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Vitamin D metabolites in childhood nephrotic syndrome

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Abstract. We measured serum levels of total and ionised calcium, phosphate, intact parathyroid hormone, 25-hydroxyvitamin D [25(OH)D], 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] and the vitamin D binding protein (DBP) in 14 children with idiopathic nephrotic syndrome and 10 healthy, age-matched controls. In all nephrotics serum DBP levels were below the normal range. Serum 25(OH)D was below 7 ng/ml in 10 of 14 nephrotic children and in the low normal range in the remaining 4 patients. The average serum 1,25$(OH)_2$D levels were lower in the nephrotic patients than in the controls. However, free 1,25$(OH)_2$D levels were normal in the nephrotic patients. Both serum 25(OH)D and 1,25$(OH)_2$D correlated positively with the concentration of DBP. There was a significant negative correlation between serum DBP levels and the urinary protein excretion and a significant positive correlation between the urinary excretions of DBP and albumin. From this study it can be concluded that the nephrotic child is capable of maintaining appropriate serum concentrations of free calcitriol despite important urinary losses of both substrate and bound calcitriol.

Key words: Nephrotic syndrome – Vitamin D – 25-hydroxyvitamin D – Parathyroid hormone – 1,25-dihydroxyvitamin D

Introduction

It is well established that (adult) patients with the nephrotic syndrome have abnormalities in calcium metabolism. Gastrointestinal absorption of calcium is impaired so that faecal calcium excretion equals or exceeds dietary calcium intake [1–3]. This leads to low plasma total and ionised calcium concentrations [4, 5] and hypocalciuria [6]. These abnormalities have been related, firstly in adult nephrotic patients, to disturbed vitamin D metabolism. In 1977, Schmidt-Gayk et al. [7] and Barragry et al. [8] demonstrated that adult nephrotic patients display urinary loss of vitamin D bound to its binding protein (DBP), which results in low serum 25-hydroxyvitamin D [25(OH)D] levels. These data were confirmed by Goldstein et al. [9] and Colston et al. [10]. Depressed levels of 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] and raised parathyroid hormone (PTH) levels were then reported in adult nephrotic patients with normal renal function [7, 9]. Subsequent studies, however, yielded conflicting results. Two groups studied the free fraction of 1,25$(OH)_2$D in nephrotic patients: Auwerx et al. [11] found them reduced, Koenig et al. [12] slightly elevated. One study of paediatric patients with the nephrotic syndrome mentions low 25(OH)D levels, together with modest hyperparathyroidism and normal plasma concentrations of 1,25$(OH)_2$D [13]. We have studied in depth the vitamin D status in 14 nephrotic children.

Patients and methods

Patients. Fourteen children (8 boys, 6 girls) with an idiopathic nephrotic syndrome were studied while nephrotic, either during their first attack ($n = 7$) or during a relapse ($n = 7$). Their ages ranged from 2 to 14 years; 10 had an idiopathic nephrotic syndrome without haematuria or hypertension and were steroid sensitive; 3 other patients had haematuria and moderate mesangial proliferation on renal biopsy; however, remission with prednisone was attained within 4 weeks. The last child had a steroid-resistant nephrotic syndrome, although her biopsy showed no lesions on both light microscopy and immunofluorescence. The control patients were 10 children aged 2–14 years, 7 girls with day-time wetting and 3 boys with nocturnal enuresis. At the time of blood sampling, the children were not fasting and none of the nephrotic patients were on steroids or ciclosporin.

