Accepted Manuscript published as EC-18-0184.R2. Accepted for publication: 05-Oct-2018

ENDOCRINE CONNECTIONS

VITAMIN D AND CRITICAL ILLNESS – what endocrinology can learn from intensive care and vice versa

Amrein K. [1,2], Papinutti A.[3], Mathew E. [3], Vila G. [4], Parekh D.[5]

1 Thyroid Endocrinology Osteoporosis Institute Dobnig, Graz, Austria
2 Department of Internal Medicine, Division of Endocrinology and Diabetology, Medical University of Graz, Graz, Austria
3 Department of General Surgery, Medical University of Graz, Graz, Austria
4 Department of Internal Medicine III, Division of Endocrinology and Metabolism, Medical University of Vienna, Vienna, Austria
5 Clinician Scientist in Critical care, Institute of Inflammation and Ageing, University of Birmingham, UK

Funding: None

Disclosures: KA received speaker fees and unrestricted research grants from Fresenius Kabi

Word count: 3511

Abstract 224

ABSTRACT

The prevalence of vitamin D deficiency in intensive care units ranges typically between 40 and 70 %. There are many reasons for being or becoming deficient in the ICU. Hepatic, parathyroid and renal dysfunction additionally increase the risk for developing vitamin D deficiency. Moreover, therapeutic interventions like fluid resuscitation, dialysis, surgery, extracorporeal membrane oxygenation,

cardiopulmonary bypass and plasma exchange may significantly reduce vitamin D levels.

Many observational studies have consistently shown an association between low vitamin D levels and poor clinical outcomes in critically ill adults and children, including excess mortality and morbidity such as acute kidney injury, acute respiratory failure, duration of mechanical ventilation and sepsis. It is biologically plausible that vitamin D deficiency is an important and modifiable contributor to poor prognosis during and after critical illness. Although vitamin D supplementation is inexpensive, simple and has an excellent safety profile, testing for and treating vitamin D deficiency is currently not routinely performed. Overall, less than 800 patients have been included in RCTs worldwide, but the available data suggest that high-dose vitamin D supplementation could be beneficial. Two large RCTs in Europe and the US, together aiming to recruit > 5000 patients, have started in 2017, and will greatly improve our knowledge in this field.

This review aims to summarize current knowledge in this interdisciplinary topic and give an outlook on its highly dynamic future.

Accepted Manuscript published as EC-18-0184.R2. Accepted for publication: 05-Oct-2018

A short history of vitamin D in critical care

Only 10 years ago, a potential link between acute illness and vitamin D, which is well known for its role in calcium and bone homeostasis, was regarded as quite absurd – how could this hormone be acutely relevant to the specialty of critical care? In fact, it now transpires that the high prevalence of vitamin deficiency in critically ill adults and children, combined with the pleiotropic effects of vitamin D, could indeed be of great importance in this patient population.

The first relevant randomized controlled trial was published in 2003 by the Belgian endocrinology-anesthesiology visionary Greet van den Berge and her team. In this trial, a “low” dose of 200 IU of vitamin D3 compared with a “high” dose of 500 IU over 10 days in 22 prolonged critically ill patients showed limited effects on inflammatory biomarkers (1). Although, in retrospect, the “high-dose” of vitamin D was quite low, this trial was ahead of its time and led the way revealing important findings of severe bone hyper-resorption and presence of vitamin D deficiency in the critically ill.

A few years of silence in the scientific community followed, but the topic rapidly regained attention after the publication of two studies in 2009: the report of high rates of vitamin D deficiency including some with undetectable levels among 42 Australian critically ill patients referred to the endocrinology department in a letter in the New England Journal of Medicine (2) and 100 children requiring ICU admission for respiratory infections by Canadian researchers (3). This was to be the beginning of subsequent years of research and debate with skeptics arguing that deficiency is purely a bystander and marker of illness severity. Despite this, the current evidence for replacement therapy is compelling, but there remain unanswered questions, including adequate dosing strategies, the effect of critical illness on vitamin D metabolomics and the optimum target vitamin D level to provide clinical benefit in critical illness.

Vitamin D status in critically ill patients

Vitamin D deficiency is common in critical illness with prevalence between 40-70% (4-7), Error! Reference source not found.. In burn patients, the prevalence appears to be even higher (8, 9). Many patients enter the ICU in a deficient state due to pre-existing malnutrition and disease. However, vitamin D metabolism is dysregulated in some critically ill patients with vitamin D levels rapidly falling after ICU admission (10, 11). The similarity between results in diverse geographical areas with variable UVB exposure suggests that the influence of individual chronic and/or acute disease on vitamin D deficiency is largely independent of sun exposure (12). A number of large observational studies from across the globe have confirmed that vitamin D deficiency (usually defined as 25(OH)D levels below 20ng/ml) is frequent in adult and pediatric critical illness (5, 6, 13-16). Vitamin D deficiency has been shown to be associated with sepsis, acute respiratory distress syndrome and acute kidney injury (17-20) and three different meta-analyses confirm that patients with low vitamin D status have a longer ICU stay and increased morbidity and mortality (18, 21, 22). Recently, substantial metabolomic differences in pathways related to glutathione metabolism and glutamate metabolism were found in an observational study in vitamin D deficient compared to non-deficient ICU patients (separated by a cutoff of 15 ng/ml) (23).

In critical illness there also is evidence of rapid falls in circulating 25(OH)D concentrations, potentially due to disrupted metabolism, fluid resuscitation, decreased synthesis of vitamin D binding protein due to hepatic dysfunction, interstitial extravasation caused by increased vascular permeability, renal wasting of vitamin D, decreased renal conversion to 1,25(OH)D3 and increased tissue conversion of 25(OH)D3 to 1,25(OH)D3 (11, 24-26). The role of free/bioavailable vitamin D remains unclear although it is possible that although vitamin D binding protein (VDBP) and thus total D decreases, circulating free D may be maintained (27). In a posthoc analysis of the VITDAL-ICU trial, free/bioavailable vitamin D was not superior to total 25(OH)D in predicting mortality neither in the placebo nor in the intervention group (28). There is also evidence that critically ill patients with very low 25(OH)D concentrations have blunted responses to vitamin D replacement possibly due to conversion into alternate metabolites and epiforms (29).

 Accepted Manuscript published as EC-18-0184.R2. Accepted for publication: 05-Oct-2018

Biological rationale

There is strong biological plausibility that supports a contributing role of vitamin D deficiency to poor outcomes, mediated by genomic and non-genomic effects (8). In the last decade, vitamin D has been implicated in the function of a wide range of tissues including the innate and adaptive immune system (30, 31). The specific nuclear vitamin D receptor (VDR) is widely expressed in many cell types and organs relevant to critically illness (32), and is known to regulate hundreds of genes (32, 33). Therefore, vitamin D has the ability to act synergistically on the immune response to acute systemic inflammation and infection (19, 34), lung epithelial function (35), muscle function and metabolism (36) and cardiac function (37), to name a few (Error! Reference source not found.). Additional information on exact mechanism of action and potential influence of vitamin D deficiency on acute critical illness is summarized in **Table 2**.

