

## Plasma exchange induces vitamin D deficiency

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### Summary

**Background:** Plasma exchange is used in the treatment of diseases mediated by pathogenic circulating proteins, or for transplant desensitization. Its non-targeted nature results in the depletion of physiologically important molecules, and it is often complicated by hypocalcaemia.

**Aim:** To determine the effects of plasma exchange on vitamin D binding protein (DBP) and associated vitamin D metabolites.

**Design:** Single-centre prospective cohort study of 11 patients.

**Methods:** DBP and vitamin D metabolites were measured before and immediately after five plasma exchanges, and 7 and 28 days after discontinuation of plasma exchange.

**Results:** Plasma exchange reduced plasma DBP concentration from  $196.9 \pm 53.2$  to  $98.5 \pm 34$   $\mu\text{g/ml}$

( $P=0.0001$ ), 1,25-dihydroxyvitamin D from  $103 \pm 52$  to  $42 \pm 4$   $\text{pmol/l}$  ( $P=0.003$ ) and 25-hydroxyvitamin D from  $49.7 \pm 29$  to  $22 \pm 9.4$   $\text{nmol/l}$  ( $P=0.0017$ ), through their removal in effluent. After 7 days, DBP and 1,25-dihydroxyvitamin D were not significantly different from baseline, but 25-hydroxyvitamin D remained significantly lower after 7 days ( $26.4 \pm 9.8$   $\text{nmol/l}$ ,  $P=0.02$ ) and 28 days ( $30.8 \pm 15.5$   $\text{nmol/l}$ ,  $P=0.048$ ). Corrected calcium decreased from  $2.23 \pm 0.11$  to  $1.98 \pm 0.08$   $\text{mmol/l}$  ( $P=0.0007$ ) immediately after five treatments. Plasma calcium was significantly associated with 1,25-dihydroxyvitamin D ( $r^2=0.79$ ,  $P<0.0001$ ).

**Conclusions:** Plasma exchange induced an acute reversible decrease in plasma 1,25-dihydroxyvitamin D, DBP, calcium and a sustained decrease in plasma 25-hydroxyvitamin D.

### Background

Plasma exchange is used for the treatment of diseases mediated by circulating pathogenic proteins and is a recommended therapy for auto-immune diseases such as severe anti-neutrophil cytoplasmic antibody-mediated vasculitis (AAV),<sup>1</sup> anti-glomerular basement membrane disease (Goodpasture's syndrome),<sup>2,3</sup> immune-mediated neuropathies<sup>4,5</sup> and myasthenia gravis<sup>6</sup>; dysproteinaemias<sup>7</sup>; thrombotic thrombocytopenic purpura and the haemolytic uremic syndrome;<sup>8,9</sup> and in HLA-sensitized or blood

group incompatible transplantation, for pre-operative desensitization and treatment of antibody-mediated organ rejection.<sup>10</sup> Plasma exchange removes the plasma component of blood by extracorporeal circulation through a cell separator or semi-permeable filter. The cellular blood components are returned to the patient with the infusion of replacement fluid, usually electrolyte balanced human albumin solution or (less frequently) donor plasma. This approach effectively removes medium and large molecular weight proteins (up to

1000 kDa) including pathogenic antibodies.<sup>11</sup> However, it results in the collateral depletion of many physiologically important molecules which may include vitamin binding protein, and its indiscriminate nature contributes to a number of well-characterized complications.

Common complications of plasma exchange include symptomatic hypocalcaemia, muscle cramp, urticaria and hypotension,<sup>12–14</sup> and occur in up to 12% of plasma exchange treatments.<sup>12</sup> Hypocalcaemia is conventionally attributed to the binding of ionized calcium to the citrate used as anticoagulant. Most patients require administration of intravenous calcium to treat or prevent symptomatic hypocalcaemia, although hypocalcaemia may still occur and persist after completion of plasma exchange.

