Palmer David (Orcid ID: 0000-0001-5208-681X)

(i) Title: Unbound Vitamin D Concentrations Are Not Decreased in Critically Ill Patients

Word Count: 2309 excluding abstract and references

(iii) Authors:

David Palmer[1], Steven Soule[1,2,3], Ravinder Reddy Gaddam[4], Peter Elder[4,5], Stephen Chambers[4], Matthew Doogue*[1,2,6]

Correspondence:

David Palmer

C/- Matthew Doogue

Department of Medicine

University of Otago, Christchurch

PO Box 4345, Christchurch 8140,

New Zealand.

Tel +64 27 251 8339

Email ddg.palmer@gmail.com

(iv) Affiliations:

[1]Department of General Medicine, Canterbury District Health Board, Christchurch, New Zealand; [2]Department of Medicine, University of Otago, Christchurch, New Zealand; [3]Department of Endocrinology Canterbury District Health Board, Christchurch, New Zealand; [4]Department of Pathology, University of Otago, Christchurch, New Zealand; [5]Canterbury Health Laboratories Christchurch, New Zealand; [6]Department of Clinical Pharmacology, Canterbury District Health Board, Christchurch, New Zealand.

Declaration of Interest, Funding, and Author Contribution

Conflicts of Interest:

The authors declare there are no conflicts of interest that could prejudice the impartiality of the research or be perceived to do so.

Funding:

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Ethical approval

The study was approved by the Health and Disability Ethics Committee, Wellington, New Zealand (protocol number 15/STH/36)

Guarantor

David Palmer

Contributorship:

Dr Palmer collected baseline data, performed data analysis, and prepared the manuscript. Dr Soule was involved in project planning, and reviewed and edited the manuscript. Dr Reddy Gaddam collected samples and baseline data. Dr Elder performed the laboratory analysis. Prof Chambers oversaw the original study where patient samples were collected. A/Prof Doogue was involved in project planning, manuscript writing, and editing.

Acknowledgments:

No acknowledgments.

(v) Keywords:

Vitamin D, Critical illness, Protein binding, Vitamin D-Binding Protein, Carrier Protein, Steroids, Tandem Mass Spectrometry

(i) Title: Unbound Vitamin D Concentrations Are Not Decreased in Critically Ill Patients

Word Count: 2309 excluding abstract and references

(iii) Authors:

David Palmer[1], Steven Soule[1,2,3], Ravinder Reddy Gaddam[4], Peter Elder[4,5], Stephen Chambers[4], Matthew Doogue*[1,2,6]

Correspondence:

David Palmer

C/- Matthew Doogue

Department of Medicine

University of Otago, Christchurch

PO Box 4345, Christchurch 8140,

New Zealand.

Tel +64 27 251 8339

Email ddg.palmer@gmail.com

(iv) Affiliations:

[1]Department of General Medicine, Canterbury District Health Board, Christchurch, New Zealand; [2]Department of Medicine, University of Otago, Christchurch, New Zealand; [3]Department of Endocrinology Canterbury District Health Board, Christchurch, New Zealand; [4]Department of Pathology, University of Otago, Christchurch, New Zealand; [5]Canterbury Health Laboratories Christchurch, New Zealand; [6]Department of Clinical Pharmacology, Canterbury District Health Board, Christchurch, New Zealand.

Declaration of Interest, Funding, and Author Contribution

Conflicts of Interest:

The authors declare there are no conflicts of interest that could prejudice the impartiality of the research or be perceived to do so.

Funding:

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Ethical approval

The study was approved by the Health and Disability Ethics Committee, Wellington, New Zealand (protocol number 15/STH/36)

Guarantor

David Palmer

Contributorship:

Dr Palmer collected baseline data, performed data analysis, and prepared the manuscript. Dr Soule was involved in project planning, and reviewed and edited the manuscript. Dr Reddy Gaddam collected samples and baseline data. Dr Elder performed the laboratory analysis. Prof Chambers oversaw the original study where patient samples were collected. A/Prof Doogue was involved in project planning, manuscript writing, and editing.