Vitamin D, rather than a vitamin or just a food supplement, is therefore in reality, a precursor to a potent steroid hormone influencing a wide range of cellular pathways in organs that are highly relevant to the effects of critical illness and may exert its beneficial effects on acute inflammation, nosocomial infection, respiratory failure, cardiogenic shock and critical illness myopathy. In summary, vitamin D may help to prevent secondary complications in a population at very high risk and there is currently no rationale to suggest that, apart from vitamin D deficiency, any particular type of ICU patients could benefit more or less. However, burn patients appear to be at particular and even long-term risk because of the necessary sun avoidance after their injury (8, 9).

Bone during and after critical illness

Recently, bone health has been recognized as important for ICU survivors and the limited available data suggest impaired bone health and high fracture risk (38-41). In addition to underlying disease, critical illness per se seems to be detrimental to musculoskeletal health in various ways: immobilization, inflammation, multiple endocrine alterations, hypercatabolism including muscle wasting, malnutrition and

some drugs all have the potential to disturb the delicate balance between bone formation and resorption (42, 43). In a post hoc analysis of the VITdAL-ICU study, vitamin D3 did not have a significant effect on the increased levels of ß-Crosslaps and osteocalcin during critical illness (44). Nevertheless, vitamin D is one of the cornerstones of osteoporosis therapy. Treatment of vitamin D deficiency with the aim to reach levels considered necessary for optimal bone health in other populations (above 20ng/ml) (45, 46) may possibly be the only easily adoptable treatment to improve skeletal consequences of prolonged critical illness besides other, more expensive, risky and/or time-consuming possibilities like antiresorptive treatment and physiotherapy. Hollander and Mechanick suggested the consideration of intravenous bisphosphonates which potently reduce bone resorption (47). However, a number of contraindications and potential side effects like hypocalcemia, renal impairment and atrial fibrillation need to be considered. In order to avoid frank hypocalcemia, vitamin D deficiency should always be treated before bisphosphonates are given. Interestingly, in a large retrospective analysis, patients pretreated with bisphosphonates had significantly better outcomes even though they were older; additional vitamin D seemed to have an additional beneficial effect (48). In summary, ICU survivors appear to be at high risk for excessive bone loss and fracture risk. Therefore, interventional studies with vitamin D and antiresorptive agents including denosumab and parenteral bisphosphonates are necessary in the near future.

Effects of enterally administered Vitamin D supplementation

Van den Berghe et al (1) tried to demonstrate that in critically ill patients an intravenous supplementation with 200 (low dose group) compared to 500 (high dose group) IU cholecalciferol results in elevated to normal vitamin D levels. Although higher levels of 25(OH)D were detected on days 2, 6 and 7 in the high-dose group compared to the low-dose group, they did not reach normal 25(OH)D levels..

Years later, Amrein et al (49) initiated a randomized controlled pilot study with an ultra-high loading dose vitamin D (540.000 IU) in ICU patients. In this trial, 25 patients were randomly assigned to vitamin D3 versus placebo. The results showed significantly elevated 1,25(OH)D levels in the intervention group and in 80%, normalized 25(OH)D levels were found. In consequence of these results Amrein et al (50) initiated the VITdAL-ICU trial, in which 475 ICU patients with vitamin D

Accepted Manuscript published as EC-18-0184.R2. Accepted for publication: 05-Oct-2018

deficiency (<20ng/mL) were randomly assigned to either high dose vitamin D3 or placebo. The regimen of the high dose group consisted of a single high dose supplementation with 540,000 IU followed by a 90,000 IU monthly maintenance dose for five months. The 25(OH)D level in the high-dose group reached sufficiency (> 30 ng/mL) in 52.2 % of the patients after seven days.

Quraishi et al (51) compared changes of 25(OH)D and cathelicidin levels in septic ICU patients. 30 patients were randomly divided into three groups (each group consisting of 10 patients). The first group received 200,000 IU cholecalciferol enterally, the second 400,000 IU enterally and the third a placebo. Blood was drawn on days 1, 3, 5 and 7. Compared to baseline, the mean change in total 25(OH)D in the placebo group on day 5 was 3 (-3 to 8)%, the 200,000 IU cholecalciferol group 49 (30-82)%, and the 400,000 IU group in 69 (55-106)% (P <0.001). The bioavailable 25(OH)D increased by 4 (-8-7)%, 45 (40-70)% and 96 (58-136)% (P <0.01).
Han et al (52) administered cholecalciferol compared with placebo in a double-blind, randomized controlled pilot study in 30 patients. Nine mechanically ventilated ICU patients received 50,000 IU cholecalciferol on five days, 11 patients received 100,000 IU daily and 10 patients were given a placebo. At baseline, 13 patients (43%) had vitamin D deficiency (25(OH)D <20 ng/mL). The 50,000 IU and 100,000 IU regimens resulted in a significant increase in the average 25(OH)D plasma levels. On day 7, the values were 45.7 ± 19.6 ng/mL and 55.2 ± 14.4 ng/mL, respectively, compared to unchanged values in the placebo group (21 ± 11.2 ng / mL, P <0.001).

Current vitamin D testing and supplementation in the ICU

The most common laboratory test to assess vitamin D nutritional status is total 25-hydroxyvitamin D serum concentration. There are a number of methods for measuring 25-hydroxyvitamin D in serum or plasma, including enzyme immunoassay, radioimmunoassay, high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC/MS), and LC/MS/MS. Laboratory professionals are often confronted with challenges related to vitamin D testing, including controversy over optimal and target vitamin D concentrations, variable reference ranges across marketed assays and reference laboratories, lack of standardization of vitamin D assays, and misordering of 1,25-dihydroxyvitamin D testing. Among possible markers, serum total 25(OH)D is currently considered to be

the best marker of vitamin D status (53). Measurement of vitamin D concentration is currently not routine practice on ICU and there is currently no consensus on definition on vitamin D deficiency, in critical illness. The role of other metabolites including free/bioavailable vitamin D remains to be clarified. Generally, progress has been made in the last years in the harmonization of various assays. However, further standardization (e.g. the definition of vitamin D deficiency and measurement of other possible markers of vitamin D status) would be sensible (54).

In the general population, it is recommended that all healthy children and adults meet a daily minimum requirement of vitamin D - the Institute of Medicine (IOM) recommends 400 to 800IU of vitamin D3 (46). The Endocrine Society increased this dose to 1500 to 2000 IU/day for individuals at risk of deficiency (45, 55). Current standard enteral nutrition formulas used in critical illness contain vitamin D2 or D3 (native vitamin D, half-life 2-3 weeks), but rarely more than 400 IU in a daily regime. Parenteral multivitamin preparations typically contain only 200 or 220 IU of native vitamin D. In healthy individuals, such doses can improve vitamin D deficiency, but this requires months of treatment. In critical illness, the optimal native vitamin D dose remains unclear. Although no standard of care has been established, it appears logical that at least the recommended daily allowances for healthy individuals should be provided (400-600IU daily for children, 600-800IU for adults). The role for additional provision of active vitamin D (calcitriol or other metabolites) is even less clear, but certainly needs to be further tested. Active and native vitamin D metabolites are very different in half-life (several hours compared to a few weeks), therapeutic range (narrow vs. broad) and costs (more expensive vs. inexpensive) (56).There is however a biological rationale that active vitamin D *on top* of high-dose vitamin D3 could be of additional benefit. Besides patients with chronic preexisting renal dysfunction, many other ICU patients appear to be unable to sufficiently activate native vitamin D to its physiologically active form calcitriol (50). To date, no trial has looked at a combined vitamin D regime.