Vitamin D may impact on patient outcomes in many plasma exchange-treated diseases. Its deficiency may potentiate steroid induced osteoporosis and increases fracture rates,<sup>15,16</sup> results in impaired innate immunity and increased infection rates,<sup>17–19</sup> and may be permissive to the development and activity of autoimmune diseases such as multiple sclerosis,<sup>20</sup> AAV<sup>21</sup> and systemic lupus erythematosus.<sup>22</sup> Taken together, these data provide a strong rationale for the prevention of vitamin D deficiency in plasma exchange-treated disease.

Vitamin D metabolites are transported in plasma by carrier proteins.<sup>23</sup> The most important of these is the 56 kDa vitamin D binding protein (DBP), which carries 99% of vitamin D metabolites in the circulation.<sup>18,24</sup> DBP binding protects vitamin D metabolites from hydroxylase-mediated catabolism, and their half-life may therefore be decreased by a reduction in DBP. Furthermore, removal of DBP and associated vitamin D metabolites would reduce plasma vitamin D metabolite concentrations directly, similar to the situation in nephrotic syndrome where urinary losses of DBP result in vitamin D deficiency through concomitant urinary loss of vitamin D metabolites.<sup>25</sup> The effect of plasma exchange on DBP is not known; however, since plasma exchange readily depletes proteins of a similar size, we hypothesized that plasma exchange would induce vitamin D deficiency through DBP depletion.

## Methods

We performed a prospective cohort study of 11 patients receiving plasma exchange at Addenbrooke's hospital, Cambridge, between June 2010 and March 2011. Patients aged 18 or older were enrolled in the study if they required five or more exchanges and were able to provide informed consent. Patients

were excluded if they had received plasma exchange within the preceding 12 months, had known genetic defects of the vitamin D endocrine system, hypercalcaemia (defined as albumin-corrected calcium >2.6 mmol/l) or were receiving treatment with any vitamin D-containing compound including cholecalciferol, ergocalciferol, vitamin D-containing calcium supplements, alfacalcidol, 1,25-dihydroxyvitamin D or paricalcitol, vitamin D-containing over-the-counter supplements, calcimimetic agents or parathyroid hormone (PTH)-related compounds. The Cambridgeshire Regional Research Ethics Committee approved the study.

Demographic and clinical data including age, gender, ethnicity, diagnosis, co-morbidity and indication for plasma exchange were recorded. All patients received single plasma volume exchanges using continuous flow centrifugation with the COBE Spectra apheresis system (Terumo, Zaventem, Belgium). One volume of Acid Citrate Dextrose (ACD-A) was used with every 10 volumes of electrolyte-balanced human albumin solution volume to prevent coagulation. All patients received Zenalb<sup>®</sup> 4.5 human albumin solution exclusively as replacement fluid; no patient received donor plasma. Zenalb<sup>®</sup> 4.5 contains 4.5% human albumin to at least 95% purity, and contains sodium 140 mmol/l, potassium, chloride citrate and sodium *n*-octanoate. Zenalb<sup>®</sup> 4.5 contains no calcium, DBP or vitamin D metabolites (Rosalind Sackey, personal communication; Bio Products Laboratory, Hertfordshire, UK). Prophylaxis against hypocalcaemia was given with a minimum of 20 ml 10% intravenous calcium gluconate per treatment and was increased if hypocalcaemia occurred.

Non-fasting blood samples were collected in Lithium Heparin or EDTA bottles, and processed as described previously.<sup>26</sup> Plasma exchange effluent was collected in Lithium Heparin bottles at the end of each treatment. Laboratory determinations included urea, creatinine, haemoglobin, albumin, calcium, phosphate, PTH, 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D and DBP. We determined these parameters before the onset of treatment and before and immediately after every plasma exchange for the first three patients, and subsequently before and immediately after the first and fifth treatment. After completion of plasma exchange, biochemical parameters were again measured after 7 and 28 days.

