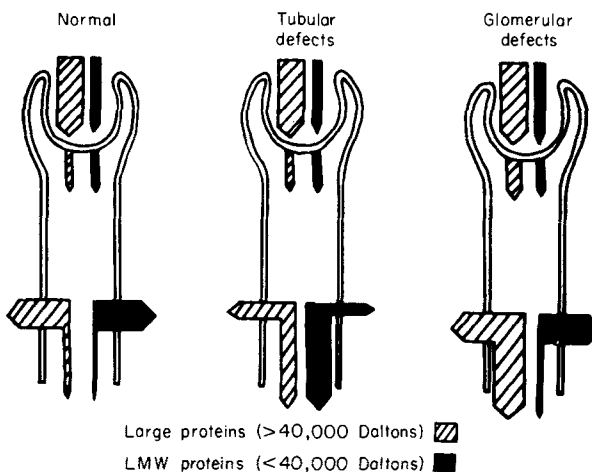


FIG. 1. Mechanisms of proteinuria. During passage through the glomeruli serum proteins are filtered, mainly in proportion to their relative size, into the primary filtrate. The reabsorption of protein in the proximal tubules is also illustrated and the relative amounts of proteins of normal, tubular and glomerular proteinuria indicated (from Berggård, I. Sv. Läkartidningen 67, 5798, 1970).

#### LOW MOLECULAR WEIGHT PROTEINURIA

The classical definition of "tubular proteinuria" is illustrated in Fig. 1. The left part of the picture shows that a large protein like albumin is present at high concentration in plasma, normally passes over into the glomerular filtrate in small amounts and is then mainly reabsorbed in the proximal tubules. Simultaneously, an LMW-protein, e.g.  $\beta_2$ -microglobulin, will pass the glomerular membrane freely and also becomes reabsorbed. Increased glomerular permeability, shown to the right, will not affect the  $\beta_2$ -microglobulin reabsorption but cause a heavy albuminuria; presumably the albumin reabsorption will become over-saturated. In the middle of the picture, with damage to the proximal tubules, the relative impact on LMW-reabsorption is more pronounced than that on the albumin excretion. Table II shows an estimate of approximate data for the two mentioned proteins during normal conditions. In glomerular proteinuria the 24 h excretion of albumin may exceed 10 Gm ( $10^4$ -increase) and in "LMW"-proteinuria  $\beta_2$ -microglobulin excretion could exceed 100 mg ( $10^3$ -increase) which allows for a very large variation of the ratio between the two proteins in urine (14).

TABLE II. Normal renal handling of a large and a small plasma protein

	Albumin	$\beta_2$ -microglobulin
Plasma concentration	40 Gm/l	2 mg/l
Glom.-filtrate	1 Gm/24 <sup>h</sup> ?	150 mg/24 <sup>h</sup>
Urinary excretion	0.010 Gm/24 <sup>h</sup>	0.1 mg/24 <sup>h</sup>

The knowledge about urinary LMW-proteins developed explosively about 20 years ago by use of electrophoretic separation, gel filtration according to size and ultracentrifugation according to weight; and later by the use of immunochemical methods and enzyme-assays. At first the dominating LMW proteins were detected, like  $\beta_2$ -microglobulin,  $\alpha_2$ -microglobulin (RBP), the "post-gamma" and the "beta-trace"-proteins and light chains of immunoglobulins. With a short plasma half-life, dependent on the GFR (15), LMW-proteins at a low concentration were hidden among the large plasma proteins and not easily found in plasma, as illustrated by Fig. 2.

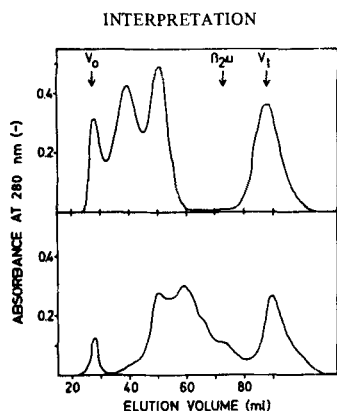


Fig 2. Protein profile patterns obtained by gel chromatography on Sephadex G-200 of serum (above) from a patient with uremia - actual  $\beta_2$ -microglobulin value being 30 times above the normal and for comparison concentrated urine (below) from a patient with renal tubular disease - chronic cadmium poisoning - excreting high amounts of LMW proteins. The column was equilibrated with 0.02 M Tris-HCl pH 8.0 containing 0.15 M NaCl.

However, enzymes like lysozyme and ribonuclease (8), and hormones like insulin and LH (20), were soon found to be handled by the kidney in similar manner.