Laboratory techniques. Serum calcium, phosphate and creatinine levels were measured using standard laboratory techniques. Serum ionised calcium was measured in whole blood with the Corning 288

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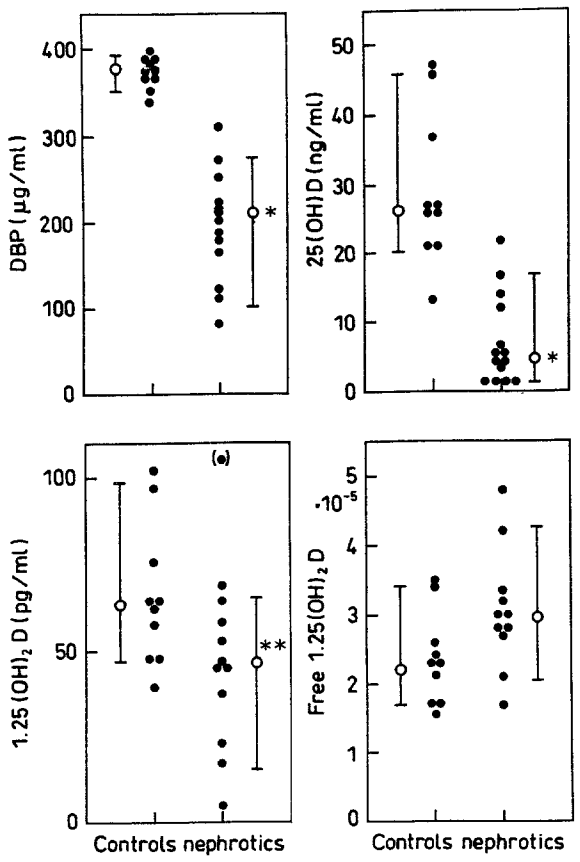

Fig. 1. Serum vitamin D binding protein (*DBP*), 25-hydroxyvitamin D [*25(OH)D*], 1,25-dihydroxyvitamin D [*1,25(OH)$_2$D*] and free 1,25(OH)$_2$D levels in nephrotic children and controls. The *closed circles* represent the individual figures. The *open circles* with bars indicate the median, 10th and 90th per centile values. * $P < 0.01$; ** $P < 0.05$

Blood Gas Analyzer. Serum PTH levels were measured as the intact molecule with an immunoradiometric assay [14]. Serum 25(OH)D levels were measured by competitive protein binding [15] and 1,25(OH)$_2$D levels by radioimmunoassay [16]. DBP was measured by single radial immunodiffusion [17]. The free 1,25(OH)$_2$D concentration is calculated from the molar ratio of 1,25(OH)$_2$D to DBP $\times 10^{-5}$. Urinary total protein excretion was measured by the Biuret method and urinary albumin by radioimmunoassay. The Mann-Whitney U test was used for statistical analysis of differences between patients and controls. The correlation analyses were performed using Pearson's test.

Results

Urinary protein excretion in the 14 nephrotic children was between 44 and 545 mg/kg per 24 h. Serum albumin, total calcium, ionised calcium, intact PTH (iPTH) and creatinine levels are given in Table 1. Serum albumin levels were between 0.89 and 3.07 g/dl (median 1.36 g/dl). Serum total calcium was between 7.2 and 9.0 mg/dl (median 8.1 mg/dl), which is significantly lower than the control values (median 9.6 mg/dl). Serum ionised calcium was between 1.06 and 1.29 mmol/l (median 1.13 mmol/l) which is significantly lower than in the controls (median 1,25 mmol/l) ($P < 0.05$). iPTH levels between 0.0 and 24.3 pg/ml (median 8.0 pg/ml) were not different from the levels measured in the control group (median 13.1 pg/ml). The median serum

Table 1. Serum albumin (S-alb), total calcium (S-Ca), ionised calcium (S-Ca^{2+}), creatinine (S-Cr) and intact PTH (S-iPTH) in 14 nephrotic children