Vitamin D metabolites and DBP were also measured in plasma effluent after the first treatment, and the effluent volume recorded. Albumin corrected calcium (normal range 2.1–2.5 mmol/l), phosphate (normal range 0.8–1.4 mmol/l) and creatinine (normal range 35–125  $\mu$ mol/l) were analysed using

an autoanalyser (Dimension, Siemens Healthcare Diagnostics, Surrey, UK). Glomerular filtration rate was estimated from plasma creatinine (eGFR) using the four-variable Modified Diet in Renal Disease study formula. 1,25-Dihydroxyvitamin D, 25-hydroxyvitamin D and DBP analyses were conducted in duplicate. 1,25-Dihydroxyvitamin D was measured by radioimmunoassay (IDS Gamma-B, Tyne and Weir, UK) with inter- and intra-assay coefficient of variation (CV) of 4% and 9%, respectively. DBP concentration was determined by ELISA (R & D Systems, Oxford, UK) with both an inter- and intra-assay CV of 6%. 25-Hydroxyvitamin D was measured by chemiluminescent assay (LiasonDiaSorin, Stillwater, MN, USA) with an inter- and intra-assay CV of 3% and 6%, respectively. PTH (reference range 16–87 ng/l) was measured on an automatic chemiluminescent assay (Siemens Healthcare Diagnostics) with an inter-assay CV of 8%. Patients at risk of Vitamin D deficiency and insufficiency were defined as a 25-hydroxyvitamin D concentration of <25 or 50 nmol/l, respectively.

Data were analysed using Stata SE v12.1 (College Station, TX, USA) and are presented as mean  $\pm$  SD or median (IQR) as appropriate for their distributions. The effects of plasma exchange on plasma 1,25-dihydroxyvitamin D, DBP and 25-hydroxyvitamin D concentrations were estimated using linear regression and are presented as coefficients  $\pm$  standard error. Since the plasma concentration of PTH is influenced by renal function,<sup>27</sup> changes in PTH were determined using mixed effects linear regression models for repeated measures of eGFR. Pre- and post-plasma exchange values were compared using a two-sided paired Student's *t*-test for parametric data, and the Wilcoxon matched-pairs signed rank test for non-parametric data. Mean values at post-plasma exchange time points (1, 7 and 28 days) were compared using repeated measures ANOVA. *P*-values <0.05 for two-sided tests and <0.025 for one-sided tests were considered statistically significant.

## Results

Eleven caucasian patients aged  $59 \pm 13$  years were included in the study, of which seven were male. Indications for plasma exchange were AAV ( $n=5$ ), myasthenia gravis ( $n=3$ ), paraneoplastic neuropathy ( $n=2$ ) and voltage-gated potassium channel antibody-mediated encephalopathy ( $n=1$ ) (Table 1). All five patients with AAV had renal impairment (eGFR <60 ml/min/1.73 m<sup>2</sup>) at entry; three had an eGFR of <15, of which one was dialysis-dependent.

The mean baseline plasma 1,25-dihydroxyvitamin D at entry was 119 (54–140) pmol/l. Baseline plasma DBP concentration was  $206.5 \pm 64.7$   $\mu$ g/ml, and 25-hydroxyvitamin D concentration was  $49.7 \pm 29$  nmol/l. Vitamin D deficiency at entry of the study was identified in two, and insufficiency identified in four patients. Baseline PTH was 161 (98–343) ng/l, with the majority of values above the reference range. There was no correlation between baseline eGFR and 1,25-dihydroxyvitamin D ( $r^2=0.37$ ,  $P=0.26$ ) or 25-hydroxyvitamin D ( $r^2=-0.19$ ,  $P=0.57$ ) concentrations, but PTH was inversely associated with eGFR ( $P=0.005$ ).

Patients received a plasma exchange dose of  $44.8 \pm 10.8$  ml/kg per treatment. All patients received purified human albumin solution exclusively as the exchange fluid. Three patients received seven treatments; eight received five treatments. There were no treatment withdrawals. One patient was lost to follow-up after completion of plasma exchange. Patient characteristics are shown in Table 2.

