

Dissertation

**VITdAL@ICU – Correction of Vitamin D deficiency in critically ill
patients: a randomized, double-blind, placebo-controlled trial**

submitted by

Dr. med. univ. Christian Schnedl

for the Academic Degree of

Doctor of Medical Science

(Dr. scient. med.)

at the

Medical University of Graz

conducted at the

Division of Endocrinology and Metabolism

Department of Internal Medicine

under supervision of

PD Dr. Karin Amrein and Prof. Dr. Harald Dobnig

Graz, June 2013

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Declaration

I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organisations that have contributed to the research for this thesis. Due acknowledgment has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the guidelines of “Good Scientific Practice”.

Graz, June 2013

Acknowledgements

This work is dedicated to my parents for their lifelong support that finally has brought me to this point.

Sincere thanks go to my doctoral father Harald Dobnig for enabling this study and his longstanding guidance and Karin Amrein for her enduring encouragement and help during the doctoral thesis.

I am deeply indebted to Helga Warnkross for her persistent work and help in finishing this study.

Moreover, I would like to thank Andrea Berghofer and Regina Riedl being responsible for the statistical analyses.

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List of Abbreviations

β CTX = carboxy terminal cross-link telopeptide of type 1 collagen

1,25(OH)₂D = 1,25-hydroxyvitamin D

25(OH)D = 25-hydroxyvitamin D

AMP = antimicrobial peptide

ASPEN = American Society for Parenteral and Enteral Nutrition

bALP = bone alkaline phosphatase

b-CTX = beta-crosslaps

CaBP = calcium-binding protein

CACRU = calcium/creatinine ratio, urine

CCI = Charlson comorbidity index

CI = confidence interval

CRP = C-reactive protein

DBP = vitamin D binding protein

DPD = deoxypyridinoline

ESPEN = European Society for Clinical Nutrition and Metabolism

FGF-23 = fibroblast growth hormone-23

FOS = Framingham Offspring Study

GH = growth hormone

HR = hazard ratio

HPFS = Health Professionals Follow-up Study

IOM = Institute of Medicine

ICU = intensive care unit

i.e. = id est

IGF-1 = insulin like growth factor 1

IFN- γ = Interferon- γ

IL-1 = Interleukin 1

LOS = length of stay

LURIC = Ludwigshafen Risk and Cardiovascular Health Study

MRSA = methicillin – resistant staphylococcus aureus

NF κ B = nuclear factor kappa B

NHANES III = Third National Health and Examination Survey

NTproBNP = NTpro brain natriuretic peptide

PAMP = pathogen-associated molecular pattern
PRR = pathogen recognition receptor
PICP = carboxy terminal propeptide of type I collagen
PINP = amino terminal propetide of type I collagen
PTH = parathyroid hormone
PYD = pyridinoline
RAAS = renin-angiotenin-aldosteron-system
RANK(L) = receptor activator of nuclear factor- κ (ligand)
RCT = randomized controlled trial
RCU = respiratory care unit
RR = relative risk
SAP = statistical analysis plan
SAPS II = Simplified Acute Physiology Score
T1D = type 1 diabetes mellitus
TISS-28 = Therapeutic Intervention Scoring Sysem
TLR = toll like receptor
TRAP = tartrat resistant acid phosphatase
TRPV6 = transient receptor potential cation channel, subfamily V, member 6
URTI = upper respiratory tract infections
VDR = vitamin D receptor
VDREs = vitamin D response elements
VDR-RXR = vitamin D receptor-retinoic acid x-receptor complex
WHI = Women`s Health Initiative

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Zusammenfassung

Hintergrund

Ein niedriger Vitamin D Status ist mit erhöhter Morbidität und Mortalität sowohl in der Allgemeinbevölkerung als auch bei IntensivpatientInnen vergesellschaftet. Dennoch hat bisher keine randomisierte, kontrollierte Studie untersucht, ob sich das klinische Outcome von PatientInnen auf Intensivstationen durch die Behandlung mit ausreichend hohen Dosen an Vitamin D verbessert.

Methoden

In dieser randomisierten, doppel-blinden, placebo-kontrollierten Studie auf fünf Intensivstationen eines großen Lehrkrankenhauses schlossen wir eine gemischte Population von 480 kritisch kranken Erwachsenen mit Vitamin D Mangel ($\leq 20\text{ng/ml}$) ein, die entweder Placebo oder hochdosiertes Vitamin D₃ erhielten. Eine primäre Auf sättigungs-dosis von 540,000 Einheiten Cholecalciferol oral oder mittels Magensonde verabreicht, wurde um 5 monatliche Erhaltungsdosen von 90,000 Einheiten in einem Beobachtungszeitraum von 6 Monaten ergänzt. Der primäre Endpunkt war die Dauer des Aufenthaltes im Krankenhaus.

Resultate

In der Interventiongruppe erhöhte sich das durchschnittliche 25-Hydroxyvitamin D von 13.0 ± 4.8 bei Studieneinschluss auf $35.5 \pm 20.6\text{ng/ml}$ nach 7 Tagen und 52.5% der PatientInnen in der Vitamin D Gruppe erreichten innerhalb dieser Zeit Werte von über 30ng/ml . Die Aufenthaltsdauer im Krankenhaus war in der Placebo Gruppe ähnlich zur Vitamin D Gruppe (Median 19.3 vs. 20.1 Tage, $p=0.981$), wie dies auch in Hinblick auf die Liegedauer auf Intensivstation der Fall war (Median 10.7 vs. 9.6 Tage, $p=0.384$). Insgesamt war ein Trend bezüglich einer niedrigeren Krankenhaus- und 6-Monats-Mortalität in der Vitamin D Gruppe im Vergleich zur Placebo Gruppe ersichtlich (28.3% vs. 35.3%, $p=0.121$ und 35% vs. 42.9%, $p=0.087$). Statistische Signifikanz wurde in der vordefinierten Subgruppe mit schwerem Vitamin D Mangel zu Studienbeginn ($\leq 12\text{ng/ml}$, $n=200$) erreicht, da sowohl Krankenhaus- als auch 6-Monats-Mortalität durch hochdosierte Vitamin D Gabe signifikant reduziert wurde (28.6% vs. 46.1%, $p=0.010$ und 34.7% vs. 50.0%, $p=0.021$). Unerwünschte Ereignisse wie Hyperkalzämie, Hyperkalziurie, Stürze und Frakturen waren im Rahmen der 6-monatigen Studiendauer zwischend den beiden Behandlungsgruppen nicht unterschiedlich.

Schlussfolgerung

Eine hochdosierte Vitamin D Gabe war nicht in der Lage die Dauer des Krankenhausaufenthaltes signifikant zu senken. Allerdings war die Krankenhaus- und 6-Monats-Mortalität in der Gesamtgruppe tendentiell und bei PatientInnen mit schwerem Vitamin D Mangel signifikant niedriger. Größere Interventionsstudien sind notwendig, um diese Ergebnisse zu bestätigen und einen möglichen schwächeren Effekt bei Patienten mit moderatem Vitamin D Mangel zu untersuchen.

ClinicalTrials.gov number: NCT01130181

Abstract

Background

A low vitamin D status is associated with increased morbidity and mortality in the general population and in critically ill patients. However, no randomized controlled trial has yet evaluated whether treatment with a sufficiently large dose of vitamin D can improve the clinical outcome of patients in an intensive care setting.

Methods

In this randomized, double-blind, placebo-controlled, single-center trial in five intensive care units of a large academic center, we assigned a mixed population of 480 critically ill adults with vitamin D deficiency ($\leq 20\text{ng/ml}$) to receive either placebo or high-dose vitamin D3. An initial loading dose of 540,000IU cholecalciferol applied orally or via feeding tube was followed by five monthly maintenance doses of 90,000IU for a total follow-up period of 6 months. The primary endpoint was length of hospital stay.

Results

In the intervention group, mean 25(OH)D increased from a baseline level of 13.0 ± 4.8 to $35.5 \pm 20.6\text{ng/ml}$ after 7 days and the percentage of patients who achieved vitamin D sufficiency ($>30\text{ng/ml}$) was 52.5% within this period. Hospital length of stay was similar in the placebo group compared with the vitamin D group (median 19.3 vs. 20.1 days, $p=0.981$), as was length of ICU stay (median 10.7 vs. 9.6 days, $p=0.384$). Overall, there was a trend towards a lower hospital- and 6-month mortality in the vitamin D group compared to the placebo group (28.3% vs. 35.3%, $p=0.121$ and 35.0% vs. 42.9%, $p=0.087$). This was statistically significant in the predefined subgroup with severe vitamin D deficiency at baseline ($\leq 12\text{ng/ml}$, $n=200$), as both hospital- and 6-month mortality were significantly reduced by high-dose vitamin D supplementation (28.6% vs. 46.1%, $p=0.010$ and 34.7% vs. 50.0%, $p=0.021$). Adverse events including hypercalcemia, hypercalciuria, falls and fractures were not different between the treatment groups during the 6-month follow-up period.

Conclusions

High-dose oral vitamin D3 did not significantly reduce hospital length of stay as compared with placebo in mixed critically ill patients. However, hospital- and 6-month mortality tended to be lower in the entire group and was significantly lower in patients with severe vitamin D deficiency. Larger intervention trials are necessary to confirm our findings and evaluate a possible weaker effect in patients with moderate vitamin D deficiency.

ClinicalTrials.gov number: NCT01130181

1. Introduction

Vitamin D, first identified as a vitamin early in the 20th century, was recognized as important for the prevention of rickets in the 1920s (1). When in the mid of the last century this severe form of vitamin D deficiency appeared to have been conquered by vitamin D supplementation and food fortification to newborns, many health care professionals thought that the major health problems resulting from vitamin D deficiency had been resolved.

However, the past decade has witnessed an extraordinary renaissance in vitamin D research based on a reevaluation of its potential benefits to human health. Beside its effects on calcium and phosphorus homeostasis the discovery that most cells in the human body are endowed with a vitamin D receptor (VDR) and that up to 3% of the human genome may be influenced by the active vitamin D (2), has provided new insights into the function of this vitamin.

Consequently, many research groups in different disciplines of medicine including intensive care draw their attention to the promising, pleiotropic effects of vitamin D under various conditions.

1.1. Sources and metabolism of vitamin D

Humans derive vitamin D from exposure to UVB by sunlight, from their diet or from supplements. Significant dietary levels of vitamin D are only present in oily fish like salmon, sardines and mackerel, fish oil, mushrooms and egg yolk and contain either vitamin D₂ or D₃. Vitamin D₂ is produced through the ultraviolet irradiation of ergosterol from yeast and a variety of plant materials while vitamin D₃ has its first synthesizing step through the ultraviolet irradiation of 7-dehydrocholesterol in the skin (3-5).

The D₂ and D₃ forms differ chemically only in their side chain structure, with the result that single doses of D₂ lead to lower levels of circulating 25-hydroxyvitamin D [25(OH)D] than single doses of D₃ (6). Nevertheless, daily administration of D₂ and D₃ maintains comparable levels of 25(OH)D and both forms function as prohormones (5, 7). As at the tissue level biologic activity appears to be similar, references to vitamin D or its metabolites will refer to both forms unless otherwise indicated with a specific subscript.

After the discovery of vitamin D, fortification of this hormone in some foods was introduced in many Western countries in order to prevent rickets. However, several cases of hypercalcemia and hypervitaminosis D in infants led to the cessation of vitamin D

fortification of milk in Great Britain in the 1950 (8, 9) and nowadays only Sweden and Finland allow to fortify milk in Europe (10, 11). Recently, two systematic reviews showed that the fortification of food in the USA improves the serum 25(OH)D concentration in the general population (12, 13) although the Western-style diet still contributes to low vitamin D levels (14).

Indeed, the major source of vitamin D (approximately 80 to 90%) is cutaneous synthesis through the effects of UV light, which induces a photochemical reaction in the epidermis. Solar ultraviolet B radiation (wavelength 290 to 315nm) penetrates the skin and converts 7-dehydrocholesterol to previtamin D₃, which is rapidly converted to vitamin D₃. Sunlight exposure never causes vitamin D intoxication because excess production of previtamin D₃ or vitamin D₃ causes a photoconversion to inactive metabolites (lumisterol, tachysterol, suprasterol I and II and 5,6 transvitamin D₃) (3, 5, 15). Skin-derived vitamin D synthesis is quite variable, depending on factors like skin pigmentation, degree of latitude, season, age, outdoor activities and sunscreen use (6, 16).

Successive hydroxylations are required to convert the vitamin D to its active form calcitriol or 1,25-hydroxyvitamin D [1,25(OH)₂D]. Circulating vitamin D is either stored in adipose tissue or enzymatically metabolized in a first substrate dependent step in the liver to 25(OH)D via CYP27A1 (mitochondrial and microsomal cytochrome P450 enzyme). Currently, 25(OH)D is the best available serum parameter reflecting the patient's vitamin D status, which comprises overall vitamin D body stores derived from sunlight exposure and dietary intake from food, fortified products and/or supplements (17, 18). 25(OH)D is rapidly released by the liver into the systemic circulation, where it normally exhibits a biological half-life of 2 to 3 weeks (19). In the kidney, 25-hydroxyvitamin D-1 α -hydroxylase (CYP27B1, a mitochondrial P450 enzyme) in the proximal renal tubule is essential to metabolize 25(OH)D to its biologically active form 1,25(OH)₂D.

Its mechanism of action is similar to that of steroid hormones and is mediated by binding to the nuclear vitamin D receptor (VDR). VDR is a member of the superfamily of nuclear hormone receptors and functions as a heterodimer with the retinoic acid X receptor (VDR-RXR) for regulation of vitamin D target genes. These heterodimeric complexes interact with specific DNA sequences [so called vitamin D response elements (VDREs)] resulting in either activation or repression of transcription (3, 6, 20).

The renal production of 1,25(OH)₂D is tightly regulated by parathyroid hormone (PTH), serum calcium and phosphorus levels (3, 5). Furthermore, fibroblast growth factor 23 (FGF-23), secreted from bone, is a potent negative regulator of 1,25(OH)₂D and thus an important

factor in the calcium and phosphorus homeostasis (21, 22). Adequate 1,25(OH)₂D serum levels are necessary to ensure efficient renal calcium reabsorption besides intestinal calcium and phosphorus absorption (5, 23). It also induces the expression of the enzyme 25-hydroxyvitamin D-24-hydroxylase (CYP24, a mitochondrial P450), which metabolizes hormonally active vitamin D metabolites into biologically inactive, water-soluble calcitroic acid (3, 5).

Circulating 25(OH)D levels are approximately 500-1000 times higher than 1,25(OH)₂D levels and both vitamin D metabolites are predominantly protein-bound in systemic circulation. Only 0.03% of 25(OH)D is free, whereas 88% is bound to vitamin D binding protein (DBP) and the remainder to albumin (24).

1.2. Definition and prevalence of vitamin D deficiency

Currently, there is no universally accepted definition of vitamin D deficiency (25). The 2011 Endocrine Society guideline that is targeted at the “at risk population” defines vitamin D deficiency as a 25(OH)D level of less than 20ng/ml (to convert from ng/ml to nmol/l, multiply by 2.496), levels between 20 to 30ng/ml as a relative insufficiency of vitamin D and a level of ≥ 30 ng/ml as a sufficient vitamin D status (5, 11). According to the guideline the following observations led to the justification of this definition: 25(OH)D levels are inversely associated with PTH until levels of 30 to 40ng/ml, at which point PTH levels are at their nadir (26-28). Furthermore, intestinal calcium transport increased by 65% in women when 25(OH)D levels increased from an average of 20 to 34ng/ml (29).

A more conservative approach was set by the American Institute of Medicine (IOM) in their 2011 published recommendation on dietary reference intakes for calcium and vitamin D for the general “healthy” population (18, 30). The work of the IOM to define human requirements for vitamin D for the general population was based on assuring bone health, being the only causative “indicator”. For the IOM, serum 25(OH)D concentrations of 16ng/ml reflect a level that is sufficient to ensure bone health for approximately half the general population and nearly all persons (97.5% of the general population) are assured bone health when serum levels of 25(OH)D are 20ng/ml. According to these conclusions, 20ng/ml displays the upper range of human requirements and reflects a level which meets the needs of 97.5% of the general population (30, 31).

The applicability of these definitions to critically ill patients is unclear as a predefined biochemical-outcome correlation has not yet been determined in this specific population.

The few studies published to date evaluating vitamin D deficiency in critical illness have applied definitions used in the general population as described in the review by Lee (32).

Prevalence of hypovitaminosis D in the general population

If prevalence rates of vitamin D deficiency are studied, the definition must be clearly kept in mind. Most studies on vitamin D deficiency refer to the threshold of 30ng/ml. Therefore, when the considerations of the IOM report are applied, the prevalence of vitamin D deficiency would be substantially lower.

The individual vitamin D status depends on a variety of factors including age, skin pigmentation, clothing, genetic factors, nutrition, seasonality, latitude, sea level, body mass index (BMI), sunscreen use, outdoor activities, pollution, comorbidities like malabsorption syndromes and drugs interfering in vitamin D metabolism such as antiseizure medication or glucocorticoids (16, 33, 34).

A very high prevalence of vitamin D deficiency has been reported in children, elderly and hospitalized patients. According to several studies, 40 to 100% of U.S. and European elderly men and women are vitamin D deficient (26-28, 35-44). 42% of Hispanic and black adolescents and 48% of white preadolescent girls had 25(OH)D levels below 20ng/ml in two U.S. observational studies (45, 46). Even in the sunniest areas hypovitaminosis D is a common condition because traditionally populations living in these regions do often avoid the sun. In Saudi Arabia, the United Arab Emirates, Australia, Turkey, India and Lebanon, 30 to 50% of children and adults had levels below 20ng/ml (47-50). In contrast, black people of two tribes in Tanzania living near the equator who are exposed to sunlight without sun protection had average vitamin D levels of 48ng/ml far beyond the threshold of 30ng/ml (51).

Prevalence of vitamin D deficiency in the critically ill

Data on vitamin D status in critically ill patients remain sparse. To date, 11 studies (52-62) evaluated vitamin D status and its prevalence in critically ill patients (Table 1). Although different criteria for deficiency/insufficiency were applied across the studies, the prevalence of vitamin D insufficiency/deficiency ranged from 38% to 100% in a total of almost 3000 patients. Mean 25(OH)D levels were below 20ng/ml in most of the studies and thus within the deficient range. Summarizing these results provide unequivocal evidence that regardless of details of definition vitamin D deficiency is highly prevalent among critically ill patients.

Table 1 Summary of vitamin D status and prevalence of vitamin D deficiency in critically ill patients

Author	Year	N	Admission diagnosis	Prevalence (definition used)	Vitamin D status (ng/ml)
Nierman et al. (52)	1998	49	Respiratory failure	92% (<16)	12.0 (6.4)
Jeng et al. (53)	2009	49	Sepsis	100% (<30)	16.0 (8.5)
			Non sepsis	92% (<30)	16.2 (7.2)
Lee et al. (54)	2009	42	Mixed	93% (<24)	16.4 (8.8)
Lucidarme et al. (55)	2010	134	Mixed	79% (<24)	12.8 [7-22]
Mata-Granados et al. (56)	2010	33	Sepsis	97% (NA)	5.3 (4.0)
McKinney et al. (57)	2011	136	Mixed	38% (<20)	24.6 (12.1)
Venkatram et al. (58)	2011	437	Mixed	78% (<20)	NA
Flynn et al. (59)	2011	66	Surgical	74% (<20)	NA
Ginde et al. (60)	2011	81	Sepsis	79% (<30)	20.8 [13-28]
Braun et al.(61)	2012	1325	Mixed	86% (<30)	18.2 (13.7)
Zajic et al. (62)*	2013	655	Mixed	60.2% (<20)	19.6 (11.3)

Vitamin D status [25(OH)D] is given as mean (SEM) or median [interquartile range]. NA = not available.

**Published only in abstract form.*

1.3. Calcium, phosphorus and bone metabolism

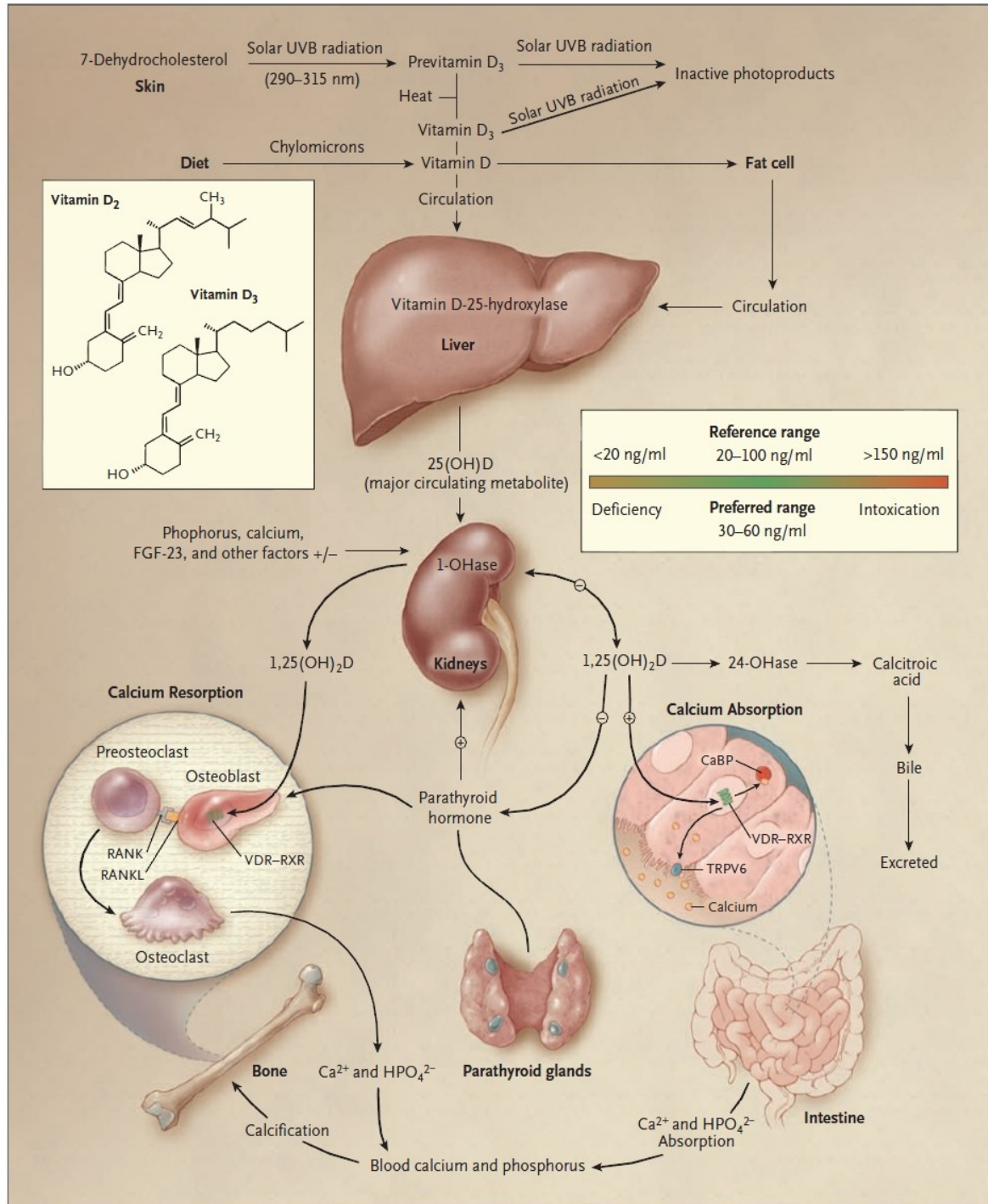
Calcitriol or 1,25(OH)₂D, the active metabolite of vitamin D, is essential to ensure intestinal calcium absorption in the small intestine by interacting with the vitamin D receptor-retinoic acid x-receptor complex (VDR-RXR) to enhance the expression of the epithelial calcium channel protein (transient receptor potential cation channel, subfamily V, member 6 [TRPV6]) and calbindin 9K, a calcium-binding protein (CaBP). Without vitamin D, only 10 to 15% of dietary calcium and about 60% of phosphorus is absorbed. Due to the effect of 1,25(OH)₂D intestinal calcium absorption is increased to 30 to 40% and phosphorus absorption to approximately 80% (5, 29).

Furthermore, 1,25(OH)₂D is recognized by its receptor in osteoblasts, inducing the expression of the receptor activator of nuclear factor- κ ligand (RANKL). RANK, the receptor for RANKL on preosteoclasts, binds RANKL, which is essential for the development of preosteoclasts to mature osteoclasts. Mature osteoclasts remove calcium

Introduction

and phosphorus from bone, maintaining calcium and phosphorus levels in the blood and assuring physiological bone turnover in the skeleton (Figure 1) (5).

Figure 1 Metabolism of vitamin D in the regulation of calcium, phosphorus and bone metabolism



Reproduced from Holick MF. Vitamin D deficiency. N Engl J Med 2007;357:266-81, with kind permission © Massachusetts Medical Society.

PTH guarantees the tubular reabsorption of calcium as it is the key stimulator to produce 1,25(OH)2D in the kidneys (5, 23). Therefore vitamin D deficiency causes a remarkable stimulation of the parathyroid glands to maintain calcitriol levels in the normal range by

activating renal 25-hydroxyvitamin D-1 α -hydroxylase. This compensatory metabolic process results in secondary hyperparathyroidism (26-28, 39, 42).

Consequently, in secondary hyperparathyroidism the mineralized skeletal collagen matrix is dissolved and bone mass is gradually lost through an excessive activation of preosteoclasts differentiating into mature osteoclasts. Elevated PTH levels also cause phosphaturia, resulting in a low or low normal serum level. Without an adequate calcium-phosphorus product, mineralization of the matrix is limited, leading to classic signs of rickets in children and osteomalacia or osteoporosis in adults (5, 63). Finally, PTH increases the metabolism of 25(OH)D to 1,25(OH)₂D, which further aggravates vitamin D deficiency. Calcitriol serum levels are tightly regulated and only at very low 25(OH)D concentrations, circulating 1,25(OH)₂D levels eventually begin to fall (64).

1.3.1. Vitamin D and skeletal health

It is well established that vitamin D is essential for bone mineralization. Nevertheless, numerous studies investigating serum 25(OH)D levels, vitamin D supplementation in randomized controlled trials (RCTs) and skeletal health have yielded conflicting and inconclusive results.

Vitamin D and antifracture efficacy

The efficacy of calcium and vitamin D in preventing fractures was best demonstrated in 3270 elderly French women given 1200mg of calcium and 800IU of vitamin D daily for 18 months, which led to a significant reduction of hip and non-vertebral fractures (43% and 32%, respectively, $p < 0.05$) (37).

According to a recent meta-analysis of high-quality RCTs, doses of vitamin D between 482 and 770IU per day reduced non-vertebral fractures in community-dwelling older individuals by 29% independently of additional calcium supplementation. Antifracture efficacy increased significantly with a higher administered dose of vitamin D and higher achieved blood 25(OH)D levels (65). Another review by the same group (Bischoff-Ferrari and colleagues) revealed a 26% respectively 23% relative risk reduction in hip and non-vertebral fractures in studies using doses of 700 to 800IU vitamin D, while supplementation of 400IU vitamin D demonstrated no positive antifracture effect in older persons (36).

The Women's Health Initiative study that compared the effects of 400IU vitamin D plus 1000mg calcium per day with placebo in more than 36,000 postmenopausal women demonstrated a small but significant improvement in hip bone density but no significant

reduction in hip fracture. Subgroup analyses of treatment adherent women over 60 years of age however, showed a 29% risk reduction in hip fractures with supplementation (66). Evaluation of the RECORD trial in over 5,200 patients showed no antifracture efficacy in elderly participants receiving 800IU vitamin D (67). But two major limitations of this intervention trial should not be neglected. Firstly, 25(OH)D levels were available only in a very small group (n=60, 1.1% of the study population). Furthermore, compliance with medication declined to 63% after 2 years and might have been as low as 45% when non-responders to the questionnaire about compliance were included, which raises questions about the generalisability of the major findings of this study (68).

The results of a 2007 meta-analysis of 29 trials of supplementation with both calcium and vitamin D or with calcium alone suggested that daily supplementation with 1200mg of calcium and at least 800IU of vitamin D resulted in reduced rates of fracture and a modest increase in bone mineral density. However, the association between serum 25(OH)D levels and skeletal outcomes was not confirmed (69).

