Urinary-peptide excretion by patients with and volunteers without diabetes

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Large quantities of peptides (1–4 g) are excreted in human urine each day. In this study we sought to analyze how peptide excretion varies with increasing albuminuria associated with diabetes, as well as to characterize the size distribution of albumin-derived peptides in urine from volunteers without diabetes and from patients with macroalbuminuria and diabetes. We detected albumin-derived peptides by injecting tritiated albumin intravenously into human volunteers and patients with diabetes. Urine was collected after 24 hours and fractionated on a size-exclusion column. This fractionation revealed peptides with molecular weights ranging from 300 to 500 Da in volunteers without diabetes. The albumin-derived peptides were of higher molecular weight in the urine of a patient with macroalbuminuria and diabetes. The molecular-weight distribution of the peptides derived from tritiated albumin peptides was paralleled by the distribution of all protein peptides (including albumin) as determined with the use of the Biuret protein assay or absorbance at 214 nm. We determined peptide-excretion rates by filtering urine from patients with diabetes through a 10,000 Da molecular-weight–cutoff membrane and then measuring the filtrate with the use of the Biuret assay. This analysis revealed that the peptide-excretion rate increased with increasing total protein excretion, regardless of whether the patient demonstrated normoalbuminuria or microalbuminuria. Among patients with macroalbuminuria, the peptide-excretion rate leveled off and even decreased in the face of an increasing albumin concentration or protein-excretion rate. This study confirms that albumin-derived and protein-derived peptides exist at high concentrations in urine. Although peptide-excretion rates are maintained at similar levels up to macroalbuminuric states, the relative proportion of peptide excretion is significantly reduced compared with total protein. (J Lab Clin Med 2005;145:239–246)

Abbreviations: AER = albumin-excretion rate; dpm = disintegrations per minute; GBM = glomerular basement membrane; HPLC = high-pressure liquid chromatography; K_{av} = fractional available volume; PBS = phosphate-buffered saline solution; V_e = elution volume; V_o = void volume; V_t = total volume

It has been assumed that proteins are filtered and excreted intact. However, it has recently been shown that filtered proteins are degraded during renal passage. In both rats[1–10] and human subjects,[11] filtered albumin undergoes extensive degradation (>95%) to produce small peptides (<10 kD) that are excreted in the urine. The degradation process appears to be partially impaired in diabetes because the ratio of intact albumin to albumin peptides increases with increasing albuminuria in both rats[4,7,9,10] and

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human subjects.[11] The exact anatomic location of the "degradation pathway" has not been determined, but it likely takes place in cells distal to the GBM, most probably in tubular epithelial cells, where albumin is subjected to endocytosis and trafficked to lysosomes. Once degraded, albumin is subjected to exocytosis into the tubular lumen and excreted in the urine. The degradation pathway appears to be nonspecific; all proteins tested to date have been shown to be degraded, including horseradish peroxidase, immunoglobulin G, transferrin, and albumin.[1–7]

Peptides in urine appear to be the result of degradation of filtered protein. Although we have not established the origin of peptides in human urine, in rats we have not observed protein-derived peptides in blood analyzed at a very high sensitivity of 0.003%.[2] Furthermore, rats whose ureters were occluded for 4 hours while the animals were under anesthesia produce no detectable albumin-derived peptides in plasma containing 10^6 dpm/mL tritiated albumin.[6] Recent studies have demonstrated a variety of peptides in normal human urine[12] and in the urine of patients with kidney disease.[13] We have demonstrated with the use of reverse-phase HPLC that human urinary peptides are not dissimilar to those obtained from the tryptic digestion of albumin[14] and that the urinary peptides are susceptible to peptidases.[15]

The cellular uptake of intact protein and regurgitation of peptides was initially observed in cultured cells and lysosomes.[16,17] Histochemical and electron-microscopic studies[18] have shown that after endocytosis, proteins of greater molecular weight are degraded within lysosomes of the epithelial cells in proximal convoluted tubules, whereas iodine-125–labeled albumin has been shown to be degraded by lysates of rat-kidney lysosomes.[19] The degradation pathway has also recently been demonstrated with the use of cultured HK-2 cells, which permit access to both the apical and basal-fluid compartments of the cell without leakage between compartments. Internalized ^{125}I-labeled albumin was degraded to peptides, which were excreted into the medium on both the apical and basolateral sides of the HK-2 monolayer.[20] Despite this observation, radiolabeled albumin fragments were not reported in the bloodstream.

Urinary peptides (<10 kD) can be detected with the use of radioactive tracers. Radioactive proteins are intravenously injected and the urine collected is fractionated by means of size-exclusion chromatography.[1–11] Because the radioactive label is regularly distributed throughout the protein molecules, most of the peptides produced are detectable on the basis of their radioactive tags. However, urinary peptides are not detected with the use of routine total protein assays, including the sulfosalicylic-acid assay, the benzethonium assay, the Bradford assay, or Coomassie-blue staining of electrophoretic gels, but they may be detected with the use of assays designed to measure peptide bonds, such as the Biuret assay.[14,15] Excretion rates obtained with the use of radioactive albumin and the Biuret assay have been shown to be similar in both normal control rats and those with proteinuria.[1,21,22] The peptides are also not detected with the use of conventional immunochemical assays, which only detect intact protein or large peptides.[4,11] In terms of excretion rates, normal individuals would excrete albumin at a rate of 25 mg/d, as determined with the use of immunochemical assays, but in reality they are excreting more than 1300 mg/d albumin-derived peptides.[15] Thus conventional immunochemical based assays severely underestimate the amount of albumin excreted in urine, and they are therefore unable to comprehensively quantify changes in urinary albumin excretion.

In this study we sought to investigate urinary peptides in terms of size distribution and to examine their excretion rate in patients with diabetes and varying degrees of proteinuria/albuminuria.

METHODS

Volunteers and patients. All studies involving human subjects were carried out in accordance with the principles of the Declaration of Helsinki, and informed consent was obtained from the subjects. Ethics clearance was approved by the Austin and Repatriation Medical Centre Human Ethics Committee.

We characterized tritiated peptides in urine from volunteers and patients with diabetes in terms of size distribution using size-exclusion chromatography. In brief, participants were injected intravenously with 1 mCi (~4 mg) of tritiated albumin and their blood and urine collected after 24 hours. Experimental methods have been previously described in detail.[11] Albumin integrity was checked before use and in the plasma after 24 hours. Urine samples from 5 healthy volunteers and 5 patients with diabetes were examined.

In a separate experiment, we collected urine samples from patients with diabetes who regularly attended the Endocrine Clinic at the Austin and Repatriation Medical Centre (Heidelberg, Australia). No attempt was made to distinguish patients on the basis of diabetes type or treatment. Urine specimens were collected over a period of 24 hours and stored at −20°C until assay. We selected urine samples randomly until we had 24 with AERs of less than 20 μg/min (normoalbuminuric), 25 with AERs of 20 to 200 μg/min (microalbuminuric), and 28 with AERs of more than 200 μg/min (macroalbuminuric) as measured on the basis of immunoturbidimetry findings.

We measured the concentration of total protein (intact protein plus peptides) and total low molecular weight protein (<10 kD) with the use of the Biuret assay in urine from patients with diabetes before and after filtration using Centricon centrifugal filters with a 10-kD molecular-weight cutoff