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Alterations of retinol-binding protein 4 species in patients with different stages of chronic kidney disease and their relation to lipid parameters

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ABSTRACT

Retinol-binding protein 4 (RBP4) is elevated in patients with chronic kidney disease (CKD) and has been discussed as marker of kidney function. In addition to an elevated concentration, the existence of truncated RBP4 species, RBP4-L (truncated at last C-terminal leucine) and RBP4-LL (truncated at both C-terminal leucines), has been reported in serum of hemodialysis patients. Since little is known about the occurrence of RBP4 species during the progression of CKD it was the aim of this study to analyse this possible association. The presence of RBP4, RBP4-L, RBP4-LL and transthyretin (TTR) was assessed in serum of 45 healthy controls and 52 patients with stage 2–5 of CKD using ELISA and RBP4 immunoprecipitation with subsequent MALDI-TOF-MS analysis. A reduction of glomerular filtration rate was accompanied by a gradual elevation of RBP4 serum levels and relative amounts of RBP4-LL. Correlation analysis revealed a strong association of the RBP4-TTR ratio with parameters of lipid metabolism and with diabetes-related factors. In conclusion, RBP4 serum concentration and the appearance of RBP4-LL seem to be influenced by kidney function. Furthermore, the RBP4-TTR ratio may provide diagnostic potential with regard to metabolic complications in CKD patients.

Introduction

The retinol-binding protein 4 (RBP4) has been supposed as new adipokine, possibly contributing to the onset of insulin resistance and type 2 diabetes mellitus in obese subjects [1,2], but mainly being influenced by kidney function [3–5].

The main function of RBP4 is the transport of retinol (ROH) to its target tissues [6,7]. To prevent the renal loss of the low-molecular weight RBP4–ROH complex (approximately 21 kDa), the transport is furthermore facilitated by the binding of transthyretin (TTR) a visceral protein with an approximate molecular weight of 55 kDa [6,8,9]. After the delivery of ROH to its target tissues, the affinity of RBP4 to TTR decreases and RBP4 is subjected to renal degradation [6,7]. Since the kidneys are the main site of RBP4 catabolism, RBP4 serum concentration is associated with renal function and has therefore been supposed as surrogate marker of kidney function [10–14]. Beside changes in the RBP4 serum concentration, changes in the appearance of RBP4 species have been described for patients undergoing hemodialysis [15,16]. For these patients increased amounts of RBP4 species have been described. Thereby the RBP4 species are characterized by a truncation at the last (further on referred to as RBP4-L) or at both C-terminal leucines (RBP4-LL) [15]. However, little is known about the importance of truncated RBP4 species in progression of chronic kidney disease (CKD). Therefore, the aim of the present study was to investigate the relative amounts of truncated RBP4 species next to RBP4 serum concentration in the progression of kidney dysfunction, namely stages 2–5 of CKD.

Materials and methods

Subjects. Serum samples of 45 subjects (27 male/18 female) without any signs of kidney disease served as controls and were obtained from the Department of Clinical Nutrition, German Institute of Human Nutrition, Nuthetal and from the Department of Endocrinology, Diabetes and Nutrition, Charité Campus Benjamin Franklin, Berlin. Additionally, serum samples of 52 patients (34

Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; ESRF, end-stage renal failure; MALDI-TOF-MS, matrix-assisted laser desorption ionization-time of flight-mass spectrometry; RBP4, retinol-binding protein 4; ROH, retinol; TTR, transtyretin.

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male/18 female) with diagnosis of CKD were obtained from the Medizinische Klinik Nephrologie, Charité Campus Benjamin Franklin, Berlin. Definition of CKD was based on kidney biopsy or reduced estimated glomerular filtration rate (eGFR <60 mL/min/1.73 m^2) for more than 3 months, proteinuria, abnormalities on the urine dipstick or sediment examination, or abnormal renal imaging studies. Renal function was quantified by eGFR which was calculated according to Modification of Diet in Renal Disease formula including serum creatinine concentration, age and gender [17]. The stages of CKD were used as grouping characteristic and were assigned in accordance to the K/DOQI guidelines [18].

Exclusion criteria were age <18 years and pregnancy. Since insulin resistance and diabetes mellitus are typical features in CKD patients, we decided to include diabetic patients in the control group. However, these diabetic patients did not show abnormalities with regard to kidney function.

The study protocol was approved by Ethics Committees of the hospitals and the University of Potsdam. Written informed consent was obtained from each subject. All anthropometric, clinical, and biochemical parameters of the participants were collected by trained personnel.

Blood was collected from an antecubital vein, immediately centrifuged, and serum was immediately frozen at −80 °C until measurement.

Measurement of RBP4 and TTR. The serum concentrations of RBP4 and TTR were quantified by non-commercial enzyme-linked immunosorband assays (ELISA) as described in detail previously [5,19]. For detection of both, RBP4 and TTR, a standard was used containing RBP4 and TTR obtained from human blood (N Protein Standard/SL OQIM, Dade Behring GmbH, Germany).

Immunoprecipitation of RBP4 and subsequent analysis by MALDI-TOF-MS. Immunoprecipitation of RBP4 was performed as described elsewhere in detail [20]. Immunoprecipitates were subsequently analyzed by matrix-assisted laser desorption ionization-time of flight-mass spectrometry (MALDI-TOF-MS) as previously described [20]. Representative MALDI-TOF-MS spectra are depicted in Fig. 1.

