Journal of Steroid Biochemistry & Molecular Biology 128 (2012) 56–61

Contents lists available at SciVerse ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Antiproteinuric treatment reduces urinary loss of vitamin D-binding protein but does not affect vitamin D status in patients with chronic kidney disease

Carolina R.C. Doorenbos[a], Milton M. de Cuba[a], Liffert Vogt[b], Ido P. Kema[c], Jacob van den Born[a], Reinold O.B. Gans[d], Gerjan Navis[a], Martin H. de Borst[a,*]

[a] Department of Internal Medicine, Division of Nephrology, University Medical Center Groningen and University of Groningen, P.O. Box 30.001, 9700 RB, Groningen, the Netherlands
[b] Department of Internal Medicine, Academic Medical Center, P.O. Box 22660, 1100 DD, Amsterdam, the Netherlands
[c] Department of Laboratory Medicine, University Medical Center Groningen and University of Groningen, P.O. Box 30.001, 9700 RB, Groningen, the Netherlands
[d] Department of Internal Medicine, University Medical Center Groningen and University of Groningen, P.O. Box 30.001, 9700 RB, Groningen, the Netherlands

ARTICLE INFO

Article history:
Received 20 June 2011
Received in revised form 26 August 2011
Accepted 8 September 2011

Keywords:
Vitamin D binding protein
Vitamin D_3
Proteinuria
RAAS blockade

ABSTRACT

Vitamin D deficiency is common in chronic kidney disease (CKD). Increased urinary loss of vitamin D binding protein (VDBP), the main transporter of 25-hydroxyvitamin D_3 in the circulation, has been postulated to contribute to vitamin D deficiency in proteinuria. To test this hypothesis we analyzed urinary and plasma levels of VDBP, 25-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3 from proteinuric patients, before and after antiproteinuric interventions.

We performed a post-hoc analysis of a clinical trial in CKD patients ($n = 13$, creatinine clearance median 60 (range 25–177) ml/min) subjected to the following study periods: washout (no antiproteinuric treatment, 4 weeks), lisinopril 40 mg QD (ACEi, 6 weeks), or indomethacin 75 mg BID (NSAID, 4 weeks) in randomized sequence. Healthy subjects screened for donation ($n = 10$) served as controls. Plasma and urine VDBP levels were measured by ELISA, 25-hydroxyvitamin D_3 levels by LC–MS and 1,25-dihydroxyvitamin D_3 levels by radioimmunoassay.

In CKD patients urinary VDBP excretion was strongly increased (median (range) 5413 (155–211,027) μg/24 h) as compared to healthy controls (64 (23–111) μg/24 h, $p < 0.001$). Both NSAID and ACEi significantly decreased urinary VDBP excretion, in proportion to proteinuria reduction. Plasma VDBP, 25-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3 levels, however, were similar between patients and controls and not affected by antiproteinuric intervention.

Urinary VDBP excretion is markedly increased in proteinuria and responds to antiproteinuric treatment. Urinary VDBP loss is not associated with plasma VDBP or vitamin D_3 levels, suggesting that urinary loss of VDBP does not affect vitamin D status.

1. Introduction

Vitamin D deficiency is common in chronic kidney disease (CKD). Beside contributing to deregulation of calcium/phosphate metabolism in CKD, more recently vitamin D deficiency has been associated with progression of CKD and mortality in CKD patients as well [1,2]. Reduced activity of the enzyme 1α-hydroxylase in damaged tubular epithelial cells is considered the main cause of 1,25-dihydroxyvitamin D_3 (active vitamin D) deficiency in CKD patients, although induction of the vitamin D-degrading enzyme 24-hydroxylase may also contribute [3,4]. However also 25-hydroxyvitamin D_3 deficiency is relatively common in CKD [5], suggesting that additional mechanisms contribute to vitamin D deficiency in CKD. Increased urinary loss of vitamin D-binding protein (VDBP), the main transporter of vitamin D_3 in the circulation, has been postulated to contribute to vitamin D deficiency in proteinuric conditions [6].

In the circulation, 25-hydroxyvitamin D_3 is bound to the albumin-like protein VDBP. The VDBP-25-hydroxyvitamin D_3 complex is filtered by the glomerulus, followed by receptor-mediated re-uptake at the brush border of tubular epithelial cells [7] involving megalin [8] and cubulin [9]. Under normal conditions urinary VDBP excretion is therefore minimal. In proteinuria, on the other hand, loss of receptor-mediated uptake at the tubular brush border may result in urinary excretion of the VDBP-vitamin D_3 complex, possibly contributing to vitamin D deficiency.