Five plasma exchange treatments reduced plasma 1,25-dihydroxyvitamin D from  $103 \pm 52$  to  $42 \pm 4$  pmol/l ( $P=0.003$ ). However, 1,25-dihydroxyvitamin D concentrations at 7 ( $83 \pm 42$  pmol/l,  $P=0.38$ ) and 28 days ( $75 \pm 34$  pmol/l,  $P=0.46$ ) after completion of plasma exchange did not differ significantly from baseline. Similarly, five treatments reduced plasma DBP levels (Figure 1) from  $196.9 \pm 53.2$  to  $98.5 \pm 34$   $\mu$ g/ml ( $P=0.0001$ ), but after 7 ( $194 \pm 47.8$   $\mu$ g/ml,  $P=0.63$ ) and 28 days ( $223.5 \pm 69.9$   $\mu$ g/ml,  $P=0.85$ ), DBP did not differ from baseline. The plasma 25-hydroxyvitamin D concentration decreased from  $49.7 \pm 29$  to  $22 \pm 9.4$  nmol/l ( $P=0.0017$ ); however, in contrast to 1,25-dihydroxyvitamin D and DBP, it remained lower than baseline after 7 ( $26.4 \pm 9.8$  nmol/l,  $P=0.02$ ) and 28 days ( $30.8 \pm 15.5$  nmol/l,  $P=0.048$ ) (Figure 1). PTH was not significantly altered after five treatments (153 [118–230],  $P=0.67$ ), and did not differ from baseline after 7 (106 [50.8–124],  $P=0.07$ ) or 28 days (59 [37–208],  $P=0.07$ ). However, in a multilevel mixed effects regression model accounting for renal function, every plasma exchange resulted in a reduction in PTH of 28 ng/l ( $P=0.001$ ).

We next sought to determine whether the changes in plasma DBP and vitamin D metabolite concentrations were due to their removal in plasma. First, we tested this by measuring DBP and 25-hydroxyvitamin D in plasma exchange effluent in a subset consisting of the first three patients enrolled in our study (specified *a priori*). For these three patients, the pre-treatment plasma DBP and plasma 25-hydroxyvitamin D concentrations were  $256 \pm 97$   $\mu$ g/ml and  $74 \pm 35$  nmol/l, respectively. The effluent contained

**Table 1** Patient characteristics

Age	Gender	Diagnosis	Number of exchanges
73	M	ANCA-associated vasculitis (MPO)	7
45	M	Myasthenia gravis	5
43	F	ANCA-associated vasculitis (PR3)	5
69	M	ANCA-associated vasculitis (MPO)	5
51	M	Paraneoplastic neuropathy (neuro-endocrine)	5
71	M	Potassium channel antibody-associated encephalopathy (VGKC-Ab)	5
64	M	Myasthenia gravis	5
63	F	ANCA-associated vasculitis (PR3)	7
73	M	Myasthenia gravis	5
63	F	Paraneoplastic neuropathy (parotid acinic carcinoma associated anti-Ma2 antibody)	5
38	F	ANCA-associated vasculitis (PR3)	7

MPO, myeloperoxidase; PR3, proteinase-3; VGKC-Ab, voltage gated potassium channel antibody.

**Table 2** Patient characteristics and biochemical profile

	Entry
Age (years)	59 ± 13
Renal function	
Creatinine (μmol/l)	106 (71–379)
eGFR (ml/min/m <sup>2</sup> )	56.9 ± 39.5
25-Hydroxyvitamin D (nmol/l)	50.6 ± 30.1
1,25(OH) <sub>2</sub> Vitamin D (pmol/l)	103 ± 52
Parathyroid hormone (pg/ml)	161 (98–343)
DBP (μg/ml)	196.9 ± 53.2
Albumin (g/dl)	32 ± 9
Albumin corrected calcium (mmol/l)	2.23 ± 0.11
Phosphate (mmol/l)	1.26 ± 0.28
Haemoglobin (g/dl)	11.4 ± 3.2