The following RBP4 variants were identified using MALDI-TOF-MS analysis: non-truncated RBP4 with a median molecular weight of 21,063 Da (interquartile range = 28.3 Da), RBP4-L with a molecular weight of 20,953 Da (interquartile range = 31.4 Da) and RBP4-LL with a molecular weight of 20,837 Da (interquartile range = 21.9 Da) [21].

Data analysis. The results were expressed as medians and ranges. The amounts of the truncated RBP4-forms RBP4-L and RBP4-LL were expressed as per cent of peak height of non-truncated RBP4. The peak heights were determined in a valley-to-valley procedure. Statistical analysis was accomplished by use of non-parametric procedure (SPSS version 15.0, SPSS Inc., Chicago, USA). The Kruskal–Wallis test was used to test for significant differences between the groups. When there was a significant effect, Mann–Whitney *U*-rank test was performed to evaluate differences in proportions between cases and control subjects. Spearman–Rho was used to calculate correlation coefficients. p values of <0.05 were considered to be statistically significant (two-tailed).

Results

Subject characteristics

The characteristics of the study population are given in Table 1. There was a steady decline of eGFR from control to CKD 5 with $p < 0.001$ between all groups. Additionally, other parameters also changed with progression of kidney disease in comparison to controls: systolic blood pressure was elevated in the groups CKD 2, CKD 3 and CKD 5 ($p < 0.05$ for all), total cholesterol levels were lower in all CKD groups ($p < 0.05$ for all), LDL-cholesterol was reduced in CKD 2 ($p < 0.05$), CKD 4 ($p < 0.01$), and CKD 5 ($p < 0.01$), triacylglycerol levels were elevated in CKD 5 ($p < 0.01$), HbA1c was significantly higher in CKD 2 ($p < 0.01$), CKD 3 ($p < 0.01$) and CKD 4 ($p < 0.05$) and borderline elevated in CKD 5 ($p = 0.052$) and haemoglobin was reduced in CKD 3, CKD 4 and CKD 5 ($p < 0.001$ for all) in comparison to control subjects. Furthermore, HDL-cholesterol was lowest in the group CKD 5 in comparison to CKD 3 and CKD 4 ($p < 0.01$ for both) and in comparison to the control group ($p < 0.001$), but there were no differences to CKD 2. Moreover, BMI was higher in CKD 2 and 4 in comparison to control subjects and CKD 5 ($p < 0.05$), but there was no difference to CKD 3.

The subjects of all five groups did not differ in age, diastolic blood pressure, fasting glucose, and total serum protein concentration. Approximately 22% of the CKD patients and 13% of the control subjects were positively diagnosed for diabetes mellitus (Table 1).

RBP4, TTR, and relative amounts of RBP4 species

The RBP4 serum concentration as well as the appearance of RBP4 species changed with progression of CKD (Table 2). RBP4 serum levels were elevated in CKD 4 and 5 in comparison to control and CKD 2 ($p < 0.01$ for both). The relative amount of RBP4-LL

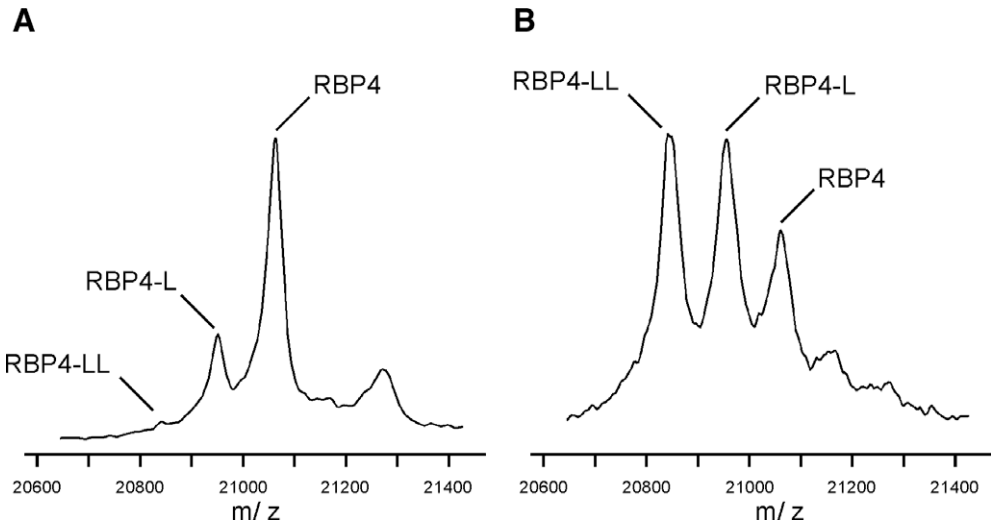

Fig. 1. Representative MALDI-TOF-MS spectra for RBP4 pattern in control subject (A) and in patients with chronic kidney disease (B). Abbreviations used: RBP4, retinol-binding protein 4; RBP4-L, RBP4 truncated at the last C-terminal leucine; RBP4-LL, RBP4 truncated at both C-terminal leucines.