To investigate whether urinary loss of VDBP might contribute to vitamin D deficiency in proteinuric patients, we measured urinary and plasma levels of VDBP, 25-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3 of patients with overt proteinuria,

* Corresponding author. Tel.: +31 50 361 6161; fax: +31 50 361 9310.
E-mail address: m.h.de.borst@umcg.nl (M.H. de Borst).

0960-0760/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2011.09.002

Table 1
Baseline characteristics of CKD patients with proteinuria and age- and gender-matched healthy controls.

	Healthy controls	Proteinuric CKD patients		
		No treatment	ACEi	NSAID
n	10	13	10	13
Age (years)	57 (41–68)	56 (41–67)	55 (41–67)	55 (41–67)
Male (%)	7 (70%)	9 (69%)	8 (80%)	8 (67%)
DM (*n*)	0	4	4	4
BMI (kg/m^2)	27 (23–29)	27 (23–38)	30 (23–38)	30 (24–38)
Systolic BP (mmHg)	119 (105–151)	143 (124–197)*	123 (107–169)	151 (122–188)*
Diastolic BP (mmHg)	70 (55–84)	83 (69–95)*	72 (60–81)#	79 (65–95)*
Proteinuria (g/24 h)	0.0 (0.0–0.2)	2.9 (1.3–13.1)*	2.0 (0.2–7.9)*,#	1.2 (0.3–12.5)*,#
Serum creatinine (μmol/l)	74 (60–92)	107 (53–292)*	142 (59–358)*	120 (59–346)*
Creatinine clearance (ml/min)	113 (59–187)	74 (31–134)*	64 (23–112)*	61 (22–134)*
Serum phosphate (mmol/l)	1.10 (0.79–1.22)	1.09 (0.84–1.41)	1.00 (0.97–1.03)	1.14 (0.78–1.48)
Serum calcium (mmol/l)	2.30 (2.16–2.43)	2.38 (2.29–2.52)*	2.42 (2.41–2.43)	2.46 (2.29–2.65)*
Serum albumin (g/l)	44 (42–47)	35 (25–38)*	36 (27–38)*	37 (32–40)*,#
Serum cholesterol (mmol/l)	5.1 (4.4–7.1)	6.6 (5.5–11.1)*	6.5 (4.0–8.4)	5.7 (4.8–8.8)*
Serum triglycerides (mmol/l)	1.1 (0.8–3.0)	2.9 (1.4–7.0)*	3.5 (1.1–6.7)*	3.0 (1.5–6.4)*
Serum PTH (pmol/l)	3.20 (3.10–3.58)	8.29 (1.19–22.50)*	8.8 (2.90–39.90)	9.51 (2.16–51.30)*

DM = number of patients with diabetes mellitus, BMI = body mass index, BP = blood pressure.
* $p < 0.05$ vs. healthy controls.
# $p < 0.05$ vs. untreated patients.

and tested the effects of proteinuria reduction by different modes of pharmacological intervention, i.e. blockade of the renin–angiotensin–aldosterone system (RAAS) or prostaglandin inhibition, respectively.

2. Material and methods

2.1. Patients

The current study is a post-hoc analysis of a previously performed clinical trial [10]. The original study by Vogt et al. was performed in Caucasian patients ($n = 16$) who fulfilled the following inclusion criteria after a six week washout period without RAAS intervention: proteinuria ≥ 2 g/day, diastolic blood pressure (BP) < 90 mmHg, creatinine clearance ≥ 30 mL/min and age 18–70 years. During the washout period BP was titrated with hydrochlorothiazide 12.5 mg QD combined with amlodipine or doxazosine if necessary; this regimen remained unchanged during the study period. One patient received calcitriol during the study period, none of the other patients received vitamin D supplements or analogues. Participants were asked to adhere to a restricted sodium intake (<100 mmol/day) and standardized protein intake (1 g/kg body weight/day). The study was approved by our local medical ethics committee, was performed in adherence to the declaration of Helsinki, and all participants provided written informed consent.