Patient no.	S-alb (g/dl)	S-Ca (mg/dl)	S-Ca^{2+} (mmol/l)	S-Cr (mg/dl)	S-iPTH (pg/ml)
1	0.89	7.3	1.06	0.61	6.9
2	1.52	7.2	1.11	0.52	20.2
3	1.19	7.6	1.12	0.38	0.0
4	2.53	8.2	–	0.53	6.5
5	1.38	7.6	1.09	0.56	10.6
6	1.30	8.5	–	0.53	24.3
7	1.95	8.1	1.13	0.65	14.5
8	3.07	8.8	1.07	–	6.1
9	2.53	8.1	–	0.69	21.0
10	1.23	8.1	1.13	0.47	5.1
11	1.12	8.4	1.29	0.41	5.5
12	2.93	9.0	1.22	0.56	9.2
13	1.21	8.1	1.17	0.40	0.0
14	1.34	7.6	1.17	0.46	19.0
Median	1.36	8.1	1.13	0.53	8.0

phosphate level was 4.6 mg/dl in the nephrotics and 4.5 mg/dl in the controls. Serum creatinine levels of the nephrotic patients were also within the normal range: 0.40–0.69 mg/dl (median 0.53 mg/dl).

Serum DBP, 25(OH)D, 1,25(OH)$_2$D and free 1,25(OH)$_2$D levels in control children and nephrotic patients are shown in Fig. 1. Median serum levels of the vitamin D metabolites in the controls were as follows: DBP was 380 µg/ml (range 341–397 µg/ml), 25(OH)D 26.6 ng/ml (range 13.2–46.9 ng/ml) and 1,25(OH)$_2$D 65 pg/ml (range 40–102 pg/ml). The median free 1,25(OH)$_2$D concentration was 2.21×10^{-5} (range $1.56–3.54 \times 10^{-5}$).

In all nephrotic patients serum DBP levels were below the normal range (median 204 µg/ml, range 79–313 µg/ml). There was a significant negative correlation between serum DBP and urinary protein excretion (Fig. 2a) and a strong positive correlation between the urinary DBP and urinary albumin (Fig. 2b). Serum 25(OH)D was undetectable (<2.5 ng/ml) in 4 patients, below 7 ng/ml in 6 further patients and within the low normal range in the remaining 4. Altogether, these levels were significantly lower than in the control group ($P < 0.01$). Serum 1,25(OH)$_2$D levels were variable: below the normal range in 4 patients, within the normal range in 7 and above the normal range in 1 nephrotic patient. If the latter is discarded, the figures in the nephrotic children are significantly lower than in the control group ($P < 0.05$). In contrast, free 1,25(OH)$_2$D levels were normal in 9 nephrotic patients and higher than normal in 2 patients.

Discussion

Like many other steroids, vitamin D and its metabolites, 25(OH)D and 1,25(OH)$_2$D, circulate in the plasma bound to a specific carrier protein, which influences the bioavailability of the hormone [18, 19]. Bound to the binding protein, steroid hormones are unable to enter the cell freely: only the free fraction is internalised and bound to specific