Circulating 25(OH)D concentrations may fall rapidly during the initial phase of severe acute illness and its treatment. Therefore, the use of a loading mega-dose for rapid restoration of vitamin D levels followed by regular supplementation appears necessary in critical illness (57). Apart from intramuscular high-dose vitamin D

Accepted Manuscript published as EC-18-0184.R2. Accepted for publication: 05-Oct-2018

formulations, no intravenous vitamin D mono-preparations are available at present.

Side effects in critically ill patients

Possible side effects after high dose supplementation include higher risk for fractures, falls and mild hypercalcaemia. Symptoms are mostly related to the effects of hypercalcemia. Vitamin D intoxication can be caused by high intake (>50 000 IU per day) and is typically linked to hypercalcemia and hyperphosphatemia. However, the intake of 10,000 IU vitamin D3 per day for up to 5 months is considered safe (58).

In ICU patients, side effects are rare and no vitamin D intoxication has been reported. However, due to the complexity of the treatment and the underlying disease, recognition of adverse events in a critically ill population is difficult. Several studies in ICU patients using mainly oral cholecalciferol in doses ranging from 200 IU to 540,000 IU, reported very limited side effects (1, 50, 51, 59, 60). In the VITDAL-ICU study, Amrein et al (50) found mild hypercalcaemia in 1% of patients, all of which were asymptomatic. In this trial, overall no significant differences in calcium, phosphorus and renal parameters in either group were found. Vitamin D levels in the treatment group were well below the level considered acutely toxic (150 ng/ml) (2). While outside of the ICU mega-doses are now obsolete because of increased fracture and fall risk (61), available evidence in critical illness from the VITdAL-ICU trial do not suggest increased risk for falls or fractures in these specific circumstances (50). Vitamin D toxicity has not been described in the ICU setting but may occur after prolonged intake of excessive doses (>10,000IU/day and 25(OH)D levels >200ng/ml) and, rarely, in individuals with mutations in CYP24A1 causing failure to metabolize 1,25-dihydroxyvitamin D (62, 63). Additional information on available vitamin D formulations is given in **Table 3.**

What endocrinology can learn from intensive care and vice versa

Sample size and power of a study

So far, many single vitamin D intervention trials have given disappointing results and many more, even relatively large trials including the recently completed VIDA and awaited VITAL trial are/will likely be negative (64). A great issue in these studies is

that despite their relatively large size including thousands of individuals, they still are underpowered. Even more problematic, they have not exclusively included vitamin D deficient subjects. It is not reasonable why patients with normal vitamin D levels are included in intervention trials; moreover, vitamin D should ideally not be given in the placebo group (65).

Recently, this concept was beautifully discussed in a German epidemiologic study showing that depending on the baseline risk of a population, the necessary sample size for a single trial to have adequate power increases sharply in low baseline risk (66). Therefore, the high prevalence of vitamin D deficiency and the inherently high morbidity and mortality in intensive care in a short time period increase the probability for an intervention trial to prove a beneficial effect of vitamin D. Therefore, we believe that currently, targeting high-risk groups and including exclusively patients with vitamin D deficiency will reduce necessary sample sizes and improve the likelihood of showing an effect.

Megadoses

To date, megadoses and long dosing intervals are considered obsolete. Besides higher risk of falls and/or fractures in multiple studies (61, 67), Martineau was also able to show a decreased efficacy of high bolus doses in the prevention of acute respiratory tract infections (68). This lack of effect appears biologically reasonable, as after a large dose, possibly vitamin D is catabolized more rapidly to inactive metabolites. Also, it is rarely necessary to increase vitamin D levels rapidly. However, in critical care, time is paramount, and vitamin D levels must be improved within days which is only possible with a megadose (57). Typical dosing regimes used in outpatients are ineffective in this short time period but a single, large vitamin D3 dose works within a few days (69). Therefore, the best approach in intensive care is probably a large loading dose followed by a regular daily or weekly maintenance dose. The optimal dosing regime is likely also dependent on individual patient factors including gastrointestinal function, underlying disease, co-medication, renal/hepatic function, genetic factors, ethnicity and body weight. In critical care, it also makes sense to determine serial vitamin D levels to guide therapy in patients with prolonged ICU/hospital stay.

 Accepted Manuscript published as EC-18-0184.R2. Accepted for publication: 05-Oct-2018

Vitamin D intervention trials in critical illness

In recent years, several vitamin D interventional trials with or without placebo groups including vitamin D deficient individuals or all-comers have been completed (**Table 4**). Given the low chance of successful normalization of vitamin D status with the traditional daily vitamin D regime (57), other supplementation strategies including mega-doses for initial loading have been used. Overall, there is substantial variation in these studies regarding treatment duration (single dose or up to 6 months), dose, route of administration (enteral, intramuscular, or intravenous) and metabolite (native vitamin D: cholecalciferol, ergocalciferol, active vitamin D: calcitriol). With the exception of the VITdAL-ICU trial (n=475) (50), these studies have been small (n<70). In the VITdAL-ICU trial, there was a non-significant absolute risk reduction in 6-month all-cause mortality in the vitamin D group (placebo : 43% vs. vitamin D3 : 35%). The findings did achieve statistical significance in the subgroup with severe vitamin D deficiency at baseline (25(OH)D <12ng/ml) corresponding to a number needed to treat of 6 (50). The primary endpoint, length of hospital stay, however, was not different between groups.

Recently, 3 independent groups published meta-analyses on the effect of vitamin D on the mortality of ICU patients (70-72). Because of the small number of additional patients besides the VITdAL-ICU trial, and the substantial heterogeneity between studies, these meta-analyses have added little additional information and maybe even caused confusion (73, 74). The conclusions drawn by the three groups of authors varied according to study selection, however the fact that currently less than 800 adult patients have been included in published RCTs makes meta-analyses problematic at this stage. Furthermore, none of these trials specifically included critically ill patients with severe vitamin D deficiency, which is the only subgroup where a significant beneficial effect of vitamin D supplementation on mortality has been shown to date. Ironically, similar to other settings, vitamin D deficiency was not an inclusion criterion in some studies. Six trials are currently registered on *clinicaltrials.gov* examining the effect of vitamin D supplementation in critically ill patients with vitamin D deficiency. One is a phase 2 study in children (NCT02452762). Three trials involve small numbers of selected sub-groups of critical ill patients (e.g. acute kidney injury, NCT02962102, neuro-critical care, NCT02881957). A single center study (n=430) in Saudi Arabia is examining the effect

of a single high dose (400,000IU) of vitamin D3 in critically ill patients with severe deficiency (25(OH)D <12ng/mL) with a primary outcome of hospital mortality (NCT02868827). The last two are large multi-center randomized placebo controlled trials that both have started in 2017 (summarized in **Error! Reference source not found.**) and will hopefully conclusively answer the question if vitamin D replacement confers clinical benefit in critical illness.