A well founded concept has evolved that LMW-proteins are largely taken up by pinocytosis in the proximal renal tubules and then degraded to the amino acid level in the lysosomes of the tubular cells (4). Thus, they are lost to the body after glomerular filtration, whether reabsorbed or not, although the amino acids may be re-used for protein synthesis. The reabsorption of large

proteins, like albumin, is widely held to be unselective and occurring in the distal parts of the proximal tubules. The more proximal reabsorption of small proteins is possibly selective and seems to be a more vulnerable process. As suggested by Maack, selectivity may depend not only by a reabsorption site selectivity but also on the type of anionic or cationic charge of the proteins and on differences in access, as determined by hindrance to the passage of proteins from tubular fluid to the endocytic sites at the base of the microvilli in the proximal tubular cells.

The major role of the kidney for the catabolism of peptide hormones is underlined by the fact that some, e.g. insulin and GH, are taken up also directly from the peritubular blood flow. i.e. at the contraluminal side of the tubular cell (12).

Interestingly, it has been shown that some peptides and proteins, like glucagon, angiotensin and bradykinin, are not reabsorbed by pinocytosis but mainly degraded in the tubular lumen by enzymes in the brush border membrane (3). Thus, their degradation products will rapidly occur in the urine.

#### $\beta_2$ -MICROGLOBULIN - A SUITABLE LMW-PROTEIN FOR CLINICAL USE ?

$\beta_2$ -microglobulin has become extensively used as a model substance for the renal handling of LMW-proteins (17). The protein essentially exists as a free monomer in plasma and urine. It has a molecular size (10.800) which should allow almost free passage over the glomerular membranes, seems to be biologically inert (a "light chain" of HLA-antigen molecules) and is constantly produced in most individuals. It can be used like S-creatinine for an estimation of the glomerular filtration rate; and as the serum level is approximately doubled at a 50% reduction of the GFR the glomerular load is almost constant, even in renal disease. There is no biological feed back, with a fluctuation of the serum level but, the production is increased in certain malignant and "autoimmune" disorders (10).

The normal excretion of  $\beta_2$ -microglobulin in adult subjects is about 0.125 mg/24<sup>h</sup> corresponding to 5/ $\mu$ g/h or 0.5-1.0 mg/mmol creatinine  $\times 10^2$ . This implies that the normal tubular reabsorption is larger than 99.9%. A small decrease of the reabsorption will thus cause a considerably increased urinary output. The determination of the protein offers few problems except the obstacle that  $\beta_2$ -microglobulin, depending probably on the presence of proteolytic enzymes, may become degraded in urine if the pH is <5.5-6.0 (5). In short time collected specimens reproducible results are, however, obtained in the urine as shown in fig. 3. It has been found that U-  $\beta_2$ - microglobulin values are better expressed as a ratio to U-creatinine than as the amount over

a time period. At a high diuresis a degradation can be avoided if the urine samples are collected in 5 ml tubes containing 0.5 ml of 10% sodium azide in a 0.07 M phosphate buffer solution (pH 6.5).

URINARY EXCRETION OF  $\beta_2$ -MICROGLOBULIN

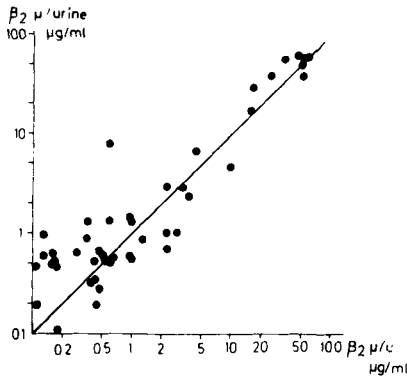


Fig 3. U- $\beta_2$ -microglobulin in 46 patients with renal disease. Two 1-3 hour collections were performed during a water load. Urinary pH was  $>5.5$

Practical studies with urinary  $\beta_2$ -microglobulin determinations in healthy subjects and in patients with renal disease have shown that the excretion over time is uninfluenced by the urinary flow at various loads of water, osmoles, sodium or bicarbonate (19). A normal excretion can be found in patients with pronounced glomerular proteinuria or with signs of distal tubular lesions, causing isostenuria. In patients with a reduced renal function; when S-creatinine is higher than 200-250  $\mu\text{mol/l}$  or S- $\beta_2$ -microglobulin  $> 4.5$ -5.0  $\text{mg/l}$  there is for practical purpose a threshold, with an obligatory leakage of the protein into the urine, together with other LMW-proteins (17).

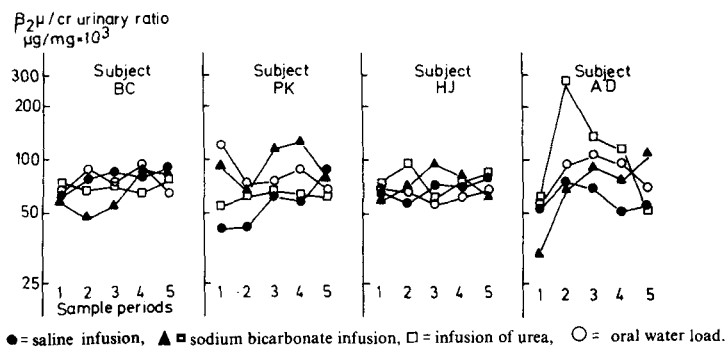


Fig 4. The urinary  $\beta_2$ -microglobulin/creatinine ratio in healthy subjects during diuresis experiments. The highest value in subject AD obtained in "pre-renal ischemia" due to a high infusion rate of hypertonic urea-solution.