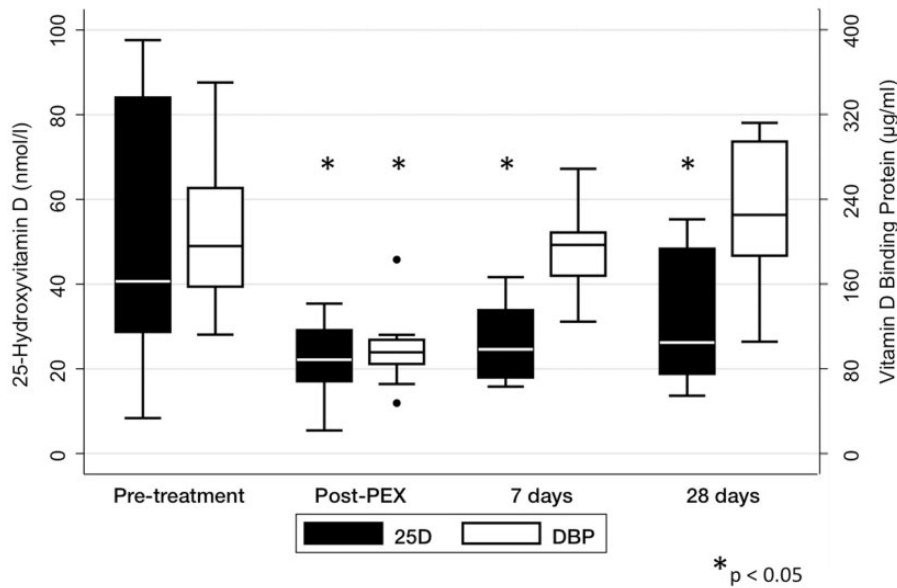
137 ± 40 μg/ml DBP and 59 ± 30 nmol/l 25-hydroxyvitamin D, confirming significant removal by plasma exchange (Figure 2). Second, we considered whether, for all patients, the number of plasma exchange treatments predicted post-treatment plasma concentrations of 1,25-dihydroxyvitamin D, DBP and 25-hydroxyvitamin D. Every plasma exchange treatment reduced plasma 1,25-dihydroxyvitamin D by 12.4 ± 3.4 pmol/l ( $P=0.002$ ), plasma DBP by 19.5 ± 4.8 μg/ml ( $P<0.001$ ) and plasma 25-hydroxyvitamin D by 5.7 ± 1.6 nmol/l ( $P=0.001$ ).

Given that we observed a marked decrease in 1,25-dihydroxyvitamin D and 25-hydroxyvitamin D concentrations and fluctuations in PTH with plasma exchange, we next asked whether depletion of vitamin D metabolites contributed to hypocalcaemia. In this study, we administered 28 ± 9 ml

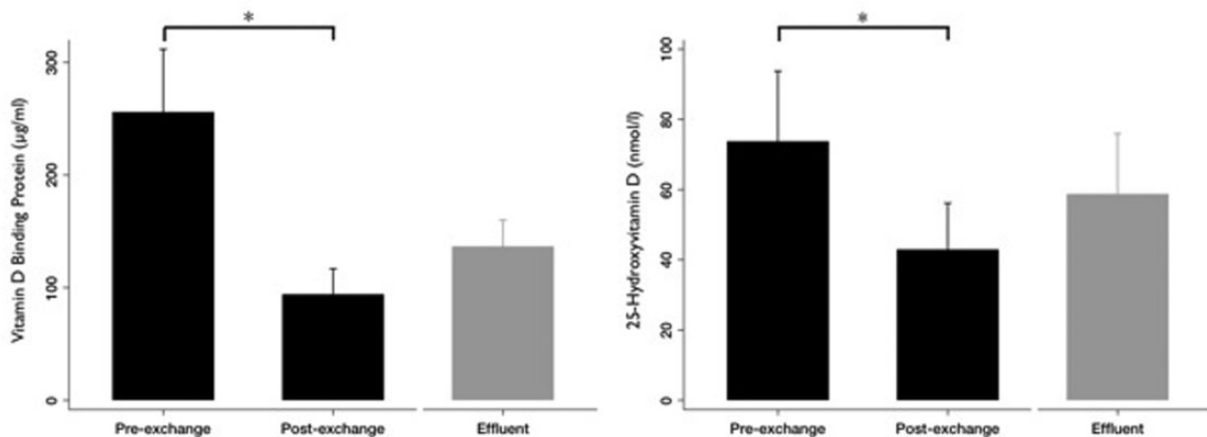
intravenous 10% calcium gluconate during each treatment. Despite this, we observed a decrease in albumin-corrected calcium from 2.23 ± 0.11 to 1.98 ± 0.08 mmol/l ( $P=0.0007$ ) after five plasma exchange treatments. Although corrected calcium tended to be lower 7 days after completion of plasma exchange (2.13 ± 0.18 mmol/l), this did not reach significance ( $P=0.23$ ). Across all time points, plasma 1,25-dihydroxyvitamin D concentrations correlated with corrected calcium ( $r^2=0.79$ ,  $P<0.0001$ , Figure 3). Corrected calcium did not correlate with PTH ( $P=0.67$ ) or phosphate ( $P=0.23$ ).