Vitamin D intervention before critical illness

In specific circumstances including intensive chemotherapy in some hemato-oncologic diseases, cardiac and other elective surgical procedures, ICU stay is foreseeable. Thus, we believe that diagnosing and treating vitamin D deficiency (besides iron and other nutritional deficiencies) appears reasonable in this subgroup, but there are currently no data to support such an approach.

Conclusion

Over the last decade, experimental, observational and clinical studies have highlighted the high prevalence of vitamin D deficiency, and its strong association with morbidity and mortality in critical illness. The scientific rationale as to why this may be the case is compelling. Supporters of vitamin D do not suggest it to be the panacea but this hormone plays an important pleiotropic role in the setting of critical illness and may support recovery from severe acute illness. We now have a better, albeit not complete understanding from clinical trials of the potential target vitamin D level and dosing strategies required for conferring benefit. Importantly, vitamin D testing and supplementation is readily available, safe, and inexpensive and could be rapidly implemented into clinical practice if the on-going trials show benefit.

Accepted Manuscript published as EC-18-0184.R2. Accepted for publication: 05-Oct-2018

REFERENCES

1. Van den Berghe G, Van Roosbroeck D, Vanhove P, Wouters PJ, De Pourcq L, Bouillon R. Bone turnover in prolonged critical illness: effect of vitamin D. J Clin Endocrinol Metab. 2003;88(10):4623-32.
2. Lee P, Eisman JA, Center JR. Vitamin D deficiency in critically ill patients. N Engl J Med. 2009;360(18):1912-4.
3. McNally JD, Leis K, Matheson LA, Karuananyake C, Sankaran K, Rosenberg AM. Vitamin D deficiency in young children with severe acute lower respiratory infection. Pediatr Pulmonol. 2009;44(10):981-8.
4. Amrein K, Amrein S, Holl A, Waltensdorfer A, Pieber T, H D. Vitamin D, parathyroid hormone and serum calcium levels and their association with hospital mortality in critically ill patients. Crit Care. 2010;14(Suppl 1):P589.
5. Braun A, Chang D, Mahadevappa K, Gibbons FK, Liu Y, Giovannucci E, Christopher KB. Association of low serum 25-hydroxyvitamin D levels and mortality in the critically ill. Crit Care Med. 2011;39(4):671-7.
6. Perron RM, Lee P. Efficacy of high-dose vitamin D supplementation in the critically ill patients. Inflamm Allergy Drug Targets. 2013;12(4):273-81.
7. Zajic P, Amrein K. Vitamin D deficiency in the ICU: a systematic review. Minerva Endocrinol. 2014;39(4):275-87.
8. Al-Tarrah K, Hewison M, Moiemen N, Lord JM. Vitamin D status and its influence on outcomes following major burn injury and critical illness. Burns Trauma. 2018;6:11.
9. Rech MA, Colon Hidalgo D, Larson J, Zavala S, Mosier M. Vitamin D in burn-injured patients. Burns. 2018.
10. Amrein K, Christopher KB, McNally JD. Understanding vitamin D deficiency in intensive care patients. Intensive Care Med. 2015;41(11):1961-4.
11. Lee P. Vitamin D metabolism and deficiency in critical illness. Best Pract Res Clin Endocrinol Metab. 2011;25(5):769-81.
12. Amrein K, Venkatesh B. Vitamin D and the critically ill patient. Curr Opin Clin Nutr Metab Care. 2012;15(2):188-93.
13. Amrein K, Zajic P, Schnedl C, Waltensdorfer A, Fruhwald S, Holl A, Purkart T, Wunsch G, Valentin T, Grisold A, et al. Vitamin D status and its association with season, hospital and sepsis mortality in critical illness. Crit Care. 2014;18(2):R47.
14. 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(9):1609-11.
15. McNally JD, Menon K, Chakraborty P, Fisher L, Williams KA, Al-Dirbashi OY, Doherty DR. The Association of Vitamin D Status With Pediatric Critical Illness. Pediatrics. 2012.
16. Rippel C, South M, Butt WW, Shekerdemian LS. Vitamin D status in critically ill children. Intensive Care Med. 2012;38(12):2055-62.
17. Braun AB, Litonjua AA, Moromizato T, Gibbons FK, Giovannucci E, Christopher KB. Association of low serum 25-hydroxyvitamin D levels and acute kidney injury in the critically ill. Crit Care Med. 2012;40(12):3170-9.