Patients were subsequently treated in random order with indomethacin 75 mg BID (retard formula; Indocid® Merck & Co., Inc., Whitehouse Station, NJ, USA) and rofecoxib (VIOXX®), both for four weeks. A subset of the original 16 patients ($n = 11$) underwent an extension study consisting of another washout period (six weeks) followed by lisinopril (40 mg QD) treatment for six weeks. Blood and urine samples were collected at the end of each study period.

Since non-specific COX inhibition would be sufficiently suitable as a non-RAAS-related antiproteinuric intervention, we did not analyze the rofecoxib study period. From the 16 patients in the original study, urine and plasma samples were available from 13 patients with the following disorders: membranous glomerulopathy ($n = 2$), primary focal segmental glomerular sclerosis ($n = 2$), IgA nephropathy ($n = 2$), non-conclusive diagnosis ($n = 2$) and (type 2) diabetic nephropathy ($n = 5$). Baseline characteristics of these patients were similar to the original population. Plasma and urine samples were available of 13 patients for the washout period, of 13 patients for the indomethacin treatment period (NSAID), and of 10 patients for the extension study with lisinopril (ACEi). Age- and gender-matched healthy Caucasian subjects ($n = 10$) who were screened for kidney donation were included as controls. None of the controls used a vitamin D supplement.

2.2. Measurements

VDBP was measured in EDTA-plasma or urine with a VDBP sandwich ELISA (Immundiagnostik, catalog # K2314, Bensheim, Germany), according to the manufacturer's instructions. Briefly, plasma (diluted 1:40,000) or urine (diluted 1:2–1:5 for controls and 1:10–1:30,000 for the other groups depending on the concentration of VDBP) was incubated in a microtiter plate coated with polyclonal anti-VDBP antibodies for 1 h. Subsequently, a polyclonal peroxidase-labeled rabbit-anti-VDBP detection antibody was added and incubated for 1 h. After washing, tetramethylbenzidine was added as substrate for 15 min. After adding a stop solution, absorbance at 450 nm was measured by a spectrophotometer (BenchMark Plus, Bio-Rad Laboratories, Veenendaal, The Netherlands). Using a standard curve generated with VDBP protein as provided by the manufacturer, final VDBP concentrations were calculated. VDBP excretion was calculated from the VDBP concentration in urine collected over a 24-h period. The detection limit of this ELISA is 1.23 ng/ml; intra-assay CV < 5.0% for 16 replicate determinations at concentrations of 24.2 and 42.9 mg/dl and inter-assay CV < 12.7% for a concentration of 19.3 mg/dl in 14 different assays on two different lots; recovery ranges from 85 to 116% and linearity was acceptable ($r^2 = 0.998$).

Plasma and urine 25-hydroxyvitamin D_3 levels were determined using isotope dilution–online solid phase extraction liquid chromatography–tandem mass spectrometry (ID-XLC–MS/MS). K_2-EDTA plasma was prepared immediately after collection and stored until analysis at −80 °C. Patient samples were analyzed in one run. First, plasma was pre-treated by proteolysis to disrupt 25-hydroxyvitamin D_3–VDBP interactions. Subsequently deuterated internal standard (IS: 25-hydroxyvitamin D_3-d6), was added and samples were mixed. Samples were extracted and analyzed by XLC–MS/MS (system described previously [11]). Quantification of