## Discussion

In this prospective cohort study, we evaluated the effect of plasma exchange on vitamin D status. A typical course of plasma exchange induced a sustained decrease in 25-hydroxyvitamin D concentration, and a transient decrease in 1,25-dihydroxyvitamin D, DBP and calcium concentrations. The mean 25-hydroxyvitamin D concentration of 22 ± 9.4 nmol/l after five plasma exchanges is within the range where clinical manifestations of vitamin D deficiency might be expected and, importantly, the 25-hydroxyvitamin D concentration remained depressed 4 weeks after plasma exchange. Furthermore, plasma exchange induced a marked reduction in 1,25-dihydroxyvitamin D, and plasma 1,25-dihydroxyvitamin D was closely associated with hypocalcaemia. Surprisingly, we found a decrease in PTH with plasma exchange and not association with hypocalcaemia. In addition to the direct removal of 25-hydroxyvitamin D with plasma effluent, two other factors are likely to



**Figure 1.** Effect of plasma exchange on 25-hydroxyvitamin D and DBP. Statistical comparisons are made with baseline values for each variable. Plasma exchange significantly reduced DBP, although this had recovered to baseline after 7 days. In contrast, 25-hydroxyvitamin D concentrations remained significantly lower than baseline after 7 and 28 days.

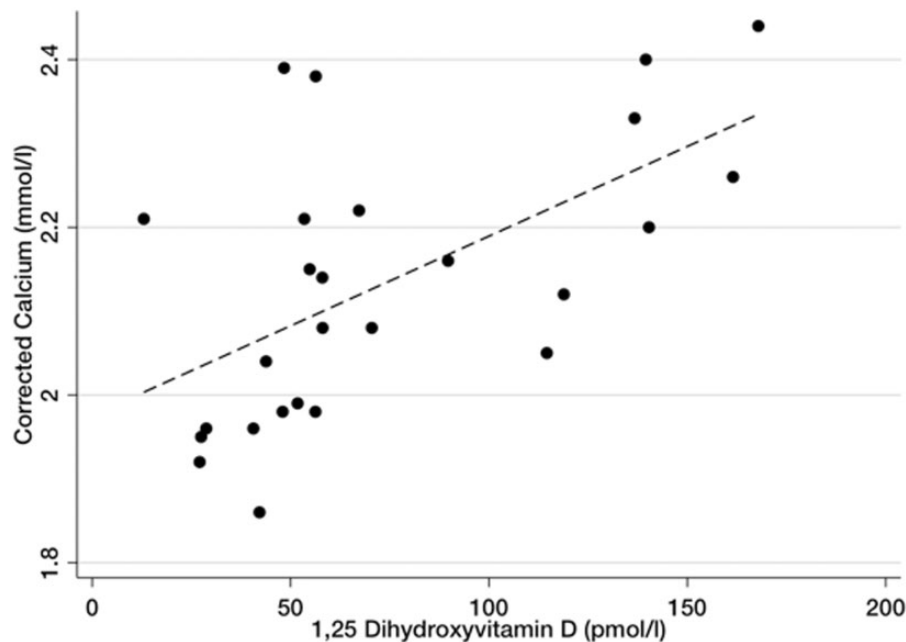


**Figure 2.** Pre-exchange, effluent and post-exchange DBP and 25-hydroxyvitamin D values in a subset of three patients. Bars show mean (standard error) values of (A) DBP and (B) 25-hydroxyvitamin D levels in plasma before and after the first plasma exchange (black), and in the effluent from this exchange (grey).