18. de Haan K, Groeneveld AB, de Geus HR, Egal M, Struijs A. Vitamin D deficiency as a risk factor for infection, sepsis and mortality in the critically ill: systematic review and meta-analysis. Crit Care. 2014;18(6):660.
19. Parekh D, Patel JM, Scott A, Lax S, Dancer RC, D'Souza V, Greenwood H, Fraser WD, Gao F, Sapey E, et al. Vitamin D Deficiency in Human and Murine Sepsis. Crit Care Med. 2017;45(2):282-9.
20. Parekh D, Thickett DR, Turner AM. Vitamin D deficiency and acute lung injury. Inflamm Allergy Drug Targets. 2013;12(4):253-61.
21. McNally JD, Nama N, O'Hearn K, Sampson M, Amrein K, Iliriani K, McIntyre L, Fergusson D, Menon K. Vitamin D deficiency in critically ill children: a systematic review and meta-analysis. Crit Care. 2017;21(1):287.
22. Zhang YP, Wan YD, Sun TW, Kan QC, Wang LX. Association between vitamin D deficiency and mortality in critically ill adult patients: a meta-analysis of cohort studies. Crit Care. 2014;18(6):684.
23. Lasky-Su J, Dahlin A, Litonjua AA, Rogers AJ, McGeachie MJ, Baron RM, Gazourian L, Barragan-Bradford D, Fredenburgh LE, Choi AMK, et al. Metabolome alterations in severe critical illness and vitamin D status. Crit Care. 2017;21(1):193.
24. Czarnik T, Czarnik A, Gawda R, Gawor M, Piwoda M, Marszalski M, Maj M, Chrzan O, Said R, Rusek-Skora M, et al. Vitamin D kinetics in the acute phase of critical illness: A prospective observational study. J Crit Care. 2018;43:294-9.
25. Nair P, Lee P, Reynolds C, Nguyen ND, Myburgh J, Eisman JA, Center JR. Significant perturbation of vitamin D-parathyroid-calcium axis and adverse clinical outcomes in critically ill patients. Intensive Care Med. 2013;39(2):267-74.
26. Quraishi SA, Camargo CA, Jr. Vitamin D in acute stress and critical illness. Curr Opin Clin Nutr Metab Care. 2012;15(6):625-34.
27. De Pascale G, Quraishi SA. Vitamin D status in critically ill patients: the evidence is now bioavailable! Crit Care. 2014;18(4):449.
28. Martucci G, Tuzzolino F, Arcadipane A, Pieber TR, Schnedl C, Urbanic Purkart T, Treiber G, Amrein K. The effect of high-dose cholecalciferol on bioavailable vitamin D levels in critically ill patients: a post hoc analysis of the VITdAL-ICU trial. Intensive Care Med. 2017;43(11):1732-4.
29. Jenkinson C, Taylor AE, Hassan-Smith ZK, Adams JS, Stewart PM, Hewison M, Keevil BG. High throughput LC-MS/MS method for the simultaneous analysis of multiple vitamin D analytes in serum. J Chromatogr B Analyt Technol Biomed Life Sci. 2016;1014:56-63.
30. Prietl B, Treiber G, Pieber TR, Amrein K. Vitamin D and immune function. Nutrients. 2013;5(7):2502-21.
31. Rejnmark L, Bislev LS, Cashman KD, Eiriksdottir G, Gaksch M, Grubler M, Grimnes G, Gudnason V, Lips P, Pilz S, et al. Non-skeletal health effects of vitamin D supplementation: A systematic review on findings from meta-analyses summarizing trial data. PLoS One. 2017;12(7):e0180512.
32. Bouillon R, Carmeliet G, Verlinden L, van Etten E, Verstuyf A, Luderer HF, Lieben L, Mathieu C, Demay M. Vitamin D and human health: lessons from vitamin D receptor null mice. Endocr Rev. 2008;29(6):726-76.
33. Campos LT, Brentani H, Roela RA, Katayama ML, Lima L, Rolim CF, Milani C, Folgueira MA, Brentani MM. Differences in transcriptional effects of 1alpha,25 dihydroxyvitamin D3 on fibroblasts associated to breast carcinomas and from paired normal breast tissues. J Steroid Biochem Mol Biol. 2013;133:12-24.
34. Hewison M. Vitamin D and immune function: an overview. Proc Nutr Soc. 2012;71(1):50-61.
35. Dancer RC, Parekh D, Lax S, D'Souza V, Zheng S, Bassford CR, Park D, Bartis DG, Mahida R, Turner AM, et al. Vitamin D deficiency contributes directly to the acute respiratory distress syndrome (ARDS). Thorax. 2015;70(7):617-24.
36. Girgis CM, Clifton-Bligh RJ, Hamrick MW, Holick MF, Gunton JE. The roles of vitamin D in skeletal muscle: form, function, and metabolism. Endocr Rev. 2013;34(1):33-83.
37. Lugg ST, Howells PA, Thickett DR. Optimal Vitamin D Supplementation Levels for Cardiovascular Disease Protection. Dis Markers. 2015;2015:864370.

Accepted Manuscript published as EC-18-0184.R2. Accepted for publication: 05-Oct-2018

38. Orford N, Cattigan C, Brennan SL, Kotowicz M, Pasco J, Cooper DJ. The association between critical illness and changes in bone turnover in adults: a systematic review. Osteoporos Int. 2014;25(10):2335-46.
39. Orford NR, Bailey M, Bellomo R, Pasco JA, Cattigan C, Elderkin T, Brennan-Olsen SL, Cooper DJ, Kotowicz MA. The association of time and medications with changes in bone mineral density in the 2 years after critical illness. Crit Care. 2017;21(1):69.
40. Rousseau AF, Foidart-Desalle M, Ledoux D, Remy C, Croisier JL, Damas P, Cavalier E. Effects of cholecalciferol supplementation and optimized calcium intakes on vitamin D status, muscle strength and bone health: a one-year pilot randomized controlled trial in adults with severe burns. Burns. 2015;41(2):317-25.
41. Orford NR, Lane SE, Bailey M, Pasco JA, Cattigan C, Elderkin T, Brennan-Olsen SL, Bellomo R, Cooper DJ, Kotowicz MA. Changes in Bone Mineral Density in the Year after Critical Illness. Am J Respir Crit Care Med. 2016;193(7):736-44.
42. Griffith DM, Walsh TS. Bone loss during critical illness: a skeleton in the closet for the intensive care unit survivor? Crit Care Med. 2011;39(6):1554-6.
43. Grimm G, Vila G, Bieglmayer C, Riedl M, Luger A, Clodi M. Changes in osteopontin and in biomarkers of bone turnover during human endotoxemia. Bone. 2010;47(2):388-91.
44. Schwetz V, Schnedl C, Urbanic-Purkart T, Trummer C, Dimai HP, Fahrleitner-Pammer A, Putz-Bankuti C, Christopher KB, Obermayer-Pietsch B, Pieber TR, et al. Effect of vitamin D3 on bone turnover markers in critical illness: post hoc analysis from the VITdAL-ICU study. Osteoporos Int. 2017;28(12):3347-54.
45. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM. Evaluation, treatment, and prevention of vitamin d deficiency: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2011;96(7):1911-30.
46. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, Gallagher JC, Gallo RL, Jones G, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab. 2011;96(1):53-8.
47. Hollander JM, Mechanick JI. Bisphosphonates and metabolic bone disease in the ICU. Curr Opin Clin Nutr Metab Care. 2009;12(2):190-5.
48. Lee P, Ng C, Slattery A, Nair P, Eisman JA, Center JR. Preadmission Bisphosphonate and Mortality in Critically Ill Patients. J Clin Endocrinol Metab. 2016;101(5):1945-53.
49. Amrein K, Sourij H, Wagner G, Holl A, Pieber TR, Smolle KH, Stojakovic T, Schnedl C, Dobnig H. Short-term effects of high-dose oral vitamin D3 in critically ill vitamin D deficient patients: a randomized, double-blind, placebo-controlled pilot study. Crit Care. 2011;15(2):R104.
50. Amrein K, Schnedl C, Holl A, Riedl R, Christopher KB, Pachler C, Urbanic Purkart T, Waltensdorfer A, Munch A, Warnkross H, et al. Effect of high-dose vitamin D3 on hospital length of stay in critically ill patients with vitamin D deficiency: the VITdAL-ICU randomized clinical trial. JAMA. 2014;312(15):1520-30.
51. Quraishi SA, De Pascale G, Needleman JS, Nakazawa H, Kaneki M, Bajwa EK, Camargo CA, Jr., Bhan I. Effect of Cholecalciferol Supplementation on Vitamin D Status and Cathelicidin Levels in Sepsis: A Randomized, Placebo-Controlled Trial. Crit Care Med. 2015;43(9):1928-37.
52. Han JE, Jones JL, Tangpricha V, Brown MA, Brown LAS, Hao L, Hebbar G, Lee MJ, Liu S, Ziegler TR, et al. High Dose Vitamin D Administration in Ventilated Intensive Care Unit Patients: A Pilot Double Blind Randomized Controlled Trial. J Clin Transl Endocrinol. 2016;4:59-65.
53. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357(3):266-81.
54. Sempos CT, Heijboer AC, Bikle DD, Bollerslev J, Bouillon R, Brannon PM, DeLuca HF, Jones G, Munns CF, Bilezikian JP, et al. Vitamin D assays and the definition of hypovitaminosis D: results from the First International Conference on Controversies in Vitamin D. Br J Clin Pharmacol. 2018.
55. Holick MF. The IOM D-lemma. Public Health Nutr. 2011;14(5):939-41.