A 2009 Cochrane meta-analysis by Avenell and colleagues testing the effects of vitamin D supplementation alone and with calcium showed no significant relationship between vitamin D supplementation alone and a reduction in the risk of fracture. However, this study group confirmed the finding of the 2007 meta-analysis that vitamin D plus calcium is effective in reducing the risk of hip fracture in older persons (RR 0.84, 95% CI 0.73-0.96) (70).

Vitamin D and falls

A recent report from the Agency for Healthcare Research and Quality (AHRQ) and Tufts Medical Center, analysing the existing data from observational trials and RCTs, concluded that there was fair evidence of an association between lower serum 25(OH)D concentrations and an increased risk of falls in institutionalized elderly (71).

Bischoff-Ferrari and colleagues reported in a meta-analysis of five randomized clinical trials that vitamin D intake reduced the risk of falls by 22% (corrected OR 0.78, 95% CI 0.64-0.92) among elderly individuals. The pooled risk difference indicated that 15 people would need to be treated with vitamin D to prevent one person from falling (72).

Pfeifer et al. could demonstrate in a double-blind, randomized and calcium controlled trial that falls were reduced by 27% after 12 months respectively by 39% after 18 months in elderly participants receiving 800IU vitamin D per day (73). Another randomized controlled trial conducted over a 5-month period in nursing home residents with 800IU vitamin D per

day plus calcium reported even a 72% reduction in the risk of falls compared with the placebo group (74).

Vitamin D and dose dependency

Several large observational studies addressed the issue, whether there is a threshold level of 25(OH)D below which adverse skeletal outcomes are more likely to occur.

In one Swedish study of 1194 elderly men, levels below 16ng/ml 25(OH)D were associated with a greater risk of fracture (75). The case-cohort study Osteoporotic Fractures in Men (MrOs) revealed similar findings: older men with serum 25(OH)D levels less than 20ng/ml had a higher risk of hip fracture compared to men in the top quartile of total 25(OH)D (≥ 28 ng/ml) (76). In a prospective, nested case-control study of older women the authors observed that women with the lowest 25(OH)D levels (< 19 ng/ml) at study entry had a significantly greater increased risk for hip fracture during the next 7 years compared to women with the highest concentrations (≥ 28 ng/ml) (77).

However, in an observational study of healthy community-dwelling women (n=1471) levels of 25(OH)D below 20ng/ml were not associated with an increased risk of adverse consequences for musculoskeletal outcomes including fracture, falls, bone density or grip strength (78). According to the 2009 meta-analysis of Bischoff-Ferrari et al. (eight high-quality RCTs, n=2426) the risk of falling is related to the amount of vitamin D administered besides achieved serum 25(OH)D concentrations. High-dose supplemental vitamin D defined as a dose of 700-1000IU per day reduced fall risk by 19%, whereas serum 25(OH)D concentrations of 24ng/ml or more resulted in a 23% fall reduction. Falls were not notably reduced by low dose supplemental vitamin D or serum 25(OH)D concentrations of less than 24ng/ml (79).

As a consequence, the Endocrine Society guideline recommends vitamin D supplementation for fall prevention relying mainly on this meta-analysis that demonstrated a dose-response between falls and serum 25(OH)D (11). This approach is heavily criticized by IOM committee members, who raise doubts about the conclusions of the meta-analysis due to significant inconsistencies and misrepresentations of results within the meta-analysis (31).

1.3.2. Calcium homeostasis and bone turnover in critically ill patients

Hypocalcemia in the intensive care unit (ICU)

Despite the tightly regulated PTH/calcitriol axis to maintain extracellular concentrations of ionized calcium within a narrow physiological range hypocalcemia is highly prevalent in critically ill patients (80, 81).

A recently published review elucidated the pathophysiology of hypocalcemia in ICU patients and concluded that beside some clear causative cases (hypoparathyroidism after thyroidectomy, chelation after pheresis, transfusion with citrated blood, acute renal insufficiency, fluid overload after dialysis, pancreatitis or alkalosis) there are also many drugs (antiresorptives, chemotherapeutic agents) associated with hypocalcemia (82).

For many patients, hypocalcemia reflects a hypoalbuminemic state (83) and sepsis is a well-recognized risk factor for this metabolic condition (84, 85). In animal studies inflammatory markers (IL-1 β , IL-6, TNF- α) induced hypocalcemia in association with decreased PTH levels and upregulated calcium-sensing receptors (CaSR) in the parathyroid glands. These observations lead to the suggestion that increased sensitivity of parathyroid cells to the extracellular calcium concentration may be the major responsible mechanism for hypocalcemia in the setting of an inflammatory process such as sepsis (82).

Nevertheless, in a study of 88 medical ICU patients the majority (>50%) had no identifiable etiology for hypocalcemia (86). Vitamin D deficiency may be a plausible explanation, which captured the attention of many intensivists (32). Vitamin D deficiency as a common and treatable cause of hypocalcemia in the intensive care setting is not well appreciated. Lee and coworkers described in two critically ill patients the clear association of vitamin D deficiency with severe hypocalcemia and acute cardiac failure in intensive care, which was reversed by vitamin D supplementation (87, 88). However, the specific contribution of vitamin D status to cardiac function has not been fully elucidated in the ICU population (82). Because of the association of hypocalcemia with worse clinical outcomes including higher mortality rates (86, 89) many intensivists focused on treatment strategies. However, a recent systematic Cochrane review identified no clear evidence that parenteral calcium supplementation impacts the outcome of critically ill patients owing to the lack of RCTs with endpoints on clinical outcomes like mortality, multiple organ dysfunction or ICU and hospital length of stay (90). Therefore, treatment of hypocalcemia in the absence of specific hypocalcemia-attributable symptoms has been called into question for concerns that such medical intervention may even aggravate the disease process in critically ill (82).

Bone hyperresorption in the ICU

Evaluation of bone turnover markers in vitamin D deficient critically ill patients revealed a pattern consistent with dysfunctional bone formation and severe bone resorption.

The study of Van den Berghe and colleagues in 2003 is of utmost interest as it investigated bone turnover markers in vitamin D deficient critically ill patients compared to a healthy control group and examined the effect of vitamin D supplementation on bone turnover markers (91).

Critically ill patients with an anticipated ICU stay of 10 days or longer (n=22) were compared with matched healthy controls and then randomized to daily intravenous vitamin D supplementation of 200IU (low dose) or 500IU (high dose) as long as parenteral nutrition was required. Serum 25(OH)D increased in the high dose vitamin D group but did not reach normal levels at any time point. At intensive care admission critically ill patients showed bone hyperresorption with 6-fold elevated carboxy terminal cross-link telopeptide of type 1 collagen (β CTX) levels and highly elevated urinary collagen cross links. The bone formation markers carboxy terminal propeptide of type I collagen (PICP) and amino terminal propeptide of type I collagen (PINP) remained stable in the high dose intervention group in contrast to a decrease in the low dose group ($p<0.05$ and $p=0.07$ respectively). The bone resorption markers urinary pyridinoline (PYD) and deoxypyridinoline (DPD) increased markedly in both study groups during ICU stay. Serum β CTX levels showed a time-dependent increase and were 3-fold higher in nonsurvivors compared with survivors on the last day of intensive care ($p<0.05$). In summary, bone formation and resorption were uncoupled in vitamin D deficient patients during prolonged critical illness (91).

Bone hyperresorption partly independent of PTH levels was also reported by Nierman and coworkers in a respiratory care unit (RCU) population. This study group could further demonstrate that calcitriol and the bisphosphonate pamidronate caused a significant decrease in bone resorption ($p<0.01$) (52, 92).

Possible reasons for accelerated bone resorption in an intensive care setting include beside vitamin D deficiency also the proinflammatory state, immobilization (93), medication (e.g. antiseizure drugs, corticosteroids, heparinoids and loop diuretics) (94, 95) and profound hormonal alterations such as impaired GH and IGF-1 secretion, low T3-syndrome, secondary hyperparathyroidism, hypogonadism and hypercortisolism (52, 92, 96, 97). This catabolic state of prolonged critical illness could contribute to impaired fracture healing including iatrogenic bone lesions like sternotomy in cardiac surgery and increased risk of new fractures (32). Recently, a large case-control study conducted by Orford and coworkers

showed an increased risk for sustaining an osteoporosis-related fracture after critical illness in elderly women. Therefore the study authors postulated an association between critical illness and subsequent substantial skeletal morbidity (98).

1.4. Nonskeletal actions of vitamin D

In the past few years, many scientists focused on the different functions of vitamin D and its metabolites in a large number of tissues. This has been stimulated by the appreciation that most tissues in the body have receptors for 1,25(OH)₂D and thus clearly are target tissues. Furthermore, a variety of these cells also contains the enzyme (25-hydroxyvitamin D-1 α -hydroxylase, CYP27B1) which is necessary for converting the major circulating metabolite of vitamin D [25(OH)D] to the active form 1,25(OH)₂D. Regulation of extrarenal vitamin D metabolism differs from renal vitamin D metabolism and is more 25(OH)D substrate dependent and not under the feedback control of the parathyroid-hormone axis. Generally, the nonclassic actions of vitamin D can be categorized into three general effects (6):

- regulation of hormone secretion
- regulation of immune function
- regulation of cellular proliferation and differentiation

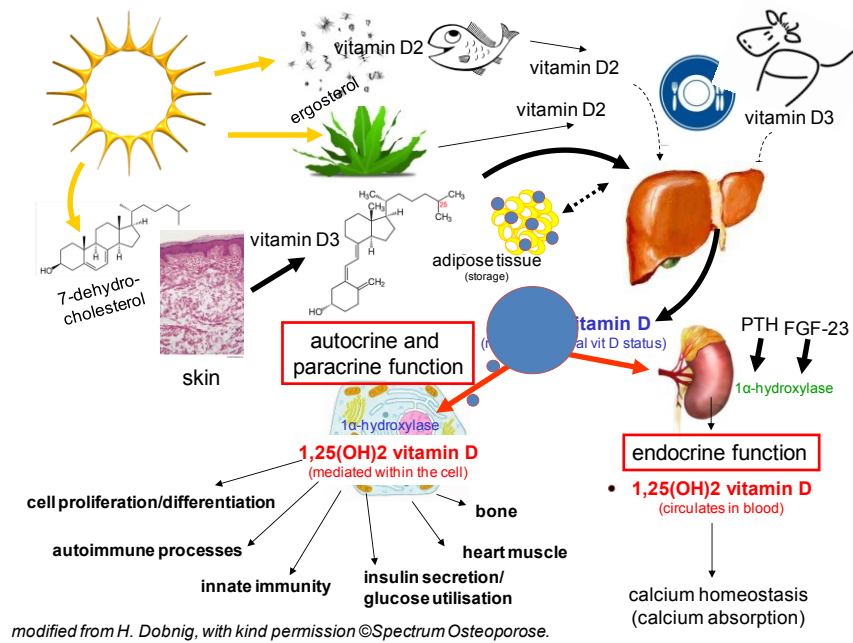
Indeed, these categories are artificial and the effects of 1,25(OH)₂D on any tissue may involve actions in more than one of these categories. Nevertheless, the categories serve as a good overview.

1.4.1. Nonclassic target tissues and vitamin D-mediated effects

Regulation of hormone secretion

The function of 1,25(OH)₂D to maintain normal bone mineral homeostasis has already been mentioned. It inhibits the synthesis and secretion of PTH and prevents the proliferation of the parathyroid gland (99). Furthermore, it stimulates the production of FGF-23 (100). Through these endocrine feedback loops it exerts an important key role in skeletal health and 1,25(OH)₂D or its analogs are able to control secondary hyperparathyroidism in chronic renal failure. Figure 2 gives an overview on autocrine and paracrine effects of 1,25(OH)₂D besides the well known metabolic pathway and endocrine functions.

Figure 2 Overview about the metabolic pathway and endocrine/autocrine functions of vitamin D



Vitamin D seems to have an important effect on glucose homeostasis, which might be of probably therapeutic importance. 1,25(OH)₂D stimulates insulin secretion, but the mechanism is not well understood (101, 102). VDR and calbindin-D28k were found in pancreatic β -cells and based on animal studies using calbindin-D28k null mice, calbindin-D28k might be essential for depolarization-stimulated insulin release (103, 104). Furthermore, calbindin-D28k seems to protect against cytokine mediated destruction of β -cells (105) and early studies have suggested that vitamin D-deficient rodents are not able to adequately secrete insulin compared with vitamin D-sufficient controls (106).

The renin-angiotensin-aldosterone-system (RAAS) is important for regulating blood pressure and mineral metabolism. Pioneering work by Li et al. established that VDR knockout mice have elevated blood pressure, cardiac hypertrophy and a high activity of the RAAS. Furthermore, mice given injections of 1,25(OH)₂D demonstrated suppression of renin mRNA expression (107).

Regulation of immune function

The physiological importance of local synthesis of 1,25(OH)₂D is clearly illustrated by the interaction between vitamin D and the immune system, which has been appreciated nearly 30 years ago with three important discoveries: first, the presence of VDRs in activated human inflammatory cells (108); second, the ability of 1,25(OH)₂D to inhibit T cell proliferation (109) and third, the ability of activated macrophages to produce 1,25(OH)₂D in patients with sarcoidosis (110).

Remarkably, a further 25 years passed before the significance of these observations, especially concerning innate immunity, was clarified. Innate immune responses involve the activation of toll like receptors (TLRs) not only in monocytes and macrophages but also in a number of epithelial cells including those of the epidermis, gingiva, intestinum, vagina, bladder and the lungs (111). TLRs are transmembrane pathogen recognition receptors (PRRs) that bind pathogen-associated molecular patterns (PAMPs) on infectious agents (i.e. *M. tuberculosis*) and consequently, trigger the innate immune response in the host. A heterodimer of TLR1 and TLR2 is known to have a key role in recognizing microbial ligands including bacterial lipopeptides and is responsible for the transcriptional induction of genes like CYP27B1 and the vitamin D receptor (VDR) (112).

This suggests that circulating serum 25(OH)D enters monocytes, is converted to active 1,25(OH)₂D by mitochondrial 25 hydroxyvitamin D-1 α -hydroxylase and then binds to the VDR. The complex of 1,25(OH)₂D bound to the VDR enables the transcriptional induction of cathelicidin expression, which is a potent antimicrobial protein, and the promotion of autophagy. Calcitriol was shown to be essential in the ability of monocytes to kill *M. tuberculosis* following phagocytic uptake of the pathogen (see also Figure 3) (113).

However, this mechanism strongly depends on sufficient availability of serum 25(OH)D, which was elegantly studied by Liu et al. who used monocytes cultured in medium supplemented with serum from populations with pronounced differences in vitamin D status. In *in vitro* experiments, TLR1-TLR2-induced cathelicidin expression was significantly higher when incubated in vitamin D sufficient serum from white North American individuals compared to vitamin D deficient serum from black North American individuals and vitamin D supplementation to cell cultures reversed this difference (113, 114).

In contrast to cathelicidin expression, which is activated by treatment with 1,25(OH)₂D alone, optimal production of β -defensin 4A, another important antimicrobial peptide, requires coincident signaling by nuclear factor kappa B (NF κ B) and Interleukin 1 (IL-1) within the cell (115). Endoscopic studies in humans have demonstrated that β -defensin 4A is secreted in the gastric mucosa after infection with *Helicobacter pylori* and may therefore constitute a major aspect of immune defense against this bacterial pathogen at the mucosal surface (116).

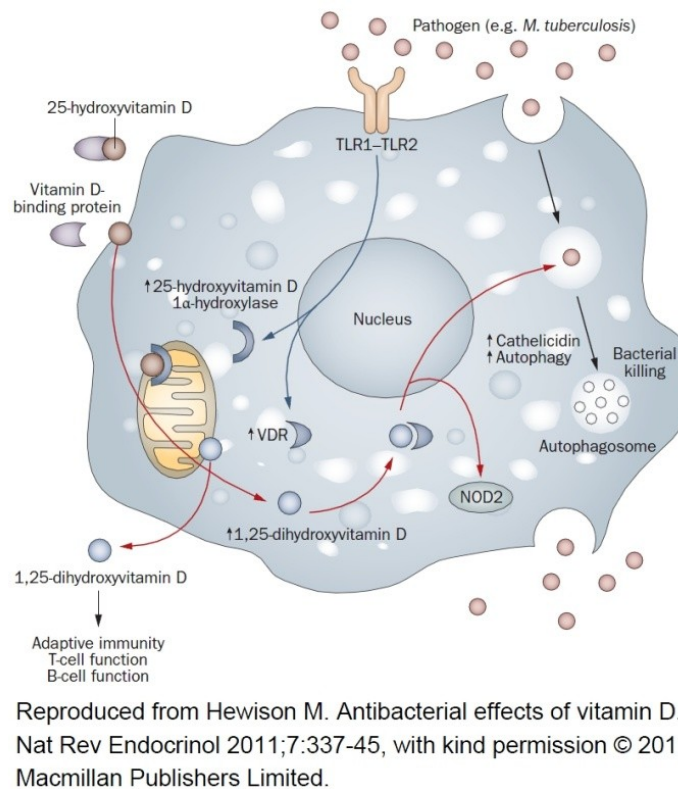
Finally, antimicrobial factors such as cathelicidin and β -defensin 4A begin the killing of internalized pathogens by promoting the fusion of antimicrobial-enriched lysosomes with phagocytic vacuoles. Recently, the induction of autophagy, which is a eukaryotic

mechanism that involves encapsulation of organelles or cell proteins in a double-membrane autophagosome, has also been proposed a role in cellular response to infection (112).

Sufficient levels of local 1,25(OH)₂D seem to be essential to induce monocyte autophagy effectively as a recent report has shown the causal relationship of 1,25(OH)₂D-induced human monocyte antibacterial response to *M. tuberculosis* infection and cellular autophagy in vitro (117).

Much of the current understanding of the antibacterial and autophagic effects of vitamin D stems from in-vitro studies of monocytes and macrophages. Nevertheless, as already mentioned, the innate immune system is widely distributed and operates not only in cells within the lymphopoietic system but also within epithelia which are in direct contact to the outside environment (i.e. skin, respiratory and gastrointestinal tract) and contributes to the protective barrier of these tissues. Therefore, the importance of vitamin D and its analogs as far as antibacterial effects are concerned should not be underestimated (6).

Figure 3 Vitamin D-induced cathelicidin expression and monocyte bacterial killing



Surprisingly, vitamin D fulfils a dual function in the human immune system by promoting antibacterial response to infection as mentioned above whilst helping to prevent an overelaboration of general inflammation. Vitamin D inhibits the adaptive immune system which is responsible for the ability of T and B lymphocytes to produce cytokines and

immunoglobulins and consequently mitigates the action of antigen presenting cells like macrophages and dendritic cells (6).

In particular, 1,25(OH)₂D suppresses immunoglobulin proliferation and production and delays the differentiation of B cell precursors into plasma cells (118).

In addition, 1,25(OH)₂D inhibits T cell proliferation, especially the T helper (Th)-1 cells (109) capable of recruiting macrophages and producing Interferon- γ (IFN- γ) and IL-2, which prevent further antigen presentation to and recruitment of T lymphocytes and T lymphocyte proliferation (119). In contrast IL-4, IL-5 and IL-10 production can be increased (120), shifting the balance to a Th-2 cell phenotype.

In summary, the ability of 1,25(OH)₂D to suppress the adaptive immune system appears to be beneficial for various, especially autoimmune conditions. Bikle emphasizes in his review that in a number of experimental models including inflammatory arthritis, autoimmune diabetes, experimental allergic encephalitis (a model for multiple sclerosis) and inflammatory bowel disease, 1,25(OH)₂D supplementation influenced the disease process positively (6). However, suppression of the immune system might be a high price if this vitamin D-mediated mechanism leads to a decreased immune surveillance.

Regulation of cellular proliferation and differentiation

Regarding vitamin D and its metabolites, the epidermis is unique because it is capable of not only generating previtamin D through ultraviolet irradiation but also converting 25(OH)D to 1,25(OH)₂D and responding to 1,25(OH)₂D within the same cell. As noted previously, 1,25(OH)₂D enables the keratinocyte to strengthen the innate immune response and to suppress the autoimmune mechanisms (6). Additionally, 1,25(OH)₂D promotes the differentiation of keratinocytes moving from the basal layer of epidermis to the enucleated corneocyte and inhibits their proliferation in the basal layer of epidermis (121).

These effects may contribute at least in part to the successful treatment of psoriasis with vitamin D.

The vitamin D endocrine system, which regulates about 3% of the human genome, has been evaluated for its potential anticancer function in animal and cell studies and there exists widespread knowledge that many malignant cell types express the vitamin D receptor (122). The accepted basis for the promising effects of 1,25(OH)₂D in the prevention and treatment of malignancy includes its antiproliferative, prodifferentiating effects on most cell types.

In particular, as mentioned in the review by Bikle, 1,25(OH)₂D stimulates the expression of cell cycle inhibitors p21 and p27 and the expression of the cell adhesion molecule

E-cadherin, while it inhibits the transcriptional activity of β -catenin (6). Epidemiological studies, which paid most attention to cancers of the breast and colon, supported the importance of vitamin D for the prevention of various types of cancers (123-125).

1.4.2. Potential clinical applications for vitamin D and its metabolites

This chapter discusses various clinical disease patterns which might be related to the vitamin D-mediated pleiotropic effects. There is great debate in the literature about the possible beneficial health effects of vitamin D supplementation and the speculation that optimal vitamin D status is able to prevent a spectrum of chronic diseases, although only few large, adequately powered RCTs are currently published for many of these diseases.

Vitamin D and all-cause mortality

The Cochrane review by Bjelakovic et al. published in 2011 analyzed the influence of vitamin D on mortality. In the 50 RCTs included, the authors investigated a total of 94,148 participants randomly assigned to either vitamin D or no treatment/placebo. Vitamin D in the form of vitamin D3 (cholecalciferol) decreased mortality significantly (RR 0.94, 95% CI 0.91-0.98; 74,789 participants) in predominantly elderly women, whereas vitamin D2 (ergocalciferol), alfalcidol or calcitriol had no statistically significant effect on mortality. Another important finding of this review was the observation that vitamin D3 was beneficial only in combination with calcium. Bjelakovic and colleagues speculated that vitamin D3 plus calcium can indirectly decrease mortality by preventing fractures, especially in elderly people (126).

This result fully concurs with findings of a recently published Cochrane review by Avenell and coworkers including 45 RCTs with a total of 24,749 participants which showed that only vitamin D concomitant with calcium could prevent hip fractures. However, this study group found no significant effect of vitamin D on mortality (70).

Recently, randomized trials and meta-analyses of randomized trials that focused on falls and fractures as primary endpoint have concluded that the reduction of risk for falls and hip and non-vertebral fractures is dose dependent (75-77, 79). In contrast to this finding, Bjelakovic et al. demonstrated that vitamin D3 had even a positive effect on mortality in dosages less than 800IU per day (126).

Four large studies in community-based settings in both genders showed a nonlinear association of vitamin D levels with all-cause mortality.

Introduction

The Uppsala Longitudinal Study of Adult Men, a prospective cohort study of elderly men, demonstrated an U-shaped association between vitamin D concentrations and total mortality. An approximately 50% higher total mortality rate was observed among men in the lowest 10% ($<18.4\text{ng/ml}$) and the highest 5% ($>39.3\text{ng/ml}$) compared with intermediate concentrations (range: 18.4 to 37.3ng/ml) (127).

Similar findings were recently reported by a Danish group who conducted a retrospective, observational cohort study (the CopD Study), analyzing serum 25(OH)D from 247,574 subjects from a general practice sector. A serum 25(OH)D level of 20 to 24ng/ml was associated with the lowest mortality risk, whereas the hazard ratios (HR) of all-cause mortality at very low ($<4\text{ng/ml}$) and high ($>56\text{ng/ml}$) serum levels of 25(OH)D were 2.13 (95% CI 2.02-2.24) and 1.42 (95% CI 1.31-1.53), respectively (128).

Effects on mortality were also seen in two large cohorts – in the NHANES III (Third National Health and Examination Survey; 16,818 participants from the non-institutionalized US population) and in the German LURIC population (3316 patients referred for coronary angiography in a tertiary care medical center) - in which an independent negative association between low 25(OH)D levels and all-cause mortality in patients was demonstrated (129, 130).

Zittermann et al. evaluated in a recently published meta-analysis associations of serum 25(OH)D and mortality in prospective observational studies among general populations. According to the data from 14 prospective cohort studies involving 62,548 participants the study group suggested a nonlinear decrease in mortality risk as 25(OH)D levels increases with optimal concentrations reported between 30 and 35ng/ml. In this meta-analysis however, it was not possible to assess the association between 25(OH)D concentrations and mortality risk above 45ng/ml (131). Therefore, a biphasic association of vitamin D levels with mortality rates as previously discussed cannot be excluded and should raise cautions about possible changes of dietary reference intake for vitamin D.

Although the underlying mechanism of how vitamin D deficiency might reduce life expectancy is not completely clear, experimental evidence showed that vitamin D receptor-knockout mice had various metabolic and cardiovascular disturbances and a shorter lifespan (122). A more general mechanism is proposed by the authors of a population-based cohort study in female twins, demonstrating longer leukocyte telomere length with higher circulating 25(OH)D concentrations. Leukocyte telomere length is a predictor of aging-related disease and positively related to longevity (132).

Vitamin D and cardiovascular disease

Cardiac myocytes and fibroblasts express a functional VDR and also the required enzyme 25-hydroxyvitamin D-1 α -hydroxylase for the vitamin D metabolism within the cell (133). Pilz et al. have recently summarized the direct and indirect effects of vitamin D on the myocardium. This comprises factors like the suppression of the cardiac renin-angiotensin-aldosterone-system, regulation of calcium flux and myocardial contractility, effects on differentiation and proliferation of cardiomyocytes and antihypertrophic effects. Indirectly, vitamin D may influence the myocardium by preventing secondary hyperparathyroidism, infectious diseases and through antidiabetic and anti-atherosclerotic effects (134).

Large clinical trials however, show inconclusive and conflicting data related to a variety of cardiovascular outcomes. One British RCT in almost 2700 elderly participants who received either vitamin D (100,000IU) every 4 months or placebo for 5 years found no statistically significant difference in event rates for various cardiovascular outcomes including cardiovascular death (RR 0.84; 95% CI 0.65-1.10) (135).

An analysis of the NHANES III cohort evaluated cardiovascular death in over 13,000 men and women regardless of baseline medical history and compared four categories of serum 25(OH)D concentrations ranging from less than 17.8ng/ml to more than 32.1ng/ml. In a multivariate adjusted analysis no significant association was found between serum 25(OH)D level and cardiovascular death and only a trend toward increased cardiovascular mortality rate was detected in the lowest quartile of 25(OH)D level (129).

The Framingham Offspring Study (FOS) evaluating 1739 persons with no history of cardiovascular disease and a mean age of 59 years found that participants with serum 25(OH)D concentrations less than 15ng/ml were about 60 percent more likely to have a cardiovascular event including myocardial infarction, coronary insufficiency, angina, stroke, transient ischemic attack, claudication and heart failure ($p=0.01$) (136).

In a nested case-control study derived from the Health Professionals Follow-up Study (HPFS), 454 men 40 to 75 years old with no cardiovascular history at baseline and who had a nonfatal myocardial infarction or coronary heart disease death over a 10 year period were matched with 1354 controls. Across four categories of men based on their serum 25(OH)D concentrations, lower concentrations were significantly associated with increased cardiovascular events. Compared with a reference category (men with levels ≥ 30 ng/ml 25(OH)D) those with the lowest levels (≤ 15 ng/ml) had an adjusted relative risk (RR) of 2.1 (95% CI 1.2-3.5; $p=0.02$) (137).

Vitamin D status and cardiovascular risk has also been evaluated in subjects with established cardiovascular disease or end-stage kidney disease. In a prospective German trial of more than 3000 subjects undergoing coronary angiography, severe vitamin D deficiency (25(OH)D <10ng/ml) had 3 to 5 times risk of dying from heart failure or sudden cardiac death during a 7-year follow-up period compared with levels of 25(OH)D above 30ng/ml (138). Furthermore, subjects in the lowest quartile for 25(OH)D had an increased multivariate-adjusted hazard ratio for cardiovascular mortality compared with subjects in the highest quartile for 25(OH)D (HR 2.22; 95% CI 1.57-3.13) (130).