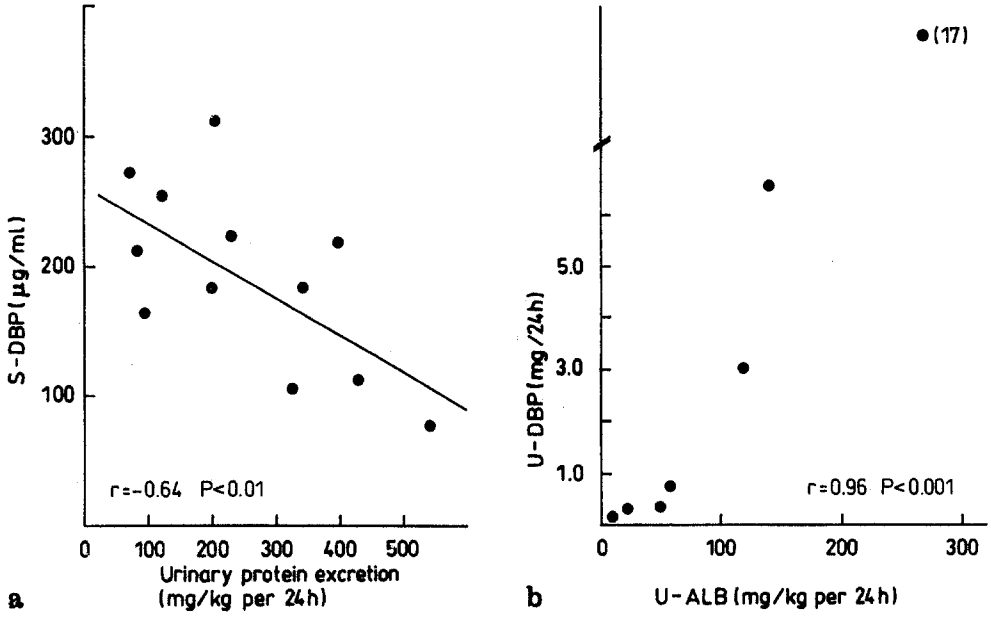

Fig. 2. Correlations between: **a** serum DBP (*S-DBP*) levels and urinary protein excretion and **b** between urinary DBP (*U-DBP*) and albumin excretion (*U-ALB*)

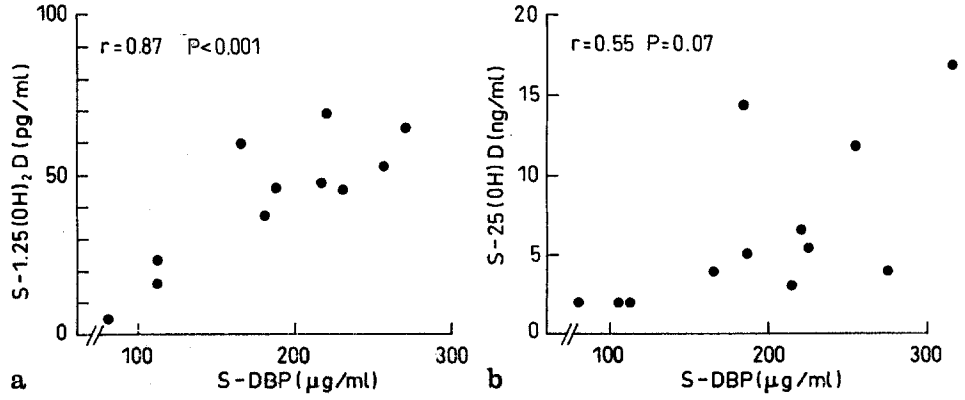

Fig. 3. Correlations between **a** S-DBP and **b** S-1,25$(OH)_2$D levels and serum 25(OH)D levels in nephrotic children

intracellular receptors. Therefore, only the free fraction and not the total 1,25$(OH)_2$D corresponds to its biological activity in vivo and in vitro. The specific binding protein for vitamin D, referred to as DBP or Gc globulin, is a low molecular weight protein (56,000 daltons) that is excreted in the urine of nephrotic adult patients [8]. This urinary loss leads to low plasma levels of both DBP and 25(OH)D [7, 8, 17]. The reported levels of 1,25$(OH)_2$D have been contradictory. Korkor et al. [20] studied six nephrotic adults with a normal glomerular filtration rate (GFR) and found low 25(OH)D levels but normal levels of 1,25$(OH)_2$D as well as of ionised calcium and PTH. In contrast, Lambert et al. [21] found low 1,25$(OH)_2$D levels in seven nephrotic patients with a normal GFR. Low 1,25$(OH)_2$D levels were attributed to urinary loss of this hormone. Differences between patient groups might be explained by the influence of additional factors, such as degree of proteinuria, duration of the illness, kidney function, serum albumin level and steroid therapy. The fact that ionised calcium and iPTH levels were normal in another study was thought to be due to normal concentrations of the free 1,25$(OH)_2$D [20]. Two studies have assessed the free fraction of 1,25$(OH)_2$D in adult nephrotics. Auwerx et al. [11] found decreased levels, Koenig et al. [12] increased levels. The discrepancy could be explained by the difference in methodology, but also by the small numbers of patients or a selection bias. For instance, it is true that the patients in the study of Auwerx et al. [11] were adults with a relatively long-standing nephrotic state.