515 56. Schnedl C, Dobnig H, Quraishi SA, McNally JD, Amrein K. Native and active vitamin D in
516 intensive care: who and how we treat is crucially important. Am J Respir Crit Care Med.
517 2014;190(10):1193-4.
518 57. McNally JD. Vitamin D as a modifiable risk factor in critical illness: questions and answers
519 provided by observational studies. J Pediatr (Rio J). 2014;90(2):99-101.
520 58. Vieth R. Vitamin D toxicity, policy, and science. J Bone Miner Res. 2007;22 Suppl 2:V64-8.
521 59. Mata-Granados JM, Vargas-Vasserot J, Ferreiro-Vera C, Luque de Castro MD, Pavon RG,
522 Quesada Gomez JM. Evaluation of vitamin D endocrine system (VDES) status and response to
523 treatment of patients in intensive care units (ICUs) using an on-line SPE-LC-MS/MS method. J Steroid
524 Biochem Mol Biol. 2010;121(1-2):452-5.
525 60. Nair P, Venkatesh B, Lee P, Kerr S, Hoechter DJ, Dimeski G, Grice J, Myburgh J, Center JR. A
526 Randomized Study of a Single Dose of Intramuscular Cholecalciferol in Critically Ill Adults. Crit Care
527 Med. 2015;43(11):2313-20.
528 61. Sanders KM, Stuart AL, Williamson EJ, Simpson JA, Kotowicz MA, Young D, Nicholson GC.
529 Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled
530 trial. JAMA. 2010;303(18):1815-22.
531 62. Jacobs TP, Kaufman M, Jones G, Kumar R, Schlingmann KP, Shapses S, Bilezikian JP. A lifetime
532 of hypercalcemia and hypercalciuria, finally explained. J Clin Endocrinol Metab. 2014;99(3):708-12.
533 63. Schlingmann KP, Kaufmann M, Weber S, Irwin A, Goos C, John U, Misselwitz J, Klaus G,
534 Kuwertz-Broking E, Fehrenbach H, et al. Mutations in CYP24A1 and idiopathic infantile
535 hypercalcemia. N Engl J Med. 2011;365(5):410-21.
536 64. Scragg R, Khaw KT, Toop L, Sluyter J, Lawes CMM, Waayer D, Giovannucci E, Camargo CA, Jr.
537 Monthly High-Dose Vitamin D Supplementation and Cancer Risk: A Post Hoc Analysis of the Vitamin D
538 Assessment Randomized Clinical Trial. JAMA Oncol. 2018:e182178.
539 65. Scragg R. Emerging Evidence of Thresholds for Beneficial Effects from Vitamin D
540 Supplementation. Nutrients. 2018;10(5).
541 66. Brenner H, Jansen L, Saum KU, Holleczek B, Schottker B. Vitamin D Supplementation Trials
542 Aimed at Reducing Mortality Have Much Higher Power When Focusing on People with Low Serum
543 25-Hydroxyvitamin D Concentrations. J Nutr. 2017;147(7):1325-33.
544 67. Smith H, Anderson F, Raphael H, Maslin P, Crozier S, Cooper C. Effect of annual intramuscular
545 vitamin D on fracture risk in elderly men and women--a population-based, randomized, double-blind,
546 placebo-controlled trial. Rheumatology (Oxford). 2007;46(12):1852-7.
547 68. Martineau AR, Jolliffe DA, Hooper RL, Greenberg L, Aloia JF, Bergman P, Dubnov-Raz G,
548 Esposito S, Ganmaa D, Ginde AA, et al. Vitamin D supplementation to prevent acute respiratory tract
549 infections: systematic review and meta-analysis of individual participant data. BMJ. 2017;356:i6583.
550 69. Ilahi M, Armas LA, Heaney RP. Pharmacokinetics of a single, large dose of cholecalciferol. Am
551 J Clin Nutr. 2008;87(3):688-91.
552 70. Langlois PL, Szwec C, D'Aragon F, Heyland DK, Manzanares W. Vitamin D supplementation in
553 the critically ill: A systematic review and meta-analysis. Clin Nutr. 2018;37(4):1238-46.
554 71. Putzu A, Belletti A, Cassina T, Clivio S, Monti G, Zangrillo A, Landoni G. Vitamin D and
555 outcomes in adult critically ill patients. A systematic review and meta-analysis of randomized trials. J
556 Crit Care. 2017;38:109-14.
557 72. Weng H, Li JG, Mao Z, Zeng XT. Randomised trials of vitamin D3 for critically ill patients in
558 adults: systematic review and meta-analysis with trial sequential analysis. Intensive Care Med.
559 2017;43(2):277-8.
560 73. Amrein K, Martucci G, McNally JD. When not to use meta-analysis: Analysing the meta-
561 analyses on vitamin D in critical care. Clin Nutr. 2017;36(6):1729-30.
562 74. McNally JD, Ginde AA, Amrein K. Clarification needed for the systematic review of vitamin D
563 trials in the ICU. Intensive Care Med. 2017;43(4):595-6.

564

Accepted Manuscript published as EC-18-0184.R2. Accepted for publication: 05-Oct-2018

565

FIGURE LEGENDS

Figure 1.

Overview of vitamin D metabolism and its classic and non-classic effects on different target organs/systems

Accepted Manuscript published as EC-18-0184.R2. Accepted for publication: 05-Oct-2018

Figure 1:

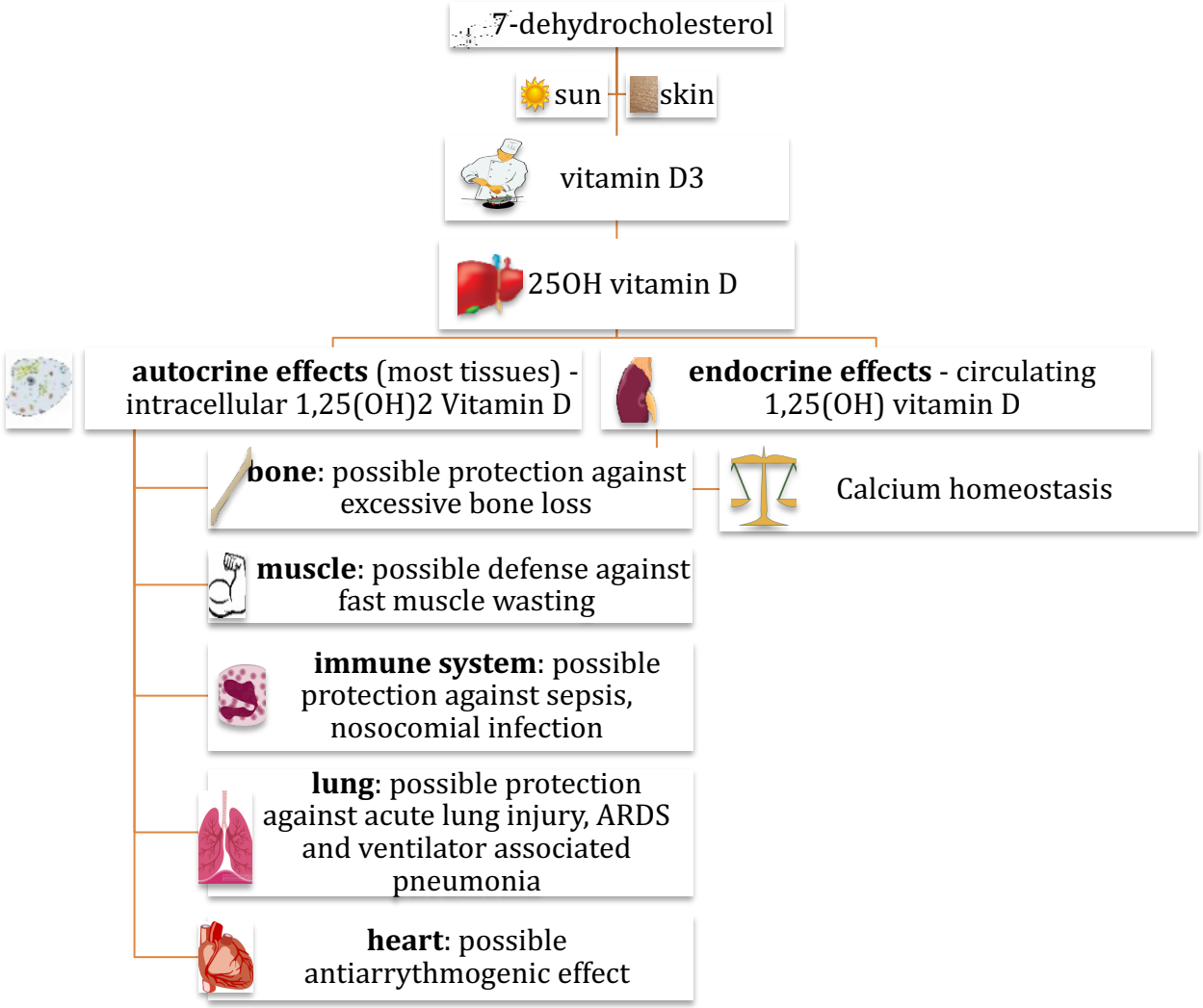

Table 1: Selected observational trials on the incidence of vitamin D deficiency in ICU patients.

Author, Journal, Year	Design Population	N of patients	Vitamin D deficiency definition	Outcomes
Braun A., Crit Care Med. 2011 Boston, Massachusetts, USA (5)	Retrospective observational study, Medical and surgical ICU patients	2399	Pre-admission 25(OH)D was categorized as deficiency in 25(OH)D (≤15ng/mL), insufficiency (16–29ng/mL) and sufficiency (≥30ng/mL)	Deficiency: 27% (637 patients) Insufficiency: 38% (918 patients) Sufficiency: 35% (844 patients)
Amrein K., Crit. Care. 2014 Graz, Austria (13)	Retrospective observational study, Medical and surgical ICU patients	655	25(OH)D was categorized as deficiency in 25(OH)D (≤20ng/mL), insufficiency (20–30ng/mL), normal (>30ng/mL)	Deficiency: 60% of patients Insufficiency: 26% of patients Normal level: 14% of patients
Matthews LR, American J of Surgery. 2012 Atlanta, USA (74)	Prospective observational study, Surgical ICU patients	258	25(OH)D was categorized as severe deficiency in 25(OH)D (≤13ng/mL), moderate deficiency (14–26ng/mL) and mild deficiency (27-39ng/mL), sufficiency (>40ng/mL)	Severe deficiency: 54% (138 patients) Moderate deficiency: 37% (96 patients) Mild deficiency: 7% (18 patients) Sufficiency: 1% (3 patients)
Venkatram S, Critical Care. 2011	Retrospective study,	437	25(OH)D was categorized as deficiency in 25(OH)D	Deficiency: 78% (340 patients)

Accepted Manuscript published as EC-18-0184.R2. Accepted for publication: 05-Oct-2018

New York, USA (75) (85)	medical ICU patients		(0-19ng/dL), insufficiency (20–29,9ng/dL) and normal levels (≥30ng/mL)	Insufficiency: 17% (74 patients) Normal level: 5% (23 patients)
Higgins DM, JPEN J Parenter Enteral Nutr. 2012 Ontario, Canada (76)	Prospective study, Medical and surgical ICU patients	196	25(OH)D was categorized as deficiency in 25(OH)D (<12 ng/ml), insufficiency (12–24ng/mL) and normal levels (>24ng/mL)	Deficiency: 26% (50 patients) Insufficiency: 56% (109 patients) Normal level: 19% (37 patients)
Nair P, Intensive Care Med. 2015 Sydney, Australia (25)	Prospective multicentre cohort study, ICU patients	100	25(OH)D was categorized as deficiency in 25(OH)D (<10ng/ml), insufficiency (10–20 ng/ml) and normal levels (>20ng/ml)	Deficiency: 21% (21 patients) Insufficiency: 55% (55 patients) Normal level: 24% (24 patients)

Table 2 : Mechanism of action on target organ systems that may influence critically ill patients.

Target organs	Mechanism of action
Immune System	Vitamin D metabolites are acting as modulators of cells of the innate and adaptive System (30, 31, 34). Innate System: 1,25-dihydroxyvitamin D3 and 3 of its analogs induce expression of the human cathelicidin antimicrobal peptide (CAMP) gene and genes involved in autophagy and phagosome maturation all of which are involved in the intracellular destruction of pathogens; promotion of an anti-inflammatory response by inhibiting the maturation of DCs; Adaptive System: VitD induces anti-inflammatory responses through direct effects on T-cells (34, 77, 78).
Cardiac function	Vitamin D may play a role in atrial fibrilation prevention by negatively regulating the renin-angiotensin-aldosterone-system (RAAS), mediating calcium homeostasis, binding to vitamin D receptors (VDR) on cardiac myocytes and furthermore by having antioxidant properties that may reduce levels of reactive oxygen species (ROS) in the atria, which contribute to inflammation and proarrhythmic substrate formation (79). The exact mechanism of action unknown but the recent research on animal models suggest that calcitriol has been shown to have a key role in enabling the maturation and differentiation of ventricular myocytes isolated from neonatal rat hearts and could therefore potentially influence heart failure (37). Vitamin D receptors are also present in all cells implicated in atherosclerosis. Those include endothelial cells, vascular smooth muscle cells and immune cells. It appears to regulate vascular cell growth, migration and differentiation; immune response modulation; cytokine expression; and inflammatory and fibrotic pathways. All of those mechanisms play a crucial role in different stages of the atherosclerotic plaque vulnerability and rupture (80).
Lung function	A lack of VDRs in the pulmonary epithelial barrier appeared to compromise its defense, leading to more severe lipopolysaccharide (LPS)–induced lung injury. Moreover, vitamin D treatment alleviated LPS-induced lung injury and preserved alveolar barrier function (35). Therefore, vitamin D may be a potential therapeutic strategy in acute lung injury and acute respiratory distress syndrome.
Muscle function and metabolism	Some molecular mechanism studies suggest that vitamin D impacts muscle cell differentiation, intracellular calcium handling, and genomic activity. Some animal models have confirmed that vitamin D deficiency and congenital