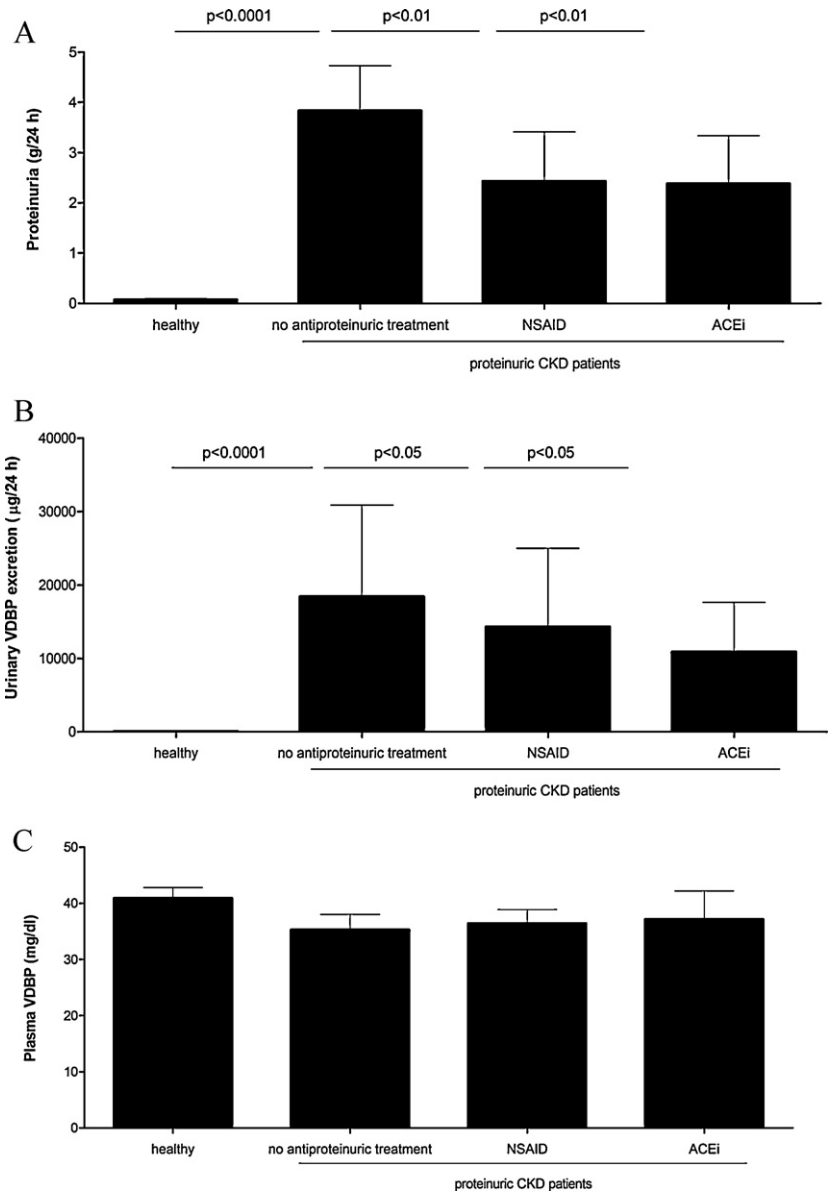

Fig. 1. Urinary VDBP excretion per 24 h and plasma VDBP levels. (A) Proteinuria (mg/24 h) in healthy controls, CKD patients without antiproteinuric treatment, with NSAID treatment and with ACE inhibitor treatment. In CKD patients without antiproteinuric treatment, proteinuria was strongly increased as compared to healthy controls. Antiproteinuric treatment (both ACEi and NDAID) significantly reduced proteinuria. (B) Urinary VDBP excretion (ug/24 h) in healthy controls, proteinuric CKD patients without antiproteinuric treatment, with NSAID treatment and with ACE inhibitor treatment. In proteinuric CKD patients, 24 h-urinary VDBP excretion was strongly increased as compared to healthy controls. Antiproteinuric treatment (both ACEi and NSAID) significantly reduced urinary VDBP excretion. (C) Plasma VDBP levels (mg/dl) in healthy controls, proteinuric CKD patients without antiproteinuric treatment, with NSAID treatment and with ACE inhibitor treatment. Antiproteinuric treatment did not affect plasma VDBP levels.

plasma 25-hydroxyvitamin D_3 was done relating analyte/IS peak area ratios in patient plasma to analyte/IS peak area ratios in blanc (pre-treated and dialysed) plasma spiked with 25-hydroxyvitamin D_3 at concentrations ranging from 0 to 280 nmol/l and IS at a fixed concentration. Method characteristics: LOQ 4.0 nmol/l; intra-assay CV < 7.2% and inter-assay CV < 14.1% for 3 concentrations between 20 and 150 nmol/L; recovery ranges from 93 to 98% and linearity was acceptable ($r^2 = 0.9972$). The accuracy of 25-hydroxyvitamin D_3 results was established using NIST (National Institute of Standards & Technology, Gaithersburg, MD) reference material to establish true values for calibration standards. Calibration standards, QC-samples and patient samples were stable for 6 days at 6 °C (CV < 11%). Samples are stable for 3 freeze-thaw cycles (CV < 3%). Levels of 1,25-dihydroxyvitamin D_3 in plasma and urine were measured by radioimmunoassay. Standard laboratory measurements were performed as described previously [10].

