Recent studies have shown that low-molecular weight proteins in serum seem to be better markers for detecting reduced glomerular filtration rate (GFR) than the conventional measurement of serum creatinine (1, 2). Several proteins, such as ribonuclease, α1-microglobulin, β2-microglobulin (B2M), and cystatin C have been compared with serum creatinine (2, 3). Whereas serum creatinine is increased only after a reduction of ~50% in GFR, the above proteins, especially cystatin C, are already increased within that so-called creatinine-blind range (1, 4–6). Recently, another low-molecular weight protein, the β-trace protein (BTP), isolated primarily from cerebrospinal fluid (7), was shown to be increased in patients with renal diseases (8, 9). However, there is no information on the relationship of BTP and a standard measure of GFR such as inulin clearance. Therefore, to investigate the potential clinical usefulness of BTP for early detection of reduced GFR, we have performed corresponding measurements of BTP and inulin clearance.

The study included 115 diabetic patients (44 women; mean age, 53.4 years; 71 men, mean age, 52.9 years); 57 had type I diabetes and 58 had type II. GFR was determined by measuring the inulin clearance. A priming dose of 2.5 g of inulin (Inutest; Laevosan) was administered intravenously within 30 s, followed by a constant infusion (15 mL/h) of inulin at a rate dependent on serum creatinine (infusion rate, 1.5 g/h for serum creatinine concentrations <130 μmol/L; 0.75 g/h for serum creatinine concentrations between 130 and 265 μmol/L; and 0.38 g/h for serum creatinine concentrations between 265 and 450 μmol/L).

After 30 min for equilibration and after the patients were instructed to empty their bladders, two spontaneously voided urine samples were collected at 60-min intervals. Blood samples were taken before bolus injection as well as at the beginning and at the end of each urine collection period. After centrifugation (1600g for 15 min), samples of serum and urine were stored at ~80 °C until analysis. A fully enzymatic assay for the quantification of inulin was used (10). GFR was calculated by taking the mean of the two clearances and correcting that value to 1.73 m² of body surface area. Serum creatinine concentrations were determined by an enzymatic method, and B2M was determined by a turbidimetric method; both methods were performed on a Hitachi 717 analyzer with reagents supplied by Boehringer Mannheim (Creatinine plus and Tina-quant β2-microglobulin). Controls with assigned values showed interassay imprecision (CV) of <5%.

For determination of BTP, a newly developed nephelometric research assay was performed on a BNA II analyzer (Dade Behring Marburg). This assay is based on the principle of latex particle-enhanced immunonephelometry using rabbit polyclonal antibodies against BTP. Calibration of the assay is based on highly purified BTP from cerebrospinal fluid characterized by amino acid sequencing and quantitative amino acid analysis. For the default sample dilution of 1:100, the basic measuring range is ~0.25–15.8 mg/L. The total analytical imprecision (intraassay plus interassay; n = 40) of the assay, calculated from two control materials and three serum samples with concentrations of 1.51–7.89 mg/L, was between 2.33% and 6.5%.

Statistical calculations were performed with SPSS 7.5 for Windows (SPSS Software). The diagnostic validity was evaluated by the ROC curve analysis. GraphROC for Windows, Ver. 2.1, was used for calculations of areas under the curves (11). Regression analysis was performed with the EVAPAK for Windows software, Ver. 3.01 (12). P < 0.05 was considered statistically significant. The study was performed in accordance with ethics standards of the Helsinki Declaration of 1975 (revised in 1985).

The GFR was <80 mL/min in 41 patients and >80 mL/min in 74 patients, which was considered as the lower cutoff limit of GFR. BTP showed curvilinear behavior in relation to GFR, as did creatinine and B2M (Fig. 1, A–C). There were significant correlations between GFR and the reciprocal concentrations of creatinine, B2M, and BTP (r = 0.666, 0.514, and 0.672, respectively; P < 0.05); the differences between the correlation coefficients, however, were not statistically significant (P > 0.05). The median values (and ranges) were 88 μmol/L (49–331 μmol/L) vs 69 μmol/L (33–137 μmol/L) for creatinine, 2.17 mg/L (0.51–12.1 mg/L) vs 1.44 mg/L (0.85–3.59 mg/L) for B2M, and 0.82 mg/L (0.44–6.44 mg/L) vs 0.52 mg/L (0.32–1.09 mg/L) for BTP and differed significantly between the two groups (Mann–Whitney U-test, P < 0.0001). For BTP, an upper 97.5% reference limit of 0.79 mg/L was calculated by both parametric and nonparametric approaches (13) considering patients with GFR values >80 mL/min as individuals with normal GFR. Fig. 1D shows ROC curves of the three analytes to discriminate between patients with normal (>80 mL/min) and reduced (<80 mL/min) GFR values. The curve of BTP is above the curves of creatinine and B2M. The area under the BTP curve is significantly higher than the areas under the two other curves, demonstrating a higher power of discrimination (P = 0.042). When the point with the highest diagnostic efficiency (0.79 for creatinine and B2M, 0.81 for BTP) was selected as the optimal decision limit, the threshold was 91 μmol/L for creatinine, 2.34 mg/L for B2M, and 0.64 mg/L for BTP. The corresponding diagnostic sensitivities and specificities were 49% and 96% for both creatinine and B2M and 76% and 84% for BTP.
In summary, our data support the view that BTP may be suitable as an indicator of reduced GFR even in the creatinine-blind range. Additional detailed studies are required, but are worthwhile because the search for improved markers of GFR continues (14).

This work was supported in part by the Fund of the German Chemical Industry (Grant 400770 to K.J.) and includes parts of the doctoral thesis of F.P.

References