In a recent published parallel-group, double-blind, placebo-controlled trial in 305 healthy postmenopausal women aged 60-70 years the daily intake of 400IU or 1000IU vitamin D3 could not reduce cardiovascular disease risk factors including blood pressure, serum lipid profile, insulin resistance and inflammatory markers (139).

Finally, the results of large placebo-controlled RCTs like the Vital-study investigating cardiovascular risk factors after daily intake of 2000IU vitamin D in over 20,000 people should be waited for to give concise and justified recommendations.

Vitamin D and infectious diseases

The link between vitamin D deficiency and susceptibility to infection has been suggested for many decades mentioning the early observation that children with rickets were more likely to experience infections of the respiratory system, leading to the coining of the term “rachitic lung” (140). The underlying mechanisms of vitamin D and especially innate immunity responses have been considerably clarified during the past 3 decades, which was described in the aforementioned subchapter. Most of our current knowledge encompasses the relationship between the pathogen *M. tuberculosis* and the human innate immunity resulting in the production of antimicrobial peptides. In vitro studies also showed that 50,000-90,000IU of vitamin D inhibited growth or killed strains of important human pathogens like *Staphylococcus aureus*, *Streptococcus pyogenes*, *Klebsiella pneumonia* and *Escherichia coli* (141).

The discovery of nonskeletal functions of this steroid hormone has reinvigorated interest in vitamin D as a potential modulator concerning infectious diseases. The seasonality of viral respiratory tract infections such as those caused by the influenza virus (“the flu”) and the rhinovirus (“the common cold”) was highlighted and Cannell et al. argued that vitamin D status may be a contributor in determining the population’s susceptibility to seasonal influenza outbreaks as well as the degree of associated morbidity and mortality. They called

it the “Hope-Simpson stimulus” dedicated to the British general practitioner and epidemiologist R.E. Hope-Simpson who proposed in 1981 that a “seasonal stimulus” intimately associated with solar radiation explained the remarkable seasonality of epidemic influenza (142).

Furthermore, epidemiologic studies revealed strong associations between seasonal variations in vitamin D levels and the incidence of various infectious diseases including respiratory infection and influenza (142). In a secondary analysis of over 18,000 US individuals of the NHANES III cohort, Ginde et al. found an inverse relationship between serum 25(OH)D concentrations and the incidence of upper respiratory infections (143).

In 2010, Sabetta and colleagues conducted a prospective cohort study showing that serum 25(OH)D concentrations of 38ng/ml or greater were associated with a 2-fold decrease in the number of upper respiratory infections and a case-control study of the NHANES III cohort found an association between low vitamin D status and increased nasal colonization with MRSA (144, 145).

In the excellent review, published by Yamshchikov and coworkers in 2009, 13 controlled intervention trials focusing on vitamin D and treatment of infectious diseases were identified (5 bacterial, 7 viral and 1 helminth). The selected clinical trials demonstrated considerable heterogeneity in baseline demographics, sample size and vitamin D supplementation strategies. Sample size ranged from 24 subjects in a pediatric tuberculosis (TB) trial to 3444 subjects participating in a study of vitamin D and infection in elderly persons. Native vitamin D replacement strategies varied in frequency and dose of therapy ranging from only 40IU vitamin D given daily for 20 years to 100,000IU vitamin D given bimonthly for a year. Only 6 of the 13 clinical trials provided information regarding the effectiveness of their selected repletion strategy by reporting baseline and follow-up 25(OH)D levels in the study population and in 11 of the 13 studies patients were enrolled irrespective of baseline vitamin D status (146).

In contrast to previous clinical tuberculosis studies reporting faster resolution of TB symptoms and significantly higher rates of sputum smear conversion (147, 148), the well-designed trial by Wejse et al. (149) (n=365) demonstrated no clear benefit of adjunctive vitamin D in TB treatment. The major limitation in the latter RCT is the fact that baseline and follow-up levels of 25(OH)D were similar in the intervention and placebo group despite the supplementation of 300,000IU vitamin D in a period of 8 months. Therefore, it is possible that the dose regimen used was insufficient.

Introduction

The effect of vitamin D therapy in viral upper respiratory tract infection was evaluated by Avenell et al. in a large sample size of 3444 community-dwelling elderly subjects who were given 800IU vitamin D or placebo for longer than 2 years, as part of the Randomised Evaluation of Calcium and Vitamin D (RECORD) trial. This study failed to show significant differences in the primary endpoint (fracture prevention) as well as in the second endpoint of self-reported infection rate. The results of the RECORD study, however, should be cautiously interpreted due to poor observed compliance with study medication in the participants and meager increases in serum 25(OH)D levels of the intervention group after vitamin D therapy (150).

Another trial by Aloia et al. included 208 healthy postmenopausal African American women who were given 800IU vitamin D daily or placebo for 2 years and then followed by 2000IU vitamin D or placebo for 12 months. A lower rate of self-reported upper respiratory tract infections or influenza was observed in the intervention group and this effect was further magnified with an increase in the vitamin D dosage from 800IU daily to 2000IU daily vitamin D (151). A follow-up RCT of the same study group around Li-Ng revealed that oral vitamin D supplementation (2000IU cholecalciferol daily) in the winter months compared to placebo treatment did not reduce the incidence and duration of severity of upper respiratory tract infections among ambulatory adults although the statistical trend appeared to favour the vitamin D receiving group (152).

As these investigations yielded inconclusive and mixed results Yamshechikov et al. declared in their review that further research of higher quality RCTs regarding adjunctive vitamin D therapy for tuberculosis, influenza and viral upper respiratory tract illnesses is warranted having in mind more effective vitamin D repletion, larger sample size and measurement of pre- and post-treatment serum 25(OH)D levels (146).

Since then 4 RCTs have been performed and extended our current knowledge of vitamin D and infection. Laaksi et al. conducted a double-blinded RCT in 164 young, healthy Finnish men who were allocated to receive either 400IU vitamin D or placebo for a period of 6 months, and evaluated the number of days absent from duty due to respiratory tract infection during this timespan. The main outcome variable did not differ between the groups but as a secondary analysis, the proportion of men remaining healthy throughout the 6-month study period defined as absent days from duty was significantly greater in the intervention group ($p < 0.05$). Of particular importance is the observation that with a daily amount of 400IU vitamin D only 30% of the participants in the intervention group achieved 25(OH)D levels greater than 32 ng/ml (10).

In a British multicenter RCT Martineau and colleagues investigated 146 adults with sputum smear-positive pulmonary tuberculosis who received either 400,000IU vitamin D or placebo during 42 days in an intensive-phase treatment for pulmonary tuberculosis. Vitamin D treatment did not significantly affect time to sputum culture conversion in the whole study population, which was the predefined primary endpoint. Interestingly, the sputum culture conversion was significantly hastened in participants of the intervention group with the tt-genotype of the TaqI vitamin D receptor polymorphism (HR 8.09, 95% CI 1.36-48.01, $p=0.02$) and this subgroup of patients with tuberculosis might derive clinical benefit from vitamin D supplementation (153).

Finally, a Swedish double-blind RCT published in 2012 demonstrated that in patients with different types of immunodeficiency, the daily intake of 4000IU cholecalciferol caused a significant reduction of infectious symptoms and eliminated pathogens in the nasal fluid, probably due to the effects of antimicrobial peptides. During the study, significantly less microbiological samples were obtained in the vitamin D group ($p<0.05$) and the number of positive findings tended to be higher in the placebo group ($p=0.052$) (154).

In 322 healthy adults however, monthly administration of 100,000IU of vitamin D did not reduce the incidence or severity of upper respiratory tract infections (URTIs) in a randomized, double-blind, placebo-controlled trial. Nevertheless, it should be clearly mentioned that the mean baseline 25(OH)D level of participants was 29ng/ml. Thus the population was vitamin D replete already at the beginning of the study (155).

Vitamin D and autoimmune diseases

Beside genetic and environmental factors it has long been postulated that vitamin D status affects the prevalence of autoimmunity by influencing the adaptive immune system. Vitamin D deficiency has been linked to the development or disease activity of multiple sclerosis, type-1 diabetes mellitus, rheumatoid arthritis, inflammatory bowel diseases such as Crohn's disease, systemic lupus erythematosus and psoriasis (156).

Nowadays, vitamin D deficiency is implicated as a risk factor for autoimmune diseases due to epidemiological and experimental observations which implicate the following key observations: firstly, higher latitudes are associated with higher prevalence rates of autoimmune diseases. Secondly, disease exacerbations are associated with the changing seasons towards the winter months and thirdly, a higher prevalence of vitamin D deficiency was reported in patients with autoimmune diseases compared with matched healthy

controls. Furthermore, attenuation and prevention of autoimmune diseases by vitamin D treatment were shown in animal models of autoimmunity (156).

Results of clinical trials however, remain conflicting. For example, regarding type-1 diabetes mellitus (T1D), Hypponen et al. found a significantly reduced risk for T1D (RR 0.22, 95% CI 0.05-0.89) over an observation period of 30 years in a Finnish birth-cohort study when high-dose vitamin D supplementation of 2000IU per day was given during infancy (157).

Similar to this finding, the case-control multicenter EURODIAB Substudy 2 showed a 33% reduction of T1D in children who received vitamin D supplementation early in life (158). Several clinical trials administering vitamin D or analogues to different groups of T1D patients including recent and latent adult onset patients demonstrated controversial results. A prospective, nonblinded and uncontrolled trial by Aljabri and coworkers showed a correlation between increased serum 25(OH)D levels and decreased glycosylated haemoglobin after supplementation of 4000IU vitamin D daily with calcium for 12 weeks (159). Li et al showed in an open label trial that after administration of calcitriol for 1 year, islet beta-cell function was better preserved as reflected by significantly higher plasma C-peptide levels (160), while another open label study demonstrated a temporarily reduced insulin requirement in recent onset diabetic patients (161).

However, two double blind, placebo controlled trials (n = 34 and 40) showed no benefit of 1,25(OH)₂D on beta cell function and insulin requirement in young adults with recent-onset T1D (162, 163).

Thus for clinical practice, there is still a need for larger, well-designed and placebo-controlled trials to determine efficacy of vitamin D supplementation as an adjunctive therapy in this subpopulation.

Vitamin D and type-2 diabetes mellitus

Type-2 diabetes (T2D) is one of the most common chronic diseases and its complications have become a major cause of morbidity and mortality worldwide. A recent review by Chagas et al. suggested that vitamin D deficiency may predispose to glucose intolerance, altered insulin secretion and the development of T2D, either through a direct action on the vitamin D receptor (VDR) or indirectly by suppressing the low-grade chronic inflammation due to the downregulation of pro-inflammatory cytokines. The latter mechanism could be a main contributor to insulin resistance in T2D (164).

Introduction

Vitamin D may stimulate insulin release by pancreatic β -cells. Furthermore elevated PTH levels were found in impaired insulin secretion. Low vitamin D levels were also associated with macrovascular events in patients with diabetes which may be attributable to effects on blood pressure, the renin-angiotensin-aldosterone-system, endothelial function including the vascular endothelial growth factor or chronic inflammation (165).

Clinically, vitamin D insufficiency and T2D share similar risk factors including obesity, physical inactivity, age, non-white ethnicity and seasonal variation in both glucose and 25(OH)D levels (166). In the previous decade, many observational studies demonstrated a link between insufficient vitamin D status with development of T2D. For example, Mitri and coworkers showed in a meta-analysis of 8 large observational cohort studies that a daily vitamin D intake of 500IU decreased the risk of T2D by 13% compared with a daily amount of 20 IU of vitamin D. Furthermore, individuals with the highest vitamin D status ($>25\text{ng/ml}$) had a 43% lower risk of developing T2D (95% CI 24-57%) compared with those in the lowest group ($<14\text{ ng/ml}$) (167).

The most recent systematic review and meta-analysis evaluating the effect of vitamin D supplementation on glycemic control and insulin resistance comprised 15 randomized controlled trials comparing vitamin D or analogues with placebo. The results of this systematic review suggested a weak effect of vitamin D supplementation in reducing fasting glucose (RR -0.32mmol/l , 95% CI -0.57 to -0.07 , $P=0.01$) and improving insulin resistance in patients (mean difference favouring vitamin D: 0.25 , 95% CI 0.03 - 0.48 , $P=0.03$) with T2D or impaired glucose tolerance. However, the effect size is small and of questionable clinical significance. Moreover HbA_{1c} , the marker of longer term glycemic control, was not significantly altered in patients with impaired glucose tolerance or T2D (165).

Furthermore, the Women's Health Initiative study could not demonstrate an impact on diabetes incidence in 33,951 post-menopausal women randomized to 1000mg calcium plus 400IU vitamin D daily or placebo over a 7 year follow-up period (HR 1.01 , 95% CI 0.94 - 1.10) (168). However, the used dose of vitamin D was very low and probably insufficient to normalize vitamin D concentrations because in a subgroup analysis ($n=3097$) 61% of the participants had 25(OH)D levels below 20ng/ml at baseline.

In a recent published double-blind, randomized, control study in 109 subjects with prediabetes and hypovitaminosis D, high doses of vitamin D supplementation (mean weakly dose of $88,865\text{IU}$) rose 25(OH)D levels rapidly from 22ng/ml to nearly 70ng/ml but had no significant effect on insulin secretion, insulin sensitivity or the development of diabetes during the 1 year study period (169).

In sum, there are only few well-designed, randomized controlled intervention trials evaluating the effects of vitamin D on glucose metabolism in vitamin D deficient patients as the vast majority of available studies is inadequate in regard to design, sample size, duration of the intervention or using vitamin D supplements that frequently did not normalize 25(OH)D concentrations (170). Therefore, well-conducted, suitably powered, randomized, controlled trials with adequate vitamin D doses and control of achievement of sufficient levels are warranted to effectively assess whether vitamin D can reduce the incidence of T2D and the rate of micro- and macrovascular complications.

Vitamin D and cancer incidence/mortality

Cancer research over the last three decades has reported growing evidence that a poor vitamin D status is associated with an increased cancer risk. In brief, observational studies showed that solar UVB radiation was inversely associated with cancer incidence and mortality of 13 cancer sites including bladder, breast, colon, esophagus, gallbladder, stomach, ovary, pancreas, prostate, rectum, kidney, uterine corpus cancer and Hodgkin's lymphoma, even after considering major confounding factors (171).

In 2006, Giovannucci et al. were among the first to show the results of a prospective study relating 25(OH)D levels to cancer mortality by analyzing data from the Health Professionals Follow-up Study (HPFS). In a multivariable Cox proportional hazards model, an increment of 10ng/ml in 25(OH)D levels was associated with a 17% reduction in total cancer incidence (RR 0.83, 95% CI 0.74-0.92) and a 29% reduction in total cancer mortality (RR 0.71, 95% CI 0.60-0.83) (172).

In 2007 Freedman et al. however, did not find a significant association of 25(OH)D levels and total cancer mortality analyzing results from the North-American NHANES III cohort. On the contrary, there was a trend of increased risk of cancer mortality in men whose baseline 25(OH)D were in the two highest categories (32ng/ml upwards; RR 1.35, 95% CI 0.78-2.31, p for trend =0.08 for the highest category) (173).

In the German LURIC population, being older and having much lower 25(OH)D levels compared to the participants of the NHANES III cohort, the risk of total cancer mortality was reduced by 55% (RR 0.45, 95% CI 0.22-0.93) in the fourth versus the first 25(OH)D quartile (174).

Trivedi et al. were the first to analyze cancer mortality as a secondary endpoint in their 5-year double-blind RCT among 2686 British community based individuals aged 65 to 85 receiving 100,000IU vitamin D or placebo orally every 4 months and they could not find a

significant difference between the vitamin D and the placebo group (RR 0.86, 95% CI 0.61-1.20) (135).

Moreover, the 7-year Women's Health Initiative (WHI) trial enrolled 36,282 postmenopausal women across the US comparing a daily supplement of vitamin D (400IU) and calcium (1000mg) with placebo and also evaluated total cancer incidence and mortality as part of multiple secondary analyses. The median serum 25(OH)D level of the study population was 16.8ng/ml and the trial did not find a significant effect of combined vitamin D and calcium supplementation on either the risk of total cancer (adjusted HR 0.98, 95% CI 0.91-1.05) or total cancer mortality (adjusted HR 0.89, 95% CI 0.77-1.03) (175). When interpreting these statistically non significant results, it should be underlined that daily vitamin D doses of 400IU are often too low to sufficiently raise 25(OH)D levels to 30ng/ml or greater, which might be necessary to evoke possible extraskkeletal benefits (11).

A highly cited study is the RCT among 1180 healthy community-dwelling postmenopausal women in Nebraska performed by Lappe et al. which aimed to determine the efficacy of vitamin D3 (1100IU/d) plus calcium (1400-1500mg/d) or calcium (1400-1500mg/d) alone compared to placebo in reducing the incidence of fractures. The vitamin D plus calcium intervention increased mean 25(OH)D levels from 28.8ng/ml to 38.5ng/ml and the risk of incidence cancer being a secondary endpoint was reduced by 60% in the vitamin D plus calcium group (RR 0.40, 95% CI 0.20-0.82, $p=0.013$). As the study group hypothesized that cancers diagnosed early in the study would have been present although unrecognized at entry, this analysis was repeated for women free of cancer after 1 year of follow-up. Then the risk of developing cancer at the end of the study for the vitamin D plus calcium group remained significant with a RR of 0.23 (95% CI 0.09-0.60, $p<0.005$).

This study did not provide data on cancer mortality but the findings of this trial are so far the best evidence to demonstrate that vitamin D supplementation may significantly reduce the risk of cancer in a randomized placebo-controlled trial (176). Nevertheless, further evidence is necessary to strengthen the notion that vitamin D has a causative role in preventing cancer.

1.5. Treatment regimens

1.5.1. Guidelines and recommendations

To date there are two widespread guidelines available which describe the needs of vitamin D supplementation from two different points of view.

Table 2 summarizes the recommended vitamin D intakes of and the differences between the two reports.

Table 2 Vitamin D intakes recommended by the IOM and the Endocrine Society

Group age	IOM recommendations			Endocrine Society recommendations	
	<i>AI^a/EAR^b</i>	<i>RDA^c</i>	<i>UL^d</i>	<i>Daily requirement</i>	<i>UL^d</i>
<i>Infants</i>					
0-6 months	400 IU ^a		1000 IU	400-1000 IU	2000 IU
6-12 months	400 IU ^a		1500 IU	400-1000 IU	2000 IU
<i>Children</i>					
1-3 yrs	400 IU ^b	600 IU	2500 IU	600-1000 IU	4000 IU
4-8 yrs	400 IU ^b	600 IU	3000 IU	600-1000 IU	4000 IU
<i>Males/Females</i>					
9-18 yrs	400 IU ^b	600 IU	4000 IU	600-1000 IU	4000 IU
19-70 yrs	400 IU ^b	600 IU	4000 IU	1500-2000 IU	10,000 IU
>70 yrs	400 IU ^b	800 IU	4000 IU	1500-2000 IU	10,000 IU
<i>Pregnancy/Lactation</i>					
14-18 yrs	400 IU ^b	600 IU	4000 IU	600-1000 IU	4000 IU
19-50 yrs	400 IU ^b	600 IU	4000 IU	1500-2000 IU	10,000 IU

AI, adequate intake; EAR, estimated average requirement; RDA, recommended dietary allowance; UL, upper limit;

^a *AI is estimated as the evidence is insufficient for development of EAR/RDA*

^b *EAR indicates the median intake needs of the general population*

^c *RDA indicates the intake that covers the needs of $\geq 97.5\%$ of the general population*

^d *UL indicates the level above which there is risk of adverse events.*

On the one hand, there is the new public health report on dietary intake requirements for calcium and vitamin D aimed at the North American general population from the Institute of Medicine (IOM), which has been released on November 30, 2010 (30).

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On the other hand, the Journal of Clinical Endocrinology and Metabolism recently published the guideline “Clinical Practice Guideline for the Evaluation, Treatment and Prevention of Vitamin D Deficiency” written by an Endocrine Society Task Force, aimed at persons at risk for vitamin D deficiency (11).

The IOM, at the request of agencies of the US and Canadian governments, assembled a committee to update the Dietary Reference Intakes (DRIs) for calcium and vitamin D on the basis of two extensive systematic reviews conducted by the Agency for Healthcare Research and Quality in 2007 and 2009. They provided evidence-based reviews of research on calcium and vitamin D in relation to both skeletal and extraskeletal chronic disease outcomes.

The IOM committee concluded that available scientific evidence supports a key role of calcium and vitamin D in skeletal health, consistent with a cause-and-effect relationship and providing a sound basis for determination of intake requirements. For extraskeletal outcomes including cancer, cardiovascular disease and autoimmune disorders, the IOM found the evidence to be inconsistent, inconclusive as to causality and insufficient to recommend nutritional intakes. Moreover, some observations suggested an U-shaped curve for several outcomes related to vitamin D (cardiovascular disease, falls, all-cause mortality) with the lowest risk at moderate levels and increased risk at both low and high levels of 25(OH)D.

For vitamin D, the 2011 DRIs are based primarily on bone health outcomes. According to scientific evidence concerning 25(OH)D concentrations, levels of 16 ng/ml meet the needs of approximately half the population (median population requirement or EAR) and levels around 20 ng/ml meet the needs of at least 97.5% of the population (akin the RDA). Intakes of vitamin D required to achieve these 25(OH)D concentrations are shown in Table 2. After age 1, the RDA is estimated to be 600IU/d for all life-stage groups except men and women being 71 and older for whom the RDA is 800IU/d. Due to the wide variation of cutaneous synthesis of vitamin D (seasonality of solar exposure, skin pigmentation, sunscreen use, latitude, outdoor activities and other factors) the DRIs are based on an assumption of minimal or no sun exposure.

Further, it is important to stress that the DRIs are developed for “normal healthy persons” in the North American population without any regard for individuals with specific disease states.

In general, vitamin D safety consists of two concepts including on the one side the safe tolerable upper intake level (UL) and on the other side the measurement of circulating

25(OH)D levels, which clearly complement each other. The IOM committee considered a variety of indicators in the determination of tolerable upper intake levels including hypercalcemia, hypercalciuria, vascular and soft tissue calcification, nephrolithiasis and for vitamin D the U-shaped relationship of some relevant clinical outcomes. For vitamin D, the ULs are now 4000IU/d for ages 9 and older but are lower for children (Table 2). According to the existing evidence intakes of 10,000IU/d or lower have not been linked to hypercalcemia or acute toxicity. However, the IOM committee stated that toxicity is not the appropriate basis for an UL that is intended to reflect long-term intake and to be used as a public health tool. Thus, the committee followed an approach to maximize public health protection and declared that the UL should be seen as a target intake and the risk for harm might begin to increase once intakes surpass this level.

Finally, the IOM committee concluded that the majority of the North American population currently meets its needs for vitamin D as mean 25(OH)D levels have been above 20ng/ml in representative samples. Nevertheless, specific subgroups might need higher doses of vitamin D, in particular those with poor nutrition, dark skin pigmentation and living at higher latitudes or in institutions (18, 30).

In contrast to the IOM report, the Endocrine Society Guideline is notably addressed at diseased populations at high risk for vitamin D deficiency and recommends 25(OH)D screening therefore in chronic kidney disease, hepatic failure, hyperparathyroidism and malabsorption syndromes (cystic fibrosis, inflammatory bowel disease, Crohn's disease, bariatric surgery and radiation enteritis) besides the classic indications rickets, osteomalacia and osteoporosis. Furthermore, some frequently used drugs such as antiseizure medications, glucocorticoids, AIDS medications, antifungals and cholestyramine significantly influence the cytochrom P450 metabolism (thus vitamin D metabolism) and should be taken into account. Some granuloma-forming disorders like sarcoidosis and tuberculosis show abnormal intestinal calcium absorption and bone resorption and should be considered as well when vitamin D therapy is initiated.

The Endocrine Task Force also concluded that African-American and Hispanic people, pregnant and lactating women, older adults with a history of falls and nontraumatic fractures and obese children and adults ($\text{BMI} > 30\text{kg/m}^2$) are prone to vitamin D deficiency and should be evaluated for vitamin D deficiency.

Contrarily to the IOM report, vitamin D sufficiency is defined as a 25(OH)D level of 30ng/ml or higher, while levels ranging from 21 to 29ng/ml are regarded as insufficient and

below 21ng/ml as deficient. Thus, the Endocrine Task Force recommends higher vitamin D intakes for the population at risk for vitamin D deficiency (Table 2) (11).

As a rule of thumb, the daily consumption of 100IU vitamin D raises circulating 25(OH)D by 1ng/ml (177). This means that a person with a baseline 25(OH)D level of 10ng/ml has to take 2000IU daily to achieve a 25(OH)D level of 30ng/ml which is considered as lower target value by the experts of the Endocrine Task Force. However, it should be clearly stated that the increment in circulating 25(OH)D depends on various factors including the body weight, the initial 25(OH)D level, the administered dose and the type of oral vitamin D (177).

The tolerable upper intake levels are 10,000IU/day for the same age group as a dose-ranging study reported that men who received 10,000IU/day of vitamin D for 5 months did not show any alteration in either serum calcium or urinary calcium excretion (178). Additionally, data on skin synthesis of vitamin D showed that in apparently healthy free-living individuals a daily amount of up to 10,000IU is safe as there have not been any reports of vitamin D intoxication after intensive solar UVB irradiation (177, 179).

Finally, the IOM committee and the Endocrine Task Force fully concur in the demand of future large-scale, well-designed RCTs to clarify the effects of vitamin D on nonskeletal outcomes as well as to identify threshold effects and possible nonhypercalcemic adverse effects, while the two disagree on target serum 25(OH)D levels and treating procedures (11, 18, 31). Taken all the aforementioned aspects into account, it makes sense to determine baseline vitamin D status in an individual, then to adjust the daily required oral vitamin D dose according to initial 25(OH)D levels and the individual's body weight and finally to reassess circulating 25(OH)D levels approximately 3-6 months later in order to change the oral dose, if necessary (177).

1.5.2. High-dose vitamin D supplementation

Most individuals who require vitamin D supplementation take either a daily tablet or daily drops, but there is concern that the doses might be frequently inadequate and that compliance with daily medication is likely to be suboptimal (67). Having the half-life of 25(OH)D (2 to 3 weeks) following oral cholecalciferol supplementation in mind (19), a high-dose vitamin D regimen seems to be a tempting option.

Two studies have recently shown safety and efficacy of a single high-dose cholecalciferol administration in rheumatologic and elderly patients (180, 181). Bacon and colleagues scrutinized the effect of 3 different vitamin D₃ regimens orally administered in an elderly

but otherwise healthy population: a 500,000IU loading dose, a 500,000IU loading dose plus 50,000IU/month or only 50,000IU/month. They demonstrated a mean increase in 25(OH)D of 23.2ng/ml in the loading and loading plus monthly groups from baseline to 1 month and thereafter, levels declined to plateaus of 27.6ng/ml and 36.5ng/ml respectively after 5 months. In the monthly group, 25(OH)D levels reached a plateau of approximately 32.1ng/ml at 3 to 5 months. Serum calcium levels were monitored in all participants and not a single case of hypercalcemia occurred. Of interest is that PTH levels were only suppressed by vitamin D treatment in those patients with baseline 25(OH)D levels below 20ng/ml (180).

Similarly, von Restorff et al. showed a mean 25(OH)D level of 32.6ng/ml (mean increase: 26.6ng/ml) after 3 months with a single loading dose of 300,000IU cholecalciferol in 33 rheumatologic patients. Two cases of mild and asymptomatic hypercalcemia (max. 2.69 mmol/l) were reported. However, this study used additional calcium supplements of 500-1000mg/d depending on the dietary calcium intake (181).

Of utmost interest relating to safety issues is the RCT by Sanders et al. in 2256 community-dwelling women using an oral annual high-dose of 500,000IU cholecalciferol. This study demonstrated an increased risk of falls and fractures especially during the first 3 months after study medication intake in the treatment group. The incidence rate for falls was 1.15 (95% CI 1.02-1.30, $p<0.05$) and for fractures 1.26 (95% CI 1.00-1.59, $p<0.05$) in the vitamin D group. The reason for these findings are unknown and serum levels of 25(OH)D were available only in a small subgroup of the study population ($n=131$ at baseline) (182). Nevertheless, taking into account the half-life of cholecalciferol (2 to 3 weeks), an annual high-dose vitamin D supplementation certainly is not an ideal treatment regimen and may even be harmful.