2.3. Statistical analysis

Results are expressed as median (range). Statistical differences between groups were assessed using the Wilcoxon non-parametric test for paired observations or the Mann–Whitney non-parametric test for unpaired observations. Non-parametric testing was used given the small sample size and the fact that urinary VDBP was not normally distributed. Data in graphs are presented as mean ± SEM. Spearman's correlation was used for correlations between continuous variables. All calculations and analyses were performed using SPSS 16.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

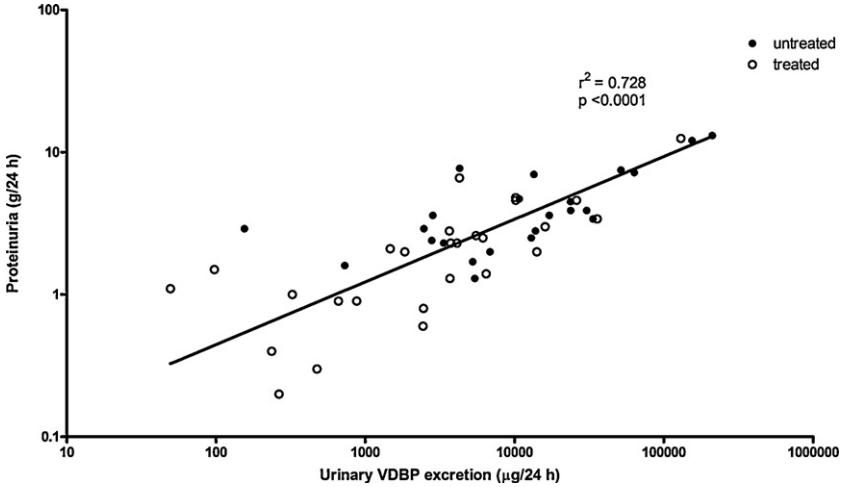

Fig. 2. Association between proteinuria and 24 h-urinary VDBP excretion. Association between proteinuria (g/24 h) and urinary VDBP excretion (ug/24 h) in treated (open circles) and untreated (closed circles) proteinuric CKD patients.

3. Results

3.1. Patient characteristics

Baseline patient characteristics are presented in Table 1. At baseline, systolic BP and serum creatinine concentration were slightly elevated in CKD patients, and creatinine clearance was lower as compared to healthy controls. CKD patients were in K/DOQI stages I–III. Both ACEi and NSAID effectively reduced proteinuria as compared to the period without antiproteinuric treatment (Fig. 1A).

3.2. Urine and plasma VDBP levels

In CKD patients without antiproteinuric treatment, urinary VDBP excretion was strongly increased as compared to healthy controls (5413 (155–211,027) vs. 64 (23–111) µg/24 h, $p < 0.001$, Fig. 1B). Urinary VDBP excretion was significantly reduced by antiproteinuric therapy, either with ACEi (4276 (50–35,800) µg/24 h, $p < 0.05$), or with NSAID (1653 (97–129,854) µg/24 h, $p < 0.05$). Urinary VDBP excretion was strongly associated with proteinuria across treated and untreated CKD patients (Fig. 2). The decline of urinary VDBP excretion was significantly associated with the antiproteinuric response (NSAID: $r = 0.776$, $p < 0.01$, ACEi: $r = 0.771$, $p < 0.05$). Plasma VDBP levels of proteinuric patients showed a trend to reduction as compared to healthy controls, but this difference did not reach statistical significance. Antiproteinuric treatment did not affect plasma VDBP levels (Fig. 1C). When data from both antiproteinuric interventions were pooled to increase statistical power, plasma VDBP levels remained similar to controls.

3.3. Urine and plasma levels of 25-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3

Plasma 25-hydroxyvitamin D_3 levels (Fig. 3A) were not significantly different between CKD patients without antiproteinuric treatment (40 (16–72) nmol/l) and healthy controls (40 (28–51) nmol/l). Antiproteinuric treatment did not affect 25-hydroxyvitamin D_3 levels (NSAID 42 (19–63), ACEi 38 (28–43) nmol/l). Similarly, 1,25-dihydroxyvitamin D_3 levels (Fig. 3B) were not different among CKD patients and healthy controls, and not affected by antiproteinuric treatment. Urinary 25-hydroxyvitamin D3 was detectable in only one CKD patient (8.0 nmol/l), namely the patient with highest proteinuria during the period without antiproteinuric treatment (proteinuria 13.1 g/24 h), and was reduced to 5.5 nmol/l by NSAID treatment, and to 2.6 nmol/l by ACEi. Although the plasma VDBP of this patient (27 mg/dL) was below the median, 25-hydroxyvitamin D_3 (74.7 nmol/l) and 1,25-dihydroxyvitamin D_3 (79.7 pmol/l) were normal. Urinary 1,25-dihydroxyvitamin D was not detectable in any patient.