contribute to a decrease in 25-hydroxyvitamin D: first, both hypocalcaemia and high PTH up-regulate the 25-hydroxyvitamin D 1 $\alpha$ -hydroxylase (CYP27B1), leading to an increased conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D. Second, the half-life of 1,25-dihydroxyvitamin D and 25-hydroxyvitamin D is markedly reduced in DBP deficiency,<sup>28</sup> through exposure of more unbound vitamin D metabolites to catabolism.

Consistent with previous reports, we observed a reduction in plasma calcium with plasma exchange. Hypocalcaemia is the commonest complication of

plasma exchange and has been attributed to the use of citrate and, to a lesser extent, the removal of calcium in the plasma effluent.<sup>29,30</sup> However, our data also demonstrate a strong association between post-plasma exchange hypocalcaemia and 1,25-dihydroxyvitamin D concentrations (Figure 3). 1,25-Dihydroxyvitamin D is indispensable for calcium homeostasis. Together with PTH, it is a key regulator of active intestinal trans-epithelial calcium absorption and renal tubular calcium reabsorption, and its deficiency results in hypocalcaemia. Our data suggest that acute 1,25-dihydroxyvitamin D deficiency may



**Figure 3.** Relationship of plasma calcium and 1,25 dihydroxyvitamin D concentrations. Scatterplot and linear regression demonstrating the correlation of plasma corrected calcium and 1,25 dihydroxyvitamin D ( $r^2=0.79$ ,  $P=0.0001$ ,  $\beta$ -coefficient=0.002). Values represent measurements for all patients from pre-treatment until immediately after the final plasma exchange.

contribute to the hypocalcaemia observed with plasma exchange. This notion is supported by the finding that hypocalcaemia may persist for several days after completion of plasma exchange,<sup>31</sup> whereas the plasma half-life of citrate is <40 min.<sup>32,33</sup>

Surprisingly, we found a decrease in PTH with plasma exchange despite reductions in calcium and 1,25 dihydroxyvitamin D and 25-hydroxyvitamin D concentrations. The expected increase in PTH is likely offset by the marked improvement in renal function observed in those subjects with significant renal impairment. Furthermore, PTH removal during plasma exchange, concomitant calcium gluconate infusion and fluctuation of other regulators of PTH secretion (such as phosphate) may have disrupted the normal parathyroid response to hypocalcaemia. Together with the removal of PTH by plasma exchange, these factors limit the inferences that can be drawn from the PTH response to plasma exchange.

Our findings should be interpreted within the limitations of our study. The study population was relatively small and heterogeneous in terms of diagnosis, renal function, season of recruitment and baseline vitamin D status. Our study did not include a control group not receiving plasma exchange. However, since we studied vitamin D status over a very short period of time and noted rapid changes in vitamin D concentrations within subjects coincident with plasma exchange, controlling for factors such

as seasonality is unlikely to markedly affect our findings. There was some variation in the volume of plasma exchange and dose of calcium gluconate. Further, we did not follow patients beyond 4 weeks after plasma exchange.

The identification of a profound and sustained reduction in 25-hydroxyvitamin D and an acute but reversible reduction in 1,25-dihydroxyvitamin D reported here are of great clinical importance. The majority of patients receiving plasma exchange are also exposed to glucocorticoids and other immune modulating therapies that increase the risk of osteoporosis, fractures and infection. Vitamin D deficiency further increases these risks. Together with the mounting evidence that vitamin D deficiency predisposes to auto-immunity or to activity of auto-immune disease, our findings provide a strong rationale for both 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D supplementation during and after plasma exchange. However, the dose response in the setting of acute DBP depletion is not known. Studies to determine the optimal dosage regimen are urgently needed.

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*Conflict of interest:* None declared.

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