To our knowledge, only one report on vitamin D metabolites in nephrotic children has been published. Freundlich et al. [13] studied 58 nephrotic patients aged 2–20 years. In this cohort, serum total calcium was low and PTH, measured as the carboxy terminal moiety, was "slightly higher in relapse than in remission", although the differences were not statistically significant. Serum 25(OH)D levels were strikingly low (mean 9.0 ng/ml, controls 14–46 ng/ml) but 1,25$(OH)_2$D levels were within the normal range (mean 46 pg/ml, controls 15–65 pg/ml). These authors concluded that "hypocalcemia, modest hyperparathyroidism and strikingly low calcidiol levels" characterise the nephrotic state but "calcitriol, the most active metabolite of vitamin D, was found to be normal". Analysis of the data of Freundlich et al. [13] is hampered by the fact that individual figures and ranges are not always given.

In our group of 14 children with idiopathic nephrotic syndrome we also measured serum ionised calcium, the iPTH, serum and urinary DBP and the free concentration of $1,25(OH)_2D$. Serum ionised calcium was significantly lower than in control children, but iPTH levels were not different from control values. Serum DBP and 25(OH)D levels were significantly lower than in healthy children. In 1 nephrotic child, serum $1,25(OH)_2D$ was above the normal range. When this patient was excluded, the median $1,25(OH)_2D$ levels in nephrotics was significantly lower than in the control group.

The significant positive correlation between both serum 25(OH)D and $1,25(OH)_2D$ levels and serum DBP are in keeping with the role and the affinity of the carrier protein. The negative correlation between serum DBP and proteinuria and the strong positive correlation between the urinary DBP and urinary albumin provide an acceptable explanation for the variation in abnormalities found within the nephrotic group.

Our findings confirm that serum calcium and 25(OH)D levels are markedly depressed in patients with the idiopathic nephrotic syndrome. We also demonstrated that the binding protein for the vitamin D metabolites is lost in the urine in amounts that strongly correlate with urine albumin. Serum DBP levels therefore are reduced in relation to the magnitude of proteinuria. Our study clarifies the controversy about serum $1,25(OH)_2D$ levels in nephrotic patients. We have indeed been able to show that there is a consistent abnormality in serum $1,25(OH)_2D$ levels: serum $1,25(OH)_2D$ levels are positively correlated with serum DBP (Fig. 3). The normal levels of serum free $1,25(OH)_2D$ is an important finding which is in accordance with one study in adults [12] but at variance with another [11]. Normal free calcitriol levels are the likely explanation for the normal iPTH levels.

Our findings suggest a disturbance in the normal feedback systems of plasma calcium homeostasis. Firstly, how can the finding that serum iPTH is not increased in the presence of low serum ionised calcium be explained? Secondly, what stimulus, other than PTH, maintains normal free calcitriol levels despite substrate deficiency and urinary loss of the bound moiety? Serum phosphate levels were similar in controls and nephrotics. Other activators of renal 1α-hydroxylase, e.g. growth hormone, insulin-like growth factor-1 and prostaglandins, have not been measured.

The different abnormalities in calcium and vitamin D metabolism observed in the nephrotic state raise the important question as to whether vitamin D supplementation is indicated in nephrotic children. Our study does not provide arguments in favour of systematically giving vitamin D supplements to nephrotic children. However, from the information available today, the following can be proposed: early in the nephrotic state, the DBP is lost and this is followed rapidly by a depletion of the 25(OH)D stores and later by reduced total $1,25(OH)_2D$. The next step, if the nephrotic state persists and no supplements are given, is an inevitable drop in free $1,25(OH)_2D$ levels also.

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