4. Discussion

In line with prior studies [12] we found that in proteinuric patients urinary VDBP excretion was strongly increased, in proportion to proteinuria. However, urinary VDBP loss was not associated with significantly reduced plasma levels of VDBP, 25-hydroxyvitamin D_3 or 1,25-dihydroxyvitamin D_3, although a trend towards lower plasma VDBP was observed in CKD. Urinary loss of VDBP was substantially and significantly reduced by antiproteinuric intervention, in proportion to proteinuria reduction, irrespective of the mode of treatment. This large change in urinary VDBP was not associated with significant changes in plasma VDBP, 25-hydroxyvitamin D_3 or 1,25-dihydroxyvitamin D_3. Taken together these data do not support the assumption that urinary loss of VDBP contributes to vitamin D deficiency in proteinuric patients.

Our findings seem to be in contrast with a previous paper by Thrailkill et al., who suggested that enhanced excretion of VDBP may play a role in vitamin D deficiency [12]. Although the sample size of that study was higher, no difference in serum 25-hydroxyvitamin D_3 levels was found between type 1 diabetic patients with or without albuminuria and healthy controls either. Several factors may account for the absence of an association between changes in urinary VDBP and circulating vitamin D. Urinary VDBP loss is normally compensated for by synthesis in the liver which is estimated at 10 mg/kg [13]. In patients with liver disease, lower plasma levels of VDBP and 25-hydroxyvitamin D_3 have been measured [14], implicating that normal liver function is required to maintain normal plasma VDBP levels. Although plasma levels of albumin were reduced in CKD patients as compared to controls, cholesterol and triglycerides were increased (Table 1), suggesting that liver function was not compromised in these patients. In addition, the occupancy of circulating VDBP by vitamin D_3 metabolites is generally lower than 5% [15], implying that only massive VDBP loss could result in 25-hydroxyvitamin D_3 deficiency. Accordingly, there was no association between plasma VDBP and vitamin D_3 levels. Only in one severely nephrotic patient, plasma VDBP was relatively low and urinary loss of 25-hydroxyvitamin D_3 could be