Similar findings were seen in the study by Smith and coworkers, where in women given an annual intramuscular injection of 300,000IU ergocalciferol, a 59% increase in fractures of proximal femur or distal forearm fractures was reported. A major limitation of this otherwise large ($n=9440$), well-designed study is that similar to the above mentioned study, 25(OH)D levels were available only in a small subgroup of 43 participants (0.45%) and this subpopulation was even vitamin D replete at baseline (mean 25(OH)D concentration 56.5ng/ml) (183).

In an intensive care setting the Spanish group around Mata-Granados demonstrated that 60,000IU cholecalciferol administered orally on day 0 and 4 evoked a significant increase of 25(OH)D and 1,25(OH)₂D ($p<0.01$ for both), whereas the application of 2 µg calcitriol

intravenously on alternate days had no impact on these levels in septic patients. Limitations of this study include its design (unclear treatment allocation, no randomization, no blinding) and the lack of relevant safety parameters like calcium levels (56).

In our own double-blind, placebo-controlled pilot RCT, 540,000IU cholecalciferol or placebo was delivered once via feeding tube or orally to 25 critically ill patients at a medical intensive care unit. Already on day 1, 25(OH)D was significantly higher in the intervention group and during the one week follow-up period, 80% of the vitamin D treated patients achieved 25(OH)D levels above 30ng/ml. Notably, the highest 25(OH)D level was 64ng/ml on day 3, which is far below the currently accepted toxicity threshold above 150ng/ml. No hypercalcemia or hypercalciuria occurred (184). Of interest is also the transient rise of 1,25(OH)₂D, which was presumably due to the boost of suddenly available 25(OH)D substrate as serum PTH and calcium levels remained unchanged during that period similar to the observations described earlier by van den Berghe (91). However, we found large, unpredictable interindividual differences in serum 25(OH)D response. Two patients in the intervention group demonstrated either a small (7ng/ml) or no increase (1ng/ml) in 25(OH)D levels (184). Obviously, vitamin D resorption was significantly reduced because of gastrointestinal dysfunction, which is common in an intensive care setting. Therefore, an individual dose finding might be preferable to an “one dose fits all”-approach. Possibly development of intravenous vitamin D formulas would also have the potential to reduce the variable intestinal absorption rates.

1.5.3. Vitamin D intoxication and adverse events

Researchers have proposed 3 major theories about the mechanism of vitamin D toxicity. All involve increased concentrations of vitamin D metabolites reaching the VDR in the nucleus of target cells and causing exaggerated gene expression. Possible mechanisms of vitamin D intoxication after excessive oral vitamin D intake include (185):

- the rise of circulating 1,25(OH)₂D into the toxic range, which increases intracellular 1,25(OH)₂D levels
- the rise of circulating 25(OH)D levels to $\mu\text{mol/l}$ concentration (above 400ng/ml) that exceeds the DBP binding capacity so that free, unbound 25(OH)D enters the cell and enhances direct gene expression
- the rise of circulating concentration of many vitamin D metabolites, exceeding the DBP binding capacity and thereby causing release of free, unbound 1,25(OH)₂D, which enters its target cells

Although all three mechanisms may exist in parallel and contribute to toxic vitamin D effects, the most important one is probably the effect of 25(OH)D itself at supraphysiological concentrations in the circulation. When toxicity due to excessive vitamin D intake is observed, it is rarely accompanied by highly elevated circulating 1,25(OH)₂D levels. This may be contributable to the tight regulation of serum levels by PTH and FGF-23 levels.

In granulomatous disorders such as sarcoidosis and tuberculosis however, consistently elevated circulating 1,25(OH)₂D levels have been described because of the overexpression of unregulated extrarenal 1 α -hydroxylase gene expression especially in macrophages in the face of normal or even low circulating 25(OH)D levels. Thus, even without vitamin D treatment, hypercalcemia may occur in these conditions (185).

In clinical practice, there is also widespread use of 1,25(OH)₂D or other active forms of vitamin D to treat secondary hyperparathyroidism in chronic kidney disease. An overexpression of target cells to these physiologically active vitamin D substances might result in symptoms such as hypercalcemia, hyperphosphaturia and soft tissue calcification (nephrocalcinosis, vascular calcification et al.).

However, this should be clearly distinguished from the oral intake of native vitamin D (177). Toxic vitamin D effects may occur only if circulating 25(OH)D concentrations are permanently above 150-200ng/ml. The hallmarks of vitamin D intoxication are hypercalcemia and hypercalciuria, and the most prominent clinical manifestations are nausea, increased thirst, polyuria, polydipsia, poor mental status, reversible normocytic anemia and renal impairment (186). Vitamin D toxicity is treated mainly by managing resultant hypercalcemia with aggressive hydration and low dietary calcium/vitamin D intake. In severe cases calcitonin, corticosteroids or bisphosphonates are a therapeutic option (187-189).

For ethical reasons, no systematic studies investigated vitamin D intoxication in humans but over the past decades numerous case reports described accidental vitamin D intoxication in patients with established osteoporosis after prescription of excessive therapeutic vitamin D doses and accidentally overfortified dairy products (185).

Araki et al. recently reported two cases of vitamin D intoxication with severe hypercalcemia due to manufacturing and labelling errors of two dietary supplements, which contained vitamin D amounts several hundred-fold higher than declared (1,864,000IU daily and 970,000IU daily, respectively). 25(OH)D concentrations were markedly increased in both patients (1220ng/ml and 645ng/ml) and it took nearly 1 year until the 25(OH)D levels

normalized, while the patients became asymptomatic after 1 month when 25(OH)D decreased below 400ng/ml and calcium fell into the normal range. Serum calcium, 25(OH)D and creatinine were positively associated, but not 1,25(OH)₂D which was maintained in a narrower range (189).

As the intake of vitamin D supplements is worldwide on the rise related to the proposed general health benefits, vitamin D intoxication should be considered in the differential diagnosis of hypercalcemia.

It is however noteworthy that the IOM committee recently stated that already circulating 25(OH)D levels above 50 ng/ml should raise concerns among clinicians about potential adverse effects. This statement is mainly based on several observational findings demonstrating an U-shaped curve with outcome variables like all-cause mortality, cardiovascular disease and falls (18, 30). As a consequence of the RCT by Sanders et al. (182) which had shown an increased risk of falls and fractures among older community-dwelling women with a high-dose vitamin D supplementation, a recent study investigated the effects of high-dose vitamin D regimen on bone turnover markers. Rossini and colleagues showed that a single oral bolus of 600,000IU vitamin D₃ resulted in an acute rise of circulating 25(OH)D levels with a mean peak increment to 67.1ng/ml after 3 days and a parallel increase of biochemical markers of bone resorption. Thus, the authors postulated that vitamin D supplementation exceeding 100,000IU might contribute to an unexpected increase in fracture rate due to acutely increased excessive bone resorption (190, 191).

Furthermore a recently published British study by Turner and coworkers scrutinized the effect of a high loading dose of vitamin D₂ (300,000IU) on vitamin D metabolites and fibroblast growth factor-23 (FGF-23) concentrations in elderly patients being on bisphosphonate therapy. Due to the boost of vitamin D₂, they found large increases in 1,25(OH)₂D and FGF-23 concentrations. In particular the latter has been accused of contributing to defective bone mineralization and extraskeletal complications such as left ventricular hypertrophy, vascular calcification and increased mortality rates in chronic kidney disease (192). However, it is noteworthy that these data do not reflect causality and the underlying mechanisms for potentially harmful vitamin D effects at circulating 25(OH)D levels between 50 and 150ng/ml have yet to be elucidated.

1.6. Vitamin D and scientific gaps in the intensive care setting

1.6.1. Vitamin D deficiency and its potential manifestation in the ICU

Vitamin D deficiency is common in ICU patients and prevalence rates range from 38% to 100% in observational studies (see chapter 1.2). Critically ill patients with prolonged stay in an intensive care unit may develop vitamin D deficiency due to a number of reasons including the lack of exposure to sunlight, malnutrition, decreased renal 1α hydroxylation in acute renal failure, increased tissue conversion of 25(OH)D to 1,25(OH) $_2$ D during acute stress and the inflammatory response (91, 193).

Vitamin D is usually supplemented along with nutritional support in critical care. Approximately 100-300IU of vitamin D2 or vitamin D3 daily is provided in standard enteral and parenteral nutrition regimens (32). This corresponds to the current American Society for Parenteral and Enteral Nutrition (ASPEN) recommendation of 200IU parenteral vitamin D per day (194), while the European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines do not specifically address vitamin D supplementation (195, 196). However, it is questionable whether this relatively small amount is sufficient.

The pleiotropic effects of vitamin D on immunity, mucosal function, glucose metabolism and calcium homeostasis (see chapter 1.3.2) have attracted the attention of many intensivists as they may be fundamental to the common morbidities seen among critically ill patients including the systemic inflammatory response syndrome, sepsis, multiorgan failure and metabolic dysfunction.

Table 3 presents a hypothetical model adapted from Lee et al. (193) elaborating how vitamin D deficiency may be an unrecognized contributor to adverse outcome in intensive care patients.

Table 3 Vitamin D deficiency and potential adverse outcomes in critically ill patients

Known functions of vitamin D	Known associations in general population	Potential manifestations in critically ill patients
Cardiac function	Myocardial infarction, cardiac failure	Cardiogenic shock
Glucose metabolism	Diabetes	Hyperglycemia
Calcium homeostasis	Osteomalacia, osteoporosis	Hypocalcemia, bone hyperresorption
Mucosal barrier function	Inflammatory bowel disease	Mucositis, translocation of bowel flora
Innate immunity	Tuberculosis, URTI	Nosocomial infections, sepsis, septic shock
Adaptive immunity	Autoimmune disease	Systemic inflammatory response syndrome
Muscle function	Myopathy, myalgia	Critical illness myopathy, weaning failure
Vitamin D deficiency Increased mortality Multi-organ dysfunction and increased mortality 		

Vitamin D and inflammation in critical illness

Markers of systemic inflammation including C-reactive protein (CRP), TNF- α and IL-6 are typically increased during ICU stay. Prolonged hypercytokinemia causes multi-organ failure and IL-6 in particular is a critical mediator in the acute response to stress and in the development of the systemic inflammatory response and consecutive multi-organ failure (193).

Van den Berghe and coworkers showed that these circulating levels of inflammation markers were 5- to 400-fold higher upon intensive care admission compared to matched healthy controls ($p < 0.01$). In prolonged critically ill patients, parenteral vitamin D₃ supplementation of 500IU per day reduced CRP and IL-6 levels by approximately 40% and 60%, respectively and the fall of the inflammatory markers was significantly more pronounced compared to the control group receiving only 200IU per day (91).

This cytokine suppressive effect may attenuate the extent and severity of systemic inflammatory response syndrome, which contributes significantly to morbidity in ICU patients.

Lucidarme et al. on the other hand were unable to demonstrate an association between 25(OH)D levels and CRP concentrations at the time of admission in a small ICU cohort (n=134) (55).

Vitamin D and sepsis in critical illness

The multiple functions of vitamin D in the immune system's response to infection lead to the suggestion that it may have an essential role in combating sepsis. Basic science data highlight vitamin D's role in the optimal functioning of the innate immune system mainly by producing antimicrobial peptides (AMP) such as cathelicidins and β -defensins and attenuating the inflammatory cascade. However, it is still uncertain whether vitamin D has a clinically detectable effect on the common pathway of sepsis. Furthermore, the potential effects of vitamin D on other physiologic systems including bone and electrolyte metabolism (bone resorption, hypocalcemia and -phosphatemia), insulin sensitivity, pulmonary and cardiovascular function, make it a challenge to isolate its relationship to sepsis. The existing literature in this field is limited to observational studies (197). Nevertheless, sepsis is highly prevalent in critically ill patients and these patients are also at high risk of additional nosocomial infections including ventilator-associated pneumonia, catheter- and cannula-related infection and antibiotic-associated colitis (32, 198).

A large retrospective cohort study of 2399 critically ill patients revealed a significant association between low serum 25(OH)D levels and the rate of blood culture positivity. However, analyzing septic patients alone, a positive correlation between vitamin D deficiency and mortality could not be verified (199).

A recent small study in 66 patients at the surgical ICU showed a trend towards higher rates of infection and sepsis in those with a serum 25(OH)D concentration of less than 20ng/ml (59). In a prospective cohort study by Cecchi et al. (n=170) vitamin D deficiency was a predictor of mortality in patients with severe sepsis and septic shock in comparison with trauma patients in the univariate analysis, while the relationship became insignificant in the multivariate analysis (200). However, this study was clearly underpowered for survival analyses.

Another study of Ginde and colleagues investigated 81 patients with suspected infection in the emergency department and demonstrated that 24 hours after admission, those with

25(OH)D levels of <30 ng/ml were more likely to have severe sepsis ($p<0.01$) and dysfunction of two or more organ systems ($p<0.05$) (60). In another study, serum cathelicidin levels were positively correlated with 25(OH)D in ICU patients (53), which is of particular interest as these antimicrobial peptides are expressed in most barrier tissues like airway, bladder and gastrointestinal epithelium.

Notably, vitamin D binding protein (DBP) was decreased in patients with sepsis compared to those without sepsis (53), which raises new questions about the role of this carrier protein in mediating vitamin D responses in the ICU.

Of interest is also the potential causal role of low vitamin D levels in the associations between African-American race and winter season on rates of infections and sepsis. In regard to race, African-Americans are more likely to develop sepsis, infections and have higher rates of organ dysfunction with sepsis when compared to Caucasians (197). Furthermore, exemplarily for the US, there is a seasonal variation of respiratory infections and sepsis with higher incidences in the winter months (201). This parallels the annual variations in serum 25(OH)D concentrations in humans reaching the lowest point after winter (5).

In summary, the results of observational studies are mixed but promising. The failure of vitamin D to demonstrate effects in the current literature may be attributable to study design and insufficient power to reveal a mortality difference in septic patients.

Vitamin D and mortality rates in critical illness

A growing body of evidence suggests that vitamin D may influence the risk of mortality in critically ill patients.

Table 4 gives an overview on the existing literature describing associations between vitamin D status and survival from critical illness (57, 58, 61, 199, 202-204).

Introduction

Table 4 Association between 25(OH)D levels and mortality in critical illness

Author	Study details	Main outcome(s)
Annweiler et al. (202), Angers, France	Design: Cross-sectional study Population: 399 elderly patients admitted to an acute care geriatric unit Primary endpoint: Association between admission 25(OH)D levels and in-hospital mortality Reference group: 25(OH)D levels ≤ 20 ng/ml	25(OH)D levels > 20 ng/ml were associated with a reduction in in-hospital mortality (OR 0.65, 95% CI 0.44-0.96)
McKinney et al. (57), Tennessee, USA	Design: Retrospective cohort study Population: 136 veterans admitted to a mixed ICU Primary endpoint: Association between 25(OH)D levels and ICU LOS and overall survival rate Reference group: 25(OH)D levels ≤ 20 ng/ml	Patients with 25(OH)D levels > 20 ng/ml had a higher survival rate (69 vs. 44%) and a lower ICU-LOS (29% vs. 58% stayed 3 days or longer)
Venkatram et al. (58), New York, USA	Design: Retrospective cohort study Population: 437 patients admitted to a medical ICU Primary endpoint: Association between 25(OH)D levels and in-hospital mortality Reference group: 25(OH)D levels ≥ 20 ng/ml	25(OH)D levels < 20 ng/ml were associated with increased risk of in-hospital mortality (OR 8.7, 95% CI 1.03-72.8)
Braun et al. (199), Boston, USA	Design: Retrospective cohort study Population: 2399 patients admitted to 2 ICUs Primary endpoint: Association between admission 25(OH)D levels taken 7-365 days prior to ICU admission and 30-days mortality Reference group: 25(OH)D level > 15 ng/ml	25(OH)D levels < 15 ng/ml were associated with increased risk of 30-day mortality (OR 1.69, 95% CI 1.26-2.26), similar OR were found for in-hospital, 90-day and 1-year mortality.
Matthews et al. (203), Atlanta, USA	Design: Prospective cohort study Population: 258 patients admitted to a surgical ICU Primary endpoint: Association between admission 25(OH)D levels and ICU LOS, ICU-related costs and ICU-related mortality Reference group: 25(OH)D levels > 26 ng/ml	25(OH)D levels ≤ 26 ng/ml were associated with longer ICU LOS ($p < 0.01$), increased ICU-related costs ($p < 0.01$) and a higher ICU-related mortality rate ($p < 0.05$)
Arnson et al. (204), Tel-Hashomer, Israel	Design: Prospective cohort study Population: 130 patients admitted to a medical ICU Primary endpoint: Association between admission 25(OH)D levels and 60-day mortality Reference group: 25(OH)D levels ≤ 20 ng/ml	25(OH)D levels > 20 ng/ml were not associated with a reduction in risk of 60-day mortality but a significant shorter average survival was noted in vitamin D deficient ICU patients.
Braun et al. (61), Boston, USA	Design: Retrospective cohort study Population: 1325 patients admitted to a medical or surgical ICU Primary endpoint: Association between 25(OH)D levels ± 7 days of ICU admission and in-hospital, 30-day, 90-day and 1-year mortality Reference group: 25(OH)D level > 15 ng/ml	25(OH)D levels ≤ 15 ng/ml were associated with increased risk of in-hospital (OR 1.77, 95% CI 1.04-3.01), 30-day (OR 1.94, 95% CI 1.17-3.21), 90-day (OR 1.78, 95% CI 1.14-2.76) and 1-year mortality (OR 1.65, 95% CI 1.12-2.43)

* adapted from Quraishi et al. (205)

25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; LOS, length of stay; OR, odds ratio;

A single definitive threshold level for 25(OH)D and mortality benefit during critical illness remains elusive and causation has not been proved yet which makes future large-scale, well-designed vitamin D supplementation RCTs necessary.

Furthermore, potential confounding factors such as age, gender, sun exposure, socioeconomic status and especially obesity should be clearly kept in mind when designing such RCTs.

1.6.2. Measurement and interpretation of vitamin D levels in the ICU

Although serum 25(OH)D is the best available biomarker to reflect an individual's overall vitamin D status which has led the IOM committee to recommend it as a screening marker (18, 30), interpretation of vitamin D status based on a random 25(OH)D measurement in acute illness should be performed with caution.

Critically ill patients may receive large volumes of intravenous fluids within a relatively short period of time to correct hypovolemia and hypotension, especially in sepsis. Acute expansion of the intravascular volume is associated with reduction in levels of various electrolytes, proteins and blood components due to hemodilution. Therefore, rapid fluid shifts may influence measured vitamin D status due to the fact that more than 99% of 25(OH)D is bound to DBP and albumin. Thus another factor in vitamin D metabolite measurement, not encountered in the general population, needs to be considered in this special setting (24).

Krishnan et al. demonstrated in a small study (n=19) that acute fluid loading of 2 litres resulted in a 35% reduction in 25(OH)D, a 45% reduction in 1,25(OH)₂D with elevation in PTH levels and a 30% reduction of albumin in patients undergoing cardiopulmonary bypass. Serum concentrations of the vitamin D metabolites may require up to 24 hours following the surgical procedure to return to baseline. Approximately 30% of patients would have been classified as being vitamin D deficient if measurement has been undertaken immediately after fluid loading (206). Many ICU patients are often water logged and unable to clear sufficiently a fluid load due to hypoalbuminemia, renal dysfunction and high ADH levels. Consequently, acute fluid load may have a longstanding effect in ICU patients.

Moreover, low levels of albumin and DBP due to inflammatory responses and decreased synthesis are thought to increase vascular permeability and interstitial extravasation. A decline of these binding proteins impairs an efficient 25(OH)D reabsorption at the renal tubule and augments renal clearance of 25(OH)D (205).

In sum, besides the fact that vitamin D correlates with severity of illness and by general definition ICU patients are truly severely ill, acute hemodilution, interstitial extravasation and/or decreased synthesis of binding proteins may explain in part the disproportionately low vitamin D levels in critically ill patients.

Recently, the same Australian group showed in a small observational study that random 25(OH)D measurements in ICU patients may not reflect the 24-h profile of vitamin D, which scrutinizes the observation of widespread vitamin D deficiency based on a single measurement in an intensive care setting (207). Therefore, intensivists should be aware of the above mentioned items in interpreting serum vitamin D levels in critically ill patients. Future studies should assess if it is necessary to perform multiple analyses of 25(OH)D. Besides, further data need to clarify if the determination of total and free 25(OH)D concentrations as well as those of albumin and DBP are helpful in the critically ill population, while it is of little clinical relevance in the general population. This may be essential to get a more realistic and sound approximation of vitamin D status in critically ill patients.

1.6.3. Functional vitamin D deficiency and future directions in the ICU

Paul Lee has recently proposed the theoretical concept of functional vitamin D deficiency in critical illness (32). This means that the clinical consequences of vitamin D deficiency are not only dependent on the severity of vitamin D depletion, but also related to tissue requirements. In such a model, the circulating 25(OH)D pool represents a substrate reservoir for conversion to active metabolites at the tissue level during times of acute stress. It is conceivable that the physiological needs with appropriate and adequate substrate conversion [25(OH)D] to active hormone [1,25(OH)₂D] is generated through a regulatory system (i.e. local/renal 1 α -hydroxylase and PTH axis) in satisfying the demand of tissue requirements. When circulating vitamin D levels are sufficient and tissue requirement is relatively low, organ function is normal and physical health maintained.

In acute stress and critical illness however, multifactorial hypocalcemia is very common and may lead to a compensatory rise in PTH, which would enforce the conversion of 25(OH)D to 1,25(OH)₂D to maintain calcium homeostasis as a result of increased bone resorption and intestinal calcium absorption, respectively. Due to secondary hyperparathyroidism, the consumption of 25(OH)D would further exacerbate vitamin D deficiency. Evidence in support of this hypothesis came from studies that demonstrated that secondary hyperparathyroidism was linked to hypocalcemia and low 25(OH)D levels in critically ill

patients (55, 59, 97). Furthermore, in contrast to the general population, in which calcitriol concentrations are maintained within a normal range even with low 25(OH)D levels (5), calcitriol levels correlate positively with 25(OH)D levels and are up to 50% lower in ICU patients, which suggests substrate deficiency may be more profound in critically ill patients (52, 56, 91, 92).

Therefore vitamin D deficiency in the ICU is a state of mismatch between substrate supply and tissue requirements. Despite stimulation of renal 1α -hydroxylase by PTH, local tissue may be unable to generate adequate 1,25(OH)₂D levels. PTH resistance associated with hypomagnesemia, acute renal failure and relative hypoparathyroidism may further compromise 1,25(OH)₂D formation (32).

Consequently, the combination of severe vitamin D deficiency and enhanced 1,25(OH)₂D tissue requirements in face of an insufficient functioning PTH axis leads to a breakdown of various homeostatic tissue mechanisms probably resulting in increased morbidity and mortality in the ICU. The clinical presentation varies depending on degree of deficiency and extent of tissue requirement and includes several organ systems (see Table 3, page 36).

Over the past decade, the awareness for the importance of vitamin D beyond maintenance of musculoskeletal health has grown substantially and a variety of research groups has focused on the multitude of pleiotropic effects of this hormone.

While serum levels of vitamin D metabolites and their binding proteins (albumin and DBP) are low in critically ill patients, the mechanisms underlying this observation are complex and likely stem from abnormalities in the metabolism and regulation of vitamin D in face of heightened tissue requirement of the active form calcitriol (32).

In contrast to several single laboratory variables such as CRP, leucocyte count, procalcitonin, IL-6, D-dimer and thromboplastin time (208), vitamin D levels may not only predict disease severity and outcome but also contribute to co-morbidities and mortality in the intensive care setting as a causal factor. Additionally, unlike modern therapeutics used in critical care medicine, vitamin D is inexpensive and readily available virtually everywhere and thus may have advantages from an economic point of view as well.

Although preliminary data suggest an association of vitamin D deficient states with adverse clinical outcomes in critically ill patients, a causative role of vitamin D remains unverified. Therefore, the primary aim of future research must be to conduct large, well designed, placebo-controlled RCTs to determine whether vitamin D supplementation improves outcome in critically ill patients.

2. Materials and Methods

2.1. Rationale of the study and hypothesis

Vitamin D plays a pivotal role in calcium homeostasis and exhibits a number of pleiotropic effects that are of special interest to the ICU population. Moreover, based on current knowledge, vitamin D deficiency is associated with disease severity and mortality in critically ill patients (32, 197).

However, so far it is unclear whether a low vitamin D status is causally linked to adverse outcomes or merely reflects poor health status. In contrast to previous clinical trials that failed to show a benefit for correcting hormonal insufficiencies in critical illness (i.e. administration of growth hormone) (209, 210), one should keep in mind that vitamin D does not only act on endocrine, but also autocrine level, which allows for tissue-specific control of 1,25(OH)₂D regulation and action that may be completely independent of circulating calcitriol levels. Additionally, vitamin D has an acceptable safety profile with a broad therapeutic window besides low costs (5, 25).

The hypothesis of this first large RCT worldwide was that supplementation with a sufficiently large dose of oral vitamin D₃ leads to a fast correction of low vitamin D status and possibly to clinical benefits, particularly relating to immune, cardiac and muscle function. Because these potential benefits likely occur at various functional hierarchies, we have chosen “length of hospital stay” as a primary surrogate marker for a change in morbidity in this patient group.

To optimize study design and ascertain safety and efficacy of the chosen loading dose of 540,000IU of cholecalciferol, a pilot trial was conducted in 25 medical critically ill patients with vitamin D deficiency (184). Adverse effects like hypercalcemia or hypercalciuria did not occur in this pilot study and vitamin D levels increased significantly in most patients within two days, although 2 out of 10 patients showed no or only a modest rise at day 7. Consequently, we chose to continue with the same loading dose, but added monthly maintenance doses of vitamin D to the study protocol.

The half-life of 25(OH)D of several weeks following oral cholecalciferol supplementation allowed the administration of high doses. Large loading doses rapidly and safely normalized 25(OH)D levels in elderly patients with vitamin D deficiency and healthy individuals (180, 181, 211). However, after this RCT had been started, it was shown that an annual high-dose

intervention was associated with a higher risk of falls and fractures in elderly patients (182). After careful consideration of potential benefits and this new potential adverse effect of a high loading dose in a different population (elderly community-dwelling women), we decided to proceed with the initial study protocol because a rapid restoration of vitamin D status may still have positive effects on immunity and even muscular function in the critical care population. It is also likely that the time period in which vitamin D may exert its potential benefits is relatively short. In summary, we felt that these possible benefits outweigh this reported risk by far. The study group was aware that smaller doses of vitamin D were likely an effective treatment option for most patients with low vitamin D status within months but this time span may be too long during critical illness. Some other relative contraindications such as sarcoidosis and other granulomatous diseases also needed to be considered before administration of a high loading dose (212, 213).

2.2. Study design and treatment protocol

The VITdAL@ICU trial was an investigator-initiated, non-commercial, double-blind, placebo-controlled randomized clinical trial. This study compared high-dose oral cholecalciferol (vitamin D3) versus placebo treatment in a mixed population of 480 critically ill patients with low 25(OH)D levels at study enrollment (≤ 20 ng/ml). Following an initial loading dose of 540,000 IU of vitamin D3, patients received 90,000 IU of vitamin D3 on a monthly basis for a period of 5 months resulting in a total treatment/observation period of 6 months.

The VITdAL@ICU study was conducted at the Medical University of Graz, Austria, a tertiary care university hospital with a catchment area covering the Southeast of Austria (population approximately 1.5 million). It recruited patients from five ICUs: medical (15 beds), neurological (8 beds), cardiothoracic surgery (13 beds) and two mixed surgery units (12 and 10 beds, respectively).

Ethical aspects and informed consent

The institutional ethical committee of the Medical University of Graz, Austria (reference number 21-214 ex 09/10, EudraCT-Nr.: 2010-018798-39) and the Austrian Agency for Health and Food Safety (AGES) approved the study before initiation. During the study period which lasted from May 2010 to September 2012, annual reports were submitted to

these institutions describing the study progress and several amendments were added in regard to safety considerations.

According to Austrian law informed consent was obtained prior to inclusion into the study. If patients were intubated content and study procedures were explained to them after regaining consciousness. The patient could withdraw from the study at any point in time without giving any reason and any impact on treatment, respectively. A register was kept of all patients evaluated for inclusion and of patients who chose to withdraw from the study.

Eligibility criteria for inclusion and recruitment

Upon admission to one of the five partaking ICUs, all adult patients underwent screening for study participation. Patients ≥ 18 years who were expected to stay at the ICU ≥ 48 hours and were vitamin D deficient [25(OH)D levels ≤ 20 ng/ml] were screened for inclusion and exclusion criteria.

The following patients were not considered eligible for inclusion:

- patients likely to die within the upcoming 24 hours
- with severely impaired gastrointestinal motility (ileus, gastric volume > 400 ml)
- contraindication for study drug application (orally or via feeding tube)
- readmission after participation in the VITdAL@ICU pilot study
- enrollment in another intervention trial
- documented hypercalcemia (total calcium > 2.65 or ionized serum calcium > 1.35 mmol/l)
- patients with a history of granulomatous disease (tuberculosis, sarcoidosis)
- recent kidney stones (≤ 1 year)
- pregnant or lactating women

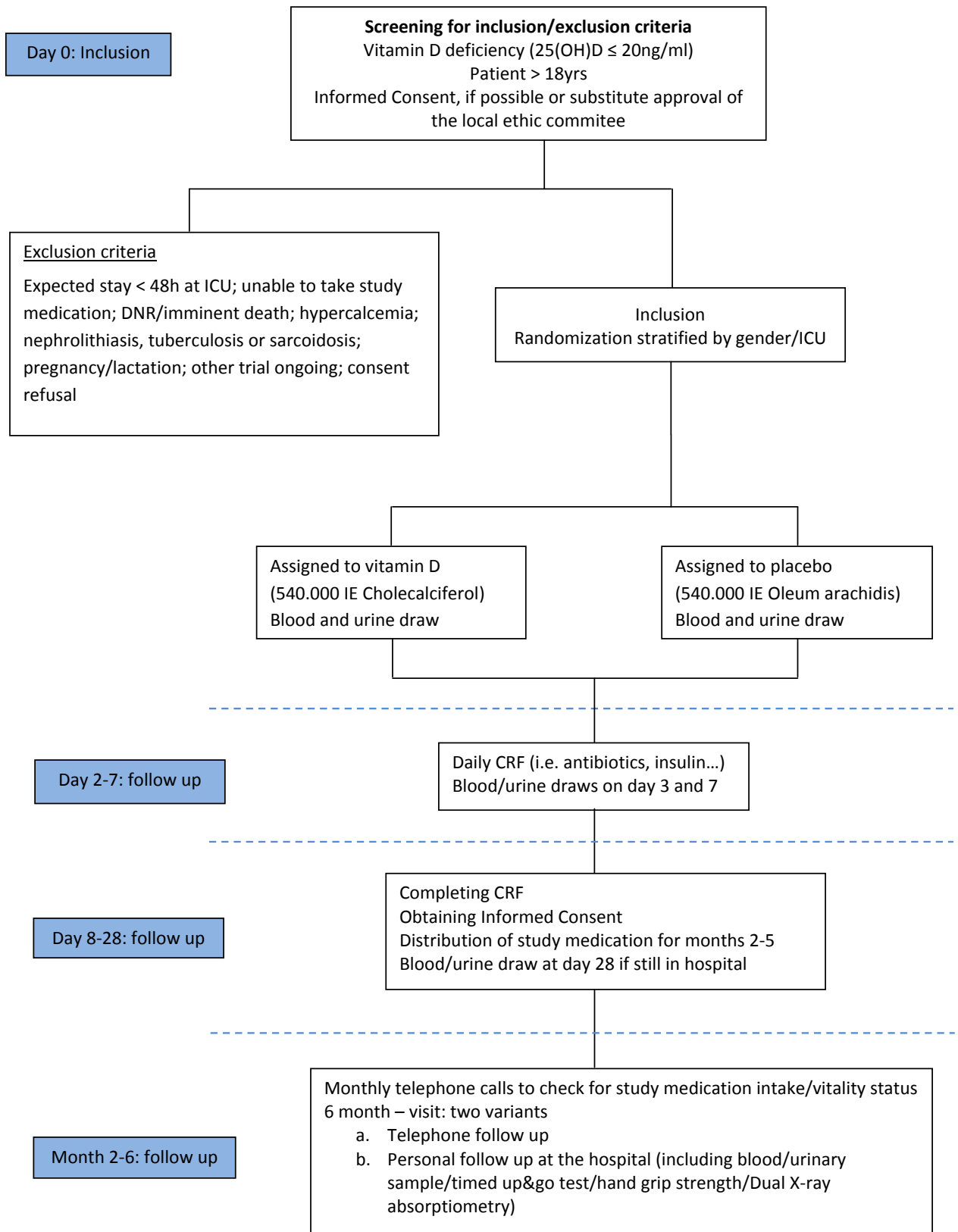
An overview of the study procedures is given in figure 4.

Patients at each of the five ICUs were randomly assigned to one of the two treatment study groups, “Vitamin D” or “Placebo”, in a 1:1 ratio, using the web-based randomization tool “Randomizer for Clinical Trials” (www.randomizer.at). Block randomization was used with patients stratified according to ICU and gender.

Patients who were readmitted to intensive care after participation in the VITdAL@ICU trial were not eligible for reinclusion.

Before study commencement the trial was registered at ClinicalTrials.gov, number: NCT01130181.

Figure 4 Trial procedures flow sheet



Study drug preparation, labelling and intake

The cholecalciferol preparation was provided by Fresenius Kabi, Austria. As placebo, oleum arachidis was used. Identical bottles of study medication containing 180,000IU of cholecalciferol in 15ml of oleum arachidis or corresponding placebo were prepared and labelled at the institutional pharmacy. The study team involved in patient care of the RCT was blinded to treatment allocation and all study-related activities were carried out in a double-blind fashion. Study medication was stored at room temperature. For usage of the study medication outside of the ICU, the patient, next of kin or continuing caregiver/s were instructed by the study team and received oral and written information on how and when to take the remaining study medication. Furthermore, they were asked to refrain from routine use of vitamin D over the following 6 months. Additionally, the patient's family practitioner and, if applicable, treating general ward or rehabilitation facility were personally informed or contacted by telephone and instructed about the study.

Patients randomized to the "Vitamin D" group received a loading dose of 540,000IU of vitamin D3 orally or via feeding tube. This dose equals three bottles of a commercially available vitamin D preparation in Austria (Oleovit D3[®] solution; concentration of cholecalciferol is 400IU vitamin D3/drop of oleum arachidis). Total carrier volume was 45ml. From month 1 to 5, patients received monthly maintenance doses of 90,000IU cholecalciferol. Patients randomized to the "Placebo" group received oleum arachidis in the same volume and time schedule as "Vitamin D" patients.

Patients in both study arms were allowed to receive regular low dose (approximately 200IU per day) vitamin D supplements via enteral and/or parenteral nutrition as deemed appropriate by the treating physicians during stay at the ICU, but were not allowed to receive any vitamin D preparations during their stay at the general ward or at home.

Data collection and management

At baseline, data on demographic and clinical characteristics of the patients were obtained. Simplified Acute Physiology Score (SAPS II), presence of comorbidities (classified according the Charlson Comorbidity Index), medical history, admission diagnosis by category and relevant medication were registered.

The following categories for diagnosis at admission and concomitant diseases were predefined:

- Surgical ICU admissions (including trauma, cardiac surgery, vascular surgery, thoracic surgery, brain surgery, transplantation, other surgical reasons)
- Medical or neurological ICU admissions (including cardiovascular, respiratory, hematological/oncological, sepsis/infectious, gastrointestinal/hepatic, renal, metabolic, neurological, other non-surgical reasons)

In addition, we recorded the need for and the number of days of hemodynamic support (Norepinephrine, Arterenol[®]) and mechanical ventilation including the necessity of tracheostomy as well as the quantity of parenteral vitamin D supplements (Cernevit[®]/Vitalipid[®]). Information on insulin requirements and use of antibiotics were registered on day 0, 2 and 6.

All patients who had left the hospital were contacted by telephone on a monthly basis to check for compliance with the monthly intake of the study drug (5 times) and vital status. The long-term follow-up appointment at 6 months was performed in two ways, dependent on the patient's mobility and preference. Variant A (estimated 80% of the study participants) corresponded to a follow-up visit by telephone while variant B (estimated 20%) included an additional ambulatory visit at the clinic. The following data set was collected in all patients:

- Compliance with intake of study drug (number of maintenance doses)
- Additional vitamin D intake
- Falls and fractures since study inclusion (number)
- Respiratory tract infections (number and use of antibiotics)
- Hospitalizations (number and cause)
- Performance status (Eastern Cooperative Oncology Group (ECOG) Score)
- Quality of life questionnaire

The following clinical examinations were only performed during a personal visit at the clinic:

- Blood and urinary testing (similar to that on days 0, 3, 7, 28)
- Timed Up & Go – Test (in seconds)
- Hand grip strength (in mmHg)
- Dual X-ray absorptiometry (DXA) with bone mineral density (at the hip and the lumbar spine)

Relevant data were manually transferred from source files into paper-based primary Case Report Forms (CRFs) identified by ascending patient numbers specific to respective ICU. Data were then checked for accuracy and transferred to an electronic CRF (“openClinica”, an open source for clinical research software provided by the Joanneum Research Company) by the clinical research assistance team. Extensive verification checks were performed throughout the study by an external study monitor.

Biochemical analyses

Venous blood samples were taken upon ICU admission and in the morning on days 0, 3, 7 and 28 if feasible. Analyses included routine serum chemistry, hematology, markers of inflammation and parameters of calcium and vitamin D metabolism. Additional measurements of markers of metabolism and inflammation as well as other endocrine parameters were planned on stored samples that were frozen and stored at -70°C.

25(OH)D was measured by ELISA (Immunodiagnostic Systems, Boldon, UK). On weekdays, 25(OH)D analyses in our institution were performed on a daily basis.

1,25(OH)₂D was analyzed with ELISA (Immunodiagnostic Systems, Boldon, UK), while parathyroid hormone (PTH) was measured by electrochemiluminescence immunoassay (Elecsys 2010, Roche Diagnostics, Mannheim, Germany).

Calcium in serum and urine was analyzed by o-Kresolphthalein-complexon (Cobas analyzer, Roche Diagnostics, Mannheim, Germany).

N-terminal prohormone brain natriuretic peptide (NT-proBNP) was measured by electrochemiluminescence (Elecsys 2010, Roche Diagnostics, Mannheim, Germany).

The inflammatory markers C-reactive protein was analyzed by immunoturbidimetric assay (Cobas analyzer, Roche Diagnostics, Mannheim, Germany), while procalcitonin was measured by electrochemiluminescence immunoassay (Thermo Scientific B R A H M S, Hennigsdorf Germany).

The actual timings of all assessments performed during the VITdAL@ICU study are given in Table 5.

Materials and Methods

Table 5 Overview of time schedule

<i>Study visit</i>	<i>Day0</i>	<i>Day2</i>	<i>Day3</i>	<i>Day6</i>	<i>Day7</i>	<i>Day28</i>	<i>6 month FU</i>
visit number	1	2	3	4	5	6	7
inclusion/exclusion criteria	x						
signed informed consent	x	x	x	x	x	x	
randomization	x						
demographics	x						
relevant medical history	x						
recent and concomitant medication	x						
<i>Safety evaluation</i>							
blood draws*	+/- 0		+/- 48 hours		+/- 48 hours	+/- 7 days	+/- 1 month
laboratory assessments	x		x		x	x	X [‡] (20%)
urinary analysis	x		x		x	x	X [‡] (20%)
vital status	x	x	x	x	x	x	x
number of falls/fractures							x
<i>Outcome variables</i>							
laboratory assessments	X		X		X	X	X [‡] (20%)
urinary analysis	X		X		X	X	X [‡] (20%)
use of insulin/antibiotics	X	X		X			
use of parenteral/enteral formulas	X	X	X	X	X	X	
duration of mechanical ventilation	X	X	X	X	X	X	
need for vasopressor therapy	X	X	X	X	X	X	
upper respiratory tract infections							X
performance status (ECOG score)							X
quality of life (SF 12)							X
timed up & go test [‡]							X (20%)
hand grip strength [‡]							X (20%)
DXA ^{‡**}							X (20%)
<i>Study medication</i>							
drug dispensing	x				x	x	
drug accountability / compliance check	x					X	x
drug return					x	x	x

* Blood draws: For timing of blood draws the tolerance for still acceptable points in time will be as follows: 48 hours for scheduled blood draws at days 3 and 7; 1 week for the scheduled blood draw at day 28; 1 month for the scheduled blood draw at month 6.

** DXA: dual energy x-ray absorptiometry

‡ Only with personal follow-up at the Medical University of Graz (estimated 20%)

2.3. Study methods

2.3.1. Patient populations and analysis sets

Primary analysis set

The intention-to-treat population included all patients who received at least one dose of medication. Study participants who did not provide an informed consent after regaining consciousness and refused to provide any more information were excluded from the study and were not included in any statistical analysis. All patients included here were analyzed according to the treatment assignment during randomization.

Safety population

The safety analyses were based on the treated set which was defined as all randomized patients who received at least one dose of trial medication. Study participants who did not provide an informed consent after regaining consciousness and refused to provide any more information were excluded from the study and will not be included in any statistical analysis. All patients were analyzed according to the treatment they actually had received.

Interim safety analyses

Three interim safety analyses were performed after 100, 240 and 360 patients had been included into the study. Because of the high dose of cholecalciferol given at study enrollment we decided to use the 28-day-mortality as a main safety parameter. The independent biometrician and the institutional review board then advised the study team to continue the study to completion because no statistical significant difference had occurred between the two groups (however, there was a trend after 240 patients; exact Fisher test, $p=0.091$).

Other safety endpoints considered were ICU and hospital mortality, 6-month mortality as well as serum 25(OH)D and calcium levels. The vital status was determined for all patients up to month 6. In cases where the exact hour of death was unknown, 12 am was used for the analysis. If there was a discrepancy between time of death reported in the hospital discharge file (openMEDOCS) and the time given in the medical record (physician report or discharge letter, time given on a chart...), the verified time given on the latter documents were considered. 25(OH)D and serum calcium levels were measured at day 0, 3, 7 and, if available, at day 28 and month 6 in order to recognize vitamin D intoxication [25(OH)D >150ng/ml], hypercalcemia (total calcium >2.65mmol/l, ionized calcium >1.35mmol/l) and/or hypercalciuria (urinary calcium-creatinin-ratio >0.60). Prompted by the study of

Sanders et al. which had demonstrated a higher risk of falls and fractures in the annual high-dose vitamin D group in elderly women, the study team decided to add the number of (self-reported) falls and fractures in the 6-month follow-up as an additional safety endpoint.

Blood sample for laboratory evaluations were collected at day 0, 3, 7, 28 and at month 6 in case of a personal follow-up. Laboratory data recorded were analyzed quantitatively and qualitatively. Qualitative analyses were done comparing the laboratory data to their reference ranges. Values outside the reference range were tabulated.

Missing data

All patients should complete all the required assessments at each visit. The eCRF database included all data items as they were recorded. All available data were used in the analyses and data summaries. There was no imputation of any missing data. To ensure that the original data were always available, any derived variables were denoted as additional variables.

2.3.2. Primary endpoint: length of hospital stay

For the present study „the length of hospital stay“ (LOS) was defined as the time elapsed between application of study drug at the ICU until discharge from the hospital or a comparable institution (in hours) or death. Further stays at other health care facilities like nursing homes or rehabilitation centers were not considered. This implied that patients who had been transferred from LKH Universitätsklinikum Graz to another hospital were carefully followed up and necessary information was obtained either by telephone call or in written form. In cases where information from a peripheral hospital only included “day” and not the “exact hour” of discharge, the point in time for analysis was arbitrarily set to 12am. The time needed for transportation from one hospital to the other was included in the calculation of the “length of hospital stay”. A listing and assignment of all institutions to either “hospital or hospital-like institutions” or “health care and rehabilitation facilities” to which participating patients had been transferred to was independently classified by two members of the study team before unblinding the study data (see Appendix).

2.3.3. Secondary endpoints

Percentage of patients with 25(OH)D levels ≥ 30 ng/ml at day 7 in the intervention group

Length of ICU stay

The length of ICU stay for this study was defined as the time elapsed between application of study drug at the ICU until discharge from ICU.

The following intermediate-care facilities were considered as “ICU-institutions”:

- Intermediate “medical/respiratory care unit” (Department of Internal Medicine)
- CK GMIÜ (Department of Surgery).

The following units were not taken into account for ICU stay because they did not fulfil the criteria of an ICU (patients were only monitored):

- CK HEIÜ (Department of Surgery, Division of Cardiac Surgery)
- CX TXIMC (Department of Surgery, Division of Transplantation Surgery)
- NK Stroke Unit (Department of Neurology).

In analogy to the above stated remarks, study patients transferred to external ICUs were carefully followed up and necessary information was obtained either by telephone calls or in written form. In cases where information from a peripheral hospital only included “day” and not the “exact hour” of discharge from ICU this point in time for analysis was arbitrarily set to 12am. The time needed for transportation from one ICU to the other was included in the calculation of the “length of ICU stay”. A listing of all ICUs to which participants of the study had been transferred to, was included in the appendix.

In cases where study participants had been transferred from an ICU to a hospital ward and were readmitted to an ICU within 48 hours, the “length of ICU stay” also included the time the patient had spent at the hospital ward. Readmissions later than 48 hours were handled and analyzed as “hospital stay”.

Need for and duration of mechanical ventilation during ICU stay

The need for and duration (in hours) of mechanical ventilation were compared between the two study groups and was defined as starting with application of study medication. It comprised only the period of the endotracheal intubation and possible alternate intubation times were added up and analyzed in total. These data were merely documented for the time spent at the ICUs of the LKH Universitätsklinikum Graz and were not considered for stays at external ICUs.

Furthermore the percentage of patients who had received a tracheostomy during ICU stay was documented and compared between the two study groups.

Need for and duration of vasopressor therapy during ICU stay

The need for and duration (in hours) of vasopressor therapy were compared between the two study groups and defined as starting with application of study medication. Only Norepinephrine (Arterenol®), the most important vasopressor, was studied. If several periods of norepinephrine were necessary, they were added up and analyzed in total. Other vasopressor agents like dobutamin, levosimendan etc. were not taken into account. These data were only documented for the time spent at the ICUs of the LKH Universitätsklinikum Graz and not collected for stays at external ICUs.

NT-proBNP levels at day 0 and 7 compared in both study groups (covariate day 0)

C-reactive protein and procalcitonin values at day 0 and 7 compared in both study groups (covariate day 0)

1,25(OH)₂D and PTH at day 0 and 7 compared in both study groups (covariate day 0)

TISS-28 score at day 0 and 7 compared in both study groups (covariate day 0)

Need for parenteral/enteral nutrition during ICU stay and number of vitamin D supplements

The need for parenteral/enteral nutrition was analyzed as a dichotomous variable (yes/no). Moreover, the mean daily dose of vitamin D supplements (Cernevit®, 220 IU and Vitalipid®, 200IU), which was 210 IU vitamin D per ampoule and routinely administered during ICU stay, related to the total time of ICU stay was compared between the two study groups.

Use of insulin and antibiotics at day 0 and 6

The requirement for insulin treatment (intravenous or subcutaneous) and the need for and number of antibiotics at day 0 and 6 were compared between the two study groups using day 0 as a covariate.

2.4. Statistical methods

All clinical and safety data collected in the study were analyzed and reported with SAS v9.2 procedures in a Windows XP environment.

Analysis of primary endpoint

The primary hypothesis:

H0: There is no clinically important difference in the length of hospital stay in participants treated with vitamin D versus placebo.

The primary analysis for comparing length of hospital stay between the two groups was made using the Mann-Whitney U-test.

Sensitivity analyses considered time to hospital discharge as survival endpoint with death as competing risk.

Analyses of secondary endpoints

For secondary endpoints, differences between the two groups were evaluated with the t test or Mann-Whitney U-test for continuous variables as appropriate. Laboratory parameters having a skewed distribution were log-transformed. For categorical variables the chi-square test or Fisher Exact test were used. Survival endpoints were displayed in Kaplan-Meier plots and compared by the log rank test.

Predefined subgroup analysis

As far as the primary and secondary endpoints are concerned, the vitamin D intervention group was analyzed separately for study patients with baseline 25(OH)D >12 and ≤ 12 ng/ml. As patients with 25(OH)D ≤ 12 ng/ml show a severe vitamin D deficiency which is even associated with osteomalacia, a high-dose supplementation may achieve a more pronounced statistical significance in the investigated endpoints.

Sample size calculation

The sample size was calculated to detect a difference in mean length of hospital stay of 2 days with 80% power and a significance level of 5%. Using the Mann-Whitney U-test for sample size calculation, a group size of 234 was needed to show a reduction of 2 days (equivalent to 14%) of the primary study outcome based on a mean hospital stay of 14 days and standard deviation of 7 days for the control group. To consider drop-outs of the study, a sample of 480 patients (240 per arm) was considered appropriate.

Demographic and baseline characteristics

In a summary of demographic, baseline and diagnostic characteristics (age, weight, height, sex, SAPS II, Charlson comorbidity index, laboratory parameters) a comparison of the treatment groups took place. To this end appropriate descriptive statistics were applied.

Presentation

Continuous data were described by means, standard deviations, medians and upper and lower quartiles as appropriate. The number of observations and minimum and maximum values were also included. Categorical data were summarized using frequencies and percentages.

My personal role in this intervention trial

I have been part of the VITdAL@ICU-study team from February 2010 to May 2013 as a student of medical science in order to prepare this thesis to obtain the academic degree “Doctor medicinae universae et scientiae medicae”. I was involved in all study related procedures, especially for the following items:

- Correspondence with the institutional ethical committee of the Medical University of Graz, Austria and the Austrian Agency for Health and Food Safety (application, amendments, annual reports)
- Introductory discussions with physicians and ICU staff of the participating ICUs
- Daily 25(OH)D screening at ICUs, recruitment, informed consent and medical follow-up (6 months) of 480 patients (start: May 2010; end: September 2012)
- Data ascertainment of external transferred study patients
- Assistance in the creation of an electronic database in cooperation with Joanneum Research Company (DI Bernd Tschapeller) and supervision of electronic data entry
- Cooperation with Mag. Berghofer (study monitor) especially for monitoring of data input and control of the trial master file
- Data transfer to the responsible statistician (Prof. Berghold, Institute for Medical Informatics, Statistics and Documentation), answers to data queries and ambiguities, transcript of the SAP, preparations of several study team internal meetings including the final Blind Review Meeting (20th December 2012) with the following participants: Prof. Dobnig (principal investigator), Prof. Pieber (investigator), PD Amrein (investigator), Dr. Schnedl (investigator), Prof. Berghold (biometrician), DI Riedl (biometrician) and PD Plank (consulting ICU physician) (start: October 2012; end: March 2013)

3. Results

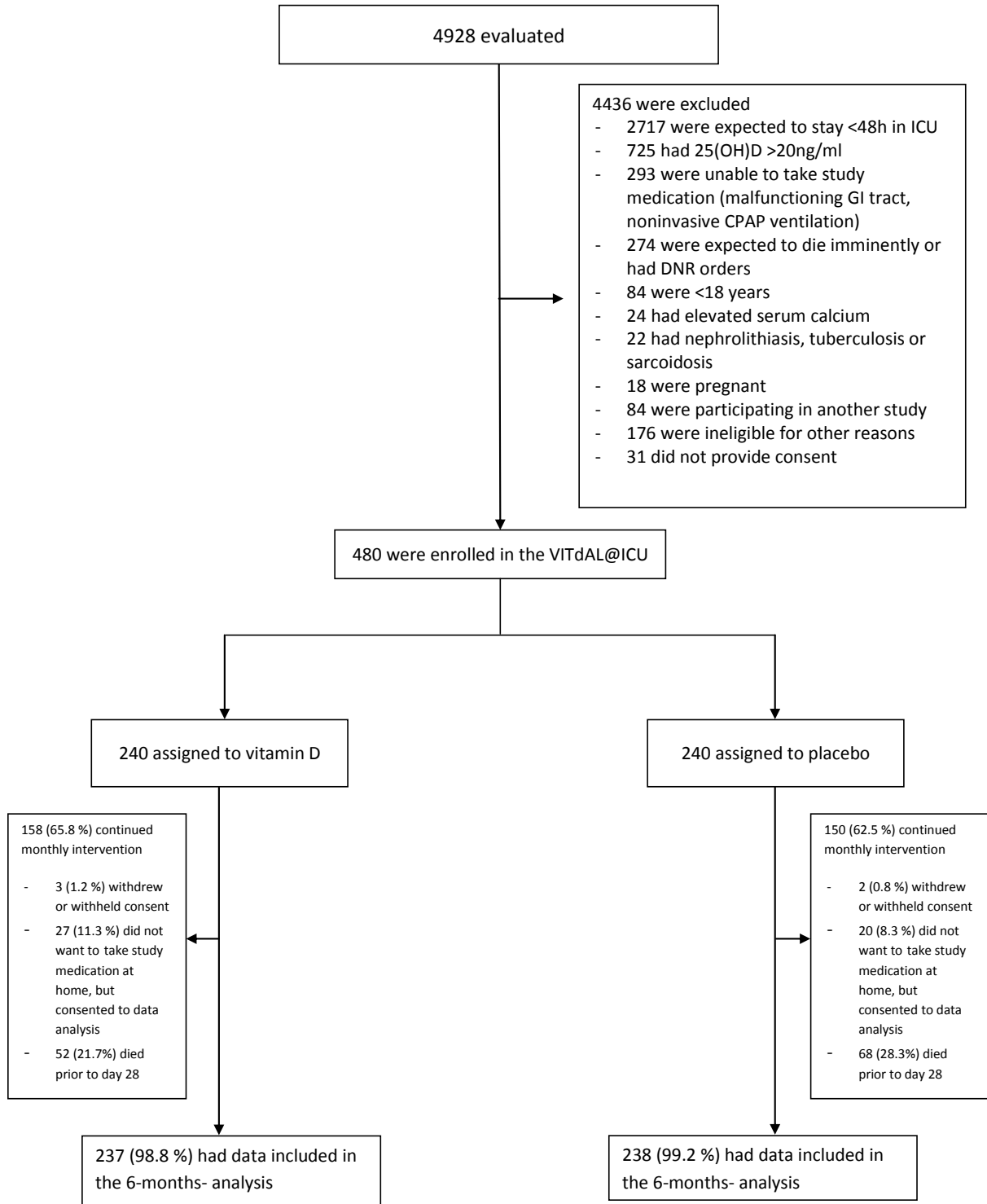
3.1. Study intervention

A total of 4928 patients were evaluated for study inclusion. 4436 were excluded because of one or several exclusion criteria. The reasons are presented in figure 5. Therefore, 480 patients were enrolled in the study and randomized to the study medication (540,000IU vitamin D or placebo). After regaining consciousness, 5 patients refused to give informed consent for further study procedures and were therefore excluded from all analyses (lost to follow-up). Consequently, a total of 475 patients are analyzed on an intention-to-treat basis. 47 patients (9.8%) did not want to continue the monthly study medication (90,000IU vitamin D or placebo), but agreed to data analysis. 120 patients (25%) died prior to day 28 after study inclusion and therefore did not take maintenance doses. 308 patients (64.2%) continued to ingest the monthly study medication and 189 of them (39.8%) took all maintenance doses up to month 5. Among the remaining 119 patients, 65 died during the follow-up period while 54 lost their study medication.

Results

Figure 5 Enrollment of patients in the VITdAL@ICU study

All adult patients admitted to five intensive care units (ICU; medical, neurological, cardiac surgery and two mixed surgery units) from May 4, 2010 onward were eligible for inclusion. GI gastrointestinal, CPAP continuous positive airway pressure



3.2. Clinical and biochemical characteristics

Clinical and biochemical characteristics of all subjects are shown in the following tables (Tables 6-10). Biochemical characteristics are furthermore analyzed according to the five study time points respective to the study medication (vitamin D or placebo). Continuous data are presented as means $\pm$ standard deviation and median [minimum-maximum] or [interquartile range], categorical data are shown in total numbers (percentages).

Table 6 Clinical baseline characteristics of the two study groups

Baseline characteristics		Placebo N=238		Vitamin D N=237		p-value
		n mean $\pm$ SD	% med [min-max]	n mean $\pm$ SD	% med [min-max]	
Center*	mixed surgical ICU	44	18.5	46	19.4	
	cardiothoracic ICU	69	29.0	68	28.7	
	mixed surgical ICU	14	5.9	13	5.5	
	medical ICU	53	22.3	52	21.9	
	neurological ICU	58	24.4	58	24.5	
Gender*	Male	155	65.1	154	65.0	
	Female	83	34.9	83	35.0	
Age (yrs)		65.3 $\pm$ 14.0	68 [20-90]	63.9 $\pm$ 15.5	66 [18-92]	0.443
Weight (kg)		79.8 $\pm$ 17.4	78.5 [45-178]	78.6 $\pm$ 16.4	79.5 [38-155]	0.707
Height (cm)		171.5 $\pm$ 8.9	172 [148-196]	167.0 $\pm$ 8.8	170 [147-190]	0.085
BMI (kg/m ²)		27.1 $\pm$ 5.5	26.2 [16.6-62.3]	27.2 $\pm$ 5.0	26.7 [14.3-48.4]	0.636
Nicotine packyears (py)		35.4 $\pm$ 19.6	37.5 [1-90]	33.3 $\pm$ 21.8	40 [3-88]	0.533
Charlson Comorbidity Index**		3.2 $\pm$ 2.2	3 [0-11]	3.0 $\pm$ 2.2	3 [0-9]	0.221
SAPS II (ICU admission)†		34.2 $\pm$ 15.7	31 [6-92]	32.4 $\pm$ 15.0	30 [6-97]	0.265
TISS 28 (study start)‡		38.0 $\pm$ 8.2	38 [17-60]	37.7 $\pm$ 7.6	38 [16-63]	0.706
Admission diagnosis	Sepsis	18	7.6	19	8.0	-
	Cardiovascular	32	13.4	28	11.8	
	Neurological	61	25.6	54	22.8	
	Other non-surgical	36	15.1	40	16.9	
	Cardiac surgery	44	18.5	45	19.0	
	Trauma	18	7.6	23	9.7	
	Other surgical	29	12.2	28	11.8	

* Stratification criteria for randomization

** The Charlson comorbidity index (CCI) includes 19 comorbid conditions to predict general health status with higher scores indicating a wider range of comorbidities (214).

† Simplified Acute Physiology Score (SAPS II) ranging from 0 to 163 points is a severity of disease classification system and a frequently used ICU scoring system (215).

‡ Therapeutic Intervention Scoring System (TISS-28), reliable indicator of the use of nursing manpower (216).

Results

Baseline demographic and clinical characteristics of the patients were well matched between the two study groups (table 6). We quantified the severity of illness according to the Simplified Acute Physiology Score (SAPS II) on a scale from 0 to 163 points with higher scores indicating a greater severity of acute illness (215). The Charlson comorbidity index (CCI) was used to describe pre-existing chronic comorbidities of the investigated study population (214). The criteria of the therapeutic intervention scoring system (TISS-28) were applied to quantify the demands of nursing activities (216). SAPS II- and TISS-28 values were extracted from routinely determined data.

Tables 7-10 show descriptively the main metabolic and endocrine biochemical parameters related to the two study groups. Continuous data are presented as means $\pm$ standard deviation or median [minimum-maximum] as appropriate. A reference table with all listed parameters is found in the appendix.

Table 7 Metabolic biochemical characteristics of VITdAL@ICU subjects – Placebo group

	Placebo group				
	Day 0 (n=218-237)	Day 3 (n=177-217)	Day 7 (n=148-196)	Day 28 (n=45-58)	Month 6 (n=39-43)
Metabolic biochemical parameters					
Calcium (mmol/l)	2.05 $\pm$ 0.20	2.11 $\pm$ 0.19	2.16 $\pm$ 0.20	2.21 $\pm$ 0.17	2.39 $\pm$ 0.11
Ion. Calcium (mmol/l)	1.09 $\pm$ 0.07	1.10 $\pm$ 0.08	1.11 $\pm$ 0.08	1.13 $\pm$ 0.07	1.15 $\pm$ 0.06
Calcium/creatinine ratio urine	0.49 $\pm$ 0.59	0.44 $\pm$ 0.57	0.51 $\pm$ 0.54	0.59 $\pm$ 0.60	0.24 $\pm$ 0.19
Phosphate (mg/dl)	3.60 $\pm$ 1.22	3.31 $\pm$ 1.05	3.25 $\pm$ 1.14	3.54 $\pm$ 1.26	3.26 $\pm$ 0.52
Creatinine (mg/dl)	1.41 $\pm$ 1.04	1.29 $\pm$ 1.06	1.21 $\pm$ 0.89	1.21 $\pm$ 1.01	1.03 $\pm$ 0.41
Bilirubin (mg/dl)	1.43 $\pm$ 1.92	1.48 $\pm$ 2.68	1.84 $\pm$ 3.91	1.96 $\pm$ 4.59	0.77 $\pm$ 0.73
Cholesterol (mg/dl)	112 $\pm$ 55	124 $\pm$ 53	143 $\pm$ 58	151 $\pm$ 51	185 $\pm$ 40
Glucose (mg/dl)	151 $\pm$ 52	130 $\pm$ 39	131 $\pm$ 51	131 $\pm$ 59	105 $\pm$ 39
CRP (mg/l)	105 [0.6-419]	88 [0.7-475]	55 [0.6-365]	51 [0.6-316]	3.5 [0.6-30]
Procalcitonin (ng/ml)	0.6 [0-363]	0.4 [0-114]	0.2 [0-95]	0.2 [0-66]	0.1 [0-0.3]
Fibrinogen (mg/dl)	465 $\pm$ 186	551 $\pm$ 175	556 $\pm$ 171	459 $\pm$ 156	354 $\pm$ 105
Hemoglobin (g/dl)	10.2 $\pm$ 1.9	10.0 $\pm$ 1.7	10.0 $\pm$ 1.8	10.3 $\pm$ 1.9	13.2 $\pm$ 1.6
Leukocytes (G/l)	11.2 $\pm$ 5.9	10.4 $\pm$ 5.3	11.2 $\pm$ 5.1	8.2 $\pm$ 3.9	6.9 $\pm$ 3.3
NTproBNP (pg/ml)	1883 [18-35.000]	2155 [26-35.000]	1744 [11-35.000]	1730 [12-35.000]	140 [12-2109]
Albumin (g/dl)	2.89 $\pm$ 0.54	2.90 $\pm$ 0.53	2.99 $\pm$ 0.57	3.13 $\pm$ 0.71	4.49 $\pm$ 0.48
Total protein (g/dl)	5.44 $\pm$ 0.94	5.76 $\pm$ 0.77	6.06 $\pm$ 0.83	6.47 $\pm$ 1.00	7.59 $\pm$ 0.44

CRP, C-reactive protein; NTproBNP, NTpro brain natriuretic peptide;

Results

Table 8 Metabolic biochemical characteristics of VITdAL@ICU subjects – Vitamin D group

	Vitamin D group				
	Day 0 (n=210-236)	Day 3 (n=179-215)	Day 7 (n=151-204)	Day 28 (n=49-72)	Month 6 (n=32-37)
Metabolic biochemical parameters					
Calcium (mmol/l)	2.07 ± 0.20	2.11 ± 0.18	2.17 ± 0.18	2.25 ± 0.15	2.44 ± 0.17
Ion. Calcium (mmol/l)	1.10 ± 0.09	1.11 ± 0.08	1.12 ± 0.07	1.14 ± 0.07	1.19 ± 0.07
Calcium/creatinine ratio urine	0.45 ± 0.59	0.42 ± 0.49	0.46 ± 0.65	0.66 ± 0.45	0.30 ± 0.27
Phosphate (mg/dl)	3.61 ± 1.32	3.41 ± 1.12	3.36 ± 1.01	3.55 ± 0.84	3.39 ± 0.47
Creatinine (mg/dl)	1.48 ± 1.21	1.45 ± 1.29	1.28 ± 1.24	1.15 ± 0.90	1.10 ± 0.39
Bilirubin (mg/dl)	1.61 ± 3.11	1.71 ± 3.98	1.69 ± 4.52	0.95 ± 1.47	0.56 ± 0.26
Cholesterol (mg/dl)	110 ± 53	125 ± 47	141 ± 46	174 ± 58	189 ± 49
Glucose (mg/dl)	153 ± 52	130 ± 36	126 ± 37	121 ± 40	100 ± 28
CRP (mg/l)	109 [0.8-449]	89 [0.6-412]	76 [0.6-356]	32 [0.6-212]	2.6 [0.6-108]
Procalcitonin (ng/ml)	0.8 [0-318]	0.4 [0-97]	0.2 [0-36]	0.1 [0-35]	0.1 [0-0.5]
Fibrinogen (mg/dl)	470 ± 195	537 ± 191	549 ± 177	438 ± 158	363 ± 109
Hemoglobin (g/dl)	10.1 ± 1.7	9.9 ± 1.7	10.0 ± 1.7	10.8 ± 1.9	13.4 ± 2.0
Leukocytes (G/l)	11.7 ± 6.2	10.7 ± 5.9	11.7 ± 5.3	9.6 ± 5.2	7.1 ± 2.6
NTproBNP (pg/ml)	1714 [27-35.000]	1595 [8-35.000]	1419 [6-35.000]	680 [13-35.000]	164 [15-4874]
Albumin (g/dl)	2.83 ± 0.56	2.81 ± 0.56	2.90 ± 0.59	3.25 ± 0.69	4.45 ± 0.46
Total protein (g/dl)	5.32 ± 0.92	5.63 ± 0.85	6.00 ± 0.90	6.72 ± 0.90	7.62 ± 0.58

CRP, C-reactive protein; NTproBNP, NTpro brain natriuretic peptide;

Table 9 Endocrine biochemical characteristics of VITdAL@ICU subjects – Placebo group

	Placebo group				
	Day 0 (n=237)	Day 3 (n=220-221)	Day 7 (n=202)	Day 28 (n=60)	Month 6 (n=38-43)
Endocrine biochemical parameters					
1,25(OH)2D (pmol/l)	42.6 ± 41.3	47.0 ± 49.2	41.5 ± 39.4	43.2 ± 36.4	98.6 ± 40.9
25(OH)D (ng/ml)	13.5 ± 4.6	13.9 ± 5.0	14.5 ± 5.1	17.3 ± 6.9	26.2 ± 12.8
PTH (pg/ml)	79.5 ± 63.2	80.4 ± 64.0	71.4 ± 60.8	58.9 ± 68.2	56.9 ± 31.2
bALP (μg/l)	22.6 ± 16.5	25.1 ± 15.5	27.9 ± 14.1	34.2 ± 21.2	29.9 ± 13.6
Osteocalcin (ng/ml)	12.9 ± 13.9	12.5 ± 13.3	12.2 ± 10.9	17.1 ± 15.4	31.4 ± 16.8
b-CTX (ng/ml)	0.67 ± 0.47	0.77 ± 0.51	0.77 ± 0.56	0.92 ± 0.54	0.39 ± 0.19
TRAP (U/l)	2.21 ± 0.97	2.51 ± 0.99	2.88 ± 1.15	3.16 ± 1.51	3.36 ± 1.38

PTH, parathyroid hormone; bALP, bone alkaline phosphatase; b-CTX, beta-crosslaps; TRAP, tartrat resistant acid phosphatase;

Table 10 Endocrine biochemical characteristics of VITdAL@ICU subjects – Vitamin D group

	Vitamin D group				
	Day 0 (n=237)	Day 3 (n=218-220)	Day 7 (n=202)	Day 28 (n=72)	Month 6 (n=31-37)
Endocrine biochemical parameters					
1,25(OH)2D (pmol/l)	43.3 ± 46.0	104.3 ± 97.6	74.5 ± 67.1	55.5 ± 51.7	107.7 ± 53.0
25(OH)D (ng/ml)	13.0 ± 4.8	33.5 ± 18.7	35.5 ± 20.6	32.7 ± 19.3	46.0 ± 17.5
PTH (pg/ml)	80.1 ± 65.5	74.6 ± 70.7	58.4 ± 46.4	40.6 ± 27.7	47.4 ± 28.1
bALP (µg/l)	22.8 ± 15.1	25.6 ± 15.2	30.3 ± 20.0	40.7 ± 33.3	31.4 ± 20.4
Osteocalcin (ng/ml)	11.6 ± 11.0	14.2 ± 21.0	14.3 ± 22.2	15.5 ± 9.7	27.6 ± 10.9
b-CTX (ng/ml)	0.65 ± 0.56	0.75 ± 0.53	0.73 ± 0.54	0.90 ± 0.73	0.38 ± 0.23
TRAP (U/l)	2.14 ± 0.89	2.48 ± 0.90	2.81 ± 1.01	3.40 ± 1.16	3.20 ± 0.96

PTH, parathyroid hormone; bALP, bone alkaline phosphatase; b-CTX, beta-crosslaps; TRAP, tartrat resistant acid phosphatase;

3.3. Primary and secondary endpoints

3.3.1. Primary endpoint

The median length of hospital stay (minimum-maximum) was not different between the placebo group [19.3 days (0.15-154.1)] and the vitamin D group [20.1 days (0.16-181.0)] ($p=0.981$).

3.3.2. Secondary endpoints

ICU stay

Similar results were observed investigating the ICU stay. The median length of ICU stay (minimum-maximum) was 10.7 days (0.15-154.1) in the placebo group and 9.6 days (0.16-181.0) in the vitamin D group, which was not statistically significant ($p=0.384$).

Mechanical ventilation

The need for mechanical ventilation following study inclusion (defined as orotracheal intubation) was similar in the two study groups being 67.7% ($n=161$) in the placebo group and 67.1% ($n=159$) in the vitamin D group ($p=0.897$). There was also no statistically significant difference in the duration of mechanical ventilation between the two groups (167 hours in both groups; $p=0.619$). The need for tracheotomy during ICU stay was higher in the placebo group but did not reach statistical significance [24.8% ($n=59$) in the placebo group versus 18.6% ($n=44$) in the vitamin D group; $p=0.100$].

Vasopressor requirement

The use of the main vasopressor agent norepinephrine (Arterenol[®]) was not different in the study population during ICU stay. 150 patients (63.0%) needed pharmacological hemodynamic support with this agent in the placebo group for a median duration of 81 hours compared to 148 patients (62.5%) in the vitamin group for a median duration of 79 hours ($p=0.896$ and $p=0.569$, respectively).

TISS 28 score

TISS-28 scores did not differ in the study population at day 7. The placebo group reached a mean score of 34.9 ± 8.1 in contrast to a mean score of 35.1 ± 6.9 in the vitamin D cohort ($p=0.609$).

Nutrition

No statistically significant difference was found in the necessity of enteral and parenteral nutrition during hospital stay. In the placebo group 191 (80.3%) patients received enteral and 176 (74.0%) parenteral nutrition, whereas 188 (79.3%) and 180 (76.0%) needed it in the vitamin D group ($p=0.801$ and $p=0.615$, respectively). The additional intravenously administered vitamin D (Cernevit[®] or Vitalipid[®]), which was routinely prescribed during ICU stay as considered appropriate by the responsible physician/s, was similar in the two study groups. The mean daily dose was 140 ± 111 IU in the placebo group compared to 141 ± 122 IU in the vitamin D group ($p=0.836$).

Insulin requirement

The application of insulin to maintain glycemic control did not differ between the two study groups at day 6 after study inclusion. 92 patients (42.6%) received a mean dose of 52.5 ± 32.2 units insulin in the placebo group as opposed to 83 patients (38.3%) with a mean dose of 52.5 ± 32.2 units in the placebo group ($p=0.357$ and $p=0.209$, respectively).

Antibiotics requirement

The use of antibiotics was similar between the two study groups at day 6 after study inclusion. 166 (78.3%) received one or more antiinfectious substances in the placebo group and 159 patients (74.0%) in the vitamin D group ($p=0.292$).

Results

Table 11 gives an overview on the main endpoints of the VITdAL@ICU-study.

Table 11 Main endpoints of the VITdAL@ICU study

Endpoints		Placebo N=238		Vitamin D N=237		p-value
		n mean ± SD	% med [min-max]	n mean ± SD	% med [min-max]	
Primary endpoint						
Hospital stay (hours)		641 ± 584	463 [4-3698]	642 ± 606	483 [4-4344]	0.981
Hospital stay (days)		26.7 ± 24.3	19.3 [0.15-154.1]	26.7 ± 25.3	20.1 [0.16-181]	
Secondary endpoints						
ICU stay (hours)		423 ± 536	256 [4-3698]	376 ± 501	231 [4-4344]	0.384
ICU stay (days)		17.3 ± 22.3	10.7 [0.15-154.1]	15.7 ± 20.9	9.6 [0.16-181]	
Mechanical ventilation	yes	161	67.7	159	67.1	0.897
	no	77	32.3	78	32.9	
Duration (hours)		319 ± 414	167 [0.4-2494]	278 ± 337	167 [0.5-1898]	0.619
Tracheotomy	yes	59	24.8	44	18.6	0.100
	no	179	75.2	193	81.4	
Vasopressor (norepinephrine)	yes	150	63.0	148	62.5	0.896
	no	88	37.0	89	37.5	
Duration (hours)		147 ± 174	81 [0.5-940]	138 ± 196	79 [1-1368]	0.569
TISS-28 (at day 7)		34.9 ± 8.1	34 [17-62]	35.1 ± 6.9	35 [13-48]	0.569
Nutrition						
Enteral	yes	191	80.3	188	79.3	0.801
	no	47	19.7	49	20.7	
Parenteral	yes	176	74.0	180	76.0	0.615
	no	62	26.0	57	24.0	
Intravenous vitamin D supplements	yes	139	58.4	145	61.2	0.537
	no	99	41.6	92	38.8	
Relative daily dose of vitamin D*		140 ± 111	112 [3-420]	141 ± 122	105 [5-630]	0.836
Use of insulin and antibiotics at day 6						
Insulin use	yes	92	42.6	83	38.3	0.357
	no	124	57.4	134	61.2	
Dose (i.v./s.c.)**		52.5 ± 32.2	48 [4-144]	59.6 ± 37.3	58 [2-204]	0.209
Antibiotics use	yes	166	78.3	159	74.0	0.292
	no	46	21.7	56	26.0	
Number of antiinfectious substances		-	1 (1-5)	-	1 (1-4)	0.968

* The mean daily dose of vitamin D supplements routinely administered during ICU stay related to the total time of ICU stay.

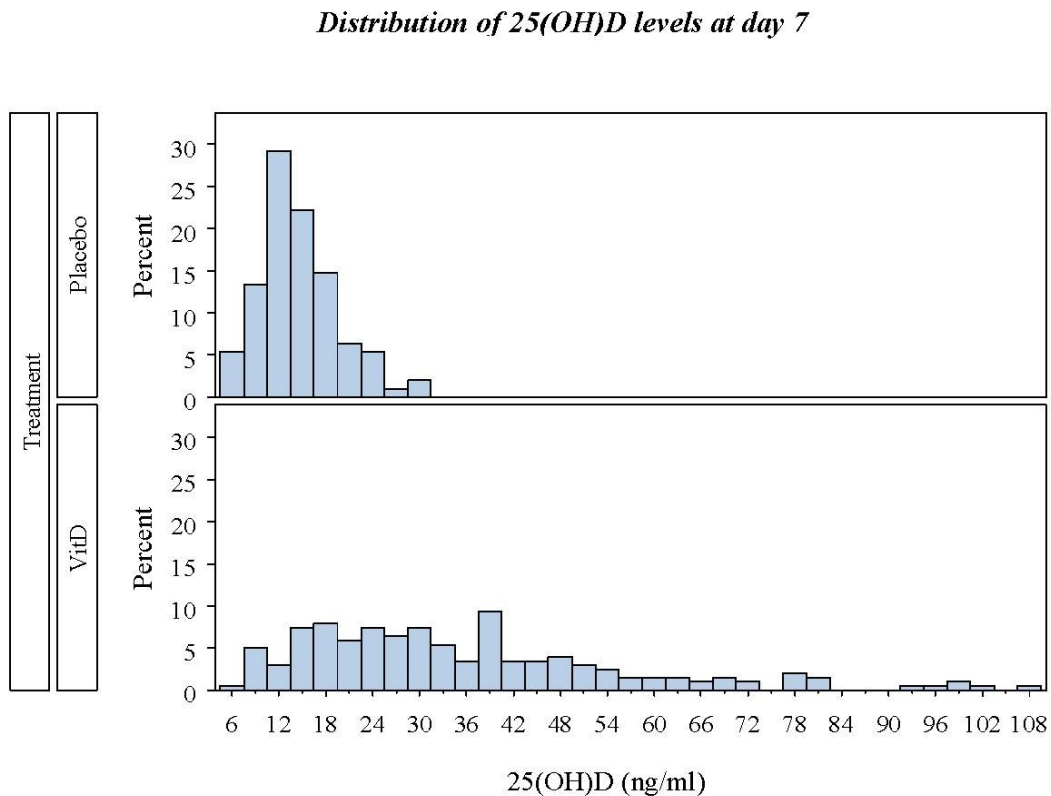
** The requirement for daily insulin treatment (intravenous and/or subcutaneous) is added up.

Biochemical evaluations

In the intervention group, 106 patients (52.5%) reached 25(OH)D levels ≥ 30 ng/ml at day 7 after study inclusion, while 96 patients (47.5%) remained in the deficient and insufficient range, respectively. There was no 25(OH)D level available for the remaining 35 patients in the vitamin D group at day 7 as this cohort either died before or was transferred to an external ICU or hospital ward.

In the placebo group mean 25(OH)D was 13.5 ± 4.6 ng/ml at day 0 and remained at this level with a mean of 14.5 ± 5.1 ng/ml at day 7. In the vitamin D group however, mean 25(OH)D increased from a mean of 13.0 ± 4.8 at day 0 to a level of 35.5 ± 20.6 ng/ml after 1 week. Figure 6 shows the distribution of 25(OH)D levels 1 week after study inclusion.

Figure 6 Vitamin D levels at day 7 in the two study groups



1,25(OH)₂D was significantly lower in the placebo group at day 7 and reached a mean level of 41.5 ± 39.4 pmol/l compared to 74.5 ± 67.1 pmol/l in the vitamin D group ($p < 0.001$). Furthermore, vitamin D led to a significant decrease of the mean PTH level in the vitamin D group at day 7 (58.4 ± 46.3 pg/ml in contrast to 71.3 ± 60.8 pg/ml in the placebo group; $p = 0.020$).

There were no differences in the inflammation markers CRP and procalcitonin after 7 days. CRP was slightly, but not statistically significant lower in the placebo group with a median

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of 54.8 [0.6-365.0]mg/l compared to 75.8 [0.6-355.7]mg/l in the vitamin D group ($p=0.293$). However, procalcitonin levels were higher in the placebo group but did not reach significance (placebo: 0.2 [0.0-95.0]ng/ml vs. vitamin D: 0.2 [0.0-35.8]ng/ml, $p=0.206$).

NT-proBNP, the main serum marker of cardiac function, did not differ between the groups after 1 week. The median was 1744 [11-35.000]pg/ml in the placebo group and 1419 [6-35.000]pg/ml in the vitamin D group ($p=0.812$).

The laboratory outcomes are described briefly in table 12.

Table 12 Secondary biochemical outcomes of the VITdAL@ICU-study, day 7

Endpoints		Placebo N=195-202		Vitamin D N=202-204		p-value
		n mean ± SD	% med [min-max]	n mean ± SD	% med [min-max]	
Biochemical evaluations at day 7						
25(OH)D ≥30ng/ml	yes	4	2.0	106	52.5	-
	no	198	98.0	96	47.5	
1,25(OH)2D (pmol/l)		41.5 ± 39.4	24.5 [1-207]	74.5 ± 67.1	58 [7-391]	<0.001
PTH (pg/ml)		71.3 ± 60.8	53.0 [8.3-486.7]	58.4 ± 46.3	44.2 [8.9-297.7]	0.020
CRP (mg/l)		80.1 ± 74.2	54.8 [0.6-365]	93.3 ± 79.8	75.8 [0.6-355.7]	0.293
Procalcitonin (ng/ml)		2.0 ± 8.5	0.2 [0.0-95.0]	1.0 ± 3.2	0.2 [0.0-35.8]	0.206
NT-proBNP (pg/ml)		5180 ± 7862	1744 [11-35000]	4673 ± 8438	1419 [6-35000]	0.812

3.4. Safety outcomes

Biochemical adverse events

The highest achieved 25(OH)D levels were 107.3ng/ml at day 7 and 106.4ng/ml after 6 months. In the vitamin D group, 13% (n=26) and 22% (n=8) of study patients with available blood samples had supranormal levels (>60ng/ml) after 7 days and 6 months, respectively. In the placebo group, the highest 25(OH)D level was 56.9ng/ml after 6 months. Thus no patient had 25(OH)D levels above the normal range at any time point.

The maximum total serum calcium level was 3.0mmol/l after 6 months. This patient was later found to have had normocalcemic primary hyperparathyroidism at study entry. 11% (n=4) of study patients who presented for a personal follow-up at study end had elevated, but asymptomatic calcium levels in the vitamin D group (range: 2.7-3.0mmol/l).

The maximum ionized serum calcium level was 1.5mmol/l at the study time points day 0, 28 and after 6 months in the vitamin D group (5 out of 753 measurements; 0.7%).

Mortality

Regarding mortality rates, we noticed a trend towards lower mortality in the vitamin D group which was strengthened with longer observation time.

During ICU stay, 63 patients (26.5%) died in the placebo group compared to 54 patients (22.8%) in the vitamin D group (HR 0.84, 95% CI 0.58-1.21, p=0.340).

During hospital stay, 84 patients (35.3%) died in the placebo group in contrast to 67 patients (28.3%) in the vitamin D group (HR 0.78, 95% CI 0.56-1.07, p=0.121).

After 6 months, 102 patients (42.9%) had deceased in the placebo group compared to 83 patients (35.5%) in the vitamin D group (HR 0.78, 95% CI 0.58-1.04, p=0.087). A Kaplan-Meier curve is presented in figure 7.

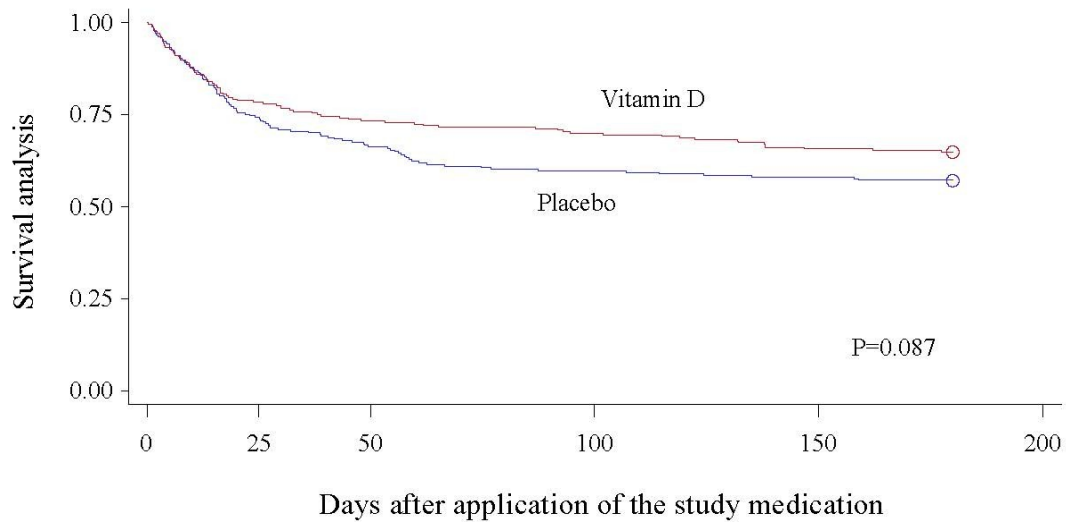
Falls and fractures

The number of falls was lower in the vitamin D group with a risk ratio of 0.73 but did not reach significance (p=0.166). In both groups, only two fractures occurred during the 6-month follow-up.

The safety evaluations including the causes of death are shown in table 13.

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Figure 7 Kaplan-Meier curve of survival among 475 ICU patients during the 6-month study period



Survival among 238 patients in the placebo group and 237 patients in the high-dose vitamin D group.

Treatment with cholecalciferol was associated with a trend of higher rate of survival ($p=0.087$ by the stratified log-rank test)

Table 13 Safety outcomes of the VITdAL@ICU-study

Safety outcomes		Placebo N=238		Vitamin D N=237		p-value
		n	%	n	%	
ICU mortality	dead	63	26.5	54	22.8	0.340
	alive	175	73.5	183	77.2	
28-day mortality	dead	68	28.6	52	21.9	0.135
	alive	170	71.4	185	78.1	
Hospital mortality	dead	84	35.3	67	28.3	0.121
	alive	154	64.7	170	71.7	
6-month mortality	dead	102	42.9	83	35.0	0.087
	alive	136	57.1	154	65.0	
Falls (N=136 PBO and 153 VIT)	yes	33	24.3	27	17.7	0.166
	no	103	75.7	126	82.4	
Fractures (N=136 PBO and 153 VIT)	yes	2	1.5	2	1.3	-
	no	134	98.5	151	98.7	
Cause of death (n=185)	Sepsis	30	29.4	26	31.3	-
	Cardiovascular	30	29.4	24	28.9	
	Neurological	19	18.6	14	16.9	
	Other	23	22.6	19	22.9	

PBO, placebo group; VIT, vitamin D group;

3.5. Subgroup analyses [25(OH)D $\leq$ 12ng/ml]

3.5.1. Clinical baseline characteristics of the subgroup

Before unblinding all relevant data, the study team decided in the “Blind Review Meeting” (December 20th, 2012) to investigate a subpopulation consisting of all study patients with a screening 25(OH)D level $\leq$ 12ng/ml. We hypothesized that critically ill patients with severe vitamin D deficiency might benefit to a greater extent from high-dose vitamin D supplementation.

The following table shows the clinical baseline characteristics in this subpopulation comprising 200 study patients (placebo: n=102; vitamin D: n=98).

Table 14 Clinical subgroup baseline characteristics of the two study groups [25(OH)D $\leq$ 12ng/ml]

Baseline characteristics*		Placebo N=102		Vitamin D N=98		p-value
		n mean $\pm$ SD	% med [min-max]	n mean $\pm$ SD	% med [min-max]	
Center	mixed surgical ICU	17	16.7	19	19.4	
	cardiothoracic ICU	30	29.4	28	28.6	
	mixed surgical ICU	6	5.9	5	5.1	
	medical ICU	27	26.5	22	22.5	
	neurological ICU	22	21.5	24	24.4	
Gender	Male	64	62.8	63	64.3	
	Female	38	37.2	35	35.7	
Age (yrs)		66.5 $\pm$ 13.6	70 [29-90]	64.2 $\pm$ 15.6	66 [21-91]	0.334
Weight (kg)		78.6 $\pm$ 17.5	77.5 [51-178]	77.9 $\pm$ 16.4	75.5 [50-155]	0.775
Height (cm)		171.1 $\pm$ 9.4	170.5 [148-196]	169.2 $\pm$ 8.7	170 [150-190]	0.147
BMI (kg/m ²)		26.9 $\pm$ 5.7	26.0 [18.2-62.3]	27.3 $\pm$ 5.4	26.8 [18.6-48.4]	0.477
Nicotine packyears (py)		35.6 $\pm$ 22.7	30 [4-90]	36.1 $\pm$ 20.6	40 [6-85]	0.967
Charlson Comorbidity Index		3.5 $\pm$ 2.2	3 [0-9]	3.2 $\pm$ 2.2	3 [0-9]	0.320
SAPS II (ICU admission)		32.8 $\pm$ 13.1	31 [6-69]	31.3 $\pm$ 15.2	28 [6-97]	0.197
TISS 28 (study start)		38.0 $\pm$ 8.0	39 [19-60]	37.1 $\pm$ 7.3	38 [16-63]	0.382
Admission diagnosis	Sepsis	5	4.9	8	8.2	-
	Cardiovascular	16	15.7	13	13.3	
	Neurologic	24	23.5	23	23.5	
	Other non-surgical	26	25.5	15	15.3	
	Cardiac surgery	20	19.6	19	19.4	
	Trauma	5	4.9	9	9.2	
	Other surgical	6	5.9	11	11.1	

* For explanation see table 6

3.5.2. Primary and secondary endpoints of the subgroup

Primary endpoint of the subgroup

Hospital stay was not significantly different between both groups (19.0 vs. 20.1 days; $p=0.404$).

Secondary endpoints of the subgroup

More patients in the placebo group needed mechanical ventilation [69 patients (67.7%) vs. 62 patients (63.3%)] and tracheotomy [24 patients (23.5%) vs. 16 patients (16.3%)] during the ICU stay in contrast to the vitamin D group but statistical significance was not obtained ($p=0.515$ and $p=0.203$, respectively).

The need for pharmacologic hemodynamic support was also statistically insignificant higher in the placebo group as 68 patients (66.7%) received norepinephrine (Arterenol[®]) with a median duration of 75.0 hours compared to 59 patients (60.2%) with a median duration of 73.4 hours in the vitamin D group ($p=0.343$ and 0.396 , respectively).

Significantly fewer patients received parenteral nutrition in the placebo group in contrast to the vitamin D group [69 patients (67.7%) vs. 79 patients (80.6%); $p=0.037$], which was accompanied by a trend for less intravenously administered vitamin D supplements [54 patients (52.9%) vs. 61 patients (62.2%), $p=0.183$].

At day 6 after study inclusion, 39 patients (43.3%) needed insulin for glycemic control in the placebo group compared to 33 patients (36.7%) in the vitamin D group ($p=0.361$). The required daily insulin dose was insignificantly lower in the placebo group than in the vitamin D group ($51.2 \pm 31.4\text{IU}$ vs. $63.0 \pm 40.7\text{IU}$, $p=0.128$).

Table 15 describes in brief the primary and secondary endpoints of the predefined subgroup.

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Table 15 Main endpoints of the VITdAL@ICU subgroup [25(OH)D ≤12ng/ml]

Endpoints *		Placebo N=102		Vitamin D N=98		p-value
		n mean ± SD	% med [min-max]	n mean ± SD	% med [min-max]	
Primary endpoint						
Hospital stay (hours)		625 ± 595	456 [25-3698]	708 ± 687	484 [5-4344]	0.404
Hospital stay (days)		26.1 ± 24.8	19.0 [1.0-154.1]	29.5 ± 28.6	20.1 [0.2-181]	
Secondary endpoints						
ICU stay (hours)		403 ± 547	218 [19-3698]	419 ± 601	233 [5-4344]	0.978
ICU stay (days)		16.8 ± 22.8	9.1 [0.8-154.1]	17.5 ± 25.0	9.7 [0.2-181]	
	yes	69	67.7	62	63.3	0.515
	no	33	32.3	36	36.7	
Duration (hours)		281 ± 383	147 [0.4-2162]	313 ± 393	157 [1.8-1898]	0.756
	yes	24	23.5	16	16.3	0.203
	no	78	76.5	82	83.7	
	yes	68	66.7	59	60.2	0.343
	no	34	33.3	39	39.8	
Duration (hours)		155 ± 191	75 [2.0-940]	156 ± 259	73 [4-1368]	0.396
TISS-28 (at day 7)		34.0 ± 7.8	33 [17-60]	34.3 ± 8.0	34 [13-48]	0.938
Nutrition						
	yes	82	80.4	77	78.6	0.750
	no	20	19.6	21	21.4	
	yes	69	67.7	79	80.6	0.037
	no	33	32.3	19	19.4	
	yes	54	52.9	61	62.2	0.183
	no	48	47.1	37	37.8	
Relative daily dose of vitamin D		140 ± 105	137 [3-420]	147 ± 129	105 [5-630]	0.906
Use of insulin and antibiotics at day 6						
	yes	39	43.3	33	36.7	0.361
	no	51	56.7	57	63.3	
Dose (i.v./s.c.)		51.2 ± 31.4	53 [4-136]	63.0 ± 40.7	68 [2-204]	0.128
	yes	67	76.1	68	77.3	0.859
	no	21	23.9	20	22.7	
Number of antiinfectious substances		-	1 (1-5)	-	1 (1-4)	0.865

* For explanation see table 11

Biochemical evaluations in the subgroup

In the subgroup with baseline 25(OH)D ≤ 12 ng/ml, 36 patients in the vitamin D group (43.4%) had 25(OH)D levels ≥ 30 ng/ml at day 7 while the majority did not reach values in the normal range (47 patients, 56.6%).

Mean 25(OH)D increased from a baseline level of 9.0 ± 2.4 ng/ml to 31.9 ± 20.7 ng/ml in the vitamin D group after 7 days. In the placebo group, mean 25(OH)D was 10.2 ± 3.4 ng/ml at day 0 and did not change after 7 days with a mean of 11.6 ± 4.1 ng/ml.

1,25(OH)₂D was significantly lower in the placebo group at day 7 with a mean of 34.8 ± 33.3 pmol/l compared to 75.8 ± 65.7 pmol/l in the vitamin D group ($p < 0.001$). PTH levels were distinctly but insignificantly higher in the placebo group at day 7 [71.5 ± 50.3 pg/ml compared to 59.5 ± 41.2 pg/ml; ($p = 0.082$)].

The two main inflammation markers did not react congruently as at day 7, CRP was slightly lower in the placebo group (placebo: 47.3 [3.9-365] mg/l vs. vitamin D: 78.2 [1.4-330.4] mg/l, $p = 0.301$) in contrast to distinctly higher levels of procalcitonin in the placebo group (placebo: 0.2 [0.0-95.0] ng/ml vs. vitamin D: 0.2 [0.0-35.8] ng/ml; $p = 0.056$) after 1 week.

NT-proBNP was insignificantly higher in the placebo group with a median of 2007 [11-35000] pg/ml as opposed to 1937 [46-35000] pg/ml in the vitamin D group ($p = 0.269$).

The following table gives an overview on the laboratory outcomes in the subgroup.

Table 16 Secondary biochemical outcomes of the VITdAL@ICU-subgroup [25(OH)D ≤ 12 ng/ml], day 7

Endpoints		Placebo N=82-83		Vitamin D N=83-86		p-value
		n mean ± SD	% med [min-max]	n mean ± SD	% med [min-max]	
Biochemical evaluations at day 7						
25(OH)D ≥30ng/ml	yes	0	0	36	43.4	-
	no	82	100	47	56.6	
1,25(OH)2D (pmol/l)		34.8 ± 33.3	19 [1-137]	75.8 ± 65.7	60 [7-260]	<0.001
PTH (pg/ml)		71.5 ± 50.3	59.1 [14.5-290.4]	59.5 ± 41.2	44.4 [8.9-205.4]	0.082
CRP (mg/l)		74.8 ± 76.7	47.3 [3.9-365]	98.0 ± 82.3	78.2 [1.4-330.4]	0.301
Procalcitonin (ng/ml)		2.0 ± 10.6	0.2 [0.0-95.0]	1.2 ± 4.1	0.2 [0.0-35.8]	0.056
NT-proBNP (pg/ml)		6075 ± 8448	2007 [11-35000]	4776 ± 8123	1937 [46-35000]	0.269

3.5.3. Safety outcomes of the subgroup

Biochemical adverse events

In the vitamin D group, the maximum 25(OH)D level was 101.8ng/ml at day 7. 13% (n=11) and 14% (n=2) of patients with available serum samples had supranormal levels (>60ng/ml) after 7 days and 6 months, respectively.

The maximum serum calcium level was 3.0mmol/l in the already mentioned patient with diagnosed primary hyperparathyroidism after 6 months and 3 patients (21%) had elevated, but asymptomatic levels at the end of the study period, while the maximum ionized serum calcium level was 1.5mmol/l after 28 days and month 6, respectively in the vitamin D group.

Mortality

We detected significantly lower mortality rates in the vitamin D group especially with a longer observation period.

During ICU stay, 34 patients (33.3%) died in the placebo group compared to 23 patients (23.5%) in the vitamin D group (HR 0.63, 95% CI 0.37-1.08, p=0.088). Regarding the following longer study periods all mortality rates became statistically significant favouring the intervention group.

In hospital, 47 patients (46.1%) deceased in the placebo group in contrast to 28 patients (28.6%) in the vitamin D group (HR 0.55, 95% CI 0.34-0.87, p=0.010). After 6 months, 51 patients (50.0%) had died in the placebo group compared to 34 patients (34.7%) in the vitamin D group (HR 0.60, 95% CI 0.39-0.93, p=0.021). Multivariate-adjusted analysis including age, sex, Charlson comorbidity index, SAPS II and PCT, total calcium and PTH at study begin even strengthened this finding with an adjusted hazard ratio of 0.54 (95% CI 0.34-0.86, p=0.009).

According to Cox regression analysis this implies that after a study period of 6 months the risk to die was 40% lower in the vitamin D group with a number needed to treat (NNT) of 6.5.

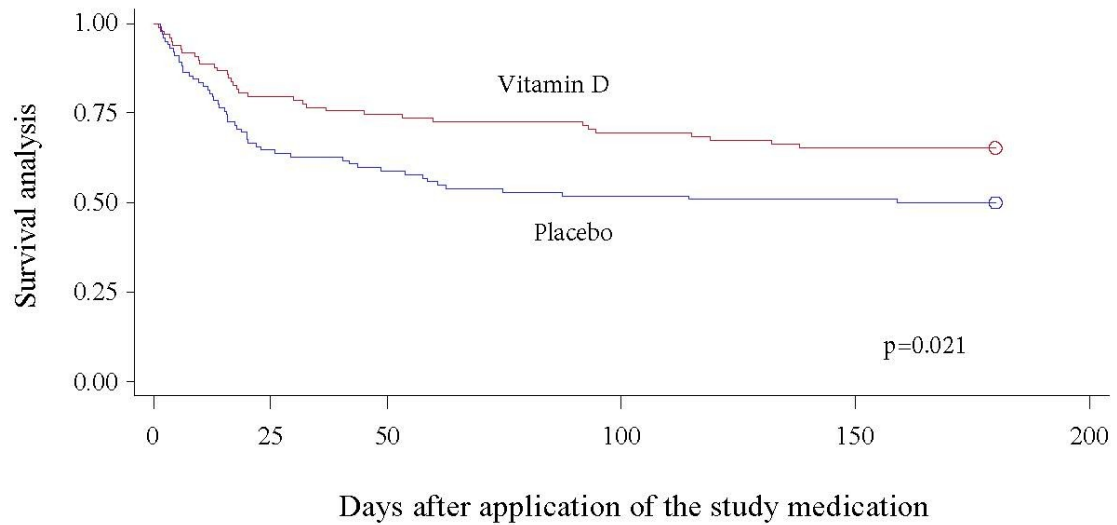
The figure below displays the Kaplan-Meier curve in the subgroup.

Falls and fractures

The number of (self-reported) falls and radiologic verified fractures was not statistically different between the two study groups. There were 14 reported falls (27.5%) in the placebo group compared to 16 (25%) in the vitamin D group (p=0.766) and 1 fracture in each group. The safety evaluations including the causes of death are shown in table 17.

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Figure 8 Kaplan-Meier curve of survival among 200 ICU patients with severe vitamin D deficiency at baseline during the 6-month study period



Kaplan-Meier curve among 102 patients in the placebo group and 98 patients in the high-dose vitamin D group (subgroup with baseline 25(OH)D levels $\leq 12\text{ng/ml}$).

Treatment with cholecalciferol was associated with a significant higher rate of survival ($p=0.021$ by the stratified log-rank test)

Table 17 Safety outcomes of the VITdAL@ICU-subgroup [25(OH)D $\leq 12\text{ng/ml}$]

Safety outcomes		Placebo N=102		Vitamin D N=98		p-value
		n	%	n	%	
ICU mortality	dead	34	33.3	23	23.5	0.088
	alive	68	66.7	75	76.5	
28-day mortality	dead	37	36.3	20	20.4	0.015
	alive	65	63.7	78	79.6	
Hospital mortality	dead	47	46.1	28	28.6	0.010
	alive	55	53.9	70	71.4	
6-month mortality	dead	51	50.0	34	34.7	0.021
	alive	51	50.0	64	65.3	
Falls (N=51 PBO and 64 VIT)	yes	14	27.5	16	25.0	0.766
	no	37	72.5	48	75.0	
Fractures N=51 PBO and 64 VIT)	yes	1	2.0	1	1.6	-
	no	50	98.0	63	98.4	
Cause of death (n=85)	Sepsis	16	31.4	12	35.2	-
	Cardiovascular	13	25.5	9	26.5	
	Neurological	8	15.7	4	11.8	
	Other	14	27.4	9	26.5	

* PBO, placebo group; VIT, vitamin D group;

4. Discussion

In this thesis, we report results of a randomized, double-blind, placebo-controlled trial evaluating high-dose vitamin D treatment in a mixed critically ill, vitamin D deficient study population. Our primary hypothesis was that vitamin D would reduce the length of hospital stay but this primary study endpoint was very similar between both groups.

Likely we did not find statistically significant differences between the well matched study groups in regards to length of ICU stay, markers of infection (CRP, PCT, use of antibiotics), the duration of mechanical ventilation and incidence of tracheotomy, markers of glycemic control (need for insulin) or cardiac function (NTproBNP, demand for vasopressors). Provided nutritional support including vitamin D supplements was also comparable in both groups.

A nonsignificant, but with longer observation time conclusive trend favouring survival in the vitamin D group was evident (mortality after 6 months 35.0% vs. 42.9%, $p=0.087$).

Focusing on biochemical effects we found as expected a significant rise of 25(OH)D levels accompanied by higher 1,25(OH)₂D and decreased PTH levels in the vitamin D group after 7 days. Surprisingly on the other hand, nearly half of the study patients in the intervention group (47.5%) did not reach sufficient 25(OH)D levels ($\geq 30\text{ng/ml}$) after one week despite the high-dose used. Critically ill patients often suffer from decreased blood flow in the gastrointestinal tract and thus from a limited resorption capacity, which may explain the unpredictable individual 25(OH)D response seen in the treatment group. Moreover, an unknown amount of 25(OH)D may have been removed from the circulation early and deposited in liver and adipose tissue.

Regarding adverse clinical effects, patients in our high-dose intervention group did not experience higher rates of falls and fractures during the 6-month follow-up period in contrast to the findings in other populations (182, 183).

The highest achieved individual 25(OH)D level in the intervention group was 107.3ng/ml and thus still remote from levels thought to be indicative of vitamin D toxicity ($>150\text{ng/ml}$) (5). Hypercalcemia quantified as total serum calcium levels $>2.65\text{mmol/l}$ was a rare event in the vitamin D group (8 out of 764 measurements, 1.0%). All patients remained asymptomatic and did not require therapeutical interventions.

As predefined in the statistical analyses plan, all statistical procedures were separately performed for severely vitamin D deficient patients ($\leq 12\text{ng/ml}$) assuming that the potential for a more pronounced health benefit could be larger in this subpopulation.

The majority of endpoints remained insignificant in these analyses as well. There was no difference in the length of hospital- and ICU stay. We observed slightly less tracheotomies, a lower demand of vasopressors, lower levels of PCT and NTproBNP in the vitamin D group at day 7 but none of these differences achieved statistical significance (from $p=0.343$ to $p=0.056$).

Changes in vitamin D metabolism were similar to the total study population with a significant rise of 25(OH)D and 1,25(OH)₂D and a trend for lower PTH levels in the vitamin D group at day 7. Only 43.5% of the patients in the intervention-arm had 25(OH)D levels ≥ 30 ng/ml at day 7 as opposed to 52.5% in the total study group.

The most important finding of our RCT is that in this predefined subgroup hospital- and 6-month mortality were significantly lower in the vitamin D group ($p=0.010$ and $p=0.021$, respectively). The administration of high-dose cholecalciferol substantially reduced all-cause mortality by 40% in the vitamin D group after 6 months.

Limitations and strengths of the trial

The major limitation of our trial is the fact that it was underpowered for mortality analyses. We nevertheless detected a benefit for improved survival in the predefined subgroup with profound vitamin D deficiency. This strong effect is mirrored by a surprisingly low number needed to treat of 7 in severely vitamin D deficient critically ill adults. One additional life would have been saved for 7 patients treated with high-dose vitamin D.

We are unable to give a reasonable explanation for the mechanisms that have led to this marked reduction in mortality rate in the vitamin D group as none of the chosen surrogate morbidity parameters showed a significant difference between the two study groups and also the causes of death were similarly reduced. Overall, the intervention group needed 25% less tracheotomies (44 vs. 59, $p=0.100$), indicating a more favourable course of illness. In severely deficient patients this effect was even more pronounced (16 vs. 24, -33%), although the rather small number of affected patients possibly precluded this result from being statistically significant ($p=0.203$).

We speculate that the cardiovascular-, infectious disease- and intensive care-related parameters were measured too early (during the first 7 days following the intervention and most exclusively during ICU stay) and that the monitoring of intervention should have been performed for a longer follow-up time to allow for a better picture of the full biologic effects of vitamin D treatment. On the other hand, we were unable to collect all data that would have been relevant or interesting due to restricted personal and financial resources.

For example, no parameter reflecting possible effects on skeletal or smooth musculature or, what is probably even more important, clinical evaluation regarding development and follow-up of infections is available.

However, this study remains the first RCT studying the effects of high-dose vitamin D on important outcome parameters in a mixed population of critically ill adults. Although the study population was heterogeneous including medical, neurological, surgical and cardiothoracic ICU patients, it was homogeneous with respect to the distribution within the two study arms which was due to an optimum randomization procedure strengthening the plausibility of the study outcomes.

Another important issue is the reliability of low 25(OH)D levels as a screening parameter in critically ill patients. Some intensivists have expressed their reservations about the validity of a single 25(OH)D measurement because ICU patients often receive substantial amounts of fluids within a short period of time. Inflammatory processes themselves may decrease binding proteins (albumin, DBP) and lead besides extravasation and different degrees of capillary leak to a profound effect on 25(OH)D levels (205, 206).

Conclusion

In summary, this is the first randomized, double-blind, placebo-controlled intervention trial that studied the effect of cholecalciferol treatment on the outcome of vitamin D deficient ICU patients. Although the primary outcome and most surrogate markers were unaffected by the treatment, it is a powerful and intriguing finding that there was a trend for improved survival in the total study population and a significant marked effect on survival in the predefined subgroup despite a relatively small sample size of the vitamin D group. This is the first direct evidence that vitamin D is not only a marker but also an important contributor for poor outcome in critical illness. Not many interventions have ever demonstrated improved survival in this highly vulnerable population. The fact that this has been achieved with an inexpensive substance that has few, if any acute adverse effects is all the more delectable.

A larger multicenter trial is now urgently warranted to confirm our findings, to clarify the underlying biologic mechanisms and to investigate whether critically ill patients with less severe vitamin D deficiency (25(OH)D levels between 12 and 30ng/ml) may also benefit from vitamin D supplementation.

While awaiting these results, it seems reasonable to screen critically ill patients for vitamin D deficiency after initial hemodynamic stabilization and provide an oral daily dose of 2,000

to 4,000 IU (at least) in profound vitamin D deficiency, as this dose is considered safe by all relevant institutions.

Key messages

- High-dose vitamin D supplementation did not alter the length of hospital- or ICU stay in a mixed population of 475 critically ill adults.
- Several cases of asymptomatic moderate hypercalcemia and vitamin D levels above the reference range were observed, but high-dose vitamin D did not increase 25(OH)D levels into the toxic range.
- Vitamin D led to significantly higher survival up to 6 months in severely vitamin D deficient ICU patients.
- The underlying pathophysiologic mechanisms for this finding are to date unclear and await to be illuminated in further research.
- A large multicenter trial powered for mortality analyses is warranted in critically ill adults.
- Similar trials are necessary to evaluate the effects of vitamin D in critically ill children.
- Although we used a high-dose of 540,000IU cholecalciferol, this single dose was unable to normalize 25(OH)D serum levels in almost half of the patients in the intervention group, probably reflecting severely impaired gastrointestinal blood flow and thus resorption of vitamin D3. Consequently, an individual approach including regular 25(OH)D measurements is probably necessary to overcome the large interindividual variation in dose requirements to correct vitamin D deficiency.
- Future studies should also investigate alternative treatment regimens including more frequent dosing and development of appropriate parenteral vitamin D formulas.

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6. Appendix

6.1. Amendments (Reference table, Case report form, vote of Ethical Committee, Correspondence)

Reference table (VITdAL@ICU-study)
Endocrinology laboratory

Parameter	Normal	Unit	Legend
1,25(OH)2D	39-193	pmol/l	1,25(OH)D2 Vitamin D3
25(OH)D	30.0-60.0	ng/ml	Vitamin D3
PTH	15.0-65.0	pg/ml	Parathormon
bALP	5.7-32.9	µg/l	Bone alkaline acid phosphatase
OC	1.0-35.0	ng/ml	Osteocalcin
b-CTX	0.08-0.46	ng/ml	β-Crosslaps
TRAP	2.18-3.94	U/l	Tartratresistente acid Phosphatase

Standard laboratory

Parameter	Normal	Unit	Legend
CA	2.20-2.65	mmol/l	Total calcium
CAION	1.15-1.35	mmol/l	Ionized calcium
CACRU	bis 0.60	mmol/mg	Calcium/creatinine ratio urine
Phosphat	2.60-4.50	mg/dl	Phosphate
CREA	0.70-1.20	mg/dl	Creatinine
BILIG	0.10-1.20	mg/dl	Bilirubin
CHOL	bis 199	mg/dl	Cholesterol
GLUC	70-115	mg/dl	Glucose
CRP	bis 5.0	mg/l	C-reactive protein
PCT	0.00-0.50	ng/ml	Procalcitonin
FIB	210-400	mg/dl	Fibrinogen
HB	13.0-17.5	g/dl	Hemoglobin
LEUKO	4.4-11.3	G/l	Leukocytes
NTproBNP	bis 100	pg/ml	NTpro-brain natriuretic peptide
ALB	3.5-5.3	g/dl	Albumin
TP	6.6-8.3	g/dl	Total protein

Medizinische Universität Graz

Universitätsklinik für Innere Medizin (Klinikvorstand: Univ.Prof.Dr. Ernst Pilger)
Klin. Abt. für Endokrinologie & Stoffwechsel (Leiter: Univ.Prof.Dr. Thomas Pieber)

A 8036 Graz, Auenbruggerplatz 15, Tel.: +43.316.385-12383, Fax: +43.316.385-13428

e-mail: endo.admin@medunigraz.at



Sehr geehrte/r Kollege/In,

Ihr/Ihre Patient/In geb. am wurde
im Rahmen des stationären Krankenhausaufenthaltes am LKH-
Universitätsklinikum Graz im 201__ in die randomisierte,
doppelblinde klinische Interventionsstudie „VITdAL@ICU“ aufgenommen.

In dieser Studie geht es um die Fragestellung, ob die Aufenthaltsdauer auf
Intensivstation, oder nachfolgende Krankenhausaufenthalte durch eine Korrektur
eines Vitamin D Mangels positiv beeinflusst werden.

Da ein Vitamin D-Mangel diagnostiziert wurde, bekam er/sie hochdosiert
Vitamin D (540.000 Einheiten Cholecalciferol/3 Fläschchen Oleovit D3®) oder
Placebo verabreicht. Im weiteren 6-monatigen „Follow up“ soll der/die Patient/In
7,5 ml (entsprechend 1/2 Fläschchen) Studienmedikation 1x pro Monat zu sich
nehmen. Diese wurden dem/der Patienten/In bei Krankenhausentlassung
mitgegeben. Um die Zuverlässigkeit der Studienmedikationseinnahme zu
gewährleisten, wird es von unserer Seite telefonische Kontakte mit dem
Patienten geben.

*Wir würden Sie höflich bitten, in den kommenden 6 Monaten von
Interventionen im Kalzium/Vitamin D-Stoffwechsel Abstand zu nehmen* (kein
Oleovit D3 oder ähnliche Präparate).

Bei Fragen stehen wir Ihnen unter folgenden Telefonnummern oder per Email gerne zur
Verfügung:

OA Dr. Karin Amrein:	0316-385-80798; karin.amrein@klinikum-graz.at
Dr. Christian Schnedl:	0660-523 8091; christian.schnedl@medunigraz.at
OA Dr. Helga Warnkross:	0676-939 2029
ao.Univ.-Prof. Dr. Harald Dobnig:	0316-385-82383

Mit freundlichem Dank für Ihre Bemühungen und kollegialen Grüßen,

Das Studienteam

Medizinische Universität Graz, Universitätsplatz 3, A 8010 Graz
Rechtsform: Juristische Person öffentlichen Rechts gemäß Universitätsgesetz 2002
Information: Mitteilungsblatt der Universität und www.meduni-graz.at
DVR-Nr.: 2109494, UID: ATU 57511179

Bankverbindung: Bank Austria Creditanstalt
Konto-Nr. 50094 840 004, BLZ: 12000
Raiffeisen Landesbank Steiermark
Konto-Nr.: 49510, BLZ: 38000

VIT_DAL@ICU – "Correction of vitamin D deficiency in critically ill patients"

Subject Nr./Initials: ____/____
Max Huber i.B. 201/MH

Date: ____/____/201____
dd/mm/yyyy

Investigator: ____
Max Huber i.B. MH

INCLUSION CRITERIA	
☐ erfüllt ☐ erfüllt ☐ erfüllt ☐ erfüllt	• ≥18 Jahre • erwarteter ICU Aufenthalt ≥ 48 Stunden • Vitamin D Mangel: 25(OH)D ≤ 20 ng/ml (____ ng/ml am ____) • Magen- oder Jejunalsonde wenn Schlucken nicht möglich
EXCLUSION CRITERIA	
☐ nicht erfüllt ☐ nicht erfüllt ☐ nicht erfüllt ☐ nicht erfüllt ☐ nicht erfüllt ☐ nicht erfüllt ☐ nicht erfüllt	• Lebenserwartung < 24 h • Hyperkalzämie (total calcium >2.65 OR ion. calcium >1.35 mmol/l) • Schwer eingeschränkte gastrointestinale Motilität (Ileus, Residualvolumen >400ml) • Bekannte kürzliche Nephrolithiasis (≤ 1 Jahr) • Granulomatöse Erkrankung (Tuberkulose, Sarkoidose) • Schwangerschaft • Patient in anderer Interventionsstudie
RANDOMISIERUNG	☐ MED ☐ NEURO ☐ ANAEST ☐ HERZ ☐ ICU3 - ____
INFORMED CONSENT	☐ ja ☐ primär nicht einwilligungsfähig, nachgeholt am ____/____/____ ☐ verstorben am ____/____/____, ____h ____an: _____
STUDIENMEDIKATION	erhalten am ____/____/____ um _____

TIMELINE	TAG 0	TAG 3	TAG 7	TAG 28	MONAT 6
DATUM	____.____.201____	____.____.201____	____.____.201____	____.____.201____	____.____.201____

PATIENT/IN	
NAME	
Geburtsdatum	____/____/____ (dd / mm / yyyy) Etikette: _____
Geschlecht	männlich ☐ weiblich ☐
Gewicht	____ kg
Grösse	____ cm
Nikotin	Nie ☐ Nicht erhebbar ☐ Ja ☐ , ____ PY St.n. ☐
TELEFONNR.	

AUFNAHMEDATUM	
AUFNAHMEDIAGNOSE	

VIT_DAL@ICU – "Correction of vitamin D deficiency in critically ill patients"Subject Nr./Initials: / /
Max Huber z.B.: 202/MHDate: / / 201
dd/mm/yyyyInvestigator:
Max Huber z.B.: MH

VORERKRANKUNGEN	_____

VITAMIN D ZUHAUSE	Nein/nicht bekannt ☐ Ja ☐ Produkt: _____
RELEVANTE MEDIKAMENTE	_____

INSULIN ☐ iv. ☐ sc. ☐ iv. ☐ sc. ☐ iv. ☐ sc.	Tag 0: Nein ☐ Ja ☐	Dosis <u> </u> U/24h
	Tag 2: Nein ☐ Ja ☐	Dosis <u> </u> U/24h
	Tag 6: Nein ☐ Ja ☐	Dosis <u> </u> U/24h
ANTIBIOTIKA	Tag 0: Nein ☐ Ja ☐	_____
	Tag 2: Nein ☐ Ja ☐	_____
	Tag 6: Nein ☐ Ja ☐	_____
SONDENERNÄHRUNG	Nein ☐ Ja ☐ Produkt: _____ Dosis <u> </u> ml/ <u> </u> von _____ bis _____ (dd/ mm/ yyyy)	
INTRAVENÖSE ERNÄHRUNG	Nein ☐ Ja ☐ Produkt: _____ Dosis <u> </u> ml/ <u> </u> von _____ bis _____ (dd/ mm/ yyyy) Vitamin D (Vitalipid, Cernevit), ☐ Nein ☐ Ja, Dosis <u> </u> Ampullen/ 24h	
BEATMUNG	Nein ☐ Ja ☐	
☐ INTUBIERT ☐ MASKE	von <u> </u> / <u> </u> / <u> </u> <u> </u> h bis <u> </u> / <u> </u> / <u> </u> <u> </u> h ☐ intermittierend	
☐ INTUBIERT ☐ MASKE	von <u> </u> / <u> </u> / <u> </u> <u> </u> h bis <u> </u> / <u> </u> / <u> </u> <u> </u> h ☐ intermittierend	
☐ INTUBIERT ☐ MASKE	von <u> </u> / <u> </u> / <u> </u> <u> </u> h bis <u> </u> / <u> </u> / <u> </u> <u> </u> h ☐ intermittierend	
VASOPRESSOREN	Nein ☐ Ja ☐	
☐ Noradrenalin	<u> </u> / <u> </u> / <u> </u> <u> </u> h bis <u> </u> / <u> </u> / <u> </u> <u> </u> h	<u> </u> / <u> </u> / <u> </u> <u> </u> h bis <u> </u> / <u> </u> / <u> </u> <u> </u> h
☐ Dobutamin	<u> </u> / <u> </u> / <u> </u> <u> </u> h bis <u> </u> / <u> </u> / <u> </u> <u> </u> h	<u> </u> / <u> </u> / <u> </u> <u> </u> h bis <u> </u> / <u> </u> / <u> </u> <u> </u> h
☐ Levosimendan	<u> </u> / <u> </u> / <u> </u> <u> </u> h bis <u> </u> / <u> </u> / <u> </u> <u> </u> h	<u> </u> / <u> </u> / <u> </u> <u> </u> h bis <u> </u> / <u> </u> / <u> </u> <u> </u> h

VIT_DAL@ICU – "Correction of vitamin D deficiency in critically ill patients"

Subject Nr./Initials: ____/____
Max Huber z.B. 20/MH

Date: ____/____/201____
dd/mm/yyyy

Investigator: ____
Max Huber z.B. MH

☐ Dopamin	____/____/____ h bis ____/____/____ h	____/____/____ h bis ____/____/____ h
☐ Milrinon	____/____/____ h bis ____/____/____ h	____/____/____ h bis ____/____/____ h
☐ andere	____/____/____ h bis ____/____/____ h	____/____/____ h bis ____/____/____ h
Optional: NEUE PNEUMONIE AB EINSCHLUSS	Nein ☐	Ja ☐ (je 1 Kriterium aus a) und b)) erfüllt
	a) - abnormaler Auskultationsbefund ☐ - Infiltrate im Th-Rö ☐	b) - produktiver Husten ☐ - pos. Befund aus BAL oder Sekret ☐ - pos. Blutkultur, Leukozytose und erhöhtes CRP ☐

Appendix

VIT_DAL@ICU – "Correction of vitamin D deficiency in critically ill patients"

Subject Nr./Initials: ____/____
Max Huber z.B.: 202/MH

Date: ____/____/201____
dd/mm/yyyy

Investigator: ____
Max Huber z.B.: MH

FOLLOW UP Monat 6		
ERHEBUNG	am ____/____/____ ☐ telefonisch mit ☐ Patient- ☐ Angehörigem ☐ Anderen, _____ ☐ persönlich	
WIEDERAUFNAHME SPITAL	☐ NEIN, ☐ JA, von ____/____/____ bis ____/____/____ ☐ ICU ☐ IMC ☐ Normalstation wegen _____	
STUDIENMEDIKATION EINGENOMMEN	☐ NEIN ☐ JA, wie viel ____ 1-5 Dosen	
VITAMIN D ZUHAUSE	☐ Nein ☐ nicht bekannt Ja ☐ Produkt: ☐ Oleovit ☐ Rocaltrol ☐ Calcium/Vit D ☐ other	
NEUE RELEVANTE MEDIKAMENTE	_____ ☐ Kategorie: _____ _____ ☐ Kategorie: _____ _____ ☐ Kategorie: _____ _____ ☐ Kategorie: _____ _____ ☐ Kategorie: _____	
STÜRZE SEIT ICU	☐ NEIN	☐ JA, wann _____ Wie _____
FRAKTUREN SEIT ICU	☐ NEIN	☐ JA, wann _____ radiologisch verifiziert ☐ JA ☐ NEIN Wie _____
ATEMWEGSINFEKTE	☐ NEIN ☐ JA, Antibiotika: ☐ NEIN ☐ JA _____	
ECOG SCORE	☐ 0: in der Lage alle Arbeiten, in dem selben Maß wie vor der Erkrankung, durchzuführen ☐ 1: Einschränkungen in körperlich anstrengenden Arbeiten, aber ambulant und in der Lage leichtere Arbeiten zu verrichten (leichtere Hausarbeit, Büroarbeit) ☐ 2: ambulant und in der Lage, sich selbst zu versorgen, aber nicht in der Lage Arbeit zu verrichten ☐ 3: nur mehr begrenzt in der Lage, sich selbst zu versorgen; mehr als 50% der wachen Zeit liegend oder sitzend ☐ 4: nur mehr an Bett und Stuhl gebunden; überhaupt nicht mehr in der Lage, sich selbst zu versorgen ☐ 5: tot	
TIMED UP & GO TEST	_____ Sekunden	
FAUSTSCHLUSSKRAFT	LINKS _____ mmHg RECHTS _____ mmHg	

VIT_DAL@ICU – "Correction of vitamin D deficiency in critically ill patients"Subject Nr./Initials: /
Max Huber z.B. 202/MHDate: / / 201
dd/mm/yyyyInvestigator:
Max Huber z.B. MH

DXA	☐ NEIN ☐ JA; T-Score L1-L4: <u> </u> T-Score Neck: <u> </u>																																								
PEAK FLOW																																									
SF 12	1. Wie würden Sie Ihren Gesundheitszustand im Allgemeinen beschreiben? <table border="1"> <tr> <td>☐ Ausgezeichnet</td> <td>☐ Sehr gut</td> <td>☐ Gut</td> <td>☐ Weniger gut</td> <td>☐ Schlecht</td> </tr> </table> Im Folgenden sind einige Tätigkeiten beschrieben, die Sie vielleicht an einem normalen Tag ausüben: Sind Sie durch Ihren derzeitigen Gesundheitszustand bei diesen Tätigkeiten eingeschränkt? Wenn ja, wie stark? 2. mittelschwere Tätigkeit, z.B. einen Tisch verschieben, staubsaugen, kegeln, Golf spielen <table border="1"> <tr> <td>☐ stark eingeschränkt</td> <td>☐ etwas eingeschränkt</td> <td>☐ überhaupt nicht eingeschränkt</td> </tr> </table> 3. mehrere Treppenabsätze steigen <table border="1"> <tr> <td>☐ stark eingeschränkt</td> <td>☐ etwas eingeschränkt</td> <td>☐ überhaupt nicht eingeschränkt</td> </tr> </table> Haben Sie in den vergangenen Wochen aufgrund Ihrer körperlichen Gesundheit irgendwelche Schwierigkeiten bei der Arbeit oder anderen alltäglichen Tätigkeiten im Beruf bzw. zu Hause? 4. Ich habe weniger geschafft als ich wollte. ☐ Ja ☐ Nein 5. Ich konnte nur bestimmte Dinge tun. ☐ Ja ☐ Nein Hatten Sie in den vergangenen 4 Wochen aufgrund seelischer Probleme irgendwelche Schwierigkeiten bei der Arbeit oder anderen alltäglichen Tätigkeiten im Beruf bzw. zu Hause, (z. B. weil Sie sich niedergeschlagen oder ängstlich fühlten?) 6. Ich habe weniger geschafft als ich wollte. ☐ Ja ☐ Nein 7. Ich konnte nicht so sorgfältig wie üblich arbeiten. ☐ Ja ☐ Nein 8. Inwieweit haben die Schmerzen Sie in den vergangenen 4 Wochen bei der Ausübung Ihrer Alltagstätigkeiten zu Hause und im Beruf behindert? <table border="1"> <tr> <td>☐ Überhaupt nicht</td> <td>☐ Ein bisschen</td> <td>☐ mäßig</td> <td>☐ ziemlich</td> <td>☐ sehr</td> </tr> </table> In diesen Fragen geht es darum, wie Sie sich fühlen und wie es Ihnen <i>in den vergangenen 4 Wochen</i> gegangen ist. (Bitte kreuzen Sie in jeder Zeile die Zahl an, die Ihrem Befinden am ehesten entspricht). Wie oft waren Sie in den vergangenen 4 Wochen: 9. ruhig und gelassen? <table border="1"> <tr> <td>☐ immer</td> <td>☐ meistens</td> <td>☐ ziemlich oft</td> <td>☐ manchmal</td> <td>☐ selten</td> <td>☐ nie</td> </tr> </table> 10. voller Energie? <table border="1"> <tr> <td>☐ immer</td> <td>☐ meistens</td> <td>☐ ziemlich oft</td> <td>☐ manchmal</td> <td>☐ selten</td> <td>☐ nie</td> </tr> </table> 11. entmutigt und traurig? <table border="1"> <tr> <td>☐ immer</td> <td>☐ meistens</td> <td>☐ ziemlich oft</td> <td>☐ manchmal</td> <td>☐ selten</td> <td>☐ nie</td> </tr> </table> 12. Wie häufig haben Ihre körperliche Gesundheit oder seelischen Probleme in den vergangenen 4 Wochen Ihre Kontakte zu anderen Menschen (Besuche von Freunden, Verwandten usw.) beeinträchtigt? <table border="1"> <tr> <td>☐ immer</td> <td>☐ meistens</td> <td>☐ ziemlich oft</td> <td>☐ manchmal</td> <td>☐ selten</td> <td>☐ nie</td> </tr> </table>	☐ Ausgezeichnet	☐ Sehr gut	☐ Gut	☐ Weniger gut	☐ Schlecht	☐ stark eingeschränkt	☐ etwas eingeschränkt	☐ überhaupt nicht eingeschränkt	☐ stark eingeschränkt	☐ etwas eingeschränkt	☐ überhaupt nicht eingeschränkt	☐ Überhaupt nicht	☐ Ein bisschen	☐ mäßig	☐ ziemlich	☐ sehr	☐ immer	☐ meistens	☐ ziemlich oft	☐ manchmal	☐ selten	☐ nie	☐ immer	☐ meistens	☐ ziemlich oft	☐ manchmal	☐ selten	☐ nie	☐ immer	☐ meistens	☐ ziemlich oft	☐ manchmal	☐ selten	☐ nie	☐ immer	☐ meistens	☐ ziemlich oft	☐ manchmal	☐ selten	☐ nie
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☐ immer	☐ meistens	☐ ziemlich oft	☐ manchmal	☐ selten	☐ nie																																				

VITdAL@ICU - Study

Listing of external hospitals or hospital-like institutions¹

- LKH Graz West
- KH der Elisabethinen Graz
- KH der Barmherzigen Brüder Graz Eggenberg
- KH der Barmherzigen Brüder Graz Marschallgasse
- Privatklinik Kastanienhof Graz
- LKH Hartberg
- LKH Hörgas-Enzenbach
- LKH Deutschlandsberg
- LKH Voitsberg
- LKH Bad Radkersburg
- LKH Bruck an der Mur
- LKH Weiz
- LKH Wagna
- LKH Mürzzuschlag-Mariazell
- LKH Judenburg-Knittelfeld
- LKH Rottenmann
- Krankenhausverbund Feldbach-Fürstenfeld
- Marienkrankenhaus Vorau
- LKH Güssing
- LKH Oberwart
- KH Gmunden
- Landeskrankenhaus Wiener Neustadt
- KH Rudolfstiftung Wien
- KH Rosenhügel
- LKH Horn
- KH Spital an der Drau
- LKH Villach
- LKH Klagenfurt
- KH Olmütz (Tschechien)

¹ This listing comprises all hospitals or hospital-like institutions to which participants of the VITdAL@ICU study have been transferred to (transfer to general ward).

Listing of external health care and rehabilitation facilities²

Rehabilitation facilities

- Privatklinik Laßnitzhöhe
- Albert-Schweitzer-Klinik (geriatric hospital)
- Kurklinik Maria Theresia Bad Radkersburg
- Landesnervenklinik Sigmund Freud Graz (neurologic rehabilitation ward)
- LKH Hörgas-Enzenbach (acute geriatric remobilisation ward)
- KH Judendorf-Straßengel (neurologic rehabilitation ward)
- LKH Judenburg-Knittelfeld (neurologic rehabilitation ward)
- LKH Rottenmann (remobilisation ward)
- LKH Klagenfurt (neurologic rehabilitation ward)
- Rehabilitationsklinik Tobelbad
- SKA Rehabilitationszentrum Bad Tatzmannsdorf
- SKA Rehabilitationszentrum St. Radegund
- SKA Rehabilitationszentrum Bad Ischl
- SKA Rehabilitationszentrum Bad Schallerbach

Health care facilities

- Pflegeheim Kalsdorf
- Pflegeheim Wagna
- Pflegeheim Birkfeld
- Pflegeheim Stegersbach
- Pflegeheim Haus der Barmherzigkeit Graz
- Pflegezentrum Grazerfeld Süd
- Caritasheim St. Peter am Ottersbach
- Geriatriezentrum Am Wienerwald
- Humanitasgruppe Unterpremstätten
- Kurhaus Bad Gleichenberg

² This listing comprises all health care and rehabilitation facilities to which participants of the VITdAL@ICU study have been transferred to.



Medizinische Universität Graz

Ethikkommission

Auenbruggerplatz 2, A-8036 Graz

ethikkommission@medunigraz.at

Tel.: +43 / 316 / 385-13928

Fax: +43 / 316 / 385-14348

VOTUM

gültig bis 26.03.2011

EK-Nummer: 21-214 ex 09/10 **EudraCT Nr.:** 2010-018798-39
Studientitel: VITdAL@ICU - Correction of Vitamin D deficiency in critically ill patients: a randomized, double-blind, placebo-controlled trial
Prüfer: *) Prof.Dr. H. Dobnig
 Univ.Klinik für Innere Medizin
Sponsor: (Prüfer)
CRO: -

*) Antragsteller

Die o.a. Studie wurde von der Ethikkommission erstmals in der Sitzung 06-09/10 am 15.03.2010 behandelt.

Die Ethikkommission ist zu folgendem Schluss gekommen:

Es besteht kein Einwand gegen die Durchführung der Studie in der vorliegenden Form.

Stimmberechtigte bzw. anwesende Mitglieder bei der Behandlung waren: Siehe beiliegende Liste vom 15.03.2010.

Kommissionsmitglieder, die für diesen Tagesordnungspunkt als befähigt anzusehen waren und daher gemäß Geschäftsordnung an der Entscheidungsfindung und Abstimmung nicht teilgenommen haben: Univ.Prof.Dr.Harald Dobnig, Univ.Prof.Dr.Andrea Berghold

Zur Beurteilung eingereichte Dokumente:

Dokumente eingegangen am 17.02.2010, begutachtet in der Sitzung 06-09/10 am 15.03.2010

✓ Antragsformular mit Kurzfassung		10.02.2010
✓ EudraCT-Formular		10.02.2010
✓ Protokoll	1.0	19.02.2010
✓ Prüfbogen (Case Report Form, CRF)	1.0	05.02.2010
Informed Consent Form	V 1.0	19.02.2010
Informed Consent Form	Nichteinwilligungsfähige, V 1.0	19.02.2010
✓ Begründung des Einschlusses nicht-einwilligungsfähiger Intensivpatienten		10.02.2010

Nachgereichte Dokumente:

Dokumente eingegangen am 17.03.2010 (in der nächsten Begutachtung mitbegutachtet)

✓ Informed Consent Form	V 1.0	17.03.2010
✓ Informed Consent Form	Nichteinwilligungsfähige, V 1.1	17.03.2010

Dokumente eingegangen am 26.03.2010, begutachtet im 'expedited Review' am 26.03.2010

✓ Versicherungsbestätigung (Wiener Städtische)	08-N811.957	26.03.2010
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Die Ethikkommission geht – rechtlich unverbindlich – davon aus, dass es sich um eine klinische Prüfung nach AMG handelt.

EK-Nummer: 21-214 ex 09/10

Votum

Seite 1 von 2

Medizinische Universität Graz, Universitätsplatz 3, A-8010 Graz. www.medunigraz.at

Rechtsform: Juristische Person öffentlichen Rechts gem. Universitätsgesetz 2002. Information: Mitteilungsblatt der Universität und www.medunigraz.at. DVR-Nr. 210 9494.
 UID: ATU 576 111 79. Bankverbindung: Bank Austria Creditanstalt BLZ 12000 Konto-Nr. 500 940 400 04, Raiffeisen Landesbank Steiermark BLZ 38000 Konto-Nr. 49510

Das Votum der Ethikkommission berührt in keiner Weise die alleinige Verantwortung der Prüferin / des Prüfers / der Prüfer für die ordnungsgemäße Durchführung der Studie unter Einhaltung aller einschlägiger gesetzlicher Bestimmungen und Richtlinien.

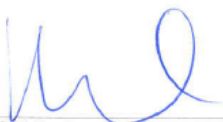
Weiters machen wir darauf aufmerksam, dass der Kommission unverzüglich zu melden sind:

- Abweichungen vom Protokoll aus Sicherheitsgründen oder Protokolländerungen
- Änderungen, die das Risiko der Teilnehmer/-innen erhöhen oder die Durchführung der Studie wesentlich beeinflussen
- Mutmaßliche unerwartete schwerwiegende Nebenwirkungen - SUSARs (AMG-Studien ab 1.5.2004) oder schwerwiegende unerwünschte Ereignisse - SAEs (andere Studien)
- Jegliche Information über sonstige Umstände, die die Sicherheit der Teilnehmer/-innen oder die Durchführung der Studie beeinträchtigen können

Begründung: Es handelt sich um eine relevante Fragestellung, die mit geeigneter Methodik beantwortet werden soll. Die vom Antragsteller vorgenommene Bewertung des Nutzen/Risiko-Verhältnisses ist plausibel.

Dieses Votum gilt für ein Jahr ab dem Datum der Ausstellung. Bei längerer Studiendauer ist rechtzeitig vor Ablauf der Gültigkeit des Votums ein Zwischenbericht vorzulegen (Berichtsformular), um eine etwaige Verlängerung zu erlangen.

Graz, 26. März 2010



Univ.Prof. DI Dr. Peter H. Rehak
Vorsitzender

Univ.Prof. DDr. Hans-Peter Kapfhammer
Stv. Vorsitzender

Achtung: Bitte bei allen das Projekt betreffende Schreiben oder telefonischen Anfragen die EK-Nummer angeben!

EK-Nummer: 21-214 ex 09/10

Votum

Seite 2 von 2

Medizinische Universität Graz, Universitätsplatz 3, A-8010 Graz. www.medunigraz.at

Rechtsform: Juristische Person öffentlichen Rechts gem. Universitätsgesetz 2002. Information: Mitteilungsblatt der Universität und www.medunigraz.at. DVR-Nr. 210 9494.
UID: ATU 575 111 79. Bankverbindung: Bank Austria Creditanstalt BLZ 12000 Konto-Nr. 500 948 400 04, Raiffeisen Landesbank Steiermark BLZ 39000 Konto-Nr. 49510.

Ethikkommission der
Medizinischen Universität Graz
LKH-Universitätsklinikum
Auenbruggerplatz 2, 3.OG
A-8036 Graz

Univ.Prof. Dr.Harald Dobnig
Universitätsklinik für Innere Medizin
Abteilung für Endokrinologie und Nuklearmedizin
Auenbruggerplatz 15
8036 Graz
Austria
harald.dobnig@medunigraz.at

10.2.2010

**Begründung des Einschlusses nicht-einwilligungsfähiger Intensivpatienten,
Darstellung der Finanzierung und Interessenskonflikte zur Studie**

"VITDAL@ICU - Correction of vitamin D deficiency in critically ill patients: a randomized, double-blind, placebo-controlled trial"

Sehr geehrter Univ.Prof. DI Dr. Rehak,

Der Einschluss nicht-einwilligungsfähiger Patienten im Rahmen des vorgeschlagenen Projektes begründet sich auf folgende Argumente:

- 1) Häufig sind es gerade nicht-einwilligungsfähige Patienten, die in einem gesundheitlich besonders betroffenen Allgemeinzustand sind im Unterschied zu „aufklärungsfähigen“ Patienten; d.h. es könnte potenziell gerade diese Patientengruppe von einer Korrektur eines Vitamin D Mangels am deutlichsten profitieren. Es geht hierbei um Effekte, die für die Prävention/Therapie nosokomialer Infektionen/Sepsis/Septischer Schock durch eine günstige Beeinflussung der Barrierefunktion der Schleimhäute erwartet werden, aber auch um die oft schwierige Entwöhnungszeit im Anschluss an eine maschinelle Beatmung, die u.a. auf eine progressive Verschlechterung der Atemmuskulatur zurückzuführen ist. Von Vitamin D sind positive Auswirkungen auf muskuläre Parameter in doppelblind-randomisierten Studien gut bekannt. Die Studienmedikation könnte gerade in dieser Population aufgrund einer Verbesserung des intrazellulären muskulären Kalziumgehaltes einen günstigen Effekt auf die Atemmuskulatur und das nachfolgende „weaning“ haben.
- 2) Patienten auf der Intensivstation sind gerade in den ersten Tagen aufgrund des neuen Ambiente, den eigenen Ängsten und des „Ausgeliefertseins“ in einer außergewöhnlichen

Grenzsituation ihres Lebens und vielmals im engeren Sinne nicht als „informed-consent“-fähig zu betrachten.

3) Bei dem vorliegenden Projekt handelt es sich zweifelsohne um ein „low-risk“ Projekt, das aus unserer Sicht die Notwendigkeit der sofortigen Aufklärung relativiert, da durch die Intervention ein bestehender Vitamin D Mangel solcherart ausgeglichen wird, dass die 25-hydroxyvitamin D Werte im physiologischen, also „Vitamin D-suffizienten“ Bereich zu liegen kommen. Nach bestem Wissen ist diese Einmaldosis, und das konnte auch in einem eigenen kleinen doppelblind-randomisierten Pilotversuch bei Intensivpatienten bestätigt werden, eine sichere Dosis, die keine negativen Auswirkungen auf die Physiologie zeigt. In allen bislang publizierten Studien, bei denen eine einmalige Aufsättigungsdosis von Vitamin D3 verwendet wurde, wie auch bei der eigenen Untersuchung konnte kein einziger Fall einer Hyperkalziämie oder eines supraphysiologisch hohen 25OHD Wertes gefunden werden. Sobald die Patienten einen psychisch stabilen Zustand erreicht haben, werden sie von uns aufgeklärt werden.

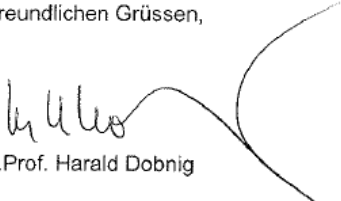
Erklärung von Interessenskonflikten, Darstellung der Finanzierung

Die Firma Fresenius-Kabi ist der Hersteller des Vitamin D3 Monopräparates „OleovitD3“, das in der angesuchten Studie Verwendung finden soll.

Neben dem zur Verfügung stellen der Studienmedikation hat sich Fresenius-Kabi auf unser Ansuchen hin bereit erklärt, das vorliegende Projekt durch Übernahme der Finanzierung von Dr. Christian Schnedl (Dissertant, Doktoratsstudium der Medizinischen Wissenschaften) sowie einer Teilzeitkraft über vorerst 2 Jahre in der Höhe von insgesamt 94.000.- Euro zu unterstützen. Frau OA. Dr. Helga Warnkross wird ehrenamtlich an diesem Projekt mitarbeiten.

Mit Fresenius-Kabi wurde eine schriftliche Vereinbarung über dieses Projekt getroffen, die im Rektorat aufliegt, und die uns freie Hand lässt sowohl bei der Datenverwaltung, wie auch der Publikation der Ergebnisse. Zu keinem Zeitpunkt wird Fresenius-Kabi Einblick in die Originaldaten haben.

Mit freundlichen Grüßen,


Univ.Prof. Harald Dobnig