#### APPLICATIONS OF LMW-PROTEIN DETERMINATIONS

It is possible to list a large number of conditions associated with tubular proteinuria, Table III.

Table III. Examples of LMW-proteinuria in the clinic

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Exposure to Cadmium (Itai-Itai)	Hypokalemia
Exposure to other heavy metals (Pb,Hg)	Renal tubular acidosis
Drug toxicity	Netrocalcinosis
Balkan endemic nephropathy	Acute tubular insufficiency
Wilson`s disease	Interstitial nephritis
Fanconi syndrome	Renal graft rejection
Cystinosis	Renal ischemia
Galactosemia	Ketoacidosis
Juvenile nephronopththisis	Large tissue trauma
Oculocerebral dystrofia	Pancreatitis
Familiar asymptomatic LMW-proteinuria	Nephropathia epidemica

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From a practical point of view the use of screening for LMW-proteinuria has proven to be of considerable value as regards the pollution problem with cadmium, e.g. in Japan (11), and for detection of the endemic Balkan nephritis (7). For the detection and study of certain rare hereditary disorders, in children, the use of urinary electrophoresis or  $\beta_2$ -microglobulin determinations have also been used. In many other clinical situations LMW-proteinuria was studied more for scientific reasons than for the clinical usefulness of this sign.

Drug toxicity is an area of potential importance. Toxic mechanisms directed towards the kidney are often primarily affecting the kidney tubules, more often the proximal than the distal tubules, litium being an exception. Typical examples are the aminoglycoside antibiotics, cyclosporin and streptozotocin (Fig. 5). It seems that LMW-proteinuria usually precedes albuminuria and S-creatinine elevations as a sensitive indicator of renal side effects. It would be reasonable to monitor new drugs routinely in this respect during the introductory trials.

## STREPTOZOTOCIN 2 Gm injections

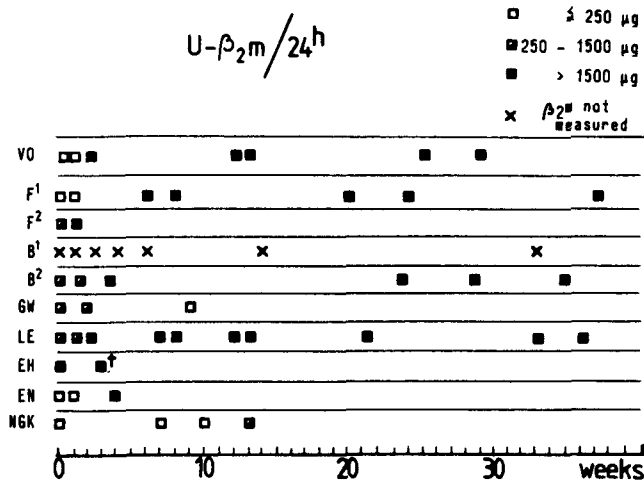


Fig. 5. Urinary excretion of  $\beta_2$ -microglobulin (24 h) during treatment with streptozotocin 2 g/week i.v. in eight patients receiving 10 courses.

A few examples of the use of LMW-protein analysis in clinical research may finally be given. Diabetic nephropathy has been defined as an almost exclusively glomerular disorder by the lack of an increased excretion of  $\beta_2$ -microglobulin. In a similar way it was possible to show that the tubular dysfunction with isostenuria, in some patients with hypercalcemia, could be classified as a purely distal phenomenon with intact proximal tubular function (18). On the other hand the demonstration of LMW-proteinuria can protect from an over-interpretation of the increased excretion of a substance: myoglobinuria in myocardial infarction was found to be an unspecific phenomenon often only indicative of LMW-proteinuria and "positive" also in cadmium exposed patients and subjects with cardiac failure due to other cause than infarction (9).

### FINAL COMMENT

It was the aim of this brief review to describe the concept of "tubular proteinuria" and to discuss the use of quantitative determinations of LMW-proteins, like  $\beta_2$ -microglobulin, for study of this phenomenon. Assay of a suitable LMW-protein here offers a sensitive tool for investigation of a



specific function of the kidneys, the reabsorption of small proteins in the proximal tubules. In severely ill patients many factors can contribute to a disturbance of this high performance process: e.g. toxemia from septic infections, circulatory failure and the presence of a tissue necrosis. It has been shown, for example, that a high glomerular load of certain basic amino acids, like lysine, causes tubular proteinuria (13). Nevertheless the quantitative determination of LMW-proteins has proven to often be useful for clinical research purposes.

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