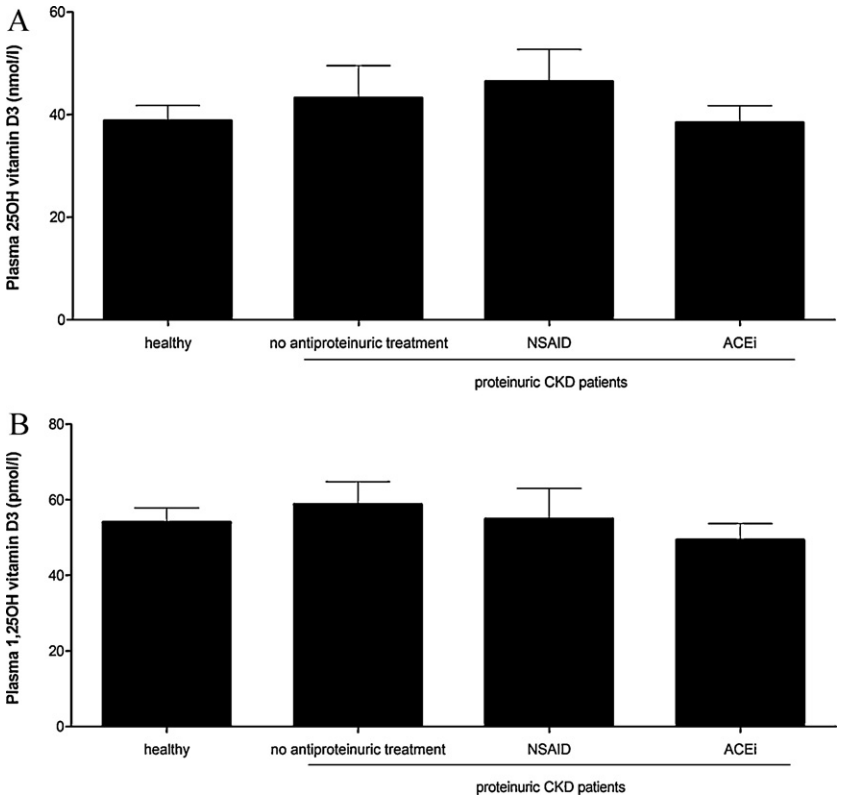

Fig. 3. Plasma 25(OH) and 1,25$(OH)_2$ vitamin D_3 levels. Plasma 25(OH) vitamin D_3 (A) and 1,25$(OH)_2$ vitamin D_3 (B) levels in healthy controls and in proteinuric CKD patients without antiproteinuric treatment, with NSAID treatment and with ACE inhibitor treatment. Antiproteinuric treatment did not affect plasma levels of 25(OH) or 1,25$(OH)_2$ vitamin D_3.

detected. This suggests that only in severe nephrotic syndrome (i.e. proteinuria > 10 g/24 h), urinary VDBP loss may contribute to lower plasma VDBP levels, although it remains very unlikely that this will affect plasma vitamin D_3 levels.

It could be argued that in our study follow-up was too short to detect significant changes in plasma VDBP and/or 25-hydroxyvitamin D_3 levels. However, the plasma half-life of VDBP is 2.5–3.0 days [16], so steady state is assumed to have been reached before the end of the treatment periods. As the plasma half-life for 25-hydroxyvitamin D_3 is longer (12 days, 15 days and even longer half-lives have been reported), it is difficult to draw conclusions on a possible treatment effect on 25-hydroxyvitamin D_3 levels. However, not even a trend to lower vitamin D levels was present within the time frame studied here, i.e. 4 and 6 week treatment periods. We cannot exclude the possibility that confounding factors such as sun exposure and dietary vitamin D intake may have been different between the study groups.

Further limitations of this study are the limited group sizes, warranting cautious interpretation of our results and requiring confirmation in larger cohorts, and the post-hoc nature of our study. The strength however, is the intervention design, and the use of different modes of intervention that enhances the robustness of our findings.

In conclusion, proteinuric CKD is associated with abundant urinary VDBP loss which can be reduced by antiproteinuric therapy. Urinary VDBP loss was not associated with significantly lower plasma levels of VDBP, 25-hydroxyvitamin D_3 or 1,25-dihydroxyvitamin D_3 in CKD patients. Antiproteinuric therapy did not affect plasma levels of VDBP, 25-hydroxyvitamin D_3 or 1,25-dihydroxyvitamin D_3. Together, these findings suggest that other factors controlling the vitamin D pathway, such as low sunlight exposure, impaired vitamin D synthesis in the skin of CKD patients or low nutritional vitamin D intake, may contribute to the more prevalent 25-hydroxyvitamin D_3 deficiency in CKD as documented in previous studies [5]. A recent review also discusses the plausible explanation that in CKD, decreased GFR reduces the amount of 25-hydroxyvitamin D_3 bound to VDBP filtered that is available for renal uptake [17]. Together, these explanations may play a more important role to the deregulation of vitamin D homeostasis in CKD patients than urinary VDBP loss.

Conflict of interest

All authors state that they have no conflict of interest.

Funding

Dr de Borst is supported by personal stimulation grants from the Dutch Kidney Foundation (grant no. KJPB.08.07) and the University Medical Center Groningen (Mandema stipend).

Acknowledgment

The authors acknowledge Else van den Berg for providing plasma and urine samples from healthy controls for this study.

References

[1] C.R. Doorenbos, J. van den Born, G. Navis, M.H. de Borst, Possible renoprotection by vitamin D in chronic renal disease: beyond mineral metabolism, Nat. Rev. Nephrol. 5 (2009) 691–700.
[2] P. Ravani, F. Malberti, G. Tripepi, P. Pecchini, S. Cutrupi, P. Pizzini, F. Mallamaci, C. Zoccali, Vitamin D levels and patient outcome in chronic kidney disease, Kidney Int. 75 (2009) 88–95.