Acknowledgments:

No acknowledgments.

(v) Keywords:

Vitamin D, Critical illness, Protein binding, Vitamin D-Binding Protein, Carrier Protein, Steroids, Tandem Mass Spectrometry

Introduction

Vitamin D has pleiotropic effects beyond its classically appreciated roles in musculoskeletal function and calcium homeostasis (1). These effects, in particular its immune mediating effects, have led to recent interest in its potential role in critical illness. Vitamin D status is commonly assessed by the measurement of 25-hydroxyvitamin D (25(OH)D) concentrations in plasma.

25(OH)D concentrations have repeatedly been shown to be lower in critically ill patients (2,3), and low 25(OH)D concentrations have been associated with adverse outcomes and increased mortality in this patient group (4–8). Evidence for supplementation of vitamin D in critical illness is limited, but a recent meta-analysis of six randomised

controlled trials of vitamin D administration in critically ill patients suggested mortality is reduced by supplementation (9).

25(OH)D is highly protein bound in human plasma, with only about 0.1 percent of total 25(OH)D unbound. The majority (85-90%) is bound to Vitamin D binding protein (VDBP), and the remainder (10-15%) is bound to albumin. Biological activity of Vitamin D is thought to follow the 'free hormone hypothesis', such that the physiological equilibrium and consequent physiological effects are determined by the free (unbound) concentration (10). VDBP concentration, and hence total 25(OH)D concentration, is markedly affected by acute and chronic disease, and by physiological changes such as pregnancy. It has been proposed that measurement of free 25(OH)D may provide a more interpretable measure of vitamin D sufficiency across a range of physiological states (11).

Direct measurement of free 25(OH)D concentration by liquid chromatography-tandem mass spectrometry (LCMS/MS) is not readily available, and immunoassay methods have previously been shown to be unreliable (12). Recently, an ELISA-based direct assay for free 25(OH)D has been developed (13), however this has not been validated in critically ill patients. Similar problems with the direct measurement of free testosterone, another highly protein-bound steroid hormone, have been reported (14,15). The free concentration can be estimated by calculation of free 25(OH)D from measurements of plasma total 25(OH)D, VDBP, and albumin concentrations (16). Despite the availability of this technique, few studies have looked at calculated free 25(OH)D in critically ill patients.

VDBP, the main binding protein of 25(OH)D in plasma, is a negative acute phase reactant (17), as is albumin. Therefore, if free 25(OH)D concentration is unchanged, bound and total concentrations of 25(OH)D will be decreased in acute illness. It is not clear whether free 25(OH)D is decreased, increased or unchanged in critical illness.

In critical illness the free concentrations of other protein-bound hormones, cortisol and thyroxine, have been shown to be increased (18), and unchanged (19) respectively while total concentrations decrease. Similarly free concentrations of highly protein-bound drugs are unchanged in critical illness despite lower total concentrations (20,21).

We hypothesised that the decrease in total 25(OH)D concentrations observed in critical illness is due to decrease in binding proteins and that calculated free vitamin D concentrations are not decreased in this setting.

Materials and Methods

Patient population and study design

This study was one of a set of prospective observational studies on a group of patients with fluid-refractory hypotension. The study was approved by the Health and Disability Ethics Committee, Wellington, New Zealand (protocol number 15/STH/36) and conducted in the Intensive Care Unit at Christchurch Hospital, New Zealand. Where patients were able to provide informed consent, this was sought prior to enrolment. However in many cases patients were unable to provide informed consent due to their condition and sedation. Where patients were unable to give consent, provisional consent was obtained from family members or friends and consent was reviewed with

the patient when they recovered. Thirty eight intensive care patients were recruited to the study. Patients included were aged over 18, were not expected to die within 72 hours, and had fluid-refractory hypotension or hypoperfusion of any cause as described previously by Gaddam et al (22). Plasma samples were collected within 24 hours of admission to ICU for measurement of 25(OH)D and binding proteins. Samples from 68 healthy controls were obtained from already-collected plasma from a reference range study approved by the Canterbury Ethics Committee, Christchurch, New Zealand. Five patients in the critically ill group were taking supplemental vitamin D prior to admission (four cholecalciferol monthly, and one calcitriol daily), and none in the healthy controls.

Laboratory analysis

Plasma 25(OH)D was analysed by LC-MS/MS after hexane extraction with deuterated 25(OH)D as a control as previously described (23). Plasma albumin was analysed using a colorimetric method (bromocresol purple) on a commercial analyser (Abbot Architect c16000).

VDBP was analysed by the direct sandwich ELISA technique using two monoclonal antibodies 3D2 and C8, raised in-house. The antibodies were raised using the technique of Kohler & Milstein (24) and purified by passage through a protein A column under normal conditions. Purified 3D2 (10ul of 2mg/mL) in 10 mls PBS (phosphate buffer saline 0.05M NaH_2PO_4, 0.15M NaCl, adjusted to pH=7.4 with 5M NaOH) was coated on a tissue culture plate (Falcon 351172) at 100ul /well and left overnight at 4°C. After washing and blocking with assay buffer (PBS containing 0.1% Tween 20 v/v and 0.1% gelatin) standards and samples were added in duplicate and incubated for 1 hr.

Standards (Sigma G8764 1mg diluted in 1ml PBS and frozen in 100ul aliquots) were diluted to 500ng/mL and serially diluted to give eight standards ranging from 500ng/mL to zero. Patient samples were diluted 1/5000 and measured in duplicate.

A second antibody C8, coupled to streptavidin was added to the plate after washing x4 with assay buffer. Incubation for 30 minutes was followed by washing and incubation with Streptavidin HRP for a further 30 minutes before the plate was washed again. Colour development with tetramethyldiamine was terminated by the addition of 1M HCl (100ul) and the plate was read at 450nm on a BMG FLUOstar Galaxy (BMG Technologies GmbH, Germany).

Estimated free 25(OH)D concentrations were calculated using the equation adapted by Powe et al from that developed by Vermeulen et al for the calculation of free testosterone concentration (16,25).

$$Free25(OH)D = \frac{total\ 25(OH)D}{1 + (6 \times 10^3 \times albumin) + (7 \times 10^8 \times DBP)}$$

Plasma calcium, corrected calcium (Calcium+0.012x(39.9-Albumin)), phosphate, and creatinine concentrations were obtained from laboratory results for the critically ill population within the eight hours prior to collection of the study blood sample. For the heathy controls, ionised calcium was recorded.

Statistical analysis

Normal distribution of data was tested with the Shapiro-Wilk test. Normally distributed data are presented as mean with standard deviation. Plasma calcium, corrected calcium, and creatinine were not normally distributed, and are presented as median with 2.5^{th} and 97.5^{th} percentiles. Total and calculated free 25(OH)D concentrations are presented as scatter plots with mean and 95% confidence intervals. Normally distributed data were compared using the unpaired t-test. The relationship between VDBP concentration and total 25(OH)D concentration is presented as a scatter plot with linear regression and calculation of Spearman's rho.

Results

Baseline characteristics

Baseline data for the 38 patients in the critical illness group and the 68 healthy controls are shown in table 1. The mean age in the critical illness group was 65 years, SD 13. 58% of the critically ill group were male, compared to 32% of the healthy controls. Median calcium corrected for albumin, and mean phosphate concentrations were both within their respective reference ranges, as was mean plasma ionised calcium in the healthy controls. As expected, VDBP concentrations were substantially lower in the critically ill group (Figure 1)

Vitamin D concentrations

Figure 2 shows the total and calculated free plasma vitamin D concentrations of the studied groups. The mean total plasma 25(OH)D was 56 nmol/L (95% CI 53–60) in the controls, and 37 nmol/L (95% CI 31–43) in the critically ill group, a difference of 19 nmol/L (95% CI for difference 13 – 26). Mean free 25(OH)D was 19 pmol/L (95% CI 18 – 20)

in the healthy controls, and 26 pmol/L (95% CI 22 – 29) in the critically ill group. The difference between means was 7 pmol/L (95% CI for difference 3 – 10).

Relationship between vitamin D binding protein and total 25-hydroxyvitamin D

Figure 3 shows the relationship between VDBP concentration and total 25(OH)D concentration. Total 25(OH)D concentration in this concentration range increases linearly with increasing VDBP concentration (Spearman's rho 0.44 (p=0.0051)).

Discussion

Total 25(OH)D plasma concentrations were lower in the critically ill patient group than the healthy controls. In contrast, calculated free 25(OH)D concentrations were not lower in the critically ill patient group than the healthy controls. This is similar to findings of other hormones in critical illness (18,19). This suggests that observational studies of decreased concentration of total 25(OH)D in critical illness may be due to decreases in VDBP and albumin concentrations rather than a decrease in free 25(OH)D.

Decreases in VDBP and albumin are predictably seen in acute illness (17), hence this result is not unexpected. This is similar to cortisol and thyroxine, the total concentrations of which fall in acute illness while free concentrations are preserved (18,19). The result is also consistent with the finding recently reported by Wang et al showing that patients with primary hyperparathyroidism have preserved free 25(OH)D concentrations despite lower total 25(OH)D concentrations caused by low VDBP concentration (26).

Our finding that calculated free 25(OH)D concentrations are not less than the general population during critical illness weakens the hypothesis that a large proportion of critically ill patients are vitamin D deficient, as suggested by previous findings of low total 25(OH)D concentrations in critical illness (2,3). This is consistent with other studies showing that vitamin D physiology is consistent with the free hormone hypothesis (27).

Other studies have also suggested that free 25(OH)D concentrations may have advantages over total concentrations as a biomarker of vitamin D sufficiency. Powe et al showed that free 25(OH)D and bioavailable 25(OH)D better correlate with bone mineral density than total 25(OH)D (16). Yu et al showed that both free 25(OH)D and bioavailable 25(OH)D are predictors of all cause mortality in people with cardiovascular disease, but total 25(OH)D is not (28). Most recently, Chen et al showed that free 25(OH)D was higher in people on dialysis who didn't develop cardiovascular events than in those who did (29) Of note, this study was not designed or powered to examine free 25(OH)D in critical illness, rather it was to examine the limitations of measuring total 25(OH)D in this population.

This study has several limitations. Firstly this is a small study examining the potential (theoretical) effect of one variable (vitamin D binding protein) on vitamin D status; the study does not examine outcomes. Secondly, it is cross-sectional, and longitudinal studies are needed for within-patient comparisons. Thirdly, haemodilution due to fluid resuscitation will have decreased the concentration of some analytes. The calculated free concentration excludes the effect of haemodilution which may account for the apparently higher mean free concentration seen in this study (figure 2). Further, there are other complexities to consider, for example VDBP has polymorphisms which affect its affinity for 25(OH)D

Of note, ELISA-based quantification of VDBP has been unreliable in certain settings due to variable antibody recognition of VDBP phenotypes, as well as assay interference by actin, which is a greater problem in the setting of tissue injury. The ELISA of VDBP used in this study has been shown to be reliable in different VDBP phenotypes as well as with differences in concentration of actin (30).

In figure 2, four outliers are notable in the critically ill group: three with conspicuously high total 25(OH)D and mid-range VDBP, and one with conspicuously high VDBP with low 25(OH)D. All three subjects with very high total 25(OH)D were taking exogenous cholecalciferol supplements, which is known to increase the concentration of 25(OH)D (31). We are unable to account for the patient with very high VDBP and low total 25(OH)D; possible causes include interference in the assay, laboratory error, clerical (transcription) error, or a particularly low affinity VDBP variant.

Conclusion

Calculated free 25(OH)D concentrations are not lower in critical illness than in healthy controls. In patients with critical illness total 25(OH)D concentration may under-represent vitamin D status. The decrease in VDBP concentration seen in acute illness is a potential confounding factor in studies examining the relationship between vitamin D concentrations and health outcomes.

References

1. Caprio M, Infante M, Calanchini M, Mammi C, Fabbri A. Vitamin D: not just the bone. Evidence for beneficial pleiotropic extraskeletal effects. Eating and Weight Disorders. 2017.

2. Lee P, Eisman JA, Center JR. Vitamin D Deficiency in Critically Ill Patients. N Engl J Med [Internet]. 2009 Apr 30 [cited 2017 Oct 16];360(18):1912–4. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMc0809996

3. Lucidarme O, Messai E, Mazzoni T, Arcade M, Du Cheyron D. Incidence and risk factors of vitamin D deficiency in critically ill patients: results from a prospective observational study. Intensive Care Med. 2010;36:1609–11.

4. Brook K, Camargo CA, Christopher KB, Quraishi SA. Admission vitamin D status is associated with discharge destination in critically ill surgical patients. Ann Intensive Care [Internet]. 2015 Dec 17 [cited 2017 Oct 16];5(1):23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26380991

5. Ala-Kokko TI, Mutt SJ, Nisula S, Koskenkari J, Liisanantti J, Ohtonen P, et al. Vitamin D deficiency at admission is not associated with 90-day mortality in patients with severe sepsis or septic shock: Observational FINNAKI cohort study. Ann Med [Internet]. 2016 Jan 8 [cited 2017 Oct 16];48(1–2):67–75. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26800186

6. Quraishi SA, McCarthy C, Blum L, Cobb JP, Camargo CA. Plasma 25-Hydroxyvitamin D Levels at Initiation of Care and Duration of Mechanical Ventilation in Critically Ill Surgical Patients. J Parenter Enter Nutr [Internet]. 2016 Feb 6 [cited 2017 Oct 16];40(2):273–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25564428

7. Moraes R, Friedman G, Wawrzeniak I, Marques L, Nagel F, Lisboa T, et al. Vitamin D deficiency is independently associated with mortality among critically ill patients. Clinics [Internet]. 2015 May 31 [cited 2017 Oct 16];70(5):326–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26039948

8. Zhang Y-P, Wan Y-D, Sun T-W, Kan Q-C, Wang L-X. Association between vitamin D deficiency and mortality in critically ill adult patients: a meta-analysis of cohort studies. Crit Care [Internet]. 2014 Dec 12 [cited 2017 Oct 16];18(6):684. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25498305

9. Putzu A, Belletti A, Cassina T, Clivio S, Monti G, Zangrillo A, et al. Vitamin D and outcomes in adult critically ill patients. A systematic review and meta-analysis of randomized trials. J Crit Care [Internet]. 2017 Apr [cited 2017 Oct 16];38:109–14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27883968

10. Chun RF, Peercy BE, Orwoll ES, Nielson CM, Adams JS, Hewison M. Vitamin D and DBP: The free hormone hypothesis revisited. Journal of Steroid Biochemistry and Molecular Biology. 2014.

11. Tsuprykov O, Chen X, Hocher CF, Skoblo R, Lianghong Yin, Hocher B. Why should we measure free 25(OH) vitamin D? J Steroid Biochem Mol Biol [Internet]. 2018;180(December 2017):87–104. Available from: https://doi.org/10.1016/j.jsbmb.2017.11.014

12. Rousseau AF, Damas P, Janssens M, Kalin S, Ledoux D, Le Goff C, et al. Critical care and vitamin D status assessment: What about immunoassays and calculated free 25OH-D? Clin Chim Acta. 2014;

13. Heureux N, Lindhout E, Swinkels L. A direct assay for measuring free 25-hydroxyvitamin D. Vol. 100, Journal of AOAC International. AOAC International; 2017. p. 1318–22.

14. Miller KK, Rosner W, Lee H, Hier J, Sesmilo G, Schoenfeld D, et al. Measurement of Free Testosterone in Normal Women and Women with Androgen Deficiency: Comparison of Methods. J Clin Endocrinol Metab [Internet]. 2004 Feb 1 [cited 2017 Oct 31];89(2):525–33. Available from:

https://academic.oup.com/jcem/article-lookup/doi/10.1210/jc.2003-030680

15. Rosner W, Auchus RJ, Azziz R, Sluss PM, Raff H. Utility, Limitations, and Pitfalls in Measuring Testosterone: An Endocrine Society Position Statement. J Clin Endocrinol Metab [Internet]. 2007 Feb 1 [cited 2017 Oct 31];92(2):405–13. Available from: https://academic.oup.com/jcem/article-lookup/doi/10.1210/jc.2006-1864

16. Powe CE, Ricciardi C, Berg AH, Erdenesanaa D, Collerone G, Ankers E, et al. Vitamin D-binding protein modifies the vitamin D-bone mineral density relationship. J Bone Miner Res [Internet]. 2011 Jul [cited 2017 Dec 1];26(7):1609–16. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21416506

17. Waldron JL, Ashby HL, Cornes MP, Bechervaise J, Razavi C, Thomas OL, et al. Vitamin D: a negative acute phase reactant. J Clin Pathol [Internet]. 2013 Jul [cited 2017 Oct 16];66(7):620–2. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23454726

18. Hamrahian AH, Oseni TS, Arafah BM. Measurements of Serum Free Cortisol in Critically Ill Patients. N Engl J Med [Internet]. 2004 Apr 15 [cited 2017 Oct 16];350(16):1629–38. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMoa020266

19. Surks MI, Hupart KH, Pan C, Shapiro LE. Normal Free Thyroxine in Critical Nonthyroidal Illnesses Measured by Ultrafiltration of Undiluted Serum and Equilibrium Dialysis*. J Clin Endocrinol Metab [Internet]. 1988 Nov 1 [cited 2017 Oct 27];67(5):1031–9. Available from: https://academic.oup.com/jcem/article-lookup/doi/10.1210/jcem-67-5-1031

20. Rowland, M, Tozer T. Clinical Pharmacokinetics and Pharmacodynamics: Concepts and Applications: Fourth edition. 2010. 96–100 p.

21. Benet, L; Hoener B. Changes in plasma protein binding have little clinical relevance. Clin Pharmacol Ther.

2002;71(3):115–21.

22. Gaddam RR, Chambers S, Murdoch D, Shaw G, Bhatia M. Circulating levels of hydrogen sulfide and substance P in patients with sepsis. J Infect [Internet]. 2017 [cited 2017 Oct 9];75:293–300. Available from: http://dx.doi.org/10.1016/j.jinf.2017.07.005

23. Lankes U, Elder PA, Lewis JG, George P. Differential extraction of endogenous and exogenous 25-OH-vitamin D from serum makes the accurate quantification in liquid chromatography-tandem mass spectrometry assays challenging. Ann Clin Biochem [Internet]. 2015 Jan [cited 2017 Dec 21];52(1):151–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24714680

24. Köhler G, Milstein C. Derivation of specific antibody-producing tissue culture and tumor lines by cell fusion. Eur J Immunol [Internet]. 1976 Jul [cited 2017 Nov 15];6(7):511–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/825377

25. Vermeulen A, Verdonck L, Kaufman JM. A Critical Evaluation of Simple Methods for the Estimation of Free Testosterone in Serum. J Clin Endocrinol Metab [Internet]. 1999 Oct 1 [cited 2017 Dec 1];84(10):3666–72. Available from: https://academic.oup.com/jcem/article-lookup/doi/10.1210/jcem.84.10.6079

26. Wang X, Shapses SA, Al-Hraishawi H. Free and Bioavailable 25-Hydroxyvitamin D Levels in Patients With Primary Hyperparathyroidism. Endocr Pract [Internet]. 2017;23(1):66–71. Available from: http://journals.aace.com/doi/10.4158/EP161434.OR

27. Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, et al. Vitamin D–Binding Protein and Vitamin D Status of Black Americans and White Americans. N Engl J Med [Internet]. 2013 Nov 21 [cited 2017 Oct 16];369(21):1991–2000. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24256378

28. Yu C, Xue H, Wang L, Chen Q, Chen X, Zhang Y, et al. Serum bioavailable and free 25-hydroxyvitamin d

levels, but not its total level, are associated with the risk of mortality in patients with coronary artery disease. Circ Res. 2018;123(8):996–1007.

29. Chen X, Lu YP, Luo T, Wu HW, Cai SF, Tian M, et al. Free 25-Vitamin D is correlated with cardiovascular events in prevalent hemodialysis patients but not with markers of renal mineral bone disease. Kidney Blood Press Res. 2019;44(3):344–53.

30. Katrina Hong, Christopher M. Florkowski, Matthew P. Doogue, Peter A. Elder JGL. A monoclonal antibody sandwich ELISA for vitamin D-binding protein (VDBP) is unaffected by Gc-globulin phenotype peptides and actin and demonstrates reduced levels in sepsis and non-sepsis intensive care patients. Clin Chim Acta. 2018;484(September 2018):7–13.

31. Shieh A, Chun RF, Ma C, Witzel S, Meyer B, Rafison B, et al. Effects of High-Dose Vitamin D2 Versus D3 on Total and Free 25-Hydroxyvitamin D and Markers of Calcium Balance. J Clin Endocrinol Metab [Internet]. 2016 Aug [cited 2017 Dec 21];101(8):3070–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27192696

Tables

Table 1. Baseline characteristics

	Critical illness (n=38)	**Healthy control** (n=68)
Age (mean ± SD)	65 ± 13	52 ± 12
Male (n (%))	22 (58%)	22 (32%)
Female (n (%))	16 (42%)	47 (68%)
APACHE III score (mean ± SD)	77 (24)	-
Plasma calcium (mean ± SD) (mmol/L)	2.0 (1.7, 2.4)	Unavailable
Plasma calcium corrected for albumin* (Median (2.5^{th} percentile, 97.5^{th} percentile)) (mmol/L)	2.2 (2.0, 2.5)	Unavailable
Plasma ionised calcium (Median (2.5^{th} percentile, 97.5^{th} percentile)) (mmol/L)	Unavailable	1.2 (1.13, 1.27)
Plasma phosphate (mean ± SD) (mmol/L)	1.4 ± 0.5	Unavailable
Plasma creatinine (Median (2.5^{th} percentile, 97.5^{th} percentile)) (mmol/L)	115 (65, 463)	Unavailable

*Corrected calcium = calcium + 0.012 x (39.9 – albumin)

Figure legends.

Figure 1. Scatter plot with mean and 95% confidence interval for VDBP concentrations in critically ill patients and healthy controls.

Figure 2. Scatter plots with mean and 95% confidence intervals of (a) total and (b) calculated free 25(OH)D concentrations in critically ill patients and healthy controls.

Figure 3. Spearman correlation scatter plot of total 25(OH)D concentration versus VDBP concentration in all subjects. Critical illness patients are shown as closed circles and healthy controls as open circles.

Figures

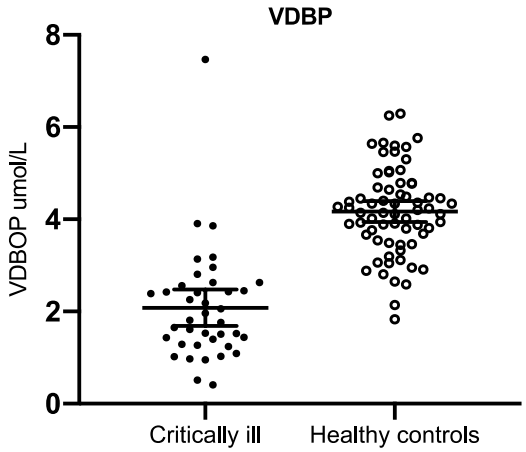

Figure 1. Scatter plot with mean and 95% confidence interval for VDBP concentrations in critically ill patients and healthy controls.

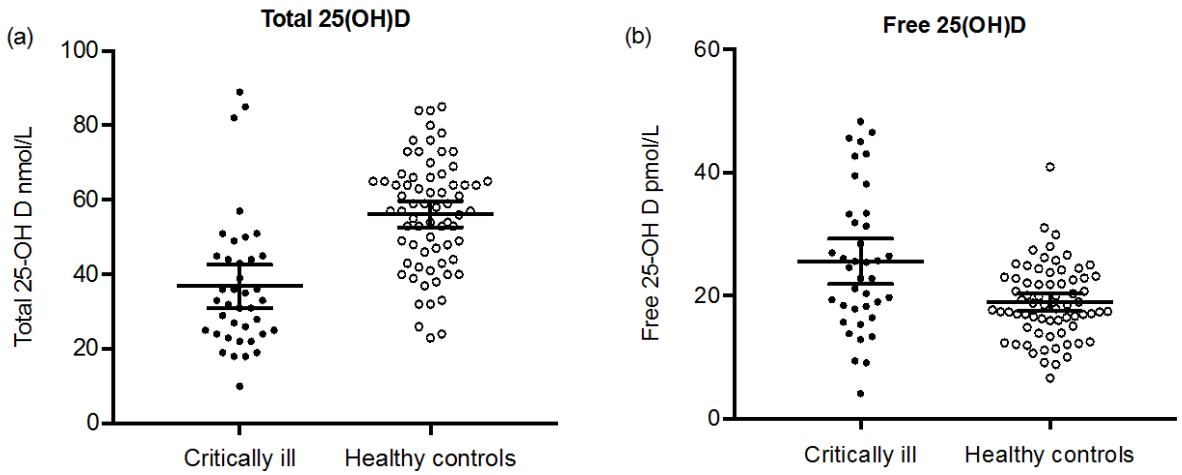

Figure 2. Scatter plots with mean and 95% confidence intervals of (a) total and (b) calculated free 25(OH)D concentrations in critically ill patients and healthy controls.

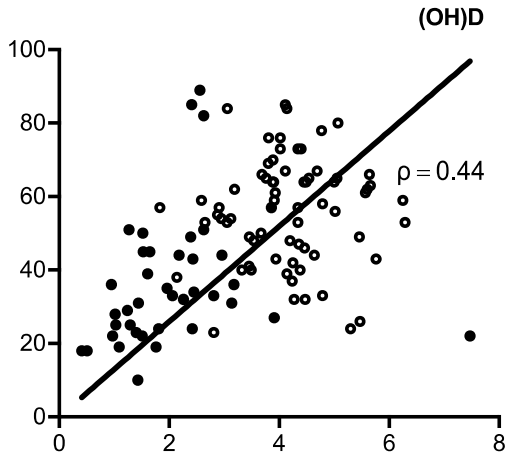

Figure 3. Spearman correlation scatter plot of total 25(OH)D concentration versus VDBP concentration in all subjects. Critical illness patients are shown as closed circles and healthy controls as open circles.

Objective Free concentrations of highly protein bound hormones, such as cortisol and thyroxine, are unchanged in critical illness despite substantial decreases in total concentration. Total 25-hydroxyvitamin D (25(OH)D) concentration is decreased in critical illness, but the free concentration of 25(OH)D has had less attention. The aim of this study was to compare total and calculated free 25(OH)D concentrations in critically ill patients with healthy controls.

Design Case control study.

Methods 38 patients with critical illness were compared with 68 healthy controls. 25(OH)D was measured by liquid chromatography tandem mass spectrometry (LCMS/MS) and vitamin D binding protein (VDBP) by direct sandwich enzyme-linked immunosorbent assay (ELISA). Total and calculated free 25(OH)D concentrations were compared using unpaired T-tests.

Results Total 25(OH)D concentrations were significantly lower in critically ill patients than controls (37 (95% CI 31 – 43) vs 57 (53 – 60) nmol/L). Calculated free concentrations of 25(OH)D were not lower in critically ill patients than healthy controls (26 (22 – 29) vs 19 (18 – 20) pmol/L).

Conclusions Calculated free 25(OH)D concentrations are not decreased in critical illness. Measuring total 25(OH)D concentrations in patients with critical illness potentially underestimates vitamin D and overestimates the number of patients who are deficient in vitamin D.