Accepted Manuscript published as EC-18-0184.R2. Accepted for publication: 05-Oct-2018

	aberrations in the vitamin D endocrine system may result in muscle weakness (36, 81, 82).
Bone	Limited available data in ICU survivors suggest impaired bone health and high fracture risk (38-41, 83). 1,25(OH)(2)D(3) is known primarily as a regulator of calcium, but it also controls phosphate (re)absorption at the intestine and kidney. Mechanism of action involve 1,25(OH)2D3, FGF23 (Fibroblast growth factor 23 – phosphaturic hormone produced in osteoblasts) and 1,25(OH)(2)D(3) via the PTH axis (84).

Table 3 : Table summerizing characteristics of available formulations of vitamin D, adjusted based on (30, 85)

Formulation	Native/ active	Recommended daily dose	On-/offset of action	Indications	Side effects	Costs
unhydroxylated, inactive form of vitamin D3 cholecalciferol **calciol**	native	400-4000IU and up to 25 000-100 000IU by hypoparathyroidismus (85)	Onset: 10-14 days Offset: 14-75 days	Vitamin D deficiency, osteoporosis therapy and prevention, hypoparathyroidism, prevention of rickets	Hypercalcemia (rare)	inexpensive
unhydroxylated, inactive form of vitamin D2 ergocalciferol **vitamin D2**	native	400-4000IU and up to 25 000-100 000IU by hypoparathyroidismus	Onset: 10-14 days Offset: 14-75 days	Vitamin D deficiency, osteoporosis therapy and prevention, hypoparathyroidism, prevention of rickets	Hypercalcemia (rare)	inexpensive
hydroxylated, active form of vitamin D **1,25(OH)2D** calcitriol **1,25-dihydroxyvitamin D3,** **1,25-dihydroxycholecalciferol**	active	0.25-1.0 µg	Onset: 1-2 days Offset: 2-3 days	secondary hyperparathyroidism in advanced CKD, hypoparathyroidism, pseudohypoparathyroidism, not in vitamin D deficiency	hypercalcemia/hyperphosphatemia is not uncommon (dose dependent), hypercalciuria, nephrocalcinosis	expensive
analog: alfacalcidol	active	0.5-3.0 µg	Onset: 1-2 days Offset: 5-7 days	secondary hyperparathyroidism in advanced CKD, hypoparathyroidism, pseudohypoparathyroidism, not in vitamin D deficiency		

Accepted Manuscript published as EC-18-0184.R2. Accepted for publication: 05-Oct-2018

other active vitamin D analogs: **paricalcitol, doxercalciferol (vitamin D2 analogs)** **falecalcitriol, maxacalcitol (vitamin D3 analogs)**	active			Secondary hyperparathyroidism in advanced chronic kidney disease	Hypercalcemia may occur, but less frequent compared with “older” active analogs	Very expensive

Table 4: Selected prospective randomized controlled trials on the effect of oral/enteral vitamin D in adult critically ill patients

Author, Journal, Year	Design Population	N of patients	Intervention	Outcomes
COMPLETED TRIALS				
Amrein K., Crit Care 2011 Graz, Austria (49)	RCT Medical ICU, 25OHD <20ng/ml	25	1x 540 000 IU D_3, enteral vs. placebo	Normalization of vitamin D levels in most patients, no adverse events; no difference in 28-d mortality or length of stay.
Amrein K., JAMA 2014 Graz, Austria (50)	RCT Mixed ICU, 25OHD <20ng/ml	475	1x 540 000 IU D_3, enteral, then 5x 90 000 IU D_3/month vs. placebo	No difference in hospital length of stay, overall no significant mortality benefit, but large and significant mortality benefit in the predefined subgroup with severe vitamin D deficiency (25OHD) < 12
Quraishi S., Crit Care Med. 2015 Boston, USA (51)	RCT ICU, sepsis	30	1x 200 000 IU D_3, enteral or 1x 400 000 IU D_3, enteral vs. placebo	Rapid correction of vitamin D deficiency, increase in LL-37 compared to the placebo group
Han JE J of Clin & transl. endocrinology	RCT ICU, mechanically	30	5x 50 000 IU D_3, enteral or 5x 100 000 IU D_3,	Shorter hospital stay, dose dependent increase of vitamin D levels and increased

Accepted Manuscript published as EC-18-0184.R2. Accepted for publication: 05-Oct-2018

2016, Nutrition 2017 Atlanta, USA (52)	ventilated		enteral vs. placebo	hCAP18 mRNA-expression compared to the placebo group
Alizadeh N, Int J Clin Pract 2016 Teheran, Iran (86)	RCT surgical ICU, stress-induced hyperglycaemia	50	600 000 IU D3, IM vs. placebo	25OHD levels increased significantly in the vitamin D group at day 7, fasting plasma adiponectin levels increased significantly in the vitamin D group, but not the placebo group
Miroliaee AE 2017, Iran J Pharm Res. Teheran, Iran (87)	RCT ICU, ventilator associated pneumonia 25OHD <30ng/ml	46	300 000 IU D3, IM vs. placebo	PCT levels significantly lower in the vitamin D group compared to placebo group, no significant difference in SOFA score between groups, mortality rate of patients in the vitamin D group was significantly lower than in the placebo group

Table 5:

Comparison between the VITDALIZE and the VIOLET trial, the two ongoing, large vitamin D3 intervention trials in acute illness

	VITDALIZE (NCT03188796)	**VIOLET** (NCT03096314)
Where	Europe, multicenter	US, multicenter
Design	Double-blind, placebo-controlled RCT	Double-blind, placebo-controlled RCT
Sample size	2400 (one interim analysis at 1200)	3000 (three interim analyses)
Intervention	Loading dose of 540,000 IU vitamin D3 (orally, enteral) Daily dose of 4,000 IU vitamin D3 (orally, enteral) up to day 90	Single dose of 540,000 IU vitamin D3 (orally, enteral)
Inclusion criteria	25(OH)D < 12ng/ml Admission to ICU (all-cause)	25(OH)D < 20ng/ml by point-of-care test Acute risk factors for ARDS and mortality contributing directly to the need for ICU admission
Primary endpoint	28-day-mortality (all-cause)	90-day-mortality (all-cause)
Recruitment started	October 2017	April 2017
Current status	Recruiting, estimated completion date 2021-2022	Stopped after first interim analysis (July 2018, ca 1400 patients)