[3] S. Pilz, S. Iodice, A. Zitterman, W.B. Grant, S. Gandini, Vitamin D Status and Mortality Risk in CKD: a meta-analysis of prospective studies, Am. J. Kidney Dis. 58 (2011) 374–382.
[4] C.F. Helvig, D. Cuerrier, C.M. Hosfield, B. Ireland, A.Z. Kharebov, J.W. Kim, N.J. Ramjit, K. Ryder, S.P. Tabash, A.M. Herzenberg, T.M. Epps, M. Petkovich, Dysregulation of renal vitamin D metabolism in the uremic rat, Kidney Int. 78 (2010) 463–472.
[5] R. Mehrotra, D. Kermah, M. Budoff, I.B. Salusky, S.S. Mao, Y.L. Gao, J. Takasu, S. Adler, K. Norris, Hypovitaminosis D in chronic kidney disease, Clin. J. Am. Soc. Nephrol. 3 (2008) 1144–1151.
[6] J.M. Barragry, M.W. France, N.D. Carter, J.A. Auton, M. Beer, B.J. Boucher, R.D. Cohen, Vitamin-D metabolism in nephrotic syndrome, Lancet 2 (1977) 629–632.
[7] A. Nykjaer, D. Dragun, D. Walther, H. Vorum, C. Jacobsen, J. Herz, F. Melsen, E.I. Christensen, T.E. Willnow, An endocytic pathway essential for renal uptake and activation of the steroid 25-(OH) vitamin D3, Cell 96 (1999) 507–515.
[8] J.R. Leheste, B. Rolinski, H. Vorum, J. Hilpert, A. Nykjaer, C. Jacobsen, P. Aucouturier, J.O. Moskaug, A. Otto, E.I. Christensen, T.E. Willnow, Megalin knockout mice as an animal model of low molecular weight proteinuria, Am. J. Pathol. 155 (1999) 1361–1370.
[9] A. Nykjaer, J.C. Fyfe, R. Kozyraki, J.R. Leheste, C. Jacobsen, M.S. Nielsen, P.J. Verroust, M. Aminoff, A. de la Chapelle, S.K. Moestrup, R. Ray, J. Gliemann, T.E. Willnow, E.I. Christensen, Cubilin dysfunction causes abnormal metabolism of the steroid hormone 25(OH) vitamin D(3), Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 13895–13900.
[10] L. Vogt, D. de Zeeuw, A.J. Woittiez, G. Navis, Selective cyclooxygenase-2 (COX-2) inhibition reduces proteinuria in renal patients, Nephrol. Dial. Transplant. 24 (2009) 1182–1189.
[11] W.H. de Jong, K.S. Graham, J.C. van der Molen, T.P. Links, M.R. Morris, H.A. Ross, E.G. de Vries, I.P. Kema, Plasma free metanephrine measurement using automated online solid-phase extraction HPLC tandem mass spectrometry, Clin. Chem. 53 (2007) 1684–1693.
[12] K.M. Thrailkill, C.H. Jo, G.E. Cockrell, C.S. Moreau, J.L. Fowlkes, Enhanced excretion of vitamin D binding protein in type 1 diabetes: a role in vitamin D deficiency? J. Clin. Endocrinol. Metab. 96 (2011) 142–149.
[13] C.J. Laing, N.E. Cooke, Vitamin D binding protein, in: D. Feldman, J.W. Pike, F.H. Glorieux (Eds.), Vitamin D, Elsevier Academic Press, Burlington, MA, 2005, pp. 117–134.
[14] D.D. Bikle, B.P. Halloran, E. Gee, E. Ryzen, J.G. Haddad, Free 25-hydroxyvitamin D levels are normal in subjects with liver disease and reduced total 25-hydroxyvitamin D levels, J. Clin. Invest. 78 (1986) 748–752.
[15] M. Speeckaert, G. Huang, J.R. Delanghe, Y.E. Taes, Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism, Clin. Chim. Acta 372 (2006) 33–42.
[16] M. Kawakami, C.B. Blum, R. Ramakrishnan, R.B. Dell, D.S. Goodman, Turnover of the plasma binding protein for vitamin D and its metabolites in normal human subjects, J. Clin. Endocrinol. Metab. 53 (1981) 1110–1116.
[17] A.S. Dusso, M. Tokoumoto, Defective renal maintenance of the vitamin D endocrine system impairs vitamin D renoprotection: a downward spiral in kidney disease, Kidney Int. 79 (